UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

DESENVOLVIMENTO DE FORMULAÇÕES NANOTECNOLÓGICAS MUCOADESIVAS PARA ADMINISTRAÇÃO SUBLINGUAL DE CARVEDILOL

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Desenvolvimento de formulações nanotecnológicas mucoadesivas para administração sublingual de carvedilol

Tese apresentada por **Paula dos Santos Chaves** para obtenção do TÍTULO DE DOUTOR em Ciências Farmacêuticas

Orientador: Prof. Dr. Ruy Carlos Ruver Beck

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“Conforme-se e o mundo será sempre o mesmo”

(Futura)

RESUMO

Introdução e objetivos: As nanocápsulas, uma vez que são produzidas com polímeros, representam sistemas mucoadesivos promissores. O uso desse tipo de sistema é importante no delineamento de medicamentos que vislumbrem a membrana sublingual como via de administração, devido ao constante fluxo de saliva. Em vista disso, esse trabalho tem como objetivos: estudar o efeito da nanoestruturação em nanocápsulas de polímeros de diferentes características iônicas, quanto as suas propriedades mucoadesivas, quando veiculadas em suspensão, hidrogel ou pós, e frente a distintas superfícies mucoadesivas (discos de mucina, mucosa vaginal ou mucosa bucal); desenvolver nanocápsulas contendo carvedilol, avaliando as suas propriedades mucoadesivas e perfil de permeação do fármaco em diferentes modelos de membrana sublingual; e produzir, a partir das nanocápsulas secas, comprimidos sublinguais contendo carvedilol nanoencapsulado. **Metodologia:** Nanocápsulas formadas por Eudragit® RS100, Eudragit® S100 ou poly(ε-caprolactona) [PCL] foram produzidas pelo método de deposição interfacial do polímero. Suas propriedades mucoadesivas foram avaliadas empregando analisador de textura. As nanocápsulas contendo carvedilol foram produzidas pelo mesmo método citado acima, utilizando Eudragit® RS100 e a PCL. A mucoadesão dessas nanocápsulas foi avaliada quanto a sua interação com moléculas de mucina, além do efeito da sua interação com a mucosa sublingual de porco na permanência do fármaco sobre a mucosa e na sua permeação, em presença de um fluxo salivar mimetizado. O transporte de carvedilol através de uma monocamada celular de células de epitélio oral (SCC4) também foi estudado. As suspensões de nanocápsulas foram, então, secas por aspersão e as propriedades das nanocápsulas redispersas foram reavaliadas. Na última etapa, foram produzidos comprimidos sublinguais pelo método de compressão direta, a partir dos pós desenvolvidos. **Resultados:** A mucoadesividade dos polímeros Eudragit® RS100, Eudragit® S100 e PCL foi potencializada pela sua estruturação em nanocápsulas. Dentre as formulações analisadas, as nanocápsulas catiônicas, formadas por Eudragit® RS100, veiculadas em gel, foram as que apresentaram melhores propriedades adesivas. Além disso, o processo de secagem não interferiu na adesividade das nanocápsulas originais. Em relação a superfície utilizada, a mucina se

mostrou uma superfície mais adesiva comparada as mucosas suínas. Entretanto, a mucina reproduziu as diferenças observadas entre as formulações. As nanocápsulas contendo carvedilol interagiram bem com moléculas de mucina, sendo essa interação mais intensa para as nanocápsulas catiônicas [Eudragit® RS100], que para as aniônicas [PCL]. No entanto, ambas as nanocápsulas melhoraram o contato do carvedilol com a mucosa sublingual suína, o que fez com que mais fármaco permeasse através da mucosa, na presença de um fluxo salivar mimetizado, em comparação com uma solução do fármaco. Além disso, as nanocápsulas controlaram a permeação do fármaco através de mucosa sublingual de porco, bem como através de monocamadas de células SCC4. A partir destes resultados, as suspensões de nanocápsulas foram secas por aspersão. As nanopartículas foram recuperadas após redispersão aquosa dos pós e mantiveram suas propriedades mucoadesivas e biofarmacêuticas. Na sequência, os comprimidos foram produzidos como forma farmacêutica final. A presença de nanoestruturas foi observada nos comprimidos, as quais foram liberadas após total desintegração destes em saliva artificial. Além disso, a liberação do fármaco partir dos comprimidos contendo as nanocápsulas apresentou um perfil controlado comparado aos comprimidos contendo o fármaco livre, reforçando a manutenção da estrutura supramolecular das nanocápsulas nos comprimidos. **Conclusão:** As nanocápsulas produzidas com Eudragit® RS100, Eudragit® S100 ou PCL apresentaram boas características mucoadesivas. As, nanocápsulas de Eudragit® RS100 e PCL também melhoraram a interação do carvedilol com a membrana sublingual de porco. Em ambos os estudos, um melhor desempenho mucoadesivo foi observado para as nanocápsulas catiônicas. Além disso, o carvedilol apresentou boa permeação através de mucosa sublingual suína e através de monocama celular de células de epitélio oral. Ainda, a secagem por aspersão das suspensões de nanocápsulas não alterou significativamente as suas propriedades. A compressão direta dos pós secos por aspersão produziu comprimidos inovadores contendo um sistema nanotecnológico mucoadesivo para administração sublingual de carvedilol, como um nanomedicamento.

Palavras chaves: Carvedilol, comprimidos, mucoadesão, nanocápsulas, permeabilidade sublingual, secagem por aspersão.

ABSTRACT

Introduction and objectives: Nanocapsules may represent promissing mucoadhesive systems since they are produced with polymers. The use of these systems is very important for drug administration by the sublingual route due to the constantly salivary flux in the oral cavity. In view of this, the objectives of this study were: to study the effect of the nanostructuration in nanocapsules on the mucoadhesiveness of polymers with different charge surface and the effect of the vehicle (suspension, hydrogel, and powder) on the mucoadhesiveness of nanocapsules as well as the effect of different mucosal surfaces (mucin, vaginal mucosa, and buccal mucosa); to develop carvedilol-loaded nanocapsules and to evaluate their mucoadhesive properties and drug permeation profiles using different models of sublingual membrane; and to produce sublingual tablets using spray-dried carvedilol-loaded nanocapsules. **Methods:** Eudragit®RS100, Eudragit®S100 or poly(ε-caprolactone) [PCL] nanocapsules were produced by interfacial deposition of the polymer method. Their mucoadhesiveness were evaluated by tensile stress tester. Carvedilol-loaded nanocapsules were produced by the method cited above and using Eudragit® RS100 or PCL as polymers. Mucoadhesiveness of nanocapsules were studied analyzing their interaction with mucin molecules and analyzing the effect of their interaction with porcine sublingual mucosa on drug retention as well on the amount of drug permeated to the receptor fluid in the presence of simulated salivary flux. The transport of carvedilol across monolayers of oral epithelial cells (SCC4) was also evaluated. In the next step, nanocapsules suspensions were spray-dried and the properties of redispersed nanocapsules were evaluated. In the last step, sublingual tablets were produced by direct compression using the spray-dried nanocapsules. **Results:** Mucoadhesiveness of Eudragit® RS100, Eudragit® S100 and PCL were improved by their structuration in nanocapsules. Among the tested formulations, the cationic Eudragit® RS100 nanocapsules formulated as a hydrogel showed the best behavior. Moreover, the drying process did not interfer in the adhesiveness of original nanocapsules. Regarding the surface substrate, mucin discs were more adhesive than porcine mucosas. However, mucin was able to reproduce the differences observed between the formulations. Carvedilol-loaded nanocapsules interacted with mucin molecules and this interaction was more

intense for cationic Eudragit® RS100 nanocapsules than for anionic PCL nanocapsules. However, both nanocapsules increased the amount of drug retained on porcine sublingual mucosa and improved the amount of drug permeated through mucosa, in comparison to the drug solution, in presence of a mimetic salivary flux was present. Furthermore, nanocapsules were able to control the drug permeation across porcine sublingual and through SCC4 monolayer. Subsequently, suitable powders were obtained by spray-drying. The original nanoparticles were recovered after aqueous redispersion of powders and the maintenance of their mucoadhesiveness and biopharmaceutics properties was observed. Moreover, sublingual tablets were produced as a final pharmaceutical form. The presence of nanometric particles in the tablets was observed and they were released after tablet disintegration in artififcial saliva. The drug was released by a controlled way from tablets containing nanocapsules when compared to tablets containing the non-encapsulated drug, reinforcing the maintenance of supramolecular structure of nanocapsules in the tablets. **Conclusion:** The Eudragit® RS100, Eudragit® S100 and PCL nanocapsules showed good mucoadhesive characteristics. Moreover, Eudragit® RS100 and PCL nanocapsules improved the carvedilol interaction with porcine sublingual mucosa. In both studies, cationic nanocapsules showed the best mucoadhesive performance. Additionally, carvedilol showed a good permeation across porcine sublingual mucosa and through oral epithelial cells monolayer. The spray-drying process did not change the properties of the original aqueous nanocapsules. Furthermore, their direct compression produced innovative tablets containing a mucoadhesive nanotechnological system for sublingual administration of carvedilol as a nanomedicine.

Keywords: Carvedilol, mucoadhesion, nanocapsules, sublingual permeability, spray-drying, tablets.

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1. INTRODUÇÃO

A nanotecnologia vem sendo amplamente explorada como uma importante estratégia para o desenvolvimento de nanocarreadores de fármacos, capazes de melhorar tanto o desempenho de medicamentos, como a qualidade de vida dos pacientes (BOSSELMANN *et al.*, 2012). A partir desta estratégia pode-se promover uma liberação controlada do fármaco no organismo, o direcionamento de fármacos para locais desejados, a diminuição da dose diária ingerida e/ou da frequência de administrações, além da diminuição nos efeitos colaterais (SCHAFFAZICK *et al*., 2003; FRANK *et al*., 2015). A partir desta ferramenta, é possível, ainda, explorar novas vias de administração que possam promover um aumento na eficácia do tratamento.

As nanocápsulas estão entre os nanossistemas poliméricos amplamente explorados para essas finalidades. Essas nanoestruturas são formadas por um núcleo oleoso, envolto por um parede polimérica, e possuem características importantes como carreadores de agentes terapêuticos lipofílicos (POHLMANN *et al*., 2013). Além do mais, as nanocápsulas podem ser utilizadas como sistemas mucoadesivos. Esse tipo de carreador é capaz de aumentar o tempo de contato dos fármacos na região de interesse, a partir de forças bioadesivas interfaciais com o muco, permitindo uma absorção mais eficiente do fármaco (CARVALHO *et al.,* 2010, SILVA *et al.,* 2012, MAZZARINO *et al.,* 2012; KLEMESTRUD *et al.,* 2013). O uso de nanocápsulas poliméricas como sistemas mucoadesivos foi relatado até o momento para administração de fármacos na mucosa vaginal (FRANK *et al*., 2014, 2017) e na mucosa nasal (FONSECA *et al.,* 2015).

A utilização de sistemas mucoadesivos é muito importante na administração de fármacos pela via sublingual, uma vez que, a cavidade oral é formada por um ambiente com constante fluxo de saliva que pode interferir na permanência de fármacos nessa região (AL-GHANANEEM *et al.,* 2007, MORALES *et al.,* 2011). Apesar dessa limitação, a cavidade sublingual é uma via de administração promissora para fármacos que devem atingir diretamente a corrente sanguínea. Além do alto suprimento vascular dessa região, ela possui uma espessura relativamente inferior às outras regiões da boca e uma camada de epitélio não-queratinizado. Essas características contribuem para

que esta superfície seja a mais permeável à passagem de fármacos (GOSWAMI *et al.,* 2008; SHIKANGA *et al.,* 2012). Sendo assim, a via sublingual pode representar uma excelente alternativa para administração de fármacos que possuem uma limitada biodisponibilidade oral devido ao metabolismo de primeira passagem.

O carvedilol é um importante agente terapêutico com características lipofílicas, que, quando administrado pela via oral, sofre um extensivo metabolismo de primeira passagem no fígado (VISHNU *et al.,* 2007; DANDAN *et al.,* 2012). Este fármaco possui uma gama de ações cardiovasculares. Ele é um antagonista não-seletivo de receptores β-adrenérgicos (terceira geração), bloqueador de receptores α1-adrenérgicos, além de possuir atividade antioxidante. Em função dessa sua múltipla ação cardiovascular, esse fármaco é uma opção importante no tratamento da hipertensão, insuficiência cardíaca congestiva leve a severa, e doenças arteriais coronarianas (RUFFOLO *et al.,* 1997; FRISCHMAN, 1998; STAFYLAS *et al.*, 2008, DANTAS *et al.*, 2013). Sua única forma farmacêutica comercialmente disponível é comprimidos para administração oral, cuja biodisponibilidade é extremamente limitada, cerca de 25 a 35% da dose administrada (DANDAN *et al.,* 2012).

Considerando o efeito do carvedilol sobre o sistema cardiovascular e no tratamento de suas doenças, é desejável a produção de uma forma farmacêutica que permita uma maior biodisponibilidade deste fármaco a partir de uma via não-invasiva. A partir disso, a hipótese deste trabalho foi baseada na capacidade mucoadesiva de nanocápsulas poliméricas para prolongar a permanência do carvedilol na mucosa sublingual, promovendo a sua absorção, e a sua formulação em sistemas de administração sublingual.

Assim, o primeiro capítulo deste trabalho compreende o estudo das propriedades mucoadesivas de nanocápsulas, quando desenvolvidas com polímeros de cargas diferentes e veiculadas em diferentes formas farmacêuticas, frente a distintas superfícies mucoadesivas. O segundo capítulo propõe o desenvolvimento de nanocápsulas poliméricas com propriedades mucoadesivas, visando aumentar o tempo de contato do carvedilol com a mucosa sublingual na presença de fluxo salivar e mellhorar, assim, sua absorção. O terceiro capítulo traz um estudo do transporte de carvedilol através

de uma monocama celular, formada com células de epitélio oral, em modelo inédito proposto nesta tese, e estabelece uma comparação com o perfil de permeação do fármaco através de mucosa sublingual de porco. O efeito da permeação do fármaco na integradidade da membrana celular também foi avaliado, assim como o potencial citotóxico das suspensões de nanocápsulas nesta linhagem celular. O quarto capítulo aborda o desenvolvimento de materiais pulverulentos, como plataforma para o desenvolvimento posterior de comprimidos sublinguais, avaliando o efeito do processo de secagem nas propriedades dos sistemas nanométricos originais. Finalmente, o quinto capítulo, trata do desenvolvimento de comprimidos sublinguais, a partir das nanocápsulas secas produzidas, como forma farmacêutica final.

Os resultados estão apresentados na forma de artigos científicos e cada capítulo compreende um artigo. Além disso, a revisão de tema dessa tese traz uma extensa revisão dos estudos que abordam a aplicação de nanopartículas poliméricas no tratamento e prevenção de doenças da cavidade oral, publicados nos últimos oito anos. Esta parte da revisão da literatura está também organizada na forma de um artigo.

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2.1. OBJETIVO GERAL

Desenvolver nanocápsulas poliméricas contendo carvedilol para administração pela via sublingual, focando nos estudos de mucoadesividade, análises de permeabilidade do fármaco através de modelos *in vitro* de mucosa oral e desenvolvimento de formas farmacêuticas sólidas intermediárias e finais.

2.2. OBJETIVOS ESPECÍFICOS

- Estudar o efeito da nanoestruturação de polímeros de diferentes cargas em nanocápsulas sobre suas propriedades mucoadesivas, veiculadas em diferentes formas farmacêuticas (suspensão aquosa, hidrogel e pós) e frente a distintas superfícies mucoadesivas (discos de mucina, mucosa vaginal e bucal);

- Desenvolver suspensões de nanocápsulas poliméricas contendo carvedilol e avaliar as suas características físico-químicas, o perfil de liberação do fármaco, o perfil de permeação do fármaco em mucosa sublingual de porco;

- Avaliar a capacidade de interação das nanocápsulas produzidas com moléculas de mucina e o efeito da sua mucoadesão na permanência do fármaco na superfície da mucosa sublingual e na quantidade de fármaco permeado através da mucosa, em presença de um fluxo salivar mimetizado;

- Estudar o transporte *in vitro* de carvedilol através de uma monocama celular de células de epitélio oral, analisando possíveis efeitos citotóxicos ou deletérios à integridade da monocamada e investigando o uso de monocamada de células SCC4 como um novo modelo de membrana sublingual;

- Desenvolver formas farmacêuticas pulverulentas a partir das suspensões líquidas, empregando a técnica de secagem por aspersão, avaliando as suas propriedades físicas, físico-químicas e morfológicas;

- Avaliar os pós quanto ao perfil de liberação do fármaco, o perfil de permeação em mucosa sublingual de porco, a mucoadesividade das partículas e o seu efeito na permanência do fármaco sobre a mucosa e na quantidade total de fármaco permeado, em presença de um fluxo salivar mimetizado;

- Desenvolver comprimidos sublinguais, a partir dos pós produzidos, avaliando a presença de nanoestruturas tanto na forma farmacêutica final, quanto no meio salivar após desintegração do comprimido, além de estudar o perfil de liberação do fármaco e o tempo de desintegração dos comprimidos em saliva artificial.

3.1. CAVIDADE ORAL

A cavidade oral compreende lábios, gengiva (mucosa oral que recobre os ossos e suporta as raízes dos dentes), bochechas, palato (teto da cavidade oral), assoalho da boca, língua e dentes. Suas funções são essenciais para a sobrevivência humana. A função primária da boca diz respeito à digestão (seleção da comida), à mastigação e à deglutição. As funções secundárias incluem a fala e a ventilação (respiração) (BERKOVITZ *et al*., 2004).

O revestimento da cavidade oral ocorre através da mucosa bucal, que é formada por uma fina membrana de muco, recobrindo uma camada de epitélio estratificado e escamoso. Abaixo do nível epitelial, está situado o tecido conectivo, constituído pela lâmina própria e submucosa (MADHAV *et al.,* 2009). Devido as suas especializações, a mucosa bucal desempenha diversos papéis como: proteção mecânica contra forças compressivas e de cissalhamento; barreira contra microorganismos, toxinas e vários antígenos; desempenha um papel na defesa imunológica, tanto humoral quanto mediada por células; as glândulas menores presentes no interior da mucosa oferecem lubrificação e tamponamento, além da secreção de alguns anti-corpos; a mucosa é ricamente inervada, fornecendo sensação de tato, propriocepção, dor e paladar (BERKOVITZ *et al.,* 2004).

A mucosa bucal pode ser classificada em três tipos: mastigatória, de revestimento e especializada. A mucosa mastigatória é caracterizada por um epitélio queratinizado e uma lâmina própria espessa. Ela recobre o palato duro e a superfície bucal da genviva. A mucosa de revestimento é não queratinizada, possui uma lâmina própria frouxa e recobre a mucosa das bochechas, lábios, alvéolo, região dento gengival, assoalho da boca, superfície ventral da língua e palato mole. O dorso da língua é uma região especializada de mucosa gustativa (BERKOVITZ *et al.,* 2004). Elas se diferenciam em relação a espessura conforme a região da boca, sendo que a buccal apresenta uma espessura de 500-600 µm, a palatal de 250 µm, a gengival de 200 µm e a sublingual de 100-200 µm (PATEL *et al.,* 2011).

A região da mucosa bucal representa uma via muito promissora para administração de fármacos. Ela possui excelente acessibilidade; evita passagem pelo trato gastrointestinal e fígado, onde fármacos podem ser degradados, entregando-os diretamente na corrente sanguínea; é uma região

com baixa atividade enzimática; a administração é indolor; possui alta aceitação pelo pacientes e pode ser utilizada tanto para ação local, quanto sistêmica (SHOJAEI *et al.,* 1998; SUDHAKAR *et al.,* 2006).

Além disso, a cavidade oral possui diversas doenças locais que afetam a saúde e a qualidade de vida das pessoas. Cárie dentária, doenças periodontais, perda de dentes, lesões da mucosa bucal, câncer orofaríngeo, doenças orais infecciosas, traumas de lesões e lesões hereditárias são exemplos de doenças orais e que representam os principais problemas de saúde pública no mundo (PETERSEN *et al.,* 2005). Segundo a Organização Mundial da Saúde (WHO, 2012) 60-90% das crianças em idade escolar e quase 100% dos adultos no mundo possuem cárie. A doença periodontal severa, que resulta em perda de dente, é encontrada em 15-20% dos adultos de meia idade (35-44 anos), enquanto que doenças orais em crianças e adultos são mais prevalentes em grupos mais pobres e desfavorecidos. Além disso, o câncer oral é o oitavo câncer mais comum no mundo (PETERSEN *et al.,* 2005).

3.2. VIA SUBLINGUAL

A região sublingual, que compreende a superfície ventral da língua e assoalho da boca, em função da suas características, se destaca em relação às outras regiões bucais, para adminstração de fármacos de ação sistêmica (GOSWAMI *et al*., 2008). A mucosa dessa região é a que apresenta menor espessura em relação as demais regiões da boca, além de ser formada por um epitélio não-queratinizado (PATEL *et al.,* 2011). O epitélio queratinizado é caracterizado pela presença de lipídeos, como ceramidas e acilceramidas, que representam uma barreira extra para a passagem de substâncias, além de ser relativamente impermeável a passagem de água. Ainda, esses epitélios se diferenciam na organização dos componentes, sendo que o epitélio não-queratinizado apresenta ausência de organização dos lipídeos nos espaços intercelulares, o que facilita a passagem de substâncias (SHOJAEI *et al.,* 1998). A menor espessura e ausência de queratinização fazem com que a mucosa sublingual apresente maior permeabilidade em relação às demais.

A cavidade sublingual é uma região com um rico suprimento de vasos sanguíneos que correm paralelos à superfície da mucosa (GOSWAMI *et al.,* 2008). A administração por essa via permite que o fármaco atinja diretamente a

circulação sanguínea, oportunizando uma absorção eficaz, sem degradações ou metabolizações prévias. Em vista disso, a via sublingual pode ser utilizada como uma excelente alternativa para administração de fármacos que possuem uma limitada biodisponibilidade oral. Haegeli e colaboradores (2007) relataram um aumento na biodisponibilidade da furosemida, um diurético que possui absorção muito variada entre os pacientes, após administração sublingual em 11 voluntários. Os autores demonstraram que a concentração máxima plasmática aumentou cerca de 43% quando o fármaco foi administrado pela via sublingual em relação a sua administração oral.

A absorção pela via sublingual é 3 a 10 vezes mais rápida em comparação com a via oral, promovendo uma ação mais imediata do fármaco, mas que pode durar menos tempo, sendo que a concentração plasmática pode declinar para níveis inferiores aos terapêuticos em poucos minutos (NIBHA and PANCHOLI, 2011). Nesse sentido, essa via é também muito explorada quando se necessita uma ação imediata do fármaco, devido a sua entrega rápida e direta na corrente sanguínea. Entretanto, ela também pode ser utilizada quando se desejam ações menos imediatas e mais prolongadas. Com esse intuito, a manutenção da concentração terapêutica de fármacos no sistema circulatório por um período mais longo e com administrações menos frequentes, pode ser obtida pelo uso de sistemas de liberação controlada (ANDREWS *et al*., 2009; MASEK *et al*., 2017).

No entanto, a cavidade oral, como um todo, incluindo a região sublingual, está exposta a um constante fluxo de saliva, que dificulta a permanência dos fármacos no seu local de absorção. Diariamente são produzidos cerca de 0,5 – 2 L de saliva, que pode influenciar o tempo de residência do fármaco na região de absorção, interferindo assim, na quantidade de ativo biodisponível (AL-GHANANEEM *et al.,* 2007; PATEL *et al.,* 2011). Para contornar essa limitação, estudos têm sugerido o uso de carreadores mucoadesivos, ou seja, sistemas capazes de aumentar o tempo de contato das formas farmacêuticas na região de interesse, a partir de forças bioadesivas interfaciais, permitindo uma absorção do fármaco mais eficiente (BREDENBERGA *et al.,* 2003; AL-GHANANEEM *et al.,* 2007; GOSWAMI *et al.,* 2008; CARVALHO *et al.,* 2010, SILVA *et al.,* 2012, MAZZARINO *et al.,* 2012; KLEMESTRUD *et al.,* 2013; PARODI *et al*., 2017).

As formas farmacêuticas para administração de fármacos por essa via necessitam de características específicas. Os parâmetros mais importantes a serem observados durante o desenvolvimento de sistemas para administração sublingual são o tempo de desintegração e a velocidade de dissolução. O ambiente sublingual possui uma pequena quantidade de saliva onde o fármaco precisa ser liberado para posterior absorção (GOSWAMI *et al.,* 2008). O volume constante de saliva presente na boca como um todo é cerca de 1,1 mL, valor bem inferior quando comparado ao presente no trato gastrintestinal (PATEL *et al.,* 2011). Estudos sugerem principalmente o uso de comprimidos sublinguais (BREDENBERG *et al.,* 2003; FUDALA *et al.,* 2003; BOLOURCHIAN *et al.,* 2009; RACHID *et al.,* 2012; MINKOWITZ *et al*., 2016; HOFFMAN *et al*., 2017), mas sprays (AL-GHANANEEM *et al.,* 2007; PARIKH *et al.,* 2013; RAUCK *et al*., 2017) e filmes (KOLAND *et al.,* 2010; SAYED *et al.,* 2013; PARODI *et al*., 2017) também tem sido propostos.

3.3. NANOPARTÍCULAS POLIMÉRICAS

Nanopartículas poliméricas são sistemas coloidais com diâmetro nanométrico e que possuem polímeros como principais componentes. Os materiais poliméricos tem características importantes que tornam esses tipos de partículas sistemas promissores para o carreamento de fármacos. Os nanocarreadores poliméricos permitem um encapsulamento eficiente de diversos agentes farmacológicos; são capazes de promover uma liberação controlada do fármaco, interferindo na sua cinética; podem ser facilmente modificados para adição de uma variedade de ligantes a sua superfície; além de que a maioria dos polímeros empregados possuem uma longa história de segurança de uso em humanos, sendo biocompatíveis e muitos até biodegradáveis (SOPPIMATH *et al*., 2001; PATEL *et al*., 2012).

Dependendo do método de produção e dos seus componentes, diferentes tipos de nanopartículas poliméricas podem ser produzidas. As principais representates desse grupo são as nanoesferas e as nanocápsulas. Nanoesferas são formadas por uma matriz polimérica, enquanto que nanocápsulas são sistemas vesiculares formados por um núcleo oleoso envolto por uma parede polimérica (REIS et al., 2006). Ambas são capazes de carrear fármacos lipofílicos tanto no seu interior quanto na sua superfície (VAUTHIER

and BOUCHEMAL, 2009). Outra representante desse grupo são as micelas poliméricas. Essas estruturas são formadas pela autoassociação de blocos de copolímeros e apresentam uma arquitetura de núcleo lipofílico, onde fármacos hidrofóbicos podem ser incorporados, e superfície hidrofílica, onde fármacos hidrofílicos podem ser associados (ELSABAHY and WOOLEY, 2012).

O uso das nanopartículas poliméricas tem resultado em benefícios terapêuticos importantes de vários fármacos solúveis e insolúveis, além de moléculas bioativas. Dentre os benefícios observados para essas partículas estão: aumento da biodisponibilidade, solubilidade e tempo de retenção em local desejado; aumento da eficácia, especifidade, tolerância e índice terapêutico de diversos fármacos; proteção contra a degradação e interação com o ambiente biológico a qual não é desejada; aumento da absorção em tecidos; assim como aumento na penetração intercelular. Como consequência, foi observada uma melhor eficácia terapêutica, as quais resultaram em redução nas despesas dos pacientes e nos riscos de toxicidade, além de uma melhor adesão ao tratamento pelos pacientes (KUMARI et al., 2010; COLSON e GRINSTAFF, 2012; ELSABAHY and WOOLEY, 2012; KULKAMI e FENG, 2013).

Além disso, tem sido observado uma crescente aplicação de nanopartículas poliméricas na área odontológica. Neste contexto, foi realizada uma revisão da literatura de estudos que abordaram o uso das nanopartículas poliméricas no tratamento e/ou prevenção de doenças da cavidade oral, abrangendo o período de 2010 a 2017, com a redação de um artigo de revisão, que se encontra no final desta seção.

3.4. NANOCÁPSULAS

Nanocápsulas poliméricas podem ser classificadas em dois tipos de sistemas, de acordo com a composição do seu núcleo oleoso: as nanocápsulas convencionais e as nanocápsulas de núcleo lipídico. A presença de monoestearato de sorbitano no núcleo dessas partículas deu origem a uma nova classe que foi chamada de nanocápsulas de núcleo lipídico (JÄGER *et al.,* 2007). Esses dois tipos de nanocápsulas apresentam algumas distinções como rigidez e capacidade de encapsular certos fármacos (POLETTO *et al.,* 2015), mas de modo geral seus benefícios são similares: controle da liberação

do fármaco e, consequentemente, aumento da eficácia; diminuição de dose e diminuição dos efeitos adversos; além disso, estão descritos também, aumento da estabilidade química ou da fotoestabilidade, aumento da captação por células e aumento da biodisponibilidade de determinados compostos (FRANK *et al.,* 2015).

Esses sistemas já foram explorados para uso em diferentes vias de administração como: oral (CATANNI *et al*., 2010), ocular (KATZER *et al*., 2014), cutânea (CONTRI *et al*., 2010, 2014), vaginal (FRANK *et al*., 2014, 2017) e nasal (FONSECA *et al*., 2015). Até o momento, não existe nenhum estudo propondo o uso de nanocápsulas, como sistemas de liberação controlada, para administração de fármacos pela via sublingual.

As nanocápsulas, além de promoverem uma liberação mais controlada e uma entrega mais eficiente do fármaco, podem representar sistemas mucoadesivos promissores, capazes de evitar a remoção de fármacos do local de absorção, pela saliva. Sua superfície é formada por polímeros e o uso de componentes com propriedades adesivas podem aumentar o tempo de contato dessas estruturas em locais específicos, como já demonstrado por alguns autores. Contri e colaboradores (2014) desenvolveram nanocápsulas formadas por Eudragit® RS 100 contendo capsaicinóides encapsulados. As formulações foram veiculadas em hidrogéis, contendo ou não quitosana, e os resultados evidenciaram a capacidade adesiva das nanopartículas na superfície da pele, empregando o teste de lavabilidade. Além disso, os autores demonstraram que as nanocápsulas foram mais eficientes em aumentar a adesão do fármaco que o hidrogel de quitosana. A combinação da encapsulação e incorporação em hidrogéis contendo quitosana, promoveu a retenção de maiores quantidades de fármaco. O efeito dessa associação também foi avaliado por Frank e co-autores (2014) em mucosa vaginal. Além de partículas formadas por Eudragit® RS 100 (polímero catiônico), os autores produziram nanocápsulas contendo Eudragit® S 100 (polímero aniônico) e demonstraram, a partir de análises de textura, que a presença das nanocápsulas promoveu um aumento no trabalho necessário para romper a ligação entre hidrogéis de quitosana e mucosa vaginal, independente do polímero utilizado. Fonseca e co-autores (2015) avaliaram a capacidade mucoadesiva de nanocápsulas de poli(Ɛ-caprolactona) funcionalizadas com copolímero metracrílico. Os autores exploraram a via

nasal para liberação cerebral de olanzapina e verificaram mudanças no potencial zeta das nanocápsulas, após contato com moléculas de mucina, sugerindo a interação entre as partículas. Além disso, os autores demonstraram, a partir do teste de lavabilidade, que a nanoencapsulação promoveu a retenção de maiores quantidades de fármaco na superfície da mucosa nasal.

3.5. MUCOADESÃO

Mucoadesão é o termo utilizado para definir a interação entre um material ou partícula e o muco, ou seja, a capacidade dessas estruturas permanecerem em contato por um determinado tempo, através de forças interfaciais (ANDREWS *et al.,* 2009). Essa propriedade vem sendo utilizada na área farmacêutica com o intuito de potencializar a absorção de fármacos em regiões de interesse. Diferentes regiões do organismo são revestidas por uma camada mucosa como a gastrointestinal, a nasal, a ocular, a bucal, a vaginal e a retal, assim como diferentes materiais e sistemas com características bioadesivas vem sendo explorados para aplicação farmacêutica (CARVALHO *et al.,* 2010).

O muco é uma película formada por proteínas e carboidratos suspensos em um ambiente aquoso e sua espessura pode variar de 40 a 300 µm. A água é o componente majoritário do muco, representanto cerca de 95 a 99% do seu peso. Entretanto, quem fornece as suas principais propriedades é a mucina, uma representante das glicoproteínas. As mucinas são macromoléculas com massa variando de 0,5 a 20 MDa e formadas principalmente por carboidratos, que representam cerca de 80 %, como N-acetilgalactoseamina, N-acetilglucosamina, fucose, galactose e o ácido siálico. No núcleo protéico do muco estão ancorados os oligossacarídeos, predominantemente a partir de ligações glicosídeas (BANSIL *et al.,* 2006). Essas estruturas são responsáveis pela carga negativa do muco em pH fisiológico (5,8 – 7,4), além de conferirem características de um gel coesivo, uma vez que possuem a tendência de se agregar e formar uma rede tridimensional. Estudos sugerem que, em função dessas características conferidas ao muco, a mucina possui um papel chave no processo de mucoadesão (PATEL *et al.,* 2011).

O mecanismo por trás do fenômeno de mucoadesão ainda não é totalmente entendido, e por isso, diferentes teorias são utilizadas para explicar esse processo: teoria eletrônica (atração entre cargas opostas), teoria da adsorção (interações hidrofóbicas, ligações de hidrogênio, forças de van der Waals), teoria da molhabilidade (capacidade da forma líquida se espalhar sobre a camada de muco), teoria da difusão (considera a penetração de moléculas na rede de muco e difusão de mucina na forma farmacêutica), teoria da fratura (dificuldade da ruptura de duas superfícies após adesão) e teoria mecânica (considera o efeito da rugosidade da superfície no aumento da área de contato) (KHUTORYANSKIY, 2011). Uma teoria apenas não é suficiente para descrever todo processo. De forma geral, a mucoadesão ocorre em dois estágios, que envolvem as diferentes teorias propostas: o estágio de contato, onde ocorre a primeira interação entre os sitemas, e o estágio de consolidação da ligação (CARVALHO *et al.,* 2010).

Os polímeros são os principais materiais utilizados no desenvolvimento de sistemas mucoadesivos. As suas capacidades adesivas se diferenciam em função da sua carga, mas, em geral, eles precisam ter fexibilidade suficiente para penetrar a rede de muco, serem biocompatíveis, não-tóxicos, além de economicamente viáveis (PATEL *et al.,* 2011). Polímeros aniônicos possuem grupos carboxílicos, que podem interagir, através de ligações de hidrogênio, com oligossacarídeos presentes na molécula de mucina e são considerados bons materiais mucoadesivos. Polímeros catiônicos, como a quitosana e polimetacrilatos sintéticos, apresentam excelentes características adesivas, sendo capazes de interagir com as moléculas negativas de mucina, através de atração eletrostática. A capacidade mucoadesiva dos polímeros não-iônicos não é tão favorável, acredita-se que esses materiais interajam com o muco pela difusão e interpenetração na camada mucosal (ANDREWS *et al.,* 2009; KHUTORYANSKIY, 2011). Diferentes sistemas mucoadesivos de liberação de fármacos, utilizando polímeros na sua composição, tem sido propostos, tais como: sistemas micrométricos (VASIR *et a.,* 2003; CILURZO *et al.,* 2005; NI et al., 2017), comprimidos (PERIOLI *et al.,* 2011; ÇELIK et al., 2017; IKEUCHI-TAKAHASHI et al., 2017), hidrogéis (XU et al., 2015; HUANG et al., 2016) além de filmes (TEJADA et al., 2017; PARODI et al., 2017) e sistemas nanométricos (FRANK *et al.,* 2014, 2017; FONSECA *et al.,* 2015).

Com o intuito de evidenciar a capacidade mucoadesiva dos sitemas desenvolvidos diferentes testes *in vitro* tem sido empregados. Dentre os mais citados, está o método que mede o trabalho necessário para romper a ligação entre a mucosa e o sistema mucoadesivo, a partir da aplicação de uma força externa e utilizando um analisador de textura (THIRAWONG *et al.,* 2007; DAS NEVES *et al.,* 2008; FRANK *et al.,* 2014; FONSECA *et al.,* 2015). Testes reológicos também são utilizados e avaliam alterações nas estruturas dos sistemas após contato com os componentes do muco (ROSSI *et al.,* 2001; CARVALHO *et al.,* 2010). O efeito mucoadesivo mimetizando fluidos biológicos pode ser analisado a partir de um teste de lavabilidade, onde a mucosa contendo uma amostra é lavada e os componentes presentes nesse lavados são quantificados (CARVALHO *et al.,* 2014; FONSECA *et al.,* 2015). Outras análises descritas para verificar a interação dos sistema com as moléculas de mucina são técnicas turbidimétricas, análises de potencial zeta, determinação de diâmetro de partícula, além do uso de ressonância plasmônica de superfície, infravermelho, raio-X e espectroscopia fotoeletrônica (ANDREWS *et al.,* 2009; KHUTORYANSKIY*,* 2011; CARVALHO *et al.,* 2014).

3.6. CARVEDILOL

O componente 1-(carbazol-4-iloxi-3-[[2-(o-metoxifenoxi)etil]-amino]-2-propanol (Figura 1) é conhecido como Carvedilol. Este fármaco é uma mistura racêmica de R(+) e S (-) enantiômeros e ambos possuem atividade farmacológica complementar. É um componente lipofílico, que apresenta logP de 3,8 e massa molecular de 406,5 g/mol. Ele possui um pKa de 7,8 devido a presença do grupo funcional amina secundária. Apresenta baixa solubilidade em água (0.02 mg/mL em pH = 7,4), que aumenta com a diminuição do pH, com saturação de 23 µg/mL em pH = 7 e de 100 µg/mL em pH = 5. Possui classificação II no Sistema de Classificação Biofarmacêutica, com baixa solubilidade e alta permeabilidade (LOFTSSON *et al*., 2008; BROOK *et al*., 2011).

