UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE FARMÁCIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

MICROPARTÍCULAS CONTENDO PANTOPRAZOL SÓDICO: DESENVOLVIMENTO TECNOLÓGICO, PRODUÇÃO EM ESCALA PILOTO E AVALIAÇÃO BIOLÓGICA

RENATA PLATCHECK RAFFIN

PORTO ALEGRE, 2007.

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE FARMÁCIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

MICROPARTÍCULAS CONTENDO PANTOPRAZOL SÓDICO: DESENVOLVIMENTO TECNOLÓGICO, PRODUÇÃO EM ESCALA PILOTO E AVALIAÇÃO BIOLÓGICA

Tese apresentada por **Renata Platcheck Raffin** para obtenção do TÍTULO DE DOUTOR em Ciências Farmacêuticas

Orientadora: Profa. Dra. Sílvia Stanisçuaski Guterres

Tese apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas, em nível de Doutorado - Produção e Controle de Qualidade de Produtos Farmacêuticos - da Faculdade de Farmácia da Universidade Federal do Rio Grande do Sul e aprovada em 05.12.2007, pela Comissão/Banca Examinadora constituída por:

Prof. Dr. Edison Luis Santana Carvalho
Universidade Luterana do Brasil

Profa. Dr. Maria Helena Andrade Santana
Universidade de Campinas

Profa. Dr. Nadia Maria Volpato
Universidade Federal do Rio Grande do Sul

Profa. Dr. Valquiria Linck Bassani
Universidade Federal do Rio Grande do Sul

R137m Raffin, Renata Platcheck

Micropartículas contendo pantoprazol sódico: desenvolvimento tecnológico, produção em escala piloto e avaliação biológica / Renata Platcheck Raffin – Porto Alegre: UFRGS, 2007. – xxvi, 298p.: il ., gráf., tab.

Tese(doutorado). UFRGS. Faculdade de Farmácia. Programa de Pós-graduação em Ciências Farmacêuticas.

1. Micropartículas. 2. Pantoprazol. 3. Evaporação de solvente. 4. Secagem por aspersão. 4. Gastro-resistência. I. Guterres, Sílvia Stanisçuaski. II. Título.

CDU: 615.453

Bibliotecária responsável:
Margarida Maria Cordeiro Fonseca Ferreira CRB10/480

Aos meus pais, irmãos e namorado que me apoiaram em todos momentos e me incentivaram a aceitar desafios.

AGRADECIMENTOS

À Profa. Silvia S. Guterres pelo incentivo à pesquisa, orientação durante todos esses anos (desde iniciação cientifica), amizade e exemplo como profissional.

À Profa. Adriana R. Pohlmann pelo exemplo de conduta profissional e pelo estímulo em todos momentos.

Ao Prof. Paolo Colombo por ter acreditado em meu trabalho e por ter me recebido em seu laboratório.

À Dra. Maria Inês Ré por ter me aberto as portas de seu laboratório e por ter me oferecido a oportunidade de trabalhar também na área de engenharia.

Aos professores Teresa Dalla Costa, Elfrides Schapoval e Cláudio Natalini pela colaboração científica.

Aos bolsistas deste projeto, Letícia M. Colomé, Cristiane R. D. Hoffmeister e Denise S. Jornada pela dedicação e amizade.

Aos colegas do Laboratório 405, IPT e Laboratorio di Tecnologia Farmaceutica (UNIPR) pela amizade, apoio e pela troca de conhecimentos.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelas bolsas de estudo de doutorado e doutorado sanduíche.

A todos aqueles que direta ou indiretamente contribuíram para a realização desta tese.

APRESENTAÇÃO

De acordo com as normas vigentes no Estatuto do Programa de Pós-Graduação em Ciências Farmacêuticas, da Universidade Federal do Rio Grande do Sul, a presente tese foi redigida na forma de capítulos, para uma melhor organização e discussão dos resultados obtidos. Assim, este exemplar está organizado da seguinte maneira:

- Introdução, incluindo a formulação da hipótese e justificativa do trabalho;
- Objetivos geral e específicos;
- Revisão de literatura;
- Capítulos 1-12: Artigos aceitos e submetidos a periódicos científicos que se referem às diferentes etapas do trabalho realizado;
- Considerações finais;
- Conclusões;
- Referências bibliográficas.

SUMÁRIO

LISTA DE FIGURAS

LISTA DE TABELAS

RESUMO

Micropartículas contendo pantoprazol foram preparadas e caracterizadas a fim de se obter sistemas multiparticulados gastro-resistentes. O trabalho foi delineado buscando-se a melhor técnica de preparação das micropartículas, assim como o estudo do processo, aumento de escala e avaliação biológica. A metodologia analítica para quantificação do pantoprazol nas micropartículas foi desenvolvida e validada. O método mostrou-se seletivo, linear, preciso e exato. A estabilidade do pantoprazol em tampão fosfato pH 7,4 foi avaliada para verificar a viabilidade da utilização deste tampão como meio de dissolução. O pantoprazol apresentou-se estável durante 6 h e considerado adequado para estudos de dissolução. A primeira técnica utilizada na preparação de micropartículas foi a evaporação de solvente, utilizando uma emulsão O/O. O polímero utilizado foi Eudragit® S100. As micropartículas apresentaram diâmetro de 56 µm e, segundo análises de DSC e IV, o fármaco apresentou-se molecularmente disperso no polímero. As micropartículas apresentaram atividade anti-ulcerogênica em modelo de ulceração gástrica em ratos por etanol, enquanto a solução aquosa de pantoprazol não apresentou atividade. Estas micropartículas foram comprimidas e permaneceram intactas no interior dos comprimidos. Quanto à proteção do pantoprazol em meio ácido, 61 % da quantidade inicial do fármaco permaneceram estáveis após 30 min em meio ácido. Uma segunda formulação utilizando a mesma técnica foi preparada coma a adição de poli(ε-caprolactona) à formulação de Eudragit® S100. O objetivo da inclusão do segundo polímero foi a obtenção de uma blenda capaz de promover liberação controlada do pantoprazol e ao mesmo tempo conferir gastro-resistência. Esta formulação também apresentou atividade anti-ulcerogênica *in vivo*. Os comprimidos contendo estas micropartículas apresentaram liberação controlada e gastro-resistência. A segunda técnica avaliada no desenvolvimento de micropartículas contendo pantoprazol foi a secagem por aspersão. Micropartículas contendo Eudragit® S100 foram produzidas e apresentaram bom rendimento, eficiência de encapsulação e estabilização do pantoprazol em meio ácido. As micropartículas foram avaliadas quanto a permeação intestinal utilizando modelo de intestino invertido. A permeação intestinal foi diretamente proporcional à liberação em tampão fosfato pH 7,4, estabelecendo uma correlação de nível A. Devido a esses fatores, estas micropartículas foram selecionadas para preparação em escala piloto. Diferentes condições operacionais foram testadas e o diâmetro médio das partículas variou entre 6.7 e 24.5 µm, influenciado pela concentração inicial de sólidos. As condições operacionais que produziram micropartículas com maior gastro-resistência foram selecionadas para estudo de estabilidade. As micropartículas apresentaram-se estáveis por 6 meses em condições aceleradas de armazenamento e não adsorveram umidade ao longo do tempo. A avaliação *in vivo* demonstrou a atividade anti-ulcerogênica desta formulação. No entanto, a formulação apresentou baixa densidade e fluxo pobre, dificultando a granulação e compressão. A forma farmacêutica desenvolvida foram aglomerados ou *soft pellets*, contendo micropartículas de pantoprazol e um excipiente de manitol e lecitina preparado por *spray-drying*. Os aglomerados apresentaram adequadas características de fluxo e rápida desintegração não afetando a gastro-resistência das micropartículas. A técnica de *spray-drying* também foi utilizada com uma blenda de Eudragit® S100 e HPMC, também visando uma liberação controlada do pantoprazol. As micropartículas apresentaram alta eficiência de encapsulação e também reduziram a formação de úlceras gástricas por etanol em ratos. Os comprimidos contendo micropartículas preparadas com a blenda apresentaram mais de 90 % de estabilização em meio ácido. Este processo também foi escalonado e as melhores condições operacionais determinadas. O processo foi reprodutível em relação ao diâmetro, densidade, eficiência de encapsulação e gastro-resistência. Esta formulação foi estável por 6 meses a 40 °C e 75 % de umidade. As quatro formulações descritas neste trabalho foram testadas quanto à estabilização do pantoprazol frente à luz UVC. O pantoprazol demonstrou ser fotoinstável tanto em solução metanólica como sólido e apenas as micropartículas preparadas com Eudragit® S100 aumentaram a fotoestabilidade do pantoprazol. Baseado no conjunto de resultados, os aglomerados contendo micropartículas de Eudragit® S100 foram selecionadas para serem testadas quanto a sua farmacocinética, em comparação com o comprimido comercial de referência. Os aglomerados demonstraram ser mais rapidamente absorvidos, reduzindo o T_{max} de 90 para 43 min, mantendo mesma biodisponibilidade oral. Desta forma, podemos concluir que o pantoprazol foi microencapsulado com sucesso e as micropartículas aumentaram a estabilidade do fármaco em meio ácido e frente à luz, além de reduzir o tempo para atingir a concentração máxima do mesmo. **Palavras-chaves:** Micropartículas,pantoprazol, evaporação de solvente, secagem por aspersão, gastro-resistência.

ABSTRACT

MICROPARTCLES CONTANING SODIUM PANTOPRAZOLE: TECHNOLOGICAL DEVELOPMENT, SCALE UP AND BIOLOGICAL ACTIVITY

The aim of the thesis is to develop, characterize and evaluate two drug delivery systems containing gastro-resistant pantoprazole microparticles, one for the prompt dissolution and the other one for controlled release of pantoprazole. First, an analytical method was developed and validated for the quantification of sodium pantoprazole by HPLC. The stability of pantoprazole in phosphate buffer at pH 7.4 was also evaluated during 22 days. The results showed that the method was specific, linear, precise and exact. Pantoprazole was stable in phosphate buffer pH 7.4 for 6 h. Then, the solvent evaporation technique was applied in the preparation of gastro-resistant pantoprazole-loaded microparticles using an O/O emulsion. Furthermore, tablets containing the microparticles were also investigated. Microparticles presented spherical and smooth morphologies and they remained intact in the inner surface of tablets. DSC and IR analyses showed that pantoprazole was physically and molecularly dispersed in the polymer. *In vivo* anti-ulcer evaluation showed that the microparticles were able to protect the rat stomachs against ulcer formation by ethanol, while the drug aqueous solution did not present activity. Concerning the acid protection, tablets showed a satisfactory drug protection in acid medium (61 % after 30 min). As a second formulation, microparticles of poly(ε-caprolactone) blended with Eudragit® S100 were prepared in order to provide controlled release and gastro-resistance. This formulation showed *in vivo* protection of stomachs against ulceration caused by ethanol in rats. These microparticles were tableted and the tablets demonstrated slower drug release and higher acid protection than the microparticles before tableting. The spray drying technique was also used to prepare pantoprazole-loaded microparticles. Microparticles containing pantoprazole and Eudragit S100® presented high encapsulation efficiency and good stabilization in acid medium. Microparticles prevented ulceration by ethanol *in vivo*. These microparticles showed more adequate characteristics for the preparation of a drug delivery system than the one prepared by solvent evaporation. The physical characteristics of pantoprazole microparticles produced in different spray dryers and operational conditions were investigated. In all conditions tested it was possible to obtain powders that presented spherical shape microparticles, with mean sizes from 6.7 to 24.5 µm. The size was mainly affected by the initial feed concentration (2.2 or 6.6% w/w). All powders presented very poor flow. Under accelerated conditions of storage, the selected microparticles were stable for 6 months. The microparticles couldn't be tableted and then, the microparticles were agglomerated with mannitol/lecithin powder. The agglomerates presented good technological properties and did not influence the drug release and the gastro-resistance of the pantoprazole microencapsulated. The spray drying technique was also used to prepare microparticles aiming to provide gastro-resistance and to control the drug release, using a blend of Eudragit S100® and HPMC. DSC analyses showed that the drug is molecularly dispersed in the microparticles, and i*n vivo* anti-ulcer evaluation demonstrated that microparticles were effective in protecting stomach against ulceration. *In vitro* gastro-resistance study showed that the microparticles stabilized pantoprazole in 62.0 % and tablets containing the microparticles in 97.5 % and provided a controlled release of the drug. This formulation was also studied in different scales of production and spray-drier designs. The microparticles were produced in different spray-driers and operational conditions at laboratory and pilot scales. The microparticles produced with two fluid nozzle atomizer and 196 kPa were prepared in three consecutive days for the process validation. The powders showed reproducible diameter, low polydispersity, similar bulk densities, encapsulation efficiency and gastro-resistance. These microparticles were evaluated for their accelerate stability. The microparticles presented less than 5 % of degradation after 180 days at 40 °C and 75 % of RH. These same microparticles were agglomerated using mannitol/lechitin spray-dried as excipient. Different amounts of lecithin and mannitol were used, but only one formulation did not alter the pantoprazole release from the microparticles, as well as the gastro-resistance. The four different formulations of microparticles characterized in this study were tested for the stabilization of pantoprazol under UVC light. Only the microparticles prepared with Eudragit® S100 improved the drug photostability. Based on the results, the agglomerates containing microparticles prepared by spray-drying with Eudragit® S100 were selected for the pharmacokinetics study in dogs. The agglomerates presented similar AUC than the reference tablet, but reduced the Tmax. In conclusion, pantopazole-loaded microparticles were successfully prepared and the stability of pantoprazol in acid medium and under light was improved. Furthermore, the time to peak plasma was reduced. **Key words:** Microparticles, pantoprazole, solvent evaporation, spray drying, gastro-resistance.

INTRODUÇÃO

O pantoprazol (PAN) é um fármaco anti-ulceroso, cuja classe terapêutica movimenta, aproximadamente, 6 bilhões de dólares ao ano no mercado mundial. É indicado para o tratamento de úlceras do trato gastrintestinal, no tratamento do refluxo gastroesofágico, na síndrome de Zollinger-Ellison e, associado a antibióticos, na erradicação do *Helicobacter pylori* (CHEER *et al.*, 2003).

Seu mecanismo de ação é a inibição da bomba de prótons, último passo da secreção ácida nas células parietais do estômago. Para tal, converte-se em meio ácido em sua forma ativa, que se liga a um resíduo de cisteína da bomba. Para converter-se apenas nos canalículos das células parietais, é necessária a passagem pelo lúmen do estômago sem degradação. Devido a isso, é comercializado em sua forma de sal sódico, como solução injetável e como comprimidos revestidos.

No entanto, formas farmacêuticas sólidas, como comprimidos revestidos, apresentam desvantagens como maior tempo de desintegração e dissolução, distribuição não uniforme pelo trato gastrintestinal, impossibilidade de fracionamento da dose e maior risco de desestabilização do sistema por rompimento do filme polimérico (LIN e KAO, 1991).

Devido a isso, outras formas farmacêuticas vêm sendo desenvolvidas para melhorar a distribuição, absorção e biodisponibilidade de fármacos, principalmente, daqueles ácido-lábeis, como peptídeos, proteínas e oligonucleotídeos. Uma das formas mais estudadas e com grande número de técnicas de preparação descritas na literatura são as micropartículas poliméricas (MP) (AMORIM *et al.*, 2001). Estudos sobre essas iniciaram no final da década de 70, mas ganharam mais força com a utilização de polímeros biodegradáveis e com propriedades especiais, como gastrorresistência, liberação controlada ou retardada.

A grande dificuldade, em geral, na preparação de MP é a encapsulação de moléculas hidrofílicas, como é o caso do PAN, pois estas técnicas geralmente utilizam água, o que leva a uma partição da molécula e baixas taxas de encapsulação do fármaco. Neste contexto, duas técnicas se destacam pela possibilidade de conduzirem a maiores taxas de encapsulação de substâncias hidrofílicas e de sua facilidade de aplicação e transposição de escala: a evaporação de solvente (O'DONNELL e MCGINITY, 1997) e a secagem por aspersão (PALMIERI *et al.*, 2001).

A evaporação de solvente utiliza o princípio de uma emulsão de solvente volátil com uma fase externa pouco volátil (água ou óleo mineral ou parafina líquida). O polímero deve ser solúvel apenas no solvente orgânico, sendo que quando este é evaporado, o primeiro precipita formando esferas de tamanho micrométrico, que são recuperadas por filtração. Para fármacos hidrossolúveis, evita-se a utilização de água na formulação, aumentando-se, assim, a possibilidade de encapsulação da substância pelo polímero (JAIN *et al.*, 1998).

A preparação por aspersão ou *spray-drying* consiste em nebulizar uma solução de polímero e fármaco, formando micropartículas homogêneas. Como há a eliminação total do solvente, pode-se usar a água para encapsular fármacos hidrossolúveis. É uma técnica rápida e de uma única etapa. Caso o polímero seja solúvel em soluções aquosas, não há a necessidade de utilização de solventes orgânicos (PALMIERI *et al.*, 2002).

A secagem por aspersão já é bastante utilizada tanto na indústria alimentícia quanto na farmacêutica para a produção de pós (BURKE *et al.*, 2004). Os estudos em escala laboratorial são inúmeros quanto à produção de micropartículas, porém dificilmente encontra-se estudos de formulação e de parâmetros de processo para o aumento da escala para piloto ou industrial. Algumas condições são necessárias para a viabilidade da produção industrial como alto rendimento e preferência da não utilização de solventes orgânicos (ONEDA e RÉ, 2003). Além disso, as características das partículas produzidas influenciam diretamente na aplicabilidade do produto como o diâmetro de partícula, área superficial e características reológicas aceitáveis para a produção de formas farmacêuticas sólidas como comprimidos e cápsulas (BIRCHAL *et al.,* 2005).

Adicionalmente, a seleção dos excipientes para o desenvolvimento de uma forma farmacêutica sólida contendo micropartículas é essencial para garantir a integridade das partículas, assim como promover a desintegração rápida a fim de manter as vantagens das micropartículas como menor tempo de residência gástrica e uniforme distribuição pelo trato gastrintestinal (SHIMIZU *et al.,* 2003).

Considerando as características do PAN, principalmente a sua degradação em meio ácido e a hidrossolubilidade, é objetivo do presente trabalho sua estabilização mediante o desenvolvimento de uma forma farmacêutica sólida contendo MP preparadas à base de polímeros gastrorresistentes, através das técnicas de evaporação de solvente e *spray-drying* e a caracterização *in vitro* e *in vivo* das micropartículas produzidas em escala laboratorial e escala piloto. Cabe ressaltar que MP contendo PAN ainda não foram descritas na literatura. Encontram-se apenas patentes em diferentes países de outras formas de estabilização do PAN e substitutos benzimidazólicos, como *pellets*, géis e comprimidos de desintegração imediata.

OBJETIVOS

OBJETIVOS

Objetivo geral

Preparar e caracterizar *in vitro* e *in vivo* micropartículas contendo pantoprazol sódico pelas técnicas de evaporação de solvente e secagem por aspersão a fim de obterem-se sistemas multiparticulados gastrorresistentes, em escala laboratorial e piloto.

Objetivos específicos

Preparar micropartículas pela técnica de evaporação de solvente em escala laboratorial usando Eudragit S100® ou sua blenda com poli(ε-caprolactona) e caracterizá-las quanto às taxas de recuperação do fármaco, morfologia e perfis de liberação.

Preparar micropartículas pela técnica de secagem por aspersão em escala laboratorial usando Eudragit S100® ou sua blenda com hidroxipropilmetilcelulose e caracterizá-las quanto às taxas de recuperação do fármaco, morfologia e perfis de liberação.

Preparar comprimidos contendo as micropartículas (sem adjuvantes) e estudar a influência da compressão sobre o perfil de liberação do pantoprazol.

Avaliar a proteção ao meio gástrico das micropartículas e dos comprimidos, através de ensaio de liberação *in vitro*.

Avaliar *in vivo* a ação anti-ulcerosa das micropartículas em ratos, após indução de úlceras por etanol.

Estudar os parâmetros do processo de secagem por aspersão para produção de micropartículas em escala piloto.

Caracterizar das micropartículas através da morfologia e de estudos de liberação *in vitro* para escolha dos melhores parâmetros.

Preparar uma forma farmacêutica final com características tecnológicas adequadas a produção.

Avaliar a biodisponibilidade dessa forma farmacêutica contendo micropartículas em cães.

REVISÃO DE LITERATURA

1 Pantoprazol

O pantoprazol é um fármaco relativamente novo, seus primeiros estudos datam de 1989 (ISI Web of Science®), foi aprovado pelo FDA em 2000 (Protonix® comprimidos revestidos) e tem especialidades farmacêuticas registradas na ANVISA desde 1995. É um derivado benzimidazólico, mais especificamente é o 5-(difluorometoxi)-2-[[3,4-dimetoxi-2-piridinil)metil]sulfinil]-1H-benzimidazol (Figura 1), análogo do omeprazol e do lansoprazol. É veiculado na forma de sal sódico sesquihidratado. Este fármaco é um inibidor da bomba de prótons, indicado para o tratamento de úlceras do trato digestivo, refluxo gastro-esofágico e na erradicação da *Helicobacter pylori* e no tratamento da doença de Zollinger-Ellison (BEIL *et al.*, 1992; FITTON *et al.,* 1996; JUNGKICKEL, 2000).

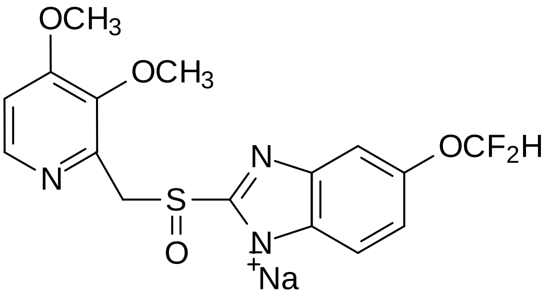


Figura 1. Fórmula estrutural do pantoprazol sódico.

Seu mecanismo de ação é a inibição irreversível da H^+,K^+ - adenosina trifosfatase (ATPase), a qual é o passo final da secreção de ácido pelas células da mucosa gástrica (BASTAKI *et al.*, 2000; KATZ, 2000). O pantoprazol é um pró-fármaco, cuja espécie ativa é uma sulfenamida tetracíclica que se liga covalentemente ao resíduo de cisteína 822, que altera a conformação da bomba de prótons e garante meia vida de recuperação da secreção ácida de 46 h. Esse tempo é muito superior ao encontrado para análogos (20 h), pois a única maneira de voltar a secreção gástrica é a síntese *de novo* de ATPases (SACHS *et al.,* 2003).

Em estudos recentes, foi mostrado que 90 % dos pacientes com úlcera duodenal e 70 % dos com úlcera gástrica são *H. pylori* positivos. Devido a isso, a erradicação desse microorganismo é essencial no tratamento desses pacientes. Para tal, uma terapia tripla é indicada, utilizando-se pantoprazol associado a antimicrobianos como claritromicina, amoxicilina ou metronidazol (CHEER *et al.*, 2003).

Zollinger-Ellison é uma síndrome associada à hipersecreção gástrica, resultando em úlceras pépticas fulminantes. Para tratamento, é necessária a administração de inibidores da bomba de prótons por longos períodos. Segundo METZ e colaboradores (2003), o pantoprazol mostrou-se efetivo no tratamento dessa síndrome, com doses de 80 a 240 mg/dia por 27 meses e com boa tolerabilidade.

Outra utilização na clínica médica do pantoprazol é no tratamento do refluxo gastro-esofágico (POOLE, 2001), que é uma doença crônica, que diminui a qualidade de vida dos pacientes, que apresentam azia constante e regurgitação. Para seu tratamento, normalmente é necessário um tratamento inicial com inibidor da bomba de prótons e, após fase aguda, é recomendável seguir com doses baixas do medicamento. O pantoprazol é o único fármaco dessa classe que não apresenta interações com o metabolismo de outros fármacos (antiácidos, cafeína, carbamazepina, diazepam, diclofenaco, digoxina, metoprolol, nifedipina, piroxican, teofilina e varfarina) (STANGHELLINI, 2003; CHEER *et al.*, 2003). Por outro lado, um estudo realizado no banco de dados do FDA (LABENZ *et al.*, 2003) concluiu que o pantoprazol não é mais seguro que outros derivados benzimidazólicos e também possui interação com antagonistas da vitamina K.

Quanto à farmacocinética, apresenta biodisponibilidade absoluta por via oral (comprimidos revestidos) de 77 % (entre 67 e 89 %), com pico de concentração plasmática $C_{máx}$ de 2,52 mg/L (administração via oral de 40 mg) e $C_{máx}$ de 5,42 mg/L (i.v. 40 mg). Há correlação linear entre dose e parâmetros farmacocinéticos. O $t_{máx}$ é de 1 a 6 h (40 mg v.o.) e a AUC de 4,61 mg.h/L. A ligação a proteínas plasmáticas é de cerca de 98 % e o volume de distribuição de 0,15 L/kg. É completamente metabolizado pelo fígado, no citocromo P450, com $t_{1/2}$ entre 0,9 e 1,9 h. A excreção dos metabólitos ocorre por via renal (CHEER *et al.,* 2003).

Além disso, segundo CULSHAW e colaboradores (1997), o pantoprazol apresenta melhor relação custo benefício que o omeprazol e a ranitidina. Em outro estudo, realizado na Holanda (HOUT *et al.,* 2003), foi demonstrado que a relação eficácia/custo é mais vantajosa para o pantoprazol comparado com o omeprazol.

A forma utilizada do fármaco é a de pantoprazol sódico sesquihidratado (PANNa.1,5 H_2O), sendo solúvel em água, pouco solúvel em tampão fosfato pH 7,4 e insolúvel em *n*-hexano. Sua estabilidade em soluções aquosas é pH-dependente. A taxa de degradação aumenta com o abaixamento do pH. Em pH 5, a meia vida de degradação é cerca de 2,8 h e em pH 7,8 é de 220 h (JUNGNICKEL, 2000).

Para poder converter-se à sua forma ativa nos canalículos das células parietais, o pantoprazol deve passar intacto pelo estômago (local onde o pH é ácido como nos canalículos). Devido a isso, a estratégia utilizada pelos produtos no mercado para contornar o seu problema de instabilidade em meio gástrico é sua formulação na forma comprimidos revestidos com polímeros gastro-resistentes. Em relação à produção técnico-científica, foram identificadas diversas patentes recentes, e nenhum artigo indexado, a respeito de outras formas farmacêuticas propostas para contornar sua degradação em meio ácido (Patente no. DE10061135-C1 – gel para administração oral, Patente No. WO200245694-A1 – pasta para administração oral - Derwent Inovation Index® e patentes USPTO® nº 6.274.173 – formulação oral de liberação retardada; USPTO® nº 6.379.705 – preparação multi-unitária). Outras patentes propõem microesferas para administração de pantoprazol. As microesferas apresentariam entre 50 e 800 μm e seriam compostas de álcoois graxos e parafina sólida (DE19925710-A) ou triglicerídeos, ésteres de ácidos graxos e parafina sólida (WO200074654-A). As microesferas seriam administradas na forma de comprimidos, cápsulas ou supositórios.

Trabalhos recentes desenvolveram metodologias de análise para o pantoprazol por espectrofotometria (KARLJIKOVIC-RAJIC *et al.,* 2003) em formas farmacêuticas por cromatografia líquida de alta eficiência (CLAE) e polarografia (MCCLEAN *et al.* 1994; MANSOUR e SOROUR, 2001; WAHBI *et al.,* 2002), em plasma (CASS *et al.*, 2001) e em presença de sais (EKPE e JACOBSEN*.,* 1999). Também há a análise do pantoprazol por voltametria diferencial, utilizando eletrodo de carbono ou de mercúrio (ERK, 2003; RADI, 2003).

Quanto à estabilidade, EKPE e JACOBSEN (1999) estudaram a estabilidade do pantoprazol em solução aquosa contendo cloreto de sódio, ácido cítrico, citrato trissódico, citrato monossódico, bicarbonato de sódio e carbonato de cálcio, em diferentes concentrações e valores de pH. Os autores demonstraram que, em pH 4, o pantoprazol não sofre degradação apenas em água pura e na presença de NaCl (após 180 min), i.e. degrada-se totalmente em presença de ácido cítrico, citrato trissódico e monossódico e bicarbonato de sódio. Em pH 5, o pantoprazol sofre degradação de 30 % em solução com bicarbonato de sódio (0,025 M), após 180 min. Armazenado por 12 dias em pH 6 e 7, em água sem adição de sais a concentração cai para 46 e 48 %, respectivamente. Para a redução da degradação, as condições de armazenamento propostas foram: pH 10 e 4 °C. Mesmo assim, comparando-se o pantoprazol com omeprazol e lansoprazol, o primeiro mostrou-se mais estável em todas condições experimentadas. De uma forma geral, os estudos permitem a conclusão geral de que a degradação do pantoprazol aumenta com a diminuição do pH e com a presença de sais em solução.

A fim de obter uma suspensão de pantoprazol extemporânea para administração oral, a estabilidade do pantoprazol também foi avaliada em solução aquosa de bicarbonato de sódio de comprimidos triturados (DETINGER *et al.*, 2002). A solução contendo 2 mg/mL de pantoprazol foi armazenada em frasco âmbar, sob refrigeração (2 a 8 °C) por 62 dias, mantendo estável mais de 90 % da quantidade inicial de pantoprazol após esse período. Esta metodologia de preparação da solução extemporânea foi utilizada em escala maior (FERRON *et al.*, 2003). Trinta e quatro comprimidos foram triturados e suspensos em 680 mL de solução de bicarbonato a 4,2 % e mantidos em seringas plásticas. A 25 ºC a suspensão foi estável por 1 dia, a 5 ºC, por 2 semanas e a -20 ºC, por até 3 meses de armazenamento.

Visando o uso intra-venoso do pantoprazol, foi avaliada a sua estabilidade após reconstituição do pó liofilizado para injeção com solução de NaCl a 0,9 % (JOHNSON, 2005). A solução foi mantida em seringas de polipropileno à temperatura ambiente por 96 h. A concentração final de pantoprazol em solução foi de 96 %, porém a solução alterou sua cor para laranja-amarelado. O autor considerou que o grau de degradação não foi significativo, não havendo alteração da estabilidade do fármaco nas condições testadas.

Todos fármacos inibidores da bomba de prótons apresentam grupamento sulfóxido quiral em comum e são comumente administrados na forma racêmica (XIE *et al.,* 2005). BRUNI (2000) demonstrou que os valores para a barreira de racemização do pantoprazol são muito altos, mostrando ser desfavorável a interconversão dos isômeros. Este resultado mostra a viabilidade de separação dos isômeros do fármaco. Por outro lado, demonstrou que a decomposição em meio ácido é favorecida. Em trabalho complementar (CASS *et al.,* 2001), foi feita análise por cromatografia líquida de alta eficiência multidimensional do pantoprazol para separação de seus enantiômeros no plasma, o que foi possível e com rendimento superior a 93%. O metabolismo do pantoprazol em microssomas hepáticos de ratos é enantiosseletivo, o que contribui para diferenças na farmacocinética dos enantiômeros do pantoprazol após administração via oral da mistura racêmica. S-pantoprazol foi metabolizado a pantoprazol sulfona e 6-hidroxi-pantoprazol, enquanto R-pantoprazol foi metabolizado a sulfeto de 4’-O-metilPAN (XIE *et al.,* 2005).

2 Micropartículas

Micropartículas poliméricas vêm sendo estudadas como formas farmacêuticas de liberação prolongada, como estratégia para a estabilização de fármacos, proteínas e DNA recombinante, frente a agentes como luz ou pH e para mascarar características organolépticas de diversas substâncias (O’DONNELL e MCGINITY, 1997; YAMAGATA *et al.,* 2003).

As micropartículas compreendem, principalmente, as microcápsulas e microesferas. Segundo RAVI KUMAR (2000), microcápsulas podem ser definidas como partículas esféricas com tamanho entre 50 nm e 2 mm contendo uma substância como núcleo. Por sua vez, microesferas são partículas esféricas matriciais. As micropartículas ainda podem ser classificadas ainda em “microballoons”, ou “microbalões“, que são microesferas ocas, utilizadas como sistemas flutuantes que permanecem mais tempo no estômago (SATO *et al.*, 2004).

Formas farmacêuticas sólidas contendo micropartículas exigem maior investimento em sua produção, porém apresentam vantagens em comparação aos sistemas unitários, entre as quais pode-se citar a distribuição rápida e uniforme pelo trato gastrintestinal, menor variação na biodisponibilidade, menor risco de desestabilização do fármaco devido ao rompimento de um sistema unitário de ação prolongada e maior reprodutibilidade de dose, além da possibilidade de fracionamento e ajuste da dose (LIN e KAO*,* 1991; BENITA, 1996).

Segundo BECKERT e colaboradores (1996), formas multiparticuladas permitem uma rápida e uniforme distribuição por toda superfície do intestino, proporcionando menor variação na absorção. Além disso, as micropartículas permanecem menos tempo no estômago, estando menos dispostas à degradação por pH ácido. O esvaziamento gástrico de *pellets* com menos de 1.4 mm de diâmetro e densidade de ate 2.4 g/cm^3 é praticamente tão rápido quanto o esvaziamento gástrico de líquidos, ocorrendo em poucos minutos e independentemente da alimentação (SCHREIRER, 2001).

2.1 Preparação das micropartículas

Diversos métodos de microencapsulação foram desenvolvidos, como a coacervação, a secagem por aspersão, a evaporação de solvente, a polimerização interfacial em meio líquido, a preparação em leito fluidizado e a extrusão (THIES, 1996). A escolha do método depende da natureza do polímero e do fármaco, da via de administração e da duração do processo. O produto final deve apresentar as seguintes características (JAIN *et al.*, 1998):

- manutenção ou melhora da estabilidade e atividade do fármaco.
- alta eficiência de encapsulação e baixa polidispersão de tamanho.
- perfil de liberação do fármaco deve ser reprodutível.
- as micropartículas devem apresentar bom fluxo e não apresentar agregação ou aderência.

No presente trabalho, duas técnicas de preparação de micropartículas foram selecionadas para a encapsulação do pantoprazol. A primeira, a emulsificação/evaporação de solvente, já foi bastante estudada em relação aos parâmetros do processo e oferece diversas alternativas para a encapsulaçao de fármacos. A segunda, a secagem por aspersão, foi descrita mais recentemente e sua maior utilização ainda é na indústria alimentícia.

2.2 Evaporação de solvente

A técnica de evaporação de solvente foi desenvolvida nos anos 70, para a preparação de microesferas de polímeros biocompatíveis e biodegradáveis. O processo baseia-se na emulsificação de um solvente volátil contendo fármaco e polímero em uma fase externa (água ou óleo) na qual o polímero é insolúvel. A emulsão é então submetida à agitação e evaporação do solvente, levando à precipitação do polímero e do fármaco, formando micropartículas, que são filtradas e secas sob condições apropriadas (JAIN *et al.*, 1998). Sua principal vantagem frente à secagem por aspersão é o fato de não haver necessidade de equipamentos especializados, apenas de uma agitação constante. Além disso, uma emulsão estável é obtida sem comprometer a atividade do fármaco (KIM *et al.*, 2002). FREITAS e colaboradores (2005) enumeram como vantagens desta técnica a ausência de temperaturas elevadas, o fácil controle do tamanho de partícula de nanômetros a muitos micrometros e a possibilidade de altas taxas de recuperação de partículas, quando comparadas a outras técnicas. É considerado um método de preparação de micropartículas fácil, compatível com a maioria dos polímeros, porém é praticamente usado apenas em pesquisas laboratoriais (VARDE e PACK, 2004).

A técnica de evaporação de solvente pode ser empregada para fármacos lipofílicos e hidrofílicos. Para o primeiro caso, são preconizadas as emulsões O/A, com a utilização de solventes orgânicos imiscíveis com água, como clorofórmio ou diclorometano (O'DONNELL e MCGINITY, 1997). Também se pode adaptar metodologias de emulsões múltiplas, como A/O/A (HERRMANN *et al.*, 1998; HOMBREIRO-PÉREZ *et al.,* 2003). O polímero é solubilizado em solvente orgânico, que é emulsificado com água contendo tensoativos. O solvente da emulsão é evaporado sob agitação mecânica ou sob pressão reduzida (O'DONNELL e MCGINITY*,* 1997).

Para encapsulação de fármacos hidrofílicos, pelo menos três técnicas estão disponíveis: (1) o fármaco pode ser "hidrofobizado" utilizando uma complexação com tensoativos iônicos; (2) partículas sólidas podem ser suspensas em soluções poliméricas e (3) utilizando emulsões múltiplas ou anidras (VARDE e PACK, 2004). No terceiro caso, é necessário adaptar a metodologia, pois, caso seja feita preparação com água, o fármaco tende a permanecer na fase aquosa e não ser encapsulado pelo polímero. Utiliza-se, então, a técnica por emulsão O/O, na qual se faz o uso de solvente orgânico polar e um óleo (parafina líquida, óleo mineral ou óleos vegetais) (O'DONNELL e MCGINITY, 1997; HERRMANN *et al.,* 1998; AMORIM *et al.,* 2001; KILIÇARSLAN *et al.,* 2003).

KILIÇARSLAN e colaboradores (2003) prepararam micropartículas contendo Eudragit® RS100 e cloridrato de verapamil a partir de emulsão de acetona/metanol e parafina líquida após evaporação dos solventes. Obtiveram uma eficiência de encapsulação de mais de 80 %, um valor extremamente alto comparado com técnicas que utilizam sistemas aquosos (37 % no caso do propranolol encapsulado por PÉREZ e colaboradores em 2000, pela técnica de emulsão A/O). ROSA e colaboradores (2003) obtiveram eficiência de encapsulação para oligonucleotídeos anti-senso entre 14 e 91 % utilizando emulsão múltipla A/O/A e poli(ácido lático-co-ácido glicólico). Em outro estudo, a nicardipina foi encapsulada com poli(ε-caprolactona) por emulsão A/O. A eficiência de encapsulação foi de 15 %, com rendimento do processo de 52 % e diâmetro médio de 5,2 μm (BARBATO *et al.*, 2001).

Micropartículas contendo propiltiouracila foram preparadas por evaporação de solvente utilizando o sistema acetona/óleo mineral (OBEIDAT e PRICE, 2005). Foram obtidas micropartículas polidispersas entre 106 e 500 μm. A viscosidade da solução inicial refletiu no diâmetro de partícula. Os autores ainda obtiveram diferenças nos perfis de liberação, sendo que as partículas com maior diâmetro apresentaram liberação mais lenta.

ZHU e colaboradores (2003) prepararam micropartículas através da técnica de evaporação de solvente utilizando uma emulsão O/O modificada a fim de encapsular fluoruracila. Foram utilizadas acetonitrila e dimetilformamida na composição da fase volátil e parafina líquida como fase contínua. Os autores, comparando a preparação de micropartículas utilizando diferentes tipos de emulsão, concluíram que emulsões múltiplas tipo A/O/A são mais eficazes para a encapsulação de peptídeos e proteínas, não sendo eficientes para o fármaco em questão. Utilizando uma emulsão O/O a taxa de encapsulação obtida foi de 85 %.

Em estudo comparativo, BODMEIER e MCGINITY (1987) demonstraram que a eficiência de encapsulação pela técnica de evaporação de solvente em sistema O/A (diclorometano foi utilizado como solvente) para diferentes fármacos é dependente da solubilidade do mesmo em água. Teofilina, cafeína e ácido salicílico não puderam ser devidamente encapsulados devido à sua completa partição em água. Já quinidina, diazepam e progesterona foram encapsuladas com rendimentos satisfatórios. As fotomicrografias de micropartículas contendo quinidina mostraram que estas são esféricas, porosas e com cristais do fármaco adsorvidos.

Apesar de FREITAS e colaboradores (2005) apontarem variações da técnica para permitir o aumento de escala, como o uso de misturadores estáticos e combinação de extração e evaporação de solvente, a evaporação de solvente apresenta algumas limitações. Pode-se citar como desvantagens da técnica a dificuldade em aumentar a escala de produção, principalmente frente à dificuldade de reproduzir tamanho de partícula e pelo elevado custo. A distribuição do tamanho de partícula pode, em muitos casos, ser muito ampla com desvio padrão relativo de 50 %, o que acarreta uma grande variabilidade na liberação do fármaco. Como o tempo de preparação é elevado, proteínas podem ser desnaturadas durante a preparação e, por fim, há a necessidade de garantir a ausência de solventes orgânicos nas micropartículas após a preparação (VARDE e PACK, 2004).

2.3 Secagem por aspersão *(spray-drying)*

As micropartículas podem ser preparadas por aspersão a partir de soluções, emulsões ou suspensões do fármaco, polímero e adjuvantes. Segundo KRISTMUNDSDÓTTIR e colaboradores (1996), microesferas são produzidas quando o fármaco é solúvel no solvente e microcápsulas, quando este está disperso. É uma técnica aplicada a fármacos e polímeros hidrofílicos e hidrofóbicos, além de permitir o controle do tamanho das micropartículas formadas (ESPOSITO *et al.,* 2002). O tamanho de partícula formado varia em torno de décimos a vários micrômetros e apresenta relativa baixa polidispersão (MASTERS, 1991).

Utilizando diferentes proporções de fármaco e polímero, KRISTMUNDSDÓTTIR e colaboradores (1996), prepararam micropartículas dissolvendo diretamente Eudragit$^{®}$ RS100 e cloridrato de diltiazem em diclorometano, com subseqüente secagem em Mini Spray-Drier Büchi$^{®}$ 190, com temperatura de entrada de 70 ^{o}C e saída de 57 ^{o}C. As microcápsulas foram produzidas dispersando o diltiazem em solução do polímero em tolueno, conduzindo à obtenção de partículas esféricas com diâmetro entre 1 e 10 μm. As microcápsulas apresentaram liberação rápida inicial, seguida por uma liberação controlada. Já as microesferas, apresentaram liberação mais uniforme, porém mais rápida.

Para preparação de micropartículas com polímeros gastro-resistentes (acetoftalato de celulose, acetotrimelitato de celulose, ftalato de hidroipropilmetilcelulose, Eudragit® S e L), PALMIERI e colaboradores (2000 e 2002) utilizaram a propriedade destes polímeros de solubilizarem-se em meio alcalino, para secá-los sem utilização de solvente orgânico. Foram estudados dois fármacos: paracetamol e cetoprofeno, sendo que em ambos casos, foi possível a obtenção de micropartículas, que foram diretamente comprimidas. Os comprimidos apresentaram boa gastro-resistência e liberação modificada. Em outro estudo (2001), este mesmo grupo de pesquisa utilizou o paracetamol com outros polímeros (Eudragit® RS e RL ou etilcelulose NF 50), solúveis em etanol, demonstrando resultados similares aos obtidos para os comprimidos. Uma observação importante é que as micropartículas na forma pulverulenta não apresentaram liberação modificada, nem diferenças em suas características, havendo apenas alterações significativas na liberação do fármaco quando as micropartículas foram comprimidas (sem utilização de adjuvantes).

Micropartículas contendo piroxicam foram preparadas utilizando Eudragit® L100 e S100 salificados (CILURZO *et al.*, 2005). As micropartículas apresentaram umidade inferior a 10 %, diâmetros médios entre 2 e 15 µm e formato irregular. As micropartículas apresentaram dissolução rápida e mucoadesividade. Em todos os casos, o fármaco e o polímero se apresentaram como uma solução sólida, melhorando a dissolução do piroxicam em relação ao fármaco micronizado.

ALVES e SANTANA (2004) prepararam micropartículas lipídicas contendo manitol e lecitina em diferentes proporções e estudaram as variáveis operacionais da operação de secagem por aspersão. Partículas cristalinas foram obtidas aumentando a concentração de fosfolipídios, fluxo de alimentação, temperatura de entrada e diâmetro da agulha. Estruturas amorfas foram obtidas principalmente quando manitol estava em maior concentração e foi incorporado como núcleo das micropartículas. Em todas amostras partículas entre 1 e 2 μm foram obtidas. As partículas também apresentaram diâmetro aerodinâmico aceitável para administração pulmonar (5 μm).

HEGAZY e colaboradores (2002) preparam micropartículas contendo piridostigmina e Eudragit® RS em diferentes proporções, utilizando um solvente ou um não solvente do fármaco. As micropartículas foram obtidas com rendimentos de 58 % a 66 % e diâmetros entre 3,7 μm e 10,3 μm. Em relação ao tempo de dissolução, as microcápsulas liberaram o fármaco após 12 h e as microesferas após 3 h. De acordo com os autores, esses resultados indicam que o fármaco encontra-se encapsulado, havendo necessidade de difusão da matriz até o meio, o que explicaria os tempos mais elevados para a liberação das microcápsulas e microesferas em comparação com a substância pura.

Outra aplicação da técnica de aspersão é no desenvolvimento de vacinas (MURILLO *et al.*, 2002). Foram preparadas micropartículas de antígeno de *Brucella ovis*, bactéria causadora de brucelose. A preparação foi eficiente quanto à fagocitose e toxicidade, mostrando que as micropartículas preparadas com poli(ε-caprolactona) são apropriadas para o uso como vacina.

Em relação à liberação sustentada, ESPOSITO e colaboradores (2002) encapsularam vitamina C por *spray-drying*, utilizando Eudragit® L100, RS100 e RL100, obtendo micropartículas com diâmetros entre 4 e 20 μm. Os estudos de liberação da vitamina C mostraram liberação sustentada, com perfil de liberação intestinal, pouco afetada pelo tipo de polimetacrilato usado.

BILLON e colaboradores (1999) estudaram a influência de polímeros derivados de celulose e adjuvantes na preparação de micropartículas contendo paracetamol por aspersão. Concluíram que o melhor polímero utilizado foi a carboximetilcelulose sódica, que aumentou o rendimento da secagem e prolongou a liberação do fármaco. A adição de polivinilpirrolidona também prolongou o tempo de cedência.

A técnica de *spray drying* permite produzir micropartículas em etapa única e sem uso de solventes orgânicos, sendo considerado um processo de baixo custo e fácil escalonamento (ONEDA e RÉ, 2003; RATTES e OLIVEIRA, 2007). O estudo do processo de secagem inclui a determinação dos parâmetros otimizados de operação para cada formulação, a fim de aumentar seu rendimento e controlar as características das partículas formadas (ESPOSITO *et al.*, 2002; RÉ, 1998). As características finais do pó a serem consideradas em um estudo de escalonamento são a umidade final, o tamanho de partícula e a polidispersão, as densidades bruta e de compactação e a coesão entre as partículas (BIRCHAL *et al.*, 2005). A distribuição do tamanho de partícula varia de acordo com a geometria da agulha, a velocidade de alimentação do *spray drier* e das condições operacionais como, por exemplo, a temperatura do ar de entrada ou a velocidade de rotação de um atomizador rotativo. Apesar da maioria dos trabalhos científicos utilizarem *spray drier* laboratoriais, a recuperação de produto final é relativamente baixa (20 – 50 %). Outra limitação dos equipamentos de pequena escala é sua capacidade reduzida de obtenção de pós com diferentes diâmetros (a grande maioria apresenta diâmetro médio entre 3 e 10 μm). Equipamentos maiores oferecem possibilidades de controle do diâmetro médio de partícula e menor polidispersão (AMERI e MAA, 2006). Mesmo mantendo parâmetros muito semelhantes em equipamentos de escalas diferentes, eles nunca são iguais e diferenças no balanço de calor e massas e no produto final são verificados quando se troca de equipamento (FOSTER e LAETHERMAN, 1995).

2.4 Produção em escala piloto

A microencapsulação de fármacos pode ser realizada por diferentes métodos, porém muitos são excessivamente demorados e difíceis de produzir industrialmente. As grandes vantagens da secagem por aspersão comparada às outras técnicas incluem o fato do processo ocorrer em apenas uma etapa, ter baixo custo e a não utilizar solventes orgânicos (ONEDA e RÉ, 2003).

Um estudo de produção de micropartículas em escala industrial foi realizado por JOHANSEN e colaboradores (2000). Os autores estudaram a encapsulação de diferentes proteínas por aspersão de emulsão A/O. Uma desvantagem deste método é a utilização de solventes orgânicos como diclorometano ou formato de etila. Foram estudados parâmetros como velocidade de alimentação, concentração de polímero e o método de coleta do pó, tendo sido concluído que o aumento de escala é viável, porém o aumento do rendimento do processo com o aumento da escala não foi observado.

Como alternativa para diminuir as perdas durante a aspersão, FREITAS e colaboradores (2004) estudaram a utilização de atomizador com ultra-som na produção de micropartículas contendo proteínas, porém o processo não obteve rendimento superior ao obtido em equipamento convencional.

Apesar da secagem por aspersão ser uma técnica vantajosa na produção de pós na área farmacêutica, alguns parâmetros deste processo ainda não totalmente elucidados (BENOIT *et al.*, 1996). Grande parte dos estudos foi realizada em escala laboratorial utilizando atomizador tipo duplo fluido. O resultado são partículas com cerca de 10 µm de diâmetro e com pouca possibilidade de controle de tamanho. Além disso, existe pouca informação sobre a influência das variáveis do processo (pressão e temperatura do ar de entrada, fluxo de alimentação e tipo de atomizador) no produto final. Alterações de paramentos do processo podem levar a alterações na cinética de dissolução de fármacos. Outra variável importante no processo é o tamanho do equipamento e da produção. A produção em maior escala requer ajuste nos parâmetros do processo porque os equipamentos apresentam diferenças significativas em relação à geometria, à capacidade de evaporação e ao fluxo de alimentação.

Os parâmetros da secagem por aspersão normalmente estudados são a temperatura de entrada, o fluxo e a pressão do ar, uma vez que os mesmos influenciam o rendimento, a umidade final do produto e o conteúdo de substância encapsulada (ZHOU *et al.,* 2004).

3 Avaliação *in vivo* de anti-ulcerosos

Testes em ratos para avaliação de fármacos anti-ulcerosos são descritos desde 1971 (GAO *et al.*, 2002 *apud* OKABE *et al.* 1971). Existem alguns ensaios descritos com a utilização de ratos ou camundongos, usando como agente causador de úlcera o ácido acético (KONTUREK *et al.,* 2002), fármacos anti-inflamatórios não esteróides (indometacina), analgésicos (ácido acetilsalicílico), (LIU *et. al*, 2001; NAKAMURA *et al.,* 2003; PETROVIC *et al.,* 2003) ou etanol (LIU *et. al*, 2001; SHAH *et. al.*, 2003; OLIVEIRA, *et. al*., 2004).

TAKEUCHI e colaboradores (1999) estudaram o efeito da administração de derivados benzimidazólicos em ratos. Pantoprazol, omeprazol e lansoprazol mostraram-se dose dependentes quanto à diminuição da secreção de ácido. A administração via oral de pantoprazol a ratos com secreção gástrica induzida por mepirizol (200 mg/kg) provocou uma diminuição de 99,2 % quando este fármaco foi administrado na dose de 3 mg/kg. Quanto à proteção contra ulcerações induzidas por ácido acético, o pantoprazol, o omeprazol e o lansoprazol diminuíram o índice lesional, sendo que o pantoprazol mostrou-se o mais efetivo (diminuição de 87 %, contra 54 % do omeprazol e 80 % do lansoprazol).

O teste de efeito anti-ulceroso dos polissacarídeos de *Ganoderma lucidum* foi realizado em ratos Wistar machos, com úlcera induzida por indometacina administrada via sub-cutânea na dose de 50 mg/kg (GAO *et al.*, 2002). Concomitantemente, o polissacarídeo foi administrado via oral (gavagem) com o intuito de avaliar o mecanismo de inibição da secreção gástrica. Os ratos do grupo controle, aos quais foi administrado somente indometacina apresentaram índice lesional de 8,6 ± 0,9 no sexto dia após administração. Já com a administração do polissacarídeo, o índice foi 6,5 ± 0,6 (dose 250 mg/kg) e 4,3 ± 0,7 (dose 500 mg/kg). O estudo demonstrou, através de análises de cultura de células do epitélio gástrico, que a diminuição da ulceração deve-se a fatores como supressão da produção de citocinas tóxicas e estimulação das células proliferativas no epitélio gástrico.

Alguns dos métodos de indução de úlceras são invasivos e cirurgias tornam os experimentos demorados e sacrificados para os animais. Devido a isso, ultimamente, o agente indutor de úlceras mais utilizado é o etanol via oral (LIU *et. al*, 2001; SHAH *et. al.*, 2003, OLIVEIRA, *et. al*., 2004). Segundo SHAH e colaboradores (2003), o pantoprazol foi efetivo na redução das úlceras causadas por etanol na dose de 20 mg/kg. LIU e CHO (2000) estudaram o modelo de indução de úlceras e de gastrite sub-crônica após administração repetida de etanol via oral. Foi verificado que a maior área lesional foi causada por apenas 1 dia de administração, contra 3 e 6 dias, quando a área lesionada diminuiu. A administração de etanol produz lesões hemorrágicas e necróticas causadas por ação de radicais livres e conseqüente formação de produtos da lipoperoxidação (SHAH *et al.*, 2003). O modelo de indução de ulceras por etanol também é considerado um bom modelo, pois o etanol não influencia a farmacocinética do pantoprazol (HEINZE *et al.,* 2001).

A indução de úlceras por ácido acético requer cirurgia sob anestesia por éter etílico, em que o estômago é exposto e o ácido acético (60 %) é colocado por 5 s na mucosa. Imediatamente, lesões e necrose são visualizadas. O abdome é suturado e o animal é submetido à administração de anti-ulceroso por até 6 dias (KONTUREK *et. al.*, 1999; LIU e CHO, 2000). Por outro lado, a indução por indometacina pode ser via subcutânea (50 mg/kg), com a administração via oral do anti-ulceroso por até 6 dias (GAO *et. al*., 2002) ou por 3 dias consecutivos e posterior tratamento (RAFFIN *et. al*., 2003). O protocolo de ulceração por etanol prevê todo estudo em curto tempo (4 horas) e administração somente por via oral, tanto do anti-ulceroso, quanto do próprio etanol (SHAH *et. al.*, 2003).

Para esses experimentos, LIU e CHO (2000) recomendam o jejum de 24 horas aos animais que serão submetidos à gavagem, uma vez que, já que a alimentação pode afetar a fisiologia do estômago e a absorção dos agentes.

4 Aglomerados

Atualmente, as micropartículas têm sido empregadas como material de enchimento de cápsulas de gelatina dura. Entretanto, esta forma farmacêutica apresenta a desvantagem de ser indivisível, fato que tem conduzido vários autores a estudarem a preparação de comprimidos a partir de micropartículas para viabilização da administração e maior proteção no trato gastrintestinal (SVEINSSON *et al.,* 1993; KIM, 1999; PALMIERI *et al.,* 2000; PALMIERI *et al.*, 2002). A compressão também foi utilizada como técnica a fim de se obter liberação controlada a partir das micropartículas. GAVINI e colaboradores (2003) preparam micropartículas por *spray-drying* contendo cetoprofeno. A compressão das micropartículas sem o uso de excipientes resultou em comprimidos com desintegração rápida. Por outro lado, o uso de adjuvantes propiciou o controle da liberação a partir das micropartículas.

As vantagens apresentadas pelos sistemas multiparticulados, especialmente as micropartículas poliméricas, são estritamente ligadas ao tamanho micrométrico. O tamanho reduzido leva a pós com baixa densidade e problemas de fluxo e manufatura. Em muitas aplicações, as partículas devem ser pequenas em função de suas propriedades biofarmacêuticas, mas grandes suficientes para facilitar a preparação de formas farmacêuticas. Em muitos casos, a transformação de micropartículas em formas farmacêuticas finais envolve processos como compactação ou granulação, que podem deformar irreversivelmente as micropartículas.

Este problema tecnológico pode ser solucionado com a preparação de aglomerados, em um processo baseado na formação de partículas maiores mantidas por forças fracas do tipo Van der Waals. Estes aglomerados devem ser rompidos por simples tubulações de ar ou contado com água, reconstituindo as micropartículas em seu tamanho original.

Aglomerados contendo cafeína foram preparados a partir de nebulizados de cafeína e excipientes para administração nasal (RUSSO *et al.*, 2004). Os pós foram mantidos sob rotação em um frasco de baquelita e os aglomerados entre 106 e 850 μm, separados. O aumento na formação de aglomerados e a melhora da performance ao insuflar foi determinado pela presença de lecitina na composição do nebulizado. A aglomeração não afetou a dissolução da cafeína.

RUSSO e colaboradores (2006) prepararam aglomerados contendo nebulizados de morfina e açúcares ou contendo cristais de morfina para aplicação nasal. Estes últimos foram preparados utilizando um excipiente composto por manitol e lecitina, que após sua preparação por *spray-drying,* foi misturado aos cristais de morfina. Todos aglomerados foram preparados através de rotação. Os aglomerados apresentaram rápida dissolução em contato com água. Apresentaram também maior permeação (teste *in vitro*) através de mucosa nasal de coelho que uma solução supersaturada de morfina.

CAPÍTULO 1: Validação metodologia analitica para quantificação do pantoprazol por CLAE

1.1 Apresentação

O pantoprazol é um fármaco inibidor da bomba de prótons, utilizado no tratamento de úlceras do trato gastrintestinal, refluxo gastro-esofágico e no tratamento do *Helicobacter pylori* (CHEER *et al*., 2003). O pantoprazol é um pró-farmaco, que se converte em sua forma ativa apenas nos canalículos das células parietais do estômago. Para tal, deve ser administrado em uma forma farmacêutica gastro-resistente. Segundo EKPE e Jacobsen (1999), a degradação do pantoprazol aumenta com a presença de sais em solução e com a diminuição do pH, o que reafirma a necessidade de administrá-lo em uma forma gastro-resistente, capaz de permitir absorção exclusivamente entérica.

O planejamento de novas formas farmacêuticas deve contemplar como etapa inicial o desenvolvimento e validação de uma metodologia analítica capaz de quantificar o fármaco contido nas formulações, bem como eventuais produtos de degradação formados, especialmente quando essas metodologias estão ausentes em códigos oficiais. Apesar do pantoprazol já ser um fármaco bastante usado na terapêutica e ter sido aprovado pelo FDA em 2000, sua monografia ainda não está descrita em compêndio oficial algum.

Desta forma, o objetivo deste capítulo foi desenvolver e validar metodologia analítica para quantificação do pantoprazol nas micropartículas através de cromatografia líquida de alta eficiência. Estes resultados foram publicados na revista Química Nova.

Publicação 01: Validação de metodologia analítica por cromatografia líquida para doseamento e estudo da estabilidade de pantoprazol sódico

Quim. Nova, Vol. 30, No. 4, 1001-1005, 2007

VALIDAÇÃO DE METODOLOGIA ANALÍTICA POR CROMATOGRAFIA LÍQUIDA PARA DOSEAMENTO E ESTUDO DA ESTABILIDADE DE PANTOPRAZOL SÓDICO

Renata Platcheck Raffin, Letícia Marques Colomé e Sílvia Stanisçuaski Guterres*
Faculdade de Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Av. Ipiranga, 2752, 90610-000 Porto Alegre – RS, Brasil
Adriana Raffin Pohlmann
Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio Grande do Sul, CP 15003, 91501-970 Porto Alegre - RS, Brasil.

Recebido em 10/3/06; aceito em 4/8/06; publicado na web em 26/3/07

VALIDATION OF ANALYTICAL METHODOLOGY BY HPLC FOR QUANTIFICATION AND STABILITY EVALUATION OF SODIUM PANTOPRAZOLE. Pantoprazole is a proton pump inhibitor used in the treatment of digestive ulcers, gastro-esophageal reflux disease and in the eradication of *Helicobacter pylori*. In this work, an analytical method was developed and validated for the quantification of sodium pantoprazole by HPLC. The method was specific, linear, precise and exact. In order to verify the stability of pantoprazole during dissolution assays, pantoprazole solution in phosphate buffer pH 7.4 was kept at room temperature and protected from light for 22 days. Pantoprazole presented less than 5% of degradation in 6 hours and the half live of the degradation was 124 h.

Keywords: pantoprazole; validation; stability.

INTRODUÇÃO

O pantoprazol [5-(difluorometoxi)-2-[[(3,4-dimetoxi-2-piridinil)metil]sulfinil]-1H-benzimidazol] é um fármaco inibidor da bomba de prótons, atualmente considerado um dos mais importantes para tratamento de uma variedade de doenças relacionadas ao trato gastrintestinal superior[1]. Este fármaco vem sendo amplamente utilizado no tratamento de úlceras gástricas, refluxo gastroesofágico e condições hipersecretórias patológicas, devido à sua capacidade de inibir irreversivelmente a bomba de prótons, diminuindo a secreção gástrica proveniente das células parietais do estômago[2]. Outra indicação é o tratamento adjunto a antibióticos para erradicação de *Helicobacter pylori*[3].

O mecanismo de ação do pantoprazol, um pró-fármaco, baseia-se na sua ativação no ambiente ácido das células parietais gástricas, após sua absorção intestinal e circulação na corrente sangüínea. A ativação leva à formação de uma sulfonamida catiônica cíclica (Figura 1), que se liga a uma ou mais subunidades catalíticas da bomba de prótons, alterando sua configuração e provocando uma inibição irreversível do transporte ácido. No entanto, para que o pantoprazol esteja apto para atuar em seu local de ação, a ativação deve ocorrer somente nos canalículos das células parietais, sendo necessária sua passagem intacta pelo lúmen do estômago[2].

O sal sódico sesquiidratado é a forma mais utilizada do fármaco ($C_{16}H_{14}F_2N_3O_4SNa.1,5H_2O$), sendo solúvel em água, pouco solúvel em tampão fosfato pH 7,4 e insolúvel em *n*-hexano[1]. A degradação do pantoprazol em soluções aquosas é dependente do pH, sendo que a taxa de degradação aumenta com o abaixamento do pH. Jungnickel[1] relatou que, em pH 5, a meia vida de degradação é de aproximadamente 2,8 h e em pH 7,8 é de 220 h.

O pantoprazol também apresenta instabilidade frente à presença de diversos sais em solução aquosa, como cloreto de sódio, ácido cítrico, citrato trissódico, citrato monossódico, bicarbonato de sódio e carbonato de cálcio, em diferentes concentrações e valores de pH, conforme descreveram Ekpe e Jacobsen[4]. Os autores observaram ainda que as condições de armazenamento mais adequadas para diminuir a degradação do fármaco são pH 10 e 4 °C.

Em outro estudo, realizado por Detinger e colaboradores[5], comprimidos foram triturados e o pó suspenso em solução aquosa de bicarbonato (2 mg/mL de pantoprazol) com o objetivo de

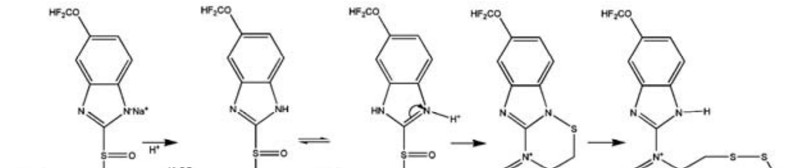


Figura 1. *Reações de ativação do pantoprazol sódico em meio ácido e ligação à bomba de prótons*

---

*e-mail: nanoc@farmacia.ufrgs.br

preparar uma forma farmacêutica líquida contendo pantoprazol. A suspensão extemporânea foi avaliada quanto à sua estabilidade por 62 dias sob refrigeração (2 a 8 °C). Após este período, mais de 90% da quantidade inicial de pantoprazol manteve-se estável. A mesma metodologia de preparação da solução extemporânea foi estudada em escala maior por Ferron e colaboradores[6]. Neste estudo, 34 comprimidos foram triturados e suspensos em 680 mL de solução de bicarbonato a 4,2% e mantidos em seringas plásticas. A 25 °C a suspensão foi estável por 1 dia (99,1%), a 5 ºC por 2 semanas (98,6%) e a –20 ºC por até 3 meses de armazenamento (98,3%).

Em outro trabalho[7], o pó liofilizado comercial de pantoprazol foi reconstituído com solução de NaCl a 0,9% com o objetivo de estudar sua estabilidade, visando o uso por via intravenosa. A solução (4 mg/mL) foi mantida em seringas de polipropileno à temperatura ambiente por 96 h. A concentração final de pantoprazol em solução foi 96% da concentração inicial, porém a solução alterou sua cor para laranja-amarelado. O autor considerou que o grau de degradação não foi significativo, não havendo alteração da concentração do fármaco nas condições testadas.

De forma geral, os estudos mostram que a degradação do pantoprazol aumenta com a presença de sais em solução e com a diminuição do pH, o que reafirma a necessidade de administrá-lo em uma forma gastrorresistente, capaz de permitir absorção exclusivamente entérica. Normalmente, o planejamento de novas formas farmacêuticas deve contemplar como etapa inicial o desenvolvimento e validação de metodologia analítica capaz de quantificar o fármaco contido nas formulações, bem como eventuais produtos de degradação formados, especialmente quando essas metodologias estão ausentes em códigos oficiais. Apesar do pantoprazol já ser um fármaco bastante usado na terapêutica e ter sido aprovado pelo FDA em 2000, sua monografia ainda não está descrita em compêndio oficial algum.

Para o controle de qualidade de formas farmacêuticas, os testes de dissolução preconizados pelos códigos oficiais são indispensáveis, sendo importantes também para orientar o desenvolvimento de novas formulações. Além disso, permitem a demonstração *in vitro* de características da formulação, as quais podem estar correlacionadas com seu desempenho *in vivo*. Em geral, estes testes são conduzidos com velocidade e agitação padronizadas e sob condições "sink", mimetizando, tanto quanto possível, as condições do trato gastrintestinal[8]. Estes podem ser conduzidos em aparatos, como dissolutores[9,10], nos quais as formas farmacêuticas são colocadas em recipientes com volume padronizado de meio de dissolução e mantidas até sua completa dissolução ou em equipamentos de células de fluxo[9], onde a amostra é submetida a um fluxo de meio de dissolução até seu esgotamento. Quando se utiliza qualquer uma destas metodologias, o baixo limite de quantificação do método analítico empregado no doseamento do fármaco é fundamental para garantir precisão na avaliação dos pontos iniciais e finais da dissolução.

Os ensaios de dissolução para novas formas farmacêuticas de liberação prolongada devem ser realizados em meio capaz de mimetizar as condições intestinais. Desta forma, os ensaios podem ser realizados empregando-se soluções tamponadas (pH 7,4) por, no mínimo, 2 h. A estabilidade da substância no meio deve ser garantida, especialmente no caso das formas farmacêuticas que utilizam polímeros de liberação colônica, cuja solubilidade se dá em solução com pH superior a 7[8,10]. No caso de formulações revestidas e/ou gastrorresistentes contendo pantoprazol, o estudo da sua estabilidade no meio de dissolução é fundamental para se detectar se há ou não degradação do pantoprazol após sua liberação/dissolução, o que ocasionaria interpretação errônea do perfil de liberação.