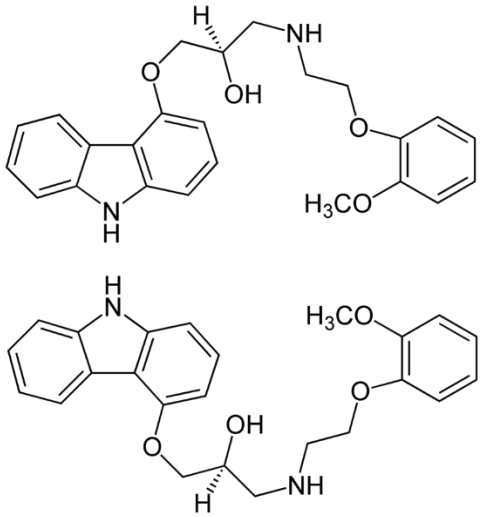

Figura 1: estrutura química do Carvedilol

O carvedilol é um betabloqueador de terceira geração, que possui uma diversificada ação sobre importantes estruturas envolvidas na geração de desordens cardiovasculares. Ele é um antagonista não-seletivo de receptores β-adrenérgicos, os quais, quando estimulados em demasia, provocam significativas alterações cardíacas (RUFFOLLO *et al.*, 1996; FRISCHMAN *et al.*, 1998; STAFYLAS *et al.*, 2008). Além disso, receptores α1-adrenérgicos também são bloqueados por esta molécula, o que resulta na obstrução da estimulação da vasoconstrição (SPONER *et al.*, 1992; FRISCHMAN *et al.*, 1998). Ainda, em função da sua atividade antioxidante, ele é capaz de combater espécies reativas de oxigênio, as quais possuem papel chave na evolução de diversas patologias cardiovasculares (FRISCHMAN *et al.*, 1998; DANDONA *et al,* 2007). Sua múltipla ação cardiovascular o torna uma opção importante no tratamento da hipertensão, insuficiência cardíaca congestiva leve a severa e doenças coronarianas arteriais (RUFFOLO *et al.,* 1997; FRISCHMAN, 1998; STAFYLAS *et al.*, 2008).

O carvedilol está comercialmente disponível na forma de comprimidos nas doses de 3,125 mg, 6,25 mg, 12,5 mg e 25 mg. Após a administração por via oral, ele apresenta uma biodisponibilidade sistêmica extremamente limitada (25-35%), devido ao extenso metabolismo hepático de primeira passagem (VISHNU *et al.,* 2007; DANDAN *et al.,* 2012). Sua absorção ocorre de forma veloz e o pico de concentração no plasma é atingido 1 a 2 h após sua administração. Ele possui um tempo de meia vida de 7 a 10 h e a posologia

indica sua administração duas vezes ao dia. Os principais efeitos adversos relatados por pacientes durante o seu uso foram dor de cabeça, hipotensão, tontura, fadiga e sonolência (MORGAN 1994; HENDERSON 2006).

Com o intuito de melhorar a adesão ao tratamento, estudos têm sido realizados com o objetivo de avaliar o uso de sistemas de liberação controlada, propostos para administração de carvedilol uma vez ao dia. Em um estudo randomizado com 77 pacientes (homens e mulheres) hipertensos de idade entre 20 e 55 anos, Henderson e co-autores (2006) compararam o uso de formulações orais de carvedilol de liberação imediata e de liberação controlada. Os autores demonstraram que ambas posologias promoveram semelhante bloqueio dos receptores β-adrenérgicos. Além disso, as formulações mostraram equivalência nos perfis farmacocinéticos. A semelhança de efeitos farmacocinéticos, da administração uma vez ao dia de carvedilol a partir de uma formulação de liberação controlada, em comparação com a administração duas vezes ao dia, empregando uma formulação de liberação imediata, foi também foi demonstrada por Kitakaze e co-autores (2012), em pacientes com insuficiência cardíaca crônica. Este estudo randomizado, multicêntrico, que contou com 41 participantes, sendo a maioria homens, demonstrou semelhança entre os valores de concentração plasmática máxima e área sobre a curva das duas formas de administração. Kim e colabordores (2015) demonstraram, ainda, em um estudo com 30 homens sadios de idades entre 20 e 55 anos, que a administração uma vez ao dia de diferentes concentrações de carvedilol de liberação controlada atingiu concentrações plasmáticas similares às terapêuticas para este fármaco.

O carvedilol vem sendo utilizado no tratamento de doenças responsáveis pelos maiores índices de morbidade e mortalidade no mundo (ZAHRA *et al.,* 2015). Devido a importânica deste fármaco, diferentes estratégias vem sendo propostas para melhorar a sua biodisponibilidade. Venishetty e co-autores (2012) exploraram o uso de nanopartículas lipídicas sólidas para administração oral. Segundo eles, esse sistema de liberação é capaz de evitar o efeito de primeira passagem, uma vez que atingem a circulação sistêmica através do sistema linfático, evitando a passagem pelo fígado. Os autores demonstraram que a área sob a curva, que representa a concentração plasmática máxima em função do tempo, aumentou significativamente quando o fármaco foi

administrado nanoencapsulado. Os valores passaram de 1,97 ± 0,09 µg/mL/h para 2,88 ± 0,23 µg/mL/h quando nanopartículas lipídicas sólidas contendo quitosana na superfície foram utilizadas e para 6,29 ± 0,23 µg/mL/h quando nanopartículas lipídicas sólidas contendo N-carboximetil quitosana sintetizado na superfície foram administradas. A adição de N-carboximetil foi realizada para evitar a liberação precoce de carvedilol no ambiente ácido do estômago.

Sistemas sólidos e líquidos autonanoemulsificáveis foram estudados por Singh e co-autores (2013), para evitar a passagem de carvedilol pelo fígado, uma vez que são sistemas que também promovem a absorção de fármacos pelo sistema linfático. Análises *in vivo* demonstraram aumento significativo da biodisponibilidade oral do carvedilol em ratos, quando comparados ao fármaco puro, na forma de suspensão de carboximetil celulose, e a uma formulação comercial de comprimidos. A concentração plasmática máxima foi cerca de 300 ng/mL para o fármaco puro, 550 ng/mL para formulação comercial e 1000 ng/mL para os sistemas sólidos e líquidos autonanoemulsificáveis, que não apresentaram diferenças entre si.

Saindane e colaboradores (2013) utilizaram nanossistemas para explorar uma nova via de administração que evitasse a passagem pelo fígado. Os autores propuseram a administração por via nasal, a partir de um spray de geleificação *in situ* contendo nanosuspensão de carvedilol. Estudos in vivo, em ratos, demonstraram que a formulação proposta aumentou significativamente 25,96% para 69,38% a biodisponibilidade do fármaco, comparada a sua administração oral, na forma de suspensão. A concentração plasmática máxima observada para o spray nasal foi de 163,99 ng/mL e para a suspensão oral foi de 67,34 ng/mL.

O carvedilol é um fármaco com características extremamente lipofílicas e é um excelente candidato para ser encapsulado em nanocápsulas. George e co-autores (2015) desenvolveram nanocápsulas formadas por PCL, monocaprilato de propilenoglicol como óleo e Lutrol F127 (Poloxâmero 407) como agente estabilizante pelo método de nanoprecipitação. Variações nas quantidades de PCL e Lutrol 127 foram avaliadas e a formulação foi otimizada. Em um estudo seguinte (GEORGE *et al*., 2016), os autores avaliaram o perfil farmacocinético do fármaco encapsulado em nanocápsulas administradas pela via oral, em ratos. Quando comparadas a uma solução do fármaco, as

nanocápsulas promoveram um aumento significativo de 221,09 % na biodisponibilidade oral do carvedilol, explicada pela absorção linfática das partículas. Outros sistemas nanométricos já propostos foram nanopartículas lipídicas sólidas (VENISHETTY *et al.,* 2013, SHAH *et al.,* 2013) e nanoemulsões (MAHMOUND *et al.,* 2009; SINGH *et al.,* 2011; POLURI *et al.,* 2011).

Appications of polymeric nanoparticles in oral diseases: a review of recent findings

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Abstract

Polymeric nanoparticles are promising drug delivery systems due to their physicochemical properties which may be explored to improve the treatment and prevention of several diseases, including oral treatments. Moreover, pharmacological effects of polymers may be improved by their nanostructuration. Therefore, this article aimed to review the studies reported between 2010 and 2017 covering the use of polymeric nanoparticles to the treatment and/or prevention of oral diseases. Their ability to improve drug antibacterial effect, to release the drug at a time-controlled way, to increase drug cellular uptake, cytotoxicity in tumor cells and solubility as well as to be formulated as mucoadhesive drug delivery systems are strategies studied in dentistry and discussed in this review. Furthermore, this report describes the application of the polymeric nanoparticles in the more prevalent oral disorders: dental carious, oral cancer, periodontal and endodontic diseases.

Keywords: application, oral diseases, polymeric nanoparticles, prevention, treatment, strategies.

INTRODUCTION

Oral diseases qualify as major public health problems due to their high prevalence and incidence in all regions of the world [1]. The most common oral diseases are dental caries, periodontal (gum) diseases, oral cancer, trauma from injuries, and hereditary lesions [2]. The distribution and severity of oral diseases vary in different parts of the world and within the same country or region. Dental caries and periodontal diseases (gingivitis and periodontitis) have historically been considered the most important global oral health burdens and are among the most common bacterial infections in humans.

Dental caries is a major health problem in most industrialized countries as it affects 60–90% of school-aged children and the vast majority of adults [3]. Over the past 25 years, studies reported that dental caries prevalence was declining on a global basis. However, during the past decade, the situation has reversed with an increase in the global prevalence of dental caries in children and adults, comprising primary and permanent teeth, as well as coronal and root surfaces [4]. Regarding to periodontal diseases, about 46% of United States adults representing 64.7 million people have periodontitis, with 8.9% having severe periodontitis [5]. In addition to socio-environmental determinants, risk factors for oral diseases include unhealthy diet, tobacco use, harmful alcohol use, and poor oral hygiene [6].

Concerning the treatment and prevention of oral diseases, local administration of drugs in the oral cavity is not a simple process. The constantly flux of saliva and involuntary swallowing may limit the quantity of drug available in the mouth [7]. Inflammatory fluids can be produced in excess in pathologic situations and interfere with drug permanence in local of action [8]. Some areas are difficult to be accessed by the conventional drugs and their action is impaired. Moreover, the oral cavity is in constant contact with the external environment, which creates a mucosal barrier that prevents the passage of diverse substances [7]. This protection limits not only the input of unwanted substances as also the access of pharmaceutical actives in the targeting site of action. Additionally, many conventional drugs have limited efficacy due to unsuccessfully action against pathogens, inadequate drug release from the pharmaceutical forms or inefficient concentration in the site of action along with the risk of important

adverse effects [9]. In order to overcome these limitations, different studies have explored the use of nanoparticles as drug delivery system, broadening the therapeutic approaches for the treatment of oral diseases.

Nanotechnology comprises a strategy involving the manipulation of particles in the nanoscale and has received great attention in the last years. It has been studied in different areas to improve the efficacy of products or systems. In the pharmaceutical area, the use of nanoparticles has been explored as drugs carrier to promote their effective delivery [10]. Furthermore, physical, chemical and biological properties of materials may be improved by the increase of their surface area when structured in nanoparticles [11]. Moreover, the large surface area allows the bind, adsorption or carrying of drugs, probes or proteins [12,13]. Nanoparticles may be formed by biological materials as phospholipids, lipids and lactic acid or by non-biological material as polymers, carbons, metals and silica [12]. Lipid nanoparticles, as liposome [14] and nanoemulsions [15] beyond polymeric nanoparticles [16] have been widely studied in the pharmaceutical area.

Polymeric nanoparticles stands out because they have good stability, high loading capacity, high control of drug release and its surface can be easily modified or functionalized [11,17,18]. Different studies have reported their properties to control drug release [19-21], to protect substances against degradation [19,21-23], to accumulate drugs in inflamed or tumor tissue [24,25], to improve cellular uptake [26-28], cellular interaction [29-31] and cellular penetration [32-34]. These properties have been explored to improve the biological effects of drugs and to decrease the risk of their side effects.

In this scenario, this article aimed to review the studies reported in the last eight years (2010-2017) on the use of polymeric nanoparticles in the treatment and prevention of oral disorders. Web of Science, PubMed and Science Direct were the data bases used in the search. The review is organized with an initial brief summary about the dental biofilm and oral diseases, followed by the discussion about the strategies to improve the treatment and prevention of these diseases using polymeric nanoparticles. Their applications in treatment of periodontal diseases, carious lesions, endodontic lesions and oral cancer are discussed.

THE DENTAL BIOFILM

The presence of microbes on all accessible surfaces of the mouth is natural, and is essential for the normal development of the physiology of the oral cavity [35]. The mouth provides a conducive environment to the colonization and growth of a diverse range of microorganisms, of which bacteria are the most common and numerous [36,37]. Although the microbiota of the oral cavity provides a beneficial environment during healthy conditions, ecological shifts may occur within the microbial community resulting in the two major oral diseases: dental caries and periodontal diseases [38,39]. A description of dental biofilm (formerly known as dental plaque) before the explanation of oral diseases is paramount.

Microorganisms have primarily been characterized as planktonic, freely suspended cells growing in nutritionally rich culture media. The biofilm mode of growth is a distinct phenotype, in which microorganisms attach to and grow universally on exposed surfaces. Microbial biofilms are ubiquitous in nature and may form on any solid surface exposed to appropriate amounts of water and nutrient [40]. These microbial cells are irreversibly associated and may not be easily removed, as by a gentle rinsing. Therefore, biofilms constitute a protected mode of growth of microorganisms that allows survival in a hostile environment.

Bacterial biofilms are more tolerant to most antimicrobials and host defenses compared with planktonic bacteria [41]. It was estimated that about 1000-fold higher drug concentrations are needed to kill bacteria in biofilms, compared to minimal inhibitory concentration (MIC) necessary for planktonic bacteria [42]. A variety of hypotheses have been proposed to explain this reduced susceptibility of biofilms to antimicrobials: the polymeric matrix of exopolysaccharide produced by bacteria [43]; the novel phenotype expressed by bacteria when growing on a surface; the slow growth rates of attached bacteria within biofilms [44]; the activation of an adaptive stress response, the physiological heterogeneity of the biofilm population; and the presence of phenotypic variants or “persisters” [45,46].

The teeth were the first location in the human body where biofilms were described [47,48], harboring the largest accumulations of bacteria in the oral cavity, since desquamation ensures lower microbial load on mucosal surfaces [36,37]. The early colonizers of the teeth are dominated by streptococci that

may comprise up to 60–90% of the initial flora [49]. These first colonizers are organisms able to withstand the high oxygen concentrations and to resist the various removal mechanisms of the oral cavity [50]. Their replication enables the subsequent adhesion of other bacterial species, mainly made up of Gram-positive rods, predominantly Actinomyces [51]. As the number of plaque layers increases, nutritional and atmospheric gradients are created, the oxygen level decreases and the anaerobes can grow and survive [52-54]. The complexity of the microbiota increases in 48 hours, as indicated by a high morphological diversity [55].

Fortunately, biofilms in the oral cavity are readily accessible allowing their mechanical removal. Dental diseases may be controlled by meticulous mechanical oral hygiene. Subjects who maintain a high standard of oral hygiene on a regular basis present a very low incidence of dental caries and periodontal disease as well as tooth loss [56]. Nevertheless, several studies evaluated effectiveness of oral hygiene instructions and revealed that there is limited adherence to daily oral hygiene regimen by patients [57,58]. Most individuals have difficulty in maintaining the necessary standards of plaque control for prolonged periods. Furthermore, oral hygiene in patients with special needs has proved inadequate and ineffective in many cases using conventional methods. The same applies to management of high caries risk and high caries activity patients. In this respect, antimicrobial substances may be used as adjunct approaches [59-61].

Antiplaque agents present in toothpastes and mouthwashes are designed to prevent the formation and/or to remove established dental biofilm. However, the length of time recommended for people to rinse with a mouthwash or the time for habitual dental brushing is generally in the order of two minutes. A major requirement of the formulation, therefore, is to deliver sufficient concentration of the inhibitor in those two minutes to ensure retention on dental and mucosal surfaces in the mouth so that the active components can be released over time at levels that will still have biological activity. This property of product retention is named substantivity, and varies markedly among antimicrobial agents [62]. With regard to the pharmacokinetic profile of orally-delivered antimicrobials, it is important to consider that higher drug concentration must present short contact

time to avoid undesirable effects, as well as low drug concentration must remain for a long contact time to be effective.

ORAL DISEASES

Dental Caries

A triad of factors is needed for the development of dental caries [63]: carbohydrates (the diet), susceptible teeth (the host) and bacteria (dental plaque). Dental plaque/biofilm is where the caries process is initiated [64-66]. Permanently metabolically active bacteria create fluctuations in pH, which leads to a loss of mineral from the tooth when the pH is dropping, or a gain of mineral when the pH is increasing [65]. The cumulative result of these de- and re-mineralization processes may be a net loss of mineral, leading to dissolution of the dental hard tissues and the formation of a caries lesion. Caries lesions initiate as a small area of subsurface demineralization beneath dental plaque (white spot lesion), and if the process is not controlled, it may progress to total deterioration of dental structure, resulting in tooth loss.

Carbohydrate-induced shifts in plaque's microbial composition toward certain specific organisms may be postulated to be a prerequisite for caries development. *Streptococcus* species*,* mainly *Streptococcus mutans,* are responsible for fermentation of carbohydrates and consequently acidification of dental plaque environment which is involved in the dental demineralization [67]. *Lactobacillus* may play a significant role only during the initiation of a low percentage of caries lesions, but may be more important in their progression [68,69]. Among the protective factors of the host involved in the caries process, the saliva is responsible for acid neutralization and induces remineralization by providing minerals that can replace those dissolved from the tooth during demineralization [70]. Among the substances applied in the treatment and prevention of caries, fluoride has the ability to prevent and arrest dental caries by changing critical pH values for dental demineralization and also by enhancing remineralization [71]. Furthermore, among various antimicrobial agents and methods tested against human dental caries, the most persistent reduction of *Streptococcus mutans* has been achieved by high concentration chlorhexidine varnishes, followed by gels and mouthwashes [72]. Noteworthy,

all preventive and treatment strategies involving mainly fluoride are not effective if a change in oral hygiene habits and exposure to sugar is implemented.

Endodontic diseases

The dental pulp is a soft connective tissue residing within the pulp chamber and root canals of teeth. Under normal circumstances, pulp tissue and its surrounding dentin are protected by dental enamel. Natural absence, caries, or iatrogenic removal of enamel exposes the dentin (and, eventually, the pulp tissue) to bacterial infection. Bacterial products or other contaminants may be introduced into the dentinal fluid as a result of dental caries, restorative procedures, or growth of bacteria beneath restorations [73]. These injurious agents can percolate into the pulp and produce an inflammatory response, or pulpitis.

Certain peculiarities are imposed on the dental pulp by the rigid mineralized dentin in which it is enclosed. The limited ability to increase in volume during episodes of vasodilatation and increased vascular permeability reduces the capacity of pulp healing after repeated insults [74]. Once the cause of the inflammation is removed and pulpitis is still reversible, pulp may return to a healthy state. Nonetheless, if reversible pulpitis is left untreated, the process evolves into a more advanced state of pulpal disease: irreversible pulpitis and pulp necrosis, two conditions that require root canal therapy, i.e. endodontic therapy [75].

Endodontic infection takes place when the root canal harbors numerous irritants as a consequence of pathologic changes in the dental pulp [76]. The root periapex becomes involved when bacterial products and deteriorating pulp tissue leak out of the root apex, evoking a chronic inflammatory response from the vessels in the periodontal ligament. A dynamic encounter between microbial factors and host defenses at the interface between infected radicular pulp and periodontal ligament at the apex results in local inflammation, resorption of hard tissues, destruction of other periapical tissues, and eventual formation of various histopathological categories of apical periodontitis, commonly referred to as periapical lesions [77]. The endodontic therapy consists in biomechanical preparation, which shapes and cleans the root canal. The irritant is removed, and the root canal is filled for healing occurrence. Although root canal treatment

is effective in most cases, complete disinfection of root canal is very difficult to achieve because of persistent microbes in anatomical complexities and apical portion of root canal [78,79].

Necrotic pulpal tissue has revealed polymicrobial flora with an average of 4-7 intra-canal species, which are often Gram-negative anaerobes [80]. Progression of infection alters the nutritional and environmental status within the root canal, making it more anaerobic with depleted nutritional levels. These changes offer a tough ecological niche for the surviving microorganisms. Because biofilm is the manner of bacterial growth, which survives in unfavorable environmental and nutritional conditions, the root canal environment will favor biofilm formation.

Periodontal diseases

Periodontal diseases usually refer to common infectious disorders known as gingivitis and periodontitis. The bacteria involved in the pathogenesis of periodontal diseases reside within biofilms both above (supragingival) and below (subgingival) the gingival margin. Local host defense against bacteria is an inflammatory response characterized by increased vascular permeability and the presence of exudate in the gingival crevice (sulcus) [81]. As the inflammatory infiltrate increases, thickness of gingival epithelium decreases and may present ulcerated areas [82]. In clinical means, the inflammatory process generates gingival swelling and bleeding, which are signs of gingivitis. Gingivitis is associated with the development of a more organized dental plaque. Such biofilms are characterized by several cell layers (100–300), with bacteria stratification arranged by metabolism and aerotolerance [83].

If the inflammatory process continues, a move is enabled to biofilm. Bacteria migrate from the supragingival to the subgingival environment with associated periodontal pocket formation and establishment of periodontitis [84]. At this time, there is a significant decrease in the *Actinomyces* species and an increase in the proportion of anaerobes such as *Tannerella forsythia, Porphyromonas gingivalis* and *Treponema denticola.* An interaction of subgingival biofilm with host immune-inflammatory response, in addition with environmental, genetic and modifying factors, will result in different clinical expressions of periodontitis [85]. In contrast to gingivitis, periodontitis leads to irreversible anatomical

changes in the surrounding tissues of the teeth, affecting periodontal ligament fibers and alveolar bone. These processes are known as periodontal attachment loss, and may result in tooth loss, if the disease is not treated. Clinical parameters involved in the diagnosis of periodontal diseases usually comprises gingival index, plaque index, bleeding on probing, pocket depth and clinical attachment loss.

Conventional treatment of periodontal diseases consists of mechanical instrumentation/scaling of dental root to reduce the total bacterial count. Supportive periodontal therapy is necessary to monitor achieved results and to maintain patterns of oral hygiene over time. If systemic antimicrobials are indicated in periodontal therapy, they should be adjunctive to mechanical debridement [86]. Local delivery of antimicrobials has also been investigated [87]. Although these alternative approaches present statistical significance in scientific studies, their clinically relevance remains questionable [88]. Multiple surgical approaches have been employed for treating intrabony defects of the periodontal attachment apparatus. However, limitations in the predictability and effectiveness of regenerative therapy are well documented in the literature [89].

The role of a high-quality root debridement along with the implementation of a risk factor modification approach (oral hygiene habits, patient's motivation and education, smoking cessation, diabetes control, healthy lifestyle changes) in the management of periodontitis is paramount [90].

Oral cancer

Cancers of the 'oral cavity and oropharynx' comprises the cancers of the lip, tongue and mouth (oral cavity), and oropharynx, excluding the salivary glands and other pharyngeal sites. Oral cancer though uncommon in developed countries is a serious and growing problem in many parts of the globe, and may arise as a significant component of the total burden of cancer [91]. Oral and pharyngeal cancer, grouped together, represents the sixth leading cancer in the world and ranks in the top three in high incidence areas. In high-risk countries, such as Sri Lanka, India, Pakistan and Bangladesh, oral cancer is the most common cancer in men and may contribute up to 25% of all new cases of cancer [91]. Etiology is multifactorial and numerous risk factors or possible causing agents of oral cancer have been described. Chemical factors like

tobacco and alcohol, biological factors like human papillomavirus (HPV), syphilis, oro-dental factors, dietary deficiencies, chronic candidiasis and viruses have been shown to be significantly associated with oral cancer [92].

Oral carcinogenesis like any other cancer is a progressive disease and normal epithelium passes through stages starting from dysplasia to finally transforming into invasive phenotypes. Although all types of carcinomas are seen in oral cavity, the most common form of oral cancer is squamous cell carcinoma, accounting for over 90 percent of oral cavity and oropharynx tumors [93]. The most common site for intraoral carcinoma is the tongue, which accounts for around 40 percent of all cases in the oral cavity proper [94].

Oral cancer remains a lethal disease for over 50% of cases diagnosed annually [91]. This is largely reflected by the fact that most cases are in advanced stages at the time of detection. Despite advances in surgery, radiation, and chemotherapy, the five-year survival rate for oral cancer has not improved significantly over the past several decades and it remains at about 50 to 55 percent [95]. Even for those surviving, quality of life remains poor.

POLYMERIC NANOPARTICLES

Nanocapsules and nanospheres are the principal representatives of polymeric nanoparticles and are different in their composition and structure. Nanospheres are composed of a polymer that forms a matrix where the drug can be adsorbed or retained [96]. On the other hand, nanocapsules have in their composition a polymer and an oil. The oily component is responsible for the formation of a core that is involucred by a polymeric wall. In this kind of particle, the drug can be adsorbed in the polymeric wall or dispersed in the oily core [97]. Surfactants are used to stabilize both systems which are prepared in aqueous media by methods of emulsification and solvent evaporation/extraction, nanoprecipitation (solvent-displacement), supercritical antisolvent method and salting-out [98,99], among others. Polymeric micelles are another representative of this kind of nanoparticle. They are formed by self-assembled copolymers blocks with hydrophobic and hydrophilic portions that compose the core and the shell, respectively. Methods used in synthesis of these nanoparticles are dialysis, oil-in water emulsion followed by solvent evaporation, solid dispersion, direct dissolution, complexation, chemical conjugation and various solvent

evaporation procedures [100]. However, in most of the studies, the type of particle is not clearly defined and they are named generally as nanoparticle. This occurred mainly for particles composed with polymers and surfactants and in some cases of particles produced with amphiphilic block copolymers. The original nomenclature is preserved in this review.

Tables 1-4 list and describe the polymeric nanosystems (nanoparticles, nanospheres, polymeric micelles and nanocapsules) in a chronological order. Among the polymeric materials composing these nanoparticles, most of them are biodegradable polymers, as chitosan, poly-d,l-lactide-co-glycolide (PLGA), polylactic acid (PLA) and poly-ε-caprolactone (PCL) [101]. Chitosan is a polymer with antimicrobial effect [102] and its use as a nanostructurated material was studied by different authors. The strategies and applications of the polymeric nanosystems listed in Tables 1-4 are discussed in the following sections.

STRATEGIES

Polymeric nanoparticles have different properties which may be explored to improve the treatment and prevention of oral diseases, including their ability to control the drug release, to increase drug antibacterial effect, cellular uptake, cytotoxicity in tumor cells and solubility as well as to form a drug delivery system with mucoadhesive property. These strategies are discussed below.

Increased antibacterial effect

As exposed early, diverse bacteria species are involved in oral infections and their combat is a key in treatment of oral diseases. Polymeric nanoparticles may interact with bacteria cell and promote a better and targeted drug delivery. Different *in vitro* studies evidenced the improved activity of drugs encapsulated in polymeric nanoparticles against bacteria involved in oral infection, either in the planktonic phase or in the biofilm phase. The nanostructuration of chitosan have been also explored.

Planktomic phase

PLGA nanoparticles were used for encapsulation of methylene blue [103] and tetracycline [104]. Their antibacterial activity was demonstrated against

Enterococcus faecalis beyond *Aggregatibacter actinomycetemcomitans* and *Prevotella nigrescens* species, respectively. Methylene blue-loaded PLGA nanoparticles containg Pluronic F-108® on the surface promoted important killing effect to polymicrobial plankton extracted from dental plaque [105,106]. Tetracycline was also encapsulated in carboxymethyl chitosan nanoparticles and it was effective against *Staphylococcus aureus* and *Escherichia coli* colonies [107]. Indocyanine green-loaded PLGA nanospheres coated with chitosan in association to light significantly reduced the number of colony forming units of *Porphyromonas gingivalis* than light and non-encapsulated indocyanine green [108]. In a subsequent study, the antibacterial effect of indocyanine green-loaded PLGA nanospheres against *Porphyromonas gingivalis* after trans-gingival light induction was reported [109]. Singh and co-workers [110] incorporated PCL nanoparticles containing satranidazole into a sodium carboxy methyl cellulose gel. The nanostructured gel showed higher antibacterial activity against *Aggregatibacter actinomycetemcomitans* than a drug solution or a gel containing the non-encapsulated drug. Farnesol-loaded nanoparticles of 2-(dimethylamino)ethyl methacrylate (DMAEMA), butyl methacrylate (BMA), and 2-propylacrylic acid (PAA) decreased the viability of *Streptococcus mutans* [111]. Encapsulation of curcumin in chitosan, alginate or starch nanoparticles decreased minimum inhibitory concentration to combat *Streptococcus mutans* [112].

Moreover, association of rose bengal in chitosan nanoparticles improved the antibacterial effect of this photosensitizer against *Enterococcus faecalis* [113,114]. Bacterial cell membrane damage was higher when rose bengal was associated to chitosan nanoparticles compared with its non-encapsulated form. Nanoparticles were able to interact with bacterial cells as shown by transmission electron microscopy analyses [114]. Furthermore, rutin-loaded chitosan nanoparticles showed better antibacterial action against *Bacillus thuringiensis*, *Bacillus pumilus*, *Pseudomonas aeurogenosa*, *Acinetobacter junii* and *Enterococcus faecalis* from dental caries than non-encapsulated rutin and unloaded chitosan nanoparticles [115]. More recently, Barreras and co-workers [116] explored the use of chitosan nanoparticles to increase the antibacterial effect of chlorhexidine. Chlorhexidine loaded-chitosan nanoparticles showed

better activity against *Enterococcus faecalis* compared to non-encapsulated chlorhexidine or the unloaded chitosan nanoparticles.

The antibacterial effect of chlorhexidine was improved by its encapsulation in nanocapsules. The growth inhibition zone of *Enterococcus faecalis* and *Streptococcus mutans* was higher for chlorhexidine-loaded nanocapsules than unloaded nanocapsules and non-encapsulated chlorhexidine. The inhibition was proportionally to drug concentration [117]. Nanocapsules were also studied for co-encapsulation of triclosan and indomethacin. The nanoparticles developed were incorporated in a primer or adhesive resin to be used in prevention of pulp inflammation after teeth restorations. The antibacterial effect of disks containing one portion of primer and two portions of adhesive were evaluated against *Streptococcus mutans*. Concentration of nanoparticles present in primer was 2% and in adhesive variated (1, 2, 5 and 10%). Antimicrobial effect in 24 h was observed for disks formed with adhesive containing 2 or 5% of nanocapsules while in 96 h the decrease of number colony forming units was observed for all concentrations of nanocapsules [118].

Biofilm phase

Chitosan has cationic characteristics and may interact electrostatically with negative charges of bacterial cells affecting its permeability and consequently its viability [102]. The use of nanostructurated chitosan may facilitate its interaction and diffusion through cellular membrane. Chitosan nanoparticles produced damaged against cellular membranes of cells forming the biofilm of *Streptococcus mutans.* The molecular weight of chitosan used in nanoparticles production influenced the cellular death in biofilm levels. Particles prepared with the lowest molecular weight chitosan were able to promote death in deepest levels [119]. Bacteria are also able to survive in acid medium because they produce an acid tolerance response [120]. In this context, chitosan nanoparticles showed the ability to alter membrane permeability of biofilm cells of *Streptococcus mutans* and to block the production of acid tolerance response, preventing the cell survive in environment with low pH [121]. Moreover, chitosan nanoparticles interfered in viability of *Enterococcus faecalis* biofilm of different strain [ATCC and OG1RF]. The decrease in colony-forming units was observed as well as undoing of biofilm structure with significantly

reduction of their thickness. Aging effect with sterile filtered saliva or phosphate-buffers saline did not interfere the antibacterial activity of chitosan nanoparticles [122]. The antibiofilm formation and a better antibacterial effect of chitosan nanoparticles suspension than chitosan solution against *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus sanguis* and *Streptococcus salivarius was also reported [123].* Curcumin-loaded chitosan, alginate or starch nanoparticles promoted a higher decrease of *Streptococcus mutans biofilm formation than non-encapsulated curcumin* [112].

Horev and co-workers [111] developed a nanocarrier able to interact with biofilm extracellular polymeric matrix and to release the drug inside this protection by means of the nanostructure disintegration in acid environment. Farnesol was encapsulated in nanoparticles formed by a diblock copolymers containing 2-(dimethylamino)ethyl methacrylate (DMAEMA), butyl methacrylate (BMA), and 2-propylacrylic acid (PAA) which are susceptible to degradation in acid environmental. Nanoparticles interacted with exopolysaccharides in biofilm surface and farnesol-loaded nanoparticles showed significantly decreased in number of colony-forming units of *Streptococcus mutans* biofilm when compared to non-encapsulated farnesol and unloaded nanoparticles.

Photodynamic therapy has been also studied as an alternative treatment against bacterial biofilm formation that involves the administration of a photosensitizer which is activated by light incidence and will increase the production of free cytotoxic radicals. However, this therapy shows some resistance, which can be associated with the poor penetration capacity of the photosensitizer agent as well as its expulsion from the bacterial biofilm [124,125]. To overcome this limitation, different authors proposed encapsulation of methylene blue in PLGA nanoparticles. Klepac-Ceraj and co-workers [105] showed the effect of cationic and anionic nanoparticles produced with cetyl trimethyl ammonium bromide or Pluronic F-108®, as surfactants, respectively, in viability of a biofilm formed with bacteria extracted from dental plaque. Treatment of polymicrobial oral biofilm was conducted with different formulations, non-encapsulated methylene blue, methylene blue-loaded cationic nanoparticles and methylene blue-loaded anionic nanoparticles followed by exposition of light. When all formulations were analyzed together, significant difference in relation to number of colony-forming units was not observed.

Methylene blue-loaded cationic nanoparticles showed just a tendency to improve the decrease of the bacterial viability. When the effect of the photo agent without and with light was compared it was significantly increased just when methylene blue was nanoencapsulated. A similar study involving the nanoencapsulation of methylene blue in PLGA nanoparticles was conducted by De Freitas and co-workers [106]. Photodynamic therapy with the nanoencapsulated photosensitizer showed a tendency to improve the decrease of the viability of a biofilm formed from human dental plaque bacteria than the photo agent free. However the results of number of colony-forming units were not different.

Rose Bengal-loaded chitosan nanoparticles showed important effects in the management of bacterial biofilm [113,114]. A higher amount of rose Bengal was internalized in *Enterococcus faecalis* when it was encapsulated in chitosan nanoparticles compared with the non-encapsulated form. The rose Bengal-loaded nanoparticles destructed the biofilm structure after exposition to light and this effect was not observed for the non-encapsulated photoactive agent [114]. Furthermore, the same research group [126] demonstrated that rose Bengal-loaded chitosan nanoparticles promoted a better antibiofilm effect against a multispecies of dentin biofilm (*Prevotella intermedia*, *Actinomyces naeslundii*, and *Streptococcus oralis*), compared with the treatment with non-encapsulated rose Bengal. Biofilm disruption and cell dead were observed.

Controlled drug release

An important property showed by polymeric nanoparticles that contributes to their use as delivery systems is the control of the drug release. The mode as the drug will be released from the polymeric nanoparticles is dependent on the polymer erosion and drug diffusion processes [127]. Sustained release allows the maintenance of drug concentration in the site of action decreasing the administration frequency and consequently improving the drug efficacy [128]. This property has been explored in different studies on the nanoencapsulation of drugs for treatment of oral diseases.

Nanoparticles were able to control the release of metronidazole benzoate [129], triclosan [130], minocycline [131], tetracycline [104,107] and lovastatin [104] intended for periodontal diseases treatment, whereas the controlled release of

silibinin [132], doxorubicin [133], alpha-TOS (analog vitamin E) [133], 5-fluorouracil [134] and curcumin [134] was explored by formulations intended for oral cancer therapeutic. The controlled release of curcumin [112] and fluoride [135] by nanoparticles was explored for dental delivery in combat or prevention of dental caries. The chlorhexidine controlled release by nanoparticles in bacterial plaque control avoided dental caries and periodontal diseases [136]. In addition, the controlled release of bovine serum albumin [137], dexamethasone [138] and miR-146ª [139] was studied for endodontic therapy. Farnesol release from nanoparticles was faster in acid environment than in basic and the release profile was enough to promote a better drug action against bacterial biofilm when compared to non-encapsulated farnesol [111]. In most cases the drugs were released from nanoparticles by a biphasic profile, which is characterized by an initial burst effect followed by an extended release. This profile is a well-known property of polymeric nanoparticles. Minocycline release was influenced predominantly by drug diffusion and the nanoencapsulation allowed a lower drug concentration decline in gingival crevice fluid when compared to drug non-encapsulated, and this behavior influenced positively the drug effect in periodontal infection treatment [131]. Doxorubicin and alpha-TOS were encapsulated in nanoparticles which is reactive species oxygen-responsive. The drug release was controlled in phosphate buffer solution medium and was accelerated in presence of potassium peroxide (KO_2). Reactive oxygen species are present in excess in tumor tissues and the use of carriers sensitive to these species promoted a more effective release of doxorubicin and alpha-TOS inside the tumor [133].

Nanocapsules were able to control the release of 15-Deoxy-D12,14-PG J2 (15d-PGJ2), a cyclopentenone-type PG used for immunomodulation of anti-inflammatory response in periodontal diseases [140] and of chlorhexidine, an metalloproteinase inhibitor and antimicrobial agent [117]. Furthermore, polymeric nanocapsules promoted the controlled release of eugenol, an oil with analgesic, anesthetic, anti-inflammatory and antibacterial activities which may be used in periodontal infection [141]. Its release from nanocapsules was evaluated in simulated gingival crevicular fluid and a burst effect was reported in the first few hours followed by an extended release. The sustained release influenced positively the drug effect in relation to its non-encapsulated form in

periodontal disease treatment. Even more, nanocapsules incorporated in adhesive resin controlled the release of indomethacin when encapsulated alone [142] or together with triclosan [118]. The release of co-encapsulated indomethacin and triclosan was also by a controlled way when the nanocapsules were incorporated in a primer [118]. These systems were designed for use in prevention of pulp inflammation after teeth restorations.

Polymeric micelles controlled the release of doxorubicin and autophagy inhibitor (LY294002) [143], cisplatin [144] and docetaxel [145] aiming their use against oral squamous cell carcinoma. Acid environment is common in some oral pathological conditions, as cancer, influencing the pH dependent drug release of formulations. Release of doxorubicin and autophagy inhibitor present in the same particle was faster in acid medium than in basic medium facilitating their release inside the tumor [143]. Docetaxel can have effective released by a controlled way in acid environmental of tumor tissue promoting a better action than non-encapsulated drug although to be released by a more controlled way in basic environment [145]. Cisplatin showed a biphasic release that was not influenced by addition of peptide (NR7) on the surface of the particles. The peptide was used to target the particles to cancer cells [144].

Active substances or drugs may have to be encapsulated on surface or inside the nanoparticle [146] and their distinct release profile may influence the result of the treatment [147]. In this way, Shrestha and co-workers [137] explored the use of chitosan nanoparticles to control the release of a growth factor, bovine serum albumin, in regenerative endodontic treatments. Two formulations were developed, one containing albumin encapsulated into the polymeric matrix and other containing albumin on the surface of the nanparticles. Around 40 % of albumin from surface was released in 10 days and around 20 % from inside the particle matrix in 30 days. Both systems were able to increase the alkaline phosphatase activity indicating odontogenic differentiation. However, the system with the best drug release control showed a higher increase in this activity. In a next study, Shrestha and co-workers [138] evaluated the influence of the localization of dexamethasone (on the surface or inside) in chitosan nanoparticles on the odontogenic differentiation. In this case, association of dexamethasone on the surface of chitosan nanoparticles promoted a better cellular differentiation than its incorporation inside the matrix polymeric due to

its faster release. In another study, tetracycline and lovastatin were incorporated on the surface and core, respectively, of PLGA nanoparticles coated with chitosan [104]. The release of higher amounts of tetracycline in the first days and a more controlled release of lovastatin was observed. The nanoencapsulation and the sequence as the drugs were released made possible the antibacterial effect and bone regeneration using only one system. Polymeric micelles were explored for sequential release of autophagy inhibitor (LY294002) and chemotherapeutic agent (Doxorubicin). The faster release of the autophagy inhibitor followed by a more controlled release of doxorubicin allows a better treatment of tumor tissue [143].

Increased cellular uptake

Nanoparticles can be internalized by tumor cells by endocytosis bypass due to their reduced size. This is an intrinsic phenomenon showed by this kind of particles that results in cellular accumulation and intracellular drug release [148]. This phenomenon was observed for polymeric micelles co-encapsulating doxorubicin and autophagy inhibitor [LY294002]. Their nanoencapsulation promoted a better cellular internalization by oral squamous carcinoma cells [CAL-27 and HN-6] followed by the drug release inside the cells [143]. In another study, nanoparticles of polyethylene glycol-polyethyleneimine [PEG-PEI] increased the cellular uptake of miRNA-146a in dental pulp cells isolated from human premolar and molar [139]. Nanoparticles of were also able to influence the cellular internalization of Rose Bengal. The photosensitizer associated to chitosan nanoparticles was visualized in cytoplasm of mouse fibroblast cells, while non-encapsulated Rose Bengal accumulated only in the cell membrane [113].

In a different approach, a specific molecule, which has cell surface receptor, may be linked to the nanoparticle surface to promote an active targeting and to increase the cellular uptake [148]. Nanoparticles of poly(ethylene glycol)–poly(lactic acid) [PEG-PLA], intended for minocycline release in periodontal diseases treatment, were uptaked by oral epithelial cells (Calu-3). This uptake was improved by the attachment of specific ligand (tripeptide arginine-glycine-aspartic acid) on nanoparticles surface [131]. Polymeric micelles of poly(lacticco-gycolic acid) – poly(ethylene) glycol [PLGA-PEG] were internalized

by oral squamous cell carcinoma cells [HN6]. The presence of NR7 peptide in the nanoparticles surface increased their cellular uptake. NR7 peptide shows affinity with epidermal growth factor expressed in oral squamous cell carcinoma [144]. Association of RGD (arginine–glycine–aspartic acid sequences), which has affinity with cellular integrin, also increased the cellular uptake of PLGA-PEG nanoparticles [133].

Increased drug cytotoxicity

Accumulation of nanoparticles in tumor cells and intracellular drug release by a controlled way may lead to a better cytotoxicity effect [148]. The improved activity of nanoencapsulated bioactive molecules against oral carcinoma cells was observed by *in vitro* assays. The better performance of nanoencapsulated antitumor agent against oral carcinoma cells in relation to its non-encapsulated form was studied by different authors. The use of nanoparticles as antitumor carrier promoted a good cytotoxic effect for cupreous complexes [149], 5-fluorouracil and curcumin [134]. The half maximal inhibitory concentration (IC50) of silibinin loaded-nanoparticles was 2.5 times lower than its not-encapsulated form. The antitumor effect of silibilin, a flavonoid compound, is associated with an increase in intracellular reactive oxygen species concentration, which results in cellular apoptosis [150]. A higher production of intracellular reactive oxygen species as well as higher apoptosis indicators (loss of mitochondrial membrane potential and morphological changes) were observed in cells treated with silibilin-loaded nanoparticles than those treated with the non-encapsulated flavonoid [132]. In addition, polymeric micelles were able to increase the cytotoxicity effect of cisplatin [144] and docetaxel [145]. The association of autophagy inhibitor (LY294002) in doxorubicin loaded-polymeric micelles improved the decrease in the cellular viability compared with the physical mixture of autophagy inhibitor and doxorubicin-loaded micelles [143].

Development of mucoadhesive drug delivery systems

Buccal cavity is a region formed by a mucosa layer which has adhesive characteristics due to presence of mucin molecules in mucus surface. Mucin is a glycoprotein with negative charge and responsible by anionic characteristics of the mucus [7]. The use of systems able to interact with mucosa surface has

been explored to strength the contact between the drug and mucosa. The better interaction may avoid the drug removing from the desired site by salivary flux and involuntary swallowing. Furthermore, periodontal disease is associated with an increased production of gingival crevicular fluid, that can difficult the permanence of the drug in the region of interest, limiting its efficacy [8]. Polymers are the most used mucoadhesive materials and their use in nanometric system has been studied to improve their adhesive performance [151]. Polymers structured as nanocapsules originated particles able to interact with vaginal [34], nasal [31] and sublingual mucosa [16]. Few studies have suggested the use of mucoadhesive polymeric nanoparticles to improve drug buccal delivery, including the encapsulation of curcumin [152], metronidazole benzoate [129] and *Punica granatum* peel extract [153]. These reports describe the formulations development and did not demonstrate the influence of mucoadhesion on drug effect. Mazzarino and co-workers [152] used PCL nanoparticles coated by chitosan, a cationic polymer that can interact with mucus layer by electrostatic interaction [34], for buccal delivery of curcumin, an antioxidant, anti-inflammatory, antitumoral and antimicrobial agent. The authors did not detail the oral disorder which curcumin could be used and only demonstrated that the developed nanoparticles were able to interact with mucin molecules. Saboktakin and co-workers [129] proposed the use of thiolated chitosan-poly (methacrylic acid) nanoparticles as metronidazole benzoate delivery system in treatment of periodontal diseases. The mucoadhesiveness of nanoparticles were observed by their interaction with mucin molecules. Polyethylenimine–dextran sulfate nanoparticles containing *Punica granatum* peel extract were produced as mucoadhesive systems for oral application in bad breathe reduction and carries prevention. The nanoparticles interacted better with buccal mucosa and retained a higher drug amount on mucosa in presence of salivary flux than the extract free in solution [153].

Improved drug solubility

Components with low aqueous solubility are difficult to be formulated and their incorporation in hydrophilic medium is a hard task, being sometimes impossible [154]. Polymeric nanoparticles are systems widely used for encapsulation of lipophilic drugs. The nanoencapsulation was essential to promote the

solubilization of curcumin into a mucoadhesive film, produced as a buccal delivery system [155]. According to the authors, this dosage form may be used for treatment of different oral disorders as gingivitis, periodontal diseases, bacterial and fungal infections, aphthous ulcers, inflammations and oral cancer, due to pharmacological activities of curcumin. However, the effect of films produced on these disorders was not evaluated yet. Nanoencapsulation improved the curcumin release from the pharmaceutical form, whereas only 1 % of free drug was released in 24 h compared to 3 % of its nanoencapsulated form. In another study, a mucoadhesive gel containing satranidazole was proposed as an alternative dosage form in the treatment of periodontitis by Bansal and co-workers [156]. The formulation showed important efficacy. However, the lipophilic characteristic of the drug affected the hydrogel stability. To overcome this problem, Singh and co-workers [110] encapsulated satranidazole in PCL nanoparticles aiming the formation of suitable mucoadhesive gel. Sodium carboxy methyl cellulose gel containing satranidazole nanoencapsulated showed a better uniformity than the gel containing the non-encapsulated drug due to the improvement of satranidazole aqueous dispersibility by the nanoencapsulation. Moreover, the nanoencapsulation improved antibacterial effect of the drug and the clinical results against periodontal diseases are showed in the following section.

APPLICATIONS

Periodontal diseases

The advantages of polymeric nanoparticles as drug delivery systems in the treatment of periodontal diseases has been demonstrated by *in vivo* studies using clinical test in humans or animal models, as dogs and rats. In general, studies have showed that administration of nanoencapsulated drugs improve their desired effects compared with its non-encapsulated form.

Polymeric micelles formed by amphiphilic block copolymer Pluronic F127 encapsulating dexamethasone and ascorbyl-palmitate promoted *in vitro* osteogenic differentiation in cultured human periodontal ligament mesenchymal stem cells, and the formulation showed potential to be used in bone regeneration [157]. 15-Deoxy-D12,14-PG J2-loaded nanocapsules were explored for immunomodulation of anti-inflammatory response in periodontal

diseases [140]. Mouse infected with human periodontal pathogen and treated with 15-Deoxy-D12,14-PG J2-loaded nanocapsules showed decrease of alveolar bone loss, of leukocyte infiltration in submandibular lymph nodes and of inflammatory markers when compared to untreated infected animals. Eugenol, a natural oil present in different plants and presenting antibacterial activity, was proposed for periodontal diseases treatment after its encapsulation in polymeric nanocapsules. The effects were evaluated in rats containing periodontal diseases induced by ligature [141]. Local administration of eugenol-loaded nanocapsules promoted continuity of epithelium of the interdental papilla significantly superior than observed for groups treated with non-encapsulated eugenol, unloaded nanocapsules and receiving no treatment. A similar effect was observed in relation to animals treated with minocycline, a reference drug. According to the histological analyses, no differences were observed in relation to alveolar bone resorption between the treatment groups. However, eugenol-loaded nanocapsules were able to avoid destruction of the alveolar bone crest, which was not observed for groups treated with non-encapsulated eugenol, unloaded nanocapsules and receiving no treatment.

The effect of nanoencapsulation of minocycline, an antibacterial agent, in PEG-PLA nanoparticles for periodontitis treatment was evaluated in beagle dogs [131]. Local administration of nanoencapsulated minocycline increased the drug concentration in gingival crevice fluid during root scaling compared with the respective drug solution and a commercial ointment. This result was explained by the controlled drug release showed by nanoparticles beyond their capacity of adherence and permeation in inflamed tissue. Furthermore, minocycline-loaded nanoparticles improved clinical parameters of periodontitis when compared with the drug solution. Non-encapsulated minocycline showed similar effect to the saline solution. The higher drug concentration in periodontal pocket improved the long-term treatment efficacy of nanoencapsulated minocycline when compared to the commercial ointment. The same research group [158] further explored the surface attachment of a specific ligand (tripeptide arginine-glycine-aspartic acid) to minocycline-loaded PEG-PLA nanoparticles aiming the targeting delivery and prolonging their permanence in periodontal pocket. This strategy increased the cellular interaction and uptake of nanoparticles. Furthermore, the presence of the peptide on the nanoparticles surface

prolonged the drug concentration at therapeutic levels in gingival crevice fluid, which influenced positively the clinical parameters of periodontitis.

A mucoadhesive gel able to retain nanoencapsulated satranidazole, an antibacterial agent, in periodontal pocket was proposed for the treatment of periodontitis. The influence of the nanoencapsulation was evaluated by human clinical studies (n = 10). The mouth was divided in two areas and the same patient received locally the treatment with gel containing non-encapsulated satranidazole or satranidazole-loaded PCL nanoparticles. After 21 days, nanoencapsulation promoted a higher reduction of periodontal markers (probing depth, plaque index and gingival index) [110]. In another study, chitosan nanoparticles were studied as carrier of siRNA as a new approach for periodontal inflammatory therapy. It was demonstrated that siRNA-loaded chitosan nanoparticles were able to recruit macrophages to inflamed tissue in mice infected with a local injection of the lipopolysaccharides from *Porphyromonas gingivalis* [159]. PLGA nanoparticles coated with chitosan were explored for co-administration of tetracycline and lovastatin for the control of periodontal infection and periodontal tissue regeneration, respectively. Beagle dogs treated with chitosan coated lovastatin+tetracycline-loaded PLGA nanoparticles showed no signal of inflammation and higher new bone formation and osteogenic activity after 8 weeks than unloaded PLGA-chitosan nanoparticles [104]. Moreover, the use of nanoparticles as carriers for photosensitizer increased the efficacy of photodynamic therapy associated to ultrasonic scaling and mechanical root planning in treatment of moderate to advanced chronic periodontitis [106]. A clinical study with 20 volunteers evidenced that the use of a nanoencapsulated photosensitizer (methylene blue-loaded PLGA nanoparticles) resulted in lower gingival bleeding index in a long-term treatment (3 months).

Endodontic diseases

Most particles studied in treatment of endodontic infection are chitosan nanoparticles and their use was explored as antibacterial agents or as carrier of antibacterial bioactives. All reports in endodontic area show the advantages of nanoencapsulation by *in vitro* studies in extracted teeth.

The use of polymeric nanoparticles as photosensitizer delivery systems has been widely studied to improve the photodynamic therapeutic in endodontic infection. Methylene blue-loaded PLGA nanoparticles associated to light activation were effective in treatment of root canals of human extracted teeth and infected with *Enterococcus faecalis*. Electronic microscopy analysis showed the presence of PLGA nanoparticles in dentinal tubules [103]. Chitosan nanoparticles as carrier of rose Bengal showed important antibacterial effect even in presence of tissue inhibitors of root canal [160]. Furthermore, this system promoted stabilization of dentin-matrix, which can be disintegrated in bacterial infection. The better effect of nanoencapsulated rose Bengal than its non-encapsulated form was observed for ultimate tensile strength. In relation to enzymatic degradation and toughness of dentin collagen, similar results were observed [113,114]. Rose Bengal encapsulated in chitosan nanoparticles neutralized lipopolysaccharides, a bacterial endotoxin present in root canal, which can aggravate the clinical symptoms of endodontic infection and impair its treatment [161]. Use of chitosan nanoparticles as carrier of rose bengal significantly potentiated its effects against inflammatory markers, like tumor necrosis factor (TNF-α) and interleukin (IL-6) [162].

Stabilization of dentin in root canal is very important for endodontically treatment success in long-term. Chitosan nanoparticles were able to stabilize the dentin-matrix by collagenase inhibition. Collagen is a structural protein of dentin, which may be degraded by bacterial enzymes. Chitosan nanoparticles and photodynamic therapy isolated reduced the collagen degradation and the effect of nanoparticles was significantly better. When the therapies were associated, the effect was potentiated and as consequence, the residual collagen was greater but not significantly different in relation to treatment isolates. Chitosan nanoparticles showed affinity with collagenases by electrostatic interaction, which explained their important inhibition effect on collagen degradation [163]. Chlorhexidine is also an inhibitor of collagenolytic enzymes and its encapsulation in nanocapsules was proposed to improve the resin-dentin bonded interface resistance when applied to demineralized dentin. Nanocapsules delivery and retention inside the dentinal tubules of extracted human molars was observed. Moreover, chlorhexidine was released from nanocapsules to demineralized dentin substrate by a controlled way. A better

antibacterial effect was observed for the drug nanoencapsulated than its non-encapsulated form [117].

Endodontic treatment also involves the use of sealers and association of antibacterial agents to this material has been proposed to improve the antibacterial effect. Association of chitosan nanoparticles into zinc oxide-eugenol sealer was more effective than the sealer without nanoparticles in the treatment of root canal of extracted bovine incisors. It was observed a significantly lower percentage of biofilm covered interface and a lower *Enterococcus faecalis* biofilm area in presence of nanoparticles [164]. Association of chitosan nanoparticles to an epoxy resin canal sealer improved their antibacterial efficacy. However, their association to calcium silicate–based root canal sealer did not change their effect, probably because of the high antimicrobial activity of calcium silicate sealer [165]. Chitosan nanoparticles also showed chelating effect in dentin as well as antibacterial effect when utilized as final irrigating of root canal [166]. For this treatment, materials usually utilized (sodium hypochlorite [NaOCl] and ethylenediaminetetraacetic acid [EDTA]) are associated with important collateral effects in dentin, like reduction of microhardness due to destruction of organic material, collagen, responsible for mechanical properties of dentin and alterations in physicochemical properties [167].

Regeneration of endodontic tissue involves disinfection of root canal, which is normally made by irrigation using EDTA followed by NaOCl that can promote cytotoxic effects in dentin cells making the regeneration difficult. Encapsulation of dexamethasone in chitosan nanoparticles was able to release the drug by slow or rapid way, depending on its localization in the nanoparticles. The use of those two particles after irrigation with NaOCl and EDTA, promoted an increase in steam cell adherence and viability in dentin at the same intensity. However, dexamethasone encapsulated on surface of chitosan nanoparticles, having a faster drug release rate, improved the odontogenic differentiation of steam cells compared with the dexamethasone encapsulated inside the particle, which showed a slower drug release profile [168].

Dental pulp cells, precursors of dental pulp tissue, infected with lipopolysaccharide as pulpal pathogen, when treated with gel containing miRNA-146a-loaded nanoparticles of polyethylene glycol-polyethyleneimine

[PEG-PEI] were able to reproduce beyond odontogenic differentiation. The effect of the gel was increased when free fibroblast growth factor was added to the gel. It was not conducted analyses with miRNA-146a non-encapsulated [139].

The production of an adhesive resin with antiinflamatory effect may be used in prevention of pulp inflammation after teeth restorations. However the resin adhesiveness may be impaired by addition of high levels of antiinflamatory agent. In this way, nanocapsules were explored for encapsulation of indomethacin and to better drug incorporation into the resin. Addition of indomethacin-loaded nanocapsules into the resin did not alter its adhesive characteristic. Moreover, the drug was able to diffuse by a controlled way through dentin of extracted healthy premolar teethes [142]. In subsequent study, the same research group co-encapsulated indomethacin and triclosan in nanocapsules to produce an adhesive resin and a primer with aniinflamatory and antimicrobial effect. The resin and primer physicochemical properties were not altered by addition of the nanocapsules and the drugs diffused by a controlled way through dentin of extracted upper premolars [118]. The controlled drug release was observed in both studies [118,142] and the antibacterial effect of disks formed by resin and primer containing the nanocapsules was observed against *S. mutans* [118].

Oral cancer

Conventional anticancer therapy is usually associated with important limitations due to the poor aqueous solubility, low permeability and low bioavailability of the anticancer agents as well as cytotoxicity effect against normal cells and aggressive adverse reactions [169]. The use of nanoparticles as anticancer delivery systems to overcome these limitations has showed interesting results due to their targeting ability [170]. This property associated to their reduced size and high surface area facilitates their permeation and accumulation in tumor cells [25]. The controlled release of antitumor agent inside the tumor cell increases its bioavailability and anticancer effect, decreasing unwanted reactions [171,172]. Polymeric nanocarriers have been reported as drug delivery systems for the treatment of oral cancer.

In this sense, polymeric micelles as cisplatin nanocarriers (cis-Diaminedichloroplatinum) promoted similar *in vivo* effect compared with the non-encapsulated drug in relation to apoptosis induction and inhibition of tumor growth in rats contaminated with human oral cells carcinoma (OSC-19). However, the use of this nanocarrier reduced the nephrotoxic effect of cisplatin and the incidence of lymphatic metastasis [173]. Nanoencapsulation of docetaxel in polymeric micelles [145] improved the *in vivo* drug antitumor effect against oral tongue squamous cell carcinoma (HSC-3) and decreased systemic toxic effect [145].

Eudragit® E nanoparticles were used for encapsulation of different flavonoids with antitumor effect but poor aqueous solubility and poor bioavailability: Naringenin (4', 5, 7-trihydroxy flavone) [174-176], Silibinin [177] and Hespertin (3',5,7-trihydroxy-4-methoxyflavanone) [178-180]. Nanoencapsulation of all these flavonoids promoted higher decrease in tumor incidence in hamsters containing induced buccal pouch carcinogenesis (7,12-dimethylbenz(a)anthracene – DMBA) than the non-encapsulated bioactive. Furthermore, the better performance of nanoencapsulated flavonoids in relation to their free form was observed in normalization of biomolecules (phospholipids, tryptophan, phenylanine, nucleic acid) or endogenous fluorophore levels (collagen, nicotinamide adenine dinucleotide, flavin adenine dinucleotide, porphyrins), whose concentrations are altered in tumor tissue. Higher redox radio values which indicate decrease of tumor metabolic activity were also observed for nanoencapsulated flavonoids. Even more, the use of nanocarrier for administration of hespertin decreases the incidence of vascular endothelial grown factor responsible by tumor progression [179].

Chitosan nanoparticles containing cupreous complexes promoted *in vivo* tumor inhibition of around 93 % without body weight loss when used in thermo-chemotherapy for treatment of rats having human oral epithelial carcinoma (KB) [149]. Encapsulation of doxorubicin in nanoparticles improved the *in vivo* drug antitumor effect and decreased systemic toxic effect since a reduced body weight loss of mice containing cells from oral tongue squamous cell carcinoma (Cal-27) was observed [133].

Carious lesion

Nanoparticles were explored as systems for release of farnesol in carious prevention [111]. Rats (15 days) orally infected with *Streptococcus mutans* and topically treated with farnesol-loaded nanoparticles of DMAEMA-BMA-PAA, unloaded nanoparticles, farnesol hydroalcoolic solution (non-encapsulated) or hydroalcoolic solution were evaluated (n = 6, per group). Treatment with farnesol-loaded nanoparticles reduced significantly the number and severity of carious lesion when compared to unloaded nanoparticles. Moreover, non-encapsulated farnesol had no effect, which was similar to its vehicle (hydroalcoolic solution). Sustained drug release and good antibacterial activity against planktonic as well as biofilm phase were reported for this polymeric system, which explained its better performance against initiation and progression of the carious lesion.

CONCLUSION

The application of polymeric nanoparticles in oral diseases is a promising research field. This review showed that polymeric nanoparticles (nanoparticles, nanospheres, polymeric micelles and nanocapsules) formulated with different polymers and using different methods were able to improve pharmaceutical properties of encapsulated drug and bioactive molecules or chitosan in the treatment or prevention of oral diseases. Their properties as controlled drug release and cellular uptake, along with the potential to increase the drug solubility, antibacterial and cytotoxicity effect and to improve the interaction with mucosa may broaden the alternatives for the treatment and prevention of oral diseases. The safety of therapies should be one of the focuses of future nanotoxicity studies. Also, further powered and well-designed randomized clinical trials for testing these applications are necessary to determine the clinical potential and efficacy of polymeric nanoparticles in the treatment or prevention of oral diseases.

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Table 1. Different polymeric nanoparticles formulations proposed for the treatment and prevention of oral diseases published in 2010-2013.

Nanoparticle type	*Polymer*	*Drug or bioactive molecule*	*Production method*	*Strategy studied*	*Application*	*Reference*
Nanoparticles	Chitosan	____	Ionic gelation	Increased antibacterial effect (biofilm)	____	Shrestha, 2010 [122]
Nanoparticles	Chitosan	Methylene blue	Solvent displacement	Increased antibacterial effect (planktonic)	Endodontic diseases (*in vitro*)	Pagonis, 2010 [103]
Nanoparticles	Chitosan	____	Ionic gelation	Increased antibacterial effect (biofilm)	____	De Paz, 2011 [119]
Nanoparticles	Chitosan	____	Ionic gelation	Increased antibacterial effect (biofilm)	____	Neilands, 2011 [121]
Nanoparticles	Thiolated chitosan-poly(methacrylicacid)	Metronidazole benzoate	Polymerization	Mucoadhesive drug delivery system Controlled the drug release	____	Saboktakin, 2011 [129]
Nanoparticles	PLGA	Methylene blue	Solvent displacement	Increased antibacterial effect (biofilm/planktonic)	____	Klepac-Ceraj, 2011 [105]
Nanoparticles	Chitosan	Rose Bengal	Ionic gelation	Increased antibacterial effect (biofilm/planktonic) Increased cellular uptake	Endodontic diseases (*in vitro*)	Shrestha, 2012 [113]
Nanoparticles	PCL	Curcumin	Nanoprecipitation	Mucoadhesive drug delivery system	____	Mazzarino, 2012 [152]
Nanocapsules	PLGA	15-Deoxy-D12,14-PG J2 (15d-PGJ2), cyclopentenone-type PG	Nanoprecipitation	Controlled the drug release	Periodontal diseases (*in vivo*[1])	Napimoga, 2012 [140]
Nanoparticles	Chitosan	____	Ionic gelation	____	Endodontic diseases (*in vitro*)	DaSilva, 2013 [164]
Nanoparticles	PCL	Triclosan	Solvent displacement	Controlled the drug release	____	Aminu, 2013 [130]
Nanoparticles	Eudragit® E	Naringenin	Nanoprecipitation	____	Oral cancer (*in vivo*[2])	Sulfikkarali, 2013 [174]
Nanoparticles	Eudragit® E	Naringenin	Nanoprecipitation	____	Oral cancer (*in vivo*[2])	Krishnakumar, 2013 A/B [175,176]
Nanospheres	PLGA	Indocyanine green	Emulsion solvent diffusion	Increased antibacterial effect (planktonic)	____	Nagahara, 2013 [108]
Polymeric micelle	[PEG-PLGA]	Cisplatin	Complexation	____	Oral cancer (*in vivo*[3])	Endo, 2013 [173]
Polymeric micelle	Pluronic F127	Dexamethasone and ascorbyl-palmitate	Microfluidics	____	Periodontal diseases (*in vitro*)	Capretto, 2013 [157]

PLGA: poly-d,l-lactide-co-glycolide, PCL: poly-ε-caprolactone, PEG: Poly(ethylene glycol). In vivo model: [1]mouse, [2]hamster, [3]rat, [4]dog, [5]human.

Table 2. Different polymeric nanoparticles formulations proposed for the treatment and prevention of oral diseases published in 2014.

Nanoparticle type	*Polymer*	*Drug or bioactive molecule*	*Production method*	*Strategy studied*	*Application*	*Reference*
Nanoparticles	Chitosan	Rose Bengal	Ionic gelation	Increased antibacterial effect (biofilm/planktonic)	Endodontic diseases (*in vitro*)	Shrestha, 2014/A [114]
Nanoparticles	Chitosan	Rose Bengal	Ionic gelation	Increased antibacterial effect (biofilm)	____	Shrestha, 2014/B [126]
Nanoparticles	Chitosan	Rose Bengal	Ionic gelation	____	Endodontic diseases (*in vitro*)	Shrestha, 2014/C [160]
Nanoparticles	Chitosan	Bovine serum albumin	Ionic gelation	Controlled the drug release	____	Shrestha, 2014/D [137]
Nanoparticles	Chitosan	_____	Ionic gelation	____	Endodontic diseases (*in vitro*)	Persadmehr, 2014 [163]
Nanoparticles	PCL	Curcumin	Nanoprecipitation	Improved drug solubility	____	Mazzarino, 2014 [155]
Nanoparticles	Eudragit® E	Silibinin	Nanoprecipitation	Controlled the drug release Increased drug cytotoxicity	____	Gohulkumar, 2014/A [132]
Nanoparticles	Eudragit® E	Silibinin	Nanoprecipitation	____	Oral cancer (*in vivo*[2])	Gohulkumar, 2014/B [177]
Nanoparticles	Eudragit® E	Hespertin	Nanoprecipitation	____	Oral cancer (*in vivo*[2])	Gurushankar, 2014 [178]
Nanoparticles	O-carboxymethyl chitosan	Tetracycline/calcium sulfate	Ionic gelation	Controlled the drug release Increase antibacterial effect (planktonic)	____	Reddy, 2014 [107]
Nanoparticles	PEG-PLA	Minocycline	Emulsion/solvent evaporation	Controlled the drug release	Periodontal diseases (*in vivo*[4])	Yao, 2014 [131]
Polymeric micelle	Hyperbranched polyacylhydrazone	Doxorubicin and autophagy inhibitor (LY294002)	Dialysis	Controlled the drug release Increased cellular uptake Increased drug cytotoxicity	____	Saiyin, 2014 [143]

PCL: poly-ε-caprolactone, PEG: Poly(ethylene glycol), PLA: poly(lactic acid). In vivo model: [1]mouse, [2]hamster, [3]rat, [4]dog, [5]human.

Table 3. Different polymeric nanoparticles formulations proposed for the treatment and prevention of oral diseases published in 2015.

Nanoparticle type	***Polymer***	***Drug or bioactive molecule***	***Production method***	***Strategy studied***	***Application***	***Reference***
Nanoparticles	Chitosan	____	Ionic gelation	____	Endodontic diseases (*in vitro*)	Del Carpio-Perochena, 2015/A/B [165,166]
Nanoparticles	Chitosan	Rose Bengal	Ionic gelation	____	Endodontic diseases (*in vitro*)	Shrestha, 2015/A [162]
Nanoparticles	Chitosan	Cupreous complexes	Ionic gelation	Increased drug cytotoxicity	Oral cancer (*in vivo*[3])	Lin, 2015 [149]
Nanoparticles	Chitosan	Dexamethasone	Ionic gelation	Controlled the drug release	____	Shrestha, 2015/B [138]
Nanoparticles	Chitosan	Rutin	Ionic gelation	Increased antibacterial effect (planktonic)	____	Patil, 2015 [115]
Nanoparticles	Chitosan	siRNA	Solvent displacement	____	Periodontal diseases (*in vivo*[1])	Ma, 2015 [159]
Nanoparticles	Chitosan	____	Ionic gelation	Increased antibacterial effect (biofilm)	____	Aliasghari, 2015 [123]
Nanoparticles	PCL	Satranidazole	Nanoprecipitation	Improved drug solubility Increased antibacterial effect (planktonic)	Periodontal diseases (*in vivo*[5])	Singh, 2015 [110]
Nanoparticles	Polyethylenimine–dextran sulfate	*Punica granatum* peel extract	Polyelectrolyte complexation	Mucoadhesive drug delivery system Controlled the drug release		Tiyaboonchai, 2015 [153]
Nanoparticles	Eudragit® E	Hespertin	Nanoprecipitation	____	Oral cancer (*in vivo*[2])	Gurushankar, 2015 [179]
Nanoparticles	PEG-PLA	Minocycline	Emulsion/solvent evaporation	Increased cellular uptake	Periodontal diseases (*in vivo*[4])	Yao, 2015 [158]
Nanoparticles	(p(DMAEMA)-b-p(DMAEMA-co-BMA-co-PAA	Farnesol	Direct dissolution	Controlled the drug release Increase antibacterial effect (biofilm)	Carious lesion (*in vivo*[3])	Horev, 2015 [111]
Polymeric micelle	PLGA-PEG	Cisplatin	Dialysis	Controlled the drug release Improved cellular uptake Increased drug cytotoxicity	____	Wang, 2015 [144]
Nanocapsules	PCL	Eugenol	Solvent displacement	Controlled the drug release	Periodontal diseases (*in vivo*[3])	Pramod, 2015 [141]

PCL: poly-ε-caprolactone, DMAEMA: 2-(dimethylamino)ethyl methacrylate, BMA: butyl methacrylate, PAA 2-propylacrylic acid, PEG: Poly(ethylene glycol), PLA: poly(lactic acid), PLGA: poly-d,l-lactide-co-glycolide. In vivo model: [1]mouse, [2]hamster, [3]rat, [4]dog, [5]human.

Table 4. Different polymeric nanoparticles formulations proposed for the treatment and prevention of oral diseases published in 2016-2017.

Nanoparticle type	***Polymer***	***Drug or bioactive molecule***	***Production method***	***Strategy studied***	***Application***	***Reference***
Nanoparticles	Chitosan	Dexamethasone	Ionic gelation	____	Endodontic diseases (*in vitro*)	Shrestha, 2016 [168]
Nanoparticles	Chitosan	Chlorhexidine	Ionic gelation	Increased antibacterial effect (planktonic)	____	Barreras, 2016 [116]
Nanoparticles	PLGA	Curcumin/5-fluorouracil	Nanoprecipitation/ Double emulsion	Controlled the drug release Increased drug cytotoxicity	____	Masloub, 2016 [134]
Nanoparticles	PLGA	Lovastatin Tetracycline	Double emulsion	Controlled the drug release	Periodontal diseases (*in vivo*[4])	Lee, 2016 [104]
Nanoparticles	PLGA	Methylene blue	Solvent displacement	Increased antibacterial effect (biofilm/planktonic)	Periodontal diseases (*in vivo*[5])	De Freitas, 2016 [106]
Nanoparticles	PLGA	Chlorexidine	Osmosis-based	Controlled the drug release	____	Chronopoulou, 2016 [136]
Nanoparticles	PLGA-PEG	Doxorubicin/ alpha-TOS	Dialysis	Control the drug release Increased antibacterial effect (planktonic) Improved cellular uptake	Oral cancer (*in vivo*[4])	Li, 2016 [133]
Nanoparticles	PEG-PEI	miR-146a	Chemical conjugation	Controlled the drug release Increased cellular uptake	Endodontic diseases (*in vitro*)	Liu, 2016 [139]
Nanoparticles	Eudragit® E	Hespertin	Nanoprecipitation	____	Oral cancer (*in vivo*[2])	Gurushankar, 2016 [180]
Polymeric micelle	Monomethoxy-PEG-bpoly(lactide)	Docetaxel	Chemical conjugation	Controlled the drug release Increased drug cytotoxicity	Oral cancer (*in vivo*[1])	Shi, 2016 [145]
Nanocapsules	PCL	Chlorhexidine	Interfacial polymer deposition	Controlled the drug release Increased antibacterial effect (planktonic)	Endodontic diseases (*in vitro*)	Priyadarshini, 2017 [117]
Nanocapsules	Eudragit® S100	Indomethacin	Interfacial polymer deposition	Controlled the drug release	Endodontic diseases (*in vitro*)	Genari, 2016 [142]
Nanocapsules	Eudragit® S100	Indomethacin and triclosan	Interfacial polymer deposition	Controlled the drug release Increased antibacterial effect (planktonic)	Endodontic diseases (*in vitro*)	Genari, 2017 [118]
Nanoparticles	Chitosan/Alginate /Starch	Curcumin	Desolvation	Increased antibacterial effect (biofilm/planktonic) Controlled the drug release	____	Maghsoudi, 2017 [112]
Nanoparticles	Chitosan	Fluoride	Ionic gelation	Controlled the drug release	____	Nguyen, 2017 [135]
Nanospheres	PLGA	Indocyanine green	Emulsion solvent diffusion	Increased antibacterial effect (planktonic)	____	Sasaki, 2017 [109]

PLGA: poly-d,l-lactide-co-glycolide, PEG: polyethylene glycol-, PEI: polyethyleneimine, PCL: poly-ε-caprolactone. In vivo model: [1]mouse, [2]hamster, [3]rat, [4]dog, [5]hum

APRESENTAÇÃO DO CAPÍTULO

O organismo é constituido por diversas regiões as quais são recobertas por uma camada de mucosa. Essa membrana pode ser explorada como uma superfície adesiva, para melhorar a interação de fármacos com seu local de absorção. Nesse sentido, nanocápsulas, quando produzidas com polímeros com capacidades adesivas, podem produzir sistemas carreadores de fármacos com propriedades mucoadesivas. Por isso, o capítulo I deste trabalho traz um estudo que avalia o efeito mucoadesivo de polímeros de diferentes cargas, quando estruturados em nanocápsulas. Além disso, a influência da forma farmacêutica (suspensão, hidrogel ou pó) em que as nanocápsulas estão veiculadas, frente a distintas superfícies adesivas (mucosa vaginal, mucosa bucal e disco de mucina), foi estudada. As propriedades mucoadesivas das partículas foram avaliadas utilizando um analisador de textura, o qual fornece o valor de trabalho necessário para romper a interação entre as formulações e a superfície adesiva. O capítulo está redigido na forma de artigo, o qual se encontra submetido para publicação.