Para a quantificação do pantoprazol em estudos de dissolução e estabilidade, o limite de quantificação é o parâmetro mais importante na escolha do método. Entre os métodos já existentes na literatura para determinação do pantoprazol em formas farmacêuticas, um método[5] de cromatografia líquida de alta eficiência desenvolvido para determinação da estabilidade de pantoprazol em suspensão oral extemporânea utilizou fase móvel em sistema isocrático, constituída de acetonitrila e fosfato de potássio dibásico 50 mM, com faixa de análise entre 6 e 24 µg/mL. Outro método cromatográfico semelhante[11] para determinação de pantoprazol em comprimidos utilizou fase móvel constituída de acetonitrila (300 mL) e tampão fosfato pH 7,4 (600 mL). Os autores utilizaram coluna octadecilsilano (C_{18}) e trabalharam na faixa de análise entre 5 e 20 µg/mL. Em ambos os métodos, o limite de quantificação não foi determinado.

Um método que empregou coluna octilsilano (C_8) e fase móvel isocrática constituída de 70:30 tampão e acetonitrila, sendo o tampão constituído por 0,02 M de fosfato de sódio monobásico e 0,03 M de fosfato de sódio dibásico, foi validado. No entanto, os autores descreveram uma concentração inferior de trabalho alta para o pantoprazol (228 µg/mL)[12]. A especificidade foi avaliada injetando-se uma mistura dos excipientes dos comprimidos comerciais de pantoprazol.

Considerando o exposto, este trabalho teve como objetivo desenvolver e validar uma metodologia analítica para doseamento de pantoprazol, através de cromatografia líquida de alta eficiência, a fim de obter um limite de quantificação, que permita a aplicação do método em estudos de dissolução *in vitro*. O trabalho visou também aplicar esta metodologia analítica na quantificação do pantoprazol em breve estudo de estabilidade do fármaco em tampão fosfato pH 7,4, a fim de investigar a presença de produtos de degradação que possam ser formados durante os ensaios de dissolução.

PARTE EXPERIMENTAL

Materiais e equipamentos

Pantoprazol sódico sesquiidratado (99,84%, Henrifarma, São Paulo, Brasil); acetonitrila grau CLAE; água purificada através de sistema Milli-Q para uso em CLAE; fosfato de potássio monobásico; fosfato de potássio dibásico. Cromatógrafo líquido de alta eficiência Perkin Elmer Série 200 acoplado a detector UV-VIS. Cromatógrafo líquido de alta eficiência Schimadzu LC-10ADV acoplado a detector de arranjo de diodo (SPD-M10AVP).

Metodologia

A validação da metodologia analítica foi realizada segundo os critérios propostos pela ICH ("International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use")[13] de 1996, USP[10] e resolução da ANVISA RE n° 899, de 29/5/2003[14]. Os parâmetros avaliados foram: linearidade, precisão intermediária, repetibilidade, exatidão, limites de detecção e quantificação e especificidade frente aos produtos de degradação, sendo os últimos ensaios realizados em cromatógrafo Perkin Elmer Série 200 com detector UV/VIS. A pureza do pico foi avaliada em cromatógrafo acoplado a detector de arranjo de diodo.

Os parâmetros cromatográficos utilizados em ambos os equipamentos encontram-se na Tabela 1.

O tampão fosfato pH 7,4, utilizado na fase móvel, foi preparado com 839 mg de fosfato de potássio monobásico e 137 mg de fosfato de potássio dibásico para 1000 mL de solução.

Para avaliação da linearidade, a faixa de concentração de

tarem de longos períodos para equilibrar a coluna, maior gasto de solvente e bombas especializadas[17].

Comparando-se as faixas de análise e limites de quantificação encontrados na literatura com os utilizados e determinados em nosso estudo, a metodologia aqui descrita reduz em 93% a faixa de análise, em relação à descrita por Badwan e colaboradores[12] que passa de 228 a 670 µg/mL para 0,5 a 20 µg/mL. Essa redução torna o método viável para sua aplicação, tanto na quantificação da liberação do fármaco a partir de formas farmacêuticas quanto em seus estudos de estabilidade.

Os resultados de repetibilidade e precisão intermediária foram expressos em termos de desvio padrão relativo (DPR) (Tabela 3). A repetibilidade apresentou DPR de 1,71; 0,59 e 1,10 para as triplicatas das concentrações de 6, 9 e 14 µg/mL, respectivamente. A precisão intermediária foi avaliada em três dias consecutivos, com amostras de mesma concentração (9 µg/mL). Os valores de DPR foram 0,59; 0,54 e 0,27 para cada dia. Para ambos os estudos os valores de desvios padrões relativos foram inferiores a 2, demonstrando repetibilidade e precisão intermediária adequadas para o método analítico em questão. No que diz respeito à exatidão, o método permitiu a recuperação de 95,39 ± 3,77% para a concentração final de 6 µg/mL; 101,13 ± 1,71% para 9 µg/mL e 101,38 ± 1,46% para 14 µg/mL, o que caracteriza o método como exato, segundo os preceitos da "International Conference on Harmonization"[13].

Tabela 3. Valores de desvio padrão relativo da avaliação da precisão intermediária e da repetibilidade do método analítico

Concentração (µg/mL)	Dia 1 DPR	Dia 2 DPR	Dia 3 DPR	Média
6	1,71			
9	0,59	0,54	0,27	0,47
14	1,10			
Média	1,13			

A análise dos cromatogramas demonstrou que há completa separação entre os picos do fármaco (6,7 min) e seus produtos de degradação em tampão fosfato (1,6 e 3,2 min), não havendo interferência dos produtos de degradação na quantificação do pantoprazol (Figura 2). A análise do pico referente ao fármaco, utilizando o detector de arranjo de diodo, demonstrou 100% de pureza no pico.

Através do estudo de estabilidade, verificou-se que a concentração de pantoprazol atinge 90% da concentração inicial em cerca de 19 h, em tampão fosfato pH 7,4, sendo que após 22 dias, a concentração atinge apenas 3,9% da concentração inicial (Figura 3). Por outro lado, o pantoprazol mantém 100% de sua concentração até 6 h, tempo normalmente limite para testes de dissolução[10], pois existe a necessidade de mimetizar condições fisiológicas de trânsito no trato gastrintestinal.

A modelagem matemática da curva de degradação foi realizada empregando o software Micromath Scientist® segundo o modelo monoexponencial (Equação 3).

$$C=100e^{-kt} \qquad (3)$$

O valor da constante observada *k* foi 0,00558 ± 0,00015 h^{-1}. O coeficiente de correlação foi de 0,9997. O tempo de meia-vida de degradação do pantoprazol em tampão fosfato foi de 124 h.

Com o objetivo de demonstrar a adequabilidade do método analítico, as micropartículas de pantoprazol empregadas para avaliação de precisão, repetibilidade e exatidão foram avaliadas quanto ao perfil de dissolução *in vitro* do pantoprazol em equipamento de células de fluxo, utilizando como meio tampão fosfato pH 7,4.

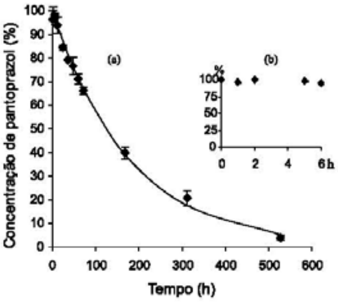


Figura 3. *Curva de degradação de pantoprazol em tampão fosfato 7,4. Os pontos indicam os dados experimentais e a linha, a modelagem matemática (a). As primeiras 6 h de análise estão representadas em escala diferente (b)*

O perfil de liberação do pantoprazol a partir das micropartículas foi determinado por 120 min (Figura 4), tendo demonstrado que neste período a totalidade de pantoprazol encapsulado foi liberada a partir das micropartículas.

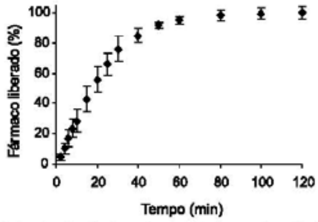


Figura 4. *Perfil de dissolução das micropartículas em tampão fosfato pH 7,4*

CONCLUSÃO

A metodologia analítica proposta para detecção e quantificação de pantoprazol por CLAE mostrou-se sensível, precisa e linear na faixa de concentração entre 0,5 e 20 µg/mL, sendo adequada para avaliação do fármaco em formas farmacêuticas como micropartículas e em soluções de tampão fosfato. Além disso, esse limite de quantificação (0,553 µg/mL) torna a metodologia adequada à utilização em ensaios de dissolução e estabilidade. A estabilidade do fármaco em tampão fosfato pH 7,4 é limitada, porém suficiente para a realização de análises normalmente conduzidas em estudos biofarmacêuticos.

AGRADECIMENTOS

CNPq/MCT, CAPES e FAPERGS.

REFERÊNCIAS

1. Jungnickel, P. W.; *Clin. Ther.* **2000**, *22*, 1268.
2. Cheer, S.; Prakash, A.; Faulds, D.; Lamb, H.; *Drugs* **2003**, *63*, 101.
3. Poole, P.; *Am. J. Health Syst. Pharm.* **2001**, *58*, 999.
4. Ekpe, A.; Jacobsen, T.; *Drug. Dev. Ind. Pharm.* **1999**, *25*, 1057.
5. Detinger, P.; Swewnson, C.; Anaizi, N.; *Am. J. Health Syst. Pharm.* **2002**, *59*, 953.
6. Ferron, G.; Ku, S.; Abell, M.; Unruh, M.; Getsy, J.; Mayer, P.; Paul, J.; *Am. J. Health Syst. Pharm.* **2003**, *60*, 1324.
7. Johnson, C.; *Am. J. Health Syst. Pharm.* **2005**, *62*, 2410.
8. Aulton, M. E.; *Delineamento de formas farmacêuticas*, Artmed: Porto Alegre, 2005.

9. *British Pharmacopoeia*; Her Majesty's Stationery Office: Londres, 2000.
10. *Pharmacopoeia of the United States of America;* 28th ed., Rockville Pharmacopoeial Convention: Estados Unidos da América, 2004.
11. Mansour, A. M.; Sorour, O. M.; *Chromatographia* **2001**, *53* suppl., S478.
12. Badwan, A. A.; Nabulsi, L. N.; Omari, A. L.; Daraghmeh, M. M.; Ashour, M.; Abdou, A. M.; Jaber, A. M. Em *Analytical Profiles of Drug Substances and Excipients;* Florey, K.; Brittain, H.G., eds.; Academic Press, Elsevier Science: USA, 2002, vol. 29, p. 213-259.
13. *Guidance for Industry – Q2B Validation of Analytical Procedures: Methodology;* European Agency for the Evaluation of Medicinal Products (ICH): Londres, 1996.
14. Agência Nacional de Vigilância Sanitária, resolução RE n° 899 de 23 de maio de 2003, Brasil.
15. Huber, R.; Müller, W.; *J. Chromatogr.* **1990**, *529*, 389.
16. Xie, Z.; Chen, X.; Jin, F.; Zhong, D.; *J. Chromatogr. Sci.* **2005**, *43*, 271.
17. Hutabarat, L. S.; Mulholland, M.; Greenfield, H.; *J. Chromatogr., A* **1998**, *795*, 377.

CAPÍTULO 2: Preparação de micropartículas contendo pantoprazol por evaporação de solvente: caracterização físico-química e avaliação biológica

2.1 Apresentação

No capitulo anterior, foi demonstrada a validação da metodologia analítica para determinação do pantoprazol em micropartículas poliméricas e em meio de dissolução.

Na continuidade dos estudos, foram preparadas e caracterizadas micropartículas gastro-resistentes contendo pantoprazol. A metodologia escolhida foi a emulsificação/evaporação de solvente. Esta técnica já foi bastante estudada e aplicada a diversos fármacos, proteínas, DNA e vacinas, utilizando também uma grande quantidade de polímeros. Em um processo de evaporação de solvente clássico, o polímero é dissolvido em um solvente imiscível com água e o fármaco é disperso ou dissolvido na solução polimérica. A dispersão ou solução resultante é emulsificada em uma fase continua aquosa contendo tensoativos. Para formar as micropartículas, o solvente orgânico deve primeiro difundir na fase aquosa e depois evaporar na interface água/ar. Enquanto o solvente evapora, as micropartículas solidificam e são recolhidas por filtração (O’DONNEL e McGINITY, 1997).

Para a encapsulação de fármacos hidrofílicos, a técnica clássica de emulsão A/O é modificada e duas alternativas são mais freqüentes, a preparação de emulsão O/O e de emulsões múltiplas tipo A/O/A ou S/A/O, ou ainda variações destes. A técnica de emulsificação O/O permite a encapsulação de fármacos em um sistema anidro, utilizando acetona emulsificada em óleo mineral ou parafina líquida.

No presente capítulo, micropartículas compostas por Eudragit® S100 e pantoprazol foram preparadas pela técnica de emulsificação/evaporação de solvente utilizando uma emulsão O/O. As micropartículas foram caracterizadas físico-quimicamente e quanto à dissolução, gastro-resistência e atividade anti-ulcerogênica *in vivo*. Estes resultados foram publicados no periódico *European Journal of Pharmaceutics and Biopharmaceutics*.

Publicação 02: Preparation, characterization and *in vivo* anti-ulcer evaluation of pantoprazole-loaded microparticles

Available online at www.sciencedirect.com
SCIENCE DIRECT®

ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 63 (2006) 198–204

European
Journal of
Pharmaceutics and
Biopharmaceutics

www.elsevier.com/locate/ejpb

Research paper

Preparation, characterization, and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles

R.P. Raffin [a], L.M. Colomé [a], A.R. Pohlmann [a,b], S.S. Guterres [a,*]

[a] *Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil*
[b] *Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

Received 10 October 2005; accepted in revised form 26 January 2006
Available online 13 March 2006

Abstract

Pantoprazole is an important drug in the treatment of acid-related disorders. This work concerns the preparation and characterization of gastro-resistant pantoprazole-loaded microparticles prepared using an O/O emulsification/solvent evaporation technique. The in vivo activity of the pantoprazole-loaded Eudragit® S100 microparticles was carried out in rats. Furthermore, tablets containing the microparticles were also investigated. Microparticles presented spherical and smooth morphologies (SEM) and they remained intact in the inner surface of tablets. DSC and IR analyses showed that pantoprazole was physically and molecularly dispersed in the polymer. In vivo anti-ulcer evaluation showed that the microparticles were able to protect rat stomachs against ulcer formation, while the drug aqueous solution did not present activity. Drug dissolution profiles from tablets demonstrated slower release than untabletted microparticles. Weibull equation was the best model for describing the drug release profiles from microparticles and tablets. As regards the acid protection, tablets showed a satisfactory drug protection in acid medium (61.05 ± 8.09% after 30 min).

Keywords: Microparticles; Pantoprazole; Emulsification/solvent evaporation; Polymer; Gastric resistance; Tablet; Release profile; In vivo ulcer evaluation

1. Introduction

Pantoprazole is an important drug in the treatment of acid-related disorders [1] and it is also very effective against *Helicobacter pylori* infections alone or associated to other drugs, like metronidazole, clarithromycin or amoxicillin [2,3]. This drug was the first water-soluble benzimidazole, 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-benzimidazole (Fig. 1), which could be administered intravenously in the form of sodium pantoprazole sesquihydrate. In low pH values, pantoprazole turns into a cationic sulfenamide, which is its active form [2,4]. This drug accumulates in the highly acidic environment of the parietal-cell canalicular lumen and it is activated. The active form, a tetracyclic cationic sulfenamide, reacts with thiol group of cysteines 813 and 822 of the transmembranal H^+/K^+ATPase [1,5]. This conversion must occur inside the gastric parietal cells, so pantoprazole must be absorbed intact by gastrointestinal tract [2].

Pantoprazole has several advantages compared to its analogues (e.g., omeprazole and lansoprazole) such as specific site of binding, greater stability in neutral pH environment, and longer duration of action [6]. Besides, it presents no potential to induce or inhibit the CYP 450 [1,2,7]. It is a more selective inhibitor of acid secretion than other proton pump inhibitors [8].

Due to the necessity to pass intact through the stomach for reaching the duodenum for absorption, the pantoprazole is formulated as solution for intravenous administration (lyophilized powder for reconstitution) or as gastric-resistant tablets (oral delayed-release dosage form). In the case of oral administration, the enteric coating prevents

---

[*] Corresponding author. Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga, 1752, Porto Alegre 90610-000, RS, Brazil. Tel.: +55 51 331 65500; fax: +55 51 33165437.
E-mail address: nanoc@farmacia.ufrgs.br (S.S. Guterres).

0939-6411/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2006.01.013

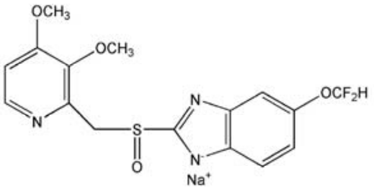


Fig. 1. Chemical structure of sodium pantoprazole.

pantoprazole from degradation in the gastric juice (at pH 1–2, pantoprazole degrades in few minutes) [9].

Up to now, no multiple-unit pharmaceutical dosage forms containing pantoprazole have been developed. As a general rule, the multiple-unit products show large and uniform distribution; they are less affected by pH and there is a minor risk of dose dumping [10]. Besides, these new drug delivery systems, as the polymeric microparticles, are also proposed to improve absorption, distribution, and bioavailability of acid labile drugs [11,12]. As they rapidly disperse in the gastrointestinal tract, they can maximize drug absorption, minimize side effects, and reduce variations in gastric emptying rates and intersubject variability [13].

The emulsification/solvent evaporation is a technique very well described in the literature [10,14] to prepare polymeric microparticles, which are useful for stabilizing drugs and to improve their distribution. This technique is an easy method, compatible with several polymers [15], and it is suitable for encapsulating both lipophilic and hydrophilic drugs [11]. Another advantage is the possibility of producing microparticles with a wide range of sizes, porosities, and shapes [16].

Eudragit® S100 is a gastro-resistant polymer used for colonic delivery, protecting drugs from pH of upper gastrointestinal tract [17]. This polymer is an anionic copolymer formed by methacrylic acid and methyl methacrylate (ratio 1:2). It is insoluble in acids and pure water, whereas it is soluble in aqueous solution presenting pH higher than 7 [18]. Microparticles prepared with this polymer can be tabletted, offering the advantages of the particulate-controlled release dosage forms [19].

Taking all the above into account, this study concerns the characterization of gastro-resistant microparticles containing sodium pantoprazole prepared using an O/O emulsification/solvent evaporation technique. Furthermore, the present study is devoted to evaluate the in vivo activity of the pantoprazole-loaded Eudragit® S100 microparticles. Additionally, tablets containing the pantoprazole microparticles were also prepared and characterized.

2. Methods

2.1. Materials

Pantoprazole sodium sesquihydrate was obtained from Henrifarma (São Paulo, Brazil). Eudragit® S100 was kindly given by Almapal® (São Paulo, Brazil produced by Rohm®, Germany). Sorbitan monooleate was obtained from Lipo Chemicals (New Jersey, USA) and mineral oil, USP grade, obtained from Fraccionata (Porto Alegre, Brazil). Acetonitrile, HPLC grade, was obtained from Fisher Chemicals (New Jersey, USA). All other chemicals were of analytical grade.

2.2. Preparation of microparticles

After dissolving the Eudragit® S100 (750 mg) in acetone (80 mL), pantoprazole sodium sesquihydrate (350 mg) was added. This suspension was emulsified (1000 rpm) with mineral oil (350 mL) containing sorbitan monooleate (1500 mg). The O/O emulsion was mechanically stirred for 2.5 h to remove the acetone. The microparticles were collected by vacuum filtration, washed with cyclohexane (2× 50 mL), and kept overnight in a desiccator.

2.3. Characterization of microparticles

2.3.1. Drug loading

An amount of the microparticles, equivalent to 10 mg of pantoprazole, was weighed and stirred with 40 mL of 0.05 M NaOH for 12 h. The volume was completed to 50 mL and drug concentration was determined after filtration (0.45 μm, Millipore®) by HPLC (Perkin-Elmer series 200; UV detector, $\lambda = 290$ nm, Shelton, USA), using a Waters® Nova Pak® column C_{18} (3.9×150 mm). Mobile phase consisted of acetonitrile/phosphate buffer, pH 7.4 (35:65 v/v).

HPLC method for pantoprazole quantification was validated [20]. Linearity (coefficient correlation) presented values higher than 0.99. The accuracy was $105 \pm 1\%$, the repeatability presented a $RSD = 0.47$, and the intermediate precision showed a $RSD = 1.17$. The detection limit was 0.5 μg/mL. To determine the reproducibility of the method and the drug purity, a liquid chromatographic system Shimadzu (LC-10ADVP, Kyoto, Japan) with a Diode Array Detector (SPD-M10AVP) was used.

2.3.2. Optical and electronic microscopies

The shape and the surface of the microparticles were analyzed by optical microscopy (Olympus®, Model BX-41, coupled with a photographic camera, Olympus®, Model PM-20, Tokyo, Japan) and scanning electronic microscopy (SEM) (Jeol Scanning Microscope JSM-5800®, Japan).

For optical microscopy analyses, samples were suspended in mineral oil. The SEM analyses were carried out using an accelerating voltage of 20 kV after they were gold sputtered (Jeol Jee 4B SVG-IN®, Peabody, USA).

2.3.3. Determination of surface area and pore diameter

The nitrogen adsorption–desorption isotherms of previous degassed organic solids, under vacuum at 40 °C, were determined at liquid nitrogen boiling point in a homemade volumetric apparatus, using nitrogen as probe. The pressure was measured using capillar mercury barometer and the results were compared to an alumina pattern. The specific surface areas of microparticles were determined by the BET multipoint technique [21] and the pore size distribution was obtained using BJH method [22].

2.3.4. Determination of particle size

The particle size distribution was determined by laser diffractometry (Mastersizer 2000, Malvern Instruments, London, UK) after dispersion in *iso*-octane. The mean diameter over the volume distribution $d_{4,3}$ was used. The span was calculated using the following equation:

$$\text{span} = \frac{d_{(v,90)} - d_{(v,10)}}{d_{(v,50)}},$$

where $d_{(v,90)}$, $d_{(v,10)}$, and $d_{(v,50)}$ are the diameters at 90%, 10%, and 50% cumulative volumes, respectively. Thus, the span gives a measure of the range of the volume distribution relative to the median diameter.

2.3.5. Determination of water content

The water content of the sample was determined by Karl Fisher titrimetry (Mettler DL 37 KF Coulometer, Switzerland). Samples were analyzed in duplicate and compared to pantoprazole.

2.3.6. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed (DSC-60 Shimadzu, Kyoto, Japan) after sealing the samples (pantoprazole, Eudragit® S100, the physical mixture, and microparticles) in aluminum pans. Calibration was carried out using indium. DSC tracings were performed from −140 to 250 °C at a rate of 10 °C/min.

2.3.7. Fourier transform infrared spectroscopic analysis

FTIR spectroscopic analysis (Shimadzu spectrometer, FTIR 8101, Kyoto, Japan) of raw materials, their physical mixture, and microparticles was carried out using KBr pellets.

2.3.8. Dissolution profile

The dissolution study was performed in flow-through cell apparatus at 37 °C using a peristaltic pump (Desaga, Heidelberg, Germany), flow rate of 1 mL/min, and phosphate buffer, pH 7.4 (PBS) as media. The samples were collected at predetermined time intervals and analyzed spectrophotometrically at 295 nm (Unicam 8625 UV/Vis spectrometer, Cambridge, England). The methodology for UV quantification was validated [20]. The profiles were analyzed by model-dependent methods (monoexponential, biexponential, and Weibull) and by model-independent method (dissolution efficiency) [23,24]. The selection of model-dependent method was based on the best correlation coefficient, the best model selection criteria (MSC), provided by Scientist® software, and the best graphic adjustment.

2.3.9. Bulk density and tapped density of microparticles

Bulk density was determined by weighing 500 mg of microparticles and measuring its volume. Tapped density was measured using a J. Engelsmann volumeter (Ludwigshafen, Germany).

2.4. In vivo anti-ulcer activity

Ulcers were induced by the oral administration of absolute ethanol (5 mL/kg) to 24 h fasted Wistar male rats ($n = 8$), weighing 200 g [25]. The groups are described in Table 1. Formulations (20 mg/kg of drug) were administered orally 1 h before the administration of ethanol. After 2 h of ethanol administration, animals were sacrificed; the stomachs were removed, opened along the greater curvature, and examined for lesion measurements. Ulcer indexes (UI) were calculated using the equation:

$$\text{UI} = \frac{10}{x},$$

where x is the total mucosal area divided by the total ulcerated area.

This protocol was approved by the Ethical Committee (deliberation number 2003247, Universidade Federal do Rio Grande do Sul, Brazil).

2.5. Preparation and characterization of tablets

2.5.1. Tabletting microparticles

Microparticles were tabletted with microcrystalline cellulose (Avicel® PH101) (28%) and magnesium stearate (0.5%). Tablets (170 mg theoretically containing 30 mg of drug) were prepared in double punch tablet machine (Korch EK0, Berlin, Germany) by individual weighing and direct compression using 8.0 mm punches.

2.5.2. Drug loading and dissolution profile

For drug loading determination, tablets were milled by a mortar and pestle. An amount of sample equivalent to 10 mg of pantoprazole was diluted with 0.05 M NaOH (40 mL) and magnetically stirred. After 12 h, the volume was completed to 50 mL and aliquots were analyzed by HPLC as described above for the microparticles.

The dissolution profile was determined using a flow-through cell apparatus as described above for microparticles.

Table 1
Groups of rats (Control 1, Control 2 and Treatment) for the in vivo anti-ulcer activity

Groups	Administered samples
Control 1	42% sodium bicarbonate solution
Control 2	Drug dissolved in water (2 mg/mL)
Treatment	Microparticles dispersed in water (mass equivalent to 2 mg/mL of pantoprazole)

2.5.3. Determination of the gastric resistance

Tablets were placed in 0.1 M HCl (3 mL) for 30 min at 37 ± 1 °C. Then, the medium was neutralized with 150 µL of 2 M NaOH and the volume was completed to 100 mL using 0.05 M NaOH. Collected samples were diluted, filtered, and analyzed by HPLC.

3. Results

Pantoprazole-loaded Eudragit® S100 microparticles were obtained using an O/O emulsion, whose anhydrous condition could prevent the degradation of drug. SEM images showed smooth spheres without crystals on the surface (Fig. 2a), presenting a hollow core after being frozen and broken (Fig. 2b). Optical microscopy analysis corroborated the capsular structure of the microparticles (Fig. 2c).

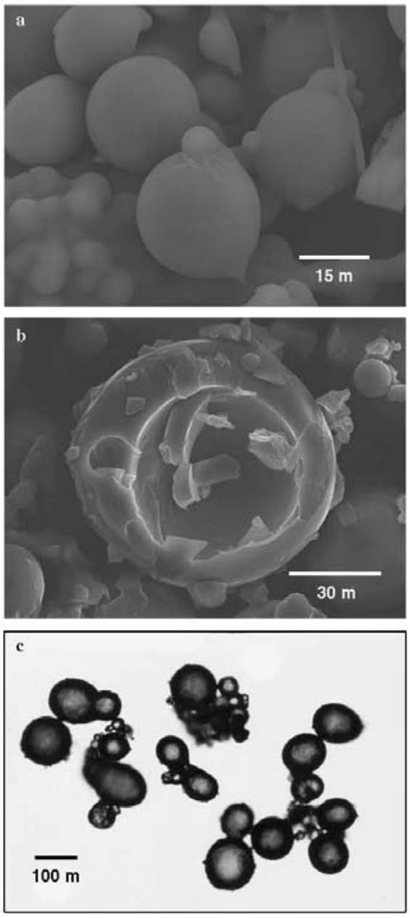


Fig. 2. SEM photomicrographs: (a) microparticles and (b) broken microparticle, and (c) optical microscopy image of microparticles.

The microparticles presented drug content of 229.5 ± 20.1 mg/g, surface area of 41 m^2/g, and pore volume of 0.04 cm^3/g. The pore size distribution in the range of 2–50 nm was close to zero, meaning the absence of mesopores [26]. The water content was 6.89% for microparticles and 6.59% for pure pantoprazole sodium sesquihydrate. The microparticles presented average size of 56.25 µm and span of 3.45.

DSC analyses were carried out for pantoprazole, Eudragit® S100, their physical mixture, and microparticles (Fig. 3). The tracing for microparticles showed a glass transition at −71.2 °C, a melting peak at 69.3 °C, and no event for pantoprazole. The results suggest that pantoprazole-loaded Eudragit® S100 microparticles are composed by a homogeneous phase, in which the polymer presents a lower degree of crystallinity than the raw material and the drug is molecularly dispersed in the polymer.

Infrared analyses (Fig. 4) were performed in order to qualify the interaction between the polymer and the drug in microparticles. The spectra of raw materials showed their characteristic bands [27–29]. When this polymer is ionized, the carboxylate band shifts from 1728 to 1560 cm^{-1}, corresponding to the anti-symmetrical vibration of COO^- [29]. The bands observed in the microparticle spectrum did not show any shift, suggesting that no new chemical bond was formed after preparing the formulation

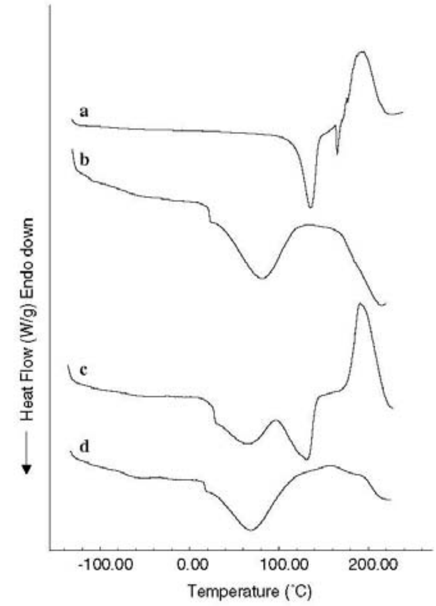


Fig. 3. DSC tracings of (a) pantoprazole, (b) Eudragit® S100, (c) physical mixture of pantoprazole and Eudragit®, and (d) microparticles.

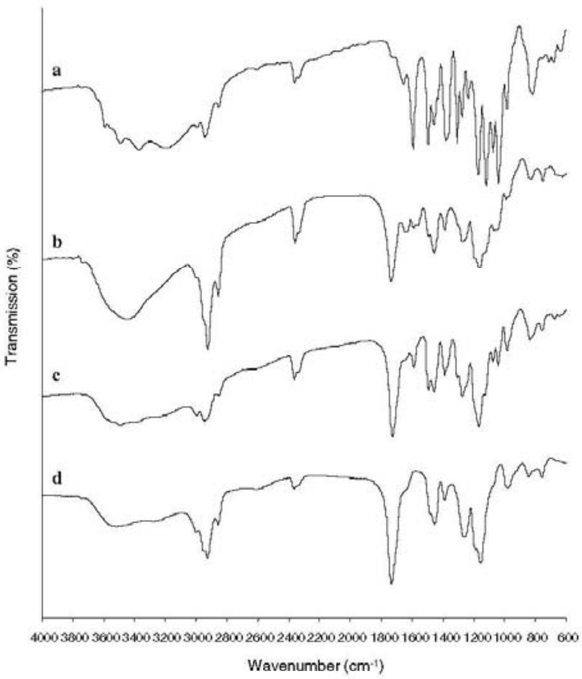


Fig. 4. Infrared spectra: (a) sodium pantoprazole sesquihydrate, (b) Eudragit® S100, (c) physical mixture of drug and polymer, and (d) microparticles.

and the results confirmed that the drug is physically dispersed in the polymer.

Regarding the gastric ulcer indexes (Fig. 5), the values were 0.42 ± 0.15 for the sodium bicarbonate solution (Control 1), 0.46 ± 0.17 for pantoprazole water solution (Control 2), and 0.07 ± 0.04 for pantoprazole-loaded Eudragit® S100 microparticles (Treatment). The Kruskal–Wallis test detected statistical differences ($p = 0.002$) among these indexes. The multiple analysis (Student–Newman–Keuls) showed that the pantoprazole-loaded Eudragit® S100 microparticles presented a gastric ulcer index statistically lower ($p < 0.05$) than those of the sodium bicarbonate aqueous solution and the pantoprazole solution groups. These results showed that the microparticles were able to protect ulcer formation by ethanol.

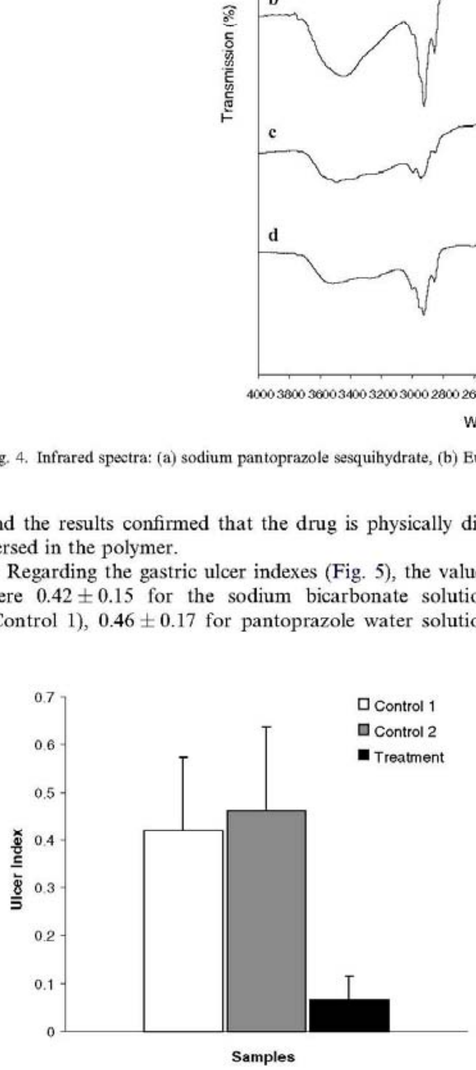


Fig. 5. Gastric ulcer indexes after administration of ethanol and sodium bicarbonate solution (Control 1), sodium pantoprazole solution (Control 2) or pantoprazole-loaded microparticles dispersed in water (Treatment).

The bulk and tapped densities of microparticles were performed and the Carr ratio and Hausner index were calculated as previously described [30]. Microparticles showed Carr ratio of 33.3 and Hausner index of 1.5. These flowability indexes demonstrated that the microparticles were cohesive and, in consequence, it was necessary to use excipients to prepare viable tablets.

The tablets presented mean weight of 169.8 ± 2.8 mg/unit corresponding to an amount of 27 mg of pantoprazole sodium sesquihydrate (equivalent to 24 mg of drug). With the aim of verifying the presence of intact microparticles in the inner of the tablets, a similar tablet formulation was prepared in the absence of the microparticles. Both tablets were broken and their insides were analyzed by SEM. Microparticles can be clearly observed in the inner of the microparticle tablets (Fig. 6).

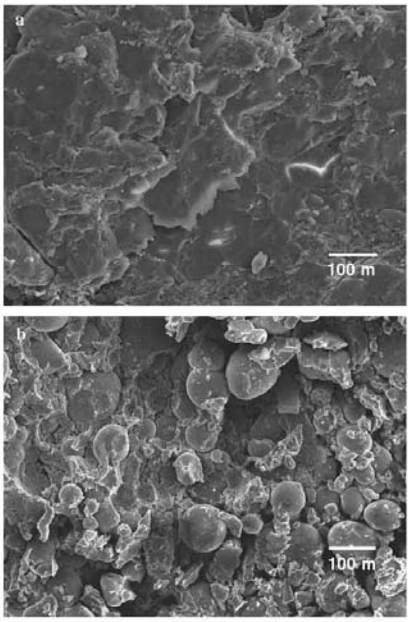


Fig. 6. SEM images. (a) Inner of a tablet prepared with the physical mixture of raw materials and (b) inner of a tablet prepared with the pantoprazole-loaded Eudragit® S100 microparticles.

Regarding the dissolution profiles (Fig. 7), 92.6 ± 2.4% of the pure pantoprazole was dissolved whereas 100.3 ± 3.9% was released from microparticles after 120 min. Drug dissolution from tablets achieved 102.1 ± 4.7% in 240 min. Dissolution efficiencies were statistically different ($p = 0.002$, ANOVA) for microparticles (81.4%) in comparison to the pure drug (73.7%). The dissolution efficiency for tablets was 67.1%.

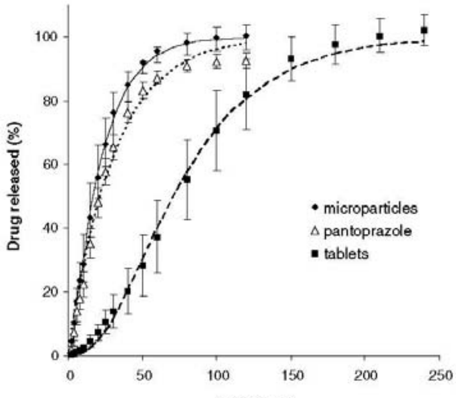


Fig. 7. Dissolution profiles (in PBS) of sodium pantoprazole sesquihydrate, pantoprazole-loaded microparticles, and tablets prepared with the microparticles. Lines show mathematical modeling for the three curves.

Considering the mathematical modeling, for pantoprazole dissolution the first-order model described the experimental data ($r = 0.9953$, $\mathrm{MSC} = 4.22$, and $k = 0.032\ \mathrm{min}^{-1}$). For the drug release from the microparticles and the tablets, the best model was that of Weibull ($D = 100[1 - \mathrm{e}^{-(t/T_{\mathrm{d}})^{\beta}}]$) presenting sigmoidal curves ($\beta =$ 1.02 and 3.02, respectively). For microparticles r was 0.9987, MSC was 5.43, and the time at which 63.2% of the drug was dissolved (T_{d}) was 19.99 min. For tablets r was 0.9985, MSC was 5.27, and T_{d} was 44.89 min.

The acid resistance experiment carried out with tablets showed that 61.05 ± 8.09% of pantoprazole remained stable presenting a satisfactory drug protection.

4. Conclusions

Pantoprazole-loaded Eudragit® S100 microparticles were successfully obtained by O/O emulsion/solvent evaporation technique. Microparticles presented spherical and smooth morphologies. DSC and FTIR analyses demonstrated that pantoprazole was physically and molecularly dispersed in the polymer forming a homogeneous phase. In vivo anti-ulcer activity evaluation showed that the microparticles were able to protect rat stomachs against ulcer formation by ethanol, while the drug aqueous solution did not present activity.

The flowability indexes (Carr ratio and Hausner index) demonstrated that the microparticles were cohesive. In this way, tabletting microparticles was possible after addition of excipients. SEM analysis showed the microparticles remained intact in the inner surface of tablets.

Drug dissolution profiles from tablets demonstrated slower release than untabletted microparticles. Weibull equation was the best model for describing the drug release profiles from microparticles and tablets. Concerning the acid protection, tablets showed a satisfactory drug protection after 30 min in acid medium. In this way, tablets obtained from pantoprazole-loaded microparticles seem to be an interesting alternative for the administration of this drug due to their ability to improve the acid protection and to control the drug release.

Acknowledgments

The authors thank Dr. M.I. Ré (IPT-USP, São Paulo) for the particle size determination and Dr. E.V. Benvenutti (IQ-UFRGS, Porto Alegre) for the surface area and pore distribution measurements. This work was supported by CNPq/MCT, Rede Nanobiotec CNPq. R.P.R. and L.C. thank CAPES (Brazil).

References

[1] D. Avner, Clinical experience with pantoprazole in gastro esophageal reflux disease, Clin. Ther. 22 (10) (2000) 1170 1185.

[2] S. Cheer, A. Prakash, D. Faulds, H. Lamb, Pantoprazole an update of its pharmacological properties and therapeutic use in the management of acid-related disorders, Drugs 63 (1) (2003) 101 132.

[3] P. Poole, Pantoprazole, Am. J. Health Syst. Pharm. 58 (11) (2001) 999 1008.

[4] G. Sachs, J.M. Shin, V. Prathaand, D. Hogan, Synthesis or rupture: duration of acid inhibition by proton pump inhibitors, Drugs Today 39 (Suppl. A) (2003) 11 14.

[5] J. Shin, M. Besancon, A. Simon, G. Sachs, The site of action of pantoprazole in the gastric H^+/K^+-ATPase, Biochim. Biophys. Acta 1148 (1993) 223 233.

[6] W. Beil, U. Staar, K. Sewing, Pantoprazole: a novel H^+/K^+-ATPase inhibitor with an improved pH stability, Eur. J. Pharmacol. 218 (1992) 265 271.

[7] A. Fitton, L. Wiseman, Pantoprazole a review of its pharmacological properties and therapeutic use in acid-related disorders, Drugs 51 (3) (1996) 460 482.

[8] K. Nishioka, T. Nagao, T. Urushidani, Correlation between acid secretion and proton pump activity during inhibition by the proton pump inhibitors omeprazole and pantoprazole, Biochem. Pharmacol. 58 (1999) 1349 1359.

[9] G.M. Ferron, S. Ku, M. Abell, M. Unruh, J. Getsy, P.R. Mayer, J. Paul, Oral bioavailability of pantoprazole suspended in sodium bicarbonate solution, Am. J. Health Syst. Pharm. 60 (13) (2003) 1324 1329.

[10] S.Y. Lin, Y.H. Kao, Tablet study of spray-dried sodium diclofenac enteric-coated microcapsules, Pharm. Res. 8 (7) (1991) 919 924.

[11] P. O'Donnell, J. McGinity, Preparation of microspheres by the solvent evaporation technique, Adv. Drug Deliv. Rev. 28 (1997) 25 42.

[12] Y. Yamagata, M. Misaki, T. Kurokawa, K. Taira, S. Takada, Preparation of a copoly(DL-lactic/glycolic acid)-zinc oxide complex and its utilization to microcapsules containing recombinant human growth hormone, Int. J. Pharm. 251 (2003) 133 141.

[13] K.A. Mehta, M.S. Kislalioglu, W. Phuapradit, A.W. Malick, N.H. Shah, Release performance of a poorly soluble drug from a novel, Eudragit®-based multi-unit erosion matrix, Int. J. Pharm. 213 (2001) 7 12.

[14] S. Benita, Microencapsulation Methods and Industrial Applications, Marcel Dekker, New York, 1996.

[15] N.K Varde, D.W. Pack, Microspheres for controlled release drug delivery, Expert Opin. Biol. Ther. 4 (1) (2004) 1 17.

[16] K.J. Zhu, J.X. Zhang, C. Wang, H. Yasuda, A. Ichimaru, K. Yamamoto, Preparation and in vitro release behaviour of 5-fluorouracil-loaded microspheres based on poly (L-lactide) and its carbonate copolymers, J. Microencapsul. 20 (6) (2003) 731 743.

[17] M. Ashford, J.T. Fell, D. Attwood, P.J. Woodhead, An in vitro investigation into the suitability of pH-dependent polymers for colonic targeting, Int. J. Pharm. 91 (1993) 241 245.

[18] D.B. Beten, M. Gelbcke, B. Diallo, A.J. Moës, Interaction between dipyridamole and Eudragit S, Int. J. Pharm. 88 (1992) 31 37.

[19] W.M. Obeidat, J.C. Price, Viscosity of polymer solution phase and other factors controlling the dissolution of theophylline microspheres prepared by the emulsion solvent evaporation method, J. Microencapsul. 20 (1) (2003) 57 65.

[20] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Validation of analytical procedures: methodology, 1996.

[21] S. Brunauer, P.H. Emmet, E. Teller, Adsorption of gases in Multimolecular Layers, J. Am. Chem. Soc. 60 (2) (1938) 309 319.

[22] E.P. Barrett, L.G. Joyner, P.P. Halenda, The determination of pore volume and area distributions in porous substances. I. Computations from nitrogen isotherms, J. Am. Chem. Soc. 73 (1) (1951) 373 380.

[23] R.C.R. Beck, A.R. Pohlmann, E.V. Benvenutti, T. Dalla Costa, S.S. Guterres, Nanostructure-coated diclofenac-loaded microparticles: preparation, morphological characterization, in vitro release and in vivo gastrointestinal tolerance, J. Braz. Chem. Soc. 16 (6) (2005) 1233 1240.

[24] P. Costa, J.M.S. Lobo, Modeling and comparison of dissolution profiles, Eur. J. Pharm. Sci. 13 (2001) 123 133.

[25] P.J. Shah, M.S. Gandhi, M.B. Shah, S.S. Goswami, D. Santani, Study of *Mimusops elengi* bark in experimental gastric ulcers, J. Ethnopharmacol. 89 (2 3) (2003) 305 311.

[26] L.B. McCusker, F. Liebau, G. Engelhardt, Nomenclature of structural and compositional characteristics of ordered microporous and mesoporous materials with inorganic hosts, Pure Appl. Chem. 73 (2) (2001) 381 394.

[27] V. Zupancic, N. Ograjsek, B. Kotar-Jordan, F. Vrecer, Physical characterization of pantoprazole sodium hydrates, Int. J. Pharm. 291 (1 2) (2005) 59 68.

[28] A.A. Badwan et al., Pantoprazole sodium, in: K. Florey, H.G. Brittain (Eds.), Analytical Profiles of Drug Substances and Excipients, vol. 29, Academic Press, Elsevier Science, USA, 2002, pp. 213 259.

[29] F. Cilurzo, P. Minghetti, F. Selmin, A. Casiraghi, L. Montanari, Polymethacrylate salts as new low-swellable mucoadhesive materials, J. Control. Release 88 (2003) 43 53.

[30] A. Schüssele, A. Bauer-Brandl, Note on the measurement of flowability according to the European Pharmacopoeia, Int. J. Pharm. 257 (1 2) (2003) 301 304.

CAPÍTULO 3: Micropartículas entéricas de liberação controlada contendo pantoprazol preparadas utilizando Eudragit S100 and Poli(ε-caprolactona)

3.1 Apresentação

No trabalho anterior, micropartículas contendo pantoprazol foram preparadas pela técnica de emulsificação/evaporação de solvente, utilizando uma emulsão O/O. Essas micropartículas preparadas apenas com Eudragit® S100 apresentaram dissolução muito similar ao fármaco puro, diferindo na gastro-resistência.

O pantoprazol é um fármaco indicado no tratamento de diferentes patologias, como refluxo gastro-esofágico e úlceras gástricas (CHEER *et al*., 2003). No caso de alívio imediato da dor causada por ulceras ou aumento na secreção acida por ingestão de medicamentos, formas farmacêuticas de liberação imediata são preferidas. Por outro lado, no caso de patologias crônicas com uso deste fármaco por períodos prolongados, formas farmacêuticas de liberação sustentadas seriam uma solução aos casos de refluxo, por exemplo. Desta forma, para atender também a esses outros casos de utilização do pantoprazol, no presente capítulo, micropartículas de liberação sustentadas foram desenvolvidas.

A mesma técnica do trabalho anterior foi utilizada, porém um segundo polímero foi adicionado à formulação, a poli(ε-caprolactona). Este polímero é um poliéster alifático, biodegradável e insolúvel em água. Suas vantagens incluem sua alta permeabilidade a pequenas moléculas, não criar ambientes ácidos durante sua degradação e excepcional capacidade de formar blendas com outros polímeros (SINHA *et al*, 2004). As micropartículas preparadas constituiram-se, portanto, de uma blenda de poli(ε-caprolactona) e Eudragit® S100, objetivando gastro-resistência e liberação controlada. Estes resultados foram publicados na revista *Pharmaceutical Development and Technology*.

Publicação 03: Enteric Controlled-Release Pantoprazole-Loaded Microparticles Prepared by Using Eudragit S100 and Poly(ε-caprolactone) Blend

Pharmaceutical Development and Technology, 12:1–9, 2007
Copyright © Informa Healthcare USA, Inc.
ISSN: 1083-7450 print / 1097-9867 online
DOI: 10.1080/10837450701556933

informa
healthcare

Enteric Controlled-Release Pantoprazole-Loaded Microparticles Prepared by Using Eudragit S100 and Poly(ε-caprolactone) Blend

Renata Platcheck Raffin, Leticia Marques Colomé, and Silvia Stanisçuaski Guterres
Faculdade de Farmácia – Universidade Federal do Rio Grande do Sul, Brazil

Adriana Raffin Pohlmann
Instituto de Química - Universidade Federal do Rio Grande do Sul, Brazil

Microparticles of poly(ε-caprolactone) and of its blend with Eudragit® S100 were prepared by emulsion/solvent evaporation technique to provide controlled release and gastro-resistance for an acid labile drug. This drug was sodium pantoprazole, a proton pump inhibitor. Both formulations were successfully prepared, but only the microparticles prepared with the blend were capable of stabilizing the drug in the acid medium. Furthermore, this formulation showed in vivo protection of stomachs against ulceration caused by ethanol in rats. These microparticles were tabletted, and the tablets demonstrated slower drug release and higher acid protection than the microparticles before tabletting.

Keywords microparticles, polymer blend, pantoprazole, gastro-resistance, solvent evaporation

INTRODUCTION

Polymeric microparticles can be prepared by using one single polymer or more than one in a blend. Polymer blends are widely used in the pharmaceutical field to obtain controlled drug delivery systems.[1–3] These formulations can improve mechanical properties, reduce toxicity, and control drug delivery.[4] Besides, they present advantages over single-unit drug delivery systems, such as improving the therapeutic effect, prolonging the biological activity, controlling the drug release rate, and decreasing the administration frequency.[5]

Poly(ε-caprolactone) (PCL) is a biodegradable hydrophobic polymer, which shows slower release of low molecular drugs and high permeability to many drugs.[6,7] This polymer is not gastro-resistant, and it does not generate an acidic environment during degradation compared to polylactides and glycolides.[7] PCL has already been blended with different polymers, such as poly(ether-ester-amide)s, poly(lactic acid), fumaric anhydride oligomer, poly(4-vinylphenol), Eudragit® RS and RL, furnishing satisfactory results for the control of drug release.[6,8–11] This polymer can also be blended with other polymers such as cellulose propionate, cellulose acetate butyrate, poly(lactide), and poly(lactide-co-glycolide), to improve stress crack resistance, dyeability, and adhesion.[7]

Eudragit® S100 is a gastro-resistant polymer used for colonic delivery, and it protects drugs from the pH of the upper gastrointestinal tract.[12] This polymer is an anionic copolymer based on methacrylic acid and methyl methacrylate (1:2 ratio). It is insoluble in acids and pure water, whereas it is soluble in aqueous solution at pH 7 or higher.[13]

The solvent evaporation process has been widely reported in recent years for the preparation of microspheres and microcapsules using biodegradable and synthetic polymers, as well as polymer blends.[5,14,15] Anhydrous systems have been used to produce microparticles using O/O emulsions.[14,16–20] This technique consists of an organic phase containing the polymer emulsified with an immiscible oil. The use of anhydrous systems can lead to higher drug entrapment efficiency and higher yields than conventional O/W emulsions.[21,22]

Drug-loaded double-walled microparticles prepared by using several methods to eliminate the initial burst and control the release of highly water-soluble drugs.[23,24] The

---

Received 24 October 2006, Accepted 27 March 2007.

Address correspondence to S.S. Guterres, Departamento de Produção e Controle de Medicamentos, Faculdade Farmácia, Universidade Federal do Rio Grande do Sul. Av, Ipiranga 2752/404, Porto Alegre, Brazil; E-mail: nanoc@farmacia.ufrgs.br

solvent evaporation method involves a single-step phase separation of composite binary polymer solutions.[25] This method presents some advantages over pan coating, fluidized beds, or spray drying in eliminating initial burst and obtaining sustained controlled release.[25]

Other biodegradable polymers, such as poly(lactide) and poly(lactide-co-glicolide), can be used in tablet formulations to control the drug release because they present good compaction properties.[26] The compression of microspheres modifies their release characteristics in either acid or neutral media. The drug release from tablets based on microparticles has been controlled by using different polymers.[27] The powders of microspheres have been easily compressed into tablets, whereas physical mixtures of drug and polymer have not been very often compressible.[27]

For this study, an acid-labile drug, pantoprazole, was chosen to be microencapsulated by the solvent evaporation technique using PCL and PCL/Eudragit® S100 blend. Pantoprazole is a proton pump inhibitor, used in the treatment of digestive ulcers.[28] It is a prodrug that degrades once protonated in acidic media.[29] So, the drug protonation for activation must occur inside the gastric parietal cells, and the tetracyclic form of pantoprazole binds irreversibly to cystein residues of the proton pump (H^+/K^+ ATPase).[30] In this way, pantoprazole must be absorbed intact before activation and, because of this, it requires an enteric drug delivery system.

In our previous work, pantoprazole-loaded microparticles were prepared with Eudragit S100® by an O/O emulsification/solvent evaporation technique.[20] These microparticles have been able to protect the stomachs of rats against ulcer formation. However, they have not controlled the drug release compared to the native drug. As far as we know, there is no report in the literature about the preparation and characterization of PCL-Eudragit® S100 blends containing pantoprazole, developed with the simultaneous objectives of providing gastro-resistance and controlling the drug release.

Taking all the findings into account, the aim of the present work was to prepare by solvent evaporation technique and to characterize the controlled-release enteric microparticles containing pantoprazole, as well as to investigate the in vivo anti-ulcer activity of the formulations. In addition, the microparticles were tabletted and characterized.

MATERIALS AND METHODS

Materials

Pantoprazole sodium sesquihydrate was obtained from Henrifarma (São Paulo, Brazil). The polymers used were Eudragit® S100 (Almapal®, São Paulo, Brazil) and poly(ε-caprolactone) (PCL) (Aldrich®, Milwaukee, USA). Sorbitan monooleate was obtained from Lipo Chemicals (New Jersey, USA). Mineral oil was USP grade and was obtained from Fraccionata (Porto Alegre, Brazil). Acetonitrile was HPLC grade and was obtained by FisherChemicals (New Jersey, USA). All other chemicals were analytic grade.

Microparticles Preparation

Single-Emulsion Microparticles (SM)

PCL was dissolved (500 mg) in acetone (80 mL) and after its complete dissolution, pantoprazole (500 mg) was added. This dispersion was emulsified (1000 rpm) with mineral oil (250 mL) containing sorbitan monooleate (750 mg). The O/O emulsion formed was mechanically stirred for 2.5 hr to allow the evaporation of all acetone. The microparticles were collected by vacuum filtration, washed two times with cyclohexane (50 mL), and dried overnight in a desiccator.

Double-Wall Microparticles (DM)

To prepare the double-wall microparticles, the suspension containing the SM microparticles in mineral oil was not filtrated. Over this suspension, Eudragit® S100 solution (500 mg) in acetone (70 mL) was emulsified. Then, after the complete solvent removal, the microparticles were collected by filtration, washed two times with cyclohexane (50 mL), and dried overnight in a desiccator.[31]

Characterization of Microparticles

Determination of Drug Loading

An amount equivalent to 10 mg of pantoprazole was accurately weighted and stirred with 40 mL of 0.05 M NaOH for 12 hr. After that, the volume was completed to 50 mL with the same solution. The pantoprazole concentration was determined after dilution and filtration through 0.22 μm (Millipore®) by HPLC (Perkin Elmer series 200 with UV detector, Shelton, USA) at $\lambda=290$ nm, LiChrosphere® RP 18 column (Merck®), and mobile phase constituted by acetonitrile:phosphate buffer pH 7.4 (35:65 v/v).

HPLC method for the quantification of pantoprazole was validated on the basisi of ICH Guideline.[32,33]

Scanning Electronic Microscopy and Energy-Dispersive X-Ray Spectroscopy

The samples were gold sputtered (Jeol Jee 4B SVG-IN®) and analyzed in Jeol Scanning Microscope JSM-5800® (Tokyo, Japan), by using an accelerating voltage of 20 kv. The SEM equipment was equipped with an energy-dispersive X-ray spectrometer (EDS Noran Instruments® model 669A-1SSS, Tokyo, Japan) for elemental analyses of in situ identification of inorganic elements. Analyses were performed by using Voyager® software. Each element produces characteristic X-rays that are displayed as peaks at one or more specific energy levels in the EDS spectrum. The element used as a marker was sulfur, present only in the drug.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was performed in DSC-60 Shimadzu (Kyoto, Japan), after sealing in aluminum pans and calibration with indium as the standard. DSC measures were performed from –140°C up to 250°C, at a rate of 10°C/min. The samples analyzed were the microparticles (SM and DM) and physical mixtures, including the components of the microparticles.

Dissolution Profile

The dissolution study was performed in flow-through cell apparatus at 37°C by using a peristaltic pump (Desaga, Heidelberg, Germany), flow rate of 1 mL/min, and phosphate buffer pH 7.4 (PBS) as medium. The samples were collected at predetermined time intervals and analyzed spectrophotometrically at 295 nm (Unicam 8625 UV/Vis spectrometer, Cambridge, England). The methodology for UV quantification was validated.[32]

Determination of Surface Area and Pore Diameter

The surface area and the porosity were determined by using the BET method (nitrogen adsorption technique).[34] The samples were desiccated and kept in liquid nitrogen temperature. The equipment used was a home-made volumetric apparatus, using nitrogen as probe. The system used a vacuum apparatus (turbomolecular Edward). The pressure was measured by using capilar mercury barometer, and the results were compared with the alumina pattern.

The pore diameter was calculated through adsoption/desorption nitrogen isotherms. The analyses were made by BJH method.[35]

Determination of Particles Size

The particle size distribution was determined by laser diffractometry after dispersion in *iso*-octane (Malvern Mastersizer 2000, Worcestershire, United Kingdom). The parameter used to present mean size was the $d_{(4.3)}$ diameter. The polydispersity (span) was calculated by the 90th percentile less the 10th divided by the arithmetical media.

In Vivo Anti-Ulcer Activity Evaluation

In vivo anti ulcer evaluation was performed according to Raffin and coworkers.[20] Ulcers were induced with absolute ethanol (5 mL/kg) orally to 24-hr fasted Wistar male rats, weighting 200 g. Solution or suspension (20 mg pantoprazole/kg) was administered orally 1 hr before the administration of ethanol. After 2 hr of ethanol administration, animals were killed, and the stomachs were removed. Sodium bicarbonate solution was used to keep the pH and the Na^+ concentration in the same level as the pantoprazole solution.

Densities of Microparticles

Bulk density was obtained weighting 500 mg of each microparticle formulation and measuring its volume. Tapped density was measured in tap apparatus (J. Engelsmann, Ludwigshafen, Germany). Compressibility index and Hausner ratio were calculated by using the values for bulk and tapped densities.[36]

Tablet Preparation and Characterization

Tableting Microparticles

Each microparticle formulation was compressed into tablets with a theoretical pantoprazole content of 40 mg. Tablets were made in double-punch tablet machine (Korch EK0, Berlin, Germany) by individual weighting. Tablets were made by direct compression with 8.0-mm punches. DM tablets contained sodium croscarmellose (1.4%), microcrystalline cellulose (Avicel® PH101) (30%), and magnesium stearate (2%). The weight was 235 mg per unit. Tablets were also prepared for comparison using a physical mixture of the polymers, drug, and excipients in the same proportion of the microparticle tablets.

Drug Loading and Dissolution Profile of Tablets

The drug content of the tablets was performed by using the same method described for the microparticles. Tablets were ground into a fine powder with a mortar and pestle. An amount equivalent to 10 mg of pantoprazole was transferred to a 50-mL volumeter flask and magnetically stirred with 0.05 M NaOH (40 mL) for 12 hr. After this, the volume was completed and analyzed by HPLC.

To compare the dissolution profiles, they were also performed in flow-through cell apparatus using the same conditions applied to the microparticles.

Determination of the Gastro-Resistance of Microparticles and Tablets

To determine the gastro-resistance, two techniques were used.

1. To verify the amount of pantoprazole protected from acid medium, tablets were placed in 0.1 M HCl (10 mL) for 60 min at 37 ± 1°C. Then, the medium was neutralized with 20 mL of 0.05 M NaOH, and the volume was completed to 50 mL by using 0.05 M NaOH. Collected samples were diluted, filtered, and analyzed by HPLC.
2. To evaluate the drug release, dissolution tests were performed for pantoprazole, SM and DM microparticles, and the tablets using variable pH media at 1.2 and 7.4. The samples were placed in the flow-through cells; and 0.1 M HCl was flowed at 1.0 mL/min (acid step) for 1 hr. During the acid step, no sample was collected for quantification because any amount of pantoprazole released at this pH is quickly degraded.[28] After the acid step, the HCl solution was replaced by phosphate buffer pH 7.4. Then, for 2 hr the samples were collected at predetermined time intervals and analyzed spectrophotometrically at 295 nm.

RESULTS

Because pantoprazole is soluble in water, it was necessary to use an anhydrous medium to prepare the emulsion. The solvent selection was based on the solubility of the formulation components.[18] Mineral oil was selected as organic external phase and acetone as organic internal phase for the preparation of the microparticles.

Both formulations showed irregular similar shape (Figure 1). However, the SM formulation was slightly smaller than the DM, and a great quantity of isolated crystals were observed on the surface of these particles (Figure 1a,b). The crystals were analyzed by EDS and sulfur was

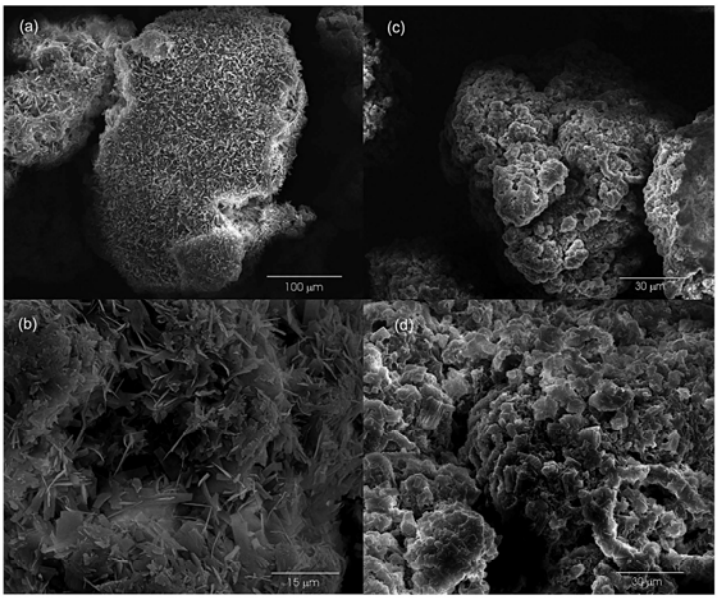


Figure 1. SEM images of SM microparticles (a and b) and DM microparticles (c and d).

detected. This result indicated that the crystals are composed by pantoprazole. On the other hand, the DM microparticles did not show any crystal, suggesting that the drug was, at least, partially encapsulated by Eudragit® S100 in the second emulsion. Concerning the DSC thermograms, PCL (Figure 2) showed an endothermic peak at 61.0°C related to its melting. Pantoprazole showed an endothermic peak (134.6°C) corresponding to its melting and an exothermic peak at 194.3°C due to its degradation. According to the literature, the melting and dehydration of pantoprazole are parallel processes in its sesquihydrate form.[37] For the physical mixture, two endothermic events were observed at 61.3 and 134.4°C, corresponding to the PCL and pantoprazole melting points and one exothermic event at 190.2°C, which corresponds to the drug degradation. The analysis of the SM microparticles showed thermic events for PCL (endothermic at 57.8°C) and pantoprazole (exothermic at 196.0°C), which indicate that polymer and drug formed heterogeneous phases. Surface crystals (Figure 1) appeared because an excess of pantoprazole was used in the formulation in relation to the amount of polymer.

Regarding the DM formulation (Figure 3), the thermogram of Eudragit® S100 showed an endothermic peak at 79.9°C, corresponding to the melting of this polymer. The curve of the physical mixture showed one endothermic peak at 60.4°C, related to the PCL melting point. However, the irregularities in the baseline indicate that the other two components are present in less quantity or show reduced entropy. For the DM microparticles, two endothermic events are observed (Figure 3). The first one, at 58.1°C, corresponds to the PCL melting point. The second one, at 125.6°C, is probably due to the decrease of the pantoprazole melting point, but no exothermic peak was observed at 190°C (pantoprazole degradation). These results suggest that pantoprazole does not form a homogeneous phase with the polymers, and the crystals are heterogeneously mixed with the polymers. To confirm this hypothesis, the DM microparticles were frozen in liquid nitrogen and broken for internal view. The broken DM showed an inner hole containing crystals (Figure 4), identified by EDS as pantoprazole.

Regarding the drug content, the SM microparticles presented 316.5 ± 10.4 mg/g of pantoprazole. These microparticles were used as cores for the preparation of the DM microparticles with final loading of 119.2 ± 3.6 mg/g of pantoprazole.

Concerning the particle size distribution, the SM microparticles showed average size of 200.8 μm and span of 1.67, whereas the DM microparticles showed larger particles (455.9 μm) and a span of 1.22. These microparticles

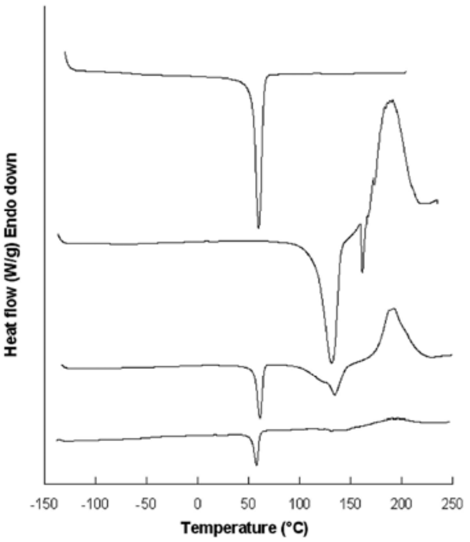


Figure 2. DSC tracings of (a) poly(ε-caprolactone), (b) pantoprazole, (c) physical mixture, and (d) SM microparticles.

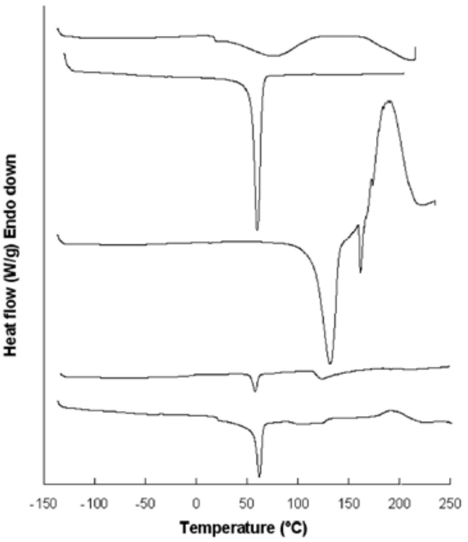


Figure 3. DSC tracings of (a) Eudragit® S100, (b) poly(ε-caprolactone), (c) pantoprazole, (d) DM microparticles, and (e) physical mixture.

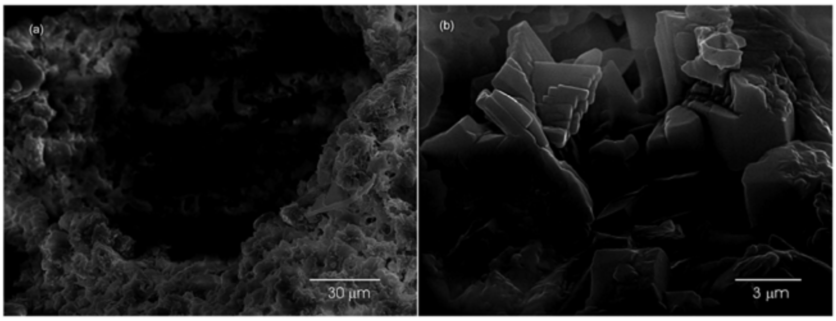


Figure 4. Broken DM microparticle showing the inner hole (a) and a portion of encapsulated pantoprazole crystals (b).

were larger than the other ones produced by the same technique.[19,38] In addition, their span values (1.67 for SM and 1.22 for DM) were lower than those observed for the microparticles containing etanidazole (2.4).[38].

To verify if 100% of the drug could be released from the microparticles, the dissolution assays were carried out in phosphate buffer pH 7.4. Pure pantoprazole, SM, and DM microparticles reached 100% of drug dissolution within 180 min (Figure 5a). Neither the microencapsulation technique nor this medium affected the stability of the drug. After the experiment, the inner residue of the cells was collected, dried under vacuum, and analyzed by EDS. The EDS spectra for both microparticles did not show the presence of sulfur. These results indicated that pantoprazole was totally released.

The in vitro gastro-resistance of encapsulated pantoprazole was determined by variable pH dissolution tests (method b). After 1 hr in acid medium, less than 0.5% (HPLC) of pure pantoprazole was stable in phosphate buffer medium (Figure 5b). The SM microparticles showed profile similar to the pure drug. After exposed to acid medium, this formulation did not protect pantoprazole against the acid degradation (0.8% remained stable when quantified at pH 7.4). On the other hand, the DM microparticles protected pantoprazole in an extension of 32% (Figure 5b). In this way, this formulation was selected for further experiments.

The DM microparticles had surface area of 36 m^2/g and mesopores were absent. Bulk and tapped densities were 0.38 and 0.49 g/cm^3. Flowability indexes (compressibility index = 23.07 and Hausner ratio = 1.3) showed that the DM microparticles have regular flow.[36]

Anti-ulcer activity evaluation in vivo (Figure 6) showed that the groups were statistically different

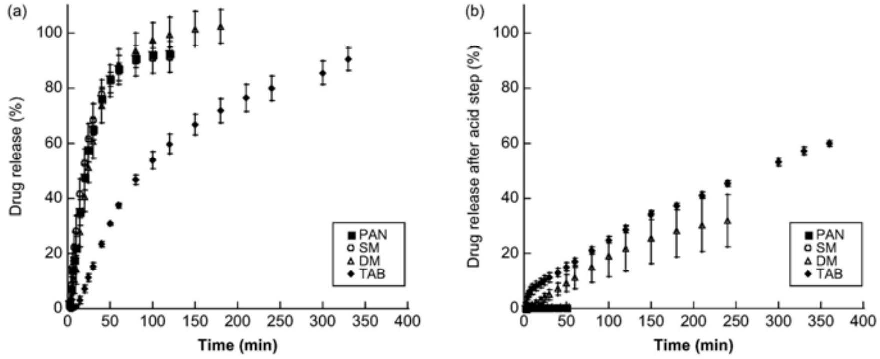


Figure 5. Dissolution profiles of pantoprazole, microparticles, and tablets in phosphate buffer pH 7.4 (a) and in variable pH (he curves represent drug release in phosphate buffer pH 7.4 after 1 hr in acid medium) (b).

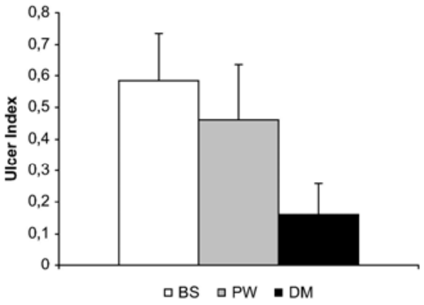


Figure 6. Ulcer indexes for the treatment with bicarbonate solution (BS), pantoprazole aqueous solution (PW), and DM microparticles suspended in water (DM).

(p=0.002) according to Kruskal-Wallis test. The bicarbonate solution group showed ulcer index of 0.58 ± 0.28, the pantoprazole aqueous solution group of 0.46 ± 0.17, and the DM group of 0.16 ± 0.09. Multiple analyses (Student-Newman-Keuls) did not differ from the group treated with the bicarbonate solution from the one treated with the pantoprazole aqueous solution. On the other hand, these two groups differed from the one treated with DM microparticles. In contrast to the in vitro results, the DM microparticles protected pantoprazole against the acid degradation and minimized ulceration by ethanol in rats.

To verify the possibility of compressing microparticles and the influence of this process in the dissolution tests, the DM microparticles were tabletted by adding sodium croscarmellose (1.4%), microcrystalline cellulose (30%), and magnesium stearate (2 %). The tablets had average weight of 232.8 ± 3.1 g, containing 7.3 ± 0.3% of pantoprazole. Tablets were broken, and the microparticles were observed in the inner part of the tablets (Figure 7). For a comparison, another tablet formulation was prepared exclusively with the physical mixture of the raw materials. The physical mixture presented poor compressibility and difficulty in handling. Polymers like PCL were very hardly compressed.[7,39] This characteristic is modified by microencapsulation. The tablets prepared with the physical mixture were very friable, presented great differences in hardness and drug release. Pantoprazole was released within 180 min presenting great variability among batches. Moreover, tablets did not disintegrate within 1 hr in phosphate buffer pH 7.4. It has already been verified an increase in compaction abilities of the microparticles in comparison with those of the corresponding physical mixtures.[39] The DM tablets disintegrated within 30 min and showed a slower release than the native drug dissolution and to the drug release from the microparticles. The tablets presented a release of more than 90% of the drug in 6 hr and no burst effect (Figure 5a). Compared to the DM microparticles, the tablets demonstrated a delay in the release of pantoprazole from 18 min to 130 min to reach approximately 60% of drug release. This result corroborates with the findings of Palmieri and coworkers,[27] who showed that the drug release from microspheres containing paracetamol was very fast, but after compression, the drug release was slower.

The gastro-resistance assay carried out for the tablets prepared with the physical mixture showed a protection of about 22% of the initial drug content. To evaluate the maximum gastro-resistance of pantoprazole, DM tablets were placed in acid medium for 1 ht. The medium was neutralized and the drug content quantified (HPLC). This evaluation showed that after neutralization, tablets presented 60 ± 5% of the initial amount of pantoprazole. In the variable pH dissolution test, pantoprazole was released from DM tablets within 360 min (Figure 5b). The tablets prepared

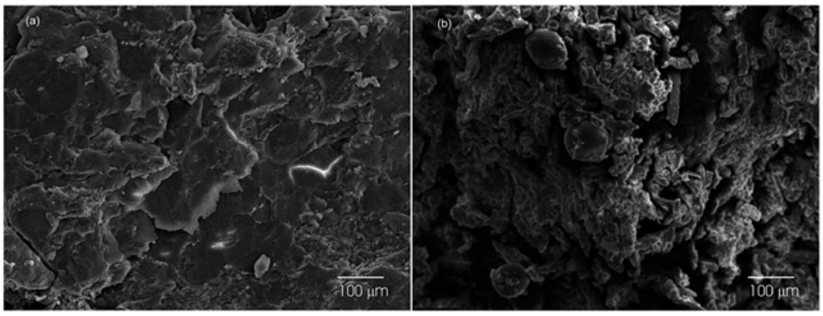


Figure 7. SEM images of (a) physical mixture tables broken (inside view) and (b) tablet containing DM microparticles.

with the DM microparticles showed both controlled release and stabilization of pantoprazole in the acid medium.

CONCLUSIONS

The SM microparticles prepared by using exclusively poly(ε-caprolactone) demonstrated a partial encapsulation of pantoprazole, but they did not protect the drug in the acid medium. Otherwise, the DM microparticles prepared with a blend of poly(ε-caprolactone) and Eudragit® S100 were able to protect more than 30% of the drug in the acid medium. These results showed the influence of Eudragit® S100 on the stability of the drug in this medium.

Even though the in vitro assay for the DM microparticles showed a partial protection of the drug in acid environment, this formulation was able to reduce the ulceration caused by ethanol in vivo. The conversion of the DM microparticles in tablets caused a decrease in the release rate of pantoprazole compared to the drug release from the DM microparticles. The stability of pantoprazole in 0.1 M HCl was increased by the compression of the DM microparticles. The tablets demonstrated a concomitant drug controlled release and high stability in the acid medium.

AKNOWLEDGMENT

The authors thank Capes, CNPq, and Fapergs for the financial support.

REFERENCES

1. Samani, S.M.; Montaseri, H.; Kazemi, A. The effect of polymer blends on release profiles of diclofenac sodium from matrices. Eur. J. Pharm. Biopharm. **2003**, *55*, 351–355.
2. Lecomte, F.; Siepmann, J.; Walther, M.; MacRae, R.; Bodmeier, R. pH-Sensitive polymer blends used as coating materials to control drug release from spherical beads: elucidation of the underlying mass transport mechanisms. Pharm. Res. **2005**, *22*, 1129–1141.
3. Nerurkar, J.; Jun, H.W.; Price, J.C.; Park, M.O. Controlled-release matrix tablets of ibuprofen using cellulose ethers and carrageenans, effect of formulation factors on dissolution rates. Eur. J. Pharm. Biopharm. **2005**, *61*, 56–68.
4. Vega-González, A.; Domingo, C.; Elvira, C.; Subra, P. Precipitation of PMMA/PCL blend using supercritical carbon dioxide. J. Appl. Pol. Sci. **2004**, *91*, 2422–2426.
5. Kim, B.K.; Hwang, S.J.; Park, J.B.; Park, H.J. Characteristics of felodipine-located polyε-caprolactone microspheres. J. Microencapsul. **2005**, *22*, 193–203.
6. Barbato, F.; Rotonda, M.I.; Maglio, G.; Palumo, R.; Quaglia, F. Biodegradable microspheres of a novel segmented polyether-ester-amides based on polye-caprolactone for the delivery of bioactive compounds. Biomaterials **2001**, *22*, 1371–1378.
7. Sinha, V.R.; Bansal, K.; Kaushik, R.; Kumria, R.; Trehan, A. Poly-ε-caprolactone microspheres and nanospheres: an overview. Int. J. Pharm. **2004**, *278*, 1–23.
8. Chandy, T.; Wilson, R.F.; Rao, G.H.R.; Das, G.S. Changes in cisplatin delivery due to surface-coated poly lactic acid-polyepsilon-caprolactone microspheres. J. Biomat. Appl. **2002**, *16*, 275–291.
9. Wang, J.; Cheung, M.K.; Mi, Y. Miscibility and morphology in crystalline/amorphous blends of polycaprolactone/poly4-vinylphenol as studied by DSC, FTIR, and ^{13}C solid state NMR. Polymer **2002**, *43*, 1357–1364.
10. Santos, C.A.; Freedman, B.D.; Ghosn, S.; Jacob, J.S.; Scarpulla, M.; Mathiowitz, E. Evaluation of anhydride oligomers within polymer microsphere blends and their impact on bioadhesion and drug delivery in vitro. Biomaterials **2003**, *24*, 3571–3583.
11. Gibaud, S.; Al Awwadi, N.J.; Ducki, C.; Astier, A. Polyε-caprolactone and Eudragit® microparticles containing fludrocortisone acetate. Int. J. Pharm. **2004**, *269*, 491–508.
12. Ashford, M.; Fell, J.T.; Attwood, D.; Woodhead, P.J. An in vitro investigation into the suitability of pH-dependent polymers for colonic targeting. Int. J. Pharm. **1993**, *91*, 241–245.
13. Beten, DB.; Gelbcke, M.; Diallo, B.; Moës, A.J. Interaction between dipyridamole and Eudragit S. Int. J. Pharm. **1992**, *88*, 31–37.
14. Zhu, K.J.; Zhang, J.X.; Wang, C.; Yasuda, H.; Ichimaru, A.; Yamamoto, H. Preparation and in vitro release behaviour of 5-fluorouracil-loaded microspheres based on poly L-lactide and its carbonate copolymers. J. Microencapsul. **2003**, *20*, 731–743.
15. Matsumoto, A.; Matsukawa, Y.; Suzuki, T.; Yoshino, H. Drug release characteristics of multi-reservoir type microspheres with polyDL-lactide-co-glycolide and polyDL-lactide. J. Control. Release. **2005**, *106*, 172–180.
16. Alavi, A.K.; Squillante, E.; Mehta, K.A. Formulation of enterosolubile microparticles for an acid labile protein. J. Pharm. Pharm. Sci. **2002**, *5*, 234–244.
17. Mateovic, T.; Ratnik, M.; Bogataj, M.; Mrhar, A. Determination of microsphere solidification time in the solvent evaporation process. J. Microencapsul. **2005**, *22*, 81–90.
18. Obeidat, W.M.; Price, J.C. Preparation and in vitro evaluation of propylthiouracil microspheres made of Eudragit RL 100 and cellulose acetate butyrate polymers using the emulsion-solvent evaporation method. J. Microencapsul. **2005**, *22*, 281–289.
19. Yang, J.F.; Qiu, L.Y.; Jin, Y.; Zhang, J.X. Thymosin-loaded enteric microspheres for oral administration: Preparation and in vitro release studies. Int. J. Pharm. **2005**, *301*, 41–47.
20. Raffin, R.P.; Colomé, L.M.; Pohlmann, A.R.; Guterres, S.S. Preparation, characterization, and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles. Eur. J. Pharm. Biopharm. **2006**, *63*, 198–204.
21. O'Donnell, P.; McGinity, J. Preparation of microspheres by the solvent evaporation technique. Adv. Drug Del. Rev. **1997**, *28*, 25–42.

22. Lamprecht, A.; Yamamoto, H.; Takeuchi, H.; Kawashima, Y. Design of pH-sensitive microspheres for the colonic delivery of the immunosuppressive drug tacrolimus. Eur. J. Pharm. Biopharm. **2004**, *58*, 37–43.
23. Lee, T.H.; Wang, J.; Wang, C. Double-walled microspheres for the sustained release of a highly water soluble drug: characterization and irradiation studies. J. Control. Release. **2002**, *83*, 437–452.
24. Rahman, N.A.; Mathiowitz, E. Localization of bovine serum albumin in double-walled microspheres. J. Control. Release **2004**, *94*, 163–175.
25. Tan, E.C.; Lin, R.; Wang, C. Fabrication of double-walled microspheres for the sustained release of doxorubicin. J. Coll. Int. Sci. **2005**, *291*, 135–143.
26. Giunchedi, P.; Gavini, E.; Bonacucina, G.; Palmieri, G.F. Tabletted polylactide microspheres prepared by a w/o emulsion-spray drying method. J. Microencapsul. **2000**, *17*, 711–720.
27. Palmieri, G.; Michelini, S.; Martino, P.; Martelli, S. Polymers with pH-dependent solubility: possibility of use in the formulation of gastroresistant and controlled-release matrix tablets. Drug Dev. Ind. Pharm. **2000**, *26*, 837–845.
28. Jungnickel, P.W. Pantoprazole, a new proton pump inhibitor. Clin. Ther. **2000**, *22*, 1268–1293.
29. Sachs, G.; Shin, J.M.; Pratha, V.; Hogan, D. Synthesis or rupture, duration of acid inhibition by proton pump inhibitors. Drugs Today **2003**, *39* (Suppl. A), 11–14.
30. Shin, J.; Besancon, M.; Simon, A.; Sachs, G. The site of action of pantoprazole in the gastric H^+/K^+-ATPase. Biochim. Biophys. Acta **1993**, *1148*, 223–233.
31. Cuña, M.; Vila Jato, J.L.; Torres, D. Controlled-release liquid suspensions based on ion-exchange particles entrapped within acrylic microcapsules. Int. J. Pharm. **2000**, *199*, 151–158.
32. *Guidance for Industry–Q2B Validation of Analytical Procedures, Methodology*; European Agency for the Evaluation of Medicinal Products (ICH): Londres, 1996.
33. Raffin, R.P.; Colomé, L.M.; Guterres, S.S.; Pohlmann, A.R. *Validação de Metodologia Analítica por Cromatografia Líquida para Doseamento e Estudo da Estabilidade de Pantoprazol Sódico.* Química Nova, **2006.** In press.
34. Brunauer, S.; Emmet, P.H.; Teller, E. Adsorption of gases in multimolecular layers. J. Am. Chem. Soc. **1938**, *60*, 309–319.
35. Barret, E.P.; Joyner, L.G.; Halenda, P.P. The determination of pore volume and area distributions in porous substances. I. Computations from nitrogen isotherms. J. Am. Chem. Soc. **1951**, *73*, 373–380.
36. *United States Pharmacopeia*; 30th Ed.; United States Pharmacopeial Convention: Rockville, MD, 2007.
37. Zupancic, V.; Ograjsek, N.; Kotar-Jordan, B.; Vrecer, F. Physical characterization of pantoprazole sodium hydrates. Int. J. Pharm. **2005**, *291*, 59–68.
38. Yip, E.Y.; Wang, J.; Wang, C. Sustained release system for highly water-soluble radiosensitizer drug etanidazole: irradiation and degradation studies. Biomaterials **2003**, *24*, 1977–1987.
39. Palmieri, G.F.; Bonacucina, G.; Di Martino, P.; Martelli, S. Spray-drying as a method for microparticulate controlled release systems; Preparation: Advantages and limits. I. Water-soluble drugs. Drug Dev. Ind. Pharm. **2001**, *27*, 195–204.

CAPÍTULO 4: Caracterização de micropartículas produzidas em spray driers de diferentes escalas de produção

4.1 Apresentação

Até o presente momento, foram descritas duas formulações de micropartículas preparadas pela técnica de evaporação de solvente. Esta técnica apresenta algumas desvantagens como a utilização de solventes orgânicos e a maior dificuldade de transposição de escala, comparada com outras técnicas descritas. A preparação de micropartículas pela técnica de *spray-drying* ou secagem por aspersão pode ser utilizada para soluções ou suspensões aquosas e equipamentos industrias já são encontrados em indústrias, principalmente alimentícias.

Neste capítulo, foram preparadas micropartículas de liberação imediata, utilizando apenas Eudragit® S100 pela técnica de aspersão. As produções em escala laboratoriais e em escala piloto foram estudadas e comparadas. Diferentes condições operacionais foram testadas na escala piloto até a obtenção de condições adequadas a fim de maximizar a gastro-resistência da formulação.

Este trabalho foi realizado no Instituto de Pesquisas Tecnológicas do Estado de São Paulo, sob supervisão da Dra. Maria Inês Ré. Os resultados foram publicados na revista *Drying Technology*.

Publicação 04: Powder Characteristics of Pantoprazole Delivery Systems Produced in Different Spray-Dryer Scales

Drying Technology, 24: 339–348, 2006

ISSN: 0737-3937 print/1532-2300 online
DOI: 10.1080/07373930600564381

Powder Characteristics of Pantoprazole Delivery Systems Produced in Different Spray-Dryer Scales

R. P. Raffin,[1] S. S. Guterres,[1] A. R. Pohlmann,[2] and M. I. Ré[3]
[1]*Faculty of Pharmacy, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre-RS, Brazil*
[2]*IPT - Institute for Technological Research of the State of São Paulo, Laboratory of Processes Chemical and Particle Technolgy, Cidade Universitária, São Paulo, SP, Brazil*
[3]*IPT - Institute for Technological Research of the State of São Paulo, Chemistry Division, Laboratory of Particles Technology, Cidade Universitária, São Paulo-SP, Brazil*

The purpose of this study is to investigate the physical characteristics of pantoprazole-loaded enteric microparticles produced in different spray dryers and operational conditions. In all conditions tested it was possible to obtain powders that presented spherical shape microparticles, with mean sizes from 6.7 to 24.5 μm. The size was affected mainly by initial feed concentration (2.2 or 6.6% w/w). The integrity of microparticles affected surface area (24 to 113 m^2/g) and drug release (71 to 98%). All powders presented very poor flow. The powder that presented higher release was produced with 6.6% solution and a rotating disc atomizer.

Keywords Pantoprazole; Powder characteristics; Spray-dryer; In vitro drug release; Enteric delivery systems

INTRODUCTION

Multiparticulate drug delivery systems have shown several advantages over single unit ones, such as more uniform transit times through the gastro-intestinal tract, less variability among individuals, and a smaller risk of dose dumping and high local concentrations.[1] Eudragit® S100 [methacrylic acid–methyl methacrylate copolymer (1 : 2)] is an enteric polymer that dissolves in aqueous solution presenting pH higher than 7.[2] Pantoprazole sodium sesquihydrate is a prodrug, protonated in acid medium of stomach parietal cells that binds irreversibly the H^+/K^+-ATPase. It is used in the treatment of digestive ulcers, gastro-oesophageal reflux disease, and *Helicobacter pylori* infections.[3] This drug should only be activated inside gastric parietal cells.[4] Therefore, it must be formulated in enteric drug delivery systems. Enteric microparticles can be prepared by the spray-drying technique using an aqueous solution of drug and polymer.[5]

From a microstructural viewpoint, the production of a formulated spray-dried powder involves the droplet formation from the atomized suspension, solution, or emulsion followed by their solidification driven by the solvent evaporation.[6] The desired microstructure can be created from the complex mixture of polymers, surfactants, and the appropriate drug that gives the in-use properties of the product. In a single operation, after atomization the suspension into the spray-dryer, the drug can be entrapped into the main component of the structurant, e.g., the polymer. This type of process permits the isolation of microspheres or microcapsules depending on the initial formulation.[7]

The pharmaceutical process evolution toward a high productivity and a simple powder formulation ensures using the spray-drying technique as the microencapsulation method. This implies a one-step process to produce microparticles free of organic solvents. Since this one-step process is easier to be scaled-up[8] and equipment is readily available, the processing costs can be reduced.

Formulation and operational conditions have a significant effect on product characteristics, which can affect drug release. The design capability of spray dryers and their mode of operation to produce dried powder of various sizes and forms do offer exciting possibilities for applying it to develop new multiparticulate delivery systems.[9]

Despite all these advantages, some drawbacks, mentioned by Benoit et al.,[10] should be recalled, as follows. The majority of studies reported in the literature originate from experiments performed in the laboratory-scale equipment with a small dimension spray-drying nozzle. In this laboratory scale, smaller particles are produced (mean particle diameter less than 10 μm). Besides, there are little or no complete data to determine a relationship between the dryer operating variables (e.g., pressures and temperatures of the compressed air, flow rate of atomized solution) and the size and the structural arrangement of produced particles.

Address correspondence to Dr. Maria Inês Ré, IPT - Instituto de Pesquisas Technológicas/Centro de Technologia de Processos e Produtos, Av. Prof. Almeida Prado, 532, Cidade Universitária, CEP 005508–901, São Paulo, Sp, Brazil; E-mail: mire@ipt.br

Changing any operating variable can lead to variations in the drug release kinetics. Therefore, knowledge about the effect of these operating variables on powder characteristics is needed to change the spray dryer scale from laboratory to industrial. These changes in scales entail modifications in the geometry and evaporation capacity of the atomizer as well as in the feed solution flow rate.

The physical characteristics of the powders depend on the design and scale of production employed. This study has been conducted to determine how pantoprazole-enteric powder characteristics vary as the spray dryer changes its scale from the laboratory to the pilot equipment. Powder characteristics examined include the particle size distribution and morphology, the bulk density and flowability as measured by angle of repose the compressibility index, and the drug dissolution kinetics.

MATERIAL AND METHODS

Pantoprazole sodium sesquihydrate has been obtained from Henrifarma (São Paulo, Brazil). Eudragit® S100 has been kindly provided by Almapal® (São Paulo, Brazil, produced by Rohm®, Germany). All other chemicals are analytical grade (Darmstadt).

The methodology adopted to prepare sodium pantoprazole suspensions formulated with Eudragit® S100 for experiments comprises two steps: (1) dissolve the polymer Eudragit® S100 (1.2 or 3.6 g) into 0.05 M NaOH (75 mL); (2) add, under stirring, sodium pantoprazole (0.3 or 0.9 g) to these aqueous solutions, until reaching 1 : 4 drug-to-polymer ratio.

Following the design of experiments shown in Table 1, each solution (total solids concentrations of 2.2 or 6.6% w/w) has been atomized into a laboratory spray dryer (Model 190 BUCHI®) and/or into a pilot spray dryer (Model S52 APV® Anhydro). The total volume sprayed was 75 mL and 2000 mL, respectively. The laboratory-scale spray dryer (model 190 BUCHI®) has the dryer cylindrical chamber of 0.13 m diameter and the total height of 0.5 m. During tests, this equipment was equipped with a two-fluid pressurized nozzle. In this nozzle atomizer, the liquid suspension is injected through a central hole of diameter $d_o = 0.7$ mm. Atomizing air is discharged through a ring area around the liquid hole. Experiments have been carried out under the following conditions: inlet air temperature 150 ± 5°C, outlet air temperature 98 ± 3°C, aspirator setting 10, suspension feed flow rate 0.24 L/h, air flow rate 500 NL/h. In this small-scale equipment, droplets flow co-currently with air flow.

The pilot spray dryer used (Model PSD 52 APV® Anhydro, Denmark) presents the cylindrical dryer chamber of 1.0 m diameter and 23 m of total height. In this equipment, three sets of experiments have been performed, as summarized in Table 1. The first set is using a rotating disc under the following operating conditions: co-current flow dryer; rotational velocity of atomizer 30,000 rpm; suspension flow rate 2 L/h; inlet and outlet air temperatures 170 ± 1°C and 85 ± 5°C, respectively. Two other sets of experiments are carried out using a two-fluid pneumatic atomizer with external mixing. In this nozzle, the liquid to be atomized is discharged through a central hole of diameter $d_0 = 1.5$ mm, whereas the atomizing air is injected through a ring area around the liquid hole. The atomizing air pressure varies from 49 to 196 kPa to generate droplets with different sizes, as shown in the previous work.[11] In one set of experiments, droplets flow in co-current with the drying air (co-current flow dryer); in the other set, droplets flow in counter-current in relation to the drying air inlet (mixed flow dryer, where the feed is sprayed upwards and the particles formed inside the dryer finish their journey in a co-current mode).

Based on these experimental data, the effect of operating variables on the powder characteristics and influence of suspension properties can be investigated in both laboratory and pilot apparatus.

Microparticle Characterization

Particle Size

The microparticle size distributions were measured by laser light diffraction (Malvern MasterSizer®, model E,

TABLE 1
Experimental conditions

Liquid properties Total feed solids concentration (% w/w)	Spray dryer configuration			
	Scale production	Atomizer	Co-current flow	Mixed flow
2.2 6.6	Laboratory	Nozzle	x	—
2.2 6.6	Pilot plant	Nozzle (two different atomizing air pressures: 49 and 196 kPa)	x	x
2.2 6.6	Pilot plant	Rotating disc	x	—

Worcestershire, UK) after redispersion in *iso*-octane. Average particle size was expressed as the mean volume diameter ($D_{4,3}$). Polydispersity was given by a span index, which was calculated by $(D_{0.9}–D_{0.1})/D_{0.5}$, where $D_{0.9}$, $D_{0.5}$, and $D_{0.1}$ are the particle diameters determined respectively at the 90th, 50th, and 10th percentile of the undersized particle distribution curve.

Surface Area and Pore Size Distribution

The nitrogen adsorption-desorption isotherms of previous degassed organic solids, under vacuum at 40°C were determined at liquid nitrogen boiling point in a homemade volumetric apparatus, using nitrogen as probe. The pressure was measured using capillary mercury barometer and the results were compared to alumina pattern. The specific surface areas of microparticles were determined by the BET multipoint technique[12] and the pore size distribution was obtained using the BJH method.[13]

Scanning Electronic Microscopy Analyses

Samples of microspheres were mounted on a sample holder, sputter-coated with a thin layer of Au, and examined with a JEOL JSM5200® scanning electron microscope (SEM) at an intensity of 15 kv using various magnifications.

Packing and Flowing Properties

Carr Index. Bulk and tapped densities of the spray-dried microparticles were determined using an automatic tapper (AutoTap, Quantachrome® Corp., Boyton Beach, FL, USA). The tapped density was measured after 1250 taps, because preliminary investigations have shown that the volumetric change after this number of taps was negligible. An average of three determinations was taken. From these measurements, the Carr index was determined. Note that the Carr index is defined as the difference between the tapped and the bulk density divided by the tapped density, expressed as a percentage.[14]

Angle of Repose. The angle of repose is the angle between the horizontal and slope of the heap. This angle is a direct indication of the potential flowability of a powder (contact and friction between particles in motion). The powder flows out from the end of a vibrating funnel as represented in Fig. 1. The angle of repose was measured in a Powder Characteristics Tester, Model PT-N (Hosokawa Microns®).

True Density

The true density of all samples was measured by helium picnometry using a Multi Pycnometer (Quantachrome®) at room temperature.

Water Content

Humidity was assayed gravimetrically in Mettler Toledo® HB 43 Halogen kept at 105°C until constant weight. Samples were assayed in duplicate.

Dissolution Kinetics

Size 0 hard gelatine capsules without coloring agent were filled with 90 mg of microparticles, corresponding to 16 mg of drug. Dissolution tests were undertaken in USP dissolution apparatus I at 50 rpm and 37°C. In order to determine if the microparticles were able to release 100% of the drug encapsulated, the dissolution was evaluated in phosphate buffer pH 7.4 for 120 min. To evaluate gastro-resistance, capsules were exposed to 300 mL of 0.1 M HCl. After 1 h, an NaOH (2.6 g) and KH_2PO_4 (6.12 g) aqueous solution (600 mL) was added in order to achieve the final pH of 7.4. The samples were collected at predetermined time intervals from 0 up to 120 min. Pantoprazole concentrations were determined by UV spectrophotometry at 295 nm.

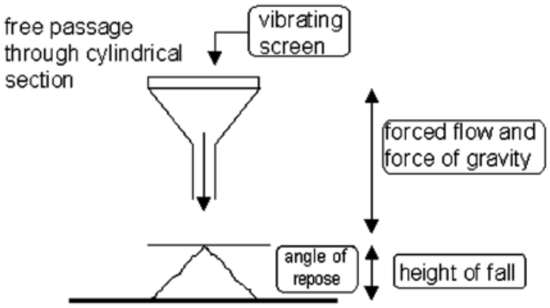


FIG. 1. Principle of the angle of repose.

TABLE 2
Characteristics of feed solution and microparticles obtained with a laboratory spray dryer

Pantoprazole-Eudragit S100 solution properties		Spray-dried powder characteristics						
Feed solid concentration (% w/w)	Solution viscosity (cp)	Particle size (µm)		Bulk density ($g{\cdot}cm^{-3}$)	Tapped density ($g{\cdot}cm^{-3}$)	Carr index	Surface area (m^2/g)	Drug loading (% w/w)
2.2	6.1 (25°C)	$D_{(4.3)}$	6.70	0.24 ± 0.02	0.40 ± 0.01	40.0 ± 1.1	86.9	17.84 ± 0.26
		$D_{(0.1)}$	1.36					
		$D_{(0.9)}$	13.03					
		span	1.95					
		S.D.	0.17					
6.6	10.4 (40°C)	$D_{(4.3)}$	9.00	0.16 ± 0.02	0.28 ± 0.01	42.8 ± 1.4	113.0	15.17 ± 0.66
		$D_{(0.1)}$	1.36					
		$D_{(0.9)}$	17.08					
		span	2.19					
		S.D.	0.68					

S.D., standard deviations for the mean diameter.

An amount equivalent to 10 mg of pantoprazole in the microparticles was weighed and dissolved in 50 mL of 0.05 M NaOH. Drug concentration was determined in a sample after filtration (0.45 µm, Millipore®) by HPLC (Perkin Elmer series 200; UV detector, $\lambda = 290$ nm, Shelton, CN), using a Merck® Lichrosphere® column C_{18} as a stationary phase. The mobile phase consisted of acetonitrile/phosphate buffer pH 7.4 (35 : 65 v/v).

RESULTS AND DISCUSSION

Effect of the Solution Properties on the Physical Characteristics of the Spray-Dried Microspheres Obtained in the Laboratory-Scale Equipment

The influence of the liquid properties on the particle size distribution has been investigated with formulated solutions containing sodium pantoprazole and Eudragit® S100 as the coating material. Table 2 summarizes the results obtained, showing that the particles obtained from the more diluted solution (2.2% w/w) are smaller than ones obtained from the more concentrated solution (6.6% w/w). The size difference is seen in Fig. 2. SEM images show spherical hollow particles with a smooth wrinkled surface without characteristic drug crystals over their surface. Hollow particles are characteristic from spray drying products of skin-forming materials.[15] Smaller particles are usually denser, and so the bulk density of this small particle size powder is higher as it can be seen in Table 2. Usually, the higher the feeds solid content, the faster the particles dried, presenting an increase of particle size and reduction of particle density.[16] Indeed, all spray-dried particles have a very poor flow according to Carr's

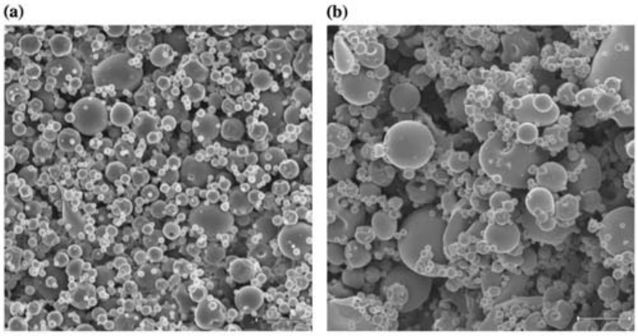


FIG. 2. SEM images of laboratory-scale microparticles produced from (a) 2.2% solution and (b) 6.6% solution.

comparison (values over 32%).[14,17] Surface area has been calculated as 86.9 and 113.0 m^2/g for 2.2% and 6.6% solution concentration, respectively.

Using both solid concentrations, it was possible to obtain powders, and all of them presented poor flow. Using the most concentrated solution, the time of the process is reduced in the same way that the amount of water and energy.

Effect of the Atomization Systems and Atomizing Conditions on the Spray-Dried Powder Characteristics

Table 3 summarizes results obtained for the two different atomizers used (two-fluid nozzle and rotary) in the pilot equipment. The more adequate value of the liquid flow rate has been fixed to produce the formulated spray-dried microparticles. For the two-fluid nozzle, the atomizing air pressure has been changed in order to investigate its effect on the spray droplet size. Operational spray-dryer variables are directly related to the product quality.[18]

The same effect observed in the laboratory scale is visible in the pilot scale. The initial solution concentration is an important factor, changing, in this case, the mean particle size from around 7 μm (laboratory scale and 2.2% w/w) to more than 20 μm (pilot scale and 6.6% w/w). In the conditions analyzed, the atomizing air pressure may not affect the particle size, as shown in Table 3.

TABLE 3
Effect of the atomizer system and atomizing conditions on the microparticles characteristics (co-current flow dryer)

			Spray-dried powder characteristics						
Atomizer	Air pressure (kPa)	Solution concentration (% w/w)	Particle size (μm)	Bulk density (g·cm^{-3})	Tapped density (g·cm^{-3})	Carr index	Angle of repose (°)	Drug loading (% w/w)	Surface area (m^2/g)
Rotating disc	—	2.2	$D_{(4.3)}$ 10.11 $D_{(0.1)}$ 1.94 $D_{(0.9)}$ 19.39 span 1.97 S.D. 1.08	0.34 ± 0.02	0.57 ± 0.04	40.3 ± 1.8	42.9 ± 1.6	17.84 ± 0.50	24.1
		6.6	$D_{(4.3)}$ 24.52 $D_{(0.1)}$ 4.86 $D_{(0.9)}$ 47.56 span 1.95 S.D. 0.98	0.21 ± 0.03	0.34 ± 0.06	38.2 ± 2.3	48.2 ± 2.9	17.36 ± 0.41	40.1
Two-fluid nozzle	49	2.2	$D_{(4.3)}$ 7.76 $D_{(0.1)}$ 1.41 $D_{(0.9)}$ 14.46 span 1.79 S.D. 0.24	0.26 ± 0.05	0.51 ± 0.04	49.0 ± 1.6	36.9 ± 1.2	17.27 ± 0.86	50.5
		6.6	$D_{(4.3)}$ 24.45 $D_{(0.1)}$ 3.94 $D_{(0.9)}$ 49.21 span 2.50 S.D. 1.90	0.11 ± 0.03	0.24 ± 0.02	51.9 ± 1.5	50.6 ± 0.8	22.60 ± 0.53	72.2
	196	2.2	$D_{(4.3)}$ 7.08 $D_{(0.1)}$ 1.58 $D_{(0.9)}$ 13.90 span 2.01 S.D. 0.71	0.23 ± 0.04	0.43 ± 0.03	45.9 ± 1.5	33.3 ± 1.9	17.30 ± 0.08	76.5
		6.6	$D_{(4.3)}$ 21.95 $D_{(0.1)}$ 3.96 $D_{(0.9)}$ 45.87 span 2.45 S.D. 0.44	0.08 ± 0.02	0.18 ± 0.01	53.9 ± 1.4	51.1 ± 1.4	17.97 ± 0.02	71.6

S.D., standard deviations for the mean diameter.

The particle size seems to be affected mainly by the initial solution concentration and, for the more concentrated solution (6.6% w/w), by the type of atomizer, too. For 2.2% solution, the mean particle size varies from 7.08 ± 0.24 and 7.76 ± 0.71 to 10.11 ± 1.08 µm when the atomizer is changed from nozzle to rotating disc. This difference can be due to the droplet size formed in each atomizer. For 6.6% solution, no difference is seen between nozzle types, since the variation of the mean particle size from 21.95 ± 0.44 to 24.52 ± 0.98 µm is in the range of experimental error.

Smaller particles are denser and presented again higher bulk and tapped densities. True densities are measured constant. All microparticles present the true density equal to $1.50 \pm 0.02\,\mathrm{g.cm^{-3}}$. This result is already expected because all microparticles are produced with the same materials.

Even though microparticles produced by rotating disc present lower Carr indexes, the angle of repose confirms that all produced powders present very poor flow, mainly due to the reduced particle size. For powder produced with the nozzle atomizer and 2.2% solution, the angle of repose is lower (36.9 ± 1.17 and 33.3 ± 1.94°, for 49 and 196 kPa, respectively), showing a better flowability than the others, but this result is not in agreement with the Carr index. The reason is that Carr is a stationary measure, whereas angle of repose is measured with flowing powder. Spray-dried powders are often cohesive caused by interparticle and friction forces and reduced particle sizes.[15]

Surface area is higher to powders produced with the nozzle atomizer. Surface area of the four powders produced varies from 50.5 to $76.5\,\mathrm{m^2/g}$. Powders produced with rotating disc atomizer presents 24.1 and $40.1\,\mathrm{m^2/g}$ for 2.2 and 6.6% solutions. This may be due to visible large holes in microparticles produced by the nozzle atomizer.

All powders presented spherical shape, smooth surface, and absence of visible pores (Fig. 3). The increase in feed solids content results in puffing or ballooning and cracking of the particles. It is also seen that pilot-scale powders presented large holes due to water rapid evaporation.[16] The mean volume diameter, particle size distribution, and bulk density do vary which might change the critical product characteristics during the scale-up.

Effect of the Spray-Air Contact Pattern on the Spray-Dried Powder Characteristics

In this study, the effect of the manner in which spray contacts the drying air on the characteristics of powders obtained in the pilot equipment is investigated (co-current flow dryer versus mixed flow dryer). The characteristics of microparticles produced by the nozzle atomizer the mixed pattern are summarized in Table 4.

Minor differences are seen between the two formulations produced in the mixed pattern. Comparing co-current and mixed flow patterns (Tables 3 and 4), there are differences in the particle size and the surface area for both 2.2 and 6.6% solutions. Particle size and surface area values of powders obtained in mixed pattern are intermediate between 2.2 and 6.6% solutions values for the nozzle atomizer at 196 kPa pressure and co-current contact. The flowability remains poor although 6.6% solution presents lower Carr index and the angle of repose remains invariable.

The most important characteristic to be compared between co-current and mixed-flow dryers is the particle moisture content. This decreases in mixed-flow dryer as shown in Table 5. As expected, the mixed pattern produces powder around 6% of moisture content, compared to 8–9% in the co-current spray-air contact. Powders with

TABLE 4
Characteristics of products prepared with nozzle atomizer, air pressure of 196 kPa (mixed flow dryer)

Solution concentration (% w/w)	Spray-dried powder characteristics							
	Particle size (µm)		Bulk density ($\mathrm{g{\cdot}cm^{-3}}$)	Tapped density ($\mathrm{g{\cdot}cm^{-3}}$)	Carr index	Angle of repose (°)	Drug loading (% w/w)	Surface area ($\mathrm{m^2/g}$)
2.2	$D_{(4.3)}$	12.73	0.20 ± 0.01	0.36 ± 0.02	44.4 ± 1.3	46.9 ± 1.1	19.00 ± 0.15	60.4
	$D_{(0.1)}$	2.01						
	$D_{(0.9)}$	23.33						
	span	1.8						
	S.D.	0.46						
6.6	$D_{(4.3)}$	15.63	0.25 ± 0.07	0.38 ± 0.03	34.2 ± 1.4	45.8 ± 1.3	18.44 ± 1.12	62.6
	$D_{(0.1)}$	2.82						
	$D_{(0.9)}$	30.82						
	span	2.25						
	S.D.	0.95						

S.D., standard deviations for the mean diameter.

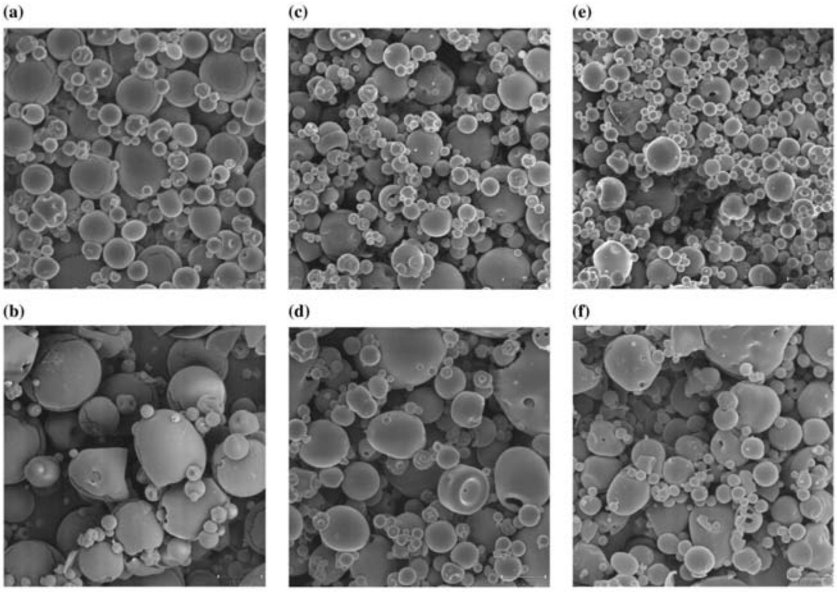


FIG. 3. SEM images of pilot-scale microparticles produced with rotating disc atomizer with (a) 2.2% solution, (b) 6.6% solution; nozzle atomizer, 49 kPa pressure, (c) 2.2% solution, (d) 6.6% solution and 196 kPa pressure, (e) 2.2% solution, and (f) 6.6% solution.

reduced water content can be stable for longer time and better characteristics when mixed to excipients.

Dissolution Profiles of Spray-Dried Microparticles

All microparticles tested presented 100% of drug release in phosphate buffer in 120 min, showing that the entire amount encapsulated were available in the medium. Concerning the gastro-resistance evaluation, the amount of pantoprazole released in 120 min is the percentage of drug that remained stable after the acid medium. The microparticles were able to protect more than 90% of initial pantoprazole quantity from the acid environment, presenting acceptable values for the administration of gastro-sensitive drugs. The higher surface area could be an indicator for reduced release, once a large area was exposed to the acid medium. On the other hand, the ideal particles should present reduced particle size, large surface area, good flow, and higher stability of the drug in acid medium together with faster release.

The drug release is significantly different from the laboratory to pilot scale for 2.2% solution. Both formulations present similar mean size (6.70 and 7.08 μm), with similar span (1.95 and 2.01) and bulk density (0.24 and 0.23 g.cm^{-3}). Even though the laboratory-scale powder presented higher surface area, it presented higher acid protection. The reason is the presence of cracking and

TABLE 5
Moisture content of microparticles

Atomizer	Spray dryer configuration	Air pressure (kPa)	Moisture content (% w/w)	
			2.2% solution	6.6% solution
Nozzle	Co-current flow	49	7.86 ± 0.12	9.07 ± 0.16
		196	8.71 ± 1.01	9.57 ± 0.30
	Mixed flow	196	6.29 ± 0.85	5.93 ± 0.61
Rotating disc	Co-current flow	—	9.58 ± 0.20	8.63 ± 0.69

holes in the microparticles produced in the pilot scale. Broken particles do not behave as the others (Fig. 4a). However, for 6.6% solution no difference is seen between the two profiles (Fig. 4b). The microparticles presented similar dissolution profiles but different physical characteristics, as size (9.00 and 21.95 µm), bulk density (0.16 and 0.08 g.cm^{-3}), and surface area (113 and 71 m^2/g). The similarity is correlated to large polidispersity (span of 2.2 and 2.45) and similar pore size distribution (data not shown). Concerning the pilot-scale powders produced with 2.2% solution, the three profiles showed similar amount of pantoprazole stable, but the one prepared with rotating disc atomizer presented faster release (Fig. 4c). Atomizing air pressure does not exert influence in drug release profiles. Particles presented similar size and span, but very different surface areas (24, 50, and 76 m^2/g). Again, the integrity of the particles affected surface area. In this case, the size and span were more important than the surface area according

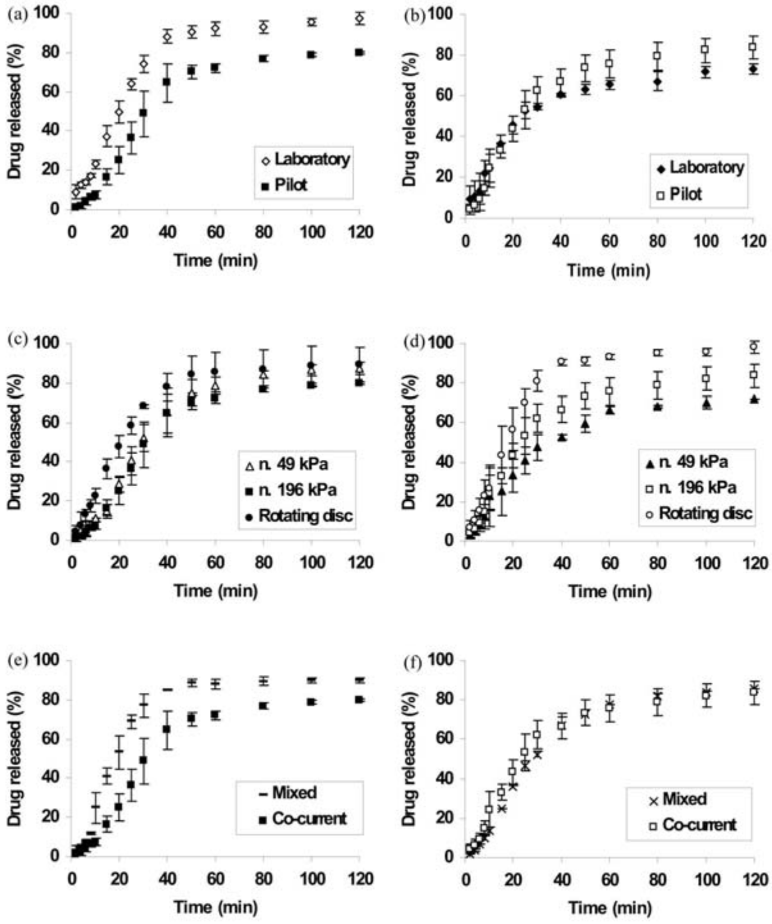


FIG. 4. Dissolution profiles of microparticles prepared in laboratory and pilot scale with nozzle atomizer and 196 kPa (a) with 2.2% solution and (b) with 6.6% solution. Comparison among pilot scale product* dissolution prepared from (c) 2.2% solution and (d) 6.6% solution. Comparison between co-current and mixed flow products sprayed from (e) 2.2% solution and (f) 6.6% solution. *n, = nozzle atomizer.

to the drug release profiles. Among the 6.6% solid concentration solutions, the faster release and the higher drug protection was achieved by the powder produced with rotating disc atomizer (Fig. 4d). This powder presented higher density (twice that of the powders produced with nozzle atomizer), reduced surface area ($40.1\ m^2/g$), and the span index showed a reduced polidispersity. On the other hand, average particle size was not different from the others (nozzle atomizer). The difference in surface area is due to the holes presented in large quantity in the powders produced with nozzle atomizer. The holes can be the reason of the lower acid protection of these powders. Significant differences are obtained for 2.2% solution also when comparing co-current and mixed flow dryers (Fig. 4e). The microparticles prepared in mixed flow dryer presented higher porosity and higher integrity than the ones produced in co-current flow process. The reduced surface area also affected the velocity of release and the percentage stable after acid stage. Among all profiles, the higher release was achieved by 6.6% solution and rotating disc atomizer, followed by 2.2% solution and mixed-flow process. The surface area and the initial feed concentration were the parameters that most affected the drug release. The feed concentration affects droplet size, residence time, surface area, and final moisture.[16] The dissolution rate and the stabilization of the drug in the microparticles are consequences from the particle size distribution. Lower span values led to straight particles size distribution and less variability among particle dissolution rate. The lower surface area also increases drug stabilization due to reduced contact to the acid medium.

CONCLUSIONS

In all conditions tested, it was possible to obtain microparticulate powders. From data obtained in this study, the initial solid concentration is the most relevant factor for determining the particle size. This effect is observed in both laboratory and pilot scales. The difference among sizes is greater when comparing 2.2 and 6.6% solution spray-dried in pilot scale and nozzle atomizer. Smaller particles are denser, but this difference does not affect the flowability. All powders present poor flow. Surface area is affected by the type of atomizer. The nozzle atomizer produces microparticles with large surface area compared to the rotating disc atomizer, probably because of the holes and cracking of the particles produced with nozzle atomizer. The mixed-flow process reduces the final particle moisture content of powders, showing an advantage compared to others. Drug release is mainly affected by the feed solids concentration. The highest release is achieved by the powder produced from 2.2% solution in the nozzle atomizer and mixed-flow dryer and from 6.6% solution with the rotating disc atomizer.

In fact, it was demonstrated here the possibility for producing drug delivery systems such as microparticles in both laboratory and pilot scales. Different powder physical characteristics are observed when operating conditions and size of the spray dryer are changed for a pharmaceutical manufacture scale-up. For a faster drug release, the microparticles should present reduced mean particle size, straight size distribution, large surface area, and good flow. In the case of pantoprazole-loaded microparticles, the gastro-resistance of the microparticles is the most important factor, but the others are still relevant. The particles produced and characterized in this article presented an ideal size, some with reduced polidispersity and with acid protection over 90%, but all of them presented very poor flow. In order to obtain capsules or tablets containing these microparticles, a selection of excipients must be done to ensure good flow and biopharmaceutical characteristics to maximize absorption. Controlling the operating conditions in the pilot scale can be useful when particle design has an important role in drug release, powder flowability, and stability.

REFERENCES

1. Lin, S.Y.; Kao, Y.H. Tablet study of spray-dried sodium diclofenac enteric-coated microcapsules. Pharmaceutical Research **1991**, *8*, 919 924.
2. Beten, D.B.; Gelbcke, M.; Diallo, B.; Moës, A.J. Interaction between dipyridamole and Eudragit S. International Journal of Pharmaceutics **1992**, *88*, 31 37.
3. Cheer, S.; Prakash, A.; Faulds, D.; Lamb, H. Pantoprazole An update of its pharmacological properties and therapeutic use in the management of acid-related disorders. Drugs **2003**, *63*, 101 132.
4. Sachs, G.; Shin, J.M.; Prathaand, V.; Hogan, D. Synthesis or rupture: duration of acid inhibition by proton pump inhibitors. Drugs of Today **2003**, *39* (Suppl. A), 11 14.
5. Palmieri, G.; Bonacucina, G.; Di Martino, P.; Martelli, S. Gastro-resistant microspheres containing ketoprofen. Journal of Microencapsulation **2002**, *19*, 111 119.
6. Goula, A.; Adamopoulos, K.; Kazakis, N. Influence of spray drying conditions on tomato powder properties. Drying Technology **2004**, *22* (5), 1129 1151.
7. Ré, M.I. Microencapsulation by spray drying. Drying Technology **1998**, *16*, 1195 1236.
8. Esposito, E.; Cervellati, F.; Menegatti, E.; Nastruzzi, C.; Cortesi, R. Spray dried Eudragit microparticles as encapsulation devices for vitamin C. International Journal of Pharmacy **2002**, *242*, 329 334.
9. Ré, M.I. Formulation of multiparticulate delivery systems by spray drying. In: *Proceedings* $20^{\text{èmes}}$ *Journées AFSIA - Association Française de séchage dans Í Industrie et Í Agriculture*, Albi, France, 2002: 147 157.
10. Benoit, J.P.; Marchais, H.; Rolland, H.; Velde, V.V. Biodegradable microspheres: Advances in production technology. In *Microencapsulation: Methods and Industrial Applications*; Benita, S., Ed.; Marcel Dekker: New York, 1996; 35 72.
11. Ré, M.I.; Messias, L.S.; Schettini, H. The influence of the liquid properties and the atomizing conditions on the physical characteristics of the spray-dried ferrous sulphate microparticles. Paper presented at the annual International Drying Symposium, August 22 25, Campinas, Brazil.
12. Brunauer, S.; Emmet, P.H.; Teller, E. Adsorption of gases in multimolecular layers. Journal of the American Chemical Society **1938**, *60* (2), 309 319.

to the drug release profiles. Among the 6.6% solid concentration solutions, the faster release and the higher drug protection was achieved by the powder produced with rotating disc atomizer (Fig. 4d). This powder presented higher density (twice that of the powders produced with nozzle atomizer), reduced surface area ($40.1\,m^2/g$), and the span index showed a reduced polidispersity. On the other hand, average particle size was not different from the others (nozzle atomizer). The difference in surface area is due to the holes presented in large quantity in the powders produced with nozzle atomizer. The holes can be the reason of the lower acid protection of these powders. Significant differences are obtained for 2.2% solution also when comparing co-current and mixed flow dryers (Fig. 4e). The microparticles prepared in mixed flow dryer presented higher porosity and higher integrity than the ones produced in co-current flow process. The reduced surface area also affected the velocity of release and the percentage stable after acid stage. Among all profiles, the higher release was achieved by 6.6% solution and rotating disc atomizer, followed by 2.2% solution and mixed-flow process. The surface area and the initial feed concentration were the parameters that most affected the drug release. The feed concentration affects droplet size, residence time, surface area, and final moisture.[16] The dissolution rate and the stabilization of the drug in the microparticles are consequences from the particle size distribution. Lower span values led to straight particles size distribution and less variability among particle dissolution rate. The lower surface area also increases drug stabilization due to reduced contact to the acid medium.

CONCLUSIONS

In all conditions tested, it was possible to obtain microparticulate powders. From data obtained in this study, the initial solid concentration is the most relevant factor for determining the particle size. This effect is observed in both laboratory and pilot scales. The difference among sizes is greater when comparing 2.2 and 6.6% solution spray-dried in pilot scale and nozzle atomizer. Smaller particles are denser, but this difference does not affect the flowability. All powders present poor flow. Surface area is affected by the type of atomizer. The nozzle atomizer produces microparticles with large surface area compared to the rotating disc atomizer, probably because of the holes and cracking of the particles produced with nozzle atomizer. The mixed-flow process reduces the final particle moisture content of powders, showing an advantage compared to others. Drug release is mainly affected by the feed solids concentration. The highest release is achieved by the powder produced from 2.2% solution in the nozzle atomizer and mixed-flow dryer and from 6.6% solution with the rotating disc atomizer.

In fact, it was demonstrated here the possibility for producing drug delivery systems such as microparticles in both laboratory and pilot scales. Different powder physical characteristics are observed when operating conditions and size of the spray dryer are changed for a pharmaceutical manufacture scale-up. For a faster drug release, the microparticles should present reduced mean particle size, straight size distribution, large surface area, and good flow. In the case of pantoprazole-loaded microparticles, the gastro-resistance of the microparticles is the most important factor, but the others are still relevant. The particles produced and characterized in this article presented an ideal size, some with reduced polidispersity and with acid protection over 90%, but all of them presented very poor flow. In order to obtain capsules or tablets containing these microparticles, a selection of excipients must be done to ensure good flow and biopharmaceutical characteristics to maximize absorption. Controlling the operating conditions in the pilot scale can be useful when particle design has an important role in drug release, powder flowability, and stability.

REFERENCES

1. Lin, S.Y.; Kao, Y.H. Tablet study of spray-dried sodium diclofenac enteric-coated microcapsules. Pharmaceutical Research **1991**, *8*, 919 924.
2. Beten, D.B.; Gelbcke, M.; Diallo, B.; Moës, A.J. Interaction between dipyridamole and Eudragit S. International Journal of Pharmaceutics **1992**, *88*, 31 37.
3. Cheer, S.; Prakash, A.; Faulds, D.; Lamb, H. Pantoprazole An update of its pharmacological properties and therapeutic use in the management of acid-related disorders. Drugs **2003**, *63*, 101 132.
4. Sachs, G.; Shin, J.M.; Prathaand, V.; Hogan, D. Synthesis or rupture: duration of acid inhibition by proton pump inhibitors. Drugs of Today **2003**, *39* (Suppl. A), 11 14.
5. Palmieri, G.; Bonacucina, G.; Di Martino, P.; Martelli, S. Gastro-resistant microspheres containing ketoprofen. Journal of Microencapsulation **2002**, *19*, 111 119.
6. Goula, A.; Adamopoulos, K.; Kazakis, N. Influence of spray drying conditions on tomato powder properties. Drying Technology **2004**, *22* (5), 1129 1151.
7. Ré, M.I. Microencapsulation by spray drying. Drying Technology **1998**, *16*, 1195 1236.
8. Esposito, E.; Cervellati, F.; Menegatti, E.; Nastruzzi, C.; Cortesi, R. Spray dried Eudragit microparticles as encapsulation devices for vitamin C. International Journal of Pharmacy **2002**, *242*, 329 334.
9. Ré, M.I. Formulation of multiparticulate delivery systems by spray drying. In: *Proceedings* $20^{\text{èmes}}$ *Journées AFSIA - Association Française de séchage dans ĺ Industrie et ĺ Agriculture*, Albi, France, 2002: 147 157.
10. Benoit, J.P.; Marchais, H.; Rolland, H.; Velde, V.V. Biodegradable microspheres: Advances in production technology. In *Microencapsulation: Methods and Industrial Applications*; Benita, S., Ed.; Marcel Dekker: New York, 1996; 35 72.
11. Ré, M.I.; Messias, L.S.; Schettini, H. The influence of the liquid properties and the atomizing conditions on the physical characteristics of the spray-dried ferrous sulphate microparticles. Paper presented at the annual International Drying Symposium, August 22 25, Campinas, Brazil.
12. Brunauer, S.; Emmet, P.H.; Teller, E. Adsorption of gases in multimolecular layers. Journal of the American Chemical Society **1938**, *60* (2), 309 319.

13. Barrett, E.P.; Joyner, L.G.; Halenda, P.P. The determination of pore volume and area distributions in porous substances. I. Computations from nitrogen isotherms. Journal of the American Chemical Society **1951**, *73* (1), 373 380.
14. Carr, R.L. Evaluating flow properties of solids. Chemical Engineering **1965**, *18*, 163 168.
15. Kwapinska, M.; Zbicinski, I. Prediction of final product properties after cocurrent spray drying. Drying Technology **2005**, *23* (8), 1653 1665.
16. Goula, A.; Adamopoulos, K. Spray drying of tomato pulp: Effect of feed concentration. Drying Technology **2004**, *22* (10), 2309 2330.
17. ASTM D6393-99. Standart test method for bulk solids characterization by Carr indices, ASTM International, www.astm.org
18. Birchal, V.; Passos, M.; Wildhagen, G.; Mujumdar, A. Effect of spray-dryer operating variables on the whole milk powder quality. Drying Technology **2005**, *23*, 611 636.

CAPÍTULO 5: Avaliação da estabilidade e da atividade anti-ulcerogênica de micropartículas gastro-resistentes contendo pantoprazol

5.1 Apresentação

No capítulo anterior, foram descritas micropartículas de Eudragit® S100 preparadas por *spray-drying*. As condições operacionais do processo foram estudadas e as melhores condições foram selecionadas. No presente capítulo, a reprodutibilidade do processo foi avaliada, em três lotes consecutivos. As micropartículas foram caracterizadas quanto ao rendimento, eficiência de encapsulação, diâmetro de partícula e densidade.

A estabilidade de produtos farmacêuticos depende de fatores ambientais como temperatura, umidade e luz, e de outros relacionados ao próprio produto como propriedades físicas e químicas do fármaco e do processo de fabricação. A estabilidade acelerada é um estudo projetado para acelerar a degradação química ou mudanças físicas de um produto farmacêutico em condições forçadas de armazenamento. Deste modo, o presente capítulo tem como objetivo avaliar a estabilidade acelerada das micropartículas, através do doseamento, análise térmica e perfil de dissolução. O terceiro objetivo do presente capítulo é a avaliação *in vivo* das micropartículas quanto à proteção da mucosa gástrica, utilizando etanol como agente indutor de úlceras gástricas. Estes resultados foram publicados na revista *The Open Drug Delivery Journal*.

Publicação 05: Gastro-Resistant Microparticles Containing Sodium Pantoprazole: Stability Studies and *In Vivo* Anti-Ulcer Activity

44 *The Open Drug Delivery Journal*, 2007, *1*, 44-51

Gastro-Resistant Microparticles Containing Sodium Pantoprazole: Stability Studies and *In Vivo* Anti-Ulcer Activity

R.P. Raffin[1], L.M. Colomé[1], E.E.S. Schapoval[1], D.S. Jornada[1], A.R. Pohlmann[*,2], S.S. Guterres[1]

[1]*Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Brazil*

[2]*Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio Grande do Sul. Av. Bento Gonçalves 9500, CP 15003, Porto Alegre, RS, Brazil, CEP 91501-970*

Abstract: The aim of the present work was to verify the *in vivo* capacity of pantoprazole-loaded microparticles to protect the gastric mucosa against ulcer formation and to evaluate their stability under accelerated conditions. Pantoprazole-loaded microparticles were prepared by spray-drying in pilot scale, using Eudragit® S100 as polymer. Transparent glass vials containing drug-loaded microparticles were stored for 6 months at 40°C and 75% RH. Photostability was tested under UVA light. Ulcers were induced by the oral administration of absolute ethanol to rats. Sodium bicarbonate solution, pantoprazole solution and drug-loaded microparticles were tested. Regarding the drug content during the accelerate stability study, samples showed complete encapsulation efficiency and were considered stable. The microencapsulation of pantoprazole reduced its photodegradation. The *in vivo* evaluation showed that the microparticles presented ulcer index lower than the solutions. Enteric microparticles had acceptable stability under accelerated conditions and were efficient in protecting the stomach against ulceration caused by ethanol.

Keywords: Pantoprazole, microparticles, stability, photodegradation, gastro-resistance, anti-ulcer activity.

INTRODUCTION

Pantoprazole is a proton-pump inhibitor used in the treatment of gastric ulcers, gastro-esophageal reflux disease and *Helicobacter pylori* infections associated to other drugs, such as metronidazole, clarithromycin or amoxicillin [1,2]. This drug was the first water soluble benzimidazole, 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-benzimidazole (Fig. **1**), which can be administered intravenously in the form of sesquihydrate sodium pantoprazole.

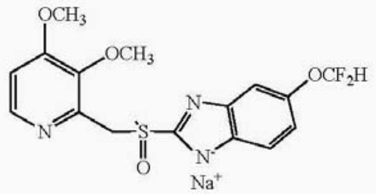


Fig. (1). Chemical structure of sodium pantoprazole.

Pantoprazole is converted to its active form inside the gastric parietal cells, binding irreversibly to the H^+/K^+ATPase. Since this conversion must occur inside the parietal-cell canalicular lumen and considering that the pro-drug is labile in the stomach environment, the pantoprazole must be absorbed intact by the gastrointestinal tract [3,4]. In this way, pantoprazole is intravenously administered after the reconstitution of lyophilized powder or orally administered as gastric-resistant tablets using enteric-coated dosage forms, which prevents pantoprazole from degradation in the gastric juice [5].

In order to administer pantoprazole by the oral route, polymeric microparticles appear to be an interesting device. Despite the more complex and onerous production of the multiple-unit systems, microparticles have several advantages in relation to the single-unit products, including ready and uniform distribution in the gastrointestinal tract, minimizing the risk of local damage caused by a dose dumping effect [6]. Furthermore, microparticles are also less affected by the pH and the gastric transit time, attain more constant plasma levels, give higher accuracy in reproducibility dose by dose and achieve a slow-release effect [7].

In our previous works, gastro-resistant microparticles were prepared using Eudragit® S100 by two techniques: emulsification/solvent evaporation and spray-drying [8,9]. These microparticles were characterized by means of their morphology, packing and flowing properties, water content and dissolution kinetics. The microparticles prepared by emulsification/solvent evaporation had higher particle sizes (56 µm) than the microparticles prepared by spray-drying (7 - 25 µm). The microparticles prepared by solvent evaporation stabilized 61% of pantoprazole content after acid exposure. However, when tested *in vivo* for the anti-ulcer activity, microparticles containing pantoprazole were able to protect the gastric mucosa against the ulceration caused by ethanol. The microparticles were prepared by spray-drying in laboratory scale and the scaling up of the process was investigated. Different parameters and spray-drier designs were tested in pilot scale. The rotating disc atomizer at co-current air/spray contact furnished the microparticles presenting the highest *in vitro* gastro-resistance (98%). These microparticles showed adequate *in vitro* characteristics, but were not tested *in vivo*.

The stability of a pharmaceutical product is a key element of quality, and regulatory authorities ensure that useful-

---

*Address correspondence to this author at the Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio Grande do Sul. Av. Bento Gonçalves 9500, CP 15003, Porto Alegre, RS, Brazil, CEP 91501-970; Tel: +55 51 33086274; Fax: +55 51 33087304; E-mail: pohlmann@iq.ufrgs.br

1874-1266/07

life claims are realistic and demonstrable. Long-term stability studies are the most reliable demonstration or exploration of a product shelf life [10]. The length of time required for such studies often renders them impractical. Therefore, a reliable alternative to real time stability testing, such as one that can predict rates of decay at temperatures of interest, is clearly required. The method of accelerated storage testing has the ability to predict shelf life at low temperatures and humidity.