Mucoadhesive properties of Eudragit®RS100, Eudragit®S100 and Poly(ε-caprolactone) nanocapsules: influence of the vehicle and the mucosal surface

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Abstract

The use of polymers as mucoadhesive materials has been explored in several drug delivery approaches. However, little attention has been given to their mucoadhesiveness when they are structured in nanocapsules. Mucoadhesion measurements are based on the use of animal mucosa or mucin discs, the glycoprotein responsible for the adhesive characteristic of mucus. Therefore, the objective of this study was to analyze the mucoadhesion of nanocapsules produced with polymers of different ionic properties, Eudragit®RS100, Eudragit®S100 or Poly(ε-caprolactone), when they are incorporated into different vehicles (suspension, hydrogel, and powder) and applied on different mucosal surfaces (mucin, porcine vaginal, and buccal mucosa). Mucoadhesion was measurement by tensile stress tester. Polymeric self-assembling as nanocapsules improved the mucoadhesion of the polymers. The best performance was shown by Eudragit®RS100-nanocapsules. Hydrogels showed higher adhesion when compared to suspensions or powders. Mucin increased mucoadhesiveness of all formulations, but reproduced the difference between them. In conclusion, this study demonstrated that Eudragit®RS100-nanocapsules interacted better with membranes. Furthermore, the vehicle influenced mucoadhesive performance (hydrogel > powders > suspensions). In addition, mucin may be used to compare formulations and in preliminary tests using tensile tester, while the use of porcine mucosa is ideal to mimic adhesion conditions considering *in vivo* experiments.

Keywords: polymeric nanocapsules, mucoadhesion, mucin disc, porcine mucosa, hydrogel, powders.

4.1. INTRODUCTION

Polymers are the most used materials in the development of mucoadhesive systems due to properties like functional group, pH, charge, and molecular weight, which may influence the form and intensity in which adhesion occurs [1]. The adhesion of polymers to mucosae has become an important subject in the development of drug delivery systems.

A mucosa is a membrane composed by an epithelial layer covered by a mucus film. Its main function is to protect an organism from the external environment. Formed by lipids, glycoproteins, and inorganic salts suspended in water, the mucus layer has a cohesive gel texture and its thickness varies between 1 and 450 µm [2]. The glycoprotein mucin is the main component of this structure, and is the main factor responsible for its adhesiveness. Mucin concentration as well as mucus properties vary in function of the mucosal location in the body, which in turn can influence the adhesive performance of the mucosa and consequently the interaction with drug delivery systems [3].

Several cavities of the human body are formed by a mucosal surface with adhesiveness characteristics like buccal, esophageal, gastric, intestinal, colonic, rectal, nasal, lung, ocular, and vaginal cavities. These regions have been explored for drug administration aiming towards a more effective local or systemic drug effect [3]. However, these sites are exposed to a constant flux of biological fluids that can remove part of the applied drug that should be absorbed, influencing drug bioavailability. The mouth, for example, produces 0.5 - 2 L of saliva per day [4] and a woman produces 2 - 3 g of vaginal fluids daily [5]. In this scenario, drug carriers that strongly interact with the mucosal surface may improve drug absorption, and are amongst the current challenges in the development of polymeric systems for mucosal application.

Previous studies proposed different theories to explain the interaction between polymeric materials and mucosal surfaces [6]. Other studies have looked into new ways to promote a stronger interaction between polymeric materials and mucosal surfaces [1]. One of the proposed alternatives to increase this interaction is the use of polymers in the form of nanocapsules [7-9], i.e. structures formed by a polymeric wall stabilized with surfactants around a lipophilic core [10]. It has been demonstrated that the structuration of polymeric

materials at the nanoscale increases the surface contact area, facilitating the binding between polymers and mucus [3]. Moreover, like nanocapsules in general, they are more likely to improve the efficacy of different drugs by controlling the release, improving stability, targeting, improving cellular uptake, modulating permeation, and/or decreasing side effects [11,12]. Another advantage is that these polymeric nanosystems can be used in different pharmaceutical forms, like suspensions, gels, or powders [12], which facilitates administration because of the flexibility of application while maintaining the original nanostructures and their properties after the drying process [13-15]. They are also included in hydrogel formulations [7, 16-19].

Eudragit®RS100 [9, 18, 20-22], Eudragit®S100 [7,21,23], and Poly(ε-caprolactone) [24-27] are examples of polymers widely used in the production of nanocapsules and they are different in relation to their chemical composition and properties. Eudragit®RS100 [Eudragit RS] is a cationic biocompatible co-polymer of poly(ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) [28,29] Eudragit®S100 [Eudragit S100] is a non-toxic anionic co-polymer of poly(methacrylic acid, methyl methacrylate) [30], and Poly(ε-caprolactone) [PCL] is a non-ionic biodegradable and biocompatible polyester [11]. Even though the adhesiveness of these three types of polymers is discussed in the literature, few attention has been given specifically to this property when they are structured in nanocapsules and how the surface charge of these nanocapsules may influence it.

An important limitation of previous studies is that mucoadhesion measurements usually comprise the use of animal mucosa. Different biological properties may occur depending on the animal source, which makes difficult to obtain reproducible and comparable data. . In addition, the removal of large areas of the mucosa may sometimes be difficult, when it is placed in inaccessible sites. In view of this, some authors have used a disc of mucin, which is the glycoprotein responsible for the adhesive characteristic of mucus, instead of mucosal tissue [8,31-33]. Animal mucin shows similar chemical and morphological structure to those of human mucin. Commercially, mucin is extracted from porcine stomach and bovine submaxillary glands [34].

Summarizing the research problem considered in this paper, the current literature lacks information about the interaction of three types of polymeric

nanocapsulas, as mucoadhseive systems, with mucin or animal mucosa models and the resulting mucoadhesiveness of such interaction. Also, there is a lack of information about how the type of vehicle used (e.g. suspension, hydrogel or powder) influences mucoadhesiveness of nanocapsules.

In this scenario, the main objective of this paper was to study the effect of the vehicle (suspension, hydrogel, and powder) on the mucoadhesiveness of Eudragit®RS100, Eudragit®S100, or Poly(ε-caprolactone) nanocapsules as well as the effect of different mucosal surfaces (mucin, vaginal mucosa, and buccal mucosa). Moreover, this paper also investigated whether mucin could be an appropriate alternative to the use of fresh animal mucosa in mucoadhesion tests of polymeric nanocapsules using a tensile stress tester.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Poly(ε-caprolactone) (Mn 80,000), sorbitan monostearate (Span 60®) and mucin from porcine stomach (type II) were acquired from Sigma-Aldrich (São Paulo, Brazil). Eudragit®RS100 was obtained from Degussa (Darmstadt, Germany), and capric/caprilic triglyceride was obtained from Dellaware (Porto Alegre, Brazil). Eudragit®S100 and hydroxyethyl cellulose were bought from Evonik Industries AG and Embacaps (Porto Alegre, Brazil), respectively. Polysorbate 80 and acetone were purchased from Vetec (Rio de Janeiro, Brazil). Ethanol and lactose were purchased from Nuclear (São Paulo, Brazil) Dinâmica (São Paulo, Brazil), respectively.

4.2.2. Preparation and characterization of nanocapsule suspensions

Nanocapsule suspensions were prepared by interfacial deposition according to the preformed polymer method [10]. An organic phase (27 mL) was injected into an aqueous phase (53 mL) formed by 0.077 g of polysorbate 80. The organic phase of particles formed of Eudragit RS (NC-RS) was composed by 0.1g of polymer and 165 µL of capric/caprilic triglyceride dissolved in acetone with magnetic stirring at 40°C. Formulations containing the polymers poly(ε-caprolactone) (NC-PCL) and Eudragit S100 (NC-S100) were produced identically, but the organic phase included 0.0385 g of sorbitan monostearate, and acetone was changed for ethanol in NC-S100. Solvents were removed by

reduced pressure (Rotavapor R-114, Büchi, Flawil, Switzerland) and the suspensions were concentrated to the final volume of 10 mL. The formulations (n = 3) were characterized in relation to diameter of particles by laser diffraction (Mastersizer 2000, Malvern Instruments Ltd., UK) to evaluate the presence of microparticles or microaggregates, inserting the formulations directly in the wet dispersion unit, and by dynamic light scattering (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK) dissolving 20 µL of formulations in 10 mL of ultrapure water to confirm the presence of only particles in the micrometer size. Zeta potential was evaluated by electrophoretic mobility, dissolving 20 µL of formulations in 10 mL of NaCl solution 10 mM (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK), and pH was analyzed using a potentiometer directly in the formulations (VB-10, Denver Instrument, USA).

4.2.3. Preparation and characterization of hydrogels

Hydrogels were produced by dissolving hydroxyethyl cellulose (2%) into nanocapsule suspensions followed by manual mixing (HG-NC-S100, HG-NC-RS, HG-NC-PCL). The formulations were maintained at 4ºC for 48 h until a hydrogel formed. The pH was determined (n = 3) after the gel was diluted in water (1:10, w/v). The morphology of the hydrogels was analyzed by scanning electron microscopy (SEM; Jeol Scanning Microscope, JSM-6060, Tokyo, Japan) operating at 10 kV, at the Microscopy Center of the University (Centro de Microscopia Eletrônica - UFRGS, Brazil). For this analysis, the samples were gold-sputtered. The differences in hydrogel morphology was analyzed in order to observe the presence of nanoparticles and to compare the different nanostructures in hydrogels. Moreover, an additional hydrogel was prepared without nanocapsules, as control (HG-HEC).

4.2.4. Preparation and characterization of spray-dried powders

The powders were prepared according to the spray-dried technique (Mini Spray-Dryer B-290 (Büchi, Flawil, Switzerland) using the following parameters: feed pump rate of 5.0 ml·min^{-1}, 100% aspiration, 0.7 mm nozzle, atomization air at 819 L·h^{-1}, and an inlet temperature of 120°C with a resulting outlet temperature of approximately 65°C. Lactose was used as drying adjuvant at 10% (w/v). It was added into nanocapsule suspensions (SD-NC-S100, SD-NC-

RS, SD-NC-PCL) prior to the drying process and kept under magnetic stirring for 10 min and during the feeding process. The morphology of powders was analyzed by scanning electron microscopy (SEM; Jeol Scanning Microscope, JSM-6060, Tokyo, Japan) operating at 10 kV, at the Microscopy Center of the University (Centro de Microscopia Eletrônica - UFRGS, Brazil). For this analysis, the samples were gold-sputtered. The difference in powder morphology was analyzed in order to observe the presence of nanoparticles in the different powders. An additional powder was produced from an aqueous dispersion of lactose in water (10 % w/v) (SD-Lac), as control.

4.2.5. Mucoadhesion measurements

A tensile stress tester (TA.XTplus Texture Analyzer; Stable Microsystem, Godalming, UK) was used to analyze mucoadhesion (n = 3) of the polymeric nanocapsules (suspension, hydrogel, and powder). Vaginal and buccal mucosa as well as mucin discs were fixed to the cylindrical probe of the equipment with double-sided adhesive tape. The mucous contacted the nanocapsules samples with a 290-mN preload force for 3 min and, then, they were removed at a constant speed of 0.10 $mm.s^{-1}$ upon complete detachment. The work (mN.mm) necessary to detach the buccal mucosa, vaginal mucosa, or mucin discs from formulations (NC-S100, NC-RS, NC-PCL, HG-NC-S100, HG-NC-RS, HG-NC-PCL, SD-NC-S100, SD-NC-RS, SD-NC-PCL) was calculated. This calculation is based on the peak of force (mN) and the maximum displacement (mm) upon complete detachment. Solutions of Eudragit S100 (S-S100), Eudragit RS (S-RS), and poly(ε-caprolactone) (S-PCL) were also analyzed for comparison. These solutions were prepared at the same polymeric concentration of the nanocapsule suspensions (0.01 $g.mL^{-1}$). Eudragit S100 was dissolved in ethanol, while Eudragit RS and poly(ε-caprolactone) were dissolved in acetone. Fresh porcine vagina and heads were obtained from Santo Ângelo slaughterhouse (Porto Alegre, Brazil). Vaginal and buccal mucous were excised using a scalpel and were immediately used. Mucin discs were produced by a compression device of the same equipment (TA.XTplus Texture Analyzer; Stable Microsystem, Godalming, UK) using a test speed of 1 $mm.s^{-1}$, post-test speed of 1 $mm.s^{-1}$, and a distance of 4 mm. Before analyses of samples, mucin

discs were hydrated with 20 µL of ultrapure water, and excess water was removed with absorbent paper.

4.2.6. Statistical analyses

The data collected were analyzed using a full factorial experiment based on the analysis of variance (ANOVA) followed by the Tukey's post-hoc test for multiple comparison of means. These analyses were performed using the SPSS statistics software, version 17.0®. Differences were considered statistically significant for a *p-value* ≤ 0.05.

4.3. RESULTS

4.3.1. Physicochemical properties of the nanocapsule suspensions and their respective hydrogels and powders

The mean size of nanocapsules suspensions was determined using the laser diffraction technique. A radar chart containing the mean diameter (d[4,3]), diameter cumulative of 10 [d(0.1)], 50 [d(0.5)] and 90 [d(0.9)] percent of particles by volume (v) and number (n) distribution is shown in Figure 1.

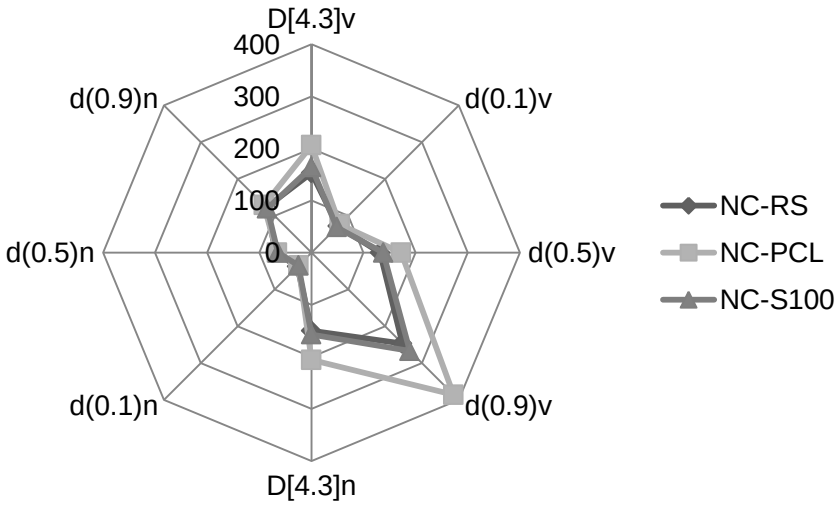

Figure 1. Radar chart of formulations.

All the formulations showed particle size distribution only in the nanoscale range as well as unimodal size distribution [35]. The d(0.9)v observed for the three formulations was lower than 400 nm and the d(0.5)n was lower than 100 nm.

The volume-weighted mean diameter (d[4,3]v) was 153 ± 25 nm, 164 ± 13 nm, and 206 ± 32 nm for NC-RS, NC-S100, and NC-PCL, respectively. Span values, which represent the polydispersity of the systems, were 1.25 ± 0.35, 1.72 ± 0.08, and 1.44 ± 0.07 for NC-RS, NC-S100, and NC-PCL, respectively. Mean diameter and polydispersity index (PDI) were confirmed by dynamic light scattering analyses (Table 1). Zeta potential was negative for NC-S100 and NC-PCL, though it was positive for NC-RS (Table 1). The pH of all formulations was slightly acid (Table 1).

Table 1. Characteristics of formulations in relation to mean diameter and polydispersity index by dynamic light scattering, zeta potential and pH (n = 3, mean ± standard deviation).

	MEAN DIAMETER (nm)	**PDI**	**ZETA POTENTIAL (mV)**	**pH**
NC-S100	154 ± 6	0.141 ± 0.014	-6.76 ± 0.30	5.66 ± 0.12
NC-RS	123 ± 6	0.108 ± 0.013	+6.62 ± 0.52	6.03 ± 0.06
NC-PCL	200 ± 2	0.124 ± 0.017	-5.27 ± 1.38	6.02 ±0.12

The pHs of HG-HEC, HG-NC-S100, HG-NC-RS, and HG-NC-PCL were 6.70 ± 0.06, 6.77 ± 0.06, 6.74 ± 0.03, and 5.86 ± 0.03 respectively. The hydrogel prepared only with hydroxyethylcellulose (Figure 2-A) had an irregular surface free of spherical particles and hydrogels prepared with nanocapsules suspension (Figure 2-B,C,D) showed a surface covered with spherical nanoparticles which varied in arrangement.

Spray-dried powders were produced using lactose as drying adjuvant. The powder produced only with lactose had irregular structures with smooth surfaces. On the other hand, the powders produced with nanocapsule suspensions showed micro-agglomerates of spherical particles, and surfaces varied according to components of the nanocapsules (Figure 3). Presence of spherical particles at the nanoscale could not be observed in all powders because these particles may be dispersed into the lactose matrix [36].

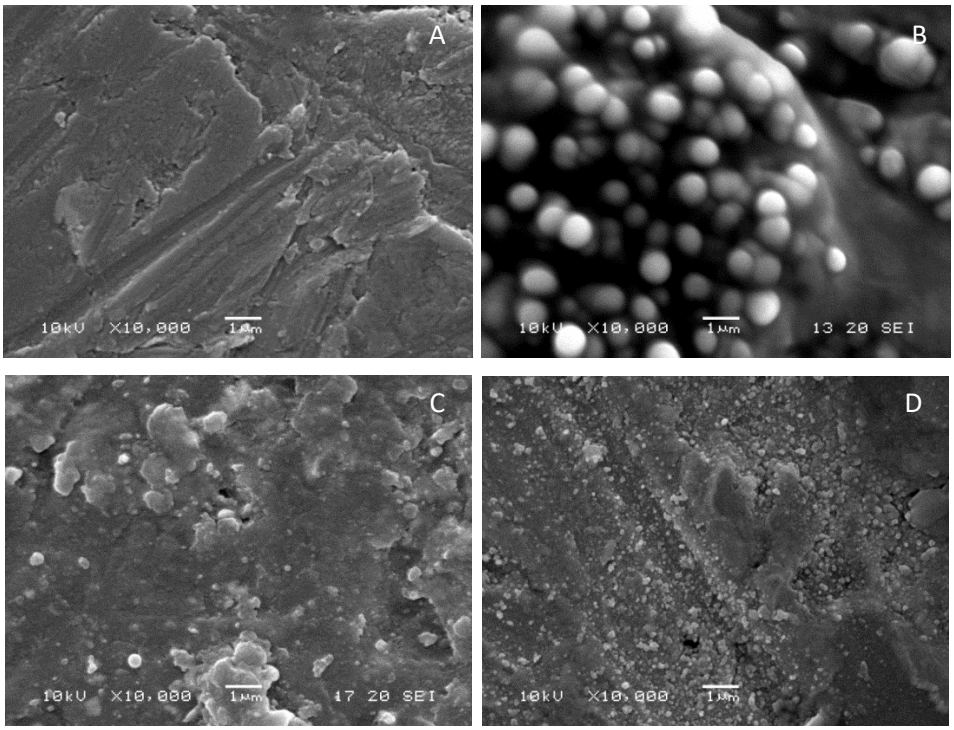

Figure 2. Scanning electron microscopy images of (A) hydrogels of hydroxyethylcellulose (HG-HEC), (B) hydrogels of hydroxyethylcellulose containing nanocapsules of Eudragit®S100 (HG-NC-S100), (C) hydrogels of hydroxyethylcellulose containing nanocapsules of Eudragit®RS100 (HG-NC-RS), (D) hydrogels of hydroxyethylcellulose containing nanocapsules of poly(ε-caprolactone) (HG-NC-PCL).

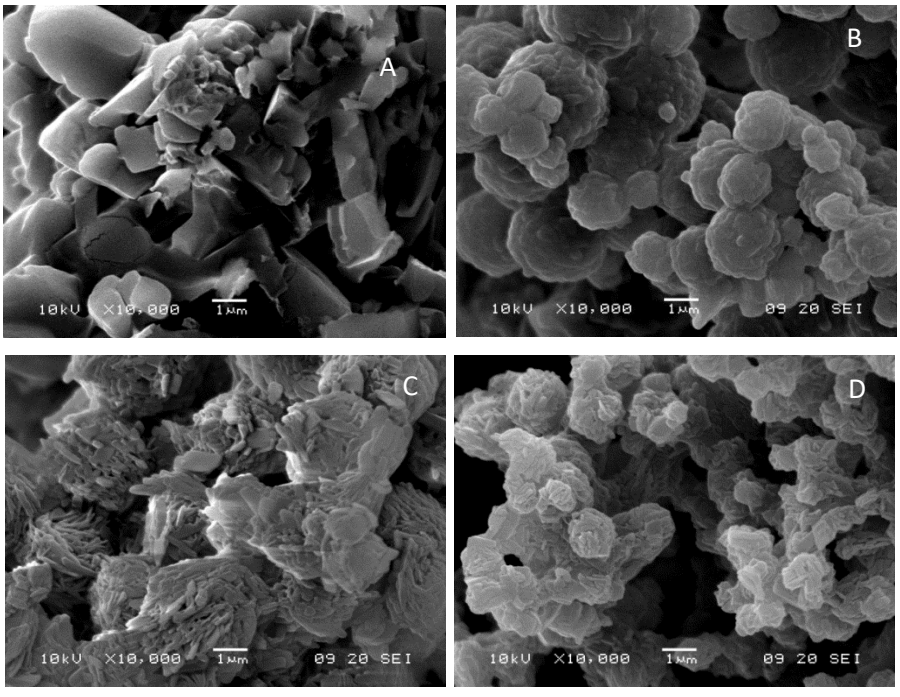

Figure 3. Scanning electron microscopy pictures. (A) lactose powder (SD-Lac), (B) lactose powder containing nanocapsules of Eudragit®S100 (SD-NC-S100), (C) lactose powder containing nanocapsules of Eudragit®RS100 (SD-NC-RS), (D) lactose powder containing nanocapsules of poly(ε-caprolactone) (SD-NC-PCL).

4.3.2. Mucoadhesion measurements

The first analysis of this study was made to compare the mucoadhesive performance of polymeric nanocapsules in suspension and the solutions of their respective wall-forming polymers. This test was designed to define if it would be necessary to consider both types of polymer structuration in the analysis. The ANOVA results showed that mucoadhesion is much stronger for formulations formed by nanocapsules than for formulations containing dissolved polymer, independently of the type of application surface or polymer used (F-value = 81.38, $p < 0.001$; $\Delta\bar{X} = 84.42$ mN.mm). In view of this, in the second part of analysis, described next, only formulations containing nanocapsules were considered.

In the next step, a three-way ANOVA was performed to study how the combination of the three factors (application surfaces, vehicles, and polymers) can influence mucoadhesiveness of formulations. Therefore, three types of application surface (mucin, vaginal mucosa, and buccal mucosa), three types of vehicles containing nanocapsules (suspension, hydrogel, and powder) and three types of polymers (Eudragit RS, Eudragit S100, and PCL) were combined. ANOVA results showed that all the main effects (i.e. the single effect of application surfaces, vehicles, or polymers) as well as the second-level interactions (i.e. the pairwise interaction between application surfaces, vehicles, and polymers) were significant ($p < 0.01$). Only the interaction at the third level (i.e. the interaction of all factors at the same time) was not significant in the experiment ($p = 0.276$). Tukey's post-hoc results are shown in Figures 4 and 5.

Regarding the post-hoc results shown in Figure 4, the interaction between the vehicle (suspension, hydrogel, or power) and the type of application surface (mucin, vaginal mucosa, and buccal mucosa) was considered. Regardless of the vehicle type, it is possible to see that the mucin application surface presents the highest mean work at $p < 0.001$: (i) mucin work for suspension = 368.48 mN.mm; (ii) mucin work for hydrogel = 949.94 mN.mm; and (iii) mucin work for

powder = 352.91 mN.mm. On the other hand, vaginal mucosa and buccal mucosa did not differ statistically for any of the three different vehicles types: (i) suspension: vaginal work = 88.327 mN.mm and buccal work = 50.88 mN.mm, p = 0.138; (ii) hydrogel: vaginal work = 246.678 mN.mm and buccal work = 219.09 mN.mm, p = 0.27; (iii) powder: vaginal work = 88.77 mN.mm and buccal work = 69.44 mN.mm, p = 0.44). Moreover, for all types of application surfaces, hydrogel was the vehicle that allowed obtaining the highest levels of mucoadhesion, while powder and suspension presented a similar behavior. This could also be corroborated statistically by comparing the main effect of the vehicles types, where the results indicated that hydrogel has a mucoadhesion level (mean work) of 471.9 mN.mm, which is significantly different at $p < 0.001$ from the other two vehicles. On the other hand, suspension (Wmean= 169.23 mN.mm) and powder (Wmean= 170.37 mN.mm) did not differ statistically ($p = 0.937$).

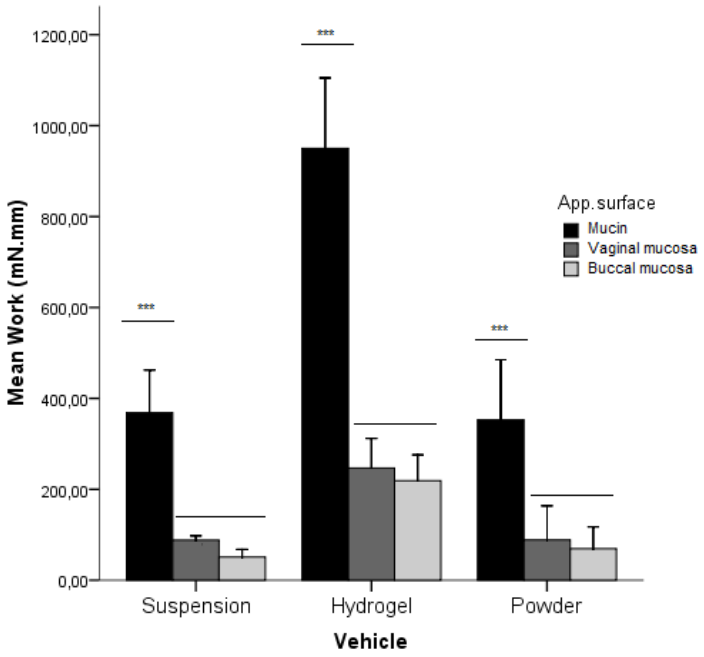

Figure 4. Mucoadhesiveness of different types of vehicles and application surfaces. *** Application surfaces that differed statistically from the others were highlighted.

Furthermore, the interaction effect between the different types of polymer (Eudragit RS, Eudragit S100, and PCL) and type of application surface (mucin, vaginal mucosa, and buccal mucosa) was analyzed, as shown in Figure 5. Again, the mucin application surface presented the highest levels of

mucoadhesion regardless of polymer type at p < 0.001: (i) mucin work for Eudragit RS = 735.94 mN.mm; (ii) mucin work for Eudragit S100 = 396.01 mN.mm; and (iii) mucin work for PCL = 539.38 mN.mm. On the other hand, vaginal mucosa and buccal mucosa did not differ statistically concerning each polymer type (p = 0.075 for Eudragit RS; p = 0.107 for Eudragit S100; and p = 0.95 for PCL). Moreover, Figure 2 illustrates the significant differences between all polymers at p<0.001 in the following order of mucoadhesion level: (i) Eudragit RS: Wmean = 375.65 mN.mm, (ii) PCL: Wmean = 249.58 mN.mm; and (iii) Eudragit S100: Wmean = 186.27 mN.mm. However, the differences between Eudragit S100 and PCL are prominent because of the mucin effect, while their behavior for vaginal and buccal mucosa seems to be very similar.

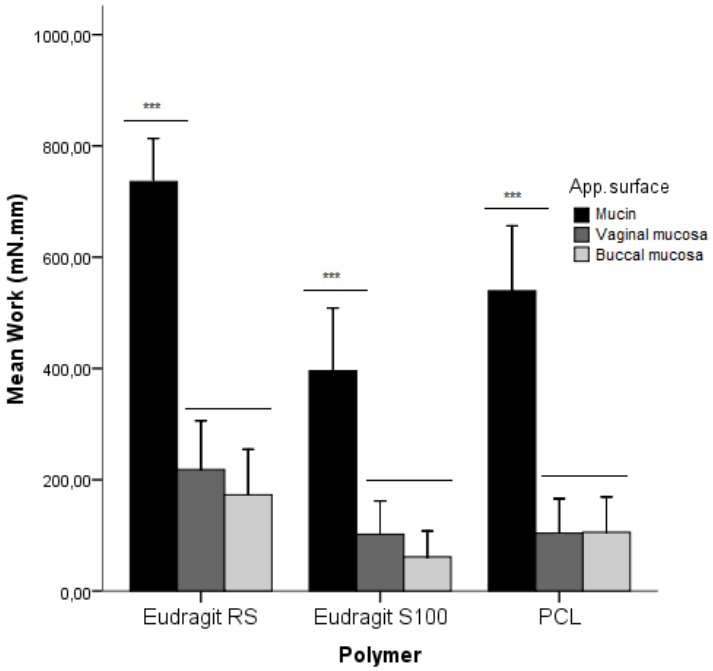

Figure 5. Mucoadhesiveness of different types of polymer and application surfaces. *** Application surfaces that differed statistically from the others were highlighted.

Figure 6 summarizes the comparative results of mucoadhesion when the different vehicle types (suspension, hydrogel, and powder) are combined with different types of polymer (Eudragit RS, Eudragit S100, and PCL). As shown in this figure, statistical differences at p < 0.01 between all types of polymers in the following order of mucoadhesion levels were observed between hydrogel and powder: (i) hydrogel: HG-NC-RS (606.54 mN.mm); HG-NC-PCL (441.72 mN.mm) and HG-NC-S100 (367.44 mN.mm); and (ii) powder: SD-NC-RS

(291.317 mN.mm); SD-NC-PCL (156.12 mN.mm) and SD-NC-S100 (63.69 mN.mm). On the other hand, concerning the suspension vehicle our findings show that NC-RS presented a mean work (mucoadhesion) of 229.09 mN.mm, which differed statistically from the other two polymers at $p < 0.001$, while NC-PCL and NC-S100 did not differ ($p = 0.355$).

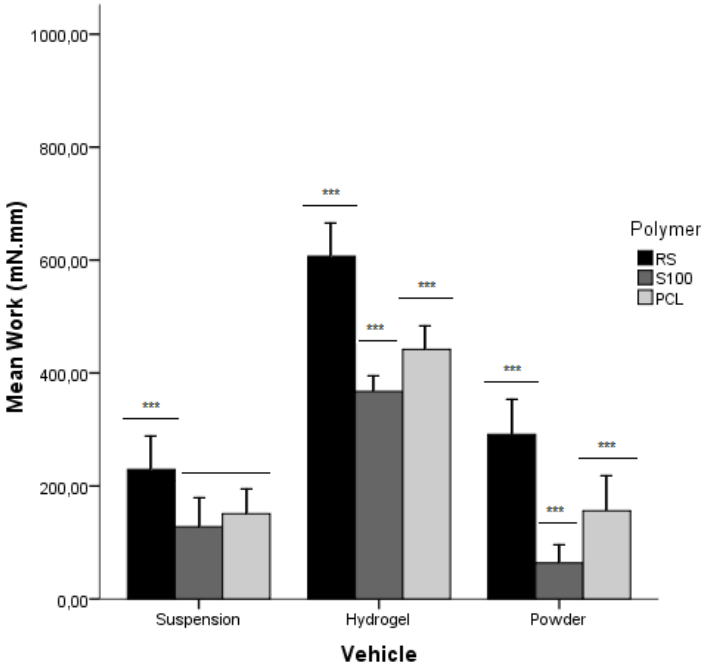

Figure 6. Mucoadhesiveness for different types of vehicles and polymers. *** Polymers that differed statistically from the others were highlighted.

4.4. DISCUSSION

In order to analyze the nanostructuration effect on mucoadhesiveness of polymers, first it is necessary to be sure of the uniformity of nanocapsules produced. In view of this, nanocapsule suspensions were analyzed regarding their size and polydispersity by laser diffraction and dynamic light scattering. All formulations showed exclusively the presence of nanometric particles with monomodal size distribution, whose values were in agreement with other studies [7,18,26]. The zeta potential, which reflects surface charge, varied according to the composition of the system. Eudragit RS nanocapsules showed positive zeta potential, while Eudragit S100 nanocapsules presented negative zeta potential due to cationic and anionic characteristics of the polymers, respectively [7]. PCL nanocapsules showed negative zeta potential due to the

presence of polysorbate 80 in the interface particle/water, since the polymer is non-ionic [37]. The pH values of all formulations were similar, and the influence of this parameter on mucoadhesion could be ignored. Therefore, according to the results obtained, the developed nanocapsules were uniform enough to afford use in the next steps of this study and in the production of hydrogels and powders. The hydrogels were produced with hydroxyethyl cellulose as thickening agent due to its non-ionic characteristics in water [38], avoiding the effect of any ionic interaction associated with hydrogel composition on mucoadhesion behavior. The semi-solid formulations showed narrow pH, which allowed ruling out its influence in mucoadhesion performance. Microscopy analysis confirmed the maintenance of original nanoparticles in the final pharmaceutical forms produced with nanocapsule suspensions. The spray-dried powders are formed by microagglomerates whose structure is influenced by nanocapsule composing the system.

The results obtained for the mucoadhesion experiments demonstrated that, regardless of the type of polymer used in the preparation of polymeric nanocapsules, they show significantly higher adhesiveness when compared to their respective solution. This may be explained by the increase of their surface area when nanostructured, allowing them to strongly interact with the layers of mucosa (either vaginal or buccal) or with mucin [7-9,18,39]. Moreover, particle size controls the ability of particles to penetrate (“fit”) the pores of the mucin mesh [40] and the structuration of polymers in nanoparticles may facilitate the “fit” with mucous pores.

The surface properties of the nanocapsules played an important role in their interaction with the mucosa, whereas mucosal surfaces have anionic properties due to the presence of negatively charged mucin molecules [3]. Eudragit RS nanocapsules had higher adhesiveness when compared to Eudragit S100 or PCL nanocapsules, regardless of mucosal surface. This better performance is observed independently of the vehicle in which nanocapsules were inserted (suspension, powder, or hydrogel). The positive charge of the polymer Eudragit RS may have produced an electrostatic interaction between the polymer and the mucosa, as previously observed by other authors [7,9,18,41]. Therefore, when polymeric nanocapsules are produced with Eudragit RS, they develop positive surface charge, as observed in zeta potential values, resulting in higher

adhesiveness for the application on mucosa [7,9,18]. On the other hand, no statistical differences were observed between the adhesiveness values of Eudragit S100 and PCL nanocapsules, regardless of the type of mucosa. A possible explanation for this result may be the fact that the formulations produced with these polymers presented similar negative zeta potential values. When these particles are inserted in hydrogel or powder, a statistical difference could be observed, and formulations containing PCL nanocapsules had higher values of adhesion than formulations containing Eudragit S100 nanocapsules. However, in this case the vehicle may have influenced the interaction with the adhesive surface. PCL has some advantages like, for example, being bioadhesive [42], biodegradable, and biocompatible [11]. Therefore, from the practical point of view it is important to assess the viability of the use of each polymer for each specific administration route. In this present work, we took into account only the technical aspect of the adhesiveness of the polymers.

In relation to the vehicle type used, hydrogels showed higher adhesion values in any kind of surface, when compared to suspension or powder vehicles. This was also previously observed by other authors [7,18,39]. An explanation for this is that a hydrogel has high viscosity and is able to interact longer with the mucosa due to its physicochemical characteristics. Hydrogels have been used in research with the aim of increasing the adhesiveness of drugs on the mucosa [43], and the results obtained demonstrate that it may be a viable option for this purpose. Polymeric nanocapsule suspensions and nanostructured powders presented higher adhesiveness to mucosa when compared to polymeric solutions. They also have other advantages compared to conventional systems, as for instance: (i) controlled drug release; (ii) capacity of drug delivery in targeting sites; (iii) increase in drug photodegradability, among others [12,44]. Therefore, these systems have been used as vehicles for different drugs such as sprays and tablets or filling of capsules, respectively. Their adhesiveness is related mainly to presence of nanometric particles. Particle sizes between 200 and 500 nm can substantially influence the diffusion of a drug present in these nanostructures, which can be transported through the mucin mesh that composes the mucosa and fluids [43,45]

Regarding the type of application surface for the *in vitro* mucoadhesion measurements, some authors have used fresh animal mucosa and these

characteristics were also evaluated by means of different methods [7,9,39,46,47]. However, given the fact that the biological properties can affect the reproducibility of the results and, depending on the mucosa location, its removal can be a limiting factor, some researchers have proposed the use of mucin discs as an alternative in the determination of the mucoadhesion of formulations [8,31-33]. In this present work, a comparative analysis between both options of surface model – fresh porcine mucosa and mucin discs - was performed to assess adhesiveness. It was observed that the use of mucin discs leads to significantly higher adhesiveness work when compared with fresh mucosa (either vaginal or buccal). When only this substance was considered in the adhesiveness experiments, the resulting values did not reproduce the values of porcine vaginal and buccal mucosa, and mucoadhesiveness was overestimated. This may have happened because the correct mucosa environment was not simulated in this situation. The mucosa has high water contents (~99 %) and around 1% of organic and inorganic materials, including glycoproteins [4]. In view of this, a mucin disc has a higher mucin concentration than physiological mucosal surfaces. However, mucin discs were able to reproduce the difference between formulations containing or not nanocapsules as well as between nanocapsules with different ionic characteristics, as observed for porcine vaginal and buccal mucosa. In view of this, for the purpose of comparing formulations, mucin discs may be a substitute model, being useful as adhesive surface for preliminary analyses of mucoadhesiveness. The two porcine mucosae used in the experiment (vaginal and buccal) did differ statistically, and appear to be a more appropriate model for mimicking *in vivo* effects.

4.5. CONCLUSION

This study demonstrated that structuration of EudragitRS100, EudragitS100, and PCL in nanocapsules improved their interaction with mucosal surface. Due to cationic characteristics, nanocapsules produced with Eudragit®RS100 showed better adhesiveness when compared to anionic nanocapsules formed by Eudragit®S100 and PCL. Incorporation of nanocapsules in hydrogels or the spray-dried process did not alter mucoadhesive profiles. Hydrogels showed higher adhesiveness than powders and suspensions. Work values of the

interaction between formulations and mucin were higher than the values observed for the interaction between formulations and porcine vaginal and buccal mucosa. However, mucin was able to reproduce the differences between formulations and vehicles, despite the fact that values were higher. In view of this, mucin discs may be an alternative mucoadhesive surface in preliminary studies. Porcine mucosa is ideal to mimic *in vivo* adhesion conditions; therefore, its use is more appropriate for studies about *in vivo* effects.

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APRESENTAÇÃO DO CAPÍTULO

Este estudo propõe a administração de carvedilol pela via sublingual, com o objetivo de melhorar a sua biodisponibilidade, uma vez que este fármaco sofre um extenso efeito de primeira passagem no fígado, quando administrado pela via oral. Sistemas nanotecnológicos poliméricos foram utilizados como carreadores do carvedilol com o intuito de explorar as potencialidades mucoadesivas dessas estruturas, bem como para aumentar o tempo de contato do fármaco no local de absorção. Sendo assim, o capítulo II deste trabalho compreendeu o desenvolvimento e caracterização de suspensões de nanocápsulas poliméricas contendo carvedilol. As partículas foram produzidas com dois tipos de polímeros, que levaram à obtenção de nanocápsulas com características de carga catiônicas e aniônicas. A capacidade de interação das nanocápsulas com as moléculas de mucina foi estudada, avaliando-se as suas propriedades adesivas e o efeito da carga de superfície nessa adesão. A viabilidade da absorção do carvedilol pela mucosa sublingual, que funciona como uma barreira à passagem de substâncias, foi avaliada, assim como o efeito da nanoencapsulação na permeação do fármaco. Por fim, foi elucidado o efeito das propriedades mucoadesivas dos sistemas desenvolvidos na permanência do fámaco na mucosa sublingual e, consequentemente, na quantidade de carvedilol permeado, em presença de fluxo salivar simulado. Este artigo está publicado no periódico "*European Journal of Pharmaceutics and Biopharmaceutics"*.

Carvedilol-loaded nanocapsules: mucoadhesive properties and permeability across the sublingual mucosa

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Abstract

Carvedilol is a drug used in heart failure, hypertension, and coronary artery diseases treatment. However, it presents low oral bioavailability (25-35 %) due to its high first-pass hepatic metabolism. The objective of this study was to develop carvedilol-loaded mucoadhesive nanocapsules as delivery systems for the sublingual administration of carvedilol. Nanocapsules were prepared using poly(ε-caprolactone) (CAR-LNC) and Eudragit® RS 100 (CAR-NC) as polymeric wall. *In vitro* interaction of formulations with mucin was performed to predict their mucoadhesion capacity. The drug's permeability and washability profiles were evaluated using porcine sublingual mucosa. Formulations showed nanometric mean diameters with low polydispersity and slightly acidic pH. Zeta potential values were positive for CAR-NC and negative for CAR-LNC. Encapsulation efficiency was higher than 87% and 99% for CAR-NC and CAR-LNC, respectively. Both formulations presented controlled drug release profiles and mucoadhesive properties. Carvedilol was able to permeate through sublingual mucosa. The nanoencapsulation improved retention time on mucosa and permeation in presence of simulated salivary flux. This study highlighted the suitability of using CAR-loaded nanocapsules in the development of innovative sublingual dosage forms.

Keywords: Carvedilol, Eudragit® RS 100, mucoadhesion, nanocapsules, poly(ε-caprolactone), sublingual permeability.

5.1. INTRODUCTION

Carvedilol (CAR) has been used for the management of important cardiovascular diseases, which are the main causes of worldwide morbidity and mortality. According to the World Health Organization (WHO), in 2012 17.5 million people died from cardiovascular diseases, and according to WHO it has been estimated that more than 22.2 million people will die of these conditions in the year 2030 [1]. CAR is a non-selective β-adrenoceptor antagonist, α1-adrenoceptor blocker, and has antioxidant effects. It has been approved for the treatment of heart failure, hypertension, and coronary artery diseases [2]. This drug is available as tablets for oral administration however its systemic bioavailability is only 25-35% due to extensive hepatic first-pass metabolism [3]. In order to increase bioavailability, different strategies have been proposed for oral and nasal administration of CAR [4-6]. The sublingual route of administration is a motivating alternative when the aim is to improve the bioavailability of drugs that undergo first-pass metabolism. Since this region is highly vascularized, the drug can enter the systemic circulation directly, bypassing hepatic metabolism. However this cavity is exposed to constant flux of saliva, which may remove part of the drug [7]. In order to prolong retention time in this area, studies have suggested the use of mucoadhesive systems, which are able to interact with the mucus layer covering the surface of buccal epithelia [8,9].

Nanoparticles are promising drug carriers that have been extensively studied. These structures can control drug release, enhancing the desired effect with fewer daily administrations, in addition to the possibility to reduce doses and mitigate side effects [10]. Polymeric nanocapsules are structures in which the drug is confined in an oily core surrounded by a polymeric wall [11]. The development of nanocapsules using polymers with mucoadhesive properties points to the potential of these structures as drug carriers to be administered through the sublingual route. Poly(ε-caprolactone) (PCL) and Eudragit® RS100 (EUD), a co-polymer of poly(ethylacrylate, methyl–methacrylate methacrylic acid ester), presents interesting bioadhesive properties [9,12]. These two polymers have been used to prepare nanocapsules for different purposes, from cutaneous administration to brain delivery [11,13-16].

In view of the considerable influence of cardiovascular disease on worldwide morbidity and mortality and the multiple cardiovascular action of CAR, the design of pharmaceutical formulations to obtain a more effective bioavailability for this drug is worth investigating. In this scenario, this study describes a nanoencapsulation process for CAR in polymeric nanocapsules with mucoadhesive properties, to improve the drug's sublingual retention and permeability. To the best of our knowledge, this is the first report on the development of polymeric nanocapsules intended to sublingual administration.

5.2. METHODS

5.2.1. Materials

Carvedilol was obtained from Henrifarma (São Paulo, Brazil). Poly(ε-caprolactone) (MW 80,000), sorbitan monostearate and mucin from porcine stomach (type II) were acquired from Sigma-Aldrich (São Paulo, Brazil). Eudragit® RS100 was supplied by Degussa (Darmstadt, Germany), and grape seed oil was obtained from Dellaware (Porto Alegre, Brazil). Polysorbate 80, acetone, and hydrochloric acid were purchased from Vetec (Rio de Janeiro, Brazil). Basic Fuchsin, sodium metabisulphite, periodic acid, and acetic acid were supplied by Dinamica (São Paulo, Brazil). Potassium phosphate was purchased from Nuclear (São Paulo, Brazil), while sodium hydroxide was bought from Cromoline (São Paulo, Brazil). HPLC grade acetonitrile was purchased from Tedia (Rio de Janeiro, Brazil).

5.2.2. Preparation of nanocapsule suspensions

Nanocapsules were prepared by interfacial deposition of preformed polymer [17,18]. For preparation of EUD nanocapsules (CAR-NC), an organic phase was prepared dissolving 0.1 g of polymer (EUD), 165 µL of grape seed oil, and 5 mg of CAR (0.5 $mg.mL^{-1}$) in 27 mL of acetone with magnetic stirring at 40 °C. To obtain PCL lipid-core nanocapsules (CAR-LNC), the organic phase was prepared in the same way, but changing EUD for PCL and adding 0.0385 g of sorbitan monostearate [18]. The organic phase was injected into 53 mL of an aqueous phase containing 0.077 g of polysorbate 80 with magnetic stirring at 40 °C. After, acetone was removed and the suspension was concentrated under

reduced pressure (Rotavapor R-114, Buchi, Flawil, Switerzland) to the final volume of 10 mL. Formulations without drug were also prepared (NC or LNC).

5.2.3. Analytical method

The CAR assay was carried out by high performance liquid chromatography (HPLC), using a method adapted from Ieggli et al (2011) [19] and validated considering the purposes of this study. Analyses were performed in a Shimadzu LC system (Kyoto, Japan) equipped with a CBM-20A system controller, a LC-20AT pump, a DGU-20A5 degasser, a SIL-20A auto-sampler, and a SPD-20AV detector (UV). A Phenomenex Luna C_{18} column (250 mm x 4.6 mm I.D., with a particle size of 5 µm) was utilized as stationary phase. The mobile phase was composed of phosphoric acid pH 3.0/acetonitrile (50:50, v/v), run at a flow rate of 0.8 $mL.min^{-1}$. UV detection was carried out at 241 nm, and run time was 10 min. For drug content and encapsulation efficiency analysis, an injection volume of 10 µL was used. For *in vitro* drug release, permeability and washability studies, the injection volume was changed to 20 µL in order to lower the quantification limit. Furthermore, the mobile phase was changed to phosphoric acid pH 3.0/acetonitrile (60:40, v/v) for the permeability and washability studies in order to improve resolution between chromatographic peaks. Specificity, linearity, intraday (n=6) and interday (n=9) precision were evaluated for all methods according to the official guidelines [20].

5.2.4. *Physicochemical characterization*

Volume-weighted mean diameters ($D_{4,3}$) and polydispersity (Span) (n=3) were analyzed by laser diffraction (LD) (Mastersizer 2000, Malvern Instruments Ltd., UK). The sample was dropped directly into the compartment disperser of equipment containing 150 mL of water until adequate obscuration index (2 – 8%). Mean particle size and polydispersity index (IPD) (n=3) were measured using dynamic light scattering (DLS) (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK) after dilution of the suspensions (20 µL) in water (10 mL) previously filtered (0.45 µm, Millipore®). Zeta potential was determined (n=3) by electrophoretic mobility (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK). Samples (20 µL) were diluted in NaCl solution 10 mM (10 mL) previously filtered (0.45 µm, Millipore®). pH (n=3) was measured by potentiometry (VB-10, Denver

Instrument, USA) directly in the formulations. The morphology was analyzed by transmission electron microscopy (TEM, Jeol JEM 1200-ExII, 100 mV, Tokyo, Japan) at the Microscopy Center of the University (Centro de Microscopia Eletrônica - UFRGS, Brazil). Samples were diluted (1:10 v/v) in ultrapure water, placed on a specimen grid (Formvar-Carbon support film, Electron Microscopy Sciences, USA), and negatively stained with uranyl acetate solution (2%, w/v).

5.2.5. Drug content and encapsulation efficiency

CAR was assayed (n=3) by HPLC, according to the method previously described, after dissolution of suspensions (1.0 mL) in acetonitrile (9.0 mL) followed by sonication (10 min). This dispersion was centrifuged at 4,120 ×*g* for 10 min. After, an aliquot (2.0 mL) of the supernatant was diluted to 10 mL in mobile phase and analyzed. Encapsulation efficiency was calculated (n=3) based on the difference between total drug and free drug contents in the ultrafiltrate. The ultrafiltrate was obtained by ultrafiltration/centrifugation technique (Ultrafree-MC 10,000 MW, Millipore, Billerica, USA) at 4,120 ×*g* for 10 min. In order to detect any interaction between the drug and the membrane, this experiment was also carried out using a solution of CAR, under the same conditions and drug recovery was determined in ultrafiltrate. The method had specificity, good linearity (r=0.999, n=3) in the range of 1.00-20.00 $\mu g.mL^{-1}$, and suitable intra (SD=1.25%) and interday (SD=1.02%) precision. Limit of detection (LoD) and limit of quantification (LoQ) were 0.296 and 0.896 $\mu g.mL^{-1}$.

5.2.6. In vitro drug release

The *in vitro* release of CAR (n=3) from nanocapsules and from a hydroalcoholic (ethanol: water 50:50 v/v, 0.50 $mg.mL^{-1}$) solution (CAR-S) was carried out using the dialysis bag method. Formulations (2 mL) were placed in a dialysis tubing cellulose membrane (flat width of 25 mm, molecular weight cut-off 14,000, Sigma-Aldrich, São Paulo, Brazil) and suspended in 100 mL of release medium (sodium phosphate buffer pH 6.8, 0.2 M). The samples were maintained in a bath at 37 °C with agitation of 70 ± 10 rpm. At predetermined time intervals, external medium (1.0 mL) was withdrawn and directly analyzed by HPLC (section 2.5). Sink condition was maintained during the whole experiment. The solubility of the drug in the medium was around 60 $\mu g.mL^{-1}$ as least 6x higher

than the expected total drug concentration after 100 % of release. Moreover, fresh medium (1.0 mL) was replaced after each sample withdrawn. The method had specificity, good linearity (r=0.997, n=3) in the range of 0.50-12.50 $\mu g.mL^{-1}$, and suitable intra (SD=2.5%) and interday (SD=2.5%) precision. Limit of detection (LoD) and limit of quantification (LoQ) were 0.062 and 0.188 $\mu g.mL^{-1}$.

5.2.7. Interactions between nanocapsules and mucin

The mucoadhesive properties of the CAR-loaded nanocapsules were evaluated using mucin from porcine stomach (type II). The soluble fraction of mucin was isolated in order to remove aggregates that could influence the analysis [21].

5.2.7.1. Particle size and zeta potential

Mean particle size and zeta potential (n=3) before and after contact (30 min) with mucin were measured to evaluate the ability of nanocapsules to interact with the compound. Mucin solutions (0.1%, 0.25%, and 0.5%, w/v) were prepared in phosphate buffer 0.02 M pH 6.8. Mean particle size was measured by DLS after dilution of the suspensions (20 µL) in mucin solutions (10 mL). Zeta potential was determined by electrophoretic mobility, and the samples (20 µL) were diluted in mucin solution containing 10 mM NaCl (10 mL). Mucin solutions were also analyzed under the same conditions.

5.2.7.2. Adsorption mucin on nanocapsules

To evaluate the amount of mucin adsorbed on CAR-loaded nanocapsules a Periodic Acid Schiff colorimetric method was used [22,23]. Mucin solutions (0.1%, 0.25%, and 0.5%, w/v) were prepared in phosphate buffer 0.02 M pH 6.8. Nanocapsules (20 µL) were added to these mucin solutions and were maintained under agitation during 30 min to allow the interaction between mucin and nanoparticles. Afterwards, the mixtures were ultracentrifuged for 20 min at 20 °C and 200,000 ×g for nanocapsules to settle [24]. The amount of free mucin in supernatant was determined. Periodic acid reagent (0.2 mL) was added to 2 mL of the supernatant and incubated at 37 °C for 2 h in a water bath. After, Schiff reagent (0.2 mL) was added and the resulting solutions were kept at room temperature (30 min). Absorbance was measured at 555 nm in a UV spectrophotometer (UV-1800 PC, Pró-Análise, Brazil). The concentration of free

mucin in supernatant was calculated from a calibration curves (n=3, r=0.998) in the range of 0.1–0.5 mg.mL^{-1}. The amount of adsorbed mucin was calculated by the difference between total mucin in the solution and free mucin after contact with nanocapsules (n=3).

5.2.8. In vitro studies using sublingual mucosa

Fresh porcine head was obtained from Santo Ângelo slaughterhouse (Porto Alegre, Brazil). Porcine sublingual mucosa was excised using a scalpel and immediately used. Tests were evaluated using modified manual Franz diffusion cell with a receptor volume of 2.5 mL and diffusional area of 0.9 cm^2. Mucosa was placed between the donor and the receptor compartment, which was filled with phosphate buffer 0.2 M pH 6.8 containing 0.1% of polysorbate 80 to better drug solubility and to reach the sink conditions.

5.2.8.1 Permeability test

For permeability of CAR (n=3) through porcine sublingual mucosa the donor compartment received 100 µL of CAR-NC or CAR-LNC or CAR-S (hydroalcoholic solution, ethanol: water 50:50 (v/v), 0.5 mg.mL^{-1}). Franz cells were maintained in a bath at 37 °C with shaking of 70 ± 10 rpm. Sink condition was maintained during the experiment. At predetermined time intervals, the receptor medium was withdrawn (40 µL) and directly analyzed by HPLC (section 2.3). The method demonstrated specificity, good linearity (r=0.999, n=3) in the range of 0.0125-12.50 µg.mL^{-1}, and suitable intra- (SD=1.48%) and interday (SD=1.54%) precision. Limit of detection (LoD) and limit of quantification (LoQ) were 0.004 and 0.011 µg.mL^{-1}.

5.2.8.2. Washability test

The effect of salivary flux on mucoadhesion of nanocapsules and CAR-S over the surface of pig´s sublingual mucosa was investigated in a washability test (n=3) [14,17]. CAR-NC or CAR-LNC or CAR-S (50 µL) was placed on the mucosa. A pre-incubation of 1h was used to allow the interaction between suspended nanoparticles and mucosa to occur [16]. Afterwards, phosphate buffer 0.2 M pH 6.8 containing 0.1% of polysorbate 80 (37 ºC) was fluxed at 0.35 mL.min^{-1} [7] to simulate action of salivary flux (Pump; Gilson; Minipuls 3,

France). The outgoing flux was collected at predetermined time intervals. CAR was assayed by HPLC (section 2.3). Samples of the CAR solution were directly analyzed, while samples of nanocapsules were subjected to an extraction process (section 2.5). At the end of the experiment, samples from the receptor compartment were directly analyzed by HPLC. The analytical method showed good linearity (r=0.999, n=3) in the range of 0.0125-5 $\mu g.mL^{-1}$. Specificity and precision results were according to the permeability studies (section 2.8). Limit of detection (LoD) and limit of quantification (LoQ) were 0.0004 and 0.0012 $\mu g.mL^{-1}$.

5.2.9. Statistical analysis

All statistical analyses were carried out by one-way analysis of variance (ANOVA). The post-hoc Turkey test was used when three or more groups were among means were considered statistically significant at a level of $p \leq 0.05$. Data are presented as the mean ± standard deviation (SD).

5.3. RESULTS AND DISCUSSION

5.3.1. Development of nanocapsule suspensions

Nanoparticles were developed using grape seed as oil component. Its use is not still approved by Food and Drug administration (FDA) however its biological effect has been studied in human due to its important antioxidant activity [25]. Oily phase used in nanoparticles development may influence their size distribution and grape seed oil has been proposed as alternative oil in nanocapsules production intended as drug delivery systems [26,27]. Nanocapsules containing medium chain triglycerides were pre tested in this study but concomitant formation of larger particles in the microscale were occurred. The use of grape seed as oil component allowed the production of particles with a narrow size distribution just in the nanoscale range. All formulations were milky bluish in aspect, and exhibited Tyndall effect. Results of mean particle size measured are shown in Table 1. All particles were in the nanometric diameter range, regardless the presence of the drug. The presence of the drug did not affect the particle size for EUD nanocapsules, since it was not observed a significant difference ($p>0.05$) in the mean diameter by LD and

DLS between CAR-NC and NC. On the other hand, the presence of drug led to a significant size decrease ($p \leq 0.05$) in CAR-LNC when compared with LNC.

Table 1. Particle size and polydispersity indices (Span and PDI) measured by laser diffraction (LD) and dynamic light scattering (DLS), zeta potential and pH of formulations.

	LD		DLS		Zeta potential ± SD (mV)	pH ± SD
	D(4,3) ± SD (nm)	Span ± SD	Z-average ± SD (nm)	PDI ± SD		
NC	162 ± 33^{a,c}	1.39 ± 0.33	142 ± 9^{a}	0.13 ± 0.01	3.9 ± 0.8^{a}	5.8 ± 0.01^{a}
CAR-NC	135 ± 3^{a}	1.21 ± 0.07	139 ± 6^{a}	0.14 ± 0.01	9.2 ± 2.4^{b}	6.8 ± 0.10^{a}
LNC	224 ± 19^{b}	1.69 ± 0.03	216 ± 8^{b}	0.13 ± 0.02	-12.2 ± 0.3^{c}	6.5 ± 0.80^{a}
CAR-LNC	161 ± 4^{c}	1.56 ± 0.03	180 ± 3^{c}	0.08 ± 0.01	-6.6 ± 0.6^{d}	6.8 ± 0.03^{a}

SD = standard deviation (n=3). Means, in column, with the same letter are not significantly different ($p > 0.05$, ANOVA).

This difference occurred because in drug presence are formed more particles with lower size. While the specific surface area was 49 ± 1 $m^2.g^{-1}$ for CAR-LNC, for LNC was 37 ± 3 $m^2.g^{-1}$. Furthermore, LNC had higher mean diameter than NC ($p>0.05$), which may be explained due to the presence of sorbitan monostearate in the core of LNC. Lipid-core nanocapsules have a diversified core structure when compared to traditional nanocapsules due to presence of sorbitam monostearate [18]. The conformation established in function of this situation may originate particles with different mean sizes. The polydispersity indexes of all formulations (Span and PDI) were suitable, signaling homogenous monomodal size distribution. TEM images demonstrated the spherical shape of nanocapsules and confirm their nanometric sizes (Figure 1). Zeta potential reflects the surface charge of particles, and, as expected, the suspensions produced with EUD exhibited positive zeta potential (Table 1). This may be explained considering the cationic nature of the polymer, which contains a quaternary ammonium group [12]. On the other hand, formulations with PCL showed negative zeta potential (Table 1), as a consequence of the non-ionic character of the polymer and the presence of polysorbate 80 at the interface particle/water [28]. These values were significantly altered by presence of the

drug ($p \leq 0.05$) and may indicate its presence on the surface of nanocapsules. Oliveira et al (2013) [29] developed an algorithm to determine drug distribution in lipid-core nanocapsules considering the drug distribution-coefficient (log D). CAR has a log D of 3.4 [30] and, according to the previous report [13], part of the drug may be adsorbed on the polymeric wall. The low values of zeta potential may not influence the stabilization of these particles since such phenomena can be explained by a steric mechanism due to the presence of polysorbate 80 on their surface, and not by electric repulsion, which would depend on the surface charge [28]. The influence of pH values on zeta potential could be refuted, since all formulations had slightly acidic pH (Table 1). Moreover, this pH is compatible with salivary pH [9].

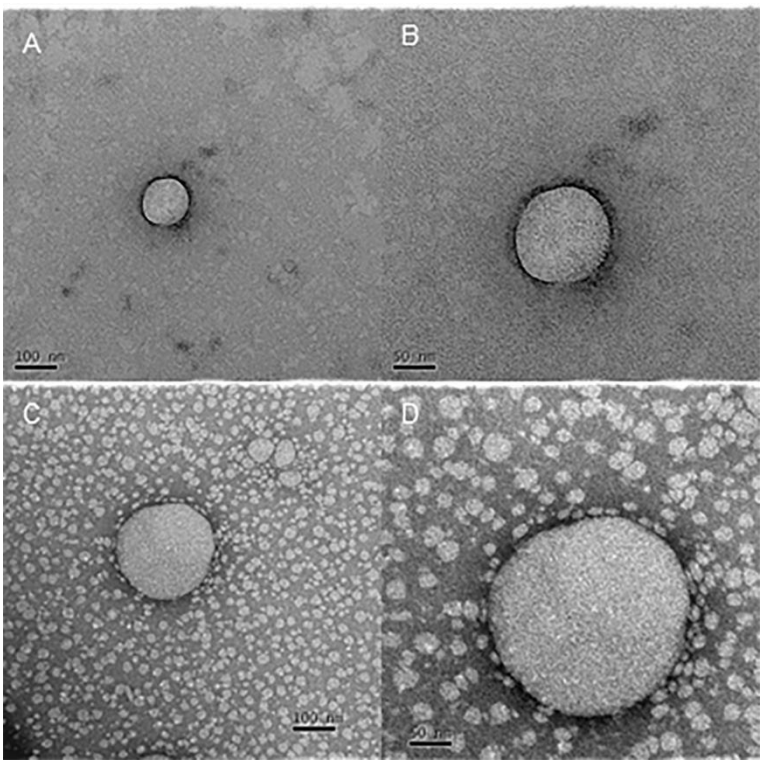

Figure 1. Transmission electron microscopy (TEM) micrographs: A (150000x) and B (300000x) Eudragit® RS100 nanocapsules (CAR-NC); C (150000x) and D (300000x) poly(ε-caprolactone) nanocapsules (CAR-LNC).

Drug content was close to 0.5 $mg.mL^{-1}$ regardless the type of polymer (CAR-NC=0.47 ± 0.08 $mg.mL^{-1}$ and CAR-LNC=0.47 ± 0.01 $mg.mL^{-1}$). A lower concentration of carvedilol was loaded in nanocapsules, when compared to marketed tablets (3.125, 6.25, 12.5 mg and 25 mg). However, a new

administration way has been proposed in this study which bypass hepatic metabolism suffered by carvedilol when orally administrated. Furthermore, polymeric nanocapsules may extend the drug release and dose maintenance in blood circulation may be prolonged, which affords to reduce both dose and administration frequency [31,32,33]. The encapsulation efficiency of CAR-NC was 88 ± 1.10 %, and 99.10 ± 0.21 % for CAR-LNC. Considering that the interaction of drug and filter was not detected (recovery of 97%), CAR-LNC showed a higher drug encapsulation efficiency than CAR-NC ($p \leq 0.05$). It is believed that this difference may be related with the core composition of particles [34]. The core of PCL-nanocapsule is formed by a dispersion of sorbitan monostearate and oil actually forming lipid-core nanocapsules [18]. The core of EUD-nanocapsule is formed only by oil, and are named nanocapsule [35]. The presence of sorbitan monostearate in the core of CAR-LNC may have facilitated drug solubilization, affording higher drug amount to be encapsulated. According to these results, the developed formulations present suitable nanometric characteristics and therefore were used in the following steps of this study.

5.3.2. In vitro drug release

One important characteristic of polymeric nanocapsules is their ability to control drug release [10]. The plasma half-life of CAR is around 7-10 h, and it was normally administered twice a day [3]. Controlled release systems of CAR may be an interesting choice to reduce the administration frequency, which may influence treatment adhesion efficiency, in addition to promoting less adverse effects. Patients using CAR reported adverse events like headache, hypotension, dizziness, fatigue, and somnolence [36]. *In vitro* drug release profiles are depicted in Figure 2. It can be observed that 88.49 ± 2.97% of the CAR diffused from CAR-S in 6 h, and that this value remained constant after 24 h. On the other hand, the drug released from CAR-NC and CAR-LNC after 24 h were 73.04 ± 3.07% and 49.47 ± 2.51%, respectively. These results demonstrated that the release of CAR from nanocapsules was slower than the diffusion of the drug in solution through the dialysis sac. PCL-nanocapsules showed a better control of drug release which may be related with the nature of the polymeric wall and/or with the presence of sorbitan monostearate in the

core. Previous studies demonstrated that the viscosity of this kind of core increases with the presence of sorbitan monostearate, consequently decreasing drug diffusive flux [17]. Furthermore, PCL has a semi-crystalline structure that may form a uniform arrangement more resistance to relaxation than EUD, an amorphous polymer [37,38]. This afforded to obtain two different drug release profiles, both of which could be interesting strategies in CAR release. Therefore, the subsequent studies were carried out to evaluate the performance of these formulations in terms of mucoadhesion.

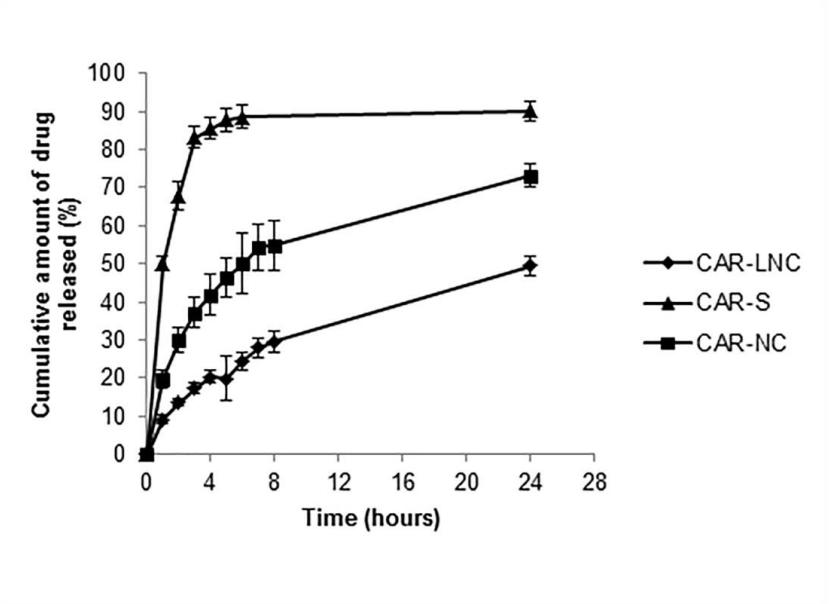

Figure 2. *In vitro* drug release profile from Eudragit® RS100 nanocapsules (CAR-NC) and poly(ε-caprolactone) nanocapsules (CAR-LNC) and from hydroalcoholic solution (CAR-S) using the dialysis bag method (n = 3).

5.3.3. Interactions between nanocapsules and mucin

In order to evaluate the mucoadhesive properties of CAR-loaded nanocapsules, the interaction of nanoparticles and mucin were analyzed. Mucin is the main component of the mucus, and is responsible for its viscous and elastic gel-like properties [39]. Commercial mucin from porcine gastric (type II) has been frequently used to analyze mucin interaction with different particles [21,40]. Moreover, according to Teubl et al (2013) [41], human and animal mucin have similar chemical and morphologic structures, suggesting that pig gastric mucin may be used like a model of human mucin. Mucin concentrations (0.1- 0.5 %) used in these analyses are in agreement with the physiological condition. The organic and inorganic materials of mucus represent around 1 % of its

constitution which is majority formed by water [7]. Furthermore, mucin is formed by diverse genes which may be expressed in different collections. The resultant collection determines the molecule conformations and how the interaction with other molecules will occur. As consequence, intensity of interaction between mucin and particles may vary for each person or physiological situation [39].

The influence on surface charge of nanocapsules after their interaction with mucin molecules was investigated. Different authors demonstrated the adsorption of mucin on the surface of particles by zeta potential changings [21,40,42]. The zeta potential of mucin solutions was -8.37 ± 1.04 mV. Mucin molecules exhibit sialic acids linked to the terminal ends of the oligosaccharide chains, which lends negative charge to the molecule [39]. CAR-NC presents a positive zeta potential, as previously discussed. However, after contact with mucin this value became negative (Table 2). On the other hand, CAR-LNC, which already had negative zeta potential, maintained charge (Table 2). The alteration in surface charge and the similar zeta potential values compared to mucin may indicate that nanocapsules form a complex with mucin molecules. Furthermore, previous studies suggested that the adsorption of mucin on the surface of particles may increase their size [40,42,43]. Therefore, mean particle size was also measured. Both formulations showed an increase in mean particle size as a function of mucin concentration (Table 2). This increase was probably due to presence of microparticles. This could be confirmed, since the granulometric size distribution (supplementary information – S1) of nanocapsules after their interaction with mucin became bimodal. Besides the main peak in the nanometric range, another small peak in the micrometer region could be observed. These nanometric and micrometer populations are similar to those observed for only nanocapsules and only mucin samples, respectively. The interaction of nanocapsules with increased concentration of mucin widened the main peak and increased the micrometer peak. These results are highlighted by the changes in polydispersity indices (Table 2), which indicate an increase in heterogeneity of size distribution. Taken together, these results suggest that part of mucin is adsorbed on the nanocapsule surface, while part is free in solution. To confirm this hypothesis, the next step was to assay the amount of mucin adsorbed on the nanocapsule surface. The amount of mucin adsorbed on the nanocapsule surface increases with the concentration

of mucin in solution, regardless of the formulation (Figure 3A). Furthermore, this assay demonstrated that there are free mucin molecules in solution, which did not adsorb on the nanocapsule surface, regardless of the initial concentration of mucin in solution, confirming the results observed by DLS. When these values are analyzed by percentage adsorbed in relation of the total amount of mucin added (Figure 3B), the values were the same for both formulations, regardless of the concentration of mucin added. These results indicated a higher ability of CAR-NC to interact with mucin molecules, when compared with CAR-LNC ($p \leq 0.05$). EUD is a cationic polymer that may interact with negative mucin molecules by electrostatic attraction [12,13]. PCL is a non-ionic polymer, a type of material that shows poor mucoadhesiveness. Their interactions with the mucosa membrane occur predominantly through diffusion and interpenetration inside the mucus [44]. The next step of this study was to evaluate the particle influence on CAR permeability across sublingual mucosa.

Table 2. Particle size (dynamic light scattering), polydispersity index (PDI) and zeta potential of formulations before and after contact with different concentrations of mucin.

	Mean diameter ± SD (nm)		**PDI ± SD**		**Zeta potential ± SD (mV)**	
Mucin concentration ($\mu g.mL^{-1}$)	CAR-NC	CAR-LNC	CAR-NC	CAR-LNC	CAR-NC	CAR-LNC
0	153 ± 11^{a}	176 ± 17^{a}	0.19 ± 0.05^{a}	0.09 ± 0.02^{a}	$+2.26 \pm 0.58^{a}$	-14.80 ± 2.69^{a}
1	155 ± 4^{a}	187 ± 16^{a}	0.22 ± 0.01^{a}	0.17 ± 0.02^{b}	-9.44 ± 2.15^{b}	-12.50 ± 1.10^{a}
2.5	$173 \pm 6^{a,b}$	$203 \pm 16^{a,b}$	$0.33 \pm 0.02^{a,b}$	0.25 ± 0.01^{b}	-9.81 ± 1.38^{b}	-12.42 ± 1.85^{a}
5	227 ± 41^{b}	240 ± 18^{b}	0.51 ± 0.15^{b}	0.44 ± 0.06^{c}	-11.81 ± 1.50^{b}	-9.54 ± 1.44^{a}

SD = standard deviation (n = 3). Means in a column followed by the same letter are not significantly different ($p > 0.05$, ANOVA).

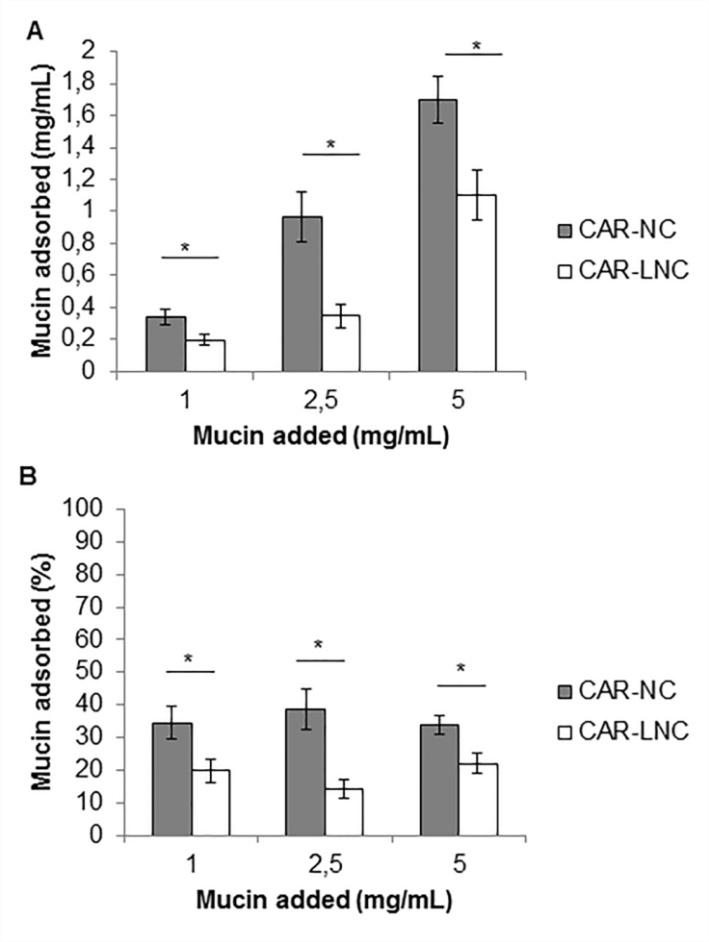

Figure 3. Concentration of mucin adsorbed on surface of nanocapsules in function of total concentration of mucin added (A). Percentage of mucin adsorbed on surface of nanocapsules in function of total concentration of mucin added (B). One asterisk (*) represents significant difference between concentration/percentage of mucin adsorbed on formulations ($p \leq 0.05$, t test).

5.3.4. In vitro sublingual mucosa permeability

Drugs administered by sublingual route should cross the mucosal membrane in order to reach blood circulation. The main role of the mucosa is the protection of oral cavity, and the structure represents an important barrier to the diffusion of some drugs [8]. Up to now, no reports describing CAR permeation through sublingual mucosa have been published. To the best of our knowledge, the study of the behavior of CAR in sublingual membrane as well as the influence of nanoencapsulation on its permeability profile was carried out.

The results demonstrate that CAR was able to permeate across sublingual mucosa. However, differences in permeation profiles were observed when CAR was in solution or nanoencapsulated (Figure 4). The solution had the highest

percent CAR permeated after 24 h (54.3 ± 2.3%), followed by CAR-NC (32.4 ± 7.7%), and CAR-LNC (8.1 ± 1.2%). These differences may be explained considering the CAR release profiles from nanocapsules (section 3.2). The solution of CAR did not have controlled release, and the only barrier to its permeation is the mucosa. Furthermore, the drug solution was produced containing 50 % of ethanol which might have facilitated the drug permeation. Ethanol is cited as permeation enhancer however they real effects against oral mucosa is contradictory and cannot be associated to ethanol concentration [45,46]. Moreover, only adding this concentration of ethanol to water made possible the obtaining of a solution with the same drug concentration as in the nanocapsules. On the other hand, when CAR is nanoencapsuled it needs firstly be released from particles before it crosses the mucosal barrier. CAR-LNC provided a more controlled CAR release than CAR-NC, in function of the differences in the structure of their core and polymeric wall, as previously discussed. These differences were reflected in the drug permeation profiles, and the control of CAR permeation was highlighted. This controlled CAR permeation, together with mucoadhesion effect, may be an important strategy to prolong the effect of the drug when administered through the sublingual route. Therefore, the next step was to investigate the effect of mucoadhesion on CAR permeability.