Taking all above into account, the aim of this work was to verify the *in vivo* capacity of pantoprazole-loaded microparticles to protect the gastric mucosa against ulcer formation and to evaluate their stability under accelerated conditions. Additionally, the work was also consecrated to determine the drug release profiles from microparticles after short term stability studies.

MATERIALS AND METHODOLOGY

Pantoprazole sodium sesquihydrate was obtained from Henrifarma (São Paulo, Brazil). Eudragit® S100 was kindly given by Almapal® (São Paulo, Brazil produced by Rohm®, Germany). The polymer average molecular weight is approx. 135,000 Da. Acetonitrile was HPLC grade and all other chemicals were analytical grade.

PREPARATION OF PANTOPRAZOLE-LOADED MICROPARTICLES

Pantoprazole-loaded microparticles were prepared in pilot scale as previously described [9]. Briefly, 48 g of Eudragit® S100 were dissolved in a NaOH solution (6 g.L^{-1}). After its complete dissolution, pantoprazole (12 g) was added. The final solution (1000 mL) was kept at 40°C and spray-dried in a pilot spray-dryer (Model PSD 52 APV1Anhydro, Denmark). Temperature and humidity in the room (24 °C and 54%, respectively) were kept constant. The atomizer used was a rotating disc under the following operating conditions: co-current flow; rotational velocity of atomizer of 30,000 rpm; suspension flow rate of 2 L.h^{-1}, inlet and outlet air temperatures of 170 ± 1°C and 85 ± 5°C, respectively. The process yield was calculated dividing the obtained mass by the sum of the masses of Eudragit® S100, pantoprazole and NaOH, expressed in percentage. Microparticles were prepared in triplicate.

DETERMINATION OF THE DRUG LOADING

The drug loading was assayed by a validated HPLC method [11] according to ICH [12]. Briefly, an amount of the microparticles, equivalent to the theoretical content of 10 mg of pantoprazole, was weighed and magnetically stirred with 40 mL of 0.05 mol.L^{-1} NaOH for 1 h in a volumetric flask. The volume was completed to 50 mL and drug concentration was determined after filtration (0.45 µm) by HPLC (Perkin Elmer serie 200) using a LiChrospher RP18 (Merck) column. Mobile phase consisted of acetonitrile/phosphate buffer pH 7.4 (35:65 v/v), the flow rate was 1 mL.min^{-1} and detector wavelength was set at 290 nm.

PARTICLE SIZE AND SCANNING ELECTRON MICROSCOPY (SEM) ANALYSES

SEM analyses were carried out using an accelerating voltage of 20 kV after gold sputtering (Jeol Scanning Microscope JSM - 6060® and JSM-5800®, Japan). The particle size distributions were measured by laser light diffraction (Malvern MasterSizer, model E, UK) after dispersion in *iso*-octane. Average particle size was expressed as the mean volume diameter. Polydispersity was given by a span index, which was calculated by equation 1.

$$Span = \frac{D_{0.9} - D_{0.1}}{D_{0.5}} \qquad (1)$$

where $D_{0.9}$, $D_{0.5}$, and $D_{0.1}$ are the particle diameters determined, respectively, at the 90th, 50th, and 10th percentile of the undersized particle distribution curve.

POWDER FLOW CHARACTERIZATION

Bulk and tapped densities were assessed according to USP 30, as well as the determination of the compressibility index. The angle of repose was assayed as previously described [9]. The angle of repose is the angle between the horizontal and slope of the heap. This angle is a direct indication of the potential flowability of a powder (contact and friction between particles in motion). The angle of repose was measured in a Powder Characteristics Tester, Model PT-N (Hosokawa Microns). Flowability was assayed according to the Ph Eur 5 [13]. Five grams of microparticles were placed inside a funnel and the time to the entire sample to flow was recorded and used as a comparative value among batches.

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

DSC was performed (DSC-4 Shimadzu, Kyoto, Japan) after sealing the samples (pantoprazole, Eudragit® S100, their physical mixture and microparticles) in aluminum pans. Calibration was carried out using indium. DSC tracings were performed from 40 °C to 250 °C at a rate of 10 °C.min^{-1}.

DETERMINATION OF WATER CONTENT

The water content of the samples was determined by Karl Fisher titrimetry (Mettler DL 37 KF Coulometer, Switzerland). Samples were analyzed in duplicate and compared to pure pantoprazole.

IN VIVO ANTI-ULCER ACTIVITY

Ulcers were induced by the oral administration of absolute ethanol (5 mL.kg^{-1}) to 24 h fasted Wistar male rats (n = 8), weighing 200 g. The groups are described in Table **1**. Formulations (20 mg.kg^{-1} of drug) were administered orally 1 h before the administration of ethanol. Prior to the oral administration, rats were anesthetized with ethylic ether. After 2 h of ethanol administration, animals were sacrificed; the stomachs were removed, opened along the greater curvature and examined for lesion measurements [8].

Ulcer indexes (UI) were calculated using equation 2.

$$UI = \frac{10}{x} \qquad (2)$$

where x is the total mucosal area divided by the total ulcerated area.

ACCELERATED STABILITY TESTS

The stability of drugs and medicines depends on environmental factors as temperature, humidity and light, as well as on the physico-chemical properties of the drug and the excipients [14]. The purpose of stability testing is to provide evidence on how the quality of a drug product varies with time and to establish a shelf life for the drug product and recommended storage conditions [10]. Accelerated stability testing are studies designed to increase the rate of chemical degradation or physical change of a drug product by using exaggerated storage conditions as part of the formal stability studies. Transparent glass vials containing 0.5 g of drug-loaded microparticles were stored for 6 months in a stability chamber at 40°C and 75% RH. Sealed and non-sealed vials were evaluated every 30 days for their drug content. The acceptance criteria for the stability tests are 5 percent change in assay from its initial value, or any degradation product's exceeding its acceptance criterion or failure to meet the acceptance criteria for dissolution [10]. Humidity was gravimetrically determined. DSC was performed for the drug-loaded microparticles after different times of storage.

Table 1. Groups of Rats (Control 1, Control 2 and Treatment) for the *In Vivo* Anti-Ulcer Activity

Groups	Administered Samples
Control 1	Sodium bicarbonate solution (4.2%)
Control 2	Pantoprazole dissolved in water (2 mg.mL-1)
Treatment	Microparticles dispersed in water (equivalent to 2 mg.mL^{-1} of pantoprazole)

IN VITRO GASTRO-RESISTANCE EVALUATION

The gastro-resistance evaluation was performed after 6 months of storage and the results compared to that profile determined just after preparation [9]. In order to ensure the homogeneity of the dispersion and no flotation of the powders, size 0 hard gelatin capsules without coloring agent were used. The capsules were filled with 90 mg of microparticles, corresponding to 16 mg of drug. Dissolution tests were undertaken in USP dissolution apparatus I at 50 rpm and 37°C. In order to determine if the microparticles were able to release 100% of the encapsulated drug, the dissolution was evaluated in phosphate buffer pH 7.4 for 120 min. To evaluate gastro-resistance, capsules were prior exposed to 300 mL of 0.1 mol.L^{-1} HCl. After 1 h, an NaOH (2.6 g) and KH_2PO_4 (6.12 g) aqueous solution (600 mL) was added in order to reach the final pH of 7.4. The samples were collected at predetermined time intervals from 0 up to 180 min. Pantoprazole concentrations were determined by UV spectrophotometry at 295 nm [8].

The profiles were analyzed by the dissolution efficiency and by model dependent methods using the software Micromath Scientist 2.01 [15,16]. Profiles were tested to fit mono and biexponential equations (equations 3 and 4).

$$C = 100(1 - e^{-kt}) \qquad (3)$$

$$C = 100\left[1 - (Ae^{-\alpha t} + Be^{-\beta t})\right] \qquad (4)$$

In order to have some insight into the drug release mechanism, a very simple and semi-empirical equation to describe drug release from polymeric systems, the power law (Korsmeyer-Peppas model), was also applied (equation 5).

$$ft = at^{n} \qquad (5)$$

In this equation, f_t is the drug dissolved fraction at time t, n is the release exponent, indicative of the mechanism of the drug release and a is the constant incorporating structural and geometric characteristics of the drug dosage form [16].

PHOTOSTABILITY DETERMINATION

Pantoprazole-loaded microparticles and pure pantoprazole were exposed to UVA light for 96 h. The light source was a fluorescent lamp UVA, 130 V, 30 W (Starlux) fixed to a chamber in a horizontal position 22 cm from the samples. The chamber was internally coated with mirrors in order to distribute light homogeneously. Pure pantoprazole powder and the microparticles powder were put in a very fine layer in watch glasses and placed inside the chamber. Samples were collected at 6, 24 and 96 h and analyzed for the pantoprazole contents by HPLC. Protected samples, completely covered with aluminum foil, were used as dark controls in order to evaluate the influence of a thermally induced drug content the total change.

STATISTICAL ANALYSIS

One-way analysis of variance was employed for the comparison of the experimental data. The non-parametric test Kruskal-Wallis was used for the *in vivo* data. Multisample comparison was performed using Student-Neuman-Keuls test.

RESULTS

Preparation OF Microparticles

The three batches of pantoprazole-loaded microparticles had yields, particle size averages and drug loading similar to those observed for microparticles previously described [9] (Table **2**). The powders yielded 52.7, 54.1, 58.0 g.

Table 2. Characteristics of the Three Batches of Microparticles

Batch	Yield (%)	Encapsulation Efficiency (%)	Humidity (%)
1	80	98.83 ± 2.07	2.3 ± 0.4
2	82	97.38 ± 3.18	2.0 ± 0.1
3	88	99.55 ± 3.09	1.7 ± 0.1

Drug loading was 179.7 ± 3.7, 177.1 ± 5.8, 181.0 ± 5.6 mg/g and all powders showed low humidity, demonstrating that the spray-drying process was efficient. The particle mean size was 23.0 ± 0.6 µm and the *span* values showed low and narrow particle size distribution (Table **3**). SEM analyses demonstrated that the microparticles presented spherical shape and blowholes (Fig. **2**).

Table 3. Particle Size Distribution of the Three Batches of Microparticles

Batch	Mean Size (µm)	$D_{0.1}$ (µm)	$D_{0.9}$ (µm)	Span
1	23.7	5.1	39.6	1.4
2	22.7	4.9	38.5	1.4
3	22.6	4.7	41.3	1.6

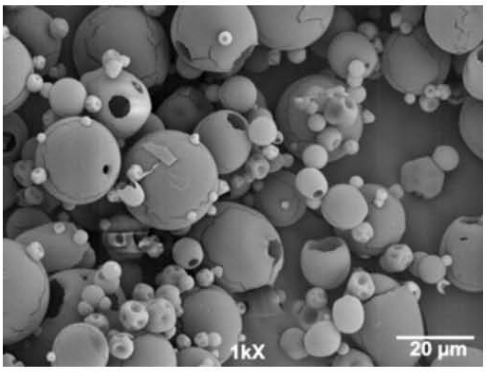


Fig. (2). Photomicrograph (photo width = 125 µm) of the microparticles just after preparation.

POWDER FLOW CHARACTERIZATION

The three batches of pantoprazole-loaded microparticles presented bulk densities of 0.27, 0.20, and 0.19 g.cm^{-3}. Tapped densities were 0.42, 0.34 and 0.34 g.cm^{-3}. All powders presented high compressibility indexes, mainly due to the reduced particle size (Table **4**). The angle of repose was 43.5 ± 3.2° corroborating with compressibility results. In the flowability test, all samples failed to flow.

Table 4. Powder Flow Properties of the Microparticles

Batch	Carr Index	Angle of Repose (°)
1	35.6 ± 0.8	40.8 ± 1.1
2	40.5 ± 1.2	42.7 ± 3.8
3	40.0 ± 1.1	47.1 ± 1.4

DIFFERENTIAL SCANNING CALORIMETRY

DSC analyses (Fig. **3**) showed an endothermic peak at 130°C, followed by degradation of pantoprazole at 190°C (exothermic). Melting and dehydratation of pantoprazole are parallel processes [17]. Eudragit® S100 (pure sample) presented an endothermic peak at 69°C, as previously observed [18]. Regarding the physical mixtures of the drug and the commercial polymer, the curve showed two endothermic peaks, one correlated to the polymer (64 °C) and the other one to pantoprazole (130 °C). In addition, an exothermic peak correlated to the pantoprazole degradation (190 °C) was also observed. For microparticles, one peak at 75 °C was observed suggesting that the drug is molecularly interacting with the polymer, as well as the microparticles stabilized the drug restraining its degradation.

ACCELERATED STABILITY TEST

Within 180 days of the stability tests, vials were weighed monthly. The increase of the weight was 0.4% for the sealed vials. Non-sealed vials had variation of weight within 5 months similar to sealed vials. In the sixth month, the weight increased 3%. These results indicated that pantoprazole microparticles are not hygroscopic, but should be stored protected from humidity.

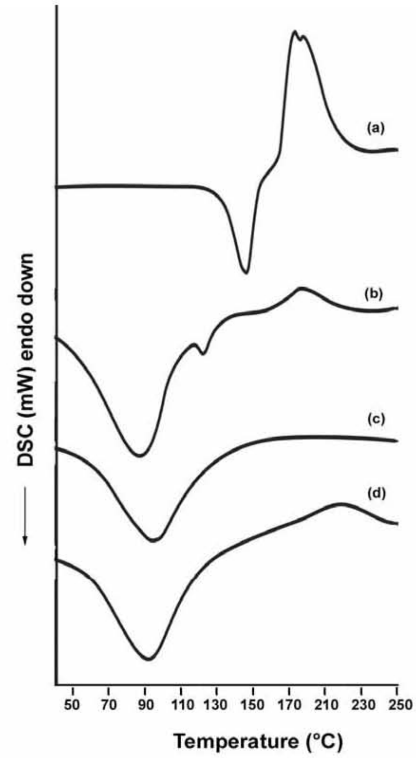


Fig. (3). DSC tracings of (**a**) PAN, (**b**) physical mixture (polymer to drug 4:1 w/w ratio), (**c**) Eudragit® S100 and (**d**) microparticles.

Regarding the drug content during the accelerated stability study, both samples, from sealed and non-sealed vials presented similar results. As shown in Fig. (**4**), samples were stable during the test.

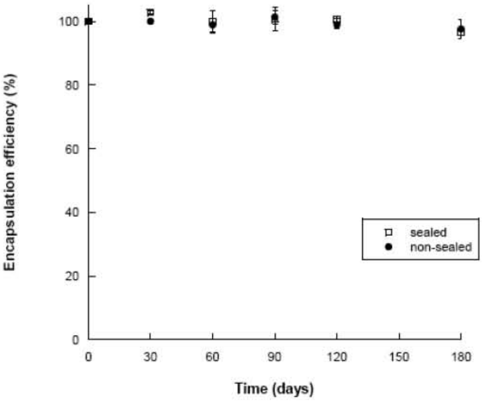


Fig. (4). Microparticles encapsulation efficiency during the accelerated stability tests for the sealed and non-sealed vials.

After 180 days of storage in the stability chamber, particles showed shape and characteristics very similar to those of the microparticles recently prepared (Fig. **2**). The microparticles seem more agglomerated after storage.

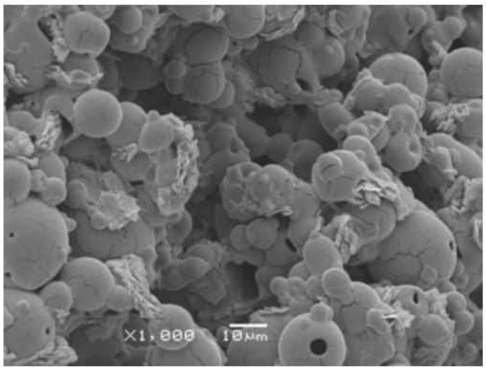


Fig. (5). Photomicrograph (photo width = 125 µm) of the microparticles after 180 days of the accelerated stability studies.

Concerning the DSC analyses during the storage period, the thermogram after 30 days did not show differences compared to that of the microparticles recently prepared. Regarding the subsequent months, an increase of specific heat was verified at 195°C, corresponding to the pantoprazole degradation. After 6 months, the specific heat increased from 0.16 to 0.75 cal.g^{-1}. No difference was verified for the peak corresponding to the polymer. No additional peaks were observed (Fig. **6**).

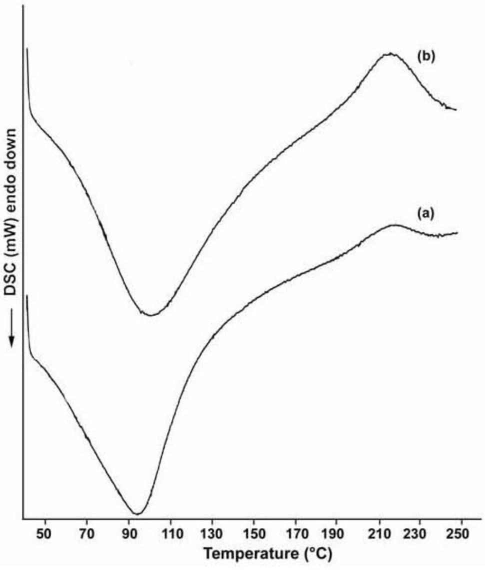


Fig. (6). DSC tracings of the microparticles during the accelerated stability studies at (**a**) 30 days and (**b**) 180 days.

GASTRO-RESISTANCE EVALUATION

Regarding the gastro-resistance evaluation before and after the stability test, it was verified a difference of 8.8 ± 2.3% in the total amount of drug released. Just after preparation, microparticles were able to stabilize 98 ± 2.1% of the drug content. After 180 days of storage, 90 ± 5.3% of the drug was stable after the acid step. Although the microparticles showed a reduction of drug content, the value is still in the acceptance criteria of the pharmacopoeias [19,20] (Fig. 7). The encapsulation efficiencies were 82.4 ± 2.5% and 76.0 ± 4.4% for times 0 and 180 days, respectively. The encapsulation efficiencies did not present significant difference (p = 0.169) within the period of the stability experiment.

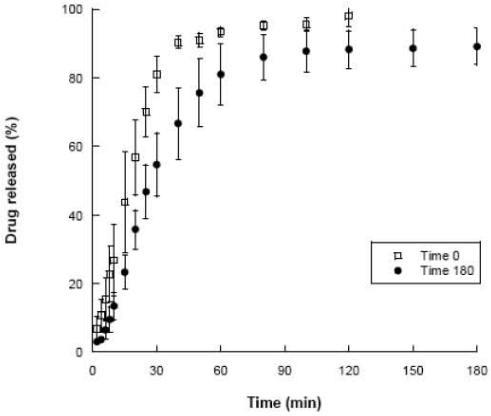


Fig. (7). Drug release from microparticles before and after the accelerated stability tests for sealed vials in phosphate buffer at pH 7.4 after 1 h in 0.1 M HCl.

The profiles were modeled using the monoexponential equation. The half-life was calculated based on the kinetic constant (k). Drug release from the microparticles at t = 0 min presented $t_{1/2}$ of 16.1 ± 3.7 min and at t = 180 min, $t_{1/2}$ of 28.9 ± 6.4 min.

The modeling of the microparticle profiles using the Korsmeyer-Peppas model (Fig. **8**) showed *n* value of 1.02 ± 0.02 for the sample recently prepared and *n* of 1.27 ± 0.10, for the microparticles after 180 days of storage.

PHOTOSTABILITY

Photostability was evaluated for 96 h (Table 5). After 6 h of light exposure no difference in the drug content was observed between pure pantoprazole or drug-loaded microparticles (98.7 and 99.2%, respectively). After 24 h, dark controls showed that pure pantoprazole was affected by temperature (drug content of 45%), but the microparticles were not (drug content of 92%). When exposed to light, pure pantoprazole was degraded almost 70%. However, drug-loaded microparticles exposed to light presented a degradation of 23%. The dark controls after 96 h of experiment showed decay in pure pantoprazole concentration of 60% and in the drug-loaded microparticles of 20%. Exposing pure pantoprazole and the pantoprazole-loaded microparticles to 96 h of

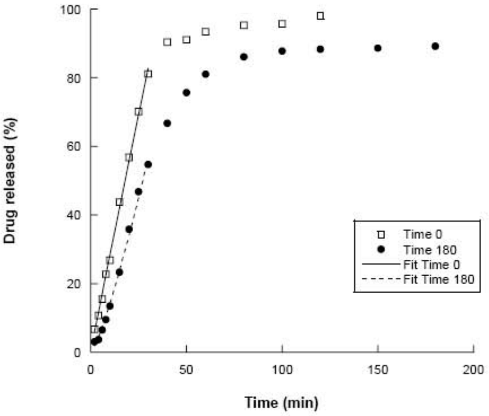


Fig. (8). Mathematical modeling of drug release profiles to the Korsmeyer-Peppas equation.

UVA light, pure pantoprazole was unstable and the sample presented only 11.6% of non-degraded pantoprazole (Table **5**). On the other hand, when exposed to light, the drug-loaded microparticles were able to protect 54.4% of the initial pantoprazole content.

Table 5. Pantoprazole Concentration After Exposure to UVA Light (130 V, 30 W)

Sample	Pantoprazole Concentration (%) After the Exposure Period		
	6 h	24 h	96 h
Pantoprazole	98.7 ± 1.0	32.6 ± 0.2	11.6 ± 2.9
Microparticles	99.2 ± 0.8	78.4 ± 1.6	54.4 ± 1.9

IN VIVO ANTI-ULCER ACTIVITY

Oral administration of ethanol to the control groups clearly showed hemorrhagic lesions developed in the glandular portion of the stomach (Fig. **9**).

The *in vivo* evaluation showed that ulcer index values were 0.58 ± 0.13 for the sodium bicarbonate solution, 0.46 ± 0.17 for the sodium pantoprazole solution and 0.13 ± 0.05 for the pantoprazole-loaded microparticles (Fig. **10**). The Kruskal-Wallis test detected statistical differences ($p = 0.002$) between the ulcer indexes. The multiple analyses (Student-Newman-Keuls) showed that the pantoprazole-loaded microparticles presented a gastric ulcer index statistically lower than the sodium bicarbonate solution ($p = 0.001$) and the sodium pantoprazole solution ($p = 0.021$). The percentages of ulceration inhibition were 21 and 78% after the administration of pantoprazole aqueous solution and microparticles, respectively.

DISCUSSION AND CONCLUSIONS

The selection of the microencapsulation technique depends largely on the physicochemical properties of the drug. For the entrapment of pantoprazole, an oil-in-oil emulsion followed by solvent evaporation was reported [8]. Pantoprazole was also microencapsulated by spray-drying using Eudragit® S100 [9,21]. These microparticles presented advantages such as high values for gastro-resistance and prompt dissolution of the drug. Three replicates were produced on three different days demonstrating the reproducibility of the technique [21]. In the present work, the spray-drying operational conditions were established based on the optimal conditions previously determined [9]. The three batches of microparticles presented acceptable and similar yields. Comparing the results with previous data [22] also produced at laboratory scale, the yields increased from 50% to 80%. Encapsulation efficiency was complete and very homogeneous, indicating that no loss of active compound occurred during spray-drying (Table **2**). Humidity was less than 2%, denoting the effectiveness of the drying process. The results of mean particle size and size distributions of microparticles recorded by laser light diffraction technique on a population basis were found to be unimodal with a narrow size distribution (Table **3**). Briefly, the microparticles presented moisture content, particle size and polydispersity adequate and similar among the three batches evaluated. In this way, the process was considered reproducible.

Fig. (9). Photographs of the stomachs opened along the greater curvature. From top to bottom: stomachs after administration of bicarbonate solution, pantoprazole aqueous solution (showing the hemorrhagic lesions developed in the glandular portion of the stomach) and microparticle aqueous dispersion (no lesions observed).

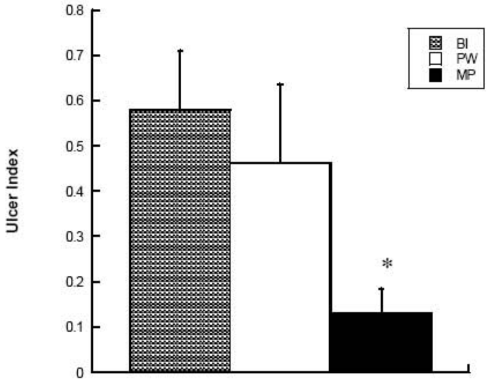


Fig. (10). Ulcer indexes for the bicarbonate solution (BI), pantoprazole aqueous solution (PW) and microparticles dispersed in water (MP). The MP group was statistical different ($\alpha = 0.05$) from the other two*.

Particles were spherical and hollow (Fig. **2**). Because of the high concentration of solids in the liquid feed, solids will come out of solution at the surface of the droplet first, leading to the formation of a crust around a hollow particle. In this case, the spray-drying process resulted in puffing or ballooning and cracking of the particles [9,23]. It is also observed that pilot-scale powders presented large holes due to the rapid evaporation of water [24]. We can classify the microparticles as hollow microspheres.

The microparticle powders presented very poor flow (Table **4**). The compressibility index, angle of repose and flowability corroborated with the low density values. These results suggest that the spray-dried microparticles are likely to have poor flowability, a constraint to be considered in further tableting experiments. This characteristic occurs as a consequence of small particle size and high interparticulate cohesiveness [25].

Concerning the DSC analyzes of the microparticles, the drug peak disappeared compared to the physical mixture (Fig. **3**). As previously reported in microparticle formulations the disappearance of melting peaks of drugs indicates their encapsulation [26]. The results suggest that pantoprazole-loaded microparticles are composed by a homogeneous phase, in which the drug is dissolved in the polymer. The major problem of solid dispersions is the result of long-term stability issues such as the appearance of crystalline drug and the resulting decrease in dissolution rate [27, 28]. Fig. (**6**) shows no endothermic peak of pantoprazole, indicating that no relevant phase separation took place during storage. The result suggests that the material agglomerated with the microparticles in Fig. (**5**) is not drug crystals.

The *in vivo* anti-ulcer evaluation demonstrated that microparticles were able to reduce ulcer formation caused by oral administration of ethanol (Fig. **9**). Ethanol-induced gastric lesions are due to stasis in gastric mucosa, which contributes to the development of the hemorrhage and necrotic aspects of the tissue injury [29]. The gastric lesions caused by ethanol have been attributed to free radical formation and subsequent formation of lipid peroxidation products [29]. The induction of ulcers by ethanol was considered a good model to evaluate the effect of pantoprazole once it has been already described that there is a lack of interaction between pantoprazole and ethanol in terms of their pharmacokinetics [30]. Ethanol-induced ulcer formation is not inhibited by cimetidine, but it is inhibited by pantoprazole and its analogous.

A shelf live of 24 months can be attributed to products that presented less than 5% of reduction of the drug during the accelerate stability tests and did not present any degradation product over limits [14]. Considering this statement and our results, the pantoprazole-loaded microparticles could have a 24 month period of shelf life, but this must be confirmed by long-term stability studies. Solid interactions between omeprazole and enteric polymers were investigated at accelerated conditions for month [31]. After 1 month of storage, omeprazole mixed to Eudragit® L100 presented less than 1% of omeprazole degradation. Eudragit® L100 and S100 are anionic copolymers formed by methacrylic acid and methyl methacrylate (ratio 1:1 and 1:2, respectively). As it was expected also for pantoprazole, the solid interaction between drug and polymer had no influence on pantoprazole stability. Indeed, acrylic polymers have shown good moisture-protective properties and the water was not available for chemical interactions between drug and polymer [31].

The release rate in monoexponential curves is dependent on the initial concentration. Considering that after 180 days, a reduction of the drug content was verified, a slower release from these microparticles was expected. The reduction of the gastro-resistance value can be explained by the presence of cracking in some microparticles after storage and consequent exposure of pantoprazole to the acid medium.

Concerning the mathematical modeling fitting the Korsmeyer-Peppas model for spherical particles, the exponent n of 0.43 indicates that the release mechanism is governed by Fickian diffusion and n higher than 0.85 it is governed by swelling of polymer (Case-II transport or super Case-II transport) [32]. The values of n between 0.43 and 0.85 for spherical particles indicates that the mechanism is governed by both phenomena (anomalous transport) [32]. The n values are obtained from the initial portion of the curve (between 60% and 80% of drug release) according to the literature [33,34]. The exponent n shows that pantoprazole release mechanism is based on super Case-II transport (non-Fickian mechanism). The mechanism of drug release did not change after 180 days of storage. The release mechanism was found to be the same that melatonin-loaded nanocapsules-coated microparticles prepared with Eudragit® S100 [16]. In both cases, drug release can be explained by the superposition of swelling, relaxation and dissolution of the polymer, which dissolves in pH values above 7.0 [35].

Regarding the photostability evaluation, pantoprazole showed great instability when exposed to UVA light. However, microparticles were able to protect the drug from light exposure. These results demonstrated that polymeric microparticles increased pantoprazole photostability, facilitating its manufacturing allowing light exposition. Lipospheres have been described to enhance photostability of molecules as melatonin [36]. However, there are no previous reports on the reduction of the photodegradation of drugs by polymeric microencapsulation as far as we know. In this way, pantoprazole-loaded microparticles were effective not only in stabilizing the drug in acid medium as increasing the *in vivo* effect and, finally, reducing the photodegradation. It should be noted that these studies were conducted at accelerated conditions intended to promote the degradation of both the pure pantoprazole and the drug-loaded microparticles. Studies under conditions representing the conventional storage (e.g., 25°C, ambient humidity) are needed to fully assess the effects of these parameters. Nevertheless, the results presented here demonstrate the potential of pantoprazole-microparticles to improve photostability of pure drug.

In conclusion, the spray-drying process to produce pantoprazole-loaded microparticles was reproducible and the microparticles showed adequate physico-chemical characteristics for drug delivery. Microparticles were formed by a homogeneous phase consisting of Eudragit® S100 and pantoprazole. The estimated shelf life for the microparticles was 24 months. The microparticles were able to stabilize pantoprazole, protecting the drug from acid exposure and light. The *in vivo* evaluation corroborated with the *in vitro* results showing that pantoprazole-loaded microparticles were efficient in protecting the stomach against ulcer formation.

ETHICAL APPROVAL OF STUDIES

The protocol of the *in vivo* experiments was approved by the Ethical Committee (deliberation number 2003247, Universidade Federal do Rio Grande do Sul, Brazil).

ACKNOWLEDGEMENTS

Authors thank CNPq/MCT and Fapergs for the financial support. R. P. Raffin and L. M. Colomé thank Capes for their fellowships.

REFERENCES

[1] Fitton A, Wiseman L. Pantoprazole - a review of its pharmacological properties and therapeutic use in acid-related disorders. Drugs 1996; 51: 460-82.

[2] Poole P. Pantoprazole. Am J Health Syst Pharm 2001; 58: 999-1008.

[3] Cheer S, Prakash A, Faulds D, Lamb H. Pantoprazole- An Update of its pharmacological properties and therapeutic use in the management of acid-related disorders. Drugs 2003; 63: 101-32.

[4] Sachs G, Shin JM, Prathaand V, Hogan D. Synthesis or rupture: duration of acid inhibition by proton pump inhibitors. Drugs Today 2003; 39: 11-4.

[5] Avner D. Clinical Experience with pantoprazole in gastro esophageal reflux disease. Clin Ther 2000; 22: 1170-85.

[6] Lin SY, Kao YH. Tablet study of spray-dried sodium diclofenac enteric-coated microcapsules. Pharm Res 1991; 8: 919-24.

[7] Fu YJ, Mi FL, Wong TB, Shyu SS. Characteristic and controlled release of anticancer drug loaded poly(D,L-lactide) microparticles prepared by spray drying technique. J Microencapsulation 2001; 18: 733-47.

[8] Raffin RP, Colomé LM, Guterres SS, Pohlmann AR. Preparation, characterization and *in vivo* anti-ulcer evaluation of pantoprazole-loaded microparticles. Eur J Pharm Biopharm 2006; 63: 198 - 204.

[9] Raffin RP, Guterres SS, Pohlmann AR, Ré MI. Powder characteristics of pantoprazole delivery systems produced in different spray-dryer scales. Drying Tech. 2006; 24: 339-48.

[10] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Stability Testing of New Drug Substances and Products (Q 1A (R2)) 2003.

[11] Raffin RP, Colomé LM, Guterres SS, Pohlmann AR. Validation of analytical methodology by HPLC for quantification and stability evaluation of sodium pantoprazole. Quim Nova 2007; 30: 1001-5.

[12] International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (ICH), "Validation of analytical procedures: methodology", 1996.

[13] European Pharmacopoeia. 5th ed., Strasbourg, France, Council of Europe 2005.

[14] Brazilian National Health Surveillance Agency. Stability testing guideline RE 1, July 29, 2005.

[15] Beck RCR, Pohlmann AR, Benvenutti EV, Dalla Costa T, Guterres SS. Nanostructure-coated Diclofenac-loaded Microparticles: Preparation, Morphological Characterization, *in vitro* Release and *in vivo* Gastrointestinal Tolerance. J Braz Chem Soc 2005; 16: 1233-40.

[16] Schaffazick SR, Pohlmann AR, Mezzalira G, Guterres SS. Development of Nanocapsule Suspensions and Nanocapsule Spray-Dried Powders Containing Melatonin. J Braz Chem Soc 2006; 17: 562-9.

[17] Zupancic V, Ograjsek N, Kotar-Jordan B, Vrecer F. Physical characterization of pantoprazole sodium hydrates. Int J Pharm 2005; 291: 59-68.

[18] Cilurzo F, Minghetti P, Selmin F, Casiraghi, A, Montanari L. Polymethacrylate salts as new low-swellable mucoadhesive materials. J Control Rel 2003; 88: 43-53.

[19] United States Pharmacopeia. Rockville, United States, United States Pharmacopeial Convention, 2007.

[20] Farmacopeia Brasileira. São Paulo, Brazil. Ed. Atheneu 1988.

[21] Raffin RP, Jornada DS, Ré MI, Pohlmann AR, Guterres SS. Sodium Pantoprazole-loaded enteric microparticles prepared by spray drying: effect of the scale of production and process validation. Int J Pharm 2006; 324: 10-8.

[22] Colomé LM, Raffin RP, Jornada DS, Pohlmann AR, Guterres SS. Pantoprazole-loaded Eudragit blended microparticles: preparation, characterization, *in vitro* gastro-resistance and *in vivo* anti-ulcer evaluation. J Drug Del Sci Tech 2007; 17: 113-8.

[23] Goula, A, Adamopoulos K, Kazakis, NA. Influence of Spray Drying Conditions on Tomato Powder Properties. Drying Technology 2004; 22:1129-51.

[24] Goula, A, Adamopoulos K. Spray drying of tomato pulp: Effect of feed concentration. Drying Technology 2004; 22: 2309-30.

[25] Billon A, Bataille B, Cassanas G, Jacob M. Development of spray-dried acetaminophen microparticles using experimental designs. Int J Pharm 2000; 203: 159-68.

[26] Ford JL, Timmins P. Drug delivery systems. In: Pharmaceutical Thermal Analysis-Techniques and Applications. New York, Ellis Horwood Limited. 1999; 190-200.

[27] Zahedia P, Lee PI. Solid molecular dispersions of poorly water-soluble drugs in poly(2-hydroxyethyl methacrylate) hydrogels. Eur J Pharm Biopharm 2007; 65: 320-8.

[28] Lovrecich M, Nobile F, Rubessa F, Zingone G. Effect of ageing on the release of indomethacin from solid dispersions with Eudragits. Int J Pharm 1996; 131: 247-55.

[29] Shah PJ, Gandhi MS, Shah MB, Goswami SS, Santani D. Study of Mimusops elengi bark in experimental gastric ulcers. J Ethnopharmacol 2003; 89: 305-11.

[30] Heinze H, Fischer R, Pfützer R, Teyssen S, Singer MV. Lack of Interaction between Pantoprazole and Ethanol A Randomised, Double-Blind, Placebo-Controlled Study in Healthy Volunteers. Clin Drug Invest 2001; 21: 345-51.

[31] Stroyer A, McGinity JW, Leopold CS. Solid State Interaction between the Proton Pump Inhibirot Omeprazole and Various Enteric Coating Polymers. J Pharm Sci 2006; 95: 1342-53.

[32] Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropylmethylcellulose (HPMC). Adv Drug Del Rev 2001; 48:139-57.

[33] Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci 2001; 13: 123-33.

[34] Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int J Pharm 1983; 15:25-35.

[35] Beten BD, Gelbcke M, Diallo B, Moës AJ. Interaction between dipyridamole and Eudragit S. Int J Pharm 1992; 88: 31-7.

[36] Tursilli R, Casolari A, Iannuccelli V, Scalia S. Enhancement of melatonin photostability by encapsulation in lipospheres. J Pharm Biomed Anal 2006; 40: 910-4.

Received: August 21, 2007 Revised: September 15, 2007 Accepted: September 17, 2007

CAPÍTULO 6: Avaliação da liberação do pantoprazol a partir de microparticulas, permeação intestinal e correlação *in vitro/ex vivo*

6.1 Apresentação

Vários modelos *in vitro* já foram descritos para avaliar a liberação de fármacos a partir das micropartículas (BECK *et al.*, 2005; VUEBA *et al*., 2005). Estes testes são muito utilizados na caracterização e no controle de qualidade de formas farmacêuticas, uma vez que a absorção de fármacos depende de sua solubilidade e permeabilidade (SOULIMAN *et al.*, 2006). Além disso, estudos de permeação intestinal envolvendo micropartículas já foram utilizados na avaliação da absorção de fármacos puros ou encapsulados (BARTHE *et al*., 1998; CARRENO-GOMEZ *et al*., 1999).

O objetivo do presente capítulo foi avaliar a liberação *in vitro* do pantoprazol a partir das micropartículas preparadas por *spray-drying* utilizando célula de fluxo como método de dissolução. Além disso, o estudo foi focado na avaliação da permeação intestinal do pantoprazol em modelo *ex vivo*, utilizando a técnica de intestino invertido. A partir dos dados de dissolução *in vitro* e permeação *in vivo* foi estabelecida uma correlação de nível A. Estes resultados foram submetidos à revista *Drug Development and Industial Pharmacy*.

Publicação 06: Eudragit S100® microparticles containing sodium pantoprazole: drug release, intestinal absorption and *in vitro/ex vivo* correlation

Eudragit S100® microparticles containing sodium pantoprazole: drug release, intestinal absorption and *in vitro*/*ex vivo* correlation

Letícia Marques Colomé[a]; Renata Platcheck Raffin[a]; Cristiane Rodrigues Hoffmeister[a]; Daniela Joice Conrado[a]; Adriana Raffin Pohlmann[b]; Sílvia Stanisçuaski Guterres[a]

[a] Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga, 2752/404, Porto Alegre, 90610-000, RS, Brazil. Telephone: 55 51 33085500, Fax: 55 51 33085437, e-mail: nanoc@farmacia.ufrgs.br

[b] Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio Grande do Sul, CP 15003, Porto Alegre, 91501-970, RS, Brazil

Running head

Eudragit® microparticles containing pantoprazole

Abstract

The objective of this work was to analyze pantoprazole-loaded microparticles prepared by spray-drying using Eudragit S100® regarding their gastro-resistance, drug release and intestinal permeation. The *in vitro* dissolution test showed that pantoprazole release was slower for the encapsulated drug. The microparticles were effective in stabilizing the drug in acid medium. The *ex vivo* absorption of pantoprazole from the microparticles using everted gut sac model was similar to the absorption of the pure drug. Absorption profiles were correlated to the profiles obtained in dissolution experiments. A level A *in vitro*/*ex vivo* correlation was established with *r* of 0.993 and 0.997 for pure drug and microparticles.

Keywords: Pantoprazole, microparticles, *in vitro* drug release, *ex vivo* intestinal absorption, *in vitro*/*ex vivo* correlation

Introduction

Pantoprazole is a proton-pump inhibitor used in the treatment of gastric disorders (Cheer et al., 2003). Pantoprazole is labile in the stomach environment and, for this reason, it must be protected from degradation in the gastric juice to be absorbed intact in the intestine. After absorption, the drug is activated inside the gastric parietal cells in the canalicular lumen (Avner, 2000).

Microparticles represent versatile carrier systems able to improve the pharmacokinetic profile of numerous drugs conferring protection against the hostile and degrading gastrointestinal tract (Carreno-Gómez et al., 1999, Palmieri et al., 2000). In this way, microencapsulation process has successfully been used for stabilizing pantoprazole in order to administer this drug by the oral route (Raffin et al., 2006a; Raffin et al., 2006b). In our previous work (Colomé et al., 2007), pantoprazole microparticles were prepared using Eudragit S100® (1:5 drug/polymer ratio) by spray-drying technique. The *in vitro* dissolution evaluation performed in a dissolutor apparatus using the basket method showed that the drug was released from microparticles slower than the pure drug was dissolved. Besides, the *in vitro* gastro-resistance assay indicated that the microparticles were effective in protecting the drug in acid medium.

Different *in vitro* models have been used to study the drug release from microparticles and to characterize the profiles regarding the release kinetics (Beck et al., 2005; Vueba et al., 2005; Raffin et al., 2006a). These tests are widely used in the characterization and in the quality control of pharmaceutical dosage forms since drug absorption depends on its solubility and permeability (Souliman et al., 2006). Furthermore, the intestinal permeation studies involving microparticles have been conducted to evaluate the uptake of pure drug or encapsulated drug (Barthe et al., 1998; Carreno-Gomez et al., 1999).

Nowadays, there is also considerable interest in the development of *in vitro* dissolution studies capable to predict the *in vivo* performance of drugs, including drug-loaded microparticles (Trapani et al., 2007). These studies can estimate the availability of the drugs after absorption and they are important to establish *in vitro/in vivo* correlations, which have been extensively discussed in the pharmaceutical field (Sunesen et al., 2005; Souliman et al., 2006).

Taking all these considerations into account, this work was focused on the *in vitro* release kinetic evaluation of pantoprazole-loaded microparticles using the flow-through cell technique, as well as on the study of the ability of these microparticles in protecting pantoprazole against acid degradation. Additionally, the study was consecrated to evaluate the *ex vivo* drug absorption of the pantoprazole-loaded microparticles using rat everted gut sac model. From the *in vitro* release and the *ex vivo* absorption results, the work aimed to establish a level A *in vitro*/*ex vivo* correlation (IVEVC). In this regarding, as far as we know no report has already been published concerning IVEVC.

Materials and Methods

Materials

Sesquihydrate sodium pantoprazole (purity of 99.84%) was obtained from Henrifarma (Brazil). Eudragit S100® was kindly given by Almapal® (Brazil; produced by Rohm®, Germany). TC199 tissue culture medium was obtained from Cultilab (Brazil). All other chemicals were of analytical grade. Microparticles were prepared using Eudragit S100® and sesquihydrate sodium pantoprazole (1:5 polymer/drug) by spray-drying technique (Spray Dryer MSD 1.0, LabMaq, Brazil).

Methods

Microparticle preparation

Eudragit S100® microparticles were prepared by spray-drying technique as previously described (Colomé et al., 2007). Briefly, microparticles were prepared dissolving Eudragit S100® in 0.1 mol.L^{-1} NaOH to give 1.5 % (w/v) solution. Subsequently, sodium pantoprazole sesquihydrate (3 mg.mL^{-1}) was added under magnetical stirring and the mixture was spray-dried (Spray Dryer MSD 1.0, LabMaq, Brazil) under the following experimental conditions: 0.8 mm nozzle, inlet temperature of 150 ºC and flow of 0.44 L.h^{-1}.

Dissolution studies

The dissolution profiles of the pantoprazole-loaded microparticles and the pure drug were performed using a flow-through cell technique. The apparatus consisted of flow-through cells connected to a peristaltic pump (Gilson Minipuls 3, USA). The flow rate was 1 mL.min^{-1}. Release experiments were carried out at 37.0 ± 0.5 ºC, using phosphate buffer pH 7.4 (PBS) as dissolution medium. An exact amount of the pure drug or the microparticle formulation (equivalent to 5 mg of pantoprazole) was placed inside each cell. The samples were collected at predetermined time intervals (2, 10, 15, 20, 30, 60 and 90 minutes) and analyzed spectrophotometrically at 290 nm (Unicam 8625 UV/Vis spectrometer, England). Experiments were carried out in triplicates.

The methodology for UV quantification was previously validated (Colomé et al., 2007). The dissolution profiles were analyzed by model-dependent methods and by model-independent method (Beck et al., 2005).

In vitro gastro-resistance evaluation

The gastro-resistance study was performed using the same apparatus used for the dissolution tests. The samples were placed inside the cells and treated with 0.1 *M* HCl pH 1.2 (1 mL.min^{-1}) (acid step). Then, after 30 min, the medium was replaced by phosphate buffer pH 7.4 and samples were collected at the same time intervals performed for the dissolution study and analyzed spectrophotometrically at 290 nm. Experiments were carried out in triplicates.

Ex vivo absorption of the pantoprazole-loaded microparticles and the pure drug in the rat everted gut sac model

Male Wistar rats (250 - 300 g weight, CREAL/UFRGS, Brazil) were used. For the everted gut sac technique (Barthe et al., 1998), animals were sacrificed by decapitation and the entire small intestine was quickly excised and flushed through several times with NaCl solution (0.9%, w/v) at room temperature. The intestine was immediately placed in tissue culture medium (TC199 with Earle’s salts, pH 7.4) at 37 °C and then gently everted over a glass rod. One end of the intestine was clamped and the whole length of the intestine was filled with fresh medium and sealed with a second clamp. The resulting large gut sac was divided into sacs of approximately 2.5 cm in length using braided suture silk.

Each experiment was carried out using the intestine from one rat. Ten sacs were prepared starting from the end of the duodenum to ensure that sacs were from the upper/mid jejunum where metabolic activity is maximal. Each sac was placed in a flask (50 mL) containing a solution of pantoprazole (0.5 mg.mL^{-1}) or a suspension of pantoprazole-loaded microparticles (equivalent to 0.5 mg.mL^{-1} of pantoprazole) in the TC199 medium. Flasks were incubated at 37 °C under magnetic stirring. At the appropriate time points, sacs were removed and washed three times in saline. The sacs were opened and the serosal fluid drained into small tubes. Samples of the medium and serosal fluid were centrifuged for 5 min at 4,193 *g* and the supernatants were collected and filtered (0.45 µm, Millipore®). The concentration was measured by the same HPLC method previously described (Colomé et al., 2007) and the absorption of pantoprazole from the mucosal to the serosal side was calculated. The experiments were carried out in triplicates.

The use of tissue culture medium ensures excellent tissue viability and metabolic activity. The integrity of the sacs by glucose measurement in both the incubation medium and the sac contents was performed using a glucose meter (Accu-check, Roche, Switzerland) as previously reported (Barthe et al., 1998).

In vitro/ex vivo correlation

A level A correlation (FDA, 1997) between the *in vitro* drug released and the *ex vivo* drug absorption for the pure drug and the pantoprazole-loaded microparticles was investigated. In this way, data obtained from the *in vitro* dissolution and the *ex vivo* absorption studies for each formulation were modeled to fit linear equation (weight factor = 1), using Micromath Scientist® software (USA). The validity of the correlation was determined by calculating the prediction errors (PE) for the *ex vivo* and *in vitro* C_{max} and AUC for the pure drug and the microparticle profiles. This estimative determines the accuracy of the IVEV correlation model in characterizing the rate and the extension of the pantoprazole absorption. The percentage prediction errors for C_{max} and AUC were calculated by Equations (1) and (2), respectively.

$$\%PE_{C\max} = \left[\frac{C\max_{abs} - C\max_{rel}}{C\max_{abs}} \right] x100 \qquad \text{(Eq. 1)}$$

where $Cmax_{abs}$ and $Cmax_{rel}$ are the *ex vivo* absorbed and the *in vitro* released drug maximum concentration, respectively.

$$\%PE_{AUC} = \left[\frac{AUC_{abs} - AUC_{rel}}{AUC_{abs}} \right] x100 \qquad \text{(Eq. 2)}$$

where AUC_{abs} and AUC_{rel} are the AUC calculated from the *ex vivo* drug absorbed and the *in vitro* drug released profiles, respectively.

For a reasonable correlation, mean absolute PE should not exceed 10 % and the prediction error for each formulation should not exceed 15 % (FDA, 1997).

Results and discussion

The microparticles presented drug content of 136.0 $mg.g^{-1}$, encapsulation efficiencies of 100.7 ± 1.7 % and mean size appropriate for oral administration (9.1 µm). SEM analysis showed that microparticles presented erythrocyte-like shape (Figure 1). The full physico-chemical characterization of these particles was previously described (Colomé et al., 2007).

Regarding the dissolution profiles at pH 7.4 (phosphate buffer) (Figure 2), 100.3 ± 1.1 % of the pure pantoprazole was dissolved in 120 minutes. The microparticles presented a pantoprazole release of 93.5 ± 2.8 % in the same period. The dissolution efficiencies were similar (p = 0.230, ANOVA) for the microparticles (77.3 ± 2.6 %) and for the pure drug (83.9 ± 1.9 %). The profiles are similar because the polymer dissolves in this medium (pH 7.4) (Beck et al., 2005). The experiment was carried out to ensure that 100 % of the drug could be released from the microparticles.

Mathematical modeling was used to analyze the dissolution profiles. The monoexponential model fit the dissolution data for the pure drug (r = 0.999, MSC = 5.14, and k = 0.037 min^{-1}), as well as for the microparticles (r = 0.998, MSC = 4.65, and k = 0.028 min^{-1}). The constant values (k) were not statistically different (p = 0.130, ANOVA) for the pantoprazole dissolution and the drug release from microparticles. The half-lives of the drug release were 19.0 min for the pure drug and 25.0 min for the microparticles.

After the acid step, the gastro-resistance study showed that 0.9 ± 0.5 % of the pure pantoprazole remained stable in pH 1.2, whereas the microparticles protected pantoprazole in 36.2 ± 3.1 %. These results demonstrate that Eudragit S100®, a gastro-resistant polymer, was able to partially protect the pantoprazole in acid medium.

Peroral drug administration requires that the compounds overcome the very efficient barriers of the gastrointestinal tract (Trapani et al., 2007). In order to predict whether the microencapsulated pantoprazole can be absorbed by the intestinal tract, everted gut sac model was used to evaluate the drug permeation comparing the pure drug and the pantoprazole-loaded microparticles. The *ex vivo* absorption profile (Figure 3) showed that 97.1 ± 11.4 % of the pure pantoprazole was absorbed in the intestinal mucosa within 60 min of incubation. The pantoprazole-loaded microparticles presented transport of 97.6 ± 7.7 % within 120 min.

Mathematical modeling was used to analyze the absorption profiles. The monoexponential model fit absorption data for the pure drug (r = 0.978, MSC = 2.30, and k = 0.040 min^{-1}), as well as for the microparticles (r = 0.999, MSC = 5.32, and k = 0.045 min^{-1}). No statistical difference (p = 0.657, ANOVA) in the constant values (k) was observed between the absorption of the pure drug and the drug from microparticles. Probably, the pH of TC199 medium (7.4), which is the same of the dissolution medium, can explain these results. At this pH, Eudragit S100® dissolves, and the released drug can cross the intestinal membrane. The half-lives of absorption were 17.2 min for the pure pantoprazole and 15.4 min for the drug from the microparticles. The sacs showed good viability during the elapsed time of experiment, as demonstrated by the active transport of glucose, which increased in the serosal side of 1.2-fold after incubation period.

The results obtained by the *in vitro* dissolution test were comparable with those obtained by the *ex vivo* studies of pantoprazole-loaded microparticles. Thus, a level A IVEV correlation was established with correlation coefficients of 0.993 and 0.997 for the pure drug and the drug-loaded microparticles, respectively (Figure 4). This type of correlation is quite important since it represents a point-to-point relationship between the compared methodologies (FDA, 1997; Souliman et al., 2006).

The prediction error evaluation gives values of 3.3 % and 2.4 % in terms of C_{max} and 3.3 % and 9.2 % in terms of AUC for the pure pantoprazole and the drug-loaded microparticles, respectively. Besides, the mean absolutes were 2.9 % for C_{max} prediction errors and 6.3 % for AUC prediction errors. These results indicated an excellent IVEV correlation.

Conclusion

This work make evident that previously prepared microparticles were able to partially stabilize the pantoprazole in acid medium, furnishing protection of the drug. The *in vitro* release kinetic evaluation showed similar profiles for the pure drug and the drug from microparticles, ensuring that the totality of the drug was released from the microparticles. Additionally, the *ex vivo* drug absorption studies demonstrated no statistical difference between the absorption of the pure drug and the pantoprazole-loaded microparticles in gut everted sacs. From the *in vitro* release and the *ex vivo* absorption results, a level A *in vitro*/*ex vivo* correlation was established making evident the effectiveness of the flow through cell dissolution model in simulating *ex vivo* absorption conditions for the studied microparticles.

Acknowledgments

The authors thank CAPES, FAPERGS, CNPq/MCT and Rede Nanocosméticos/ CNPq for supported this work.

References

Avner, D. (2000). Clinical experience with pantoprazole in gastroesophageal reflux disease. *Clin. Ther.,* 22, 1169-1181.

Barthe, L., Bessouet, M., Woodley, J. F. & Houin, G. (1998) The improved everted gut sac: a simple method to study intestinal P-glycoprotein. *Int. J. Pharm.*, 173, 255–258.

Beck, R. C. R., Pohlmann, A. R., Benvenutti, E., Dalla Costa, T. C. & Guterres, S. S. (2005) Nanostructure-coated diclofenac-loaded microparticles: Preparation, morphological characterization, *in vitro* release and *in vivo* gastrointestinal tolerance. *J. Braz. Chem. Soc.*, 16, 1233-1240.

Carreno-Gómez, B., Woodley, J. F. & Florence, A. T. (1999). Studies on the uptake of tomato lectin nanoparricles in everted gut sacs. *Int. J. Pharm.,* 183, 7-11.

Cheer, S., Prakash, A., Faulds, D. & Lamb, H. (2003). Pantoprazole: An update of its pharmacological properties and therapeutic use in the management of acid-related disorders. *Drugs*, 63, 101-132.

Colomé, L. M., Raffin, R. P., Jornada, D. S., Pohlmann, A. R. & Guterres, S. S. (2007). Pantoprazole-loaded Eudragit blended microparticles: preparation, characterization, *in vitro* gastro-resistance and *in vivo* anti-ulcer evaluation. *J. Drug Del. Sci. Tech*., 17, 113-118.

Guidance for industry, Extended release oral dosage forms: development, evaluation, and application of in vitro/in vivo correlation, Food and Drug Administration, Center for Drug Evaluation and Research (1997).

Palmieri, G. F., Michelini, S., Di Martino, P. & Martelli, S. (2000). Polymers with pH-Dependent Solubility: Possibility of Use in the Formulation of Gastroresistant and Controlled-Release Matrix Tablets. *Drug Dev. Ind. Pharm.*, 26, 837-845.

Raffin, R. P., Colomé, L. M., Pohlmann, A. R. & Guterres, S. S. (2006)a. Preparation, characterization, and *in vivo* anti-ulcer evaluation of pantoprazole-loaded microparticles. *Eur. J. Pharm. Biopharm.*, 63, 198–204.

Raffin, R. P., Jornada, D. S., Pohlmann, A. R. & Guterres, S. S. (2006)b. Sodium pantoprazole-loaded enteric microparticles prepared by spray-drying: Effect of the scale of production and process validation. *Int. J. Pharm.*, 324, 10-18.

Souliman, S., Blanquet, S., Beyssac, E. & Cardot, J. M. (2006) A level A in vitro/in vivo correlation in fasted and fed states using different methods: Applied to solid immediate release oral dosage form. *Eur. J. Pharm. Sci.*, 27, 72-79.

Sunesen, V., Pedersen, B., Kristensen, H. & Müllertz, A. (2005). In vivo in vitro correlations correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. *Eur. J. Pharm. Sci.*, 24, 305-313.

Trapani, A., Laquintana, V., Denora, N., Lopedota, A., Cutrignelli, A., Franco, M., Trapani, G. & Liso, G. (2007). Eudragit RS 100 microparticles containing 2-hydroxypropyl-β-cyclodextrin and glutathione: Physicochemical characterization, drug release and transport studies. *Eur. J. Pharm. Sci.*, 30, 64-74.

Vueba, M. L., de Carvalho, L. A. E. B., Veiga, F., Sousa, J. J. & Pina, M. E. (2005). Role of cellulose ether polymers on ibuprofen release from matrix tablets. *Drug Dev. Ind. Pharm.*, 31, 653-665.

Fig 1. SEM photomicrographs showing microparticles in two magnifications (A) 2,500 x and (B) 6,500 x.

Fig. 2. Dissolution profiles (in PBS) of sesquihydrate sodium pantoprazole and pantoprazole-loaded microparticles.

Fig. 3. Transport of sesquihydrate sodium pantoprazole and pantoprazole-loaded microparticles across everted gut sac in the incubation medium.

Fig. 4. *In vitro*/*ex vivo* correlation for (A) pantoprazole and (B) microparticles.

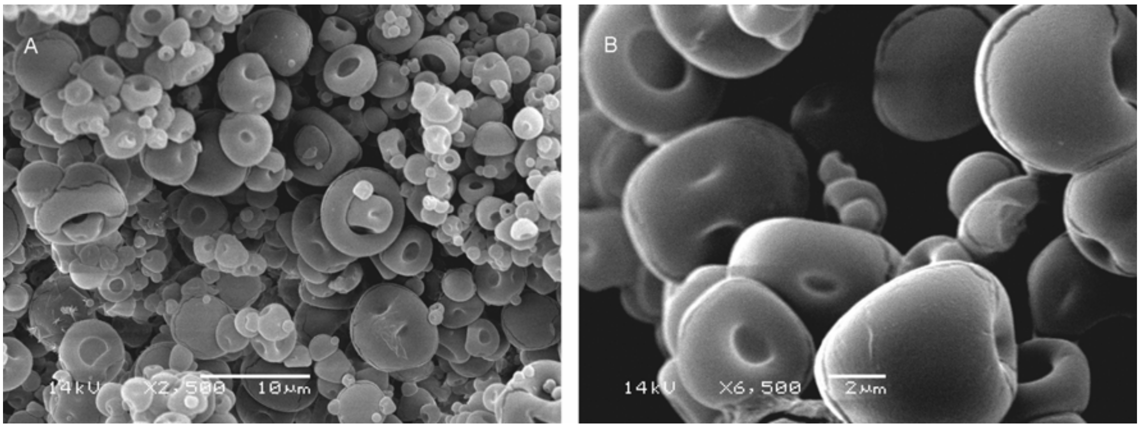


Figure 1

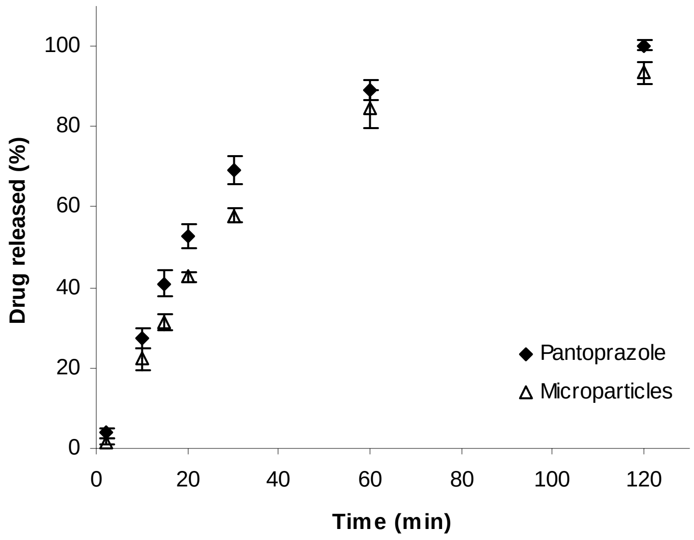
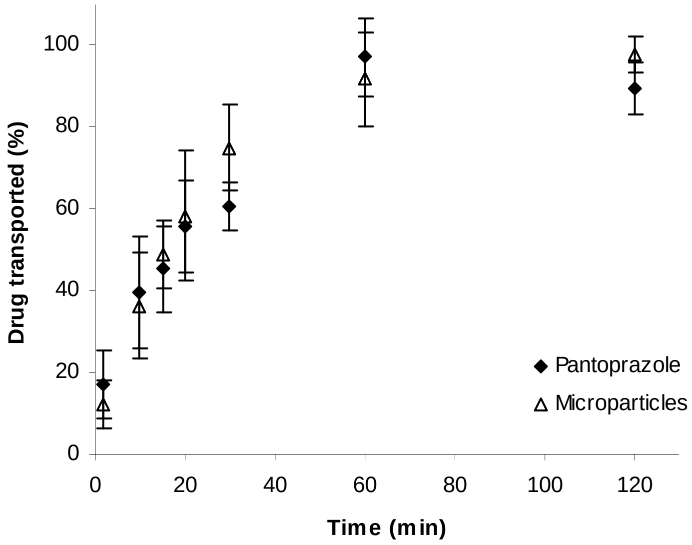

Drug released (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (min)
◆ Pantoprazole
△ Microparticles

Figure 2



Drug transported (%)
0
20
40
60
80
100
0
20
40
60
80
100
120
Time (min)
◆ Pantoprazole
△ Microparticles

Figure 3

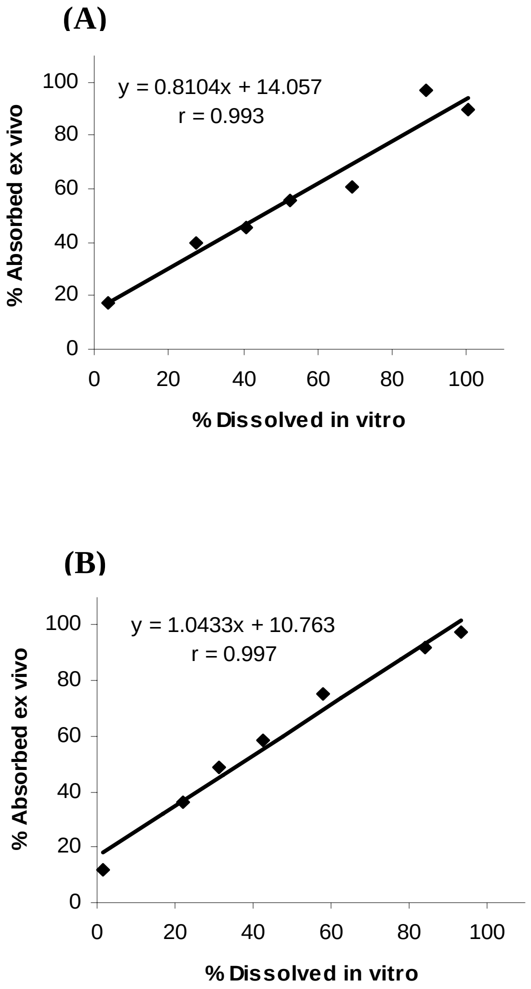

(A)
y = 0.8104x + 14.057
r = 0.993
% Absorbed ex vivo
0
20
40
60
80
100
0
20
40
60
80
100
% Dissolved in vitro
(B)
y = 1.0433x + 10.763
r = 0.997
% Absorbed ex vivo
0
20
40
60
80
100
0
20
40
60
80
100
% Dissolved in vitro

Figure 4

CAPÍTULO 7: Desenvolvimento de aglomerados contendo micropartículas de pantoprazol

7.1 Apresentação

As micropartículas preparadas em escala piloto (capitulo 5), utilizando Eudragit® S100, apresentaram baixa densidade e baixo fluxo, dificultando sua aplicação industrial. Para obter uma forma farmacêutica final, tentativas de granular ou comprimir as micropartículas resultaram em rompimento das estruturas e redução da gastro-resistência. Assim, uma forma farmacêutica cujo processo de produção não envolva compactação seria desejada.

A preparação de aglomerados ou *soft pellets* pode ser realizada sem o uso de líquidos ligantes e sem processos de compactação (RUSSO *et al.*, 2006). Os aglomerados são grandes suficientes para apresentar características tecnológicas adequadas, mas em contato com água retomam o tamanho inicial das micropartículas.

Neste trabalho, duas técnicas de aglomeração são descritas. A primeira envolve a preparação de aglomerados utilizando um pó nebulizado de manitol e de lecitina como ligantes através da técnica de rotação (*tumbling*). A segunda, uma técnica inédita, é a preparação através da vibração sobre tamises. Este trabalho compreende também a caracterização dos aglomerados através de análise morfológica, resistência, fluxo, conteúdo de fármaco e gastro-resistência. Esta parte da tese foi realizada no Laboratório de Tecnologia Farmacêutica, na Università degli Studi di Parma, Itália, sob a supervisão do Prof. Paolo Colombo, como parte do estagio de doutoramento sanduíche. Os resultados foram aceitos para publicação no periódico *Journal of Drug Delivery Sciences and Technology*.

Publicação 07: Soft Agglomerates of Pantoprazole Gastro-resistant Microparticles for Oral Administration and Intestinal Release

Soft Agglomerates of Pantoprazole Gastro-resistant Microparticles for Oral Administration and Intestinal Release

Renata P. Raffin[1*], Paolo Colombo[2], Fabio Sonvico[2], Fernanda S. Polleto[1], Gaia Colombo[3], Alessandra Rossi[2], Adriana R. Pohlmann[4], Silvia S. Guterres[1]

[1] Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmacia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.
[2] Dipartimento Farmaceutico, Università degli Studi di Parma, Parma, Italy.
[3] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Ferrara, Ferrara, Italy
[4] Departamento de Química Orgânica, Instituto de Quimica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

* Corresponding author
e-mail: reraffin@farmacia.ufrgs.br
Phone: +55-51-33085215
Fax: +55-51-33085437

Abstract

Soft agglomerates containing pantoprazole gastro-resistant microparticles were prepared for an oral delayed-release solid dosage form. A new technique was performed to agglomerate the microparticles: enteric microparticles of pantoprazole, non-agglomerating per se, were blended with mannitol/lecithin spray-dried microparticles, i.e., excipient microparticles. The blend was agglomerated by tumbling or sieve vibration. In order to elucidate the agglomerate formation, the effect of factors such as the amount of lecithin in the excipient microparticles, the ratio between pantoprazole and excipient microparticles and the agglomeration method were investigated by factorial design. Twelve batches of agglomerates presenting differing yield, drug loading, morphology, mechanical and release properties were prepared. The concentration of lecithin in the excipient microparticles was crucial for the agglomeration process. The biopharmaceutical characteristics of pantoprazole microparticles, i.e. their delayed-release properties, were not affected by the agglomeration process.

Key words: pantoprazole, agglomerates, lecithin, delayed - release, microparticles.

I. INTRODUCTION

Polymeric drug microparticles cover a relevant position among drug delivery systems, considered that they are employed to control drug release, to modify drug uptake or to improve biological availability of drug. The attainment of these biopharmaceutical attributes is opposed by the small size of particles that leads to powders with bulk volume and flow problematic for dosage forms manufacturing [1,2]. In several pharmaceutical applications, particles might be fine for drug delivery, but coarse enough for facilitating solid dosage form preparation. Often, the transformation of microparticles in solid dosage forms involves granulation and compaction, leading to irreversible modifications of the microparticle size. This technological size problem could be tackled by soft agglomeration, a process in which the powder size is enlarged by constructing weak clusters of primary microparticles [1]. In fact, soft agglomerates are easily broken down by air turbulence or water uptake, reconstituting the original size of microparticles. A similar concept has already been applied in inhalation product manufacturing [1,3,4,5]. Therefore, agglomerates should be weak to reconstitute the primary particle size, but resistant enough to be transported and processed, allowing accurate metering in dosing devices. Weak cohesion bonds due to capillary, Van der Waals or electrostatic forces, hold together the primary particles in soft structures [6]. The quantity and nature of these interactions, as well as the method of production, determine the agglomerate structures [5].

Pantoprazole, a proton pump inhibitor drug, is orally administered in enteric dosage forms, due to its instability in acidic environment [7]. In a previous paper, pantoprazole-loaded microparticles prepared by spray drying with methacrylic acid copolymer have been described [8]. These gastro-resistant microparticles kept the stability of encapsulated pantoprazole in acid. However, the technologic properties of the powder were very poor especially for dose metering. Granulation and compaction damaged microparticles, thus exposing the drug to acid environment. In order to circumvent these obstacles, soft agglomeration of the pantoprazole microparticles was considered a suitable strategy for maintaining their integrity in dosage form. Unfortunately, these pantoprazole gastro-resistant microparticles were not able to agglomerate.

Recently, a new procedure for agglomerating microparticles has been described [3]. Morphine crystals have been agglomerated in soft clusters by processing the physical mixture of drug with spray-dried microparticles of mannitol/lecithin. The lecithin was used as binder to improve the interparticle cohesion, reinforcing the internal structure of agglomerates [9]. This procedure could be applied for preparing soft agglomerates of pantoprazole enteric coated microparticles.

Thus, the purpose of this research was to study the agglomeration feasibility of pantoprazole gastro-resistant microparticles blended with spray-dried mannitol/lecithin microparticles (excipient microparticles). The goal was to make available an oral delayed-release powder dosage form to be directly swallowed or dispersed in water. The variables affecting the agglomeration of these microparticles were studied. The effect of composition and amount of excipient microparticles and of two alternative procedures of agglomeration, i.e., classical tumbling or sieve vibration, were investigated by factorial design. The soft agglomerates of gastro-resistant pantoprazole microparticles were studied in terms of micrometrics, mechanical and drug delayed-release properties.

II. MATERIALS AND METHODS

1. Materials

Sesquihydrate sodium pantoprazole was purchased from Henrifarma (São Paulo, Brazil). Methacrylic acid copolymer (Eudragit S100®) was kindly given by Almapal (São Paulo, Brazil). Mannitol (Ph. Eur.) was a gift of Lisapharma (Como, Italy) and lecithin (Lipoid S45) was supplied by Lipoid AG (Ludwigshafen, Germany). All other chemicals were of analytical grade.

2. Methods

2.1 Preparation of pantoprazole gastro-resistant microparticles

Pantoprazole microparticles were prepared in a pilot scale spray-dryer (Model PSD 52 APV Anhydro, Soeborg, Denmark) using a centrifugal atomizer at 30,000 rpm, inlet temperature of 180 °C, outlet temperature of 65 °C and feed rate of 2 L/h. The sprayed solution was prepared by dissolving Eudragit S100® and pantoprazole in 0.05 mol/L NaOH aqueous solution at drug to polymer ratio 1:4 [8]. The particle size distribution was determined by laser diffractometry Beckman Coulter® LS 13 320

(Beckman Instruments, Fullerton, USA) by dry dispersion. Average particle size was expressed as the mean volume diameter.

2.2 Preparation and characterization of spray-dried excipient microparticles

Mannitol was dissolved in 90 ml of water. Lecithin was dissolved in 10 ml of ethanol at 40 °C and mixed with mannitol solution giving an opalescent mixture. Mannitol and lecithin ratios used were 90:10, 87.5:12.5 and 85:15 (w/w) and the solid concentration was 4 % (w/v). All the solutions were spray-dried using a Buchi Mini Spray Dryer B-191 (Buchi Laboratoriums-Tecnik, Flawil, Switzerland) in the following conditions: inlet temperature 90 °C, outlet temperature 38-40 °C, feed rate 6.0 ml/min, nozzle diameter 0.7 mm, drying air flow 600 L/h.

The particle size distribution of spray-dried excipient microparticles was measured using laser light scattering apparatus (Mastersizer X, Malvern Instruments Ltd., Malvern, U.K.) suspending the particles in ethyl acetate. Particle size was expressed as median volume diameter.

The morphology and surface elemental composition of the spray-dried excipients was assessed with a scanning electron microscope (JSM 6400, Jeol Ltd., Tokyo, Japan) equipped with an EDS detector for x-ray microanalysis and using accelerating voltage of 15 kV.

The surface morphology of the spray-dried excipient microparticles was analyzed by atomic force microscopy (AFM) Digital Nanoscope III (Digital Instruments, California, USA) in tapping mode using Phosphorus (n) doped Silicon cantilever (Veeco Instruments SA.S., France) with a nominal spring constant of about 40 N/m and a resonance frequency of about 256 kHz. The microparticles were placed on a bi-adhesive tape fixed on a metallic support. Scanning was performed at a scan speed of 0.7 Hz with a resolution of 512 × 512 pixels. Images were processed using the Nanoscope (R) III 5.12r5 software (Veeco Digital Instruments, California, USA).

Flowability, as well as bulk and tapped densities, was measured in accordance with the European Pharmacopoeia [10]. The compressibility index was calculated in accordance with the United States Pharmacopoeia [11].

2.3 Soft agglomerate preparation

Six different mixtures of pantoprazole microparticles and excipient microparticles were prepared in Turbula apparatus (Wab, Basel, Switzerland) using a 300 ml PET container. The rotation was continued for 90 minutes and drug homogeneity was assessed by HPLC analysis of pantoprazole content. Each mixture was split into two portions and agglomeration was performed by two techniques. The ambient temperature was between 20°-22°C and the relative humidity between 65-85%.

2.3.1. Tumbling

Five grams of the mixture of pantoprazole and excipient microparticles were rolled into a Bakelite cylindrical jar (diameter 5.0 cm, length 4.4 cm), rotating at 45 rpm on the cylinder axis tilted at 90°. At intervals of 30 min, the tumbling was stopped and the agglomerates between 106 and 850 μm were collected by sieving [3]. The entire process lasted 3 h.

2.3.2. Vibration

Five grams of the mixture of pantoprazole and excipient microparticles were put on the top of a stack of two sieves with nominal apertures of 850 and 106 μm respectively (10 cm diameter sieves, Endecotts Ltd, London, U.K.), which was vibrated for 5 minutes on a laboratory sieve shaker (amplitude 3-4; Analysette 3 Fritz model, Fritsch GMBH, Idar-Oberstein, Germany). Agglomerates between 106 and 850 μm were collected. Reprocessing the non-agglomerated powder and forcing the larger agglomerates through the sieve, the process was repeated 5 times.

2.4 Factorial design

A multilevel factorial design was created using *StatGraphics Plus 5.1* (Herndon, USA). The factors considered for the statistical analysis were the preparation method (tumbling or vibration), the lecithin concentration in the excipient microparticles (10.0, 12.5 and 15.0 % w/w) and the ratio between the pantoprazole microparticles and excipient microparticles (1:1 and 1:2) (Table I). The percentage of lecithin in the agglomerates is shown in Table I. Twelve batches of agglomerates were prepared in triplicate. The analyzed variables were agglomeration yield, bulk density, tensile strength, disintegration time and dissolution rate. The same software was used to perform multi-sample comparison, using ANOVA with Tukey's test (α = 0.05).

2.5 Agglomerate characterization

The agglomeration yield was calculated by dividing the weight of the agglomerates in the range of 860-106 µm by the total weight of powder before agglomeration, multiplied by 100.

The drug loading was assayed by a validated HPLC method [12]. Briefly, an amount of the agglomerates, equivalent to 10 mg of pantoprazole, was carefully weighed and magnetically stirred for 1 h after dispersion in 50 ml of 0.05 mol/L NaOH. The drug concentration was determined after filtration (0.45 μm) by HPLC (LC-10 AT, Shimadzu, Japan) using a NovaPak C_{18} (3.9 x 150 mm, Waters, USA) column. Mobile phase consisted of acetonitrile/phosphate buffer pH 7.4 (35:65 v/v), the flow rate was 1 ml/min and detector wavelength was 290 nm.

The water content of the microparticles and agglomerates was determined by Karl Fisher titration .

The agglomerates were examined under an optical stereomicroscope (magnification 20 x) (Citoval 2, Jena, Germany) connected to a video camera (JVC, Tokyo, Japan) and by scanning electron microscopy as described before. Agglomerates were broken and the internal surfaces of fragments were analyzed by SEM.

Bulk (∂_b) and tapped (∂_t) densities were determined in accordance with the European Pharmacopoeia [13]. True density (∂_{true}) was measured using a helium pycnometer (Micromeritics Multivolume Pycnometer 1305, Milan, Italy). Porosity (ε) was calculated from true and bulk densities.

After size measurement, to determine the tensile strength a single agglomerate was placed on a mobile platform under the measuring head of a calibrated load cell (514 QD, DS Europe, Milan, Italy) [2]. The very slow movement of the platform caused the crushing of the agglomerate against the measuring head. The force-time curve was recorded by means of a suitable software (Scope v 3.5 AdInstruments

Ltd., Oxfordshire, U.K.). From the crushing force (F) the tensile strength (σ), was calculated applying equation 1 [1].

$$\sigma = \frac{2.8F}{\pi d^2} \qquad \text{Eq.1}$$

where d is the agglomerate diameter. The tensile strength was measured for 8 to 15 agglomerates from each replicate.

The agglomerate disintegration in buffer solutions was recorded under an optical stereomicroscope (magnification 20 x) connected to a video camera. The disintegration tests were performed by placing the agglomerate (425-500 μm) over a microscope glass and wetting it with 50 μL of phosphate buffer pH 7.4 or 0.1 N HCl (37°C). The disintegration time was measured on 25-30 agglomerates as the time for deagglomeration of the globular structure.

The drug release was assayed on flow-through cell apparatus [15] (Sotax CH 4008, Sotax, Basel, Switzerland) using two different methods. First, the total pantoprazole released from the agglomerates was determined in a dissolution test using phosphate buffer pH 7.4 as medium (37 °C and flow rate of 8.0 ± 0.5 ml/min) (Sotax CY 7, Sotax, Basel, Switzerland). Samples were collected at pre-determined times up to 60 min and analyzed by a validated UV method at 295 nm (Jasco V430, Tokyo, Japan) [16]. Pantoprazole dissolution profiles were analyzed by a first order equation using MicroMath Scientist® software (Salt Lake City, UT, USA). In the second method the samples were placed in the flow-through cells and 0.1 N HCl was pumped at 8.0 ml/min for 1 h (acid step). During the acid step no sample was collected for quantification because pantoprazole released in this pH quickly degraded [7]. After the acid step, the acid solution was replaced by phosphate buffer pH 7.4 and samples were collected at predetermined time intervals for 2 h and analyzed spectrophotometrically.

III. RESULTS AND DISCUSSION

1. Primary microparticle characteristics

Pantoprazole gastro-resistant microparticles had mean diameter of 15.6 ± 0.9 μm and contained 20 % (w/w) of pantoprazole. The powder bulk volume was high (bulk density 0.25 ± 0.07 g/cm^3) and the flow was very poor (compressibility index: 34.2 ± 1.4; angle of repose: 45.8° ± 1.3°). Granulation and compaction were not considered an option for manufacturing the dosage form, since pantoprazole-loaded microparticles could be damaged. Taking into account that in soft agglomerates weak forces bind small particles in large clusters, agglomeration was applied to improve the poor packing and flow of pantoprazole microparticle powders. The objective was to maintain the powdered size and the intestinal release properties in the final dosage form.

Since pantoprazole microparticles could not be directly agglomerated, blends of mannitol/lecithin spray-dried powders with pantoprazole gastro-resistant microparticulate powder were prepared in order to manufacture soft agglomerates, in accordance with the composition and method presented in Table I. Three excipient microparticulate powders were prepared by spray-drying three different solutions of mannitol and lecithin. The lecithin contents of the spray-dried powders obtained were 10.0, 12.5 and 15.0 % (w/w) respectively. The median volume diameters of the three powders were 3.6, 3.7 and 3.7 μm respectively, five times smaller than the size of pantoprazole microparticles. Also these spray-dried powders presented high bulk volumes (bulk density around 0.2 g/cm^3) typical of fine powders, poor packing (compressibility index between 22 and 31) and did not flow under the conditions of Ph.Eur flowability test [10]. The excipient powders prepared showed typical spray-dried round particles and evidenced a tendency to form clumps as the content of lecithin increased.

2. Soft agglomerate preparation and characterization

Table II summarizes the yield and physico-chemical characteristics of the twelve agglomerated powders obtained from blends between pantoprazole and excipient microparticles in 1:1 ratio and in 1:2 ratio (see also Table I). The agglomerates T1 and V1, prepared respectively using tumbling and vibration of 1:1 ratio microparticle mixtures and containing the lowest amount of lecithin, presented quite low agglomeration yields (16.0 and 25.5 % respectively). In addition, pantoprazole microparticles were poorly incorporated into these agglomerates, as the low loading values indicated (34.9 and 49.3 %). Hence, these agglomerates were not considered for further characterizations. The agglomerates T3 and V3 prepared at ratio 1:1 using the excipient microparticles containing more lecithin (12.5 % w/w) showed a higher yield (approx. 64 %) than the agglomerates T1 and V1; however, the drug loading was still incomplete (83.2 and 80.8 % respectively). Furthermore, the agglomerates T5 and V5, prepared with 1:1 ratio of pantoprazole microparticles and excipient microparticles with a lecithin concentration of 15.0 % w/w, showed yields similar to agglomerates T3 and V3, but drug loading was fairly complete (95.6 and 95.0 % respectively). The remaining six agglomerates, in which the ratio between the two populations of microparticles was 1:2 (T2, V2, T4, V4, T6 and V6), gave agglomeration yields between 61.5 and 84.0 % and the pantoprazole microparticles were completely embedded into agglomerates (drug loading higher than 96 %).

Summarizing, as the amount of lecithin in the blend increased, due to either the lecithin content in the excipient microparticles or to the increased ratio of excipient microparticles, the agglomeration yield and pantoprazole loading of these laboratory scale batches improved. Moreover, the process was more rapid and efficient performing the agglomeration by vibration, as showed by higher yields reported in Table II. This is due to the immediate size separation on the vibrating sieves of the agglomerates during formation.

The agglomerates produced were characterized by means of various techniques. Firstly, the agglomerates were studied by optical microscopy (Figure 1). The agglomerates prepared by tumbling presented quite a rounded shape (Figure 1, left column). The globule surface was smooth, with no appreciable non-agglomerated particles on the surface. The agglomerates prepared by vibration (Figure 1, right column) were less rounded and the surface appeared rougher due to the presence of particles not completely embedded in the globule structure.

The water content of pantoprazole microparticles was 5.97 % and for the excipient microparticles containing 10.0, 12.5 and 15.0 % of lecithin, 0.95, 1.02 and 1.14 %, respectively. The agglomerates presented water content between 4.71 and 5.30 % for all formulations and batches, showing that the relative humidity was not influent the preparation of the agglomerates.

The packing characteristics of agglomerated powders were determined by measuring bulk and tapped densities and porosity (Table II). The agglomerates showed values of bulk densities around 0.30 g/cm^3, higher than values observed for the primary microparticles but still corresponding to a loose packing arrangement of particles, as exhibited by a very porous powder bed. The tapped density values of agglomerates slightly improved the bulk values, and the compressibility indexes on average were around 16. In this way, the compressibility index, related to powder flowability, was improved by agglomeration. Agglomerate powder beds are very porous, with values ranging between 76% and 82%, a condition that could favor fast water penetration. Thus, the agglomeration process, determining the organization of particles in the globular structure, favored the packed arrangement of powder bed over primary microparticle powders. Pantoprazole gastro-resistant microparticles, as well as the three different spray-dried excipients, presented flow in infinite time (the entire samples failed to flow under conditions prescribed for the flowability test). In contrast, the agglomerates flowed very well and all products could be classified as free-flowing powders. In summary, the agglomerates showed characteristics similar to close packing arrangement and flowing ability more favorable for handling and metering than the microparticles.

The mechanical resistance is a relevant property for agglomerate use. In addition, it reflects differences in agglomerate structure as a result of their composition. Tensile strength was used to estimate the agglomerate resistance to crushing under load. Pantoprazole soft agglomerates had a very low resistance to crushing, and the tensile strength values (Table III) were between 30 and 52 mN/mm^2, similar to those reported by Russo and co-workers [3]. The samples prepared with 1:2 ratio of pantoprazole microparticles and excipient microparticles had higher tensile strength values. In particular, the agglomerate T6 presented the highest tensile strength (52.6 mN/mm^2). Tumbling produced more flowable, compact and resistant agglomerates, but the production rate was slower than with vibration procedure. In summary, the agglomerates prepared presented good resistance during flowing and poor resistance when compressed. Based on these features, they are suitable for filling hard gelatin capsules in view of oral administration.

In order to understand the different strengths obtained with the two agglomeration methods and to elucidate the agglomerate structure, SEM analysis was performed. Photomicrographs of agglomerate surface evidenced that they consist of an assembly of small (excipient) and larger (pantoprazole) microparticles. Figure 2 illustrates the agglomerates V4 and T4 selected as examples, at two different magnifications. The surface of agglomerates V4 is rough, confirming the preliminary observation by optical microscopy (Figure 2a). The detail of this surface (Figure 2b) shows small excipient particles surrounding larger pantoprazole particles, without visible bridges among them. All the agglomerates prepared by vibration showed this particular arrangement of the small and larger particles.

Differently, the surface of agglomerates T4 prepared by tumbling was smoother (Figure 2c). In this case a different arrangement of the pantoprazole microparticles and excipient microparticles was observed in SEM photogaphs, since the inter-particle space was filled of apparently melted material, likely lecithin present in excipient microparticles. This created solid bridges between the particles (Figure 2d). The differences observed between the samples V4 and T4 were similarly verified when the agglomerates of the vibrating series were compared to the tumbling series. In the tumbling series agglomerates, the lecithin in excipient microparticles was spread in the interstices between particles, so establishing solid bridges between them. This was particularly evident for the agglomerates containing the excipient microparticles with higher content of lecithin. This suggested that, in the mannitol/lecithin microparticles, lecithin could be located at the surface. In order to detect the presence of lecithin on particle surface, we analyzed the excipient microparticles containing the highest amount of lecithin by means of AFM and x-ray microanalysis. In comparison with particles made of mannitol alone, the surface of the particle containing lecithin appeared coated with a curly layer of substance, probably lecithin (Figure 3). This was also confirmed by x-ray microanalysis searching phosphorus on the surface of the microparticles mannitol/lecithin. The results obtained revealed the presence of phosphorus peak among the components of the particle surface in comparison with the mannitol alone particles where there was no P peak. This peak was more intense in case of microparticles with the highest lecithin content. Thus, lecithin accumulated on the microparticle surface, in particular in those particles having the highest lecithin content.

In order to verify if the different particle organization at the surface existed also internally, some agglomerates were broken and the fragments examined by SEM. Figure 4 shows the interior of agglomerates V3 and T3. The photomicrographs show that in the sample V3, the internal surface of fragment had the same organization of the surface i.e., distinct and individual microparticles of excipients around the pantoprazole microparticles (Figure 4a). The tumbled agglomerates T3 presented an internal structure in which the interstices between the agglomerated particles were filled by lecithin more evidently than on their surface (Figure 4b).

3. Agglomerate disintegration behavior and drug release

The effect of agglomeration on drug release was evaluated measuring the disintegration behavior of agglomerates and the dissolution rate of pantoprazole. The penetration of pH 7.4 buffer solution was fast for all agglomerates batches and the time for disaggregation ranged from 19 to 60 s (Table III). It was observed that the agglomerates obtained from 1:2 pantoprazole/excipient blends presented longer disaggregation times. On the contrary, the agglomerates were not disintegrated in HCl 0.1 N: in these conditions, they were slowly penetrated and slightly swollen by solvent, maintaining the globular structure.

To understand the influence of lecithin on the disintegration behavior, agglomerates constituted only of excipient microparticles were tested at the two pHs as well. These agglomerates disintegrated within 120 s in pH 7.4 phosphate buffer, a time longer than pantoprazole containing agglomerates. Again, in acid medium these excipient agglomerates did not disintegrate, despite the solvent penetrated the structure. Therefore, the disintegration behavior of pantoprazole agglomerates in acid medium was strongly dependent on the presence of mannitol/lecithin excipient microparticles.

The dissolution rate determination was conducted at pH 7.4 in flow-through apparatus. All samples released 100 % of pantoprazole in 60 minutes (Figure 5) and the release profiles were similar in shape. Fitting the release data to first order equation, considered an appropriate model for this microencapsulated product, the half-life ($t_{1/2}$) of drug release was calculated (Table III). All the samples exhibited $t_{1/2}$ values lower than 16.2 minutes; however, the agglomerates prepared with excipient microparticles containing 15.0 % of lecithin (T5, T6, V5 and V6), exhibited the highest $t_{1/2}$ values.

Considered that the preparation was gastro-resistant and had to dissolve in enteric environment, a dissolution test in conditions of variable pH was also performed. The objective was to study the effect of the acid penetration in the agglomerate on the successive drug release in intestinal conditions. In a previous work [8], the pantoprazole enteric microparticles practically released the drug (98 %) only in intestinal environment, after an acid step. The agglomerates containing the pantoprazole enteric microparticles showed a similar behavior, since practically 100% of drug was released only after the shift of pH at 7.4 value. However, compared to the direct dissolution in phosphate buffer, the pH variation dissolution test showed different drug release kinetic from agglomerates: the acid step slowed down the successive dissolution rate at pH 7.4 and the complete release of drug required at least 120 min. It was observed in the flow-through cell that the agglomerate mass was not disintegrated by the acid solution; the globular structure was disintegrated and dissolved only after the change of the pH to 7.4 at 60 minutes. As result, the release kinetics changed. The release profile was quite linear probably because the acid treatment created a globular reservoir slowly dissolved by the intestinal fluid (Figure 6).

4. Factorial design

From the statistical analysis on the influence of process variables on agglomerate properties, several significant effects of variables and their combinations were assessed. The ratio between pantoprazole microparticles and excipient microparticles ($p < 0.001$) and the concentration of lecithin in mannitol/lecithin microparticles ($p = 0.006$) exerted a significant influence on agglomeration yield. However, an excess of lecithin in the agglomerates caused a reduction in the process yield ($p < 0.001$). The preparation method did not significantly influence the yield ($p = 0.155$), as well as its interaction with the ratio between microparticles or the percentage of lecithin ($p = 0.923$ and 0.838, respectively). The lecithin content can be modulated in order to increase yield or design different agglomerates. In this study, the maximum yield was observed when agglomerates were prepared by vibration using the excipient microparticles with 12.5 % of lecithin at 1:2 ratio.

The ratio of pantoprazole microparticles and excipient microparticles was the factor that significantly affected the bulk density of agglomerated powders ($p < 0.001$). On increasing the proportion of excipient microparticles, the packing of agglomerates improved. Furthermore, the preparation method did not influence the bulk density of the agglomerates ($p = 0.270$). In this characterization, the percentage of lecithin in the excipient microparticles was not a determinant factor ($p = 0.828$). The low bulk density and poor flow of the pantoprazole microparticles were changed to more desirable technological characteristics of the agglomerate powder. The interaction between the concentration of lecithin and the ratio of the excipient microparticles significantly influenced the tensile strength ($p = 0.044$). The morphology of the tumbling series was not determinant in the resistance of the agglomerates (again the method was not significant, p = 0.351). Even though this analyses presents high standard deviations, the replicates were not statistical different (p = 0.345).

The disintegration time at pH 7.4 was dependent on the ratio between pantoprazole microparticles and excipient microparticles as well (p < 0.001). The agglomerates prepared using 1:2 ratio presented disintegration times significantly longer than the ones prepared with 1:1 ratio. The factor influencing the dissolution rate was the concentration of lecithin in excipient microparticles (p = 0.008). Agglomerates prepared with 15.0 % of lecithin in the excipient microparticles presented a slower release rate than the other formulations. The ratio between pantoprazole microparticles and excipient microparticles was not significant at 95 %, but influence was significant at 90 % (p = 0.084). The agglomerates prepared with 1:1 ratio presented faster drug release, compared to 1:2 ratio agglomerates. This was a consequence of the significant influence of the factor in the disintegration time. The release of pantoprazole from agglomerates was dependent on the amount of lecithin used, in terms of its concentration in excipient microparticles and ratio between excipient and pantoprazole microparticles. The disintegration was mainly affected by the ratio, but the half-life of drug release by the lecithin concentration in the excipient. In this way, the faster pantoprazole release could be achieved using 1:1 (w/w) ratio and 10.0 or 12.5 % of lecithin in the excipient microparticles.

In summary, the significant factor that affected the characteristics of the agglomerates was related to the concentration of lecithin in the formulation (in terms of percentage in the excipient or ratio between pantoprazole and excipient microparticles). The two preparation techniques produced agglomerates with different morphology that did not influence their mechanical characteristics. Agglomerates prepared with higher quantities of lecithin presented higher agglomeration yield, bulk density and tensile strength. These agglomerates, on the other side, presented slower disintegration and drug release. The concentration of 12.5 % of lecithin seemed the optimized formulation for the excipient and the 1:2 ratio guarantees higher agglomeration yield as well as resistance for the filling process.

*

Agglomeration of pantoprazole gastro-resistant microparticles blended with excipient microparticles is a technique successfully applied to size enlargement of micronized products that could be damaged by granulation or compaction. These agglomerates can be used as oral delayed-release dosage forms administered as they are or dispersed in a liquid. The composition and quantity of the excipient microparticles resulted to be the crucial factors for the agglomerate quality. Therefore, adjusting the content of lecithin used as binder, it is possible to agglomerate microparticles of materials that could not be agglomerated per se. Increasing the lecithin concentration in the spray-dried excipient microparticles or increasing the fraction of these excipient microparticles in the blend, the agglomeration was improved.

The two agglomeration techniques gave different structures. By tumbling, lecithin on microparticle surface was spread to fill the inter-particle interstices giving rise to more resistant agglomerates. On the other hand, this phenomenon was not present by vibration and the agglomerates resulted less resistant. However, the fabrication in this case was quicker.

Independently on the structure, the agglomerates presented prompt disintegration and fast dissolution at pH 7.4. However, when the agglomerates were submitted to a pH variability test, the acid penetrated in the agglomerate slowed down disintegration and dissolution on pantoprazole microparticles

These soft agglomerates reinforced by lecithin solid bridges can have applications in several administration routes where the original size recovery of primary microparticles is not strictly compulsory. The agglomerates as dosage forms makes feasible the delivery of particulate systems designed for the oral delayed–release, like the pantoprazole case. The use of alternative sugars or different bridging substances can optimize the preparation in presence of other microparticulate substances.

AKNOWLEDGEMENTS

The authors are grateful for the financial support of Capes, CNPq/MCT and Fapergs. The financial support of the Italian Ministry for University and Research is also gratefully acknowledged (PRIN 2006 program).

REFERENCES

[1] Russo P., Buttini F., Sonvico F., Bettini R., Massimo G., Sacchetti C., Colombo P., Santi P. - Chimeral agglomerates of microparticles for administration of caffeine nasal powders - J. Drug Deliv. Sci. Tech., 14, 449-454, 2004.

[2] Kim B.K., Hwang S.J., Park J.B., Park H.J. - Characteristics of felodipine-located poly(ε-caprolactone) microspheres - J. Microencapsulation, 22, 193-203, 2005.

[3] Russo P., Sacchetti C., Pasquali I., Bettini R., Massimo G., Colombo P., Rossi A. - Primary microparticles and agglomerates of morphine for nasal insufflation - J. Pharm. Sci., 95, 2553-2561, 2006.

[4] Adi H., Larson I., Chiou H., Young P., Traini D., Stewart P. - Agglomerate strength and dispersion of salmeterol xinafoate from powder mixtures for inhalation - Pharm. Res., 23, 2556-2565, 2006.

[5] Boerefijn R., Ning Z., Ghadiri M. - Disintegration of weak lactose agglomerates for inhalation applications - Int. J. Pharm., 172, 199-209. 1998.

[6] Tsantilis S., Pratsinis S.E. - Soft- and hard-agglomerate aerosols made at high temperatures - Langmuir, 20, 5933-5939, 2004.

[7] Cheer S., Prakash A., Faulds D., Lamb H. - Pantoprazole-An Update of its pharmacological properties and therapeutic use in the management of acid-related disorders – Drugs, 63, 101-132, 2003.

[8] Raffin R.P., Guterres S.S., Pohlmann A.R., Re M.I. - Powder characteristics of pantoprazole delivery systems produced in different spray-dryer scales - Drying Tech., 24, 339-348, 2006.

[9] Moreno-Atanasio R., Ghadiri M. - Mechanistic analysis and computer simulation of impact breakage of agglomerates: Effect of surface energy - Chem. Eng. Sci., 61, 2476-2481, 2006.

[10] European Pharmacopoeia (5th ed.), Council of Europe, Strasbourg, France, 2005, Flowability, p.p. 242-243.

[11] United States Pharmacopeia (30th ed.), United States Pharmacopeial Convention, Rockville, United States, 2007, Measures of powder compressibility, p. 243.

[12] Raffin R.P., Colome L.M., Guterres S.S., Pohlmann A.R. - Validation of analytical methodology by HPLC for quantification and stability evaluation of sodium pantoprazole. Quim. Nova, 30, 1001-1005, 2007.

[13] European Pharmacopoeia (5th ed.), Council of Europe, Strasbourg, France, 2005, Density of solids, p.p. 64-65.

[14] European Pharmacopoeia (5th ed.), Council of Europe, Strasbourg, France, 2005, Friability of uncoated tablets, p.p. 234-235.

[15] European Pharmacopoeia (5th ed.), Council of Europe, Strasbourg, France, 2005, Flow – through cell apparatus, p. 229.

[16] Raffin R.P., Colome L.M., Pohlmann A.R., Guterres S.S. - Preparation, characterization, and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles - Eur. J. Pharm. Biopharm., 63, 198-204, 2006.

Table I: Composition and agglomeration method of the twelwe agglomerate batches.

Agglomerate code*	Preparation method	Lecithin in excipient microparticles (%)	Pantoprazole/ excipient microparticles ratio	Percentage of lecithin in the agglomerates (%)
T1	Tumbling	10.0	1:1	5.0
T2	Tumbling	10.0	1:2	6.7
T3	Tumbling	12.5	1:1	6.3
T4	Tumbling	12.5	1:2	8.3
T5	Tumbling	15.0	1:1	7.5
T6	Tumbling	15.0	1:2	10.0
V1	Vibration	10.0	1:1	5.0
V2	Vibration	10.0	1:2	6.7
V3	Vibration	12.5	1:1	6.3
V4	Vibration	12.5	1:2	8.3
V5	Vibration	15.0	1:1	7.5
V6	Vibration	15.0	1:2	10.0

* The odd and even numbers in the code correspond to 1:1 and 1:2 ratios, respectively, and the letter (T and V) to the preparation method.

Table II: Characteristics of the agglomerates

Code	Yield (%)	Drug loading (%)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Porosity (%)	Flowability (s)
T1	16.0 ± 5.6	34.9 ± 0.02	--	--	--	--
T2	61.5 ± 3.5	96.7 ± 0.86	0.34 ± 0.01	0.40 ± 0.02	76 ± 1	30.8 ± 0.2
T3	64.0 ± 1.4	83.2 ± 2.10	0.27 ± 0.01	0.35 ± 0.01	81 ± 1	28.1 ± 0.9
T4	79.1 ± 4.1	95.8 ± 0.58	0.36 ± 0.01	0.41 ± 0.01	76 ± 1	32.0 ± 2.5
T5	62.0 ± 6.3	95.6 ± 1.07	0.28 ± 0.04	0.35 ± 0.03	81 ± 2	37.9 ± 6.2
T6	70.5 ± 0.7	97.6 ± 0.47	0.32 ± 0.02	0.38 ± 0.02	76 ± 1	23.8 ± 2.3
V1	25.5±12.0	49.3 ± 0.26	--	--	--	--
V2	76.2 ± 0.8	99.1 ± 0.78	0.32 ± 0.01	0.38 ± 0.01	79 ± 1	25.5 ± 6.4
V3	64.2 ± 4.2	80.7 ± 1.52	0.29 ± 0.01	0.36 ± 0.02	80 ± 1	22.5 ± 0.8
V4	84.0 ± 2.8	99.7 ± 1.30	0.33 ± 0.01	0.38 ± 0.01	79 ± 1	35.6 ± 1.5
V5	69.5 ± 2.1	94.9 ± 0.17	0.26 ± 0.01	0.33 ± 0.01	82 ± 1	28.4 ± 4.3
V6	79.5 ± 3.5	100.2 ± 1.93	0.33 ± 0.02	0.37 ± 0.03	78 ± 1	28.3 ± 1.0

Table III: Mechanical properties and biopharmaceutical characteristics of the agglomerates

Code	Friability (%)	Tensile strength (mN/mm^2)	Disintegration time pH 7.4 (s)	Drug release - $t_{1/2}$ (min)
T1	--	--	--	--
T2	0.11	38.6 ± 2.3	56.6 ± 2.9	9.0 ± 0.5
T3	3.90	36.0 ± 1.2	21.9 ± 3.0	11.2 ± 0.7
T4	0.07	38.9 ± 3.5	41.3 ± 2.8	7.4 ± 0.5
T5	0.51	29.6 ± 4.3	25.1 ± 4.4	13.7 ± 0.7
T6	0.32	52.6 ± 3.6	46.8 ± 3.0	13.3 ± 0.8
V1	--	--	--	--
V2	1.83	30.4 ± 1.6	48.5 ± 5.4	7.6 ± 0.5
V3	1.70	34.6 ± 0.9	23.3 ± 1.9	8.8 ± 0.4
V4	0.16	41.5 ± 1.6	59.3 ± 6.8	9.2 ± 0.6
V5	2.90	34.1 ± 3.6	19.1 ± 1.2	16.2 ± 0.4
V6	0.60	40.8 ± 5.8	50.4 ± 6.5	14.4 ± 0.8

Figure 1: Optical microscopy pictures of the agglomerates (magnification 20 x).

Figure 2: SEM images of agglomerates V4 and T4: (a) V4 at 100 x; (b) V4 at 1,000 x; (c) T4 at 100 x and (d) T4 at 1,000 x.

Figure 3: AFM images of microparticle surface: (a) manitol spray-dried; and (b) mannitol/lecithin 85:15 microparticles.

Figure 4: Inner structure of the agglomerates V3 (a) and T3 (b) (magnification 2,000 x).

Figure 5: Drug released from the agglomerates in phosphate buffer pH 7.4: (a) by tumbling: ▽ (T2), □ (T3), ◇ (T4), △ (T5), ○ (T6); (b) by vibration: ▽ (V2), △ (V3), ○ (V4), □ (V5), ◇ (V6).

Figure 6: Drug released from the agglomerates at variable pH (60 min in acid medium and from 60 to 180 min in phosphate buffer pH 7.4): (a) by tumbling: △ (T2), ▽ (T3), □ (T4), ◇ (T5), ○ (T6); (b) by vibration: △ (V2), ▽ (V3), □ (V4), ◇ (V5), ○ (V6).

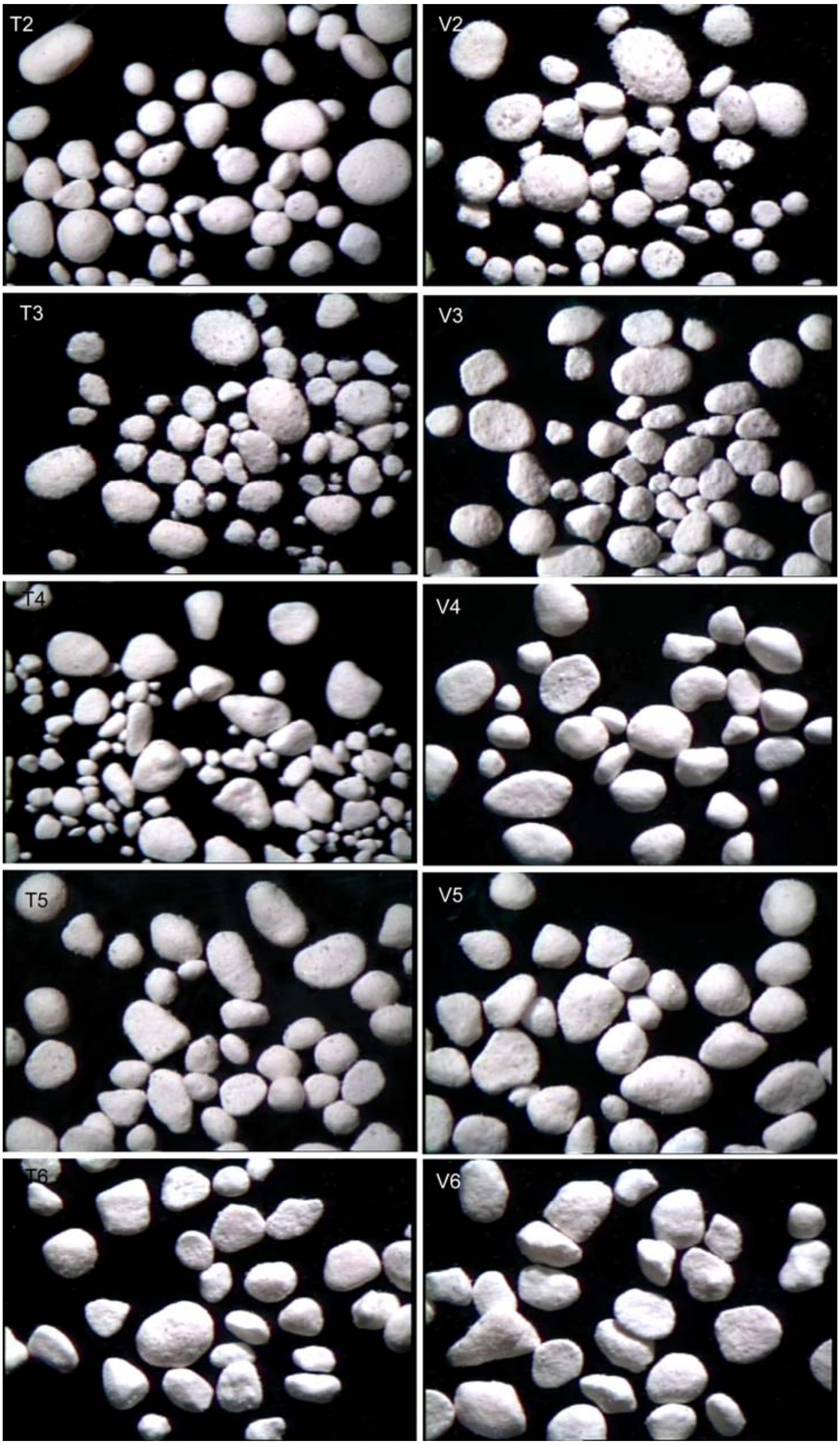


Figure 1

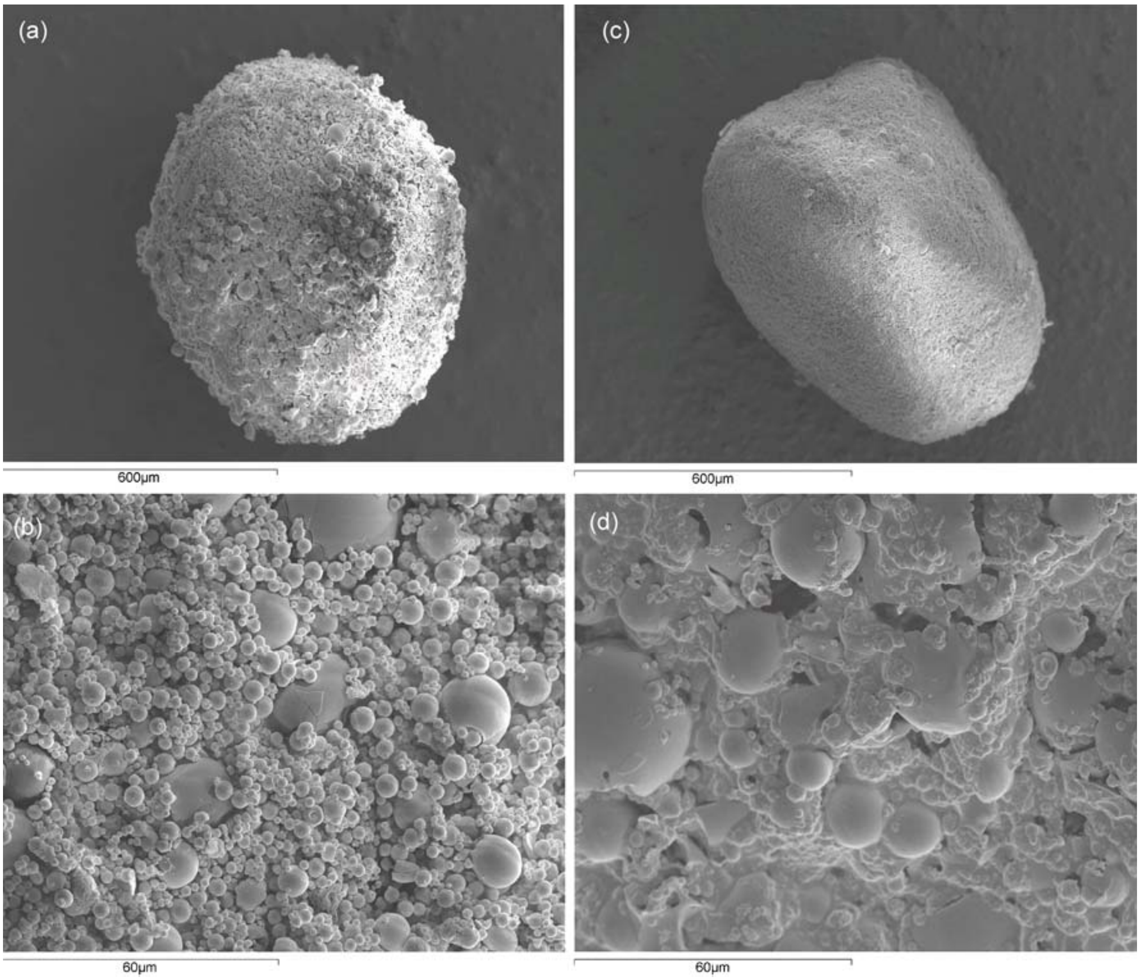


Figure 2

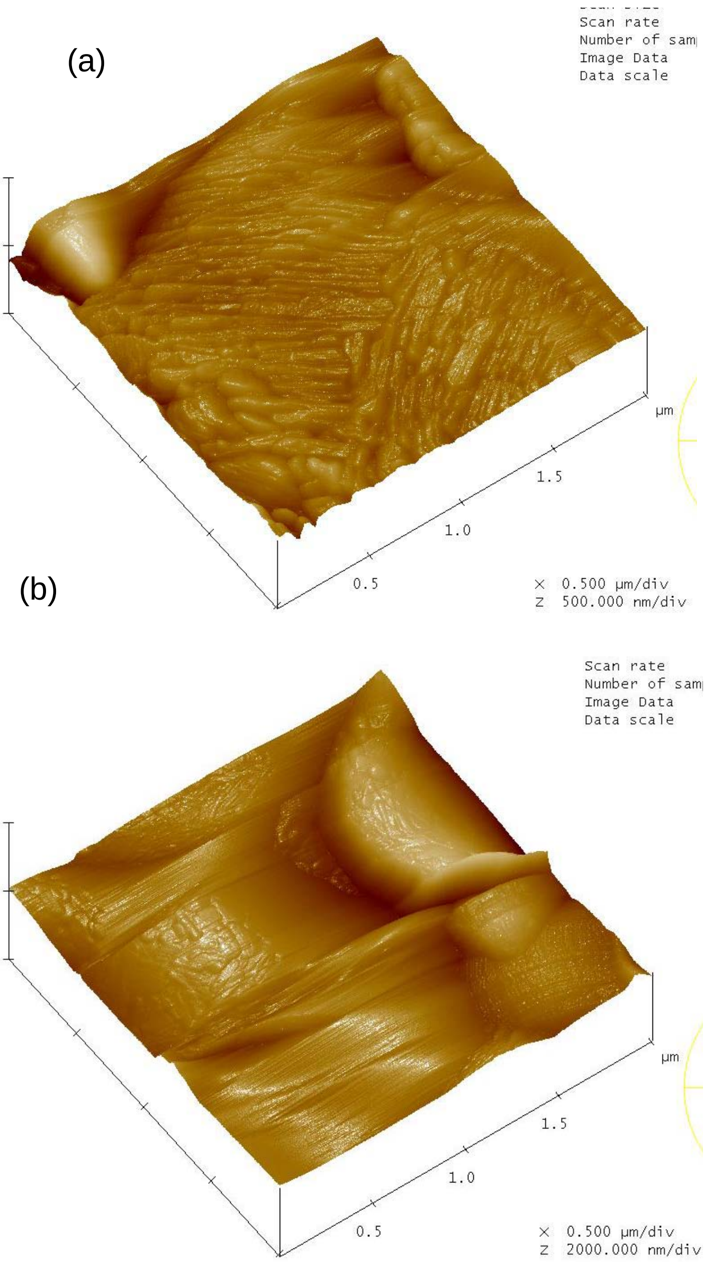

(a)
Scan rate
Number of sam
Image Data
Data scale
µm
1.5
1.0
0.5
X 0.500 µm/div
Z 500.000 nm/div
(b)
Scan rate
Number of sam
Image Data
Data scale
µm
1.5
1.0
0.5
X 0.500 µm/div
Z 2000.000 nm/div

Figure 3

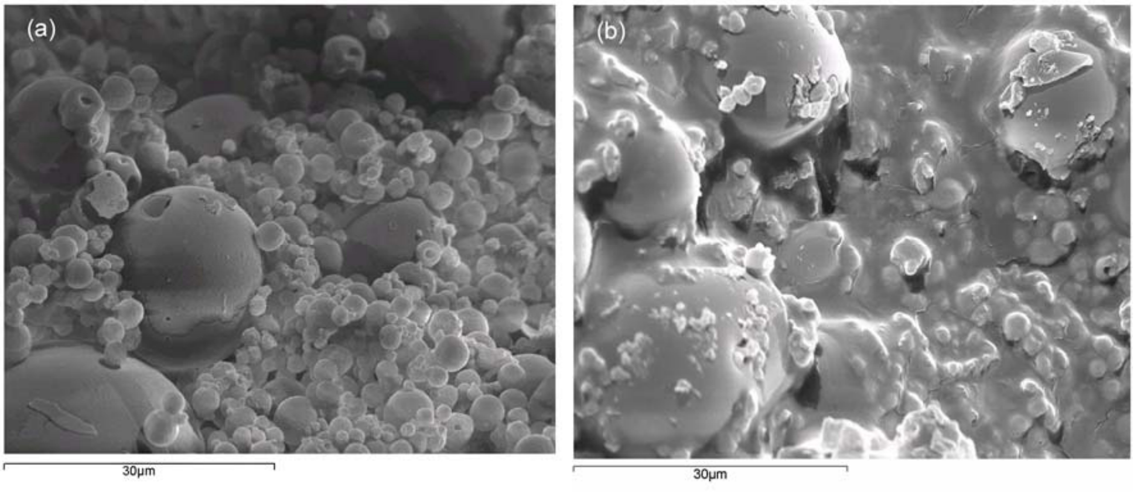


Figure 4

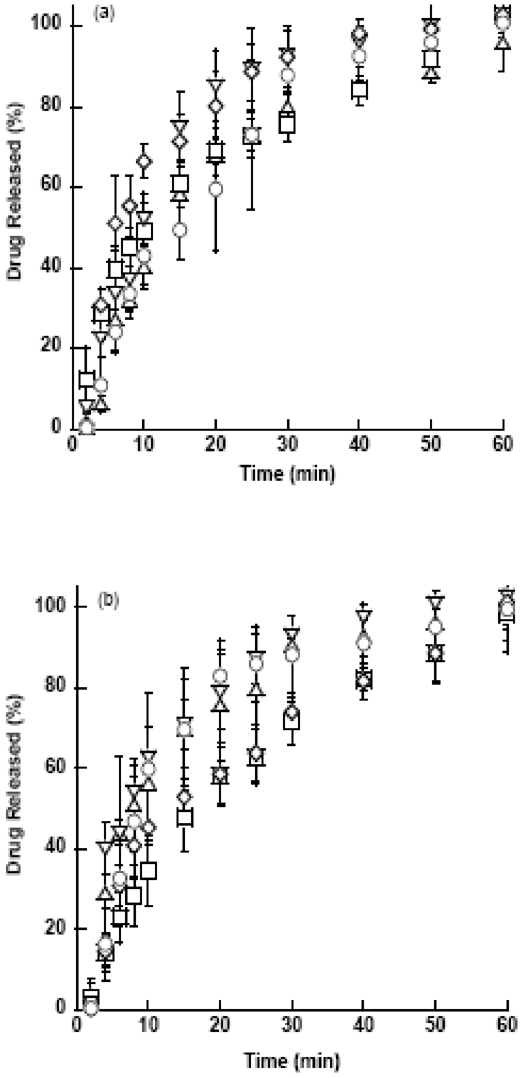

(a)
Drug Released (%)
0
20
40
60
80
100
Time (min)
0
10
20
30
40
50
60
(b)
Drug Released (%)
0
20
40
60
80
100
Time (min)
0
10
20
30
40
50
60

Figure 5

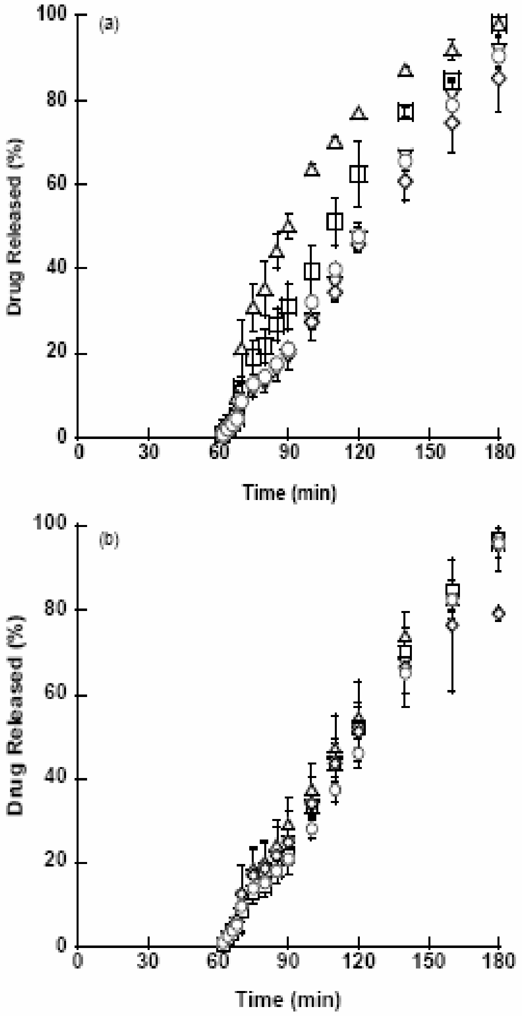

(a)
Drug Released (%)
0
20
40
60
80
100
Time (min)
0
30
60
90
120
150
180
(b)
Drug Released (%)
0
20
40
60
80
100
Time (min)
0
30
60
90
120
150
180

Figure 6

CAPÍTULO 8: Preparação de micropartículas de liberação controlada utilizando uma blenda de Eudragit S100 e Methocel F4M

8.1 Apresentação

No capítulo 3, foram descritas micropartículas de liberação controlada contendo pantoprazol, preparadas através de emulsificação/evaporação de solvente. Baseado no fato que as micropartículas preparadas por *spray-drying* descritas no capítulo 4 apresentam gastro-resistência maior que as mesmas preparadas por evaporação de solvente (capítulo 2), no presente capítulo, micropartículas de liberação controlada foram produzidas pela técnica de secagem por aspersão.

Uma das vantagens da técnica de *spray-drying* é a possibilidade de se trabalhar com sistemas aquosos. Devido a isso, torna-se inviável a preparação de micropartículas de poli(ε-caprolactona) por esta técnica. O polímero escolhido, então, para constituir a blenda juntamente com o Eudragit® S100 foi a hidroxipropilmetilcelulose (HPMC). A HPMC é um derivado solúvel da celulose, muito utilizado em comprimidos matriciais com finalidade de controlar a liberação de fármacos. A HPMC hidrata e intumesce, formando uma fase viscosa que controla a entrada de água para o interno da partícula ou matriz e a difusão de fármacos ao meio externo. Consideramos também que ainda não foram descritas na literatura micropartículas preparadas com blendas destes dois polímeros.

As micropartículas forma preparadas em escala laboratorial e caracterizadas através do doseamento do fármaco, de calorimetria diferencial exploratória e análises morfológicas. Os perfis de liberação e gastro-resistência também foram avaliados e modelados matematicamente e a atividade anti-ulcerogênica foi avaliada segundo modelo descrito no capítulo 2. Este trabalho foi publicado no periódico *Die Pharmazie*.

Publicação 08: Development of HPMC and Eudragit S100® blended microparticles containing sodium pantoprazole

Programa de Pós-Graduação em Ciências Farmacêuticas[1], Faculdade de Farmácia, Departamento de Química Orgânica[2],
Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Development of HPMC and Eudragit S100® blended microparticles containing sodium pantoprazole

R. P. Raffin[1], L. M. Colomé[1], S. E. Haas[1], D. S. Jornada[1], A. R. Pohlmann[2], S. S. Guterres[1]

Received April 28, 2006, accepted September 29, 2006

Faculdade de Farmácia, Av. Ipiranga, 2752, Porto Alegre, 90610-000, RS, Brazil
nanoc@farmacia.ufrgs.br

Pharmazie 62: 361–364 (2007) *doi: 10.1691/ph.2007.5.6077*

Pantoprazole is used in the treatment of acid related disorders and *Helicobacter pylori* infections. It is activated inside gastric parietal cells binding irreversibly to the H^+/K^+-ATPase. In this way, pantoprazole must be absorbed intact in gastro-intestinal tract, indicating that enteric delivery systems are required. The purpose of this study was to prepare pantoprazole-loaded microparticles by spray-drying using a blend of Eudragit S100® and HPMC, which can provide gastro-resistance and controlled release. Microparticles presented acceptable drug loading (120.4 mgg^{-1}), encapsulation efficiency (92.3%), surface area (49.0 m^2g^{-1}), and particle size (11.3 μm). DSC analyses showed that the drug is molecularly dispersed in the microparticles, and *in vivo* anti-ulcer evaluation demonstrated that microparticles were effective in protecting stomach against ulceration. Microparticles were successfully tabletted using magnesium stearate. *In vitro* gastro-resistance study showed that microparticles stabilized pantoprazole in 62.0% and tablets in 97.5% and provided a controlled release of the drug.

1. Introduction

Multi-particulate drug delivery systems based on polymer blends have shown several advantages over single unit ones, such as more uniform transit times through the gastro-intestinal tract, less variability among individuals, smaller risk of dose dumping and high local concentrations of drug (Lin and Kao 1991; Beckert et al. 1996). In this sense, polymer blend formulations have been widely studied because they can improve polymer mechanical properties, reduce drug toxicity and control drug delivery (Vega-Gonzalez et al. 2004). Eudragit S100 [poly(methacrylic acid-co-methyl methacrylate) (1 : 2)] is an enteric polymer and hydroxypropylmethylcellulose (HPMC) is a hydrophilic derivative of cellulose commonly used as drug release rate-controlling polymer. Both polymers are largely used in pharmaceutical formulations (Alvarez-Fuentes et al. 2004; Li et al. 2005). However, up to now, blends of these polymers have not been reported as microparticulate drug delivery systems.

Pantoprazole is a prodrug used in the treatment of digestive ulcers, gastro-esophageal reflux disease and *Helicobacter pylori* infections (Cheer et al. 2003). This prodrug is activated inside gastric parietal cells binding irreversibly to the H^+/K^+-ATPase. In this way, it must be absorbed intact in the gastro-intestinal tract, indicating that an enteric drug delivery system is required for its oral administration (Sachs et al. 2003). In consequence, enteric tablets containing pantoprazole are commercially available.

In our previous work, pantoprazole-loaded microparticles have been prepared with Eudragit S100 by an O/O emulsification/solvent evaporation technique (Raffin et al. 2006a). These microparticles were able to protect the stomachs of rats against ulcer formation. The main drawback of this technique is the difficulty of scaling up the production of microparticles as well as controlling their shape and size. So, recently, we have studied the spray-drying technique for the preparation of microparticles containing pantoprazole (Raffin et al. 2006b). This technique was adequate for the preparation of microparticles in both laboratory and pilot scales. Besides, the shape and the size of the particles have been controlled by changing the composition of the formulation and the spray-drier operational conditions.

As far as we know, no multiple-unit system based on HPMC and Eudragit S100 blended microparticles containing pantoprazole has already been developed. Taking into account all this, the purpose of the present study was to prepare pantoprazole-loaded microparticles by a spray-drying technique using the blend of Eudragit S100 and HPMC. The blend would be able to provide simultaneously gastric protection and controlled release of the drug, due to the physico-chemical characteristics of those polymers. Eudragit S100 is insoluble in acid media and pure water, whereas it is soluble in intestinal media from pH 7 upwards. HPMC hydrates, swells, coalesces, and forms a viscous phase around the exterior of the particle. This viscous layer can control both the influx of water and the efflux of drugs. Additionally, this work was dedicated to characterize the microparticles by DSC, HPLC, SEM, and surface area, as well as to verify the effectiveness of this multiparticulate system in protecting the gastro-intestinal tract against ulceration induced by ethanol in rats. The *in vitro* gastro-resistance of microparticles and tabletted microparticles was also evaluated.

2. Investigations, results and discussion

It has already been described in the literature (Riegel and Leopold 2005) that omeprazole is unstable in some solutions and suspensions containing enteric polymers. As pantoprazole is a benzimidazole analog to omeprazole its stability in the polymeric solution must be evaluated before the preparation of the microparticles. The solution containing both polymers and pantoprazole was kept at room temperature and absence of light for 24 h. No degradation was detected in 24 h demonstrating that this formulation can be used to prepare microparticles. Microparticles prepared by spray-drying were obtained with a yield of 36 ± 1% as off-white powders.

Microparticles presented concave spherical shape with visible folding and shrivelling (Fig. 1a). This morphology is formed by uneven shrinkage forces during the drying of droplets, depending on the viscosity of the liquid feed (Foster and Laetherman 1995). The addition of HPMC to the formulation increases its viscosity and the tendency to shrive or fold is also increased.

The drug content assayed by HPLC was 120.4 ± 11.9 mgg ^{1} of pantoprazole in the microparticles resulting in an encapsulation efficiency of 92.3%. The microparticle surface area was 49 m^{2}g ^{1} at an average particle size of 11.3 μm (span = 2.6). Microparticles prepared with Eudragit S100 containing pantoprazole (Raffin et al. 2006b) presented an increase in particle size with the increase of feed's viscosity. The microparticles produced with a feed with viscosity of 6.1 cP presented an average size of 6.7 μm and those produced with a viscosity of 10.4 cP presented a mean size of 9.0 μm. The formulation produced with HPMC presented a viscosity of 15.6 cP and as expected a higher average size. In accordance to the relationship between size and surface area, larger particles presented lower surface area. The surface area decreased from 86 to 49 m^{2}g ^{1} comparing the formulations without and with HPMC.

In DSC analysis, pantoprazole (Fig. 2) showed an endothermic peak at 130 °C, followed by its degradation above 170 °C. The peak at 130 °C corresponds to the melting and the dehydratation of pantoprazole, which are parallel processes (Zupancic et al. 2005). In pantoprazole DSC, the position of the melting endotherm strongly depended on the heating rate (Rosenblatt et al. 2005), even though this effect is less pronounced than with omeprazole presenting a melting range only slightly above the onset temperature for decomposition. Eudragit S100 presented an endothermic peak at 69 °C (melting) and HPMC showed an endothermic peak at 67 °C, which corresponds to the loss of adsorbed moisture or solvent from the macromolecule (Jug and Becirevic-Lacan 2004). Regarding the physical mixtures of drug and polymers the tracings showed two endothermic peaks, one correlated with the polymers (64 °C) and the other one with the drug (108 °C). On the other hand, for the microparticles only one peak at 83 °C was observed which corresponds to the melting of the blend. These results suggest that pantoprazole is molecularly dispersed in the blend. According to the literature, the disappearance of any event of the drug indicates its encapsulation (Ford and Timmins 1999).

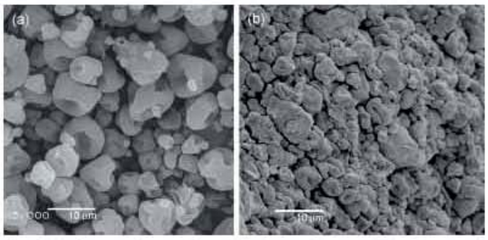


Fig. 1: SEM photomicrographs of (a) microparticles and (b) tabletted microparticles (broken section)

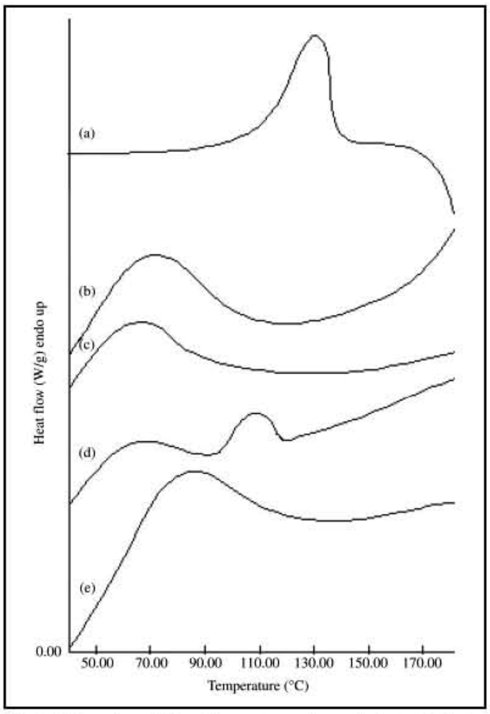


Fig. 2: DSC tracings of (a) sodium pantoprazole sesquihydrate, (b) Eudragit S100, (c) HPMC, (d) physical mixture of raw materials and (e) microparticles

Ulcers were induced by ethanol which induced large hemorragic bands that were evaluated measuring the affected area. The *in vivo* evaluation showed that ulcer index values were 0.74 ± 0.34 for the sodium bicarbonate solution, 0.46 ± 0.17 for sodium pantoprazole solution and 0.06 ± 0.07 for pantoprazole-loaded microparticles. Student-Neuman-Keuls analyses showed that the pantoprazole-loaded microparticles presented a gastric ulcer index statistically lower than the sodium bicarbonate solution ($p = 0.007$) and the sodium pantoprazole solution ($p = 0.013$). These results demonstrated that the microparticles were able to protect the stomach against ulceration induced by ethanol.

Microparticles were successfully tabletted using magnesium stearate (0.5%) as excipient and the tablets presented hardness of 34 N. Furthermore, SEM analyses showed intact microparticles inside the broken tablets (Fig. 1b).

The stability evaluation in phosphate buffer pH 7.4 showed that the pure pantoprazole, the microparticles and the tablets reached 100% of pantoprazole dissolution after 500 min. These results indicate that neither the spray-drying technique nor the medium used in the release experiments affect the stability of the drug.

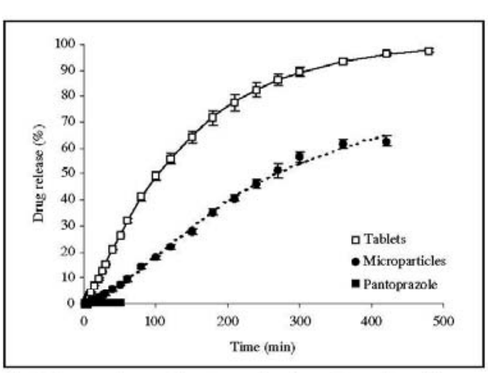


Fig. 3: Gastro-resistance of pantoprazole: drug release after acid stage (1 h). Lines show mathematical modeling for the three curves

As regards the gastro-resistance study, after the acid stage, 0.5% of pure pantoprazole remained stable, whereas the microparticles protected pantoprazole in 62.7% and tablets in 97.5% (Fig. 3).

The dissolution efficiencies were 0.40 ± 0.10% for pure pantoprazole, 43.73 ± 1.58% for microparticles and 71.41 ± 1.37% for tablets. ANOVA test indicated statistical differences ($p = 2.10^{-8}$) among the groups.

Mathematical modeling of the pantoprazole release showed that microparticle and tablet profiles fit the Weibull model (Eq. (1)).

$$C = X[1 - e^{-(t/Td)\beta}] \qquad (1)$$

where C is the percentage dissolved at time t, X is the maximum percentage dissolved after the acid stage, Td is the time at which 63.2% of the material is dissolved and β is the shape parameter. Both profiles showed sigmoidal curves ($\beta = 1.67$ and 1.14 for microparticle and tablet profiles, respectively). The parameters X and Td were 77.35% and 180.7 min for the microparticles and 100.63% and 129.9 min for the tablets, respectively. These results indicate that microparticles presented a slower release than the tablets, since it lasts 180 min to reach 49% of drug release from microparticles and 129 min to reach 64% of drug release from the tablets.

The percentage of drug dissolved is proportional to the initial concentration of the drug after the acid stage. In this sense, the tablets were able to protect the pantoprazole in a higher extension due to the smaller surface area than the microparticles. Even though the amount of drug was the same before the acid stage for each sample, after 1 h in acid medium, the drug concentration was different for pure pantoprazole, tablets and microparticles. The tablets release pantoprazole faster than the microparticles because the initial drug concentration was higher in the former than in the latter. These results are due to the lower surface area of the tablets in comparison with the microparticles, which caused a higher protection of the drug in the tablets during the acid stage.