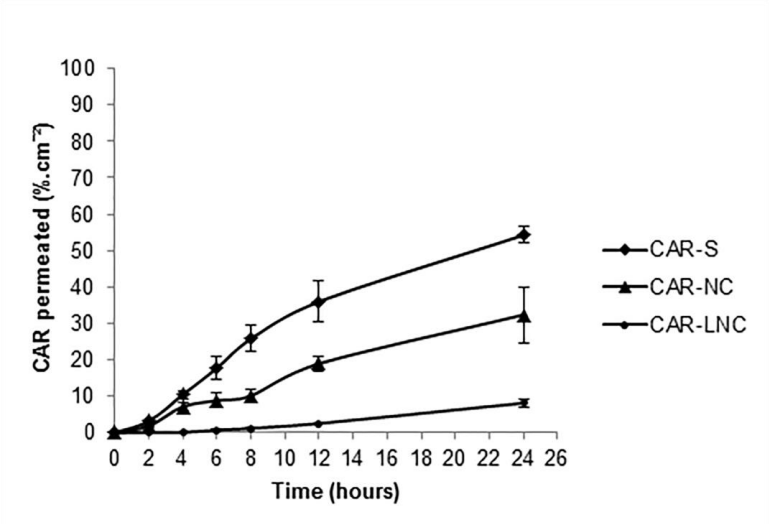

Figure 4. Mucosa permeation of carvedilol incorporated in Eudragit® RS100 nanocapsules (CAR-NC), poly(ε-caprolactone) nanocapsules (CAR-LNC) and from hydroalcoholic solution (CAR-S).

5.3.5. Washability test

The buccal cavity receives 0.5 – 2 L of saliva per day, and this constant flux interferes with the retention of dosage forms in the sublingual region [7]. CAR-NC and CAR-LNC showed an important capacity to interact with mucin molecules, as previously discussed in this study. In order to evaluate if this adhesion is enough to sustain drug levels on porcine sublingual mucosa in the presence of constant simulated salivary flux as well as to increase the drug permeated to the receptor fluid, the washability test was carried out. Results represent the amount of CAR assayed in the outgoing flux (Figure 5). The amount of CAR adhered on mucosa was higher when nanoencapsulated CAR was used, regardless of the type of the polymeric wall. The total CAR washed from CAR-S after 3 h (87 ± 1%) was slightly higher ($p \leq 0.05$) than the amount released from CAR-NC (81 ± 2%) and CAR-LNC (79 ± 4%). Therefore, nanoencapsulation promoted higher adherence on mucosa at different times. Experiments of mucoadhesion previously discussed highlighted the better performance of CAR-NC, when compared with CAR-LNC, and this result was also observed here. CAR-NC retained higher amounts of drug than CAR-LNC at all times, for up to 1 h of washing.

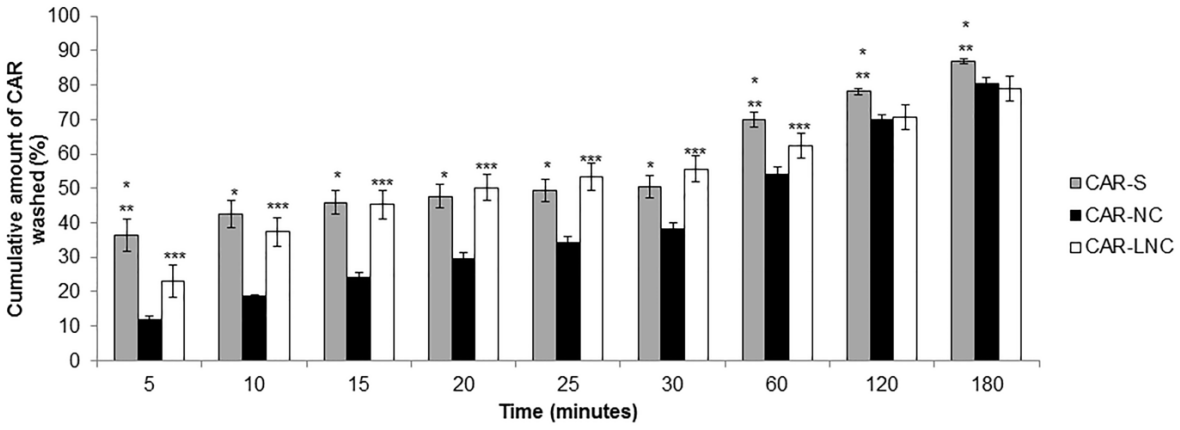

Figure 5. Washability profiles of carvedilol incorporated in Eudragit® RS100 nanocapsules (CAR-NC), poly(ε-caprolactone) nanocapsules (CAR-LNC) and from hydroalcoholic solution (CAR-S). Asterisks express significant difference and are present in formulations with higher level considering the following comparisons: *CAR-S versus CAR-NC, **CAR-S versus CAR-LNC and ***CAR-NC versus CAR-LNC ($p \leq 0.050$, t test).

The effect of mucosa adherence on drug permeation was also evidenced. Samples of the receptor medium were analyzed at the end of the experiment. Nanoencapsulation increased significantly the amount of drug permeated in presence of simulated salivary flux compared to the drug solution ($p \leq 0.05$), regardless of the formulation. The concentration of permeated CAR was 0.72 ± 0.04 $\mu g.mL^{-1}$ from CAR-NC, 0.52 ± 0.13 $\mu g.mL^{-1}$ from CAR-LNC and 0.10 ± 0.02 $\mu g.mL^{-1}$ from CAR-S. Due to the interaction of nanocapsules with the sublingual mucosa, higher amount of drug lasted on the mucosa surface compared with the solution (CAR-S), affecting the concentration of permeated drug. The amount of CAR permeated from the two different formulations (NC or LNC) was not statistically different ($p>0.05$). Such result may be explained due to their performance in the adherence on mucosa. Although CAR-NC shows a better interaction with mucosa in the first time of the experiment, the total amount of CAR washed from the mucosa was the same for both nanocapsules. Consequently similar CAR concentration was able to permeate to the receptor fluid. Washability test simulated a situation more close to reality since a salivary flux was mimetic. On the other hand drug release and permeability test were evaluated in a static situation and difference in profiles may be observed.

Frank *et al* (2014) [13] demonstrated that nanocapsules containing the cationic polymer Eudragit® RS 100 reached more deeply inside the vaginal mucosa than nanocapsules containing Eudragit® S100, an anionic polymer. The authors attributed such result to the higher electrostatic interaction of the cationic polymer with the vaginal mucosa. Fonseca *et al* (2014) [21] evaluated the mucoadhesive properties of films containing PCL or PCL functionalized with a methacrylic copolymer, and showed that the inclusion of copolymer with cationic characteristics improved the adhesive characteristics of PCL (non-ionic polymer) on the surface of nasal mucosa. Similar differences in the interaction of the mucosa with nanoparticles with opposite charges were observed here. This study revealed that nanoencapsulation is an important approach to the sublingual administration of CAR, since it was essential to increase its retention on sublingual mucosa and to improve its permeation in presence of simulated salivary flux.

5.4. CONCLUSION

Nanocapsules containing CAR produced with different polymers and core structure showed suitable nanometric and mucoadhesive properties. The nanoencapsulation of CAR improved its adherence on porcine sublingual mucosa, increasing its permeation in the presence of simulated salivary flux. Positive nanocapsules showed a higher interaction with sublingual mucosa when compared to negative nanocapsules. The present technological strategy opens promising perspectives for further studies to produce different final dosage forms containing nanoencapsulated CAR for sublingual administration.

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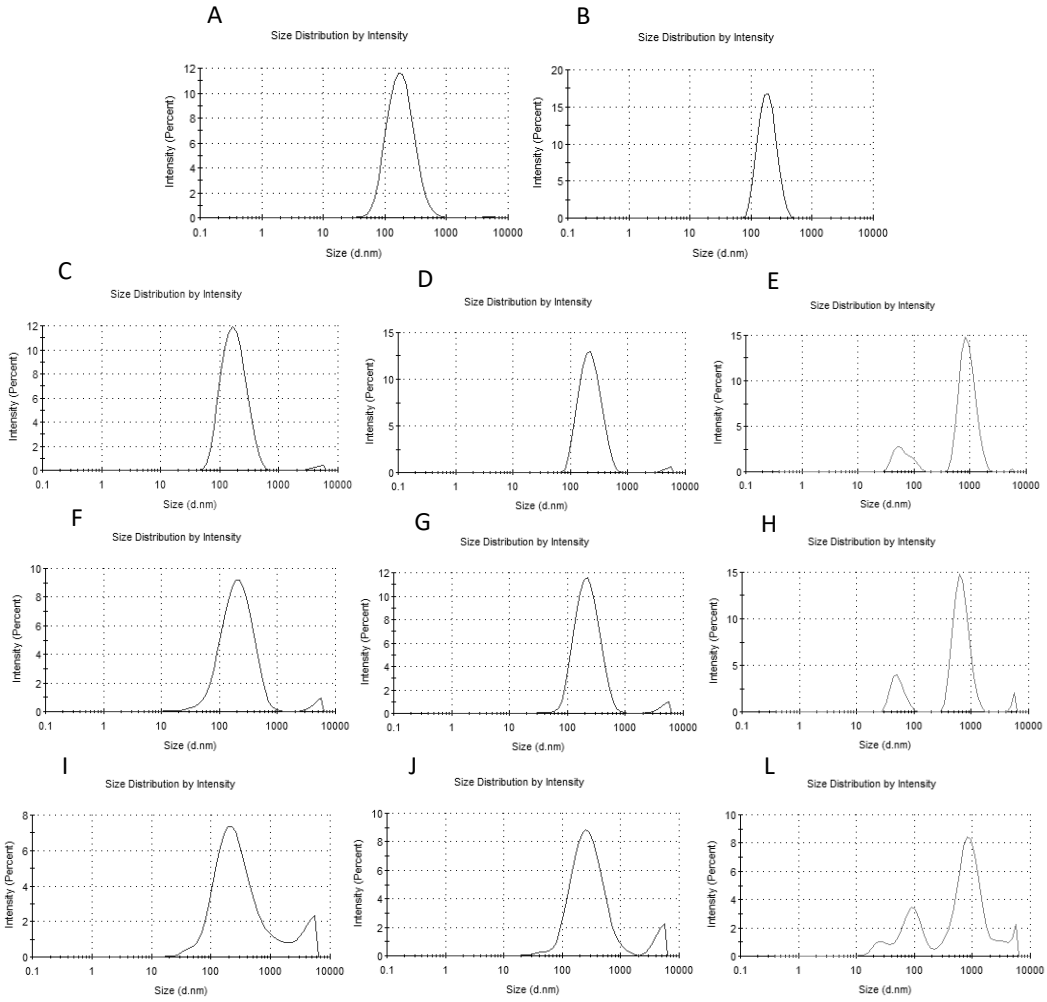
Supplementary information:



S1. Granulometric size distribution by dynamic light scattering analysis of CAR-NC isolates (A); CAR-LNC isolates (B); CAR-NC after interaction with 0.1 % of mucin (C); CAR-LNC after interaction with 0.1 % of mucin (D); mucin solution 0.1 % (E); CAR-NC after interaction with 0.25 % of mucin (F), CAR-LNC after interaction with 0.25 % of mucin (G); mucin solution 0.25 % (H); CAR-NC after interaction with 0.5 % of mucin (I); CAR-LNC after interaction with 0.5 % of mucin (J); mucin solution 0.5 % (L).

APRESENTAÇÃO DO CAPÍTULO

A partir do estudo relatado no capítulo anterior, demonstrando a potencialidade do uso de nanocápsulas poliméricas catiônicas para a administração de carvedilol pela via sublingual, o terceiro capítulo deste trabalho foi devotado à avaliação do transporte do fármaco, a partir das nanocápsulas de Eudragit RS100, através de de uma monocamada de células de epitélio oral. O uso da linhagem celular SCC4, extraída de carcinona humano de língua, foi investigado como um novo modelo de mucosa sublingual para estudo de transporte de fármacos. Para isso, foi estabelecido um estudo comparativo entre o perfil de permeação do fármaco através da monocama celular e através da mucosa sublingual de porco. Além disso, a integridade da monocamada celular após a permeação do fármaco foi monitorada, assim como o possível efeito citotóxico das suspensões de nanocápsulas foi avaliado frente a essa linhagem celular. Este capítulo está organizado na forma de uma “Short Communication” a ser submetida para publicacão.

Carvedilol-loaded nanocapsules intended for sublingual administration: drug transport across SCC4 cell monolayers

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Abstract

Carvedilol is used for cardiovascular diseases and has limited oral bioavailability due to hepatic first pass metabolism. Mucoadhesive carvedilol-loaded nanocapsules (CAR-NC) were previously proposed for the administration of this drug by sublingual route. Carvedilol nanoencapsulation controlled its permeation across porcine sublingual mucosa. In the present study, SCC4 cell monolayers were evaluated for the first time as an alternative sublingual barrier model drug transport studies. Carvedilol-loaded cationic nanocapsules were prepared by deposition of a preformed polymer. Drug permeation studies were carried out in Transwell® inserts. The integrity of cell monolayers after the drug transport was assessed by transepithelial electric resistance. Compatibility of the CAR-NC with the SCC4 cells was evaluated by the Sulforhodamine B assay. The drug permeated across cell monolayer by a controlled way when nanoencapsulated and this profile had a linear relation with to those observed in porcine sublingual mucosa. The integrity of the cell monolayer was maintained after drug permeation and CAR-NC was no cytotoxic to SCC4 cells. Thus, nanoencapsulated carvedilol permeated by a controlled and safe way by SCC4 cell monolayer. SCC4 cells monolayers may be used as *in vitro* model for sublingual drug transport studies in the development of novel formulations.

Keywords: carvedilol, Eudragit®RS100, nanocapsules, cytotoxicity, SCC4 cells, transport studies.

6.1. INTRODUCTION

Carvedilol is a drug orally administrated in treatment of heart failure, hypertension, and coronary artery diseases. However, it has a low oral bioavailability (25-35%) due to its extensive hepatic metabolism [1]. This drawback may be overcomed by its sublingual administration, which avoids the first-pass metabolism. Sublingual mucosa is a protective barrier to absorption of unwanted substances and the oral cavity is exposed to constant flux of saliva, which may remove part of the drug before its systemic absorption [2]. Therefore, mucoadhesive carvedilol-loaded nanocapsules were formulated intended for sublingual administration [3]. Eudragit®RS100 nanocapsules retained higher amount of drug on the surface of porcine mucosa in presence of simulated salivary flux than a drug solution and controlled the drug permeation through porcine sublingual mucosa [3].

The use of animal excised tissue in permeability studies have some limitations as finite viability and subotimal stirring conditions [4]. In view of this, other epithelial barrier models has been studied as cell monolayer. This membrane is able to morphologically and functionally resemble the barrier properties of the epithelium [4] and may be used to study compactibility of formulations with biological tissues [5]. Correlation of porcine sublingual mucosa and cell monolayer permeability is poor described in the literature [6]. SCC4 are cells of oral epithelium extracted from human tongue squamous cell carcinoma. The formation of a monolayer by these cells are cited by Murdoch and co-workers [7], however, their use in drug transport studies as sublingual layer model was never studied.

In view of the exposed, the objective of this study was to propose the use of SCC4 cells as oral epithelial cell monolayers to evaluate the carvedilol transport across sublingual membrane in its nanoencapsulated or non-encapsulated form. The correlation between the permeation data across the SCC4 cell monolayers and across the porcine mucosa was assessed. In addition, cytotoxicity of carvedilol-loaded nanocapsules in the oral epithelial cells was evaluated.

6.2. MATERIALS AND METHODS

6.2.1. Materials

Carvedilol was purchased from Henrifarma (São Paulo, Brazil) and Eudragit® RS100 was supplied by Degussa (Darmstadt, Germany). Grape seed oil was obtained from Dellaware (Porto Alegre, Brazil). Polysorbate 80 was acquired from Vetec (Rio de Janeiro, Brazil). SSC4 and HaCat cells were acquired from ATCC (The American Type Culture Collection, USA). Dulbecco's modified Eagle's medium, fetal bovine serum L-glutamine, penicillin/streptomycin were purchased from Gibco (Thermo Scientific, Waltham, Massachusetts, USA). Sulforhodamine B, trichloroacetic acid, and acetic acid were acquired from Sigma-Aldrich (St.Louis, MO, USA). All other chemicals and solvents were of analytical grade and used as received.

6.2.2. Preparation of carvedilol-loaded nanocapsule suspension

Carvedilol-loaded nanocapsule suspensions (CAR-NC) containing 0.50 mg of carvedilol per ml were prepared by interfacial deposition of preformed polymer method as described by Chaves and co-workers [3]. Unloaded nanocapsules (NC) were produced by same way, omitting the drug. Mean particle diameter of the formulations was measured by dynamic light scattering (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK). Zeta potential was measured by electrophoretic mobility (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK). Drug content was determined by liquid chromatography (LC) [3].

6.2.3. SCC4 cell culture

Oral epithelial cells from tongue squamous cell carcinoma SCC4 were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine and 0.05% penicillin/streptomycin (Invitrogen) at 37°C with 5% CO_2. Human immortalized keratinocytes from skin HaCaT were cultures in same conditions.

For the transport assay, SCC4 cells were seeded on the top of Transwell® inserts (0.4 µm pore size, 1.12 cm^2 surface area) of 12 well plates (Corning Costar Inc., NY, USA) at a density of 5 x 10^4 cells/well. The culture medium (0.5 ml in the apical compartment, 1.5 ml in the well) was replaced every 48 h. Cell resistance (R, Ω) was measured daily using an epithelial voltohmmeter (EVOM,

WPI Inc., USA) to assess the formation of the cell monolayer. Only monolayers with R values > 150 Ω were used for transport studies.

6.2.4. Drug transport across SCC4 cell monolayer

SCC4 cell monolayers were used 7-10 days after seeding. 200 µL of CAR-NC or an ethanol:water (1:1 v/v) carvedilol solution (CAR-HS) was added on the apical side plus 300 µL of cellular medium (DMEM+FBS+L-glutamine+Invitrogen). Both formulations had a drug concentration of 0.5 $mg.ml^{-1}$. At predetermined times (1, 2, 3, 4, 5, 6, 7, 8 and 24 h), 200 µL of the basolateral medium was withdrawn and the drug content assayed by LC. After each sampling, 200 µL of fresh cellular medium was added to the basolateral side. Collected samples were analyzed (20 µL) by LC using a Phenomenex Luna C_{18} column (250 mm x 4.6 mm I.D., particle size of 5 µm), as stationary phase, and phosphoric acid pH 3.0/acetonitrile (60:40, v/v), as mobile phase, at a flow rate of 0.6 $mL.min^{-1}$. The method was specific, linear (r=0.999, n=3) in the range of 0.005 to 50 $\mu g.mL^{-1}$, and precise (RSD of 2.7% and 2.3% for intra- and inter-day precision, respectively). Apparent permeability (Papp) of carvedilol was calculated according to Equation 1 and using the linear values of the curve (1- 5h, r=0.993 ± 0.002 [CAR-NC], r=0.993 ± 0.002 [CAR-SH]):

$$Papp = dQ/dt \; x \; 1/A.C0 \quad (1)$$

where dQ/dt is the rate of appearance of the drug on the basolateral side ($\mu mol.s^{-1}$), $C0$ is the initial concentration of the drug in apical side (mM) and A is the area of the monolayer (cm^2). Permeability of carvedilol from CAR-NC and CAR-HS across Transwell® inserts without the presence of cell monolayers was also evaluated and the apparent permeability calculated (1 – 5h, r=0.957 [CAR-NC], r=0.955 [CAR-SH].

The integrity of the cell monolayers at the end of the experiment (24 h) was evaluated comparing the calculated transepithelial electrical resistance (TEER) pre- and post-experiment. TEER values were calculated according to Equation 2:

$$\text{TEER } (\Omega.\text{cm}^2) = [\text{R (insert with SCC4)} - \text{R (insert without SCC4)}] \text{x A} \quad (2)$$

where, R is the resistance (Ω) and A is the growth area (cm^2).

6.2.5. Citotoxicity study

The cytotoxicity study of CAR-NC against oral epithelial cells was carried out by means of the sulforhodamine B (SRB) colorimetric assay (n=3). SCC4 cells were seeded onto 96-well plates at density of $5x10^3$ cells per well and allowed to attach overnight. The cells were treated with CAR-NC at different carvedilol concentrations (31.25, 62.50 and 125 $\mu g.mL^{-1}$) during 72 h to discard any effect in the long-term. Afterwards, cells were fixed with 10% trichloroacetic acid, stained with 0.4% SRB in 1% acetic acid, and plates were read at 560 nm. Results were normalized against controls. The following controls were used: unloaded nanocapsules (NC), carvedilol in solution (CAR-HS) [hydroalcoholic solution, ethanol:water 1:1 v/v, CAR 0.5 $mg.mL^{-1}$] and hydroalcoholic vehicle (HV). In parallel, cytotoxicity studies were carried out using a non-tumoral cell line (HaCaT), under the same protocol.

6.3. RESULTS AND DISCUSSION

6.3.1. Carvedilol-loaded nanocapsule suspensions

The production and complete physicochemical characterization of carvedilol-loaded nanocapsules was previously reported [3]. The new batches of CAR-NC produced for this study had mean diameter of 138 ± 5 nm, cationic surface (zeta potential of +8 ± 3 mV) and drug content of 0.48 ± 0.03 $mg.mL^{-1}$. Unloaded nanocapsules (NC) had similar values of mean diameter (137 ± 1 nm) and zeta potential (+4 ± 1 mV). These results are in agreement with those previously reported [3].

6.3.2. SCC4 cells transport studies

The mass of drug permeated across SCC4 cell monolayer from CAR-NC or CAR-SH are showed in Figure 1. Higher amount of carvedilol permeated through the monolayer when the drug was non-encapsulated, while the nanoencapsulation promoted a most controlled drug transport. Apparent permeability values reproduced this diference. The calculated Papp of CAR-NC was 3.45 ± 0.31 x 10^{-7} $cm.s^{-1}$, while CAR-HS had a Papp of 1.63 ± 0.54 x 10^{-6} $cm.s^{-1}$. This controlled trasport may be an important strategy to prolong the carvedilol plasmatic concentration when administered through the sublingual route, taking into account the mucoadhesion properties and resistance to the

salivar flux afforded by the cationic nanocapsules [3]. Drug absorption by sublingual membrane is very fast, however, the duration effect is short due to rapid decline of drug plasmatic concentration [8]. Similar profiles were observed in carvedilol permeability across porcine sublingual mucosa study [3]. Moreover, a linear correlation ($r > 0.95$) between the concentration of drug permeated across porcine sublingual mucosa and across cell monolayer was found (Figure 2). However, the total amount of the drug permeated across these two membrane was different. Epithelium of sublingual mucosa due to its 8-12 cell layers [2] is a thicker barrier to drug permeation than cell monolayer, which may explain the lower amount of drug permeated through its structure. However, as SCC4 cells monolayers were never used to evaluated drug transport, apparent permeability across the filters without cell monolayers were assessed. These apparent permeability were of 5.44×10^{-7} cm.s^{-1} and 2.72×10^{-6} cm.s^{-1} for CAR-NC and CAR-SH, respectively . The results evidence the higher apparent drug permeability across the filters without the cells and the suitable barrier to the permeation of carvedilol showed by SCC4 cell monolayers. Furthermore, maximum calculated TEER value showed by SCC4 cells (37.33 ± 10.59 Ω.cm^2) was closely to those reported for other oral epithelial cells (H376: 31.40 ± 4.28 Ω.cm^2, TR146: 50.02 ± 2.87 Ω.cm^2) [9]. Thus, SCC4 cell monolayer may be used as substitute of porcine sublingual mucosa in prelimires studies of transport to compare formulations since they are able to reproduced the diference of drug permeated when it is in solution or nanoencapsulated.

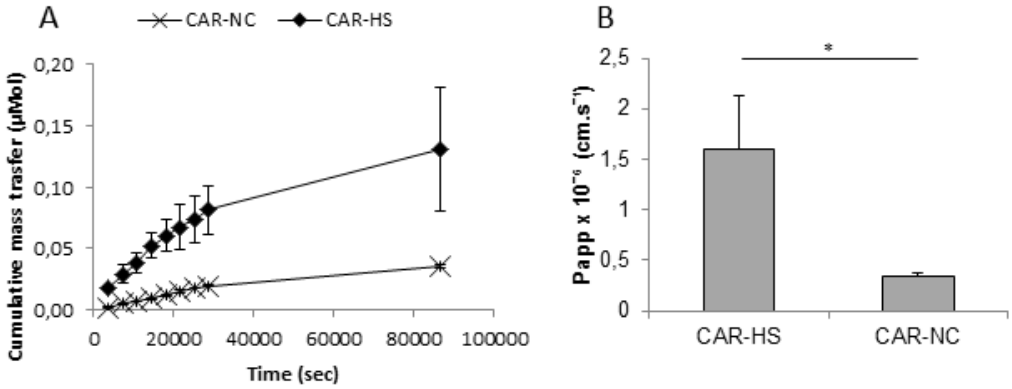

Figure 1. A: Carvedilol transport across SCC4 cells monolayer from carvedilol-loaded nanocapsule suspension (CAR-NC) and from carvedilol-hydroalcoholic solution (CAR-

HS). B: Apparent permeability values of CAR-HS and CAR-NC, asterisks (*) express significant difference ($p \leq 0.050$, t test).

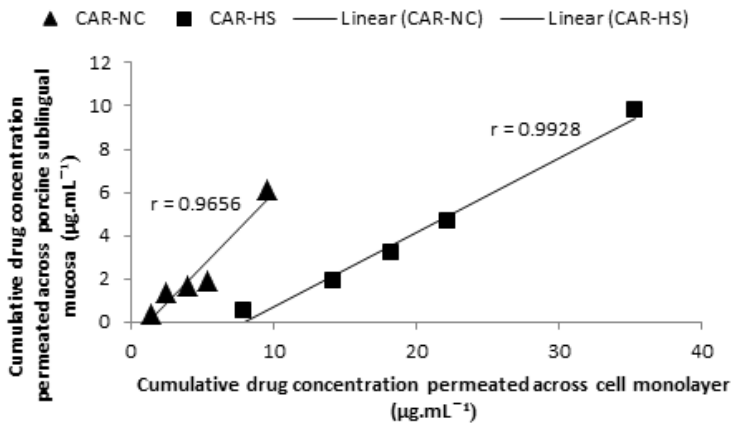

Figure 2. Correlation between cumulative drug concentration permeated across porcine sublingual mucosa and cell monolayer from from carvedilol-loaded nanocapsule suspension (CAR-NC) and from carvedilol-hydroalcoholic solution (CAR-HS).

6.3.3. Monolayer integrity (TEER) and cytotoxicity assays

The drug transport across cell monolayers did not alter significantly ($p>0.05$) the TEER values independently of drug being nanoencapsulated or in solution. The initial calculated TEER value was 37.33 ± 10.59 $\Omega.cm^2$ and post-transport was 34.60 ± 3.99 $\Omega.cm^2$ for CAR-NC and 27.63 ± 4.53 $\Omega.cm^2$ for CAR-HS. No variation in TEER after drug permeation means absence of damage in the cell monolayers integrity [8]. Moreover, cellular viability data are shown in Figure 2. The cell viability was calculated based on the cells with no treatment, which represented 100% of viability. CAR-NC as well as NC were no toxic to oral epithelial cells, regardelss of concentration studied (Figure 3A). Unlike this, interesting the cells were able to reproduce in presence of CAR-NC or NC and the viability was higher than the cells kept in culture medium without treatment. The presence of the drug did not influence the cellular behavior effect of CAR-NC and NC ($p > 0.05$). On the other hand, the drug dissolved in a hydroalcoholic solution decreased the cell viability. This effect may be explained by a low cytotoxic effect of the hydroalcoholic solution since there is no difference between CAR-HS and HV, regardless of the concentration. As SCC4 cells are extracted from squamous cell carcinoma of tongue, the cytotoxicity

screening of formulations was also evaluated using human keratinocyte cells from skin (HaCat) in order to discard the influence of carcinogenesis in cells resistance. As shown in Figure 3B, nanocapsules suspensions and hydroalcoholic solutions, containing or not the drug, did not show any cytotoxic effect at the three tested concentrations, in agreement with the data from SCC4 cells. Therefore, CAR-NC does not have potentially cytotoxic effect enabling their further study for application in the sublingual cavity.

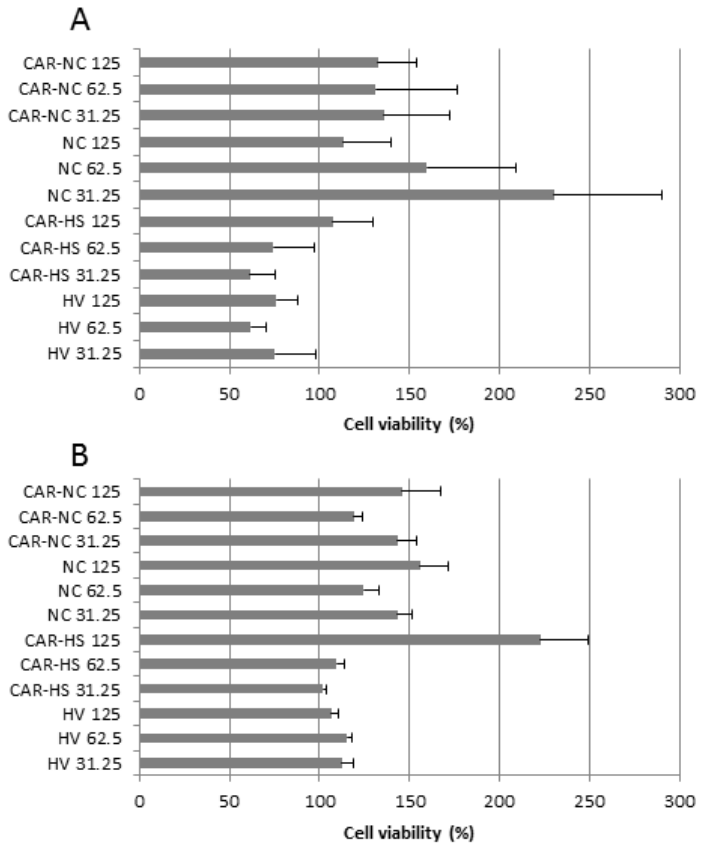

Figure 3. Cell viability of SCC4 [A] and HaCaT [B] after treatment with carvedilol-loaded nanocapsule suspension (CAR-NC), unloaded nanocapsules (NC), carvedilol-hydroalcoholic solution (CAR-HS) and hydroalcoolic solution (HV) at 31.25, 62.5 and 125 $\mu g.mL^{-1}$.

6.4. CONCLUSION

Carvelidol permeated across cell monolayer by a controlled way when it is nanoencapsulated. Data had linear correlation to those reported for porcine sublingual mucosa. Therefore, SCC4 cells monolayers may be used as *in vitro*

model for screening studies of drug permeation in the development of novel sublingual formulations. Furthermore, carvedilol permeation and nanocapsules suspension were not harmful to SCC4 cells allowing their further study on the use by sublingual route.

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APRESENTAÇÃO DO CAPÍTULO

A forma farmacêutica comprimido é a mais utilizada para administração de fármacos pela via sublingual. Essas formas sólidas são obtidas, em geral, a partir de materiais pulverulentos, previamente granulados ou não. Portanto, suspensões de nanocápsulas podem ser previamente secas para a sua formulação na forma de comprimidos. Desse modo, neste capítulo foram produzidos pós a partir da secagem por aspersão das suspensões de nanocápsulas, empregando adjuvantes de secagem com características hidrossolúveis para facilitar a sua redispersão em saliva. Novos estudos de avaliação das características mucoadesivas foram realizados para as nanocápsulas secas, a fim de verificar se essa propriedade poderia ser afetada pelo processo de secagem. Também foram conduzidos estudos para avaliação da permeação em mucosa sublingual e aderência das nanocápsulas à superfície da mucosa sublingual em presença de fluxo salivar mimetizado, avaliando o seu efeito na permeação do fármaco. O capítulo IV foi redigido na forma de artigo científico, que encontra-se em fase final de redação para posterior submissão a periódico científico.

Redispersible spray-dried powders containing carvedilol-loaded nanocapsules: mucoadhesive properties and drug permeability in sublingual mucosa

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Abstract

The aim of this study was to produce spray-dried carvedilol-loaded nanocapsules and to evaluate their mucoadhesive properties as well as their permeability performance across sublingual mucosa in presence or absence of mimicked salivary flux. Carvedilol-loaded nanocapsules with distinct charge were produced with Eudragit® RS 100 and poly(ε-caprolactone) as polymers. Powders containing nanocapsules were produced by spray-drying using a mixture of lactose/polyvinylpyrrolidone as drying adjuvant. The recovery of the original nanoparticles after aqueous redispersion was investigated by scanning electron microscopy and laser diffraction. The mucoadhesive characteristics of the formulations were assessed by texture analysis and determination of the mucin amount adsorbed on the nanoparticle surface. Permeability across porcine sublingual mucosa and the effect of mucoadhesion on this parameter in presence of simulated salivary flux was evaluated using modified Franz diffusion cells. The powders redispersion showed the recovery of the original nanoparticle, considering their size, morphology and mucoadhesiveness properties. The drug permeability was controlled by powders containing carvedilol nanoencapsulated. Nanocapsules improved the amount of drug retained on mucosa and promoted an increase of the amount of permeated drug in presence of simulated salivary flux. This study highlighted the suitability of using spray-dryied powders containing nanocapsules as a platform for the development of innovative sublingual solid dosage forms for carvedilol administration.

Key words: carvedilol, mucoadhesion, nanocapsules, powders, spray-drying, sublingual permeability.

7.1. INTRODUCTION

First-pass effect is an important limitation showed by several drugs administered orally. The drug is metabolized before reaching the systemic circulation and the concentration available to be absorbed is reduced. This metabolism occurs mainly in the liver and circumventing passage of this organ can be a promising alternative to improve the drug bioavailability (POND and TOZER, 1984, CUSTODIO *et al.,* 2008). Sublingual route of administration is richly vascularized and promotes drug absorption directly into the blood circulation avoiding the hepatic degradation. This region is protected by a thin mucosa representing the principal barrier to drug permeation (NIBHA and PANCHOLI, 2012).

Carvedilol (CAR) is an example of drug having reduced bioavailability due to fist-pass metabolism. This drug is a non-selective β-adrenoceptor antagonist, α1-adrenoceptor blocker and also has antioxidant effects. It has been used for management of heart failure, hypertension and coronary artery diseases (DANDONA *et al.,* 2007) by oral tablets. However just 25-35 % of dose is absorbed into the systemic circulation due to extensive first-pass metabolism in liver (MORGAN *et al.,* 1994). In order to improve the bioavailability of CAR our prior research has proposed its administration by sublingual route using nanotechnology to deal with the possible limitations of this route (CHAVES *et al.*, 2017). Sublingual region is exposed to constant flux of saliva that can remove parts of the drug to be absorbed (AL-GHANANEEM *et al.,* 2007). Buccal epithelium is covered by a mucus film with adhesiveness characteristics due to the presence of mucin molecules (SUDHAKAR *et al.,* 2006). The use of drug carriers with mucoadhesive properties has been proposed to prolong the residence time in this cavity (BREDEMBER *et al.,* 2003; AL-GHANANEEM *et al.,* 2007).

Nanostructures have been extensively studied because of their properties as drug delivery systems by different routes of administration (FRANK *et al*., 2015). However, their potential as carrier for sublingual drug administration has been little explored. Poly (lactic-co-glycolic) acid (PLGA) nanoparticles were studied for enhance sublingual immunotherapy (SALARI *et al.,* 2015), liposomes were explored for sublingual administration of vaccines against influenza antigens (OBEROI *et al.,* 2015) and alginic acid nanoparticles were developed for insulin

sublingual delivery (PATIL *et al.,* 2014). In our previous study it was proposed the use of polymeric nanocapsule systems as mucoadhesive carriers for sublingual CAR administration (CHAVES *et al.*, 2017). Polymeric nanocapsules are formed by a polymeric wall and a lipophilic core stabilized by surfactants (POHLMANN *et al.*, 2013). Polymers with mucoadhesive characteristics [Poly(ε-caprolactone) and Eudragit® RS100] were used for their production. These nanostructures, as liquid suspensions, showed promising mucoadhesive characteristics and increased the CAR permeation through sublingual mucosa in presence of simulated salivary flux (CHAVES *et al.*, 2017).

However, the main pharmaceutical dosage form for sublingual administration is tablets (RACHID *et al.,* 2012), prepared from powder components. Powders can be prepared from liquid nanocapsules using the spray-drying technique (GUTERRES *et al*., 2000; MÜLLER *et al.,* 2000; POHLMANN *et al.* 2002*,* SCHAFFAZICK *et al.,* 2006; MARCHIORI *et al.,* 2012; ZUGLIANELLO *et al.,* 2017). This process may produce redispersible dry powders in one step starting of a liquid suspension containing dissolved drying adjuvants. It is a fast method of low cost, produces powders with low water content and maintains the supramolecular structure of components (BECK *et al.*, 2012).

In this scenario, the objective of this study was to produce redispersible spray-dried carvedilol-loaded nanocapsules and to evaluate the mucoadhesive properties of the powders, the effect of their redispersion on the drug release and drug permeability across sublingual mucosa as well as the effect of salivary flux on mucoadhesion and drug permeability.

7.2. MATERIALS AND METHODS

7.2.1. Materials

Carvedilol was acquired from Henrifarma (São Paulo, Brazil). Poly(ε-caprolactone) (MW 80,000), sorbitan monostearate (Span 60®) and mucin from porcine stomach (type II) were supplied from Sigma-Aldrich (São Paulo, Brazil). Eudragit® RS100 was obtained from Degussa (Darmstadt, Germany) and grape seed oil from Dellaware (Porto Alegre, Brazil). Polysorbate 80, acetone and hydrochloric acid were acquired from Vetec (Rio de Janeiro, Brazil). Basic Fuchsin, sodium metabisulphite, periodic acid and acetic acid were purchased from Dinamica (São Paulo, Brazil). Potassium phosphate was obtained from

Nuclear (São Paulo, Brazil) and Sodium Hydroxide from Cromoline (São Paulo, Brazil). HPLC grade acetonitrile was purchased from Tedia (Rio de Janeiro, Brazil).