In conclusion, pantoprazole-loaded microparticles presented acceptable drug loading, encapsulation efficiency and particle size distribution. DSC analysis showed that microparticles are formed by a blend of Eudragit S100 and HPMC, as well as that pantoprazole is molecularly dispersed in the particles. The *in vivo* anti-ulcer evaluation in rats showed that microparticles provided a significant protection of stomach against ulcer formation. Furthermore, the *in vitro* gastro-resistant study showed that the microparticles and the tablets were able to stabilize the pantoprazole and provide a controlled drug release.

3. Experimental

3.1. Materials

Pantoprazole sodium sesquihydrate was obtained from Henrifarma (São Paulo, Brazil). Eudragit S100 was kindly given by Almapal® (São Paulo, Brazil produced by Rohm®, Germany). Methocel® F4M was provided by Colorcon® (São Paulo, Brazil, produced by Dow Chemical, USA). Acetonitrile, HPLC grade, was obtained from FisherChemicals (New Jersey, USA). All other chemicals were analytical grade.

3.2. Microparticle preparation

Eudragit S100 (1.2 g) was dissolved in 0.05 M NaOH (75 mL). Subsequently, HPMC (0.6 g) was added and the mixture was magnetically stirred. The solution was kept at 10 °C for 24 h. Sodium pantoprazole sesquihydrate (0.3 g) was added in the mixture before spray drying (MSD 1.0, LabMaq, Brazil). The experimental conditions were: 0.8 mm nozzle, inlet temperature of 150 °C and flow of 0.44 Lh^{-1}.

3.3. Drug loading and encapsulation efficiency

An amount of the microparticles, equivalent to 10 mg of pantoprazole, was weighed and stirred with 40 mL of 0.05 M NaOH for 1 h. The volume was completed to 50 mL and drug concentration was determined after filtration (0.45 μm, Millipore®) by HPLC (Perkin Elmer serie 200; UV detector, $\lambda = 290$ nm, Shelton, USA), using a LiChrospher® 100 RP_{18} (5 μm) (Merck®). Mobile phase consisted of acetonitrile/phosphate buffer pH 7.4 (35 : 65 v/v) and the flow used was 1 $mLmin^{-1}$. The HPLC method for pantoprazole quantification was previously validated in terms of linearity, precision, reproducibility, accuracy and specificity. The concentration range was 0.5 to 20.0 μgmL^{-1}. Linearity was 0.999 and the detection limit was 0.55 μgmL^{-1}. The accuracy was 95.39 ± 3.77% to 6 μgmL^{-1}, 101.13 ± 1.71%, to 9 μgmL^{-1} and 101.38 ± 1.46% to 14 μgmL^{-1}. The reproducibility presented a RSD = 0.47, and the intermediate precision showed a RSD = 1.17.

3.4. Scanning electron microscopy

The shape and the surface of the microparticles were analyzed by scanning electron microscopy (SEM) (Jeol Scanning Microscope JSM-5200®, Japan). The SEM analyses were carried out using an accelerating voltage of 15 kV after they were gold sputtered (Jeol Jee 4B SVG-IN®, Peabody, USA).

3.5. Determination of surface area and pore size distribution

The nitrogen adsorption-desorption isotherms of previous degassed organic-solids, under vacuum at 40 °C were determined at liquid nitrogen boiling point in a homemade volumetric apparatus using nitrogen as probe. The pressure was measured using capilar mercury barometer and the results were compared to an alumina pattern. The specific surface areas of microparticles were determined by the BET multipoint technique (Brunauer et al. 1938) and the pore size distribution was obtained using BJH method (Barrett et al. 1951).

3.6. Determination of particle size

The particle size distribution was determined by laser diffractometry (Mastersizer 2000, Malvern Instruments, London, UK) after dispersion of powders in *iso*-octane. The mean diameter over the volume distribution $d_{4.3}$ was used. The span was calculated using the Eq. (2).

$$\text{span} = \frac{d_{(v,90)} - d_{(v,10)}}{d_{(v,50)}} \qquad (2)$$

where $d_{(v,90)}$, $d_{(v,10)}$ and $d_{(v,50)}$ are the diameters at 90%, 10% and 50% cumulative volumes, respectively. Thus, the span gives a measure of the range of the volume distribution relative to the median diameter.

3.7. Differential scanning calorimetry (DSC)

DSC was performed (DSC-4 Shimadzu, Kyoto, Japan) after sealing the samples (pantoprazole, Eudragit S100, HPMC, the physical mixture and the microparticles) in aluminum pans. Calibration was carried out using indium. DSC tracings were performed from 40 °C to 180 °C at a rate of 10 $°Cmin^{-1}$.

3.8. In vitro gastro-resistance evaluation

The gastro-resistance study (37 °C) was performed in flow-through cell apparatus at 37 °C using a peristaltic pump (Desaga, Heidelberg, Germany). The samples were collected at predetermined time intervals and

Table: Groups of rats (control 1, control 2 and treatment) for the *in vivo* anti-ulcer activity test

Groups	Administered samples
Control 1	4.2% sodium bicarbonate solution
Control 2	Pantoprazole dissolved in water (2 $\mathrm{mgmL^{-1}}$)
Treatment	Microparticles dispersed in water (mass equivalent to 2 $\mathrm{mgmL^{-1}}$ of pantoprazole)

analyzed spectrophotometrically at 295 nm (Unicam 8625 UV/Vis spectrometer, Cambridge, England). The methodology for UV quantification was validated in terms of linearity, precision, reproducibility, accuracy and specificity. The concentration range was 4.0 to 30.0 $\mu\mathrm{gmL^{-1}}$. Linearity was 0.9999. Accuracy was $102 \pm 2.09\%$, $97.24 \pm 1.61\%$ and $100.37 \pm 2.56\%$, for the concentrations of 8, 11 and 17 $\mu\mathrm{gmL^{-1}}$, respectively. The reproducibility presented RSD of 0.68, and the intermediate precision showed RSD of 0.36. The samples were placed in the cells and treated with 0.1 M HCl (1 $\mathrm{mLmin^{-1}}$) (acid stage). Then, after 1 h, the medium was replaced by phosphate buffer pH 7.4 and samples were collected at predetermined time intervals and analyzed. The profiles were analyzed by model-dependent methods (monoexponential, biexponential, power law and Weibull) and by a model-independent method (dissolution efficiency) (Costa and Lobo 2001; Beck et al. 2005). The selection of the model-dependent was based on the best correlation coefficient, the best model selection criteria (MSC), provided by Scientist® software, and the best graphic adjustment.

In order to verify the stability of pantoprazole in phosphate buffer pH 7.4, a dissolution experiment was conducted.

3.9. *In vivo anti-ulcer activity*

Ulcers were induced by the oral administration of absolute ethanol (5 $\mathrm{mLkg^{-1}}$) to 24 h fasted Wistar male rats ($n = 8$), weighing 200 g (Gombosova et al. 1993; Shah et al. 2003). The groups are described in the Table. Formulations (20 $\mathrm{mgkg^{-1}}$ of drug) were administered orally 1 h before the administration of ethanol. Two hours after ethanol administration, the animals were sacrificed; the stomachs were removed, opened along the greater curvature and examined for lesion measurements.

Ulcer indexes (UI) were calculated using the Eq. (3).

$$\mathrm{UI} = \frac{10}{x} \tag{3}$$

where x is the total mucosal area divided by the total ulcerated area.

This protocol was approved by the Ethical Committee (deliberation number 2003247, Universidade Federal do Rio Grande do Sul, Brazil).

3.1. *Preparation and characterization of tablets*

Microparticles were tabletted with magnesium stearate (0.5%). Tablets (theoretically containing 40 mg of drug) were prepared in a double punch tablet machine (Korch EK0, Berlin, Germany) by individual weighing and direct compression using 8.0 mm punches. In order to evaluate the integrity of the microparticles after the compression, one tablet was fractured and the inner face was analyzed by SEM.

For drug loading determination, tablets were milled by a mortar and pestle. An amount of sample equivalent to 10 mg of pantoprazole was diluted with 0.05 M NaOH (40 mL) and magnetically stirred. After 12 h, the volume was completed to 50 mL and aliquots were analyzed by HPLC as described above for the microparticles. The dissolution profile was determined using a flow through cell apparatus as described above for microparticles.

Acknowledgement: The authors thank CAPES, CNPq, Rede Nanocosmeticos/CNPq and FAPERGS for the financial support.

References

Alvarez-Fuentes J, Fernandez-Arevalo M, Gonzalez-Rodriguez ML, Cirri M, Mura P (2004) Development of enteric-coated timed-release matrix tablets for colon targeting. J Drug Target 12: 607–612.

Barrett EP, Joyner LG, Halenda PP (1951) The determination of pore volume and area distributions in porous substances. I. computations from nitrogen isotherms. J Am Chem Soc. 73: 373–380.

Beck RCR, Pohlmann AR, Benvenutti EV, Dalla Costa T, Guterres SS (2005) Nanostructure-coated diclofenac-loaded microparticles: preparation, morphological characterization, *in vitro* release and *in vivo* gastrointestinal tolerance. J Braz Chem Soc 16: 1233–1240.

Beckert T, Lehmann K, Schmidt P (1996) Compression of enteric-coated pellets to disintegrating tablets. Int J Pharm 143: 13–23.

Brunauer S, Emmet PH, Teller E (1938) Adsorption of gases in multimolecular layers. J Am Chem Soc 60: 309–319.

Cheer S, Prakash A, Faulds D, Lamb H (2003) Pantoprazole – an update of its pharmacological properties and therapeutic use in the management of acid-related disorders. Drugs 63: 101–132.

Costa P, Lobo JMS (2001) Modeling and comparison of dissolution profiles. Eur J Pharm Sci 13: 123–133.

Ford JL, Timmins P (1999) Drug delivery systems. in: pharmaceutical thermal analysis-techniques and applications, New York, Ellis Horwood Limited, p. 190–200.

Foster TP, Laetherman MW (1995) Powder characteristics of proteins spray-dried from different spray-dryers. Drug Dev Ind Pharm 21: 1705–1723.

Gambosova I, Valentova J, Tumova I, Svec P (1993) The influence of carbamate local-anesthetics on the occurrence of experimental gastric-ulcers. Pharmazie 48: 212–215.

Jug M, Becirevic-Lacan M (2004) Multi component complexes of piroxicam with cyclodextrins and hydroxypropyl methylcellulose. Drug Dev Ind Pharm 30: 1051–1060.

Li CL, Martini LG, Ford JL, Roberts M (2005) The use of hypromellose in oral drug delivery. J Pharm Pharmacol 57: 533–546.

Lin SY, Kao YH (1991) Tablet study of spray-dried sodium diclofenac enteric-coated microcapsules. Pharm Res 8: 919–924.

Raffin RP, Colomé LM, Pohlmann AR, Guterres SS (2006) Preparation, characterization, and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles. Eur J Pharm Biopharm 63: 198–204.

Raffin RP, Guterres SS, Pohlmann, AR, Ré MI (2006) Powder characteristics of pantoprazole delivery systems produced in different spray-dryer scales. Drying Tech 24: 339–348.

Riedel A, Leopold CS (2005) Quantification of omeprazole degradation by enteric coating polymers: an UV-VIS spectroscopy study. Pharmazie 60: 126–130.

Rosenblatt KM, Bunjes H, Seeling A, Oelschlager H (2005) Investigations on the thermal behavior of omeprazole and other sulfoxides. Pharmazie 60: 503–507.

Sachs G, Shin JM, Pratha V, Hogan D (2003) Synthesis or rupture: duration of acid inhibition by proton pump inhibitors. Drugs Today 39(Suppl. A): 11–14.

Shah PJ, Gandhi MS, Shah MB, Goswami SS, Santani D (2003) Study of Mimusops elengi bark in experimental gastric ulcers. J Ethnopharmacol 89: 305–311.

Vega-Gonzalez A, Domingo C, Elvira C, Subra P (2004) Precipitation of PMMA/PCL blends using supercritical carbon dioxide. J Appl Pol Sci 91: 2422–2426.

Zupancic V, Ograjsek N, Kotar-Jordan B, Vrecer F (2005) Physical characterization of pantoprazole sodium hydrates. Int J Pharm 291: 59–68.

CAPÍTULO 9: Preparação de micropartículas preparadas com blenda de Eudragit® S100 e Methocel® F4M em *spray drier* piloto e validação do processo de produção

9.1 Apresentação

Em um processo de secagem por aspersão, tanto a formulação quanto as condições operacionais dos *spray-driers* apresentam efeito significativo nas características finais das micropartículas. A capacidade do equipamento e seu modo operacional produzem partículas de diferentes tamanhos, permitindo otimizar perfis de liberação e características tecnológicas dos pós.

As micropartículas preparadas com a blenda de Eudragit® S100 e HPMC foram preparadas em escala laboratorial e piloto e as condições operacionais foram estudadas. Os produtos obtidos foram caracterizados através de análise morfológica, fluidez e gastro-resistência. A formulação e as condições operacionais que geraram micropartículas com as melhores características foram escolhidas para testar a repetibilidade do processo produtivo.

Este trabalho foi parcialmente realizado no Instituto de Pesquisas Tecnológicas do Estado de São Paulo, sob supervisão da Dra. Maria Inês Ré. Os resultados foram publicados no periódico *International Journal of Pharmaceutics*.

Publicação 09: Sodium Pantoprazole-Loaded Enteric Microparticles Prepared by Spray Drying: Effect of the Scale of Production and Process Validation

Available online at www.sciencedirect.com
ScienceDirect

International Journal of Pharmaceutics 324 (2006) 10–18

INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Sodium pantoprazole-loaded enteric microparticles prepared by spray drying: Effect of the scale of production and process validation

Renata P. Raffin[a,*], Denise S. Jornada[a], Maria Inês Ré[b], Adriana R. Pohlmann[c], Silvia S. Guterres[a]

[a] *Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, 90610-000 Porto Alegre, Brazil*
[b] *Centro de Tecnologia de Processos e Produtos, Instituto de Pesquisas Tecnológicas, Av. Almeida Prado 532, 05508-901 São Paulo, Brazil*
[c] *Instituto de Química, Universidade Federal do Rio Grande do Sul, CP 15003, 91501-970 Porto Alegre, Brazil*

Received 16 February 2006; received in revised form 29 June 2006; accepted 29 June 2006
Available online 5 July 2006

Abstract

Pantoprazole is a prodrug used in the treatment of acid related disorders and *Helicobacter pylori* infections. It is activated inside gastric parietal cells binding irreversibly to the H^+/K^+-ATPase. In this way, pantoprazole must be absorbed intact in the intestinal tract, which indicates that enteric drug delivery systems are required for its oral administration. The purpose of this study was to investigate the physical characteristics of enteric pantoprazole-loaded microparticles prepared by spray drying using a blend of Eudragit S100® and HPMC. The microparticles were produced in different spray dryers and operational conditions at laboratory and pilot scales. Microparticles produced with two fluid nozzle atomizer and air pressure of 196 kPa presented satisfactory encapsulation efficiency and gastro-resistance. Microparticles produced with the same atomizer but using 49 kPa of air pressure presented strings in the powder. The microparticles produced in mixed flow presented very high polydispersity and the ones produced with rotating disc atomizer presented drug crystals adsorbed on the particle surfaces. The microparticles produced with two fluid nozzle atomizer and 196 kPa were prepared in three consecutive days for the process validation. The powders showed reproducible diameter, polydispersity, densities, encapsulation efficiency and gastro-resistance profile.

Keywords: Pantoprazole; Spray drying; Microparticles; Scale up; Gastro-resistance

1. Introduction

A spray dryer converts a liquid feed into solid particles with specific characteristics modeled by the equipment design, the operating conditions and the process variables (Gibson, 2001). The droplet size formed by the atomizer is directly proportional to the final particle size (Gibson, 2001). In addition, the droplet size on atomization depends upon the mode of atomization, the physical properties of the feed and the feed solid concentration (Goula and Adamopoulos, 2004). The two most common atomizers used in the pharmaceutical field are the two fluid nozzle and the rotary atomizer (Gibson, 2001). Concerning the pressure nozzle, the orifice size is chosen to control the particle size. The increase in atomizer pressure produces finer particles. On the other hand, in the rotary atomizer, the speed of the wheel controls the particle size, which reduces with the increase of the speed.

The exact characteristics expected for the final product are the first step to optimize the spray drying process. Based on these characteristics, the design and the operation variables will change. The characteristics include mainly moisture content, particle size and polydispersity, bulk and tapped densities and cohesion (Birchal et al., 2005).

Spray drying has gained more importance as a method of microencapsulation. This method has already been used to prepare microparticles with polyesters, polymethacrylates, cellulose derivatives and biopolymers containing both hydrophilic and lipophilic drugs and macromolecules. The major advantages over solvent evaporation techniques are the one-step process, the easiness to control and scale up, and the possibility of being free of organic solvent (Giunchedi et al., 2001).

The major difficulties in scaling up the spray drying process include the thermal exchange and losses, variable yields and, mostly, geometries of atomizers or turbines, drying chambers

---

[*] Corresponding author. Tel.: +55 51 33165500; fax: +55 51 33165437.
E-mail address: reraffin@farmacia.ufrgs.br (R.P. Raffin).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.06.045

and cyclones. Several reports in the literature are based on laboratory scale production (Benoit et al., 1996; Palmieri et al., 2002; Oster and Kissel, 2005). The effects of process variables are difficult to assess in general terms, due to the lack of information in the literature and to the specific drying nature of most materials (Goula and Adamopoulos, 2004).

Spray dried particle distribution varies depending on the nozzle geometry, the feeding rate and the operating conditions. Although these parameters are kept very similar, they are never identical and changes are observed among different equipments (Foster and Laetherman, 1995).

The encapsulation of hydrophilic acid labile drugs by spray drying has the advantage of no necessity of organic solvents. In this case, enteric polymers can be used to prepare microparticles because they are soluble in pH higher than 6 or 7 and can be dissolved in alkaline solutions (Palmieri et al., 2002). The resulting microspheres should be able to protect acid labile drugs from gastric juice.

Sodium pantoprazole is a prodrug that inhibits the proton pump and, consequently, the acid release in gastric lumen. This prodrug is used in the treatment of digestive ulcers, gastro-oesophageal reflux disease and as auxiliary in the eradication of the *Helicobacter pylori* (Cheer et al., 2003). This prodrug reacts in acid medium. When this conversion occurs in the parietal cell canalicular lumen, it is activated by conversion to a cyclic sulfonamide, which is the active form (Cheer et al., 2003; Sachs et al., 2003). The active form, the tetracyclic cationic sulfenamide, reacts with the thiol groups of cysteines 813 and 822 of the transmembranal H^+/K^+-ATPase (Shin et al., 1993; Avner, 2000). This conversion must occur inside the gastric parietals cells, so pantoprazole should be absorbed intact by the intestinal tract, needing an enteric drug delivery system to be administered. When the pantoprazole reacts with acid in the stomach lumen before absorption, the substance is degraded and no activity is observed (Cheer et al., 2003).

Pantoprazole-loaded microparticles have been prepared using a blend of Eudragit® S100 and Methocel® F4M in laboratory scale. In vivo anti-ulcer activity evaluation has demonstrated that the microparticles were able to protect rats against ulcers induced by ethanol, while the pantoprazole aqueous solution did not present activity (data not shown).

Taking all of these into account, the aim of this work was to produce enteric microparticles containing sodium pantoprazole by spray drying in both laboratory and pilot scales and to study and validate the production process in pilot scale. The microparticles have been characterized in terms of their morphology, flowability, encapsulation efficiency and ability of stabilizing pantoprazole in acid medium.

2. Materials

Sodium pantoprazole sesquihydrate has been obtained from Henrifarma (São Paulo, Brazil). Eudragit® S100 has been kindly provided by Almapal® (São Paulo, Brazil, produced by Rohm®, Germany). Methocel® F4M was provided by Colorcon® (São Paulo, Brazil, produced by Dow Chemical, USA). All other chemicals are analytical grade.

3. Methods

3.1. Preparation in laboratory scale and characterization of microparticles

For the laboratory production scale, three batches were prepared increasing total solids concentration in the solution feed. Eudragit® S100 and NaOH were added to 100 mL of water, in the following amounts: 1.2 and 0.2, 1.5 and 0.25, and 1.8 and 0.3 g, respectively. After the solubilization, Methocel® F4M was added (0.60, 0.75 and 0.90 g) and the solution was kept at 10 °C for 24 h. Sodium pantoprazole (0.30, 0.38 and 0.45 g) was added in the solution for spray drying. The formulations were named L1, L2 and L3, respectively.

The viscosity at 25 °C of the three solutions was measured using a Brooksfield Digital Viscosimeter (model DV-II), using spindle 01. The solutions were atomized into a laboratory spray drier (Model 190 Büchi®). The equipment is equipped with a two fluid pressurized nozzle with diameter $d_0 = 0.7$ mm. Experiments were carried out under the following conditions: inlet air temperature 150 ± 5 °C, outlet air temperature 98 ± 3 °C, aspirator setting: 10, suspension feed flow rate: 0.24 L/h, airflow rate: 500 N L/h. In this small-scale equipment, droplets flow co-currently with airflow.

Shape and surface were analyzed by scanning electron microscopy (JEOL JSM5200®) after gold sputtering using accelerating voltage of 15 kV. The particle size distribution was determined by laser diffractometry Beckman Coulter® LS 13 320 (Beckman Instruments) by dry dispersion. Average particle size was expressed as the mean volume diameter ($D_{4,3}$). Polydispersity was given by a span index, which was calculated by $(D_{0.9} - D_{0.1})/D_{0.5}$, where $D_{0.9}$, $D_{0.5}$ and $D_{0.1}$ are the particle diameters determined respectively at the 90th, 50th and 10th percentile of the undersized particle distribution curve.

The specific surface areas of microparticles were determined by the BET multipoint technique (Brunauer et al., 1938). The nitrogen adsorption–desorption isotherms of previous degassed organic-solids, under vacuum at 40 °C, were determined at liquid nitrogen boiling point in a homemade volumetric apparatus, using nitrogen as probe. The pressure was measured using capilar mercury barometer and the results were compared to alumina pattern.

To determine the drug content in the microparticles, an amount equivalent to 10 mg of pantoprazole in the microparticles was weighed and dissolved in 50 mL of 0.05 M NaOH. Drug concentration was determined in each sample after filtration (0.45 μm, Millipore®) by HPLC (Perkin-Elmer series 200; UV detector, λ = 290 nm, Shelton, USA), using a Merck® Lichrosphere® column C_{18} as stationary phase. Mobile phase consisted of acetonitrile/phosphate buffer pH 7.4 (35:65 v/v). This method was validated for specificity, linearity, accuracy, precision and detection and quantitation limit according to ICH (1996).

Size 0 hard gelatin capsules without coloring agent were filled with 90 mg of microparticles, corresponding to 16 mg of drug. Dissolution tests were conducted in USP dissolution apparatus I at 50 rpm and 37 °C. In order to determine if the microparticles were able to release 100% of the drug encapsulated, the dissolution was evaluated in phosphate buffer pH 7.4 for 480 min.

To evaluate gastro-resistance, capsules containing pantoprazole were exposed to 300 mL of 0.1 M HCl. After 1 h, a NaOH (2.6 g) and KH_2PO_4 (6.12 g) aqueous solution (600 mL) was added into the medium in order to reach pH 7.4. The samples were collected in pre-determined time intervals from 0 up to 480 min. Pantoprazole concentrations were determined by UV at 295 nm (Unicam 8625 UV/VIS spectrometer). The analytical method was validated for linearity, precision, specificity and quantitation limit according to ICH (1996).

3.2. Preparation in pilot scale and characterization of microparticles

Microparticles presenting higher amount of initial solids concentration was chosen to conduct the study in pilot scale. The pilot spray drier (Model S52 APV® Anhydro) was used with three sets of atomizers. The first set used a rotating disc under the following operating conditions: co-current flow dryer; rotational velocity of atomizer 30,000 rpm; suspension flow rate 2 L/h; inlet and outlet air temperatures 170 ± 1 and 85 ± 5 °C, respectively. Two other sets of experiments were carried out using a two fluid pneumatic atomizer with external mixing. In this nozzle, the liquid to be atomized is discharged through a central hole of diameter $d_0 = 1.5$ mm, whereas the atomizing air is injected through a ring area around the liquid hole. The atomizing air pressure varied from 49 to 196 kPa to generate droplets with different sizes (Ré et al., 2004). In one set of experiments, droplets flow in co-current with the drying air (co-current flow dryer); in the other set, droplets flow in counter-current in relation to the drying air inlet (mixed flow dryer, where the feed is sprayed upwards and the particles formed inside the dryer finish their journey in a co-current mode). Fig. 1 shows diagrams of both co-current and mixed flow apparatus used. During all the processes the room temperature and humidity were controlled in 24 ± 1 °C and 54 ± 2% of relative humidity. The dried formulation was prepared by solubilizing 36 g of Eudragit® S100, 18 g of Methocel® F4M, 9 g of pantoprazole in a solution prepared with 6 g of NaOH and 2000 mL of water. Microparticles were produced in duplicate.

Humidity was assayed gravimetrically in Mettler Toledo® HB 43 Halogen kept at 105 °C until constant weight. Microparticles were characterized by SEM, particle size distribution, specific surface area, porosity, dissolution profiles in phosphate buffer and gastro-resistance as described above for laboratory scale production. The gastro-resistant profiles were compared by f_1/f_2 method.

Rheological characteristics of the powders were also determined. Bulk and tapped densities of the spray-dried microparticles were determined using an automatic taper (AutoTap, Quantachrome® Corp.). The tapped density was measured after 1250 taps, because preliminary investigations have shown (data not shown) that the volumetric change after this number of taps was negligible. An average of three determinations was taken. From these measurements, the Carr index was determined. Note that the Carr index is defined as the difference between the tapped and the bulk density divided by the tapped density, expressed in percentage (Carr, 1965). The angle of repose was measured in Powder Characteristics Tester, Model PT-N (Hosokawa Microns®). The angle of repose is the angle between the horizontal and slope of the heap. This angle is a direct indication of the potential flowability of a powder (contact and friction between particles in motion).

3.3. Pilot scale process evaluation

The following conditions were chosen to produce microparticles: two fluid atomizer, co-current air spray contact and air pressure of 196 kPa. Three batches of 11 L were dried in three

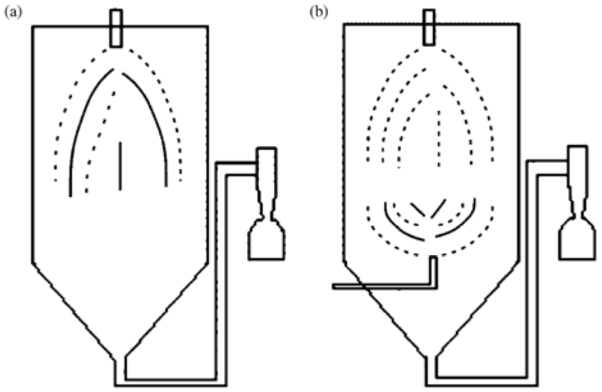


Fig. 1. Diagrams of the two types of air/spray contact in the pilot spray drier. Co-current contact used with rotating and two fluid nozzle atomizers (a) and mixed flow used with two fluid nozzle atomizer (b). Full lines indicate liquid feed and dashed lines indicate air feed.

consecutive days and the microparticles were separately analyzed. To confirm the stability of pantoprazole in the solution of the polymers (Eudragit® S100 and Methocel® F4M), samples of the solution feed were kept at room temperature in absence of light for 24 h. Samples were analyzed every 2 h by HPLC. The process yield was calculated by the obtained mass divided by the total solid raw materials in solution multiplied by 100. Microparticles were characterized by SEM, particles size distribution, humidity, surface area, flowability, drug content and gastro-resistance profiles. The true density of all samples was measured by Helium Picnometry using a Multi Pycnometer (Quanta Chrome®) at room temperature. DSC was performed in Mettler Toledo 822^{e} from −70 to 250 °C at 10 °C/min and N_2 flow rate of 30.0 mL/min. For the thermal analysis, Eudragit® S100 (3.6 g) and Methocel® F4M (1.8 g) were solubilized in NaOH solution (0.3 g in 100 mL) and spray dried in mini spray drier Buchi in the same conditions described above for the laboratory scale microparticles. The statistical test of ANOVA was used to compare the values obtained from each characterization.

4. Results

4.1. Preparation in laboratory scale and characterization of microparticles

The control of viscosity of liquid feed allows the liquid to be converted into droplets. The maximum recommended viscosity of the solutions that can be spray dried is 250 cP (Gibson, 2001). Even though all solutions presented values lower than the limit, an increasing of viscosity was observed with the increase of total solid concentration. The viscosity values of L1 (2.3%), L2 (2.9%) and L3 (3.4%) were 15.6, 28.7 and 58.5 cP, respectively.

In this way, the three solutions were able to be spray dried, and in all cases off-white powders were obtained. All three powders (L1, L2 and L3) were analyzed by SEM and the microparticles presented surface shriveling and folding (Fig. 2). This morphology is formed by uneven shrinkage forces during the drying of droplets, depending on the viscosity of the liquid feed. The tendency to shrive or fold increases with the increase of feed viscosity (Foster and Laetherman, 1995).

The particle size distribution showed differences among the three microparticles (7.50, 8.45 and 8.78 μm, corresponding to L1, L2 and L3, respectively). In general, particle size is assumed to increase with an augmentation in feed concentration, while an increase in feed solid concentration may cause a reduction of particle density presumably due to rapid crust formation, which hinders water reaching the surface, thus building up internal pressures (Goula and Adamopoulos, 2004).

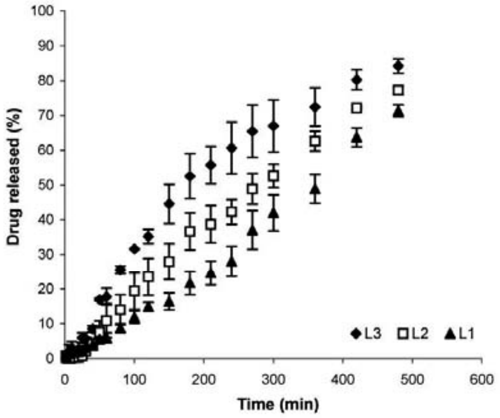


Fig. 3. Gastro-resistance profiles of the microparticles produced in laboratory scale: L1 (2.3%), L2 (2.9%) and L3 (3.4%).

The specific surface area showed similar values for the three formulations L1, L2 and L3 (70, 65 and 66 m^2/g, respectively). Although the mean diameter had increased with the increase of liquid viscosity, this variation was not observed so clearly in the surface area values, due to the precision of the measure that is 10 m^2/g.

The drug was totally released (100%) at pH 7.4 in 480 min from the three microparticle formulations. On the other hand, after the acid stage followed by release at pH 7.4 (gastro-resistance profiles), different amounts of pantoprazole were stable after 480 min. The L3 produced with the highest solid concentration solution, showed the highest protection of pantoprazole from acid medium (Fig. 3).

Considering these results, the L3 formulation was chosen to be spray dried in pilot scale due to the higher solid concentra-

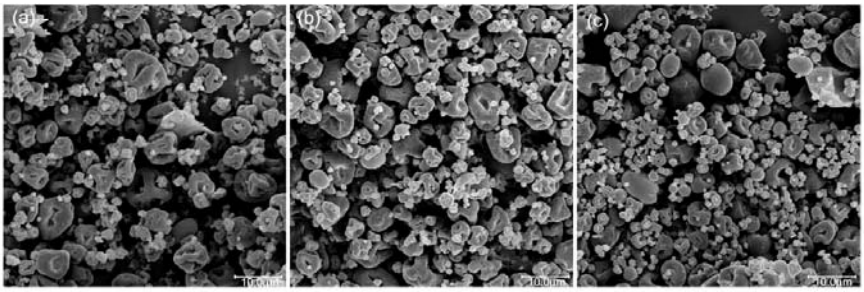


Fig. 2. SEM images of microparticles produced in laboratory scale: (a) L1 (2.3%), (b) L2 (2.9%) and (c) L3 (3.4%).

tion in the liquid feed (3.4%) and the highest stabilization of pantoprazole after acid stage.

4.2. Preparation in pilot scale and characterization of microparticles

Four different sets of atomizer, air pressure and air spray contact were tested: (i) rotating disc atomizer (RO), (ii) two fluid nozzle atomizer using air pressure of 49 kPa (N1), (iii) 196 kPa (N2) and (iv) two fluid nozzle atomizer and 196 kPa of air pressure in mixed flow (MF). Table 1 summarizes the characteristics of all four powders.

The RO-microparticles presented mean size of 38.7 μm, polydispersity (span) of 2.4 and surface area of 70 m^2/g. SEM image showed spherical and irregular particles (Fig. 4a), as well as in few particles blowholes could be observed. Furthermore, drug crystals, identified by the presence of sulfur (EDS), were visualized on the particle surface suggesting an incomplete encapsulation of pantoprazole.

The microparticles produced using two fluid nozzle atomizer (N1-microparticles and N2-microparticles) presented smaller average particle size and higher surface area than RO-microparticles (Table 1). N1-microparticles presented mean diameter of 25.7 μm, span of 2.3 and surface area of 93 m^2/g, whereas N2-microparticles presented average size of 30.8 μm, span of 3.0 and surface area of 96 m^2/g. When analyzed by SEM, N1-microparticles were formed by microparticles and strings or threads like cotton candy (Fig. 4b). Similar results have been obtained by Clarke et al. (1998) preparing microparticles by spray drying, which presented a mixture of concave microparticles and fibrous powder. The authors attributed the formation of the filaments to an insufficient force to enable the liquid filament to be broken into droplets. The formation of these filaments is influenced by the air pressure, geometry of the atomizer and the flow rate (Benoit et al., 1996). In addition, N2-microparticles presented mainly spherical particles with smooth surface and few cases of blowholes and shriveling (Fig. 4c). The reduced particle size of N2-microparticles (30.8 μm compared to 38.7 μm of RO-microparticles) led to an increase of the specific surface area of the N2-microparticles.

On the other hand, MF-microparticles prepared using the air pressure in mixed flow presented average diameter of 137.1 μm, span of 4.2 and surface area of 57 m^2/g, indicating a very high polydispersity compared to the other three powders and a reduced surface area. These results are in accordance with the photomicrography (Fig. 4d) in which very different sizes of microparticles have been observed, including particles presenting less than 10 μm and over 90 μm of diameter in great quantity. The increase in the average particle size between laboratory (8 μm) and pilot scales (30 μm) has been also described by Foster and Laetherman (1995) showing approximately a two-fold to a three-fold increase in particle size.

Bulk and tapped densities (Table 1) were similar for RO-microparticles and N1-microparticles. These formulations presented different densities when compared to N2-microparticles or MF-microparticles. The higher density of RO-microparticles can be attributed to the presence of crystals on the parti-

Table 1
Characteristics of the powders produced in pilot scale in different sets of atomizers and air pressure

Atomizer	Air pressure (kPa)	Particle size (μm)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr index	Humidity (%)	Surface area (m^2/g)	Drug content (%)
Rotating disc	–(RO)	$D_{4,3}$ 38.7, span 2.4, S.D. 0.52	0.115 ± 0.002	0.221 ± 0.005	48	4.00 ± 0.24	70.58	13.14 ± 0.23
Two fluid nozzle and co-current flow	49 (N1)	$D_{4,3}$ 25.7, span 2.3, S.D. 0.13	0.122 ± 0.002	0.255 ± 0.006	48	4.02 ± 0.27	93.13	11.49 ± 0.81
	196 (N2)	$D_{4,3}$ 30.8, span 3.0, S.D. 1.68	0.064 ± 0.002	0.125 ± 0.003	48	3.37 ± 0.12	95.83	12.81 ± 0.05
Two fluid nozzle and mixed flow	196 (MF)	$D_{4,3}$ 137.1, span 4.2, S.D. 23.1	0.060 ± 0.000	0.098 ± 0.001	39	2.84 ± 0.09	57.04	12.48 ± 0.28

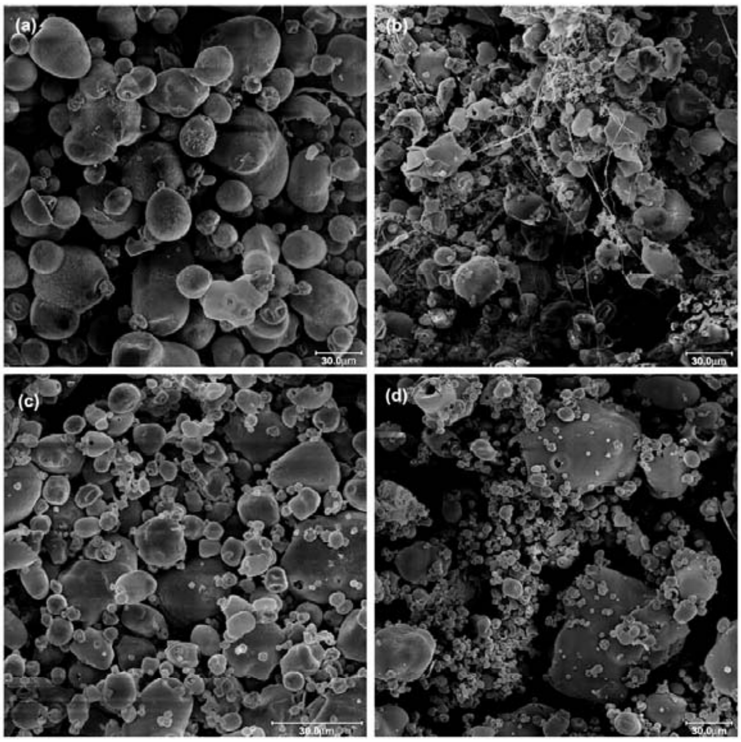


Fig. 4. Photomicrographies of the powders produced in pilot scale: (a) rotating disc atomizer (RO-microparticles), (b) two fluid nozzle atomizer and air pressure of 49 kPa (N1-microparticles), (c) two fluid nozzle atomizer and air pressure of 196 kPa (N2-microparticles) and (d) two fluid nozzle atomizer in mixed flow (MF-microparticles).

cle surface or to the large microparticles shell, since hollow microparticles were obtained (Fig. 4a). N1-microparticles presented higher bulk density (0.122 g/cm^3) than N2-microparticles (0.064 g/cm^3) or MF-microparticles (0.060 g/cm^3) probably due to the presence of the threads. The bulk densities of N2-microparticles and MF-microparticles are in accordance with previous reports for casein and NaCMC microparticles produced by spray drying containing teophylline (Foster and Laetherman, 1995; Wan et al., 1992). Tapped densities were higher for RO-microparticles (0.221 g/cm^3) and for N1-microparticles (0.255 g/cm^3) than for N2-microparticles (0.125 g/cm^3) and MF-microparticles (0.098 g/cm^3). Carr indexes (Table 1) indicated that all powders presented very poor flow (Carr, 1965).

All microparticles (RO, N1, N2 and MF) presented humidity below 4% showing the effectiveness of the drying process. The drug contents were 11.49 ± 0.81% (N1-microparticles), 12.48 ± 0.28% (MF-microparticles), 12.81 ± 0.05% (N2-microparticles) and 13.14 ± 0.23% (RO-microparticles), corresponding to the encapsulation efficiencies of 88.1%, 95.7%, 98.2% and 100.1%, respectively. However, for RO-microparticles the encapsulation was not complete according to the SEM analysis that showed unencapsulated crystals (Fig. 4a). In the case of N1-microparticles, the low value of encapsulation efficiency was probably due to the lost of drug and the formation of strings during the drying process.

Concerning the drug release in phosphate buffer at pH 7.4, all powders presented a complete release (100%) after 480 min. On the other hand, in the gastro-resistance evaluation (Fig. 5), the formulations presented different profiles. Comparing these profiles using f_1/f_2 method, N2-microparticles and MF-microparticles were similar. Both stabilized 94% of the initial pantoprazole content and presented dissolution efficiency of 64.9% and 63.2%, respectively. N1-microparticles presented faster release attributed to the presence of strings (dissolution efficiency of 72.9%) and RO-microparticles presented 90% of pantoprazole stabilization and dissolution efficiency of 60.5%.

Taking into account the characteristics of microparticles concerning the encapsulation efficiency, the average particle size, the morphology and the gastro-resistance, the N2-microparticles, produced with two fluid nozzle atomizer and air pressure of 196 kPa, were chosen in order to validate the process.

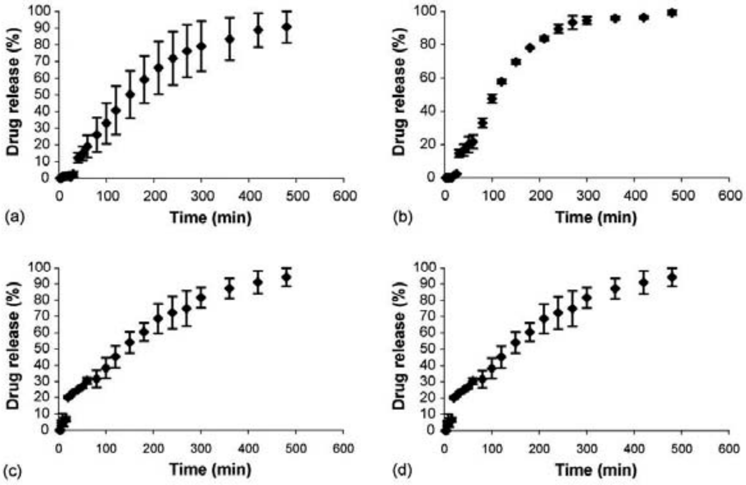


Fig. 5. Gastro-resistance profiles of microparticles produced in different sets of atomizers/pressure: (a) rotating disc atomizer (RO-microparticles), (b) two fluid nozzle atomizer and air pressure of 49 kPa (N1-microparticles), (c) two fluid nozzle atomizer and air pressure of 196 kPa (N2-microparticles) and (d) two fluid nozzle atomizer in mixed flow (MF-microparticles).

4.3. Pilot scale process evaluation

In order to evaluate pantoprazole stability during the preparation of microparticles, a stability study was conducted. The stability of pantoprazole dissolved in the solution of the polymers was evaluated before spray drying at room temperature and in the absence of light for 24 h. Every 2 h a sample was collected and no decrease in the pantoprazole concentration was observed by HPLC.

Three different batches of 11 L were spray dried in three consecutive days keeping constant room temperature and humidity (24 °C and 54%, respectively). The yields were 61.4%, 63.7% and 56.3% in the three consecutive days, respectively. The three powders were analyzed by SEM (Fig. 6) and no difference in the shape of microparticles has been detected among the batches.

Microparticles presented real density of 1.37, 1.36 and 1.38 g/cm^3 (Table 2) from batches 1, 2, and 3, respectively. Bulk density values were 0.061, 0.064 and 0.073 g/cm^3 and tapped density values were 0.108, 0.110 and 0.148 g/cm^3, corresponding to the three batches. Real, bulk and tapped densities were not significantly different among batches ($p = 0.39$, $p = 0.06$ and $p = 0.07$, respectively). The angles of repose were similar among samples ($p = 0.48$) and confirmed the poor flow of the powders (over 40°) (Carr, 1965). The encapsulation efficiencies were 98.9%, 99.5% and 100.6% for the three batches, respectively. The powders presented specific surface areas around 100 m^2/g. The particle size distributions of the three powders were very close showing reproducibility in the mean size (22 μm).

DSC analysis of unloaded microparticles (produced without the drug) showed one endothermic peak at 87 °C (Fig. 7). No event was observed for Methocel® F4M at the temperatures investigated. DSC analysis of sodium pantoprazole sesquihydrate showed an endothermic peak at 156 °C, and an exothermic peak at 198 °C (degradation) (Fig. 7). According to the literature, pantoprazole melting and dehydration are parallel processes in the case of sesquihydrate form (Zupancic et al., 2005).

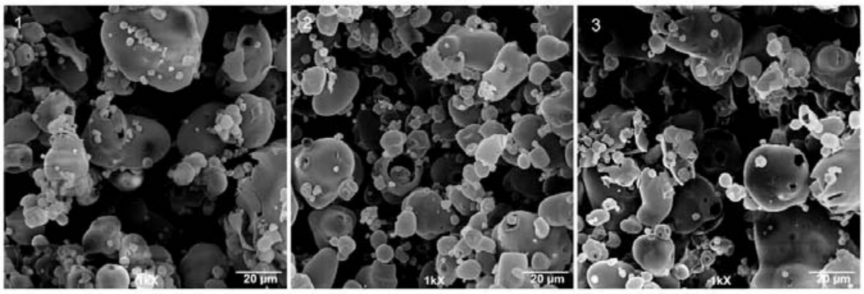


Fig. 6. SEM images of the microparticles produced in pilot scale in three different days showing the similarity among the batches.

Table 2
Characteristics of the three batches of microparticles prepared in pilot scale

Batch	Particle size (μm)	Real density (g/cm^3)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr index	Angle of repose (°)	Humidity (%)	Surface area (m^2/g)	Drug content (%)
1	$D_{4,3}$ 21.73, span 2.14, S.D. 1.49	1.37 ± 0.02	0.061 ± 0.003	0.108 ± 0.016	44	43.2 ± 2.3	2.11 ± 0.03	93.35	12.90 ± 0.08
2	$D_{4,3}$ 22.36, span 2.00, S.D. 1.42	1.36 ± 0.02	0.064 ± 0.002	0.110 ± 0.000	42	40.9 ± 2.1	2.26 ± 0.16	100.53	12.97 ± 0.97
3	$D_{4,3}$ 22.86, span 2.11, S.D. 1.82	1.38 ± 0.02	0.073 ± 0.003	0.148 ± 0.005	51	42.2 ± 2.1	3.6 ± 0.14	96.78	13.13 ± 0.43

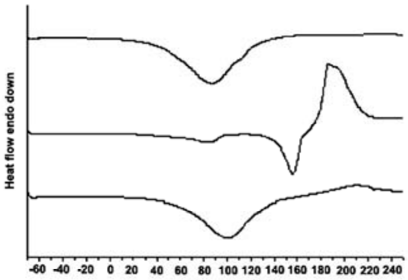


Fig. 7. Thermograms of (up to down): microparticles prepared without drug (spray dried Methocel® F4M and Eudragit® S100), sodium pantoprazole sesquihydrate and batch 2 of pantoprazole-loaded microparticles.

The exothermic event is the degradation of the drug. In the pantoprazole-loaded microparticle thermogram, one endothermic event appears at 100 °C, which corresponds to the melting of the polymer blend. The results suggest that pantoprazole-loaded microparticles are composed by a homogeneous phase, in which the drug is molecularly dispersed in the blend. According to the literature, the disappearance of any event of the drug indicates its encapsulation (Ford and Timmins, 1999).

The three batches presented complete release of pantoprazole after 480 min in phosphate buffer at pH 7.4. Furthermore, the powders presented very similar gastro-resistance profiles (Fig. 8) and the same total amount of pantoprazole stabilized in acid medium (91.4%, 90.2% and 92.3% for batches 1, 2 and 3, respectively). Dissolution efficiencies were 58.7 ± 2.5% for batch 1, 55.7 ± 2.1% for batch 2 and 60.2 ± 1.1% for batch 3. Statistical analyses showed no significant differences among the batches ($p = 0.40$).

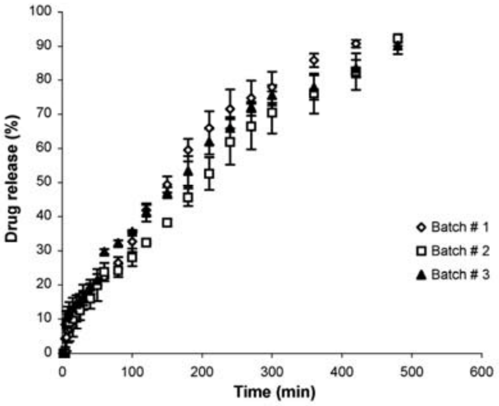


Fig. 8. Gastro-resistance profiles of the three batches of pantoprazole-loaded microparticles prepared to verify the process reproducibility.

5. Conclusions

Pantoprazole-loaded microparticles were successfully prepared by spray drying in both laboratory and pilot scales. In laboratory scale, the viscosity of the solutions fed into the spray dryer affected the particle size and the drug release. The microparticles produced with higher solid concentration were chosen to be spray dried in pilot scale because this formulation presented the highest stabilization of pantoprazole in the gastro-resistance study.

At pilot scale, among the four sets of microparticles prepared varying the atomization and the air pressure, in three of them free microparticles were obtained. The microparticles prepared with rotating disc atomizer or two fluid atomizer and mixed flow (RO-microparticles and MF-microparticles) presented either crystals on the particle surface or very high polydispersity, respectively. Using two fluid nozzle and air pressure of 49 kPa (N1-microparticles) the product obtained was not adequate because it presented strings in the powder. Using the same atomizer but air pressure of 196 kPa (N2-microparticles) the microparticles presented high encapsulation efficiency and the highest stabilization of formulation in acid medium. N2-microparticles were chosen for the pilot scale evaluation.

The three batches of pantoprazole-loaded microparticles prepared to validate the process showed reproducible diameter, polydispersity, densities, encapsulation efficiency and gastro-resistance profile.

Acknowledgements

The authors thank FAPERGS, CNPq/MCT and CAPES.

References

Avner, D., 2000. Clinical experience with pantoprazole in gastro esophageal reflux disease. Clin. Ther. 22, 1170–1185.

Benoit, J.P., Marchais, H., Rolland, H., Velde, V.V., 1996. Biodegradable microspheres: advances in production technology. In: Benita, S. (Ed.), Microencapsulation: Methods and Industrial Applications. Marcel Dekker, New York, pp. 35–72.

Birchal, V.S., Passos, M.L., Wildhagen, G.R.S., Mujumdar, A.S., 2005. Effect of spray-dryer operating variables on the whole milk powder quality. Drying Technol. 23, 611–636.

Brunauer, S., Emmet, P.H., Teller, E., 1938. Adsorption of gases in multimolecular layers. J. Am. Chem. Soc. 60, 309–319.

Carr, R.L., 1965. Evaluating flow properties of solids. Chem. Eng. 18, 163–168.

Cheer, S., Prakash, A., Faulds, D., Lamb, H., 2003. Pantoprazole—an update of its pharmacological properties and therapeutic use in the management of acid-related disorders. Drugs 63, 101–132.

Clarke, N., O'Connor, K., Ramtoola, Z., 1998. Influence of formulation variables on the morphology of biodegradable microparticles prepared by spray drying. Drug Develop. Ind. Pharm. 24, 169–174.

Ford, J.L., Timmins, P., 1999. Drug delivery systems. In: Pharmaceutical Thermal Analysis—Techniques and Applications. Ellis Horwood Limited, New York, pp. 190–200.

Foster, T.P., Laetherman, M.W., 1995. Powder characteristics of proteins spray-dried from different spray-dryers. Drug Develop. Ind. Pharm. 21, 1705–1723.

Gibson, S.G., 2001. How to optimize your spray dryer's performance. Powder Bulk Eng. 15, 31–41.

Giunchedi, P., Conti, B., Genta, I., Conte, U., Puglisi, G., 2001. Emulsion spray-drying for the preparation of albumin-loaded PLGA microspheres. Drug Develop. Ind. Pharm. 27, 745–750.

Goula, A.M., Adamopoulos, K.G., 2004. Spray drying of tomato pulp: effect of feed concentration. Drying Technol. 22, 2309–2330.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 1996. Validation of analytical procedures: methodology.

Oster, C.G., Kissel, T., 2005. Comparative study of DNA encapsulation into PLGA microparticles using modified double emulsion methods and spray drying techniques. J. Microencapsul. 22, 235–244.

Palmieri, G.F., Bonacucina, G., Di Martino, P., Martelli, S., 2002. Gastro-resistant microspheres containing ketoprofen. J. Microencapsul. 19, 111–119.

Ré, M.I., Messias, L.S., Schettini, H., 2004. The influence of the liquid properties and the atomizing conditions on the physical characteristics of the spray-dried ferrous sulphate microparticles. In: Annals of International Drying Symposium, August 22–25, Campinas, Brazil.

Sachs, G., Shin, J.M., Prathaand, V., Hogan, D., 2003. Synthesis or rupture: duration of acid inhibition by proton pump inhibitors. Drugs Today 39, 11–14.

Shin, J., Besancon, M., Simon, A., Sachs, G., 1993. The site of action of pantoprazole in the gastric H^+/K^+-ATPase. Biochim. Biophys. Acta 1148, 223–233.

Wan, L.S.C., Heng, P.W.S., Chia, C.G.H., 1992. Spray drying as a process for microencapsulation and the effect of different coating polymers. Drug Develop. Ind. Pharm. 18, 997–1011.

Zupancic, V., Ograjsek, N., Kotar-Jordan, B., Vrecer, N., 2005. Physical characterization of pantoprazole sodium hydrates. Int. J. Pharm. 291, 59–68.

CAPÍTULO 10: Preparação de aglomerados de microparticulas contendo pantoprazol de liberação controlada preparadas por *spray-drying* em escala piloto

10.1 Apresentação

Nos capítulos anteriores (8 e 9), foram descritas a preparação e a caracterização de micropartículas contento pantoprazol preparadas com a blenda de Eudragit® S100 e Methocel® F4M. Estas micropartículas foram produzidas em escala piloto e as condições operacionais do *spray-drier* foram otimizadas e o processo validado. Como continuidade dos estudos, no presente capítulo apresentamos o estudo de estabilidade acelerada das micropartículas.

Estas micropartículas apresentaram características adequadas de liberação do fármaco, mas, por outro lado, possuem baixa densidade, alto ângulo de repouso e fluxo pobre. Na tentativa de granulação e compressão, foram danificadas e a gastro-resistência diminuída, assim como ocorreu com as micropartículas de Eudragit® S100 (capítulo 7). A forma farmacêutica desenvolvida para evitar danificar as micropartículas foi a de aglomerados. Estes aglomerados foram preparados através de mistura com um excipiente composto por manitol e lecitina e aglomeração por vibração. No presente capítulo, apresentamos a preparação e caracterização destes aglomerados, assim como a avaliação dos perfis de liberação e modelagem matemática dos mesmos. Estes resultados foram submetidos ao periódico *Journal of Microencapsulation*.

Publicação 10: Development of agglomerates containing controlled release pantoprazole microparticles

Development of agglomerates containing controlled release pantoprazole microparticles

RENATA P. RAFFIN[1], PAOLO COLOMBO[2], FABIO SONVICO[2], DENISE S. JORNADA[1], ADRIANA R. POHLMANN[3], SILVIA S. GUTERRES[1].

[1]Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [2]Dipartimento Farmaceutico, Università degli Studi di Parma, Parma, Italy, and [3]Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Abstract

Pantoprazole-loaded microparticles were prepared using a blend of Eudragit® S100 and Methocel® F4M. An accelerated stability study during 6 months was carried out for these microparticles. In order to improve the technological characteristics of the pantoprazole-loaded microparticles, soft agglomerates were prepared aiming an oral delayed-release and gastro-resistant solid dosage form. The agglomeration was performed by mixing the microparticles with spray-dried mannitol/lecithin. To elucidate the agglomerate formation, the effects of factors such as the amount of lecithin in the excipient microparticles, the ratio between pantoprazole and excipient microparticles were evaluated. Pantoprazole-loaded microparticles were stable during 6 months at 40 °C and 75 % RH. The agglomerates presented different yields, drug loading, morphology, mechanical and release properties. The high lecithin concentration in the mannitol/lecithin powders was crucial for the agglomeration process. The biopharmaceutical characteristics of pantoprazole microparticles, i.e. their delayed-release properties, were not affected by the agglomeration process.

Key words: pantoprazole, agglomerates, lecithin, delayed - release, microparticles, gastro-resistance.

Correspondence: Renata P. Raffin, Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. CEP 90610-000. Fax: +5551 33085437. Email: renata.raffin@ufrgs.br

Introduction

Polymeric drug delivery systems can offer potential therapeutic advantages in comparison with conventional forms: reducing side-effects, improving the therapeutic effect, prolonging the biological activity, controlling the drug release rate, and decreasing the administration frequency. As a microencapsulation process, the spray-drying technique has some advantages such as one-stage continuous process, ease scaling-up and it is only slightly dependent upon solubility of drug and polymer (Masters, 1991, Beck et al. 2004). The size of microparticle prepared by spray-drying ranges from microns to several tens of microns with a relatively narrow distribution (Masters, 1991). The microparticles prepared by spray-drying can be used as oral dosage forms (dry powders, granules or agglomerates) and nasal powders (He et al. 1999, Raffin et al. 2006a, Russo et al. 2006, Raffin et al. 2007b).

Among the various drug delivery devices used to sustain drug release, hydrophilic matrix systems are generally preferred because of their ability to release drugs at a constant rate. The matrix is often composed of a drug mixed with a gelling agent (hydrophilic polymer). Cellulose derivatives are often chosen to develop such systems because of their low toxicity and low cost. Methacrylate copolymers (Eudragit®) are interesting candidates for the production of microparticles by spray drying since they are inert and freely soluble in organic solvents (Esposito et al. 2002, Palmieri et al. 2002). Among the different types of commercialised Eudragit®, the S100 type is a pH-dependent enteric copolymer composed of methacrylic acid and methacrylic acid methyl ester monomers, rending its solubility in pH higher than 7 (Beten et al. 1992). As a consequence, Eudragit® S100 is insoluble in the mouth and in the stomach, but it starts to dissolve in the duodenum (pH around 6). In previous works, Methocel® F4M (hydroxypropylmethylcellulose) was blended to Eudragit® S100 and the aqueous solution spray-dried to obtain pantoprazole-loaded microparticles (Raffin et al. 2006b, Raffin et al. 2007b). Pantoprazole is a prodrug that inhibits the H^+/K^+ATPase and, consequently, the acid release in gastric lumen. This prodrug is used in the treatment of digestive ulcers, gastro-oesophageal reflux disease, as well as it is an auxiliar in the eradication of the *Helicobacter pylori* (Cheer et al., 2003). It is unstable in the acid environment of the stomach lumen and must be administered using a gastro-resistance drug delivery system. Pantoprazole-loaded microparticles showed both characteristics of gastro-resistance and controlled release (Raffin et al. 2006b). These microparticles also demonstrated an anti-ulcer activity in an ethanol-induced ulcer *in vivo* model (Raffin et al. 2007b). The scaling up of the spray-drying process was studied varying process parameters, such as total solid concentration in the solution feed, type of atomizer, air pressure and air/spray contact. The following conditions were selected to produce microparticles: two fluid atomizer, co-current air spray contact and air pressure of 196 kPa (Raffin et al. 2006b).

The attainment of microparticles biopharmaceutical attributes is opposed by the small size of particles that leads to powders with bulk volume and problematic flow for dosage forms manufacturing (Russo et al. 2004, Kim et al. 2005). In several pharmaceutical applications, particles might be fine for drug delivery, but coarse enough for facilitating solid dosage form preparation. Often, the transformation of microparticles in solid dosage forms involves granulation and compaction, provoking irreversible modifications of the microparticle range size (Fu et al. 2001). In particular, this technological limitation can be solved using soft agglomeration, a process in which the powder size is enlarged by constructing weak clusters of primary microparticles (Russo et al. 2004). Soft agglomerates are easily broken down by air turbulence or water uptake, reconstituting the original size of microparticles. Weak cohesion bonds due to capillary, van der Waals or electrostatic forces hold together the primary particles in soft structures (Tsantilis & Pratsinis, 2004). The quantity and the nature of these interactions, as well as the method of production, determine the agglomerate structures (Boerefijn et al. 1998). Recently, a new procedure for agglomerating microparticles has been described (Russo et al. 2006). Morphine crystals have been agglomerated in soft clusters by processing the physical mixture of drug with spray-dried microparticles of mannitol/lecithin. The lecithin was used as binder to improve the interparticle cohesion reinforcing the internal structure of agglomerates (Moreno-Atanasio et al. 2006). In this way, we hypothesized that procedure could be applied for preparing soft agglomerates of pantoprazole-loaded microparticles.

The stability of drugs and medicines depends on the environment factors as temperature, humidity and light, and on the physico-chemical properties of the drug and the excipients (ICH, 2003). The purpose of stability testing is to provide evidence on how the quality of a drug product varies with time and to estimate a shelf life for the drug product and recommended storage conditions (ICH, 2003). Thus, the purpose of this research was to study the accelerated stability of Eudragit® S100 and Methocel® F4M blended microparticles, as well as to prepare and characterize soft agglomerates containing these microparticles.

Materials and methods

Materials

Sesquihydrate sodium pantoprazole has been obtained from Henrifarma (São Paulo, Brazil). Eudragit® S100 has been kindly gifted by Almapal® (São Paulo-Brazil, produced by Rohm®, Germany). Methocel® F4M was provided by Colorcon® (São Paulo, Brazil, produced by Dow Chemical, USA). All other chemicals are analytical grade.

Methods

Preparation of pantoprazole-loaded microparticles

Pantoprazole-loaded microparticles were prepared in pilot scale as previously described (Raffin et al. 2006b). The solution was spray-dried in pilot scale equipment (Model PSD 52 APV1Anhydro, Denmark) presenting the cylindrical dryer chamber of 1.0 m diameter and 2.3 m of total height. A two-fluid pneumatic atomizer with external mixing was used. In this nozzle, the liquid to be atomized is discharged through a central hole diameter of d_o=1.5 mm, whereas the atomizing air is injected through a ring area around the liquid hole. The pressure of the atomizing air was 196 kPa and the inlet temperature was 170 ± 1 ºC and the flow rate was 2 $L.h^{-1}$. During all the processes the room temperature and humidity were controlled (24 ± 1 ºC and 54 ± 2 % of relative humidity). For spray-drying, the solution consisted of 36 g of Eudragit® S100 in 2000 mL NaOH solution (3 $g.L^{-1}$). After complete dissolution, Methocel® F4M (18 g) was added and the solution was kept at 10 ºC for 24 h. Sodium pantoprazole (9 g) was added before spray-drying.

Accelerated stability tests

Transparent glass vials containing 0.5 g of microparticles were stored for 6 months in a stability chamber at 40 °C and 75 % RH. Sealed and non-sealed vials were evaluated every 30 days for their drug content. Humidity was gravimetrically determined.

Preparation and characterization of spray-dried mannitol/lecithin microparticles

In order to circumvent the poor flow and high bulk density of the microparticles, agglomerates were prepared after adding proper excipients for agglomeration.

An ethanol solution of lecithin (60 mL) was added to an aqueous solution of mannitol (340 mL) to achieve final ratios of 85:15, 82.5:17.5 and 80:20 (w/w). The resulting solutions contained 15 % of ethanol and 4.5 % of solids. The solutions were spray-dried in a laboratorial spray-drier Buchi Mini Spray Dryer B-191 (Buchi Laboratoriums-Tecnik, Flawil, Switzerland) using flow rate of 6.5 mL.min^{-1}, inlet temperature of 90 ± 2 °C, aspiration set in 100 % and air flow of 500 NL.h^{-1}. The three powders of mannitol/lecithin microparticles were used as excipients for agglomeration of microparticles and they were characterized by preparation yield, humidity content, morphology and specific surface area.

The yields, expressed in %, were calculated by the ratio between the mass obtained and the mass of mannitol and lecithin added to the solution.

The particle size distributions of spray-dried excipient microparticles were measured using laser light diffraction apparatus (series 2600 Malvern Instruments Ltd., Spring Lane South Malvern, Worcestershire, U.K.) suspending the particles in ethyl acetate, a non-solvent of these materials. Particle size was expressed as median volume diameter.

After gold sputtering, the morphology of the spray-dried excipients was assessed by scanning electron microscopy (SEM) using accelerating voltage of 15 kV (JSM 6400, Jeol Ltd., Tokyo, Japan).

The specific surface areas of microparticles were determined by the BET multipoint technique (Brunauer et al., 1938). The nitrogen adsorption-desorption isotherms of previous degassed organic-solids, under vacuum at 40 ºC, were determined at liquid nitrogen boiling point in a homemade volumetric apparatus, using nitrogen as probe. The pressure was measured using capilar mercury barometer and the results were compared to an alumina pattern.

Water content was assayed by Karl Fisher titration (Titro Matric 1S, Crison, Alella, Spain).

Flowability, as well as bulk and tapped densities, were measured according to the European Pharmacopoeia (2005). The compressibility index was calculated according to USP (2007).

Preparation of the agglomerates

Pantoprazole microparticles and spray-dried mannitol/lecithin microparticles were mixed in a Turbula apparatus (Wab, Basel, Switzerland) for 3 h. The homogeneity was verified by HPLC, meaning the homogeneity in the drug content.

Five grams of the mixture of pantoprazole microparticles and spray-dried mannitol/lecithin microparticles were put on the top of two sieves stack with nominal apertures of 106 and 850 μm, respectively (10 cm sieves, Endecotts Ltd, London, U.K.), which was vibrated for 10 minutes on a laboratory sieve shaker (amplitude 2-3; Analysette 3 Fritz model, Fritsch GMBH, Idar-Oberstein, Germany). Agglomerates between 106 and 850 μm were collected. Reprocessing the non-agglomerated powder and crushing the larger agglomerates, the process was repeated 8 times.

The ratios tested were 1:1, 1:2 and 1:3 (w/w). The agglomeration was not possible using lower quantity of lecithin. The formulations that could be prepared are described in Table I.

[Insert Table I here]

Drug loading

The drug loading was assayed using a validated HPLC method (Raffin et al. 2007a). Briefly, an amount of the microparticles or the agglomerates, equivalent to 10 mg of pantoprazole, was weighed and magnetically stirred with 40 mL of 0.05 $mol \cdot L^{-1}$ NaOH solution for 1 h in a volumetric flask. The volume was completed to 50 mL and drug concentration was determined after filtration (0.45 μm) by HPLC (Perkin Elmer serie 200) using a LiChrospher RP_{18} (Merck) column. Mobile phase consisted of acetonitrile/phosphate buffer pH 7.4 (35:65 v/v). The flow rate was 1 $mL \cdot min^{-1}$ and the drug was detected at 290 nm.

Agglomerate characterization

The agglomeration yield was calculated by dividing the weight of the agglomerates 106-850 µm by the total weight of powder before agglomeration, multiplied by 100.

The agglomerates were examined by scanning electron microscopy, as described before, for the microparticles. Mean size distribution was verified by sieving. The average diameter was calculated determining the mass retained in each sieve. The specific surface area was calculated by BET method (Brunauer et al., 1938). The nitrogen adsorption-desorption isotherms were obtained as described for the mannitol/lecithin microparticles.

Flowability and compressibility index were obtained using the same procedure described above for the mannitol/lecithin powders.

Agglomerates (2 g) were tested for resistance using a friabilometer operating at 25 rpm for 4 min (Ph. Eur 2005). The agglomerates were separated from the powder during the test using a 106 µm sieve. The recovered agglomerates were weighed and the percentage of powder loss was calculated.