7.2.2. Preparation and characterization of nanocapsule suspensions

The method used for nanocapsule preparation was the interfacial deposition of preformed polymer (JAGGER *et al.*, 2009; VENTURINI *et al.*, 2011). This technique consists of injection of an organic phase into an aqueous phase followed by solvent evaporation. For EUD nanocapsules production (CAR-NC) the organic phase was prepared by dissolving 0.1 g of polymer (EUD), 165 µL of grape seed oil and 5 mg of CV (0.5 $mg.mL^{-1}$) in 27 mL of acetone under magnetic stirring at 40°C. For the preparation of PCL lipid-core nanocapsules (CAR-LNC) the organic phase was prepared in the same way, but changing EUD for PCL and adding 0.0385 g of sorbitan monostearate. The composition of the aqueous phase was in both cases 0.077 g of polysorbate 80 and 54 mL of ultrapure water. Subsequent to the injection step acetone and part of the water were eliminated at reduced pressure (Rotavapor R-114, Büchi, Flawil, Switzerland) until reaching a final volume of 10 mL.

After the preparation the particles (n = 3) were analyzed according to size distribution and polydispersity by laser diffraction (LD) (Mastersizer 2000, Malvern Instruments Ltd., UK) by adding the sample directly to distilled water in the wet dispersion unit and by dynamic light scattering (DLS) (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK) by diluting the suspensions (20 µL) in 10 mL of water previously filtered with a hydrophilic membrane (0.45 µm, Millipore®). The zeta potential was determined by electrophoretic mobility (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK) and for this analyze the samples (20 µL) were diluted in 10 mL of 10 mM NaCl solution previously filtered with a hydrophilic membrane (0.45 µm, Millipore®). The pH was measured directly in the formulations by potentiometry using a calibrated potentiometer (VB-10, Denver Instrument, USA). Drug content was determined by Liquid Chromatography (LC), using a method adapted from Ieggli and co-workers (2011) and validated according to our purposes (CHAVES *et al.*, 2017). The analysis were performed on a Shimadzu LC system (Kyoto, Japan) equipped with a CBM-20A system controller, LC-20AT pump, DGU-20A5 degasser, SIL-

20A auto-sampler and a SPD-20AV detector (UV). A Phenomenex Luna C_{18} column (250 mm x 4.6 mm I.D., with a particle size of 5 µm) was utilized as stationary phase. The mobile phase was composed of phosphoric acid solution pH 3.0/acetonitrile (50:50, v/v), run at a flow rate of 0.8 mL.min^{-1}, detection wavelength of 241 nm and injection volume of 10 µL. Samples of suspensions (1.0 mL) were dissolved in acetonitrile (9.0 mL) and sonicated for 10 min. This dispersion was centrifuged at 4,120 ×*g* for 10 min. After, an aliquot (2.0 mL) of the supernatant was diluted to 10 mL in mobile phase and analyzed.

7.2.3. Atomic Force Microscopy

Nanocapsule morphology was analyzed by atomic force microscopy (AFM) in tapping mode at Saarland University, Saarbruecken, Germany. Samples were prepared by placing undiluted nanocapsule suspension onto a freshly cleaved mica surface (Plano GmbH, Wetzlar, Germany) and removing the remaining liquid after 5 minutes of incubation with a paper tissue. Measurements were performed in air, using a BioScope BS3-Z2 AFM with a Nanoscope IV controller (Bruker Corporation, Billerica, USA) and a silicon cantilever with tetrahydral tips (OMCL-AC160TS - Olympus, Shinjuku, Japan) with a nominative force constant of 42 Nm^{-1} and a resonance frequency of around 300 kHz. Images were analyzed with the NanoScope Analysis 1.40 software (Bruker Corporation, Billerica, USA).

7.2.4. Preparation and characterization of spray-dried powders containing carvedilol-loaded nanocapsules

Powders containing nanocapsules (CAR-NC-SD and CAR-LNC-SD) were produced using the spray-drying technique with a mixture of lactose (Lac) and polyvinylpyrrolidone (PVP) as drying adjuvants (ZUGLIANELLO *et al.*, 2017). For this purpose 1.25 g of lactose and 1.25 g of PVP were dissolved in 25 mL of ultrapure water under magnetic stirring. After complete solubilization of the adjuvants, the solution was mixed with 25 mL nanocapsule suspension and the resulting dispersion was dried using a Mini Spray-Dryer B-290 (Büchi, Flawil, Switzerland). The process parameters were: feed pump rate of 5.0 ml·min^{-1}, 100% aspiration, 0.7 mm nozzle, atomization air at 819 L·h^{-1} and an inlet temperature of 120 °C with a resulting outlet temperature around 65 °C. For

comparison, powders containing only the drying adjuvants (LacPVP-SD) or using a CAR hydroalcoholic (water:ethanol, 1:1, v/v; 0.5 $mg.mL^{-1}$) solution (CAR-SD) instead of the nanocapsules suspension were also prepared.

Characterization (n = 3) consisted of the determination of yield, particle size and size distribution, loss of drying, drug content and morphology. Yield was determined by relating the weight of obtained powder after the spray-drying process to the weight of all solid components presents in the formulation. Laser diffraction measurements (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK) were performed by wet dispersion method to evaluate the particle size recovery after aqueous redispersion. The powder samples were inserted directly into the wet dispersion unit containing distilled water. Loss of drying was evaluated by infrared moisture balance (MB45OHAUS, Parsippany, US). Around 1 g of powder was deposited in the balance and heated until 105 °C during 1 minute (FARMACOPÉIA BRASILEIRA, 2010). The drug content ($mgCAR.g^{-1}$) was determined by HPLC as described in section 2.2. Powders were dispersed in mobile phase followed by 15 min of shaking, 1 h of ultrasound, centrifugation (4120 xg, 10 minutes) and filtration with a hydrophilic membrane (0.45 µm, Millipore®). Morphological analyzes were carried out by scanning electron microscopy (SEM; Jeol Scanning Microscope, JSM-6060, Tokyo, Japan) operating at 10 kV, at the Microscopy Center of the University (Centro de Microscopia Eletrônica - UFRGS, Brazil). Powder samples and redispersions were analyzed after they had been gold sputtered (Jeol Jee 4B SVG-IN, Tokyo, Japan). For redispersion, a silica wafer was fixed on conductive tape. Onto the silica wafer a powder sample was added and covered with water. After 5 min the water was removed together with dissolved excipients with a paper tissue.

7.2.5. Mucoadhesion measurements

7.2.5.1. Determination of suspensions and powders adhesion on the sublingual mucosa

The adhesion of suspensions (CAR-NC and CAR-LNC) and powders (CAR-NC-SD and CAR-LNC-SD) on sublingual mucosa were evaluated by tensile stress tester (n = 3) (TA.XTplus Texture Analyzer; Stable Microsystem, Godalming, UK). Powders containing only the drying adjuvants (LacPVP-SD) were also

analyzed to evaluate the effect of adjuvants on mucoadhesion. Samples were deposited on a support and discs of porcine sublingual mucosa (10 mm) were attached on a movable probe with double-sided tape. Fresh porcine heads were obtained from Santo Ângelo slaughterhouse (Porto Alegre, Brazil). Porcine sublingual mucosa was excised from pigs using a scalpel and immediately used. Mucosa contacted the samples with a force of 290 mN during 3 min. After this the probe was removed at a constant rate of 0.10 mm/s until complete detachment. Peak of force (mN), displacement (mm) and work of mucoadhesion (mN.mm) were determined by software equipment (Exponent).

7.2.5.2. Determination of mucin concentration adsorbed on spray-dried nanocapsules

Periodic Acid Schiff colorimetric method (MANTLE and ALLEN, 1978) was used for the quantification of mucin molecules. Powders (10 mg of CAR-NC-SD or CAR-LNC-SD) were mixed during 30 minutes with different concentrations of mucin solutions (0.1%, 0.25% and 0.5%). Solutions of lyophilized mucin from porcine stomach (CHAVES *et al.*, 2017) were prepared in phosphate buffer 0.02 M pH 6.8. Concentration of mucin that did not interact with the nanoparticles was determined. For sedimentation of nanocapsules and isolation of free mucin, the mixtures were ultracentrifuged during 20 minutes at 20 °C and 200,000 xg (TEIXEIRA *et al.*, 2005). Periodic acid reagent (0.2 mL) was added to 2 mL of supernatant, which was first diluted in phosphate buffer two, four and ten times for 0.1%, 0.25% and 0.5% of mucin solution, respectively. Periodic acid reagent was freshly prepared by adding 100 µL of periodic acid solution 5% (w/v) to 7 ml of acetic acid solution 7% (v/v). The resultant solutions were incubated at 37 °C for 2 h in a water bath. Subsequently, Schiff reagent (0.2 mL) was added and the resulting solutions were kept at room temperature during 30 minutes. Schiff reagent stock solution was prepared by adding 20 ml of 1 M HCl in 100 ml of basic Fuchsin aqueous solution 1% (w/v) and was kept on refrigeration. Before the analyses, 0.1 g of sodium metabisulphite was added to 6 ml of Schiff reagent and the resultant solution was incubated at 37 °C until it became pale yellow (around 1.5 hours). Absorbance was measured at 555 nm in an UV spectrophotometer. The equipment was zeroed with a resuspension of LacPVP-SD. The amount of free mucin in the supernatant was calculated from

calibration curves (n = 3) with linearity (r = 0.998) in the range of 0.1 – 0.5 $mg.mL^{-1}$. The amount of mucin that adsorbed onto the surface of nanocapsules (n = 3) was determined by the difference between total mucin and free mucin.

7.2.6. In vitro drug release

The *in vitro* release of CAR (n = 3) from the powders (CAR-NC-SD, CAR-LNC-SD and CAR-SD) was carried out by dialysis bag method. To evaluate the effect of the aqueous medium, the powders were dispersed in artificial saliva (TEUBL *et al.,* 2013) or in water for this study. However, the powder containing the free drug (CAR-SD) was dispersed in hydroalcoholic solution (1:1 v/v) instead of water for complete drug solubilization. All powders were dispersed to obtain a final CAR concentration of 0.5 $mg.mL^{-1}$. Two mL of each formulation was placed into a dialysis tubing cellulose membrane with flat width of 25 mm (Sigma-Aldrich, São Paulo, Brazil). The bags were suspended in 100 mL of release medium (sodium phosphate buffer pH 6.8 0.2 M) previously filtered using a hydrophilic membrane (0.45 µm, Millipore®) and maintained in a bath at 37 °C under agitation of 70 ± 10 rpm. Sink conditions were maintained during the experiment. A sample of the external medium (1.0 mL) was withdrawn at predetermined time intervals (1, 2, 4, 6, 8, 12 and 24 hours) and directly analyzed by HPLC with the method described in section 2.2. An injection volume of 20 µL was used to reduce the quantification limit. The method was previous validated (CHAVES *et al.*, 2017).

7.2.7. In vitro permeability across sublingual mucosa

Measurements of drug permeability (n = 3) across sublingual membrane were evaluated using modified manual Franz diffusion cells. Fresh porcine sublingual mucosa (0.9 cm^2) was placed between the donor and the receptor compartment as previously proposed (CHAVES et al., 2017; FRANK et al., 2017). On this membrane 100 µL of the redispersed powders (CAR-NC-SD, CAR-LNC-SD and CAR-SD) in artificial saliva (TEUBL *et al.,* 2013) or in water at a concentration of 0.5 $mg.mL^{-1}$ were applied. The receptor compartment received 2.5 mL of phosphate buffer 0.2 M pH 6.8 containing 0.1% of polysorbate 80, previously filtered using a hydrophilic membrane (0.45 µm, Millipore®). Sink conditions were kept during the experiment and the diffusion cells were maintained in a

bath at 37 °C under agitation of 70 ± 10 rpm through all the experiment. At intervals of 2 hours (2, 4, 6, 8, 12 and 24 h), 40 µL of the receptor medium was withdrawn and directly analyzed by HPLC as described in section 2.2. The mobile phase consisted of phosphoric acid pH 3.0/acetonitrile (60:40, v/v) and the injection volume of 20 µL as the method previous validated by Chaves and co-workers (2017).

7.2.8. Washability test

A washability test was carried out to verify the effect of salivary flux on the mucoadhesion of particles and on the drug permeability as previous described by Chaves and co-workers (2017). The same Franz cells as for the permeability measurements (section 2.7) were used. Powders (CAR-NC-SD, CAR-LNC-SD or CAR-SD) were redispersed in artificial saliva at a concentration of 0.5 $mg.mL^{-1}$. The redispersion (50 µL) were deposited onto porcine sublingual mucosa placed between donor and receptor compartment and kept during 1 hour for interaction. After this time, phosphate buffer 0.2 M pH 6.8 containing 0.1% of polysorbate 80 (37 ºC) was fluxed over the mucosa at 0.35 $mL.min^{-1}$ (GOSWAMI *et al.*, 2008) to simulate the action of salivary flux (Pump; Gilson; Minipuls 3, France). The outgoing flux was collected at predetermined time intervals (5, 10, 15, 20, 25, 30, 60, 120 and 180 min). Samples from the powder prepared with the CAR hydroalcoholic solution were directly assayed by HPLC (section 2.2) and samples from powders containing nanoencapsulated CV were subjected to extraction process with mobile phase (please, see section 2.4). To assay the total amount of CV permeated at the end of the experiment (180 min) samples from the receptor compartment were directly analyzed by HPLC with method previous validated by Chaves and co-workers (2017). Mobile phase consisted of phosphoric acid pH 3.0/acetonitrile (60:40, v/v) and the injection volume of 20 µL.

7.2.9. Statistical analysis

Statistical analyses were done by one-way analysis of variance (ANOVA) with post-hoc turkey test when three or more groups were compared. When two groups were analyzed the Student's *t* test was used. Means were considered

statistically significant different at a level of $p \leq 0.05$. Data are presented as the mean ± standard deviation (SD).

7.3. RESULTS AND DISCUSSION

7.3.1. Preparation and characterization of nanocapsule suspensions

Carvedilol-loaded nanocapsules were extensively characterized in our previous report (CHAVES *et al.*, 2017) and the formulations showed suitable nanoscopic characteristics. The aim of this study was to produce spray-dried carvedilol-loaded nanocapsule. However, some parameters of the new batches of the nanocapsules suspension were checked before the spray-drying process. CAR-LNC showed diameters of 191 ± 27 nm by LD technique and 182 ± 7 nm by DLS method and CAR-NC of 149 ± 1 nm and 139 ± 6 nm, by respective methods. Span values of 1.3 ± 0.1 and 1.8 ± 0.2 by LD and a polydispersity of 0.14 ± 0.01 and 0.09 ± 0.02 by DLS were calculated for CAR-NC and CAR-LNC, respectively. These results show their narrow size distribution at the nanoscale. The zeta potential values were positive for CAR-NC (+7.3 ± 3 mV) and negative for CAR-LNC (-7.6 ± 1 mV) due to the presence of quaternary ammonium groups in EUD molecules and oxygen atoms on the interface of particles formed by the non-ionic polymer (PCL) (PIGNATELLO *et al.*, 2002; CATTANI *et al.*, 2010). The pH was close to the salivary being 6.8 ± 0.1 for CAR-NC and 6.9 ± 0.1 for CAR-LNC. The drug content was close to the expected value (0.5 $mg.mL^{-1}$) for both particles (0.48 ± 0.01 $mg.mL^{-1}$ and 0.47 ± 0.002 $mg.mL^{-1}$, for CAR-NC and CAR-LNC, respectively). According to these results, the formulations presented adequate characteristics corroborating our previous findings (CHAVES *et al*., 2017), being the new batches suitable for the production of the spray-dried powders.

7.3.2. Atomic Force Microscopy

Nanocapsules were visualized by atomic force microscopy (AFM). As can be seen from the height images (Figure 1), both types of nanoparticles showed a smooth surface and mostly spherical shape. Particle analysis with the NanoScope Analysis 1.40 software revealed nanocapsule sizes of 111.7 ± 46.3 nm for CAR-LNC (n = 94) and 104.0 ± 46.3 nm for CAR-NC (n = 30). The particle sizes are smaller than those determined by dynamic light scattering.

This is most likely due to the water shell (hydrodynamic radius) measured by DLS and the AFM measurement under ambient conditions resulting in collapsed polymeric structures (SCHNEIDER, *et al*., 2003, HÖFL *et al*., 2007).

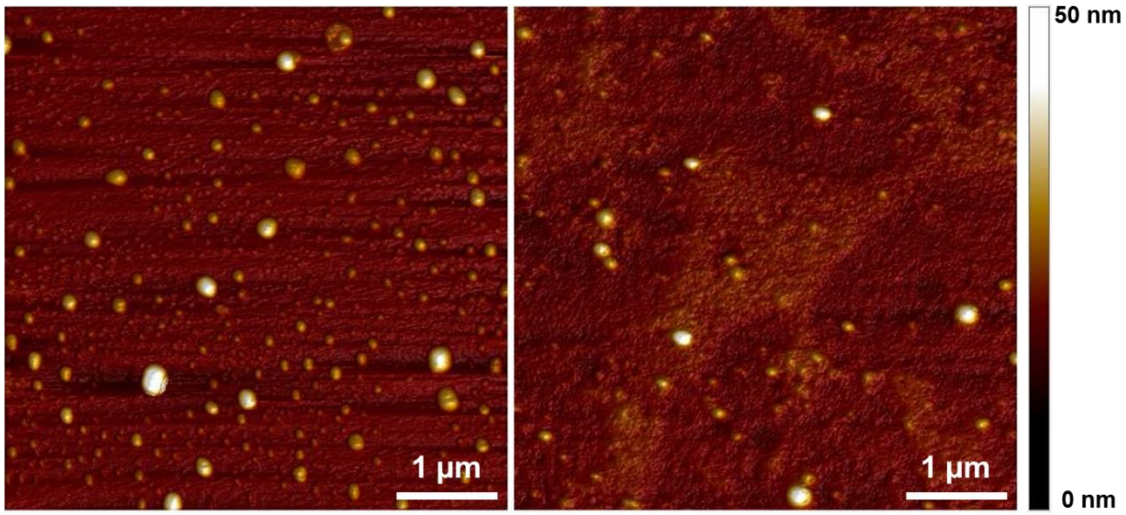


Figure 1. Nanocapsules visualized by atomic force microscopy; A: carvedilol-loaded PCL-nanocapsules (CAR-LNC) and B: carvedilol-loaded PCL-nanocapsules (CAR-NC).

7.3.3. Preparation and characterization of powders containing carvedilol-loaded nanocapsules

Powders were produced using Lac and PVP as drying adjuvants due to their aqueous solubility (ROWE *et al.,* 2009; BOURLINOS *et al.,* 2009). Sublingual administration needs a pharmaceutical dosage form with rapid dissolution time in small quantity of saliva (NIBHA and PANCHOLI, 2012). Furthermore, the mixture of Lac and PVP was previously studied as adjuvant for spray-drying of polymeric nanocapsules by Zuglianello and co-workers (2017). This previous study demonstrated that the resulting powder showed adequate characteristics and the nanometric size of particles is recovered after their aqueous dispersion. Table 1 shows the physico-chemical properties of the produced spray-dried powders.

Table 1. Physico-chemical properties of spray-dried powders containing carvedilol-loaded EUD-nanocapules (CAR-NC-SD), carvedilol-loaded PCL-nanocapsules (CAR-LNC-SD), carvedilol hydroalcoholic solution (CAR-SD) and drying adjuvants (LacPVP-SD), (mean ± SD).

Formulations	Yield (%)	Loss of drying (%)	Drug content ($mg.g^{-1}$)	Drug content recovered (%)
CAR-NC-SD	67 ± 3^{a}	2.3 ± 0.3^{a}	3.66 ± 0.16	99 ± 2
CAR-LNC-SD	64 ± 4^{a}	$2.5 \pm 0.5^{a,b}$	3.53 ± 0.15	102 ± 4
CAR-SD	31 ± 6^{b}	$3.3 \pm 0.3^{b,c}$	4.62 ± 0.53	97 ± 9
LacPVP-SD	48 ± 10^{b}	3.8 ± 0.3^{c}	_____	_____

SD = standard deviation (n = 3). Means, in column, with the same letter are not significant different ($p > 0.05$, ANOVA).

The yield of the process was around 65% for both formulations produced with the nanocapsules, similar to previous reports (SCHAFFAZICK *et al.,* 2006; HOFFMEISTER *et al.,* 2012). These values were higher for powders containing the polymeric nanocapsules than the powders containing the pure drug or only the drying adjuvants. In addition, powders prepared without the nanostructures showed higher water content and were more difficult to be collected. Presence of higher particle concentration contributed for better powder characteristics. The drug content was recovered for all powders without drug lost during the process. Granulometric distributions of the nanocapsules after the aqueous redispersion of powders are shown in Figure 2. This analyze was carried out to verify the recovering of original nanoparticle size distribution. The nanometric diameter of CAR-LNC was partially recovered when analyzed by volume (Fig. 2-A) and totally recovered when analyzed by number of particles (Fig. 2-B). Volume and size of particles have a cubic relationship and this analyze can give diameter values higher than diameters analyzed by number of particles (HOFFMEISTER *et al.,* 2012). For CAR-NC the nanometric size was recovered in analyzes of volume and number (Fig. 2-C; 2-D). The mean sizes d(0.1), d(0.5) and d(0.9) which represent the diameters at 10%, 50% and 90% of cumulative volume and number distribution are shown in Table 2. The results show the presence of some particles with a micrometric volume-weight in CAR-LNC-SD redispersion that influenced for bimodal granulometric distribution. Furthermore the diameters values evidence the presence of particles with

nanometric size after aqueous redispersion for both formulations. In order to further evaluate the ability of these powders to recover the original nanocapsules after their aqueous redispersion, their morphology was analyzed by scanning electronic microscopy (Figure 3). The presence of spherical particles in the nanoscale range was observed after the powders dispersion only for those produced with the nanocapsule suspensions (Figure 3-A, B), corroborating with previous analysis of laser diffraction. On the other hand, images of the redispersed CAR-SD (Figure 3-C) and LacPVP-SD (Figure 3-D) show the presence of particles with a diameter higher than 1 µm that probably are leavings of spray-dried powders did not solubilize.

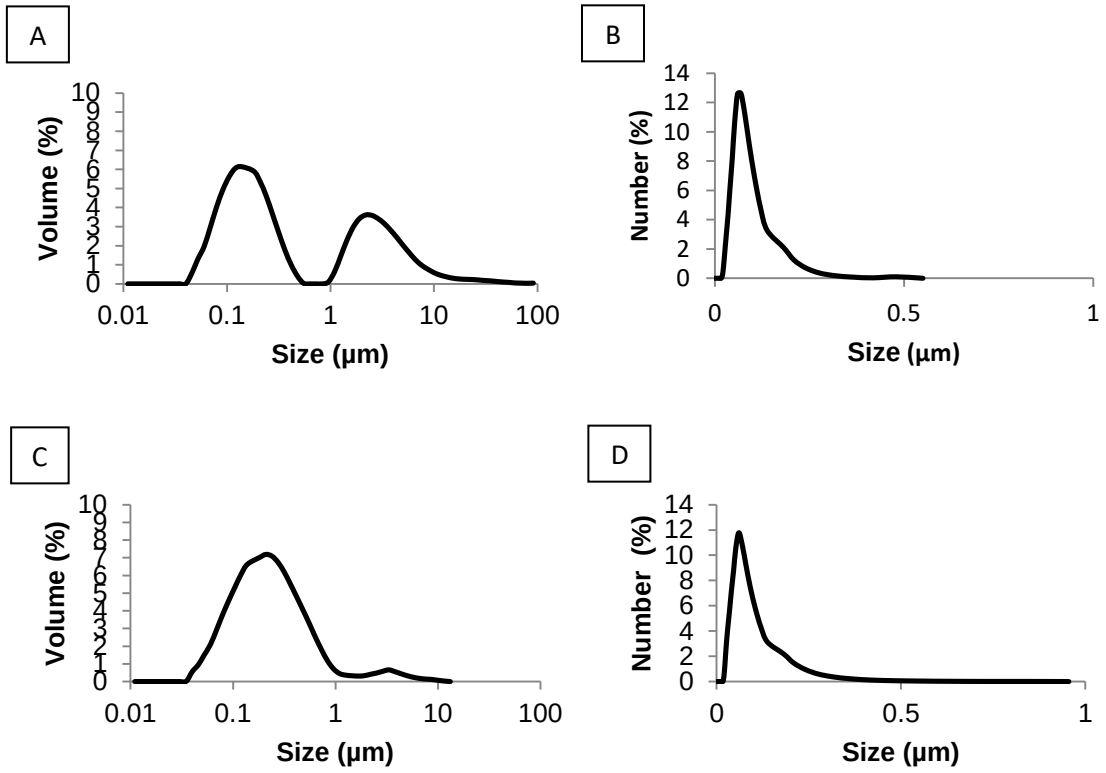


Figure 2. Granulometric distribution of volume or number by laser diffraction of nanocapsules after powders aqueous redispersion, A and B: powders containing carvedilol-loaded PCL-nanocapsules (CAR-LNC-SD); C and D: powders containing carvedilol-loaded EUD-nanocapsules (CAR-NC-SD) (n = 3).

Table 2. Diameters of volume or number by laser diffraction of nanocapsules after aqueous redispersion of powders containing carvedilol-loaded PCL-nanocapsules (CAR-LNC-SD) and of powders containing carvedilol-loaded EUD-nanocapsules (CAR-NC-SD) (mean ± SD, n = 3).

Formulations	Volume (µm)			Number (µm)		
	d(0.1)	d(0.5)	d(0.9)	d(0.1)	d(0.5)	d(0.9)
CAR-NC-SD	0.086 ± 0.002	0.214 ± 0.010	0.725 ± 0.070	0.036 ± 0.001	0.067 ± 0.001	0.134 ± 0.003
CAR-LNC-SD	0.089 ± 0.011	0.656 ± 0.764	5.809 ± 4.303	0.046 ± 0.013	0.075 ± 0.012	0.132 ± 0.013

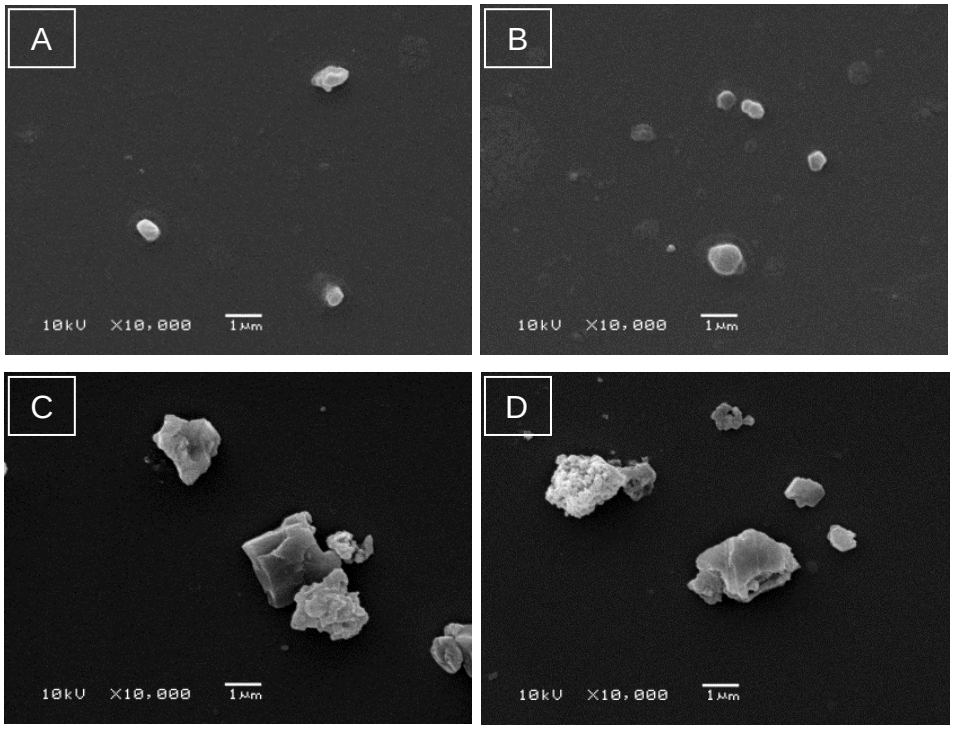
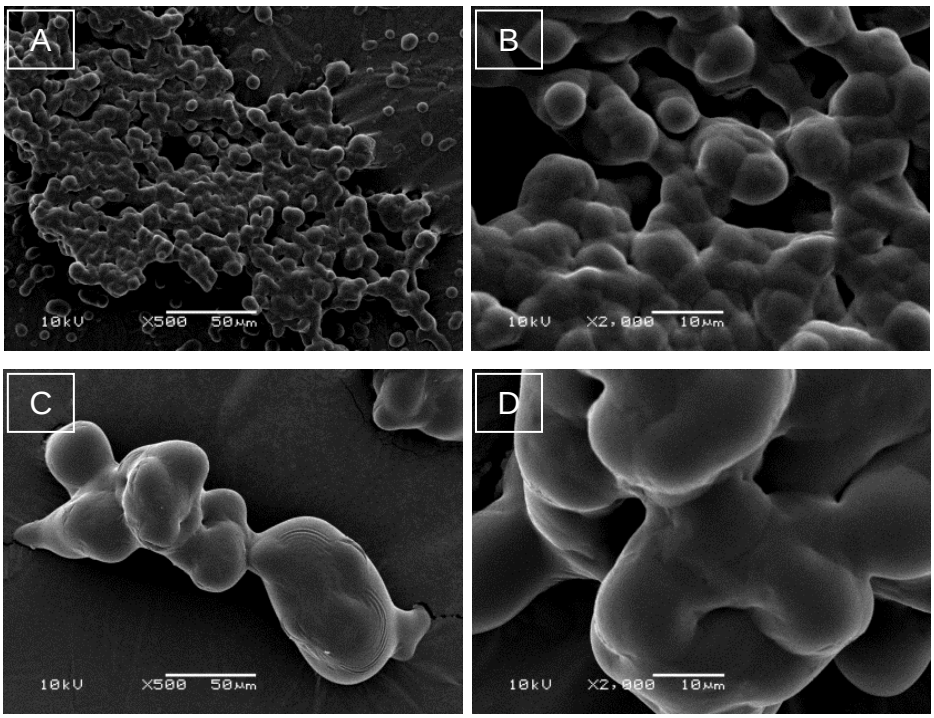


Figure 3. Scanning electron microscopy images of powders after aqueous redispersion, A: powder containing carvedilol-loaded EUD-nanocapsules (CAR-NC-SD), B: powder containing carvedilol-loaded PCL-nanocapsules (CAR-LNC-SD), C: powder containing carvedilol hydroalcoholic solution (CAR-SD), D: powder containing the adjuvants (LacPVP-SD).

Morphology of powders was evaluated by scanning electronic microscopy. The presence of spherical particles can be observed in all spray-dried powders (Figure 4). Dried adjuvants showed agglomerates of particles with regular surface (Figure 4-A,B). Powders containing the drug not-encapsulated are formed by bigger agglomerates with more irregulars forms (Figure 4-C,D). On

the other hand, powders produced with nanocapsules showed smaller particles with differences in arrangement and surface structure, as observed in figures 4-E, F, G, H. CAR-LNC-SD is arranged in form of micro-agglomerates and the presence of pores can be observed on their surface. While CAR-NC-SD showed the presence of isolate particles with more uniform surfaces. This structure can be favored the aqueous dissolution and consequently the total recovery of original EUD nanocapsules. Zugglianelo and co-workers (2017) reported the obtaining of spray-dried powder prepared from PCL nanocapsules suspensions using the mixture Lac/PVP as drying adjuvants. This powder showed similar characteristics of structure (presence of micro-agglomerates and pores on surface) and similar partial recovery of original nanostructures by volume analysis. On the other hand, the present study is the first report on the development of spray-dried powder containing nanocapsules formed with EUD RS100 using this adjuvant mixture. Considering the results of aqueous redispersion and morphological analysis, powders prepared with both nanocapsules suspensions were considered suitable for the next steps of this study, regarding the evaluation of mucoadhesive properties.



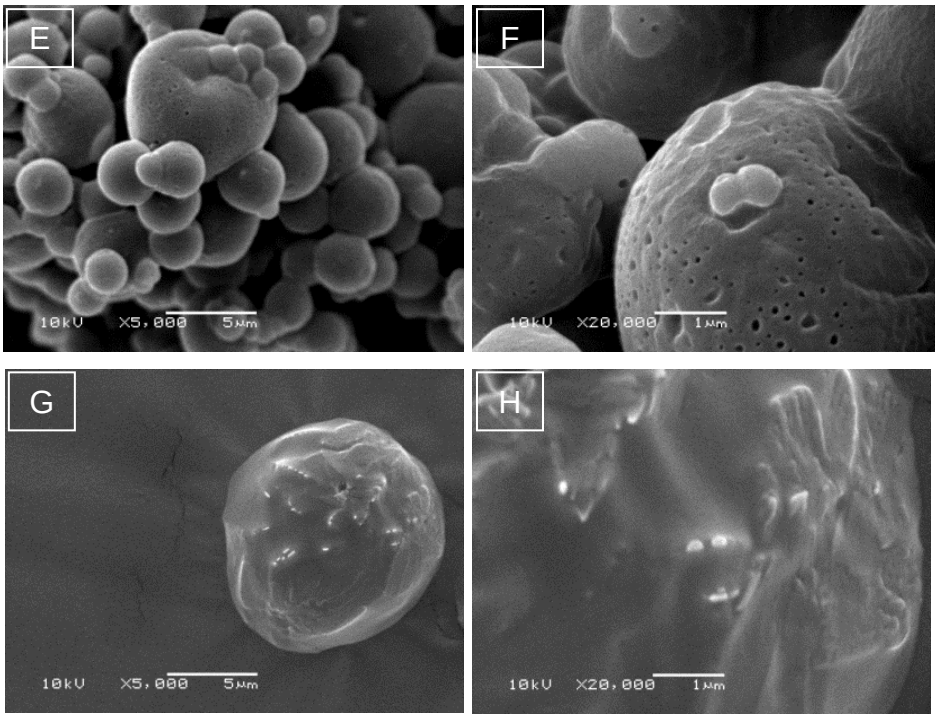


Figure 4. Scanning electron microscopy images of powders, A and B: powder of adjuvants (LacPVP-SD), C and D: powder of carvedilol hydroalcoholic solution (CAR-SD), E and F: powder of carvedilol-loaded PCL-nanocapsules (CAR-LNC-SD), G and H: powder of carvedilol-loaded EUD-nanocapsules (CAR-NC-SD).

7.3.4. Mucoadhesion measurements

The use of mucoadhesive systems has been proposed to avoid the fast drug withdraw by saliva in the buccal cavity. In our previous research (CHAVES *et al.,* 2017) the mucoadhesive properties of polymeric nanocapsules were evaluated, showing their important role to improve the drug permeability in presence of simulated salivary flux. In view of this, the next step of the present study was to evaluate the mucoadhesive properties of the systems development. The ability of powers (CAR-NC-SD, CAR-LNC-SD and LacPVP-SD) to adhere on porcine sublingual mucosa was determined by texture analysis. This technique is based on a measurement of tensile strength and determines the work necessary to separate two surfaces in contact for a determined time based on force and distance for displacement (THIRAWONG *et al.*, 2007; FRANK *et al*., 2014). The work necessary to rupture the interaction between sublingual mucosa and original nanocapsule suspensions was determined for purpose of comparison. The calculated parameters by the software program are shown in Table 3. A relative work was necessary to break

off the interaction between sublingual mucosa and nanocapsule suspensions. CAR-NC and CAR-LNC showed a similar peak of force, but the distance covered till the displacement was longer for EUD nanocapsules than for PCL and consequently the work was higher. A similar difference in the mucoadhesive capacity of nanocapsules was observed in our previous study and this result was attributed to the different ionic characteristics of the polymers (CHAVES *et al.*, 2017). EUD is a cationic polymer with a better capacity to interact with negative mucus than PCL, a non-ionic polymer (KHUTORYANSKIY *et al.*, 2011). In addition, the powders showed lower values of distance for detachment from mucosa than the suspensions probably due to their poor elastic properties. However the force needed to break the interaction was higher and consequently more work was necessary. Powders containing just the adjuvants showed adhesive characteristics which potentiated the mucoadhesive property of systems formed with EUD. On the other hand, powders containing PCL nanocapsules showed higher values of work than its liquid form but demonstrated similar work when compared to LacPVP-SD. In view of this, the mucoadhesive effect of CAR-LNC-SD can be attributed to adhesive characteristics of drying adjuvants. This is the first time that mucoadhesion of powders containing nanocapsules was evaluated by this technique.

Table 3. texture analyses parameters of interactions between sublingual mucosa and carvedilol-loaded EUD-nanocapsules (CAR-NC), carvedilol-loaded PCL-nanocapsules (CAR-LNC), powders containing carvedilol-loaded EUD-nanocapsules (CAR-NC-SD), powders containing carvedilol-loaded PCL-nanocapsules (CAR-LNC-SD) and powders containing just the adjuvants (LacPVP-SD) (mean ± SD).

Formulations	Force peak (mN)	Distance (mm)	Work (mN.mm)
CAR-NC	10 ± 0^{a}	5.73 ± 0.30^{a}	24.67 ± 3.79^{a}
CAR-LNC	10 ± 0^{a}	3.76 ± 0.42^{b}	10.67 ± 0.58^{b}
CAR-NC-SD	90 ± 20^{b}	2.13 ± 0.84^{c}	75.00 ± 14.14^{c}
CAR-LNC-SD	30 ± 10^{c}	2.11 ± 0.95^{c}	25.00 ± 9.85^{a}
LacPVP-SD	23 ± 15^{c}	1.97 ± 0.64^{c}	20.33 ± 8.33^{a}

SD = standard deviation (n = 3). Means, in column, with the same letter are not significant different ($p > 0.05$, ANOVA).