To determine the tensile strength, a single agglomerate (n = 8) was placed on a mobile platform under the measuring head of a calibrated load cell (514 QD, DS Europe, Milan, Italy) (Russo et al. 2004). The very slow movement of the platform caused the compression of the agglomerate against the measuring head. The force-time curve was recorded using the Scope v 3.5 software (AdInstruments Ltd., Oxfordshire, U.K.). From the crushing force (*F*) the tensile strength (σ), was calculated (equation 1).

$$\sigma = \frac{2.8F}{\pi d^2} \qquad (1)$$

where *d* is the agglomerate diameter.

The agglomerate disintegrations in aqueous media (phosphate pH 7.4 or 0.1 M HCl) were recorded under an optical stereomicroscope (magnification 20 x) (Citoval 2, Jena, Germany) connected to a video camera (JVC, Tokyo, Japan). The disintegration tests were performed by placing the agglomerate (425-500 µm) over a microscope glass and wetting it with 50 µL of each medium: phosphate buffer pH 7.4

or 0.1 M HCl at 37°C. The disintegration time was measured on 25-30 agglomerates as the time for deagglomeration of the globular structure.

To determine the drug release profile, size 00 hard gelatine capsules without colouring agent were filled with a mass of agglomerates corresponding to 15 mg of drug. Dissolution tests were conducted in USP Dissolution Apparatus II at 150 rpm at 37 ºC. In order to determine if the agglomerates were able to release 100 % of the encapsulated drug, the dissolution was evaluated in phosphate buffer pH 7.4 for 480 min.

To evaluate gastro-resistance, capsules containing the agglomerates (A to E) were exposed to 300 mL of 0.1 *M* HCl. After 1h, an aqueous solution (600 mL) composed of NaOH (2.6 g) and KH_2PO_4 (6.12 g) was added into the medium in order to reach pH 7.4. The samples were collected in pre-determined time intervals from 0 up to 600 min. Pantoprazole concentrations were determined by UV at 295 nm (Vankel UV/Vis spectrometer). The analytical method was previously validated. Gastro-resistance profiles were modelled to fit monoexponential and biexponential models, using MicroMath Scientist® software (Salt Lake City, UT, USA). In order to gain some information about the release mechanism, the Korsmeyer-Peppas equation (Equation 2) was also used.

$$Ft = at^n \qquad (2)$$

Results and discussion

Preparation of pantoprazole microparticles

Pantoprazole microparticles were obtained as off-white powders with yield of 62 %. Drug loading and humidity were evaluated (drug content was 13 % and residual moisture was 2 %) and considered acceptable comparing with the data of this process validation (Raffin et al. 2006b).

Accelerated stability tests

During the 180 days of the stability tests, vials were weighed monthly. No increase in the weight was verified during these period either to sealed or non-sealed vials. These results indicate that pantoprazole microparticles are not hygroscopic, even if NaOH was used in the preparation. Regarding the drug content during the accelerated stability, both samples (sealed and non-sealed vials) presented similar results. Samples were stable during 180 days (Figure 1). The decay in pantoprazole content was less than 5 % after 6 months of storage. A shelf-live of 24 months can be attributed to products that present less than 5 % of drug reduction during the accelerate stability test (ICH, 2003).

[Insert Figure 1 here]

Characterization of spray-dried mannitol/lecithin powders

Since pantoprazole microparticles could not be directly agglomerated, blends of mannitol/lecithin spray-dried powders with pantoprazole gastro-resistant microparticulate powder were prepared in order to manufacture soft agglomerates.

The mannitol/lecithin powders presented contents of lecithin of 15.0, 17.5 and 20.0 % w/w. The yield of the spray-drying process was not affected by the lecithin concentration (approximately 55 % for all formulations). Particle sizes were not influenced by the lecithin concentration, and the mean diameters of the three types of excipients were close to 3.7 μm, evidently smaller than the size of pantoprazole microparticles (22 μm). The mannitol/lecithin microparticles presented mean diameters compatible to other described in the literature using the same equipment (Atuah et al., 2003, Oster & Kissel, 2005). All the powders showed round particles and a tendency to form aggregates as the content of lecithin increased (Figure 2).

[Insert Figure 2 here]

This tendency was probably due to the presence of lecithin that did not influence the moisture content of the powders (1.8, 1.7 and 1.5 %, respectively). The microparticles presented a spherical shape, with a rough surface (Figure 3a), as the mannitol is crystalline even after the spray-drying process (verified by DSC, data not shown). These spray-dried powders presented bulk density between 0.2-0.3 g/cm^3, poor packing (compressibility index between 22-31) and did not flow.

[Insert Figure 3 here]

Characterization of agglomerates

Agglomerates were obtained with yields from 35 to 79 % (Table II). Drug loading varied from 58 to 100 %. Using the mannitol/lecithin powder containing 15 % of lecithin, agglomerates were prepared exclusively with 1:3 (w/w) ratio. The agglomerates A presented satisfactory yield, but drug loading of 85.3 %.

Mannitol/lecithin powders containing 17.5 % of lecithin were also prepared. Agglomerates B prepared with a 1:2 (w/w) ratio presented lower yield and lower drug loading than the agglomerate A. Less than 60 % of the drug was incorporated in the clusters. The 1:3 (w/w) ratio was also tested (agglomerates C). In this case, agglomerates C presented the highest agglomeration yield (79 %) and complete drug loading (100 %).

Increasing lecithin content to 20 % in the mannitol/lecithin powder, agglomeration of this powder with the pantoprazole-loaded microparticles was easier and yields of 62.4 and 76.9 % (Table II) were obtained using lower ratios (1:1 and 1:2 w/w). Otherwise, agglomerates D and E presented complete drug loading.

[Insert Table II here]

The agglomerates were studied by scanning electron microscopy. The agglomerates presented quite a rounded shape (Figure 4). The globule surface was smooth with a very small quantity of non-agglomerated particles on the surface.

[Insert Figure 4 here]

A closer analysis of the agglomerate surfaces showed that they were formed by a mixture of small (mannitol/lecithin) and large (pantoprazole-loaded microparticles) particles. The agglomerate surface was characterized by small mannitol/lecithin particles embedding larger pantoprazole particles without evident bridges among them (Figure 5). In detail, some material, likely lecithin, was spread out over the particles leading to particles more closely connected (Figure 3b). This find was particularly evident for the agglomerates containing the mannitol/lecithin powder prepared with higher content of lecithin (agglomerates C and E). The result suggested that, in the mannitol/lecithin powder, lecithin could be located at the surface. The presence of lecithin around all particles was confirmed by x-ray microanalysis searching phosphorus on the surface of the mannitol/lecithin powders. In the agglomerates, the only raw material containing phosphorus is lecithin. The results revealed the presence of phosphorus by observing a peak among the components of the particle surface in comparison with the pure sample of pantoprazole-loaded microparticles in which no phosphorus is present. Thus, lecithin accumulated on the pantoprazole-loaded microparticle surface. The specific surface area of the agglomerates was measured (Table III). The surface areas of the microparticles (close to 98 $m^2.g^{-1}$) and of the mannitol/lecithin powders (close to 60 $m^2.g^{-1}$) were used to calculate the expected surface area of the agglomerates, considering the ratio between the components. In all cases, measured and expected areas were very close, meaning that no changes in the particles structure occurred during the agglomeration process.

[Insert Figure 5 here]

The packing characteristics of the agglomerates were determined by measuring bulk and tapped densities (Table II). The agglomerates showed values of bulk densities around 0.20 $g.cm^{-3}$, higher than those observed for the pantoprazole microparticles (0.06 $g.cm^{-3}$), but still indicating a loose packing arrangement of particles. The concentration of lecithin did not influence the density. However, the ratio between pantoprazole-loaded microparticles and mannitol/lecithin powders was the major determinant for the density values. The bulk densities were higher for the agglomerates prepared with higher amounts of spray-dried mannitol/lecithin powder. The tapped density values of agglomerates slightly improved the bulk values, and the compressibility indexes on average were around 11 for the samples A, C and D. Agglomerates B and E presented a slightly higher value for the compressibility index (18 and 19, respectively). This difference is a consequence of the variation in the agglomerate size distributions. These samples (B and E) had higher quantities (42 and 43 %) of small agglomerates (less than 425 μm). Agglomerate mean diameter was calculated based on the size distribution and showed that agglomerates C presented the highest size (463 μm) and agglomerates D the smallest mean diameter (376 μm). Agglomerates A, B and E had average sizes of 404, 396 and 386 μm, respectively. Thus, the agglomeration process, determining the organization of particles in the globular structure, favoured the packed arrangement of powder bed over primary microparticle powders. The compressibility index, related to powder flowability, was improved by the agglomeration. Pantoprazole microparticles, as well as the three different spray-dried mannitol/lecithin powders, presented flow in infinite time (the entire samples failed to flow under conditions prescribed for the flowability test). In contrast, the agglomerates flowed well (Table II). The agglomerates A, B and C presented higher flowability than the agglomerates D and E ($p < 0.01$). However, all products could be classified as free-flowing powders (Ph. Eur. 5). In summary, the agglomerates showed characteristics linked to packing arrangement and flowing ability more favourable for handling and filling than the microparticles.

The mechanical resistance is a relevant property for agglomerate use. In addition, it reflects differences in agglomerate structure as a result of their composition. The friability test was used to evaluate powder loss during the transportation of the agglomerates. Friability varied from 1.06 to 2.48 % of loss (Table III) and was statistically similar ($p = 0.32$). However, the agglomerates prepared with 1:2 (w/w) ratio presented lower friability. Increasing the amount of mannitol/lecithin, the friability was also increased, demonstrating that there were some particles not embedded in the globular structure. Tensile strength was used to estimate the agglomerate resistance to crushing under load. Pantoprazole agglomerates had a very low resistance to crushing, and the tensile strength values (Table III) were between 44 and 69 mN/mm^2, similar to those reported by Russo and co-workers (2006). The samples prepared with 1:2 (w/w) ratio of pantoprazole microparticles and spray-dried mannitol/lecithin powders had higher tensile strength values. The agglomerates prepared with 1:1 (w/w) ratio presented lower values for tensile strength as well as with 1:3 (w/w) ratio. The 1:2 (w/w) ratio seemed the optimal composition in order to improve resistance. In summary, the agglomerates presented good resistance during flowing and poor resistance when compressed. Based on these features, they are suitable for filling hard gelatine capsules in view of oral administration of these drug delivery systems.

[Insert Table III here]

Agglomerate disintegration and dissolution

The effect of agglomeration on drug release was evaluated measuring the disintegration behaviour of agglomerates and the dissolution rate of pantoprazole. In phosphate buffer pH 7.4, agglomerates were slowly penetrated and slightly swollen by solvent, maintaining the globular structure. Only agglomerates prepared with 1:3 (w/w) ratio (A and C) disintegrate after 2 minutes. The agglomerates B and E, prepared with 1:2 (w/w) ratio, disintegrate more slowly in about 5 – 10 min. The agglomerates D did not disintegrate within 20 min.

In order to understand the influence of lecithin on the disintegration behaviour, agglomerates constituted only of mannitol/lecithin were tested at this pH. These agglomerates disintegrated within 120 s in pH 7.4 phosphate buffer, showing that the behaviour is dependent on the presence of HPMC and the lecithin did not exert influence on the disintegration.

The stability of all samples (pantoprazole microparticles and agglomerates) in phosphate buffer pH 7.4 was evaluated, showing that the pantoprazole microparticles and the agglomerates reached 100 % of pantoprazole dissolution after 500 min. These results indicate that neither the spray-drying and agglomeration techniques nor the medium used in the release experiments affect the stability of the drug.

Concerning the gastro-resistance evaluation, the agglomerates showed different results from the pantoprazole microparticles in terms of dissolution profile and total amount of drug after acid step (Figure 6). Pantoprazole microparticles showed 92 % of pantoprazole after exposure to acid medium. The agglomerates D, prepared with 1:1 (w/w) ratio and 20.0 % of lecithin, presented 70 % of pantoprazole after acid step. This value is similar to those reported for the same microparticles prepared in laboratory scale, before the scale up study (Raffin et al. 2007b). In this case, after a scale up study, an increase in the agglomeration yield and in the gastro-resistance could be achieved. Using the scale up study, pilot scale pantoprazole microparticles were able to stabilize 30 % more pantoprazole than the laboratory scale microparticles (Raffin et al. 2006b). The agglomerates B, prepared with 1:2 (w/w) ratio and 17.5 % of lecithin presented the lowest value of gastro-resistance (51 %). These agglomerates presented also low agglomeration yield and incomplete drug loading. The agglomerates E, prepared with 1:2 (w/w) ratio and 20.0 % of lecithin, showed 91 % of gastro-resistance. These agglomerates did not change the amount of pantoprazole stabilized by the microparticles. This result indicates that pantoprazole microparticles were not broken or deconstructed by the agglomeration process and that the mannitol/lecithin powder did not interact with them. The agglomerates prepared with 1:3 (w/w) ratio and 15.0 % (A) and 17.5 % (C) of lecithin presented very similar profiles and the amount stabilized was approximately 87 %.

[Insert Figure 6 here]

The profiles were mathematical modelled to fit mono or biexponential equations. The best fit was chosen by the higher model selection criteria (MSC, given by the software), higher determination coefficient and best graphic adjustment. Pantoprazole microparticles fit the monoexponential model (MSC = 4.38 $r^2 = 0.996$). The half-life of drug release was 155.8 min. Drug delivery systems containing water-soluble drug that follow the monoexponential model release the drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish (Costa & Lobo, 2001). On the other hand, all agglomerates profiles fit the biexponential model (Table IV).

[Insert Table IV here]

The release profiles have two different rates of release (initial burst and controlled release phase). The initial burst was higher for the agglomerates A and C (about 60 %). On the other hand, the agglomerates B, D and E showed longer controlled release phases (70 – 87 %). In all cases, the mechanism of drug release and the rate were affected by the agglomeration process. In order to gain some input into about the release mechanism, the profiles were modelled to fit the Korsmeyer-Peppas equation (2). As the agglomerates profiled fitted the biexponential model, the burst effect was added to equation 1 (3).

$$Ft = at^n + b \qquad (3)$$

For spherical systems, when the exponent n assumes a value of 0.43, between 0.43 and 0.85, 0.85 or greater than 0.85 the drug release rate is governed by fickian diffusion, anomalous transport, Case-II transport, or super Case-II transport, respectively. The parameters a and n were obtained from the initial portion of the curve (from time 0 to 100 min). Pantoprazole microparticles presented the value for n equal to 0.68, indicating that the release mechanism is the anomalous transport and no burst effect occurred. The anomalous transport has intermediate characteristics between the fickian diffusion and the non-fickian Case-II transport. It is often identified in swelling/controlled release systems since both diffusion and dissolution are coupled (Arifin et al., 2006).

On the other hand, the agglomerates A and C presented considerable burst effect (*b* values of 17.4 and 19.3, respectively) and fickian diffusion as release mechanism (*n* = 0.42 and 0.39, respectively). The agglomerate B profile could be only modelled by equation 1 and the release mechanism was diffusion (*n* = 0.14). The agglomerates E presented values for *n* of 0.30 and for *b* of 2.0, showing fickian diffusion and little or negligible burst effect. Only the agglomerates D presented the same release mechanism of the microparticles (*n* = 0. 53) and no burst effect.

Briefly, the presence of mannitol/lecithin powders strongly influenced the pantoprazole release mechanism. The agglomerates presented faster release as the ratio between pantoprazole microparticles and mannitol/lecithin increased. The high solubility and prompt disintegration of this excipient altered the gel layer formation around the microparticles and caused faster disintegration and water penetration inside the microparticles. The drug release mechanism and the release rate remained similar to those observed for the microparticles when 1:1 (w/w) ratio was used. However, these agglomerates were not capable of stabilizing more than 90 % of pantoprazole as required by the Pharmacopoeia (USP). Furthermore, the agglomerate E containing 1:2 (w/w) ratio of mannitol/lecithin (80:20) presented high gastro-resistance values and an intermediate release rate, but still considered as controlled release (half-life of release of 108.8 min).

Conclusions

The spray-drying was reproducible to obtain pantoprazole microparticles, which were stable under accelerate condition of storage within 6 months. Agglomeration of pantoprazole microparticles blended with mannitol/lecithin powders is a technique successfully applied to size enlargement of micronized products that could be damaged by granulation or compaction. These agglomerates can be used as oral delayed-release dosage forms administered themselves or dispersed in a liquid. The composition and quantity of the mannitol/lecithin powders resulted to be the crucial factors for the agglomerate quality. Therefore, adjusting the content of lecithin used as binder, it is possible to agglomerate microparticles of materials that could not be agglomerated per se. The presence of mannitol/lecithin strongly influenced the disintegration and drug release from the agglomerates. The agglomerates with more adequate mechanical and biopharmaceutical characteristics were prepared with 1:2 (w/w) ratio of pantoprazole microparticles and mannitol/lecithin (80:20) powder.

Acknowledgement

The authors are grateful for the financial support of Capes, CNPq/MCT and Fapergs. The financial support of the Italian Ministry for University and Research is also gratefully acknowledged. We thank Prof. Edilson Benvenutti for the BET analysis.

References

Arifin DY, Lee LY, Wang CH. 2006. Mathematical modeling and simulation of drug release from microspheres: Implications to drug delivery systems. Advanced Drug Delivery Reviews 58: 1274–1325.

Atuah KN, Walter E, Merkle HP, Alpar HO. 2003. Encapsulation of plasmid DNA in PLGA-stearylamine microspheres: a comparison of solvent evaporation and spray-drying methods. Journal of Microencapsulation 20: 387-399.

Beck RCR, Pohlmann AR, Guterres SS. 2004. Nanoparticle-coated microparticles: preparation and characterization. Journal of Microencapsulation 21: 499–512.

Beten DB, Gelbcke M, Diallo B, Moes AJ. 1992. Interaction between dipyridamole and Eudragit S. International Journal of Pharmaceutics 88: 31-37.

Boerefijn R, Ning Z, Ghadiri M. 1998. Disintegration of weak lactose agglomerates for inhalation applications. International Journal of Pharmaceutics 172: 199-209.

Brazilian National Health Surveillance Agency. Stability testing guideline RE 1, July 29, 2005.

Brunauer S, Emmet PH, Teller E. 1938. Adsorption of gases in multimolecular layers. Journal of the American Chemical Society 60: 309–319.

Cheer S, Prakash A, Faulds D, Lamb H. 2003. Pantoprazole- An Update of its pharmacological properties and therapeutic use in the management of acid-related disorders. Drugs 63:101-132.

Costa P, Lobo JMS. 2001. Modeling and comparison of dissolution profiles. European Journal of Pharmaceutical Sciences 13: 123-133.

Esposito E, Cervellati F, Menegatti E, Nastruzzi C, Cortesi R. 2002. Spray dried Eudragit microparticles as encapsulation devices for vitamin C. International Journal of Pharmaceutics 242: 329–334.

European Pharmacopoeia (5th ed.), Council of Europe, Strasbourg, France, 2005.

Fu YJ, Mi FL, Wong TB, Shyu SS. 2001. Characteristic and controlled release of anticancer drug loaded poly (D,L-lactide) microparticles prepared by spray drying technique. Journal of Microencapsulation 18: 733-747.

He P, Davis SS, Illum L. 1999. Chitosan microspheres prepared by spray drying. International Journal of Pharmaceutics 187: 53–65.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Stability Testing of New Drug Substances and Products (Q 1A (R2)) 2003.

Kim BK, Hwang SJ, Park JB, Park HJ. 2005. Characteristics of felodipine-located poly(ε-caprolactone) microspheres. Journal of Microencapsulation 22: 193-203.

Masters K. 1991. The spray drying handbook. New York: Longman Scientific and Technical.

Moreno-Atanasio R, Ghadiri M. 2006. Mechanistic analysis and computer simulation of impact breakage of agglomerates: Effect of surface energy. Chemical and Engineering Science 61: 2476-2481.

Oster CG, Kissel T. 2005. Comparative study of DNA encapsulation into PLGA microparticles using modified double emulsion methods and spray drying techniques. Journal of Microencapsulation 22: 235–244.

Palmieri GF, Bonacucina G, Di Martino P, Martelli S. 2002. Gastro-resistant microspheres containing ketoprofen. Journal of Microencapsulation 19: 111-119.

Raffin RP, Colomé LM, Guterres SS, Pohlmann AR. 2007a. Validation of analytical methodology by HPLC for quantification and stability evaluation of sodium pantoprazole. Quimica Nova 30: 1001-1005.

Raffin RP, Colomé LM, Haas SE, Jornada DS, Pohlmann AR, Guterres SS. 2007b. Development of HPMC and Eudragit S100® blended microparticles containing sodium pantoprazole. Pharmazie 62: 361-364.

Raffin RP, Guterres SS, Pohlmann AR, Re MI. 2006a. Powder characteristics of pantoprazole delivery systems produced in different spray-dryer scales. Drying Technology 24: 339-348.

Raffin RP, Jornada DS, Ré MI, Pohlmann AR, Guterres SS. 2006b. Sodium Pantoprazole-loaded enteric microparticles prepared by spray drying: effect of the scale of production and process validation. International Journal of Pharmaceutics 324: 10-18.

Russo P, Buttini F, Sonvico F, Bettini R, Massimo G, Sacchetti C, Colombo P, Santi P. 2004. Chimeral agglomerates of microparticles for administration of caffeine nasal powders. Journal of Drug Delivery Science and Technology 14: 449-454.

Russo P, Sacchetti C, Pasquali I, Bettini R, Massimo G, Colombo P, Rossi A. 2006. Primary microparticles and agglomerates of morphine for nasal insufflation. Journal of Pharmaceutical Sciences 95: 2553-2561.

Tsantilis S, Pratsinis SE. 2004. Soft- and hard-agglomerate aerosols made at high temperatures. Langmuir 20: 5933-5939.

United States Pharmacopeia (30th ed.), United States Pharmacopeial Convention, Rockville, United States, 2007.

Table I: Composition of the agglomerates, as well as the final amount of lecithin present in the agglomerates.

Agglomerate	Lecithin in excipient microparticles (%)	Pantoprazole/ excipient microparticles ratio	Percentage of lecithin in the agglomerates (%)
A	15.0	1:3	11.25
B	17.5	1:2	11.67
C	17.5	1:3	13.12
D	20.0	1:1	10.00
E	20.0	1:2	13.33

Table II. Characteristics of the agglomerates.

Agglomerate	Yield (%)	Drug loading (%)	Bulk density (g.cm^{-3})	Tapped density (g.cm^{-3})	Compressibility (%)	Flowability (s)
A	59.4 ± 1.6	85.3 ± 4.6	0.24 ± 0.01	0.28 ± 0.01	11.8 ± 1.0	122.2 ± 22.0
B	35.3 ± 4.8	57.9 ± 0.8	0.21 ± 0.01	0.26 ± 0.01	18.1 ± 0.9	135.6 ± 33.8
C	79.0 ± 0.9	101.0 ± 2.3	0.22 ± 0.02	0.23 ± 0.03	11.3 ± 2.8	131.6 ± 14.8
D	62.4 ± 2.5	100.3 ± 3.2	0.15 ± 0.01	0.17 ± 0.01	11.8 ± 0.5	237.9 ± 29.8
E	76.9 ± 3.7	95.5 ± 1.3	0.19 ± 0.01	0.24 ± 0.01	19.5 ± 0.1	276.8 ± 30.2

Table III. Specific surface area and mechanical properties of the agglomerates.

Agglomerate	Specific surface area ($m^2.g^{-1}$)	Friability (%)	Tensile Strength ($mN.mm^{-2}$)
A	76	2.44 ± 1.37	44.0 ± 8.6
B	85	1.06 ± 0.67	61.6 ± 4.2
C	70	2.47 ± 0.36	54.1 ± 7.7
D	73	1.67 ± 0.44	52.3 ± 6.7
E	78	1.14 ± 0.60	69.3 ± 5.5

Table IV. Mathematical model of the agglomerate dissolution profiles and fit to the biexponential equation.

Agglomerate	Biexponential equation parameters				r^2	MSC
	A	α	B	β		
A	0.42	0.043	0.27	0.0021	0.999	5.9
B	0.21	0.075	0.61	0.0007	0.992	3.8
C	0.39	0.036	0.29	0.0023	0.999	5.8
D	0.12	0.057	0.87	0.0017	0.999	6.7
E	0.25	0.034	0.53	0.0026	0.998	5.5

Figure 1. Drug content of the microparticles during 180 days of accelerate conditions storage. Sealed and non-sealed vials were evaluated.

Figure 2. The spray-dried mannitol/lecithin powders prepared with 15.0 (a), 17.5 (b) and 20 % (c) of lecithin.

Figure 3. Spray-dried mannitol/lecithin containing 17.5 % of lecithin (a) and agglomerate C (b).

Figure 4. SEM images of the agglomerates A, B, C, D and E.

Figure 5. Photomicrograph of the surface of the agglomerate C.

Figure 6. Gastro-resistance of pantoprazole microparticles (MP) and agglomerates (A to E). Drug release is reported in phosphate buffer pH 7.4 after exposure of 1 h in 0.1 M HCl.

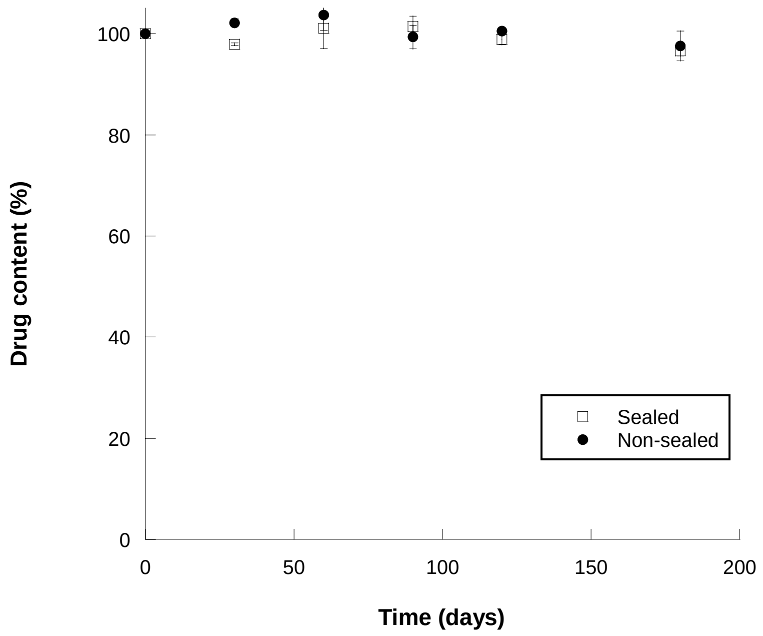

100
80
60
40
20
0
Drug content (%)
0
50
100
150
200
Time (days)
□ Sealed
● Non-sealed

Figure 1

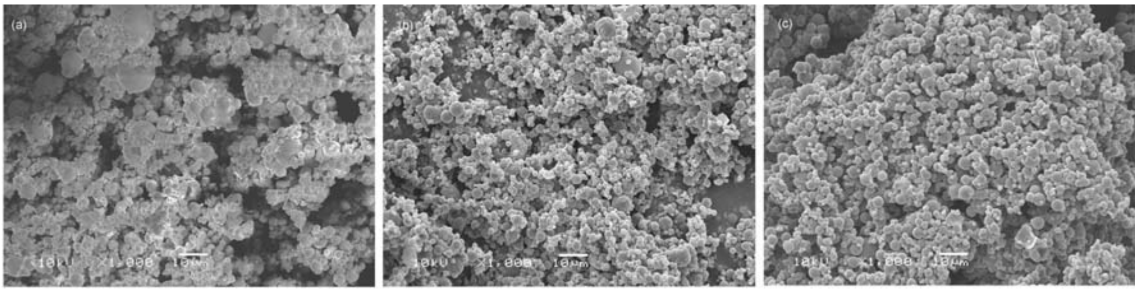


Figure 2

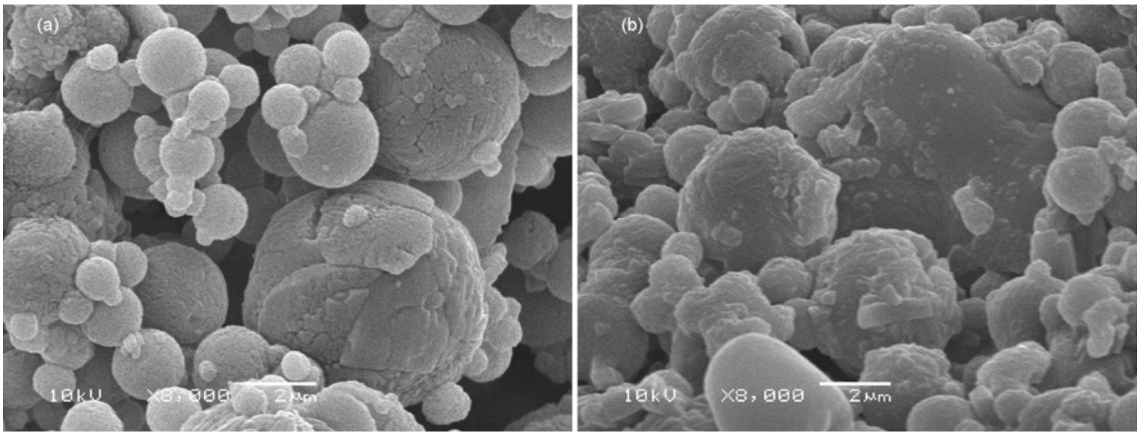


Figure 3

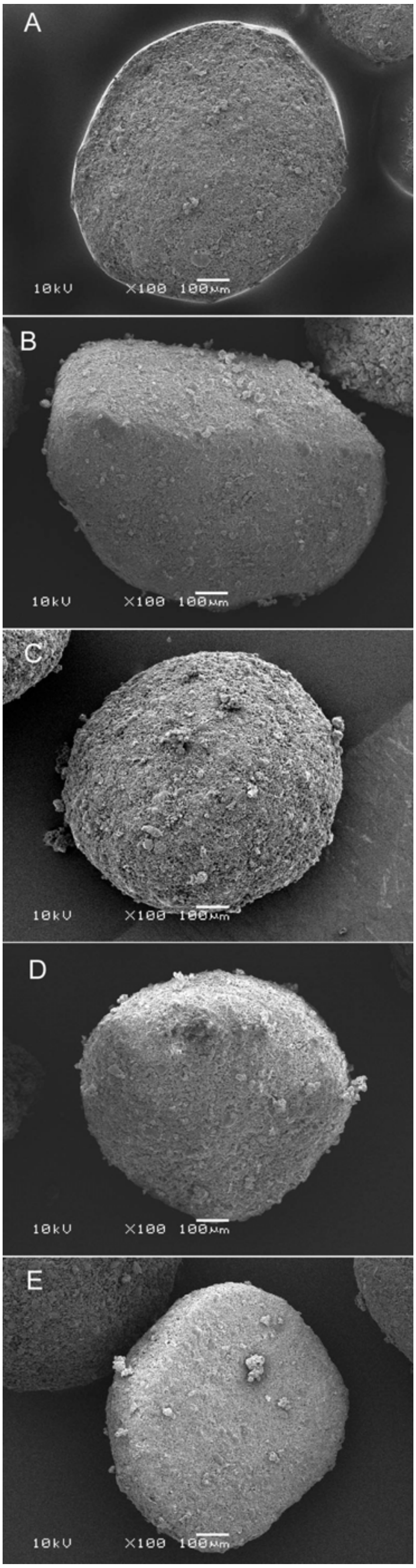

A
10kV ×100 100μm
B
10kV ×100 100μm
C
10kV ×100 100μm
D
10kV ×100 100μm
E
10kV ×100 100μm

Figure 4

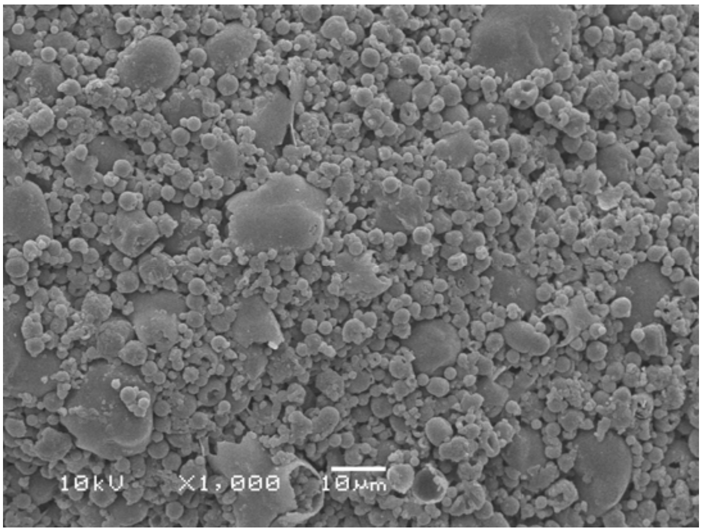


Figure 5

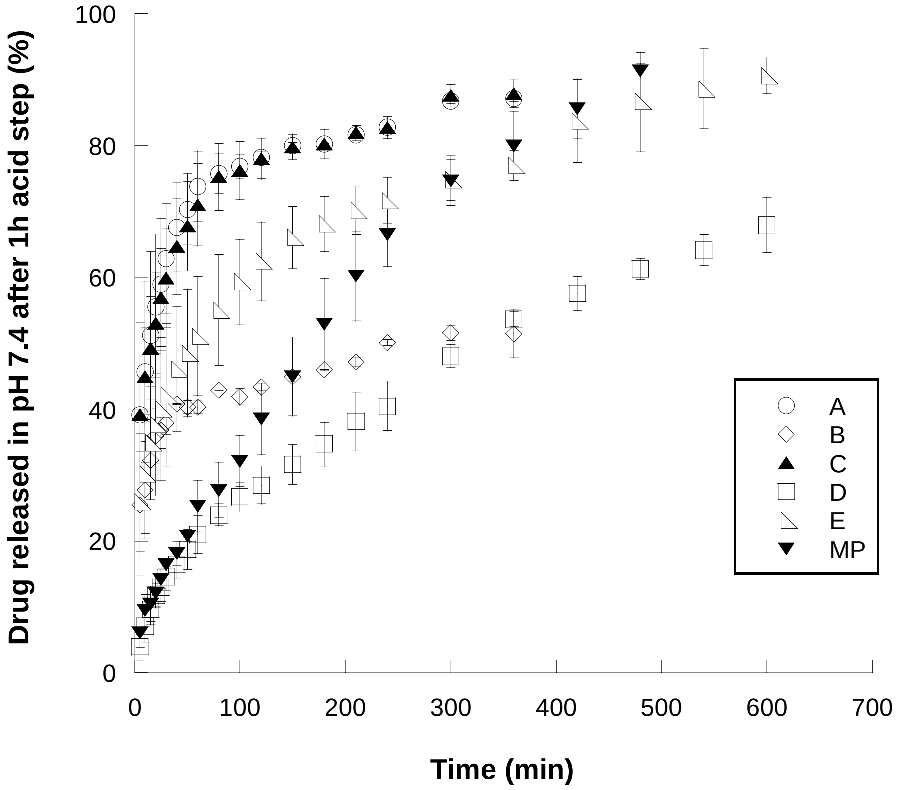

Drug released in pH 7.4 after 1h acid step (%)
100
80
60
40
20
0
0
100
200
300
400
500
600
700
Time (min)
A
B
C
D
E
MP

Figure 6

CAPÍTULO 11: Avaliação da cinética de fotodegradação do pantoprazol. Avaliação da fotoestabilidade do pantoprazol encapsulado em diferentes micropartículas

11.1 Apresentação

A degradação fotoquímica pode ser um fator importante na estabilidade de formas farmacêuticas. A radiação ultravioleta tem alto nível de energia e pode ser a causa de muitas reações de degradação. De acordo com o ICH, a fotoestabilidade intrínseca de fármacos deve ser avaliada para demonstrar que a exposição à luz não causa modificações inaceitáveis no produto. O pantoprazol é um fármaco lábil em meio ácido e na presença de sais, mas sua fotoestabilidade ainda não foi estudada. Deste modo, o objetivo do presente capítulo é avaliar a fotoestabilidade do pantoprazol frente à luz UVC, tanto na forma líquida (solução metanólica) quanto na forma sólida. Além disso, o estudo compreende a determinação da cinética de fotodegradação do pantoprazol.

A encapsulação de fármacos foi uma estratégia utilizada para o aumento da fotoestabilidade da melatonina (TURSILLI *et al.*, 2006) e do butilmetoxidibenzoilmetano (IANNUCCELI *et al.*, 2006). Nestes casos, foram preparadas lipoesferas de triacetina e incorporadas em cremes antes da exposição à luz. Nos capítulos anteriores foram descritas diferentes micropartículas contendo pantoprazol, produzidas por duas diferentes técnicas. As micropartículas descritas nos capítulos 2 e 3 foram preparadas através da técnica de evaporação de solvente utilizando Eudragit® S100 ou sua blenda com poli(ε-caprolactona). Já nos capítulos 4 e 8 foram descritas micropartículas preparadas através da secagem por aspersão utilizando Eudragit® S100 ou sua blenda com HPMC. Neste presente capítulo, estas quatro formulações de micropartículas foram avaliadas frente à luz UVC, com intuito de estudar a fotoestabilidade do pantoprazol nelas encapsulado. A cinética de fotodegradação também foi determinada. Os resultados deste estudo foram submetidos ao periódico *European Journal of Pharmaceutics and Biopharmaceutics*.

Publicação 11: Increasing of sodium pantoprazole photostability by microencapsulation: effect of the polymer and the preparation technique

Increasing sodium pantoprazole photostability by microencapsulation: effect of the polymer and the preparation technique.

Raffin, R. P.[a]; Colomé, L. M.[a]; Schapoval, E. E. S.[a], Pohlmann, A. R.[a,b]; Guterres, S. S.[a*]

[a] Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga, 1752, Porto Alegre, 90610-000, RS, Brazil. Telephone: 55 51 33085500, Fax: 55 51 33085437 (e-mail: nanoc@farmacia.ufrgs.br)

[b] Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio Grande do Sul, CP 15003, Porto Alegre, 91501-970, RS, Brazil

Abstract

Pantoprazole sodium is a proton pump inhibitor, used in acid related disorders, like peptic ulcers and gastroesophageal reflux. This drug is unstable in acid solution and in the presence of salts. The aim of this work was to study the photostability under UVC radiation of pantoprazole and to determine its kinetics. A methanol solution and the solid pantoprazole were evaluated by HPLC within 120 min and 10 days, respectively. The work was also dedicated to evaluate and compare the ability of microencapsulation in stabilizing pantoprazole after UVC radiation. Pantoprazole-loaded microparticles prepared by emulsification/solvent evaporation or spray drying were compared. Pantoprazole was encapsulated using isolated Eudragit S100® or its blend with poly(ε-caprolactone) or HPMC. In methanol solution, pantoprazole was completely degraded after 120 min and presented zero order kinetic with $t_{1/2}$ of 6.48 min. In the solid form, after 10 days, pantoprazole concentration was reduced to 27 % following zero order kinetic. The microparticles prepared only with Eudragit S100® demonstrated an increase of the drug photostability. After 10 days of irradiation, 56 and 44 % of the drug was stable when encapsulated by emulsification/solvent evaporation and spray drying, respectively. The use of polymer blends did not improve the pantoprazole photostability.

Key-words: pantoprazole, photostability, HPLC, microencapsulation, spray drying, solvent evaporation

1. Introduction

Pantoprazole, 5-(difluoromethoxy)-2-[[3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole, is a substituted benzimidazole derivative that inhibits gastric acid secretion by irreversibly binding the proton pump (H^+/K^+-ATPase) in the gastric parietal cells [1]. It is a prodrug that is activated in the acid environment of the canaliculi of the parietal cells. Pantoprazole binds specifically to a region of the proton pump that is crucial for ATPase activity and acid transport [2]. It is indicated in the treatment of *Helicobacter pilory* infections in a triple therapy consisting in its association with clarithromycin and metronidazole. It is very effective in the management of the gastro-esophageal reflux disease and in the treatment of digestive ulcers, reducing the acid output [3].

Pantoprazole has been successfully microencapsulated by two techniques and using different polymers. The solvent evaporation technique was applied in the preparation of gastro-resistant pantoprazole-loaded microparticles using an O/O emulsion. The *in vivo* activity of the pantoprazole loaded- Eudragit S100® microparticles was carried out in rats and showed that the microparticles were able to protect rat stomachs against ulcer formation, while the drug aqueous solution did not present any activity [4]. Using the same technique, microparticles of poly(ε-caprolactone) (PCL) and of its blend with Eudragit S100® were prepared in order to provide drug controlled release and gastro-resistance [5].Only the microparticles prepared with the blend were capable to stabilize the drug in the acid medium. The spray drying technique was also employed to prepare pantoprazole-loaded microparticles. The Eudragit S100® microparticles presented high encapsulation efficiency and good drug stabilization in acid medium [6]. A blend of Eudragit S100® and HPMC was also tested [7,8]. Microparticles presented acceptable drug loading, encapsulation efficiency, surface area and particle size.

The photodegradation of drug dosage forms in solid state depends on particle size surface area, crystal structure and polymorphism [9]. Only the absorbed radiation participates in photodegradation and the dilution of the drug in the excipient, as well as the coating can affect the photostability [10]. Drugs have already been stabilized in coated tablets, colored gelatin capsules, liposomes, lipospheres and by the complexation with cyclodextrines [9]. Tablets containing nifedipine with particle size of 25 or 220 μm were tested regarding the photostability. The tablets with the smaller particles presented drug loss 5-10 % higher [11]. In the same work, two dosages of nifedipine were tested and the photoinstability of nifedipine tablets decreases by increasing the drug content. Lipidic microparticles (lipospheres) were prepared and tested in order to prevent the melatonin photodegradation [12]. Creams containing lipospheres or just melatonin were exposed to light for 2 h. Only the tristearin lipospheres reduced the melatonin loss. In the case of butylmethoxydibenzoylmethane encapsulated in lipospheres of tristearin, the formulations prepared dissolving the drug with ethanol or acetone or dispersing it in the melted lipid were incorporated into a cream and irradiated in solar simulator. All formulations enhanced the drug photostability, but the one avoiding organic solvent was considered the optimum formulation [13].

There are many reports about the pantoprazole instability in acid medium [1,3,14], as well as in the presence of salts [15,16]. However, there is a lack of information about the stability of pantoprazole under UV-Vis radiation. For the other benzimidazoles, omeprazole and lansoprazole, the photodegradation under sunlight simulator has been reported [17]. Both drugs resulted unstable in solution at different pH values when exposed to solar light, which induced significant degradation. The photodegradation kinetic study under UVC light of rabeprazole showed that in methanol solution the degradation was very fast and followed zero order kinetics [18]. The purpose of this work was to determine the kinetics of photodegradation under UVC radiation of pantoprazole in a methanol solution and in the solid form using an HPLC method. In addition, the work was also dedicated to evaluate and compare the ability of microencapsulation in stabilizing pantoprazole after UVC radiation.

2. Materials and methods

2.1 Materials

Sodium pantoprazole sesquihydrate was obtained from Henrifarma (São Paulo, Brazil). Eudragit S100® was kindly gifted by Almapal® (São Paulo, Brazil, produced by Rohm®, Germany). Methocel F4M® was provided by Colorcon® (São Paulo, Brazil, produced by Dow Chemical, USA). Poly(ε-caprolactone) was obtained from Aldrich®, (Milwaukee, USA). All other chemicals were of analytical grade.

2.2 Methods

2.2.1 High-performance liquid chromatography

The stability-indicating HPLC method consisted of a Perkin Elmer serie 200 liquid chromatograph equipped with an UV/Vis detector. Detection was made at 290 nm. The stationary phase was a 150 x 3.9 mm NovaPak C_{18} octadecyl silane column (4 μm particle size) (Waters, Ireland). A security guard cartridge C_{18} (4 x 3 mm) (Phenomenex) was used. The mobile phase was prepared by mixing phosphate buffer pH 7.4 and acetonitrile (65:35 v/v). Then, the mobile phase was filtered using a 0.45 μm membrane filter (Milipore, USA). The injection volume was 20 μL and the flow rate was 0.9 $mL.min^{-1}$. The method was validated for specificity, linearity, precision and accuracy [19].

2.2.2 Preparation of the microparticles by emulsification/solvent evaporation

After dissolving the Eudragit S100® in acetone, pantoprazole sodium sesquihydrate (2:1 w/w polymer to drug ratio) was added (MP1). This suspension was emulsified with mineral oil containing sorbitan monooleate (0.4 %). The O/O emulsion was mechanically stirred for 2.5 h to remove the acetone. The microparticles were collected by filtration and washed with cyclohexane [4]. Another formulation was prepared by dissolving the PCL in acetone and adding the pantoprazole (ratio 1:1 w/w) prior to the emulsification with mineral oil containing sorbitan monooleate (0.4 %) (MP2). After the evaporation of the acetone, a solution of Eudragit S100® in acetone was added to the primary microparticle suspension. The solvent was evaporated and the microparticles collected by filtration and washed with cyclohexane [5].

2.2.3 Preparation of the microparticles by spray drying

Isolated Eudragit S100® (MP3) or blended with Methocel F4M® (MP4) were dissolved in 0.05 M NaOH solution. Pantoprazole was added and the solutions were spray dried (Mini Spray Drier, MSD 1.0, LabMaq, Brazil). The experimental conditions were: 0.8 mm nozzle, inlet temperature of 150 °C and flow rate of 0.44 Lh^{-1} [6,8].

2.2.4 Photodegradation kinetics of pantoprazole in solution

For the photodegradation studies, the light source was an UV fluorescent lamp model Ecolume®, 30W, emitting radiation at 254 nm, fixed to a chamber in a horizontal position. The chamber was internally coated with mirrors in order to distribute the light uniformly. The effect of light was studied exposing the methanol sample solutions in 1 cm quartz cells (duplicates). The temperature was controlled in the chamber (around 25 °C). The photodegradation kinetics of sodium pantoprazole was evaluated in methanol (800 μg.mL^{-1}). The samples were placed horizontally to provide maximum area of exposure to the light source. Considering the UV absorption of pantoprazole, the irradiation was carried out at different time intervals (0, 15, 30, 45, 60, 90 and 120 min). After sampling, each solution was diluted with the mobile phase to give the final concentration of 10 μg.mL^{-1}. The samples were assayed by HPLC. The mathematical modeling of the pantoprazole degradation profiles was performed using Micromath Scientist® software to fit a zero-order (1), a first order (2) or second order (3) kinetics. The best model was chosen based on the highest regression coefficient and the model selection criteria as well as the best graphic adjustment.

$$C = C_o - kt \qquad \text{Eq. (1)}$$

$$\ln C = \ln C_o - kt \qquad \text{Eq. (2)}$$

$$1/C = 1/C_o + kt \qquad \text{Eq. (3)}$$

2.2.5 Photostability evaluation of solid pantoprazole and microencapsulated pantoprazole

The microparticles MP1, MP2, MP3 and MP4 and the drug powder were placed in a very thin layer in watch glasses. Two watch glasses were prepared for each time interval for each formulation. The samples were placed inside the mirror chamber and exposed to UV light for a maximum of 10 days. The samples were collected after 1, 2, 4, 6, 8 and 10 days and evaluated for the drug content.

After sampling, the drug content was evaluated in all samples, according to the method previously described for the pantoprazole solution. The microparticles were suspended in a mixture of 0.05 M NaOH and acetonitrile 1:1 (v/v), kept under magnetic stirring for 3 h protected from light, diluted, filtered and quantified by HPLC.

2.2.6 Statistical analysis

A one-way analysis of variance was employed in the comparison of the experimental data. Post-hoc multiple comparisons were performed by Tukey´s test for significance at p–values less than 0.05.

3. Results and discussion

3.1 High-performance liquid chromatography

The comparison between the chromatograms obtained for pantoprazole or for pantoprazole in the presence of photodegradation products showed that the method was specific for pantoprazole. Linearity was obtained in range of 0.5 and 15.0 $\mu g.mL^{-1}$. The method accuracy was determined by investigating the recovery of pantoprazole at three concentrations. Results indicated recoveries from 99.2 to 104.0 %. Precision was determined for pantoprazole solutions by performing six replicates of the same concentration on three different days. Precision was adequate (RSD = 0.93 %).

3.2 Kinetics of photodegradation of pantoprazole

The methanol solutions developed a yellow color during the experiment, which intensified with time. The concentration of pantoprazole was reduced to 1.7 % after 120 min (Figure 1). The degradation kinetics could be described by zero order kinetic in the experimental conditions of this study. The correlation coefficient was 0.996 and the MSC was 4.1. The apparent degradation rate constant k was 1.424 min^{-1} and the $t_{1/2}$ was 35.11 min.

Pantoprazole was more photostable than rabeprazole, which degraded 88 % in 30 min [18]. Rabeprazole presented zero order kinetics in methanol solution and UVC light.

3.3 Photostability evaluation of solid pantoprazole and microencapsulated pantoprazole

Pantoprazole concentration was reduced much slower than in the methanol solution, but, after 10 days, the drug content was reduced to 27 % (Figure 2). The photodegradation of the solid pantoprazole also followed an apparent zero order kinetic, with degradation constant rate of 7.710 days^{-1}. The $t_{1/2}$ of degradation was 6.5 days.

The drug loading in each microparticle formulations is described in Table 1. In the formulations using only Eudragit S100® (MP1 and MP3), higher drug loadings were achieved. After 10 days of exposure, pantoprazole concentration in MP1 microparticles was 55.7 ± 1.5 % and in MP3 was 44.2 ± 3.1 %, while pure pantoprazole showed 27.2 ± 0.4 % (Figure 3). According to Tukey's test, MP1 and MP3 were statistically different from pure pantoprazole ($p < 0.001$). The microparticles MP1 were able to protect significantly more the drug than the microparticles MP3 ($p = 0.003$). The microencapsulation increased in 2 folds the pantoprazole stabilization.

The microparticles have different particle size, polydispersion and shell thickness. Also, the internal structures are different. The microparticles prepared by emulsification/solvent evaporation are larger (Table 1). The MP1 microparticles are homogeneous and hollow, formed by a solid solution of Eudragit S100® and pantoprazole [4]. On the other hand, the microparticles prepared by spray drying are smaller (less than 10 μm) and the shell is thinner. Furthermore, the spray drying microparticles present blowholes formed by the expansion of the water after the shell rapid solidification [6,7]. The higher pantoprazole content was obtained for the MP1 microparticles (220 mg.g^{-1}). The MP3 microparticles had drug loading of 173 mg.g^{-1}. In this way, we can presume that both factors (particle size and drug content) affected the microparticles, being in agreement with the literature. The MP1 microparticles presented the higher pantoprazole content after irradiation.

When pantoprazole was encapsulated by a polymer blend, different results were obtained. The addition of PCL to the microparticles (MP2) caused a reduction of pantoprazole photostability. The final amount of pantoprazole, after 10 days, was 32.9 ± 0.3 % (Figure 3). These microparticles did not present an increase of pantoprazole photostability ($p = 0.421$) compared to the pure drug. These MP2 microparticles are also hollow, but they are formed of portions of crystalline pantoprazole coated by the two polymers [5]. The addition of HPMC to the microparticles prepared by spray drying caused a reduction of the photostability of pantoprazole ($p < 0.001$). The MP4 microparticles showed that after 10 days of irradiation, only 12.6 ± 0.3 % of the drug was stable (Figure 3). These microparticles had small particle size (7.5 µm) and high surface area (70 m^2g^{-1}).

Concerning the mathematical modeling of the photodegradation of encapsulated pantoprazole, the microparticles MP1, MP2, MP3 and MP4 followed second order kinetics. The degradation rate constants were 0.00098, 0.00154, 0.00119, 0.00396 days^{-1}, respectively and the half-lives of degradation are showed in Table 1.

4. Conclusions

Under UVC light, pantoprazole was demonstrated to be very unstable. In methanol solution, the photodegradation followed zero order kinetic and was completely degraded after 120 min. In the solid form, pure pantoprazole concentration was reduced to 27 % after 10 days of exposure.

The pantoprazole-loaded microparticles showed different effect on the photostability of pantoprazole. The microparticles prepared only with Eudragit S100® demonstrated an increasing of the photostability of the drug. After 10 days of irradiation, 56 and 44 % of the drug was stable when encapsulated by emulsification/solvent evaporation and spray drying, respectively. The use of polymer blends did not improve the pantoprazole photostability. The microparticles formed by a solid solution of the polymer and the drug stabilized the drug, whereas the microparticles containing drug crystals did not.

Acknowledgment

Authors thank Capes, Cap/MCT and Fapergs for the financial support.

References

[1] P.W. Jungnickel, Pantoprazole: a new proton pump inhibitor, Clin. Ther. 22 (2000) pp.1268--1293.

[2] J. Shin, M. Besancon, A. Simon, G. Sachs, The site of action of pantoprazole in the gastric H^+/K^+-ATPase, Biochim. Biophys. Acta 1148 (1993) pp. 223--233.

[3] S. Cheer, A. Prakash, D. Faulds, H. Lamb, Pantoprazole- An Update of its pharmacological properties and therapeutic use in the management of acid-related disorders, Drugs 63(1) (2003) pp.101--132.

[4] R.P. Raffin, L.M. Colomé, A.R. Pohlmann, S.S. Guterres, Preparation, characterization and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles, Eur. J. Pharm. Biopharm. 63 (2006) pp. 198 -- 204.

[5] R.P. Raffin, L.M. Colomé, S.S. Guterres, A.R. Pohlmann, Enteric controlled release pantoprazole-loaded microparticles prepared using Eudragit S100 and poly(ε-caprolactone) blend, Pharm. Dev. Tech. (2007) DOI: 10.1080/10837450701556933.

[6] R. P. Raffin, S.S. Guterres, A.R. Pohlmann, M.I. Ré, Powder characteristics of pantoprazole delivery systems produced in different spray-dryer scales, Drying Tech. 24 (2006) pp. 339 -- 348.

[7] R.P. Raffin, D.S. Jornada, M.I. Ré, A.R. Pohlmann, S.S. Guterres, Sodium Pantoprazole-loaded enteric microparticles prepared by spray drying: effect of the scale of production and process validation, Int. J. Pharm. 324 (2006) pp. 10--18.

[8] R.P. Raffin, L.M. Colomé, S.E. Haas, D.S. Jornada, A.R. Pohlmann, S.S. Guterres, Development of HPMC and Eudragit S100® blended microparticles containing sodium pantoprazole, Pharmazie 62 (2007) pp. 361--364.

[9] K. Thorma, Photodecomposition and stabilization of compounds in dosage forms, in: H.H. Tennes (Ed), Photostability of Drugs and Drug Formulations, CRC Press, London, UK, 1996, pp. 111--140.

[10] B.D. Glass, C.S. Novák, M.E. Brown, The Thermal and Photostability of Solid Pharmaceuticals: A review. J. Therm. Anal. Calorim. 77 (2004) pp.1013--1036.

[11] W. Aman, K. Thoma, The influence of formulation and manufacturing process on the photostability of tablets. Int. J. Pharm. 243 (2002) pp. 33--41.

[12] R. Tursilli, A. Casolari, V. Iannuccelli, S. Scalia, Enhancement of melatonin photostability by encapsulation in lipospheres. J. Pharm. Biomed. Anal. 40 (2006) pp. 910--914.

[13] V. Iannuccelli, N. Sala, R. Tursilli, G. Coppi, S. Scalia, Influence of liposphere preparation on butyl-methoxydibenzoylmethane photostability. Eur. J. Pharm. Biopharm. 63 (2006) pp. 140--145.

[14] Z.A. El-Sherif, A.O. Mohamed, M.G. El-Bardicy, M.F. El-Tarras, Reversed-Phase High Performance Liquid Chromatographic Method for the Determination of Lansoprazole, Omeprazole and Pantoprazole Sodium Sesquihydrate in Presence of Their Acid-Induced Degradation Products. Chem. Pharm. Bull. 54 (2006) pp. 814--818.

[15] A. Ekpe, T. Jacobsen, Effect of Various Salts on the Stability of Lansoprazole, Omeprazole, and Pantoprazole as Determined by High- Performance Liquid Chromatography. Drug Dev. Ind. Pharm. 25 (1999) pp.1057--1065.

[16] R.P. Raffin, L.M. Colomé, S.S. Guterres, A.R. Pohlmann, Validation of analytical methodology by HPLC for quantification and stability evaluation of sodium pantoprazole, Quim. Nova 30 (2007) pp 1001--1005.

[17] M. DellaGreca, M.R. Iesce, L. Previtera, M. Rubino, F. Temussi, M. Brigante, Degradation of lansoprazole and omeprazole in the aquatic environment. Chemosphere 63 (2006) pp. 1087--1093.

[18] C.V. Garcia, N.S. Nudelman, M. Steppe, E.E.S. Schapoval, Structural Elucidation of Rabeprazole Sodium Photodegradation Products. J. Pharm. Biomed. Anal. doi:10.1016/j.jpba.2007.09.002

[19] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Validation of analytical procedures: methodology, 1996.

Table 1. Drug loading, particle size, surface area and half-life of degradation of the microparticles.

Microparticles	Drug loading ($mg.g^{-1}$)	Particle size (μm)	Specific surface area (m^2g^{-1})	Photodegradation $t_{1/2}$ (days)
MP1	220.0 ± 15.8	56.2[4]	41[4]	10.2
MP2	159.8 ± 8.3	455.9[5]	36[5]	6.5
MP3	172.7 ± 6.3	6.7[6]	87[6]	8.4
MP4	131.8 ± 1.0	7.5[7]	70[7]	2.5

Figure 1. Pantoprazole concentration in the methanol solution after exposure to UVC light.

Figure 2. Pantoprazole concentration after exposure of the solid drug to UVC light.

Figure 3. Pantoprazole concentration after exposure of the pure solid drug (▲), microparticles MP1 (□), MP2 (▽), MP3 (○) and MP4 (◇).

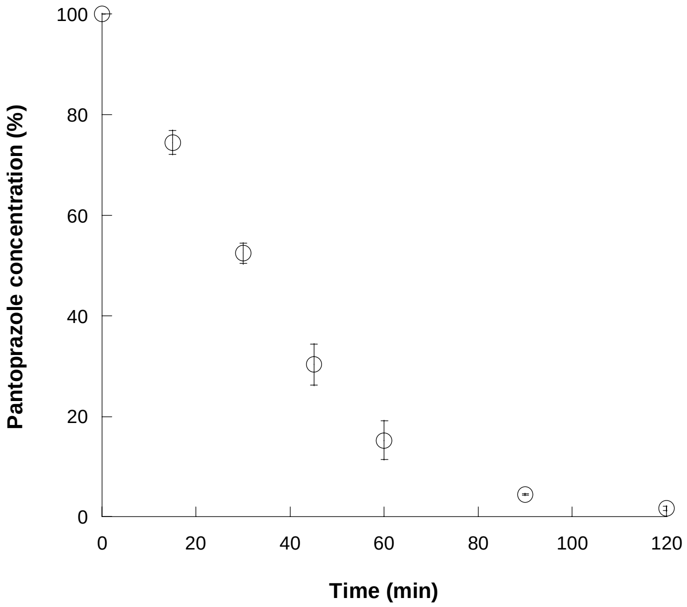

Pantoprazole concentration (%)
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (min)

Figure 1

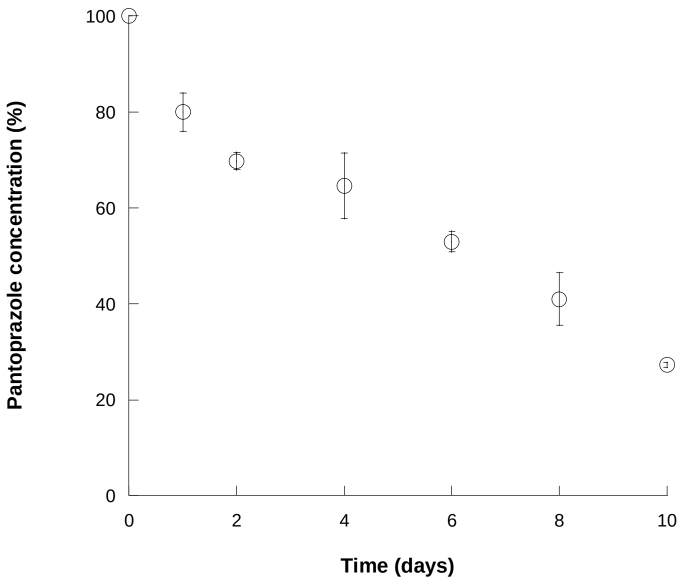

Pantoprazole concentration (%)
100
80
60
40
20
0
0
2
4
6
8
10
Time (days)

Figure 2

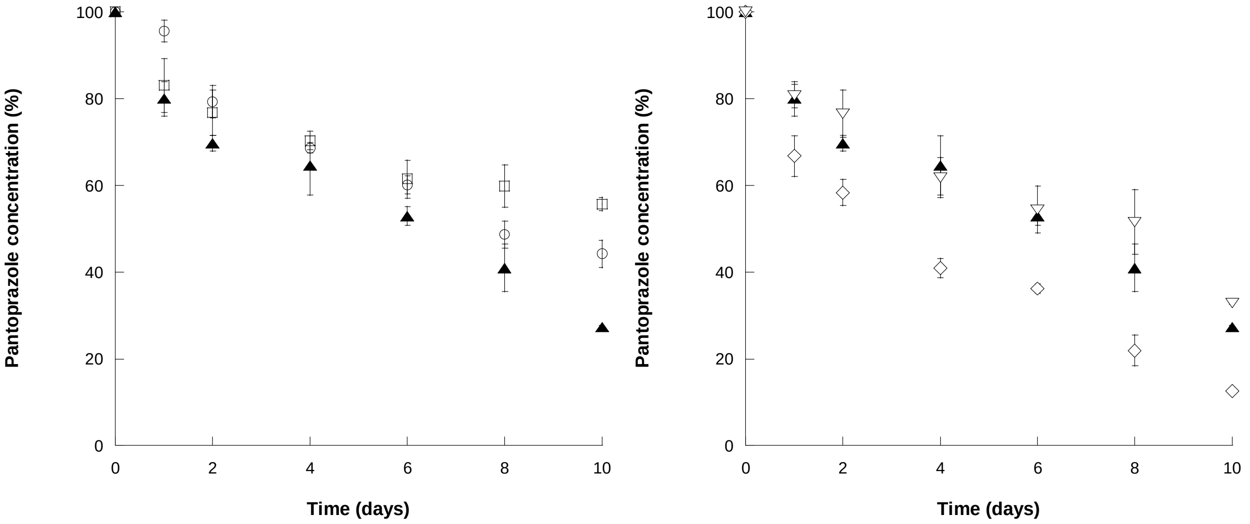

Pantoprazole concentration (%)
0
20
40
60
80
100
0
2
4
6
8
10
Time (days)
Pantoprazole concentration (%)
0
20
40
60
80
100
0
2
4
6
8
10
Time (days)

Figure 3

CAPÍTULO 12: Avaliação farmacocinética de aglomerados contendo pantoprazol em cães

12.1 Apresentação

O último capítulo desta tese compreende a avaliação farmacocinética de uma formulação desenvolvida e apresentada nos capítulos anteriores. A formulação selecionada foram os aglomerados contendo micropartículas preparadas por *spray drying* com Eudragit® S100 (capítulo 7). Estes aglomerados apresentaram rápida desintegração e não interferiram na alta gastro-resistência apresentada pelas micropartículas de pantoprazol. Estas micropartículas apresentaram-se estáveis em estudo sob condições aceleradas de armazenamento e aumentaram a estabilidade do pantoprazol frente à luz. O objetivo do presente capítulo é a avaliação da biodisponibilidade relativa dos aglomerados em relação ao comprimido comercial de pantoprazol em cães, com intuito de se obter uma forma farmacêutica gastro-resistente e de liberação imediata. O artigo contento estes resultados encontra-se em fase de redação.

Publicação 12: Immediate-release agglomerates containing enteric pantoprazole-loaded microparticles: pharmacokinetic study

Immediate-release agglomerates containing enteric pantoprazole-loaded microparticles: a pharmacokinetic study in dogs

Renata P. Raffin[1], Letícia M. Colomé[1], Cristiane R. D. Hoffmeister[1], Paolo Colombo[2], Fabio Sonvico[2], Lucas M. Colomé[3], Claudio Natalini[3], Adriana R. Pohlmann[4], Silvia S. Guterres[1*], Teresa Dalla Costa[1]

[1]Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.
[2]Dipartimento di Tecnologia Farmaceutica, Università degli Studi di Parma, Parma, Italy.
[3]Departamento de Farmacologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.
[4]Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.
*silvia.guterres@ufrgs.br

Abstract

The aim of the present study was to evaluate the bioavailability of a new drug delivery system containing pantoprazole. Gastro-resistance pantoprazole-loaded microparticles were prepared by spray-drying, mixed with the excipient compounded of mannitol and lecithin and agglomerated by vibration. The agglomerates presented complete drug loading and high production yield. One single oral dose (40 mg) was administered to 6 dogs. Each dog received either the reference tablet (Pantozol®) or the agglomerates with one week wash out period. The AUC, Cmax and ke of the agglomerates were not statistical different from the reference. The relative bioavailability was 94 %. The Tmax after the agglomerate administration was shorter than the reference tablet. The reference tablet present zero order absorption and the agglomerates presented a flip-flop condition and a first order absorption. The agglomerates were effective in reducing the time to peak plasma and can be used as a platform to prepared immediate-release enteric formulations.

Key words: pantoprazole, microparticles, agglomerates, pharmacokinetics, immediate-release, gastro-resistance

Introduction

Enteric dosage forms can be formulated as single units or as multiple unit systems. However, most enteric-coated formulations are formulated as a single unit. More recently, multiple unit systems have been developed, due to their rapid dispersion in the gastrointestinal tract, the maximization of drug absorption, reduction of peak plasma fluctuations, minimization of potential side effects without lowering drug bioavailability (Lin & Kao, 1991). They also reduce variations in gastric emptying rates and overall transit times and minimize differences in plasma levels among individuals. They are also less susceptible to dose dumping than single-unit dosage forms (Ghebre-Sellassie, 1989, Mehta 2001, Shimizu 2003)

Polymeric microparticles can be produced by several techniques, including physical and chemical methods, as coacervation, solvent evaporation and spray drying. The spray drying technique has some advantages over other methods as the absence of organic solvents during the preparation, the one step process and the facility of scaling up (Beck et al., 2004).

Pantoprazole is a proton pump inhibitor, which inactivates the final step in the gastric acid secretion pathway in gastric parietal cells of the stomach and it is indicated in the treatment of digestive ulcers, gastroesophageal reflux disease and in association with other drugs in the eradication of the *Helicobacter pylori* (Jungnickel, 2000). Pantoprazole is more stable than its analogues, omeprazole, lansoprazole and especially rabeprazole under neutral to moderately acidic conditions (Cheer et al., 2001). The greater pH selectivity of pantoprazole suggests that it less susceptible than the analogues to accumulate in the body. In the acidic environment of the gastric parietal cells, pantoprazole is activated in the form of cyclic sulfenamide that binds to one or more cysteine residues of the proton pump, which leads to irreversible inhibition of acid transport. Pantoprazole binds specifically to cysteine 822, while omeprazole, lansoprazole and rabeprazole bind to one or two additional sites (Cheer et al., 2001). Concerning pantoprazole pharmacokinetics, after single or multiple oral doses a marked interindividual variability in many pharmacokinetic parameters (time to reach peak concentration (tmax) ranged from 1 to 6 h). The Cmax was observed between 2 and 4 h (median 2.8 h) and the absolute bioavailability was calculated to be 77 % (from 67 to 89 %) after a single administration of 40 mg tablet. The absorption of pantoprazole was delayed by food for up to 8 h. Plasma protein binding of pantoprazole is 98 % and the Vd/F was calculated to be from 0.11 to 0.17 L/kg (Klotz, 2000).

The different enteric coatings, which are necessary to protect the proton pump inhibitors from acid degradation in the stomach, have the disadvantage of delaying its absorption. In this way, some strategies to circumvent this limitation have already been studied. A lansoprazole orally disintegrating tablet was developed based on tablets containing enteric-coated pellets. The oral pharmacokinetics of lansoprazole after the administration of this tablet were identical to those obtained after administration of conventional capsules of enteric-coated granules (Baldi et al., 2003, Shimizu et al., 2003). Recently, an immediate-release omeprazole formulation (powder for oral suspension) was launched in the marked. The formulation consists of pure omeprazole powder 40 mg or 20 mg per unit with 1680 mg of sodium bicarbonate to be reconstituted with water. This formulation displayed shorted tmax and higher Cmax that delayed release omeprazole pellets. After the administration of an omeprazole solution without bicarbonate, the AUC was reduced 10 times compared to the immediate-release formulation (Howden, 2005).

In order to obtain a gastro-resistant formulation that presented an immediate release of pantoprazole, we microencapsulated this drug by spray drying using Eudragit S100 as enteric polymer (Raffin et al., 2006). These microparticles had physico-chemical characteristics adequate for oral drug delivery, caused a prompt dissolution of the drug in pH 7.4 and showed less than 10 % of drug degradation after acid exposure for 1 h. The process parameters and the scale up of these microparticles were also evaluated and the optimum operational conditions were chosen. In another study (Raffin et al., 2007a), the stability of microparticles containing pantoprazole was evaluated in a stability chamber for 6 months. Microparticles were stable at 40 °C and 75 % RH. In the *in vivo* anti-ulcer evaluation, the microparticles significantly reduced the ulcer formation compared to the pantoprazole aqueous solution. Nevertheless, these microparticles did not present adequate technological characteristics to produce tablets, due to their poor flow and high compressibility index. In addition, after compression, these microparticles were damaged and the gastro-resistance reduced. The alternative to overcome those drawbacks was to develop soft agglomerates containing the pantoprazole-loaded microparticles, held together by the addition of an excipient prepared with mannitol and lecithin (Raffin et al., *in press*). These agglomerates showed immediate disintegration and fast release, without reducing the gastro-resistance of the microparticles. Taking into account those findings, the aim of the present study was to determine in dogs the pharmacokinetics of the agglomerates containing pantoprazole-loaded microparticles and to compare the pharmacokinetics parameters with a commercial delayed release pantoprazole tablet formulation.

Materials and methods

Materials

Sesquihydrate sodium pantoprazole was purchased from Henrifarma (São Paulo, Brazil). Methacrylic acid copolymer (Eudragit® S100) was kindly given by Almapal (São Paulo, Brazil). Mannitol (Ph. Eur.) was a gift of Lisapharma (Como, Italy) and lecithin (Lipoid S75) was supplied by Lipoid AG (Ludwigshafen, Germany). All other chemicals were of analytical grade.

Methods

Preparation of the agglomerates containing pantoprazole-loaded microparticles

Pantoprazole-loaded microparticles were prepared in a pilot scale spray-dryer (Model PSD 52 APV Anhydro, Soeborg, Denmark) using a centrifugal atomizer at 30,000 rpm, inlet temperature of 180 °C, outlet temperature of 65 °C and feed rate of 2 $L.h^{-1}$. The sprayed solution was prepared by dissolving Eudragit® S100 (48 g) and pantoprazole (12 g) in NaOH aqueous solution (6 $g.L^{-1}$) and the final volume was 1 L. The microparticles were prepared in duplicates.

The agglomeration excipient was prepared by dissolving mannitol in 90 mL of water. Lecithin was dissolved in 10 mL of ethanol at 40 °C and mixed with mannitol solution (87.5:12.5 w/w) giving an opalescent mixture. Final solid concentration was 4 % (w/v) and the batch volume was 500 mL. Two replicates were spray-dried using a Buchi Mini Spray Dryer B-190 (Buchi Laboratoriums-Tecnik, Flawil, Switzerland) in the following conditions: inlet temperature 90 °C, outlet temperature 38-40 °C, feed rate 5.0 $mL.min^{-1}$, nozzle diameter 0.7 mm, drying N_2 flow 300 $L.h^{-1}$.

The pantoprazole-loaded microparticles and the excipient were mixed at 1:1 (w/w) ratio in a 300 mL PET container rotating at 40 rpm for 120 min. The mixtures (15 g each) were placed on the top of a stack of two sieves with nominal apertures of 106 and 850 μm respectively (25 cm diameter sieves, Granutest, Brazil), which was vibrated for 5 minutes on a sieve shaker (amplitude 1-2; Bertel, Brazil). Agglomerates between 106 and 850 μm were collected. Reprocessing the non-agglomerated powder and crushing the larger agglomerates, the process was done 5 times (Raffin et al., *in press*).

Characterization of the microparticles and the agglomerates

The spray-drying yield was calculated by the ratio between the sum of all solids weights and the obtained mass, expressed in percentage. The agglomeration yield was calculated by the ratio between the initial mixture weight and the mass of the agglomerated obtained, expressed in percentage. The residual moisture was measured by Karl Fisher titration (DL31, Mettler Toledo). The drug loading was assayed by a validated HPLC method (Raffin et al., 2007b). Briefly, an amount of the microparticles or agglomerates, equivalent to 10 mg of pantoprazole, was weighed and magnetically stirred with 40 mL of 0.05 mol.L-1 NaOH for 1 h in a volumetric flask. The volume was completed to 50 mL and drug concentration was determined after filtration (0.45 μm) by HPLC (Perkin Elmer series 200) using a NovaPak C18 column (4 μm particle size) (Waters, Ireland). A security guard cartridge C18 (4 x 3 mm) (Phenomenex) was used. Mobile phase consisted of acetonitrile/phosphate buffer pH 7.4 (35:65 v/v), the flow rate was 0.9 mL.min-1 and detector wavelength was set at 290 nm. The particle size distribution was determined by laser diffractometry (Mastersizer 2000, Malvern Instruments, London, UK) after dispersion in *iso*-octane. The mean diameter over the volume distribution $d_{4.3}$ was used. The cohesiveness of the pantoprazole-loaded microparticles and the mannitol/lecithin excipient, as well as the mixtures in different times of the mixing process was measured in a Powder Characteristics Tester, Model PT-N (Hosokawa Microns), based on the ability of the powder to pass through sieves under vibration. More cohesive powders form agglomerates and do not pass the sieves.

Pharmacokinetics study

The study was designed as one single dose, randomized, crossover studies, with a 7-day washout period. After an overnight fasting (12 h), a single dose of either formulation (reference Pantozol® - Altana Pharma, or agglomerates of pantoprazole) was administered to 6 female dogs weighting 10-15 kg. In order to administer the agglomerates, hard gelatin capsules 00 were filled with agglomerates. The dose of

40 mg was given with 50 mL of water. The dog's legs were shaven and cannulated through the cephalic vein using a 22-gauge catheter. Blood samples (2 mL) were withdrawn at pre-dose and up to 12 h post-dose in each period. During all the study dogs had free access to water and after 3 h of the administration of the dose, they received a standard meal (200 g, Royal Canan). The blood was centrifuged at 5,000 rpm for 7 min and the plasma was frozen in liquid nitrogen prior to the HPLC analysis. The protocol was approved by the University Ethics Committee # 2007668.

Analytical method

300 μL of icy methanol containing lansoprazole 1 $\mu g.mL^{-1}$ (internal standard) was added to 300 μL of plasma. The samples were vortexed for 1 min and centrifuged at 12,000 rpm for 15 min. The supernatant was collected and analyzed by HPLC (Perkin Elmer series 200) using a NovaPak C_{18} column (4 μm particle size) (Waters, Ireland). A security guard cartridge C_{18} (4 x 3 mm) (Phenomenex) was used. Mobile phase consisted of acetonitrile/phosphate buffer pH 7.4 (30:70 v/v), the flow rate was 0.9 $mL.min^{-1}$, the detector wavelength was set at 290 nm and the injection volume was 50 μL. The method was validated according to ICH for the concentrations between 0.04 and 4.0 $\mu g.mL^{-1}$.

Pharmacokinetic and statistical analysis

Pharmacokinetic parameters were calculated employing noncompartmental methods. Cmax, the maximum observed concentration, and Tmax, the time of observed peak concentration, were determined for each dog and for each treatment. The area under the curve (AUC) was calculated by the linear trapezoidal rule plus extrapolation for the terminal part of the curve. To calculate the elimination rate constant (Ke), regression analyses were performed on the ln of plasma concentration values versus time. The apparent half-life (t1/2) was calculated as 0.693/Ke. The relative bioavailability (f_{rel}) was calculated by equation (1), where D is the dose and "test" and "ref" correspond to the reference tablet and the agglomerates, respectively.

$$f_{rel} = \left(\frac{AUC_{test} * D_{ref}}{AUC_{ref} * D_{test}} \right) * 100 \qquad \text{Eq. 1}$$

The statistical analysis was assessed by means of an analysis of variance (ANOVA) and the confidence intervals were 95 %. For each formulation, mean percent absorbed–time plots were obtained by deconvolution of the corresponding mean concentration versus time plots using the Wagner–Nelson method. These plots allowed the construction of percent unabsorbed–time plots, which were used for the evaluation of the absorption rate constants (Gibaldi and Perrier, 1982).

Results

Pantoprazole-loaded microparticles were obtained with yield of 78 %. The residual moisture was low (2.4 %) and the mean diameter was in agreement with that previously described for batches of this formulation (28.3 μm) (Raffin et al., 2006, Raffin et al., 2007a). Drug loading was 161.1 ± 0.8 mg.g^{-1} and the encapsulation efficiency was 97.9 ± 0.5 %. Microparticles cohesiveness was very low (12 %), and that did not form agglomerates. For this reason, in a previous work, the excipient composed of mannitol and lecithin was developed.

The mannitol/lecithin excipient was prepared presenting a yield of 57 %, residual moisture of 1.3 % and the mean size of 5.02 μm. The powder presented high cohesiveness of 44 %.

During the mixing process, after 30, 60, 90 and 120 min, the cohesiveness of the mixture was measured. An increase of the mixture cohesiveness was achieved with a maximum after 120 min of mixing (32 %). Then, the mixture was transferred to the sieves and vibrated for the formation of the agglomerates. The agglomeration yield (80.5 %) was higher than the previous report (76.2 %) (Raffin et al., 2007a). The agglomerates presented drug loading of 80.3 ± 0.4 mg of pantoprazole per gram of the agglomerates. The size distribution of the agglomerates after redispersion in iso-octane presented two populations, one of 5 µm corresponding to the excipient of mannitol and lecithin original size and the other at 30 µm corresponding to the pantoprazole-loaded microparticles original size. No additional peaks were observed.

Validation of the analytical method for the determination of pantoprazole in plasma

The analytical method presented a limit of quantification of 0.05 µg.mL^{-1} and a linear calibration curve from 0.05 to 4.00 µg.mL^{-1}, with correlation coefficient of 0.998. Three quality controls (0.08, 0.40 and 1.80 µg.mL^{-1}) were selected. The precision had relative standard deviations for the quality controls of 1.28, 1.34 and 1.73 %, respectively. The accuracy was 106.63 ± 0.02, 102.44 ± 0.43 and 105.15 ± 1.48 %, respectively. Mean plasma recovery was 95 %. These results were in accordance with the criteria of ICH.

The mean plasma profiles obtained after oral administration of a single dose (40 mg) in showed in Figure 1.

The noncompartimental analysis demonstrated that the plasma concentrations after administration of pantoprazole presented large variability among individuals, as previously reported for humans (Campos et al., 2007). The pharmacokinetic parameters are shown in Table I.

There was no significant difference between the $AUC_{0-\infty}$ from the tablet and the agglomerates and the relative bioavailability was 94 %. The microencapsulation and the agglomeration processes did not influenced the extension of the absorption of pantoprazole. The ke did not present significant difference between the reference tablet and the agglomerates, as well as the Cmax. On the other hand, the Tmax was reduced after the agglomerate administration ($p = 0.017$). Both formulations presented the same AUC, showing that the microparticles protected the drug in the same extension as the tablets did. However, the time to reach the peak plasma was reduced showing an advantage of the multiple-unit systems. This difference in the Tmax can be explained by the gastric emptying. In the case of large particles, as conventional single-unit tablets, the emptying from the stomach appears to be delayed, which can be attributed to the influence of peristalsis on gastric emptying and the GI. On the other hand, the gastric emptying of pellets with a size less than 1.4 mm is almost as fast as liquid emptying, occurring within minutes of intake (Schreier, 2001).

The Wagner-Nelson method was applied to the data in order to obtain the absorbed fraction profile. The absorption of the reference tablet followed zero order and 100 % was absorbed in 90 min. The drug was absorbed in the rate of 1.6 % per minute, showing a 30 min of lagtime after administration. Concerning the agglomerates administration, the profile is a flip-flop condition and after 1 h of the administration, 70 % of the dose is absorbed. The absorption followed first order.

Conclusions

The pantoprazole-loaded microparticles were agglomerated with mannitol/lecithin spray-dried. The agglomerates presented high yield and complete drug loading. After redispersion, the primary particle sizes were recovered. The analytical method for the determination of pantoprazole in plasma was validated for linearity, precision and accuracy. There was no difference between the reference tablets and the agglomerates concerning the AUC, Cmax and ke. The Tmax of the agglomerates was lower than the tablets, reducing the time to reach the peak plasma.

Acknowledgement

Authors thank Capes, Fapergs and CNPq/MCT. Authors also thank M.I. Ré for the support with agglomerate preparation. M. Nudelman and L. Suné are acknowledged for the assistance with the pharmacokinetics experiments.

References

C. W. Howden, Review article: immediate-release proton-pump inhibitor therapy-potential advantages Aliment Pharmacol Ther 22 (2005) 25–30.

D. Campos, N. R. Vieria, G. Bernasconi, F. A. Barros, E. Meurer, M. A. Marchioretto, E. Coelho, S. A. Calafatti, C. Sommer, J. Couto, S. Buranello, A. Silva, A. Amarante, E. Abib, J. Pedrazzoli, Bioequivalence of Two Enteric Coated Formulations of Pantoprazole in Healthy Volunteers under Fasting and Fed Conditions. Arzneimittel-Forschung/Drug Research 57 (2007), 309-314.

F. Baldi, P. Malfertheiner, Lansoprazole Fast Disintegrating Tablet: A New Formulation for an Established Proton Pump Inhibitor Digestion 67 (2003) 1–5.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 1996. Validation of analytical procedures: methodology.

K. A. Mehta, M. S. Kislalioglu, W. Phuapradit, A. W. Malick and N. H. Shah, Release performance of a poorly soluble drug from a novel, Eudragit®-based multi-unit erosion matrix, *Int. J. Pharm.* 213 (2001) pp. 7-12.

M. Gibaldi, D. Perrier 1982. Pharmacokinetics, second ed. (Revised and expanded). Marcel Dekker, New York (Chapter 4).

P.W. Jungnickel, Pantoprazole: a new proton pump inhibitor, Clin. Ther. 22 (2000) 1268-1293.

R. C. R. Beck, A. R. Pohlmann, S. S. Guterres, Nanoparticle-coated microparticles: preparation and characterization J. Microencapsulation, 21(2004) 499–512.

R. P. Raffin, S.S. Guterres, A.R. Pohlmann, M.I. Ré, Powder characteristics of pantoprazole delivery systems produced in different spray-dryer scales, Drying Tech. 24 (2006) pp. 339 - 348.

R.P. Raffin, L.M. Colomé, E.E.S. Schapoval, D.S. Jornada, A.R. Pohlmann, S.S. Guterres, Gastro-Resistant Microparticles Containing Sodium Pantoprazole: Stability Studies and *In Vivo* Anti-Ulcer Activity, The Open Drug Delivery Journal, 1 (2007) 44-51.

R.P. Raffin, L.M. Colomé, S.S. Guterres, A.R. Pohlmann, Validation of analytical methodology by HPLC for quantification and stability evaluation of sodium pantoprazole, Quim. Nova 30 (2007) pp 1001-1005.

R. P. Raffin, P. Colombo, F. Sonvico, F. S. Polleto, G. Colombo, A. Rossi, A. R. Pohlmann, S. S. Guterres, Soft Agglomerates of Pantoprazole Gastro-resistant Microparticles for Oral Administration and Intestinal Release, Journal of Drug Delivery Systems and Technology, in press.

S. Cheer, A. Prakash, D. Faulds, H. Lamb, Pantoprazole-An Update of its pharmacological properties and therapeutic use in the management of acid-related disorders. Drugs 63 (2001) 101-132.

H. Schreier (Ed.). *Drug Targeting Technology: Physical, Chemical, and Biological Methods.* New York, NY, USA: Marcel Dekker Incorporated, 2001. p 22.

T. Shimizu, Y. Nakano, S. Morimoto, T. Tabata, N. Hamaguchi, Y. Igari Formulation Study for Lansoprazole Fast-disintegrating Tablet. I. Effect of Compression on Dissolution Behavior *Chem. Pharm. Bull.* 51(8) (2003) 942-947.

Table I. Mean pharmacokinetic parameters of pantoprazole for both reference tablet and agglomerates after oral administration (40 mg).

Parameter	Reference tablet	Agglomerates
$AUC_{0\text{-}t}$ (μg.h/mL)	298 ± 50	214 ± 201
$AUC_{0\text{-}\infty}$ (μg.h/mL)	305 ± 48	219 ± 202
ke (min^{-1})	0.015 ± 0.004	0.022 ± 0.009
$t_{1/2}$ (min)	47.5 ± 12.0	34.1 ± 10.1
C_{max} (μg/mL)	2.83 ± 0.79	2.8 ± 2.1
T_{max} (min)	90 ± 0	43 ± 16

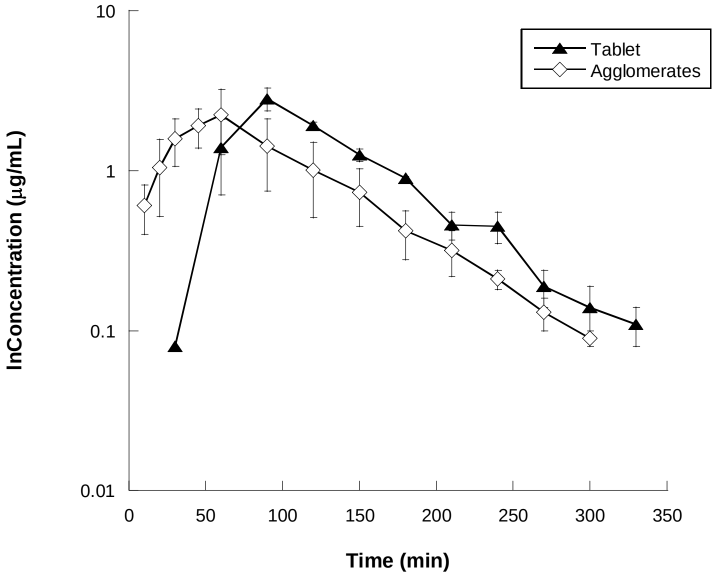


Figure 1. Mean plasma concentration after oral administration of 40 mg of pantoprazole.

CONSIDERAÇÕES FINAIS

O presente trabalho objetivou desenvolver sistemas multiparticulados contendo pantoprazol a fim de obter sua estabilização frente ao meio ácido estomacal, com conseqüente liberação entérica. As formulações foram desenvolvidas com duas finalidades; uma planejando uma liberação imediata do fármaco e outra de liberação controlada.

A etapa inicial do trabalho foi a validação da metodologia analítica para quantificação do fármaco por cromatografia líquida de alta eficiência. Os resultados demonstraram que o método utilizado foi linear, preciso, exato, específico e reprodutível para a faixa de análise utilizada (0,5 a 20 µg/mL). A grande vantagem encontrada no método desenvolvido foi o baixo limite de quantificação (0,51 µg/mL) comparado com os já descritos (MANSOUR e SOROUR, 2001, BADWAN *et al*., 2002), o que permite maior precisão nos ensaios de dissolução e estabilidade. Foi realizado também um estudo de estabilidade do fármaco em tampão fosfato pH 7,4, a fim de investigar a presença de produtos de degradação que possam ser formados durante ensaios de dissolução, uma vez que já foi descrito por EKPE e JACOBSEN (1999) que o pantoprazol é instável na presença de sais. O estudo da estabilidade mostrou que o pantoprazol apresenta meia vida de degradação de 124 h em tampão fosfato, o que garante que menos de 2 % seja degradado em 6 a 8 h, tempo necessário para os ensaios de dissolução de formas farmacêuticas de liberação controlada.

Iniciou-se o desenvolvimento das micropartículas pela técnica de evaporação de solvente, utilizando um sistema anidro (emulsão O/O) a fim de se obter maiores taxas de encapsulação do fármaco. Eudragit$^{®}$ S100 é um polímero pH dependente, solúvel em soluções aquosas em pH acima de 7 (BEIL *et al.,* 1992) e usado no revestimento entérico de comprimidos. Duas proporções de polímero e fármaco foram estudadas, porém apenas as micropartículas preparadas com a proporção 2:1 (m/m) (Eudragit$^{®}$ S100:pantoprazol) conferiram proteção ao fármaco frente ao meio ácido. As micropartículas apresentaram proteção inferior ao preconizado pela Farmacopéia Brasileira 4^{a} edição (1988), que é de 90 %, porém a formulação, quando administrada em ratos, protegeu a mucosa gástrica da ulceração causada por etanol. Além disso, utilizando as técnicas de calorimetria diferencial exploratória (DSC) e espectroscopia na região do infra-vermelho, pôde-se verificar que o fármaco apresentou-se molecularmente disperso no polímero, indicando sua encapsulação (FORD e TIMMINS, 1999).

Considerando que as indicações terapêuticas do pantoprazol incluem doenças bastante distintas, diferentes micropartículas com características específicas podem ser preparadas a fim de atender as necessidades de cada uma. Por exemplo, quando o pantoprazol é utilizado para o tratamento de pacientes com gastrite, em que a dor inicia, muitas vezes, após ingestão de alguns alimentos e a secreção gástrica deve ser interrompida o mais rapidamente a fim de aliar a dor, comprimidos de liberação imediata são mais recomendados. Por outro lado, em pacientes com refluxo gastro-esofágico, o refluxo é mais intenso pela manhã, ao acordar ou durante a noite. Nestes casos, comprimidos de liberação prolongada garantiriam liberação do fármaco até 6 a 8 horas após administração. Neste intuito, foi adicionado à formulação anterior um segundo polímero, a poli(ε-caprolactona) (PCL). Este polímero é um poliéster biodegradável, utilizado para retardar a liberação de diversos fármacos (SINHA *et al.*, 2004). Foram preparadas micropartículas com apenas PCL e com duplo revestimento de PCL e Eudragit® S100. Apenas as micropartículas preparadas com a adição dos dois polímeros apresentaram proteção do fármaco em ambiente ácido. A análise térmica demonstrou que o pantoprazol não forma uma fase homogênea com os polímeros e os cristais de fármaco estão recobertos pelos dois polímeros. Essas micropartículas apresentaram maior diâmetro do que as preparadas apenas com Eudragit® S100 (456 μm) e apresentaram redução na formação de ulceras induzidas por etanol em ratos, em comparação com a solução aquosa de pantoprazol. Os comprimidos contendo estas micropartículas conferiram 60 % de estabilização ao pantoprazol após 1 h em meio ácido e liberação controlada por 6 h. No entanto, as micropartículas preparadas por evaporação de solvente não se apresentaram tecnologicamente promissoras, porque a técnica é de difícil aumento de escala, consome elevada quantidade de solvente orgânico e gera um grande volume de resíduo de óleo mineral.

Micropartículas contendo pantoprazol foram, então, preparadas por aspersão, utilizando-se apenas Eudragit® S100, porém dissolvido em solução aquosa de hidróxido de sódio em substituição à acetona. A proporção utilizada de polímero e fármaco foi de 4:1 (m/m). As micropartículas produzidas se apresentaram esféricas, sem cristais visíveis na superfície das partículas (indicativo de fármaco encapsulado) e com baixa polidispersão em relação ao tamanho de partícula. Estas micropartículas apresentaram alta proteção do pantoprazol frente ao meio ácido em relação às micropartículas obtidas por evaporação de solvente, além de redução do diâmetro médio (de 56,25 µm para 6,70 µm) e aumento na área superficial (de 41 m^2/g para 87 m^2/g). As análises por DSC demonstraram que o fármaco apresentava-se molecularmente disperso no polímero, indicando tratarem-se de microesferas (sistemas matriciais em que há apenas uma fase, não havendo núcleo).

A etapa seguinte do trabalho foi o estudo das condições do processo em escala piloto a fim de se otimizar a produção de micropartículas nesta escala. A primeira variável de processo avaliada foi a porcentagem de sólidos na solução de alimentação do *spray drier*. Foram testadas concentrações crescentes (2,2 %, 4,4 % e 6,6 % de sólidos) baseadas na concentração anteriormente empregada (2,2 %). Considerando que, em escala laboratorial, não foram verificadas diferenças entre os pós produzidos com 4,4 % e 6,6 % de sólidos, apenas as porcentagens mais alta e mais baixa foram avaliadas na escala piloto. Foram produzidas micropartículas utilizando quatro configurações do equipamento piloto: [1] atomizador duplo fluido, ar/*spray* contato co-corrente e pressão do ar de 49 kPa; [2] atomizador duplo fluido, ar/*spray* contato co-corrente com pressão do ar de 196 kPa; [3] atomizador duplo fluido, contato tipo misto entre ar e *spray* e pressão de 196 kPa e [4] atomizador rotativo a 30000 rpm e contato co-corrente. O fator que mais influenciou o diâmetro das partículas foi a concentração de sólidos na solução de alimentação, sendo que os diâmetros variaram entre 7 µm (2,2 %) a 30 µm (6,6 %). Por outro lado, o fator que mais influenciou a capacidade protetora das micropartículas em relação ao fármaco em meio ácido foi a integridade das partículas, ou seja, a ausência ou a presença em pequena quantidade de orifícios, rachaduras e partículas fraturadas. Com base na porcentagem de proteção em meio ácido (98 %), as condições consideradas ideais foram 6,6 % de sólidos na solução, atomizador rotativo com contato co-corrente.

A reprodutibilidade entre lotes foi avaliada preparando-se 3 lotes destas micropartículas por aspersão em escala piloto sob as seguintes condições: contato co-corrente, atomizador rotativo e concentração total de sólidos de 6,6 %. Os três lotes apresentaram valores similares de rendimento do processo, teor de fármaco, diâmetro médio e fluidez. As micropartículas apresentaram rápida liberação do fármaco e os perfis de dissolução foram modelados matematicamente. Os perfis de liberação apresentaram curva monoexponencial e a velocidade de liberação foi dependente da concentração inicial do pantoprazol. Quanto à modelagem utilizando a equação de Korsmeyer-Peppas, o mecanismo de liberação indicado foi transporte super caso II (não fickiano), sendo que a liberação do fármaco pode ser explicada pelo inchamento, relaxação e dissolução do polímero, solúvel em pH acima de 7.0. A avaliação *in vivo* também mostrou resultados satisfatórios de proteção frente à ulceração por etanol em ratos. Foi realizado estudo de estabilidade acelerada das micropartículas a 40 °C e 75 % de umidade relativa, em frascos lacrados ou não. Ambas amostras de micropartículas apresentaram menos de 5 % de redução no teor em 6 meses e apenas as micropartículas não lacradas apresentaram aumento de umidade em 3 %. A estabilidade das micropartículas também foi avaliada frente à luz UVA. A microencapsulação do pantoprazol aumentou sua estabilidade de 11,6 % para 54,4 % em 96 h.

Na seqüência do trabalho, visando a obtenção de micropartículas de liberação controlada, foram preparadas formulações utilizando uma blenda de Eudragit® S100 e um tipo de hidroxipropilmetilcelulose, o Methocel® F4M pela técnica de *spray drier*. As micropartículas apresentaram diâmetro médio de 11 µm, compatível com a escala laboratorial, alta taxa de encapsulação e proteção em meio ácido similar às partículas produzidas apenas com Eudragit® S100. A liberação do pantoprazol a partir dessas micropartículas ocorreu em 480 min, um aumento de 4 vezes no tempo da liberação das micropartículas preparadas com Eudragit® S100 por *spray drying* (120 min). Da mesma maneira que para as formulações anteriores, as análises por DSC demonstraram a encapsulação do fármaco, neste caso, na blenda polimérica. O ensaio *in vivo* demonstrou novamente que as micropartículas foram efetivas na proteção da mucosa gástrica frente à ulceração por etanol.

O próximo passo foi o estudo do aumento de escala de produção desta formulação de micropartículas. Da mesma forma que para a formulação anterior, a concentração de sólidos foi aumentada (de 2,3 % para 3,4 %). Neste caso, com o aumento da concentração de sólidos na solução de alimentação ocorreu aumento da quantidade de pantoprazol estabilizado nas micropartículas. Foi, então, selecionada a maior concentração de sólidos (3,4 %) para produção em escala piloto. Entretanto, a quantidade de sólidos não foi aumentada na mesma proporção que para a formulação anterior devido ao aumento de viscosidade da solução. Soluções muito viscosas dificultam o processo de secagem e os produtos formados podem não ser homogêneos ou apresentar umidade muito alta. As mesmas quatro configurações ([1] atomizador duplo fluido, ar/*spray* contato co-corrente e pressão do ar de 49 kPa; [2] atomizador duplo fluido, ar/*spray* contato co-corrente com pressão do ar de 196 kPa; [3] atomizador duplo fluido, contato tipo misto entre ar e *spray* e pressão de 196 kPa e [4] atomizador rotativo a 30000 rpm e contato co-corrente) foram testadas, porém para apenas uma concentração de sólidos (3,4 %). Dos quatro produtos obtidos, o pó produzido com a configuração [1] apresentou fios de polímero misturados às micropartículas, decorrência da baixa pressão de ar utilizada, insuficiente para a formação de gota de uma solução mais viscosa. A configuração [2] propiciou a obtenção de micropartículas com com baixa polidispersão, esféricas, com baixa incidência de orifícios e fraturas. As micropartículas preparadas com a configuração [3] apresentaram alta polidispersão e diâmetro médio acima de 100 µm e as micropartículas preparadas na configuração [4] apresentaram cristais de pantoprazol adsorvidos na superfície. Em decorrência destes resultados e por apresentarem alta proteção em meio ácido, as micropartículas preparadas com atomizador duplo fluido, contato co-corrente e pressão de ar de 196 kPa [2] foram selecionadas para a validação do processo de produção. Foram, então, preparados três lotes em três dias consecutivos e comparados em termos de diâmetros médios, densidades, fluidez, taxas de encapsulação, áreas superficiais e umidade. Todas as características avaliadas não apresentaram diferenças significativas entre os lotes, demonstrando a reprodutibilidade do processo.

Todas micropartículas descritas acima e produzidas em escala laboratorial foram comprimidas utilizando o mínimo de adjuvantes necessários (estearato de magnésio e celulose microcristalina) a fim de se verificar a influência da compressão na liberação do pantoprazol. As micropartículas produzidas por evaporação de solvente foram comprimidas utilizando estearato de magnésio e celulose microcristalina como adjuvantes, pois a presença de tensoativo ou óleo que não foi removido impediu a compressão apenas com estearato de magnésio. Os comprimidos apresentam proteção ao meio ácido 3 vezes superior que as micropartículas não comprimidas. Por outro lado, para as micropartículas preparadas por *spray drying*, foi utilizado apenas estearato de magnésio para compressão. Os comprimidos aumentaram a proteção ao meio ácido acima dos 90 % preconizados pela Farmacopéia Brasileira IV. A liberação do pantoprazol foi concentração-dependente, porque nos comprimidos, nos quais a concentração total de pantoprazol era maior, a liberação foi mais rápida que a partir das micropartículas. Esse resultado não era esperado uma vez que os comprimidos possuem uma fase de desintegração ausente nas micropartículas. Em todos os comprimidos produzidos as micropartículas permaneceram integras em seu interior, de acordo com a análise por microscopia eletrônica de varredura.

Por outro lado, na tentativa de comprimir as micropartículas obtidas em escala piloto, o baixíssimo fluxo e as más características de compressão dificultaram a preparação de comprimidos viáveis industrialmente. Além disso, a tentativa de granular ou diretamente comprimir as micropartículas também não foi efetiva pois muitas foram danificadas e a gastro-resistência foi diminuída. A solução encontrada para a preparação de uma forma farmacêutica sólida final foi o desenvolvimento de aglomerados ou “soft pellets”. Estes aglomerados são partículas com diâmetro médio de 450 μm formadas pela adição de um excipiente nebulizado de manitol e lecitina às micropartículas. Estes aglomerados são facilmente desintegrados em contato com água, retomando o tamanho inicial das micropartículas (RUSSO *et al.*, 2005). As micropartículas preparadas por *spray drying* em escala piloto utilizando apenas Eudragit® S100 ou a mistura deste com HPMC foram aglomeradas com diferentes excipientes de manitol e lecitina. Em ambos casos, os aglomerados incorporaram praticamente todas as micropartículas e apresentaram uma grande melhora nas características tecnológicas, como fluxo, por exemplo. A adição do excipiente não afetou a integridade das micropartículas, nem reduziu a gastro-resistência das mesmas.

A fim de avaliar o comportamento *in vivo* dos aglomerados, foi realizado um estudo comparativo da farmacocinética em cães deste e do comprimido comercial (liberação modificada). Os aglomerados contendo as micropartículas preparadas com Eudragit® S100 apresentaram uma rápida absorção e *Tmax* menor que da formulação comercial. A biodisponibilidade relativa foi de 94 %.

Análogos do pantoprazol, como omeprazol, lansoprazol e rabeprazol, apresentam fotoinstabilidade e, em solução, são rapidamente degradados (DELLAGRECA *et al.*, 2006, GARCIA *et al.*, 2008). Em relação ao pantoprazol, não havia relatos na literatura de sua cinética de fotodegradação. Foram analisados o pantoprazol em solução metanolica e na forma sólida frente à luz UVC. Em solução o pantoprazol é muito instável, sendo totalmente degradado em 120 min. Na forma sólida, a degradação foi de 73 % em 10 dias. A cinética de degradação foi de ordem zero em ambas formas. As 4 formulações de micropartículas preparadas em escala laboratorial pelas técnicas de evaporação de solvente e *spray drying* foram testadas em relação à estabilidade frente à luz UVC. As micropartículas de Eudragit® S100 (pelas duas técnicas utilizadas) apresentaram aumento (2 x) da estabilidade do pantoprazol encapsulado em relação ao pantoprazol sólido, demonstrando que essas formulações além de protegerem o fármaco do meio ácido, aumentam sua fotoestabilidade.

A secagem por aspersão mostrou-se mais rápida e dispensou o uso de solventes orgânicos. Entretanto, esta técnica limita-se a tamanhos fixos de partícula (pela formação do *spray* da amostra e passagem pela abertura do atomizador), quando produzida em escala laboratorial. Por outro lado, diferentes tamanhos de partículas foram obtidos na escala piloto.

Ambas as formulações preparadas por *spray-drying* apresentaram características viáveis à produção em escala piloto e foram produzidas com a melhor configuração do equipamento a fim de aumentar a estabilidade do pantoprazol em meio ácido. A compressão das micropartículas aumentou o tempo de liberação e aumentou em mais de 3 vezes a proteção ao meio ácido. A diminuição da área superficial e a redução da erosão contribuíram para esse resultado.

As micropartículas apresentaram adequada estabilidade frente ao calor, umidade e luz. As características de fluxo foram melhoradas com a utilização da técnica de aglomeração. Os aglomerados reduziram o tempo de absorção do pantoprazol em relação ao comprimido comercial e não alteraram a biodisponibilidade do mesmo, sendo uma interessante forma farmacêutica de liberação imediata para fármacos ácido-lábeis.

CONCLUSÕES

- A metodologia analítica desenvolvida para a análise do pantoprazol por CLAE demonstrou ser específica, linear entre 0.05 e 15 µg/mL, precisa e exata, atendendo aos requisitos da legislação vigente.
- O pantoprazol sódico foi microencapsulado com sucesso, pela técnica de emulsificação/evaporação de solvente.
- As micropartículas preparadas com Eudragit® S100 apresentaram rápida dissolução e capacidade de proteger a mucosa gástrica *in vivo* da ulceração frente ao etanol.
- As micropartículas preparadas com a blenda de Eudragit® S100 e poli(ε-caprolactona) apresentaram liberação lenta do fármaco e os comprimidos preparados a partir destas estabilizaram o pantoprazol em meio ácido.
- A técnica de secagem por aspersão produziu micropartículas com características adequadas para administração via oral, sem o uso de solventes orgânicos.
- A adição de HPMC à formulação de Eudragit® S100 controlou a liberação do pantoprazol a partir das micropartículas.
- O estudo dos parâmetros do processo de secagem por aspersão, bem como a transposição para a escala piloto, foram fundamentais para a obtenção de micropartículas com maior gastro-resistência.
- O mecanismo de liberação do pantoprazol a partir das micropartículas de Eudragit® S100 produzidas por *spray-drying* foi a sobreposição de inchamento, relaxação e dissolução do polímero, solúvel em pH acima de 7.0.
- As micropartículas produzidas em escala piloto apresentaram características de fluxo pobres e foram danificadas com a compressão ou granulação.
- A preparação de aglomerados ou “soft pellets”, utilizando excipiente nebulizado de manitol e lecitina, mostrou ser uma alternativa interessante para veicular as micropartículas, uma vez que o tamanho inicial das mesmas é retomado ao contato com líquidos.
- O processo de aglomeração não alterou a gastro-resistência e a liberação do pantoprazol a partir das micropartículas.
- Os aglomerados apresentaram rápida absorção e *tmax* menor e mais homogêneo que do comprimido comercial. Não houve diferença na biodiponibilidade dos aglomerados e do comprimido comercial.

- O pantoprazol apresentou alta instabilidade frente à luz UVC tanto em solução metanólica quanto na forma sólida.
- A encapsulação do pantoprazol aumentou a fotoestabilidade do mesmo quando o polímero utilizado foi o Eudragit® S100, independentemente da técnica de preparação. A utilização de blendas poliméricas não aumentou a fotoestabilidade do fármaco.
- Os aglomerados contendo micropartículas de pantoprazol apresentaram-se bioequivalentes aos comprimidos comerciais.
- Os aglomerados demonstraram ser uma forma farmacêutica de liberação imediata e gastro-resistente, reduzindo o Tmax em relação ao comprimido comercial.

REFERÊNCIAS BIBLIOGRÁFICAS

ALVES, G. P., SANTANA, M. H. A. Phospholipid dry powders produced by spray drying processing: structural, thermodynamic and physical properties. **Powder Technology**, v.145, p.139-148, 2004.

AMERI, M.; MAA, Y. F. Spray Drying of Biopharmaceuticals: Stability and Process Considerations. **Drying Technology**, v.24, p.763–768, 2006.

AMORIM, M.; FERREIRA, J. Microparticles for delivering therapeutic peptides and proteins to the lúmen of the small intestine. **European Journal of Pharmaceuticals and Biopharmaceuticals**, v.52, p.39-44, 2001.

BADWAN, A. A.; NABULSI, L. N.; OMARI, A. L.; DARAGHMEH, M. M.; ASHOUR, M.; ABDOU, A. M.; JABER, A. M.; Pantoprazole sodium. In: Florey, K.; Brittain, H.G. (Ed.). **Analytical Profiles of Drug Substances and Excipients** vol. 29, Academic Press, Elsevier Science: USA, 2002. p. 213-259.

BARBATO, F.; ROTONDA, M.I.; MAGLIO, G.; PALUMO, R.; QUAGLIA, F. Biodegradable microspheres of a novel segmented poly(ether-ester-amide)s based on poly(ε-caprolactone) for the delivery of bioactive compounds. **Biomaterials**, v.22, p.1371-1378, 2001.

BARTHE, L., BESSOUET, M., WOODLEY, J. F.; HOUIN, G. The Improved everted gut sac: a simple method to study intestinal P-glycoprotein. **International Journal of Pharmaceutics**, v.173, p.255–258, 1998.

BASTAKI, S, CHANDRANATH, I.; GARNER, A. Comparison of five antisecretory agents acting via gastric H+/K+- ATPase. **Journal of Physiology**, v.94, p.19-23, 2000.

BECK, R. C. R., POHLMANN, A. R., BENVENUTTI, E., DALLA COSTA, T. C.; GUTERRES, S. S. Nanostructure-coated diclofenac-loaded microparticles: Preparation, morphological characterization, *in vitro* release and *in vivo* gastrointestinal tolerance. **Journal of the Brazilian Chemical Society**, v.16, p.1233-1240, 2005.

BECKERT, T.; LEHMANN, K.; SCHMIDT, P. Compression of enteric-coated pellets to disintegrating tablets. **International Journal of Pharmaceutics**, v.143, p.13-23, 1996.

BEIL, W.; STAAR, U.; SEWING, K. Pantoprazole: a novel H^+/K^+-ATPase inhibitor with an improved pH stability. **European Journal of Pharmacology**, v.218, p.265-271, 1992.

BENITA, S. (Ed.) **Microencapsulation: Methods and Industrial Applications**. Marcel Dekker, Inc. New York, 1996, preface.

BENOIT, J. P., H. MARCHAIS, H. ROLLAND,; V. V. VELDE. Biodegradable Microspheres: Advances in Production Technology. In BENITA, S. (Ed.). **Microencapsulation: methods and industrial applications**. New York: Marcel Dekker, 1996. p. 35-72

BILLON, A.; PETIT, M.; DOKO, M.;BATAILLE, B.; JACOB, M. Effects of cellulose derivatives and additives in the spray-drying preparation of acetaminophen delivery systems. **Drug Development and Industrial Pharmacy**, v.*25*, p.1149-1156, 1999.

BIRCHAL, V.; PASSOS, M. L.; WILDHAGEN, G.; MUJUMDAR, A. Effect of Spray-Dryer Operating Variables on the Whole Milk Powder Quality. **Drying Technology**, v.23, p. 611–636, 2005.

BODMEIER, R.; MCGINITY, J. W. The preparation and evaluation of drug-containing poly(*dl*-lactide) microspheres formed by solvent evaporation method. **Pharmaceutical Research**, v.4, p.465-471, 1987.

BRUNI, A. T. **Estudos teóricos aplicados à farmacologia: omeprazol e compostos análogos.** 2000. Tese (doutorado) Instituto de Química, Universidade Estadual de Campinas, Campinas, SP, 2000.

CASS, Q.; DEGANI, A.; CASSIANO, N.; PEDRAZOLLI JR., J. Enantiomeric determination of pantoprazole in human plasma by multidimensional high-performance liquid chromatography. **Journal Chromatography B**, v.766, p.153-160, 2001.

CARRENO-GÓMEZ, B., WOODLEY, J. F.; FLORENCE, A. T. Studies on the uptake of tomato lectin nanoparricles in everted gut sacs. **International Journal of Pharmaceutics**, v.183, p. 7-11, 1999.

CHEER, S.; PRAKASH, A.; FAULDS, D.; LAMB, H. Pantoprazole- An Update of its pharmacological properties and therapeutic use in the management of acid-related disorders. **Drugs**, v.63, p.101-132, 2003.

CILURZO, F.; SELMIN, F.; MINGHETTI, P.; RIMOLDI, I.; DEMARTIN, F.; MONTANARI, L. Fast-dissolving mucoadhesive microparticulate delivery system containing piroxicam. **European Journal of Pharmaceutical Sciences**, v.24, p.355–361, 2005.

CULSHAW, K., VIERQUTZ, S., EDWARDS, A.; SCHLANDER, M. A decision analysis comparing the cost-effectiveness of pantoprazole in England P077. **Gut**, v.41, n.4S, p.97A-98A, 1997.

DELLAGRECA, M., IESCE, M., PREVITERA, L., RUBINO, M., TEMUSSI, F.; BRIGANTE, M. Degradation of lansoprazole and omeprazole in the aquatic environment. **Chemosphere**, v.63, p.1087-1093, 2006.

DENTINGER, P.; SWENSON, C.; ANAIZI, N. Stability of pantoprazole in an extemporaneously compounded oral liquid. **American Journal of Healthy-System Pharmacy**, v.59, n.10, p.953-956, 2002.

EKPE, A.; JACOBSEN, T. Effect of various salts on the stability of lansoprazole, omeprazole and pantoprazole as determined by high-performance liquid chromatography. **Drug Development and Industrial Pharmacy**, v.25, p.1057-1065, 1999.

ESPOSITO, E.; CERVELLATI, F.; MENEGATTI, E.; NASTRUZZI, C.; CORTESI, R. Spray dried Eudragit microparticles as encapsulation devices for vitamin C. **International Journal of Pharmacy**, v.242, p.329-334, 2002.

FERRON, G.; KU, S.; ABELL, M.; UNRUH, M.; GETSY, J.; MAYER, P. R.; PAUL, J. Oral bioavailability of pantoprazole suspended in sodium bicarbonate solution. **American Journal of Health-System Pharmacy**, v.60, p.1324-1329, 2003.

FITTON, A.; WISEMAN, L. Pantoprazole- a review of its pharmacological properties and therapeutic use in acid-related disorders. **Drugs**, v.51, n.3, p.460-482, 1996.

FORD, J. L.; TIMMINS, P. **Pharmaceutical Thermal Analysis–Techniques and Applications.** New York: Ellis Horwood Limited, 1999. 190-200 p.

FOSTER, T.; LAETHERMAN, M. Powder characteristics of proteins spray-dried from different spray-dryers. **Drug Development and Industrial Pharmacy**, v.21, p.1705-1723, 1995.

FREITAS, S.; MERKLE, H. P.; GANDER, B. Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. **Journal of Controlled Release**, v.102, p.313–332, 2005.

GAO, Y.; ZHOU, S.; WEN, J.; HUANG, M.; XU, A. Mechanism of the antiulcerogenic effect of *Ganoderma lucidum* polysaccharides on indomethacin-induced lesions in the rat. **Life Sciences**, v.72, p.731-745, 2002.

GARCIA, C. V., NUDELMAN, N. S., STEPPE, M., SCHAPOVAL, E. E. S. Structural Elucidation of Rabeprazole Sodium Photodegradation Products. **Journal of Pharmaceutics Biomedical Analysis**, v.46, n.1, p.88-93, 2008.

GAVINI, E.; SANNA, V.; JULIANO, C. ; GIUNCHEDI, P. Compressed biodegradable matrices of spray-dried PLGA microspheres for the modified release of ketoprofen. **Journal of Microencapsulation**, v.20, n.2, p.193-201, 2003.

HEGAZY, N.; DEMIREL, M.; YAZAN, Y. Preparation and in vitro evaluation of pyridostigmine bromide microparticles. **International Journal of Pharmaceutics**, v.242, p.171-174, 2002.

HERRMANN, J.; BODMEIER, B. Biodegradable, somatostatin acetate containing microspheres prepared by various aqueous and non-aqueous solvent evaporation methods. **European Journal of Pharmaceuticals and Biopharpharmaceuticals**, v.45, p.75-82, 1998.

HOMBRERO-PÉREZ, M.; SIEPMANN, J.; ZINUTTI, C.; LAMPRECHT, A.; UBRICH, N.; HOFFMAN, M.; BODMEIER, R.; MAINCENT, P. Non-degradable microparticles containing a hydrophilic and/or lipophilic drug: preparation, characterization and drug release modeling. **Journal of Controlled Release**, v.88,p.413-428, 2003.

HOUT, B.; KLOK, R.; BROUWERS, J.; POSTMA, M. A pharmacoeconomic comparison of the efficacy and costs of pantoprazole and omeprazole for the treatment of peptic ulcer or gastroesophageal reflux disease in the Netherlands. **Clinical Therapeutics**, v.25, n.2, p.635-646, 2003.

IANNUCCELLI, N. SALA, R. TURSILLI, G. COPPI, S. SCALIA, S. Influence of liposphere preparation on butyl-methoxydibenzoylmethane photostability. **European Journal of Pharmaceutics Biopharmaceutics**, v.63, p.140-145, 2006.

JAIN, R.; SHAH, N.; MALICK, A.W.; RHODES, C. Controlled Drug Delivery by Biodegradable Poly(ester) Devices: Different Preparatives Approaches. **Drug Development and Industrial Pharmacy**, v.24, p.703-727, 1998.

JOHANSEN, P.; MERKLE, H. P.; GANDER, P. Technological considerations related to the up-scaling of protein microencapsulation by spray-drying. **European Journal of Pharmaceutics and Biopharmaceutics**, v.50, p.413-417, 2000.

JOHNSON, C. E. Stability of pantoprazole in 0.9% sodium chloride injection in polypropylene syringes. **American Journal of Health-System Pharmacy**, v.62, p.2410-2412, 2005.

JUNGNICKEL, P.W. Pantoprazole: a new proton pump inhibitor. **Clinical Therapeutics**, v.22, n.11, p.1268-1293, 2000.

KARLJIKOVIC-RAJIC, K.; NOVOVIC, D.; MARINKOVIC, V.; AGBABA, D. First-order UV-derivative spectrophotometry in the analysis of omeprazole and pantoprazole sodium salt and corresponding impurities. **Journal of Pharmaceutical and Biomedical Analysis**, v.32, p.1159-1166, 2003.

KATZ, P. Pharmacological basis of pantoprazole dosing. **Current Therapeutics Research**, v.61, n.8, p.507-522, 2000.

KILIÇARSLAN, M.; BAYKARA, T. The effect of the drug/polymer ratio on the properties of the verapamil HCl loaded microspheres. **International Journal of Pharmaceutics**, v.252, p.99-109, 2003.

KIM, B.; HWANG, S.; PARK, J.; PARK, H. Preparation and characterization of drug-loaded polymethacrylate microspheres by an emulsion solvent evaporation method. **Journal of Microencapsulation**, v.19, p.811-822, 2002.

KIM, C. Release kinetics of coated, donut-shaped tablets for water soluble drugs. **European Journal of Pharmaceutical Sciences**, v.7, p.237-242, 1999.

KONTUREK, P.; BRZOZOWSKI, T.; KWIECIEN, S.; DROZDOWICZ, D.; HARSCH, I.; MEIXNER, H.; STACHURA, J.; HAHN, E.; KONTUREK, S. Effect of *Helicobacter pylori* on delay in ulcer healing induced by aspirin in rats. **European Journal of Pharmacology**, v.451, p.191-202, 2002.

KRISTMUNDSDÓTTIR, T., GUDMUNDSSON, O.; INGVARSDÓTTIR, K. Release of diltiazem from Eudragit microparticles prepared by spray-drying. **International Journal of Pharmaceutics**, v.137, p.159-165, 1996.

LABENZ, J.; PETERSEN, K.; RÖSCH, W.; KOELZ, H. A summary of Food and Drug Administration-reported adverse events and drug interactions occurring during therapy with omeprazole, lansoprazole and pantoprazole. **Alimentary Pharmacology and Therapeutics**, v.17, p.1015-1019, 2003.

LIN, S.Y.; KAO, Y.H. Tablet study of spray-dried sodium diclofenac enteric-coated microcapsules. **Pharmaceutical Research**, v.8, n.7, p.919-924, 1991.

LIU, E. S.; CHO, C.H. Relationship between ethanol-induced gastritis and gastric ulcer formation in rats. **Digestion**, v.62, p.232-239, 2000.

LIU, X. M.; ZAKARIA, M. N. M.; ISLAM, M. W.; RADHAKRISHNAN, R.; ISMAIL, A.; CHEN, H. B.; CHAN, K.; AL-ATTAS, A. Anti-inflamatory and anti-ulcer activity of *Calligonum comosum* in rats. **Fitoterapia**, v.72, p.487-491, 2001.

MANSOUR, A.; SOROUR, O. High performance liquid chromatographic determination of pantoprazole in tablet dosage form. **Chromatographia**, v.53, p.S-478-S-479, 2001.

MASTERS, K.. **Spray drying Handbook.** New York:Halsted Press, 1985.

McCLEAN, S.; O'KANE, E.; RAMACHANDRAN, V.; SMYTH, W. Differential pulse polarographic study of the degradation of H^+/K^+ ATPase inhibitors SK&F 95601 and omeprazole in acidic media and the subsequent reaction with thiols. **Analytica Chimica Acta**, v.292, p.81-89, 1994.

METZ, D.; SOFFER, E.; FORSMARK, C.; CRYER, B.; CHEY, W.; BOCHENEK, W.; PISEGNA, J. Maintenance Oral Pantoprazole Therapy Is Effective for Patients With Zollinger-Ellison Syndrome and Idiopathic Hypersecretion. **The American Journal of Gastroenterology**, v.98, n.2, p.301-307, 2003.

MURILLO, M.; GAMAZO, C.; GOÑI, M.; IRACHE, J.; BLANCO-PRÍETO, M. Development of microparticles prepared by spray-drying as a vaccine delivery system against brucellosis. **International Journal of Pharmaceutics**, v.242, p.341-344, 2001.

NAKAMURA, C.; OTAKA, M.; ODASHIMA, M.; JIN, M.; KONISHI, N.; HORIKAWA, Y.; MATSUHASHI, T.; WATANABE, S. Rolipram, a specific type IV phosphodiesterase inhibitor, ameliorates indomethacin-induced gastric mucosal injury in rats. **Pathophysiology**, v.9, p.195-200, 2003.

OBEIDAT, W. M.; PRICE, J. C. Preparation and in vitro evaluation of propylthiouracil microspheres made of Eudragit RL 100 and cellulose acetate butyrate polymers using the emulsion-solvent evaporation method. **Journal of Microencapsulation**, v.22, n.3, p.281-289, 2005.

O'DONNELL, P.; MCGINITY, J. Preparation of microspheres by the solvent evaporation technique. **Advanced Drug Delivery Reviews**, v.28, p.25-42, 1997.

OLIVEIRA, F.; VIERIA-JÚNIOR, G.; CHAVES,M.; ALMEIDA, F. FLORÊNCIO, M. LIMA Jr, R. C. P.; SILVA, R. M.; SANTOS, F. A.; RAO, V. S. Gastroprotective and anti-inflammatory effects of resin from *Protium heptaphyllum* in mice and rats. **Pharmacological Research**, v.49, p.105-111, 2004.

ONEDA, F.; RÉ. M. I. The effect of formulation variables on the dissolution and physical properties of spray-dried microspheres containing organic salts. **Powder Technology**, v.130, p.377-384, 2003.

PALMIERI, G.; MICHELINI, S., MARTINO, P; MARTELLI, S. Polymers with pH-dependent solubility: possibility of use in the formulation of gastroresistant and controlled-release matrix tablets. **Drug Development and Industrial Pharmacy**, v.26, p.837-845 2000.

PALMIERI, G., BONACUCINA, G.; DI MARTINO, P; MARTELLI, S. Spray-Drying as a method for microparticulate controlled release systems preparation: advantages and limits. I. Water-soluble drugs. **Drug Development Industrial Pharmacy**, v.27, p.195-204, 2001.

PALMIERI, G.; BONACUCINA, G.; DI MARTINO P.; MARTELLI, S Gastro-resistant microspheres containing ketoprofen. **Journal of Microencapsulation**, v.19, n.1, p.111-119, 2002.

PÉREZ, M. H.; ZINUTTI, C.; LAMPRECHT, A.; UBRICH, N.; ASTIER, A.; HOFFMAN, M.; BODMEIER, R.; MAINCENT, P. The preparation and evaluation of poly(ε-caprolactone) microparticles containing both a lipophilic and a hydrophilic drug. **Journal of Controlled Release**, v.65, p.429-438, 2000.

PETROVIC, S.; DOBRIC, S.; BOKONJIC, D.; NIKETIC, M.; GARCIA-PIÑERES, A.; MERFORT, I. Evaluation of *Tanacetum larvatum* for an anti-inflammatory activity and for the protection against indomethacin-induced ulcerogenesis in rats. **Journal of Ethnopharmacology**, v.87, p.109-113, 2003.

POOLE, P. Pantoprazole. **American Journal of Health-System Pharmacy**, v.58, n.11, p.999-1008, 2001.

RAFFIN, R.;OBACH, E.; MEZZALIRA, G.; POHLMANN, A.; GUTERRES, S. Nanocápsulas poliméricas secas contendo indometacina: estudo de formulação e de tolerância gastrintestinal em ratos. **Acta Farmacêutica Bonaerense**, v.22, n.2, p.163-172, 2003.

RATTES, A. L. R.; OLIVEIRA, W. P. Spray drying conditions and encapsulating composition effects on formation and properties of sodium diclofenac microparticles. **Powder Technology**, v.171, n.1, p.7-14, 2007.

RAVI KUMAR, M. Nano and microparticles as controlled drug delivery devices. **Journal of Pharmacy and Pharmaceutical Sciences**, v.3, p.234-258, 2000.

ROSA, G.; BOCHOT, A.; QUAGLIA, F.; BESNARD, M.; FATTAL, E. A new delivery system for antisense therapy: PLGA microspheres encapsulating oligonucleotide/polyethyleneimine solid complexes. **International Journal of Pharmaceutics**, v.254, p.89-93, 2003.

RUSSO P., BUTTINI F., SONVICO F., BETTINI R., MASSIMO G., SACCHETTI C., COLOMBO P., SANTI P. Chimeral agglomerates of microparticles for administration of caffeine nasal powders. **Journal of Drug Delivery Science and Technology**, v.14, p.449-454, 2004.

RUSSO P., SACCHETTI C., PASQUALI I., BETTINI R., MASSIMO G., COLOMBO P., ROSSI A. Primary microparticles and agglomerates of morphine for nasal insufflation. **Journal of Pharmaceutical Sciences**, v.95, p.2553-2561, 2006.

SACHS, G.; SHIN, J.M.; PRATHA, V.; HOGAN, D. Synthesis or rupture: duration of acid inhibition by proton pump inhibitors. **Drugs of Today**, v.39, n.suppl. A, p.11-14, 2003.

SATO, Y.; KAWASHIMA, Y.; TAKEUCHI, H.; YAMAMOTO, H. In vitro evaluation of floating and drug releasing behaviors of hollow microspheres (microballoons) prepared by the emulsion solvent diffusion method. **European Journal of Pharmaceutics and Biopharmaceutics**, v.57, p.235-243, 2004.

SCHREIER, H. (Ed.). **Drug Targeting Technology: Physical, Chemical, and Biological Methods***.* New York, NY, USA: Marcel Dekker Incorporated, 2001. 22 p.

SHAH, P. J.; GANDHI, M. S.; SHAH, M. B.; GOSWAMI, S. S.; SANTANI, D. Study of *Mimusops elengi* bark in experimental gastric ulcers. **Journal of Ethnopharmacology**, v.89, p.305-311, 2003.

SINHA, V.R.; BANSAL, K.; KAUSHIK, R.; KUMRIA, R.; TREHAN, A. Poly-ε caprolactone microspheres and nanospheres: an overview. **International Journal of Pharmaceutics**, v.278, p.1-23, 2004.

SOULIMAN, S., BLANQUET, S., BEYSSAC, E.; CARDOT, J. M. A level A in vitro/in vivo correlation in fasted and fed states using different methods: Applied to solid immediate release oral dosage form. **European Journal of Pharmaceutical Sciences**, v.27, p.72-79, 2006.

STANGHELLINI, V. Management of Gastroesophageal Reflux Disease. **Drugs of Today**, v.39, n.supl. A, p.15-20, 2003.

SVEINSSON, S.; KRISTMUNDSDÓTTIR, T.; INGVARSDÓTTIR . The effect of tableting on release characteristics of naproxen and ibuprofen microcapsules. **International Journal of Pharmaceutics**, v.92, p.29-34, 1993.

TAKEUCHI, K.; KONAKA, A.; NISHIJIMA, M.; KATO, S.; YASUHIRO, T. Proton Pump Inhibitor: Effects of pantoprazole, a novel H+/K+-ATPase inhibitor, on duodenal ulcerogenic and healing responses in rats: a comparative study with omeprazole and lanoprazole. **Journal of Gastroenterology and Hepatology**, v.14, p.251-257, 1999.

THIES, C. **A survey of microencapsulation processes** In BENITA, S. (Ed.). **Microencapsulation: methods and industrial applications**. New York: Marcel Dekker, 1996. p. 1-19.

TURSILLI, R., CASOLARI, A., IANNUCCELLI, V.; SCALIA,S. Enhancement of melatonin photostability by encapsulation in lipospheres. **Journal of Pharmaceutics and Biomedical Analysis**, v.40, p.910-914, 2006.

VARDE, N. K.; PACK, D. W. Microspheres for controlled release drug delivery. **Expert Opinion on Biological Therapy**, v.4, p.1-17, 2004.

VUEBA, M. L., DE CARVALHO, L. A. E. B., VEIGA, F., SOUSA, J. J.; PINA, M. E. Role of cellulose ether polymers on ibuprofen release from matrix tablets. **Drug Development and Industrial Pharmacy**, v.31, p.653-665, 2005.

WAHBI, A.; ABDEL-RAZAK, O.; GAZY, A.; MAHGOUB, H.; MONEEB, M. Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations. **Journal of Pharmaceutical and Biomedical Analysis**, v.30, p.133-142, 2002.

YAMAGATA, Y., MISAKI, M., KUROKAWA, T., TAIRA, K.; TAKADA, S. Preparation of a copoly(dl-lactic/glicolic acid)-zinc oxide complex and its utilization to microcapsules containing recombinat human growth hormone. **International Journal of Pharmaceutics**, v.251, p.133-141, 2003.

XIE, Z., CHEN, X., JIN, F.; ZHONG, D. Simultaneous Determination of pantoprazole and Its Two Metabolites in Dog Plasma by HPLC. **Journal of Chromatographic Science**, v.43, p.1-5, 2005.

ZHOU, X.; CHEN, S.; YU, S. Effects of Spray Drying Parameters on the Processing of a Fermentation Liquor. **Biosystems Engineering**, v.88, n.2, p.193-199, 2004.

ZHU, K. J.; ZHANG, J. X.; WANG, C.; YASUDA, H.; ICHIMARU, A.; YAMAMOTO, K. Preparation and in vitro release behaviour of 5-fluorouracil-loaded microspheres based on poly (L-lactide) and its carbonate copolymers. **Journal of Microencapsulation**, v.20, n.6, p.731-743, 2003.

ANEXO: Pareceres da banca examinadora

BIOGRAFIA

Nome: Renata Platcheck Raffin

Data de nascimento: 23/01/1980

Graduação:

Farmácia (1998-2002) – Universidade Federal do Rio Grande do Sul

Farmácia Industrial (2003-2007) - Universidade Federal do Rio Grande do Sul

Pós-graduação:

Nível Doutorado (com transposição do mestrado) (2003-2007)

Programa de Pós-Graduação em Ciências Farmacêuticas

Universidade Federal do Rio Grande do Sul

Lista de Publicações

RAFFIN, R. P., COLOMÉ, L. M., HAAS, S. E., JORNADA, D. S., POHLMANN, A. R., GUTERRES, S. S. Development of HPMC and Eudragit S100® blended microparticles containing sodium pantoprazole. **Die Pharmazie**, v.62, p.361 - 364, 2007.

RAFFIN, R. P., COLOMÉ, L. M., POHLMANN, A. R., GUTERRES, S. S. Enteric Controlled-Release Pantoprazole-Loaded Microparticles Prepared by Using Eudragit S100 and Poly(-caprolactone) Blend. **Pharmaceutical Development and Technology**, v.12, p.463 - 471, 2007.

RAFFIN, R. P., COLOMÉ, L. M., SCHAPOVAL, E.E.S., JORNADA, D. S., POHLMANN, A. R., GUTERRES, S. S. Gastro-Resistant Microparticles Containing Sodium Pantoprazole:Stability Studies and In Vivo Anti-Ulcer Activity. **The Open Drug Delivery Journal**, v.1, p.44 - 51, 2007.

COLOMÉ, L. M., RAFFIN, R. P., JORNADA, D. S., POHLMANN, A. R., GUTERRES, S. S.Pantoprazole-loaded Eudragit® blended microparticles: preparation, characterization, in vitro gastro-resistance and in vivo anti-ulcer evaluation. **Journal of Drug Delivery Science and Technology**, v.17, p.113 - 118, 2007.

RAFFIN, R. P., COLOMÉ, L. M., POHLMANN, A. R., GUTERRES, S. S. Validação de metodologia analítica por cromatografia líquida para doseamento e estudo da estabilidade de pantoprazol sódico. **Química Nova**, v.30, p.1001 - 1005, 2007.

RAFFIN, R. P., POHLMANN, A. R., GUTERRES, S. S., RÉ, M. I. Powder characteristics of pantoprazole delivery systems produced in different spray-dryer scales. **Drying Technology**, v.24, p.339 - 348, 2006.

RAFFIN, R. P., COLOMÉ, L. M., POHLMANN, A. R., GUTERRES, S. S.Preparation, characterization and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles. **European Journal of Pharmaceutics and Biopharmaceutics**, v.63, p.198 - 204, 2006.

RAFFIN, R. P., JORNADA, D. S., RÉ, M. I., POHLMANN, A. R., GUTERRES, S. S. Sodium pantoprazole-loaded enteric microparticles prepared by spray drying: Effect of the scale of production and process validation. **International Journal of Pharmaceutics**, v.324, p.10 - 18, 2006.

RAFFIN, R. P., GUTERRES, S. S., NASCIMENTO, L. P. Aspectos atuais sobre a segurança no uso de produtos antiperspirantes contendo derivados de alumínio. **Infarma**, v.16, p.64 - 70, 2004.

RAFFIN, R. P., GUTERRES, S. S., POHLMANN, A. R., Mezzalira, G., Obach, E. S. Nanocápsulas poliméricas secas contendo indometacina: estudo de formulação e de tolerância digestiva. **Acta Farmaceutica Bonaerense**, v.22, p.163 - 172, 2003.

RAFFIN, R. P., MYLIUS, L. C., SILVA, K., PONTE, C. I. R. V. Perfil parasitológico de crianças de vilas periféricas de Porto Alegre, RS. **Revista Brasileira de Farmácia**, v.84, p.29 - 31, 2003.