Elucidated the mucoadhesive properties of powders produced, the next step was to verify if the adhesive capacity of nanocapsules was altered or not by drying process. Powders were redispersed in aqueous solution to evaluate the interaction of recovered nanocapsules with mucin molecules. Mucin is a glycoprotein present in the mucus and responsible for its adhesiveness properties (BANSIL *et al.,* 2006). The concentration of mucin, which is present in the supernatant, was determined by colorimetric method and the concentration adsorbed on nanocapsules surface was calculated. Results are showed in Figure 5. Different concentrations of mucin were utilized since the mucin expression may vary for each person or physiological situation (BANSIL *et al.,* 2006). When recovered nanocapsules (CAR-NC and CAR-LNC) interacted with a 1 $mg.mL^{-1}$ mucin solution, no mucin was detected in the supernatant, regardless the formulation. These results show that all mucin added are interacting with nanocapsules and evidence the mucoadhesive properties of both structures, as demonstrated in previous work (CHAVES *et al.,* 2017). When more mucin was added, its presence in the supernatant was observed as well as the difference of mucoadhesive properties of nanocapsules. CAR-NC was able to interact with higher mucin amounts than CAR-LNC, since low quantity of mucin was detected on the supernatant, confirming the texture results previously discussed and the results of previous work (CHAVES *et al.,* 2017). CAR-NC has favorable ionic characteristics to interact with negative mucin molecules (GOSWAMI *et al.,* 2008). The better adhesiveness of cationic formulations in comparison to anionic formulations was as well evidenced by other authors (DÜNNHAUPT *et al.,* 2011 FRANK *et al.,* 2014). Effect of drying-adjuvants on absorbance values was discarded because the equipment was zeroed with a resuspension of powders formed by just adjuvants. These results highlighted the mucoadhesive characteristics of recovered nanocapsules and demonstrated the maintenance of their adhesive properties after the drying process.

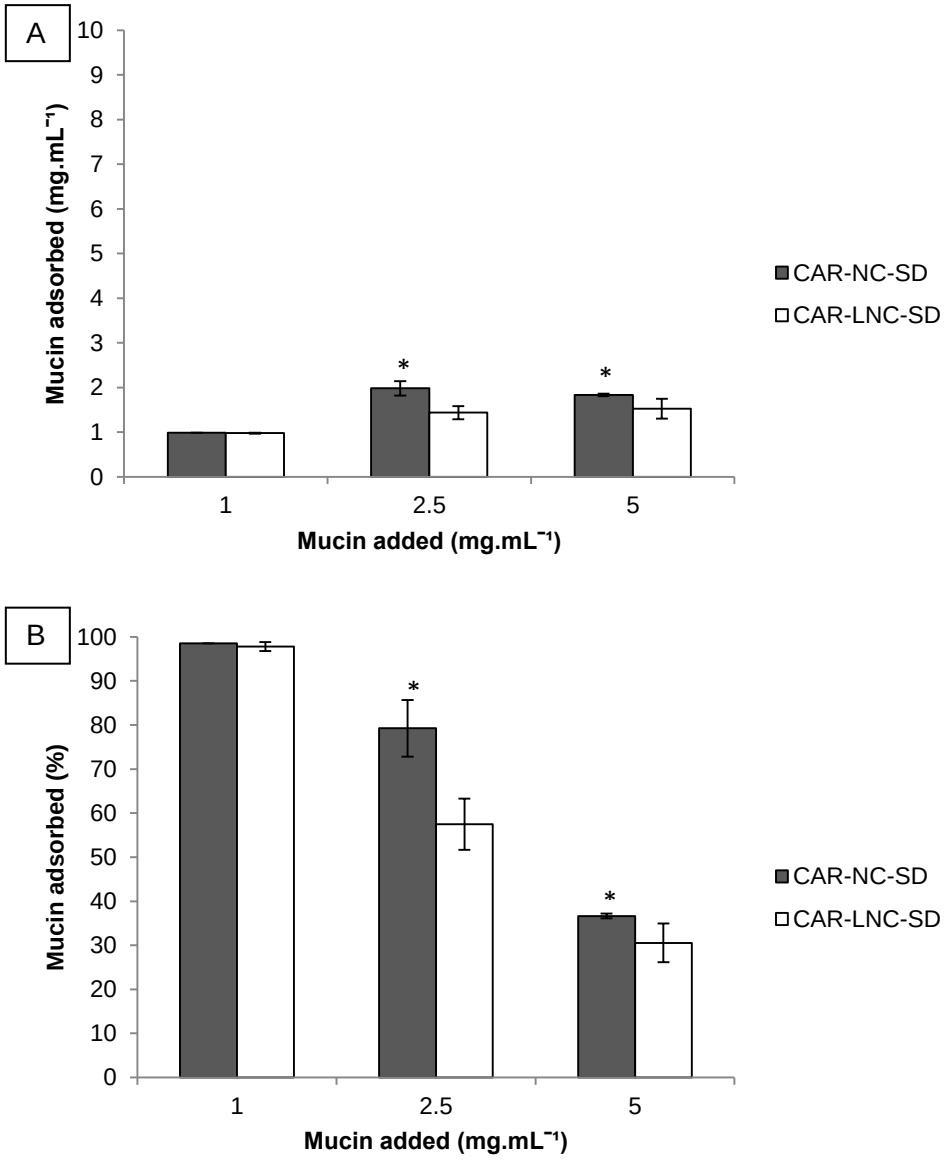
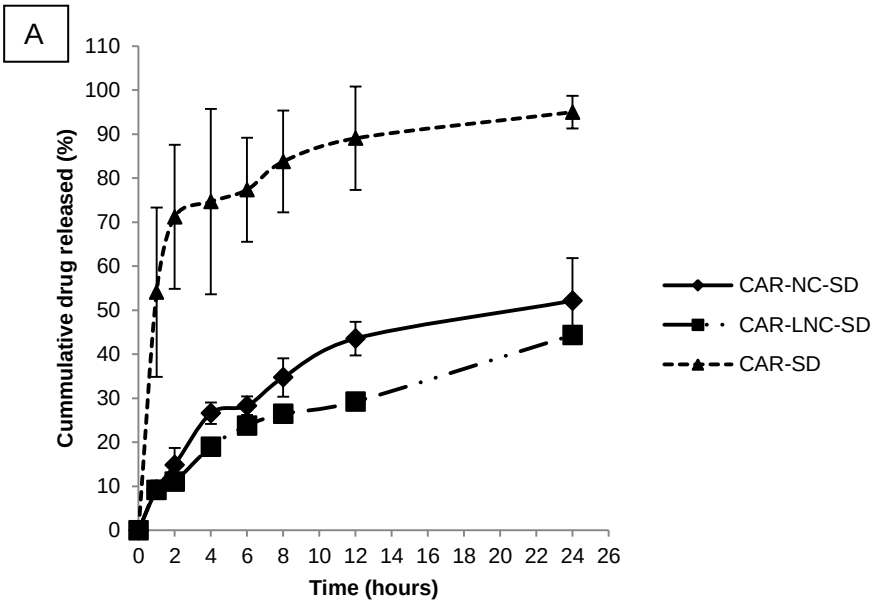


Figure 5. Concentration of mucin adsorbed on surface of nanocapsules after aqueous redispersion of powders containing carvedilol-loaded EUD-nanocapsules (CAR-NC-SD) and of powders containing carvedilol-loaded PCL-nanocapsules (CAR-LNC-SD) in function of total concentration of mucin added (A). Percentage of mucin adsorbed on the surface of nanocapsules in function of total concentration of mucin added (B). One asterisk (*) expresses significant difference and are present in formulations with higher level ($p \leq 0.05$, t test) (n = 3).

8.3.5. In vitro drug release

In vitro drug release profiles after redispersion of powders were evaluated by the dialysis bag method. Nanocapsules have the ability to control the drug release in function of their structure and drug distribution inside of this system.

In a previous study (CHAVES *et al.*, 2017) this ability was proven for nanoencapsulated carvedilol by dialysis bag method for liquid suspensions. In comparison with a hydroalcoholic drug solution, CAR release from nanostructures was slower and CAR-LNC was able to control the drug release in a more efficient way than CAR-NC. This difference was attributed to the structure of nanoparticles. CAR-LNC is a lipid-core nanocapsule since its core is formed by sorbitan monostearate dispersed in grape seed oil forming an organogel (POLETTO *et al.,* 2015). On the other hand CAR-NC is a nanocapsule and its core contains just grape seed oil. Higher core viscosity of lipid-core nanocapsule was associated with decreases in the drug diffusive flux (JAGER *et al.*, 2009). The effect of drying process on this property was evaluated as well as the influence of the aqueous medium of redispersion. Solid formulations need to dissolve in saliva in order to promote the drug dissolution and its absorption by the sublingual route. In this way, powders containing the drug nanoencapsulated (CAR-NC-SD and CAR-LNC-SD) were dispersed in artificial saliva (TEUBL *et al.,* 2013) or highly purified water to have their drug release properties evaluated. Additionaly, dispersions of powders formed by free drug (CAR-SD) were prepared in saliva as well as in drug hydroalcoholic solution to study the influence of the nanoencapsulation and the spray-drying process. Results are shown in Figure 6.



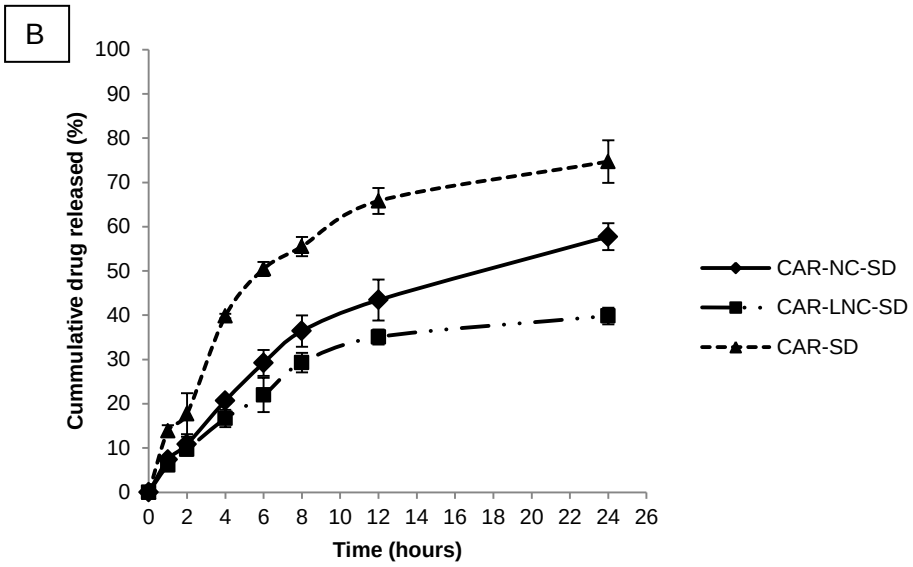


Figure 6. *In vitro* drug release profiles from powders containing carvedilol-loaded EUD-nanocapsules (CAR-NC-SD), powders containing carvedilol-loaded PCL-nanocapsules (CAR-LNC-SD) and powders containing free drug (CAR-SD) after aqueous (A) or salivary (B) redispersion by dialysis bag method (n = 3).

Drug release profiles from powders containing the nanoencapsulated CAR showed the same pattern as the original nanocapsule suspensions reported in our previous study (CHAVES *et al*., 2017). These results confirm the spray-dried process does not alter the drug release profile of the formulation. However, the total drug released from the powder redispersions was a little bit lower than those values obtained for the original suspensions or the drug solution. Whereas the aqueous redispersion powders showed values of 82.67 ± 3.45%, 52.13 ± 9.68% and 44.35 ± 1.35% for total CAR released from CAR-SD, CAR-NC-SD and CAR-LNC-SD, respectively, after 24 h, these values were 90.06 ± 0.02%, 73.04 ± 3.07% and 49.47 ± 2.51% for CAR-S, CAR-NC and CAR-LNC, respectively (CHAVES *et al.*, 2017). This effect probably occurred in function of an increase in the viscosity of the system when the drying adjuvants were solubilized together with the nanocapsules or free drug (CONTRI *et al.*, 2010). Furthermore, the drug release from powders after their dispersion was slower from redispersions containing the nanocapsules than from drug solutions independent of the aqueous medium. In view of these, the results reinforced that the spray-drying process did not modify the ability of nanocapsules to

control the drug release independent of the redispersion medium. The maintenance of this property indicates that the original structure of nanocapsules was not affected after the drying process. This result corroborates with those of the morphological and laser diffraction analyses (previously discussed), which showed the presence of particles in the nanoscale range after aqueous redisersion of powders. Furthermore, this result may evidence that the particle structure is the main parameter for the control of drug release. These results are in agreement with previous report in the literature, showing that spray-dried nanocapsules maintained their drug release control as well as the increase in this ability by powders when compared to suspensions (HOFFMEISTER *et al*., 2012). Furthermore, the redispersion of CAR-NC-SD and CAR-LNC-SD in saliva did not change the drug release profile when compared with their aqueous redispersions. However, differences in CAR release profiles when the drug is not encapsulated were observed. Salivary redispersion controlled more the CAR release than aqueous. This occurred probably due to the presence of ethanol in the aqueous redispersion that facilitated the drug solubility accelerating the drug diffusion.

As the maintenance of drug release control after the drying process was evidenced, the following step was to evaluate the powder performance on the drug permeability across the sublingual mucosa.

7.3.6. In vitro drug permeability

Sublingual mucosa is the most permeable buccal mucosa due to its tenacity and non-keratinized epithelium (NIBHA and PANCHOLI, 2012). However not all drugs are able to cross this barrier and to reach the blood circulation. Furthermore the presence of saliva can influence the drug permeation through mucosa. Ong and co-workers (2009) demonstrated that the presence of saliva decreased the permeation of quinine across the ventral surface of the tongue. The authors related this result to drug dissolution in solute. In our previous study (CHAVES *et al.*, 2017) we demonstrated for the first time the ability of CAR to cross sublingual mucosa and the control of drug permeation by its nanoencapsulation. Figure 7 shows the CAR permeability profiles from powders containing the free and nanoencapsulated drug, after aqueous or salivary redispersion. In the previous section it was observed that the redispersion of the

powder containing the pure drug prepared from a hydroalcoholic solution showed the fastest drug release. This property was reflected in the drug permeation. The drug amount for this powder instantly available for diffusion across sublingual mucosa was the highest and this system provided a less controlled CAR permeation. Independent of the redispersion medium, nanocapsules controlled the drug permeation more than the systems containing

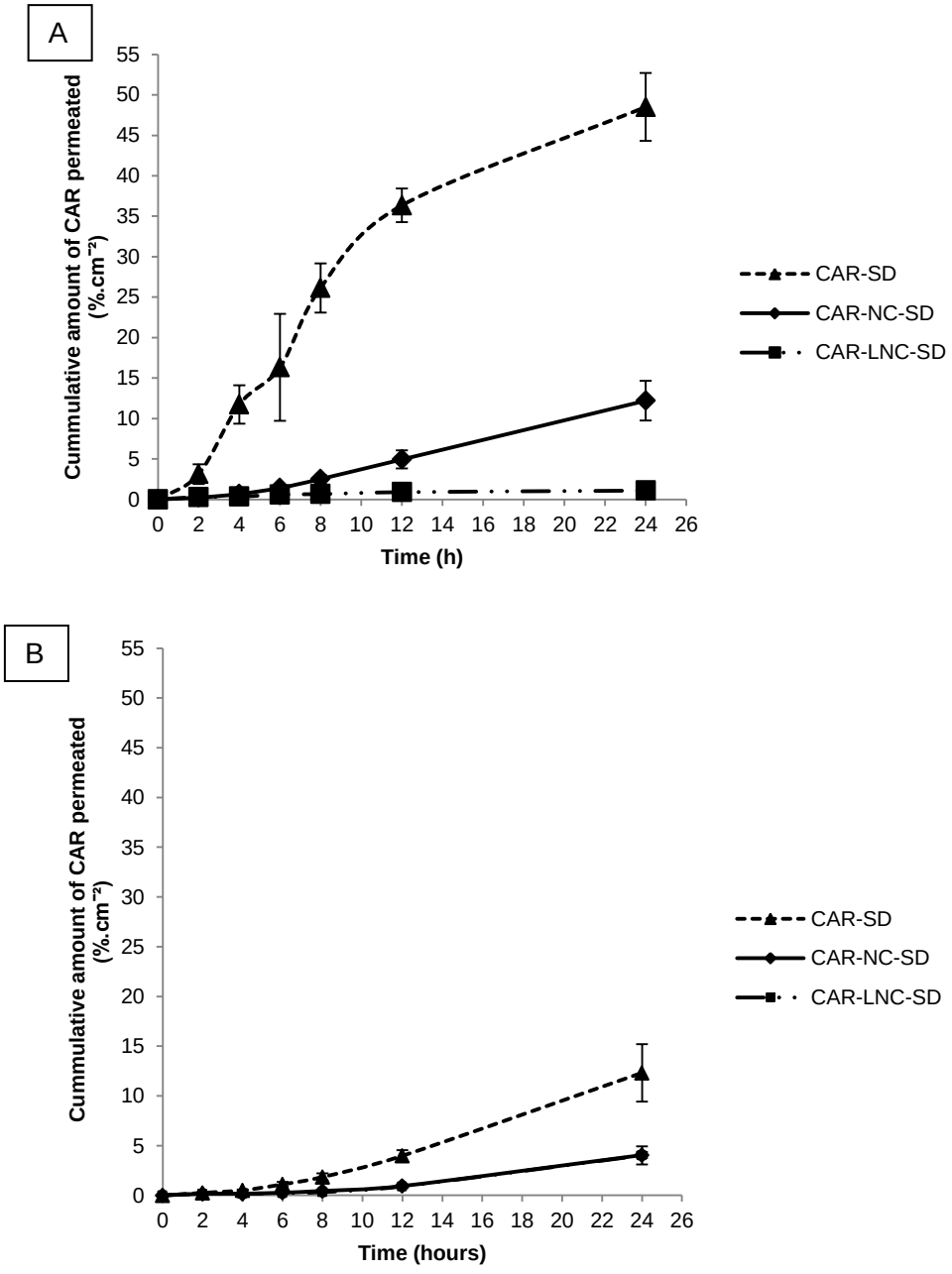


Figure 7. *In vitro* drug permeability across sublingual mucosa powders containing carvedilol-loaded EUD-nanocapsules (CAR-NC-SD), powders containing carvedilol-

loaded PCL-nanocapsules (CAR-LNC-SD) and powders containing free drug (CAR-SD) after aqueous (A) or salivary (B) redispersion (n = 3).

the non-encapsulated drug. This control is in agreement with those results reported in our previous work (CHAVES *et al.*, 2017) for the original liquid nanocapsules suspension. However, the total permeated amount was lower from powders. Analysis of the drug release profiles, as discussed before, showed that redispersed powders controlled more the CAR release than the original suspensions and consequently lower amount of drug was able to permeate across the sublingual mucosa. In another work the support of permeability results by the drug release studies was shown (HOFFMEISTER *et al.,* 2012). The redispersion medium did not influence the drug release rate (section 3.5.) from nanocapsules. However, the drug permeation profiles were altered by the presence of water or saliva. For CAR-NC-SD the total amount of permeated drug was higher from aqueous redispersion than from salivary. On the other hand, CAR-LNC-SD showed a higher cumulative amount of CAR permeated from salivary redispersion than in water. Furthermore drug permeation profiles from powders containing nanocapsules (CAR-NC-SD and CAR-LNC-SD) redispersed in saliva were the same, regardless of nanoparticle composition. In view of this, the CAR permeation was dependent on drug solution in saliva and not influenced by drug release profile, differently of suspensions and powders redispersed in water. These results evidenced the ability of CAR to cross sublingual mucosa regardless of the pharmaceutical dosage form, considering here the evaluated liquid or solid forms. So, the next step was to verify the effect of mucoadhesion on drug permeation.

7.3.7. Washability test

The washability test was used to evaluate the effect of nanoencapsulation on the adhesion of the systems onto the surface of porcine sublingual mucosa in presence of simulated salivary flux. Furthermore, the effect of mucoadhesion on the total amount of permeated CAR was studied. The main limitation of drug administration by sublingual route is the constant salivary flux which can remove part of drug to be absorbed. In this study the adhesion of the spray-dried nanosystems onto sublingual mucosa and to mucin molecules was

already proven. In this experiment the salivary flux was mimicked and the amount of washed drug was determined in outgoing flux (Figure 8). The results demonstrated that powder containing the nanoencapsulated drug promoted the retention of higher drug amounts after redispersion in water than the powder containing the non-encapsulated drug. Powders produced with EUD nanocapsules demonstrated the better performance of adhesion, due to the cationic charge of the particles, retaining a higher quantity of CAR above the sublingual mucosa. Previously adhesion analyses highlighted the better mucoadhesion characteristics of CAR-NC-SD in comparison to CAR-LNC-SD, supporting the results observed here. Due to their anionic charge, PCL nanocapsules show poor adhesiveness properties. Furthermore, these results evidenced that mucoadhesive properties of particles were not changed by dried process allowing the use of dried nanocapsules and corroborating with previous results discussed before. The redispersed powders promoted retention of higher drug amounts on porcine sublingual mucosa in the presence of constant simulated salivary flux than suspensions. The total amount of CAR washed from liquid suspensions or solution after 3 h of experiment was 87.0 ± 0.8%, 80.6 ± 1.7% and 79.0 ± 3.7% for CAR-S, CAR-NC and CAR-LNC, respectively (CHAVES *et al.,* 2017). This result is clearly higher than the value observed for the powders which promoted the wash of 8.7 ± 0.7%, 5.8 ± 0.1% and 7.3 ± 0.1% for CAR-SD, CAR-NC-SD and CAR-LNC-SD, respectively. This difference can be attributed to increase in system viscosity with presence of drying adjuvants dispersed together. Contri and co-workers (2014) analyzed by washability test the skin adhesion of nanocapsules containing capsaicinoids when in suspension or incorpored in hydroxyethyl cellulose gel. The authors showed that the increase of system consistence resulted in higher drug residence time, since hydroxypropyl methylcellulose does not have adhesive properties. Furthermore, results of tensile tester (section 3.4) demonstrated that LacPVP-SD had interaction with sublingual mucosa and may be influenced for better retention of the drug on the mucosa.

At the end of the washability test, the amount of CAR in the receptor medium was assayed in order to evaluate the influence of drug retention in presence of simulated salivary flux on drug permeation. From CAR-SD 0.027 ± 0.001 $\mu g.mL^{-1}$ of CAR permeated, from CAR-NC-SD 0.037 ± 0.005 and from CAR-LNC-SD

0.029 ± 0.004 µg.mL^{-1}. The nanoencapsulation influenced significantly the drug amount permeated when carvedilol was present in nanocapsules of EUD. The drug concentration in receptor medium was higher for CAR-NC-SD than CAR-SD ($p \leq 0.05$). CAR-NC-SD retained higher amount of drug and consequently more drug was available to permeate. The influence of system formed with PCL, on the other hand, was similar to drug in solution ($p > 0.05$). This result may be attributed with adhesive effect showed by powders formed just with drying adjuvants (LacPVP-SD), as demonstrated previous by tensile tester. The mucoadhesive of LacPVP-SD was similar to showed by CAR-LNC-SD. The results together evidenced that the use of mucoadhesive systems is essential to overcome the washing effect of salivary flux and increase drug permeation. Furthermore, they highlighted that nanoencapsulation may be a promote mucoadhesive system manly when the particles have cationic characteristics, corroborating with the results of mucoadhesion testes observed and discussed above.

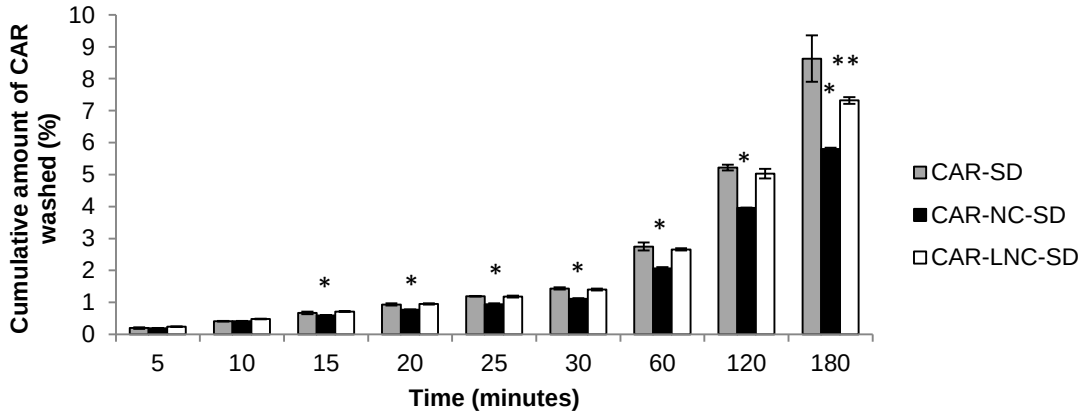


Figure 8. Washability profiles of carvedilol from powders containing carvedilol-loaded EUD-nanocapsules (CAR-NC-SD), powders containing carvedilol-loaded PCL-nanocapsules (CAR-LNC-SD) and powders containing free drug (CAR-SD) after aqueous redispersion (n = 3). One asterisk (*) represent significant difference and are present in formulation with less level ($p \leq 0.05$, ANOVA). Two asterisks (**) represent statistical difference between CAR-SD and CAR-LNC-SD and are present in formulation with less level ($p \leq 0.05$, t test).

7.4. CONCLUSION

Spray-drying powders containing carvedilol-loaded polymeric nanocapsules produced with the mixture lactose/PVP showed suitable properties and the recovery of the original nanometric diameter after their aqueous redispersion. Furthermore, the spray-dried powders showed important mucoadhesive properties and the adhesiveness characteristic of nanocapsules were not altered by the drying process. EUD and PCL nanocapsules controlled by same way the drug permeation across sublingual mucosa after redispersion of their powders in artificial saliva. Nanoencapsulation improved drug adherence on porcine sublingual mucosa and promoted an increasing in amount of drug permeation in presence of simulated salivary flux. Powders containing cationic nanocapsules showed a better performance than anionic nanocapsules. The present study developed a promising powder material containing a nanotechnological system for sublingual administration of carvedilol. This material will be used for the development of sublingual tablets in futures studies.

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APRESENTAÇÃO DO CAPÍTULO

Comprovada a manutenção das propriedades adesivas das nanocápsulas após o processo de secagem por aspersão e a potencialidade das nanocápsulas catiônicas como sistemas mucoadesivos, o último capítulo deste trabalho compreendeu o desenvolvimento de comprimidos sublinguais, a partir dos pós produzidos contendo carvedilol encapsulado em nanocápsulas de Eudragit RS 100. Os comprimidos foram caracterizados e a presença das nanocápsulas na forma farmacêutica final foi avaliada, além do perfil de desintegração dos comprimidos em saliva artificial. Essa é a primeira vez que comprimidos sublinguais contendo nanopartículas são desenvolvidos utilizando materiais pulverulentos obtidos pela técnica de aspersão. Este capítulo deu origem a um manuscrito na forma de "Short Communication", que se encontra em fase final de redação para posterior submissão a periódico científico.

Sublingual tablets containing spray-dried carvedilol-loaded nanocapsules

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Abstract

The aim of this study was to develop tablets containing spray-dried nanocapsules for sublingual administration of carvedilol. Spray-dried carvedilol-loaded nanocapsules were compressed into tablets. The force and work necessary in the tableting process were determined. Tablets were characterized comprising their dimensions and weight, morphology and drug content. The drug release profile from the tablets was evaluated using dialysis bag method. Furthermore, the time for tablet desintegration in artificial saliva was evaluated and the presence of redispersed nanocapsules in artificial saliva was analysed by dynamic light scattering. Tablets of 6 mm x 2.7 ± 0.2 mm, weighting 44 ± 4 mg and containing 0.164 ± 0.017 mg of carvedilol were produced using a force of 4.7 ± 1.6 Kg and a work of 3.2 ± 1.0 Kg.sec. Nanoparticles were observed by electron microscopy on the surface and inner compartment of tablets, which were released after tablets disintegration in artificial saliva. The drug was released from tablets containing nanocapsules in a controlled way confirming the maintenance of original nanostructures in the final dosage form. Tablets disintegration in artificial saliva took 24 min. To the best of our knowledge, this is the first report about tablets produced from spray-dried nanocapsules. These tablets are proposed an innovative nanomedicines for sublingual administration of carvedilol.

Keywords: Eudragit® RS100, carvedilol-loaded nanocapsules, direct compression, spray-dried powders, sublingual tablets.

8.1. INTRODUCTION

Carvedilol is a drug used in treatment of heart failure, hypertension, and coronary artery diseases and is commercially available for oral administration by tablets. However, by oral route its bioavailability is extremely limited (25-35 %) due to hepatic metabolism (MORGAN, 1994). Therefore, recently carvedilol-loaded cationic nanocapsules were proposed as drug delivery systems for sublingual administration (CHAVES *et al*., 2017a). This formulation has mucoadhesive properties, which improves drug adherence on porcine sublingual mucosa, increasing its permeation in the presence of simulated salivary flux compared to the non-encapsulated drug in solution.

However, tablets are the main pharmaceutical dosage forms used for sublingual drug administration. In this scenario, this carvedilol-loaded nanocapsules formulation should be converted in tablets using a technological strategy. This conversion is poor described in the literature. Friedrich and co-workers (2010) used dexamethasone-loaded nanocapsules as the binder liquid in the production of granules, which were used to produce the tablets. More recently, Beck and co-workers (2017) produced 3D printed tablets containing nanocapsules as a suitable plataform to increase the drug loading in tablets and to customize the drug release rate. However, to the best of our knowledge there is not any report about the use of spray-dried drug-loaded nanocapsules in the production of tablets, as final dosage forms, although spray-drying has been widely applied to the production of dried nanocapsules (BECK *et al.*, 2012).

Taking into account the needing of a technological strategy to convert the carvedilol-loaded nanocapsules into an intermediate pharmaceutical form to produce sublingual tablets, a previous study reported the production of the spray-dried carvedilol-loaded nanocapsules, with good redispersible behavior and mucoadhesive properties of the recovered nanocapsules (CHAVES *et al*., 2017b).

In view of the exposed above, this study aimed to develop novel sublingual tablets by direct compression using spray-dried carvedilol-loaded nanocapsules and to study the effect of compressing process on nanocapsules structure and properties. For this, the tablets were analyzed in relation to physical properties, morphology and drug content. Moreover, the maintenance of nanocapsules in final pharmaceutical dosage form and their release were analysed. These

tablets would represent an innovative nanomedicine for sublingual administration of carvedilol. The schematic representation of the expected behavior of the tablets in the sublingual mucosa is showed in Figure 1. Tablets containing carvedilol-loaded nanocapsules dispersed in water-soluble carriers (lactose and polyvinylpyrrolidone), in contact with saliva, disintegrates in their subunits. In the following step, the water-soluble carriers dissolve and carvedilol-loaded nanocapsules are released. These particles, due to their mucoadhesive properties, adhere to the mucosa and retain the drug in the sublingual cavity, which may be absorbed to the blood circulation.

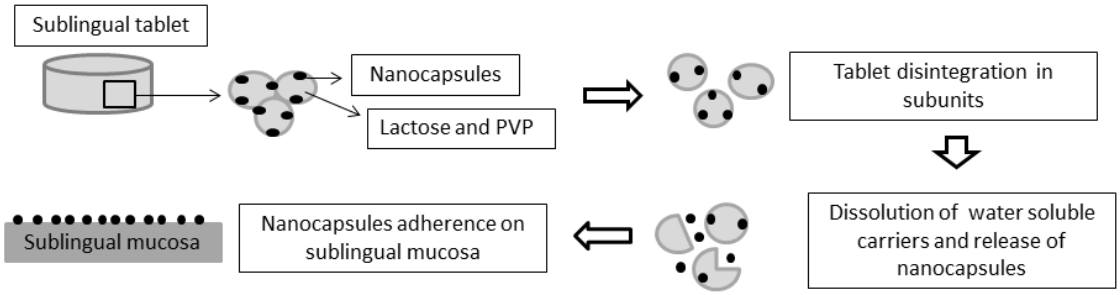

Figure 1. Schematic representation of the tablet behavior in the sublingual cavity.

8.2. METHODS

8.2.1. Preparation of carvedilol-loaded nanocapsule suspension

Carvedilol-loaded nanocapsule suspensions (CAR-NC) formed with Eudragit® RS100 and 0.5mg.ml^{-1} of carvedilol were prepared by interfacial deposition of preformed polymer method as described in Chaves, 2017a. Their mean diameter was measured by dynamic light scattering (DLS) (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK), wheres zeta potential was measured by electrophoretic mobility (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK). Drug content was determined by liquid chromatography (LC) (CHAVES *et al.*, 2017a).

8.2.2. Preparation of spray-drying powders containing carvedilol-loaded nanocapsules

Powders containing carvedilol-loaded nanocapsules (CAR-NC-SD) were produced by spray-drying a nanocapsule suspension (Mini Spray-Dryer B-290, Büchi, Flawil, Switzerland), using a mixture of lactose and polyvinylpyrrolidone (1:1 w/w, 10 %) as drying adjuvants (CHAVES *et al*., 2017b). Additionaly, powders containing non-encapsulated carvedilol were prepared by the spray-drying of a hydroalcoholic solution (ethanol:water 50:50 v/v, 0.50 $mg.mL^{-1}$) and named as CAR-HS-SD. The drug content (mg/g) in the powders was assayed by LC (CHAVES *et al.*, 2017a).

8.2.3. Preparation and characterization of sublingual tablets

Tablets containing nanoencapsulated carvedilol (CAR-NC-T) or its non-encapsulated form (CAR-HS-T) were prepared by direct compression of the spray-dried powders CAR-NC-SD and CAR-HS-SD, respectively. The powder compaction rig of a texture analyzer equipment (TA.XTplus Texture Analyzer; Stable Microsystem, Godalming, UK) was used as a model of tabletting. Around 50 mg of powder were deposited in the compartment of the rig. The probe was placed around 3 mm of the rig and the powder was compacted with the following parameters: distance 6 mm, test speed and post-test speed of $1mm.seg^{-1}$. The force (Kg) and work ($Kg.sec^{-1}$) necessary to produce the tablets (n = 10) was determined by the equipment software (Exponent; Stable Microsystem, Godalming, UK).

After preparing, tablets were weighed (n = 10) separately using a calibrated analytical balance and the mean weight was calculated. Diameter and thickness were evaluated using a ruler. Drug content (mg/tablet) was assayed by LC. Morphology of the surface and cross-section of the tablets were analysed by scanning electron microscopy (SEM; Jeol Scanning Microscope, JSM-6060, Tokyo, Japan) operating at 10 kV, at the Microscopy Center of the University (Centro de Microscopia e Microanálises - UFRGS, Brazil).

8.2.4. In vitro drug release from sublingual tablets

The *in vitro* carvedilol release (n = 3) from the tablets (CAR-NC-T and CAR-HS-T) was evaluated by the dialysis bag method. A tablet and 1 ml of artificial saliva (TEUBL *et al.,* 2013) were placed into the dialysis tubing cellulose membrane (25 mm flat width, Sigma-Aldrich, São Paulo, Brazil). The bags were suspended

in 30 mL of release medium (sodium phosphate buffer pH 6.8 0.2M) and kept in a bath at 37 °C under agitation of 70 ± 10 rpm. Sink conditions were maintained during all the experiment. A sample of the external medium (0.2 mL) was withdrawn at predetermined time intervals (1, 2, 4, 6, 8, 12 and 24 h) and analyzed by LC (CHAVES *et al.*, 2017a).

8.2.5. In vitro nanocapsule release from sublingual tablets

Tablets (CAR-NC-T and CAR-HS-T) were dissolved in 1 mL of artificial saliva and, after their total disintegration, samples of the medium were collected and analyzed by DLS without previous filtration (ZetaSizer Nano ZS, Malvern Instruments Ltd., UK).

8.2.6. Tablet disintegration in artificial saliva

Tablets were suspended in 1 mL of artificial saliva and the time for total tablet disintegration was monitored. Sample of the medium (50 µL) was withdrawn at predetermined time intervals until complete tablet disintegration and analyzed in relation to drug content by LC. The samples containing nanocapsules were diluted in mobile phase (phosphoric acid solution pH 3.0/acetonitrile, 50:50, v/v) and sonificated (10 min) previous analyses for drug extraction from the particles. Samples with drug free were diluted in mobile phase. Fresh artificial saliva (50 µL) was replaced after each sample withdrawn. The method had specificity, good linearity (r=0.999, n=3) in the range of 1 - 40 $\mu g.mL^{-1}$, and suitable intra (SD=2.9%) and interday (SD=3.1%) precision.

8.2.7. Statistical analysis

Statistical analyses were carried out by Student's *t* test ($p \leq 0.05$) using the SPSS statistical software, version 17.0® (IBM, Nova Iorque, EUA). Data are presented as the mean ± standard deviation (SD).

8.3. RESULTS AND DISCUSSION

8.3.1. Preparation of carvedilol-loaded nanocapsule suspension

The mean diameter of carvedilol-loaded nanocapsules was 138 ± 5 nm. Surface charge was positive (+8 ± 3 mV) and the drug content was closely to theoretical

(0.48 ± 0.03 $mg.mL^{-1}$). The results corroborated with those previously reported (CHAVES *et al.*, 2017a).

8.3.2. Preparation of spray-dried powders

The extensively characterization of the powders produced with lactose and PVP as drying adjuvants was described previously by Chaves and co-workers (2017b). Here, three new batches were produced under the same condition and the drug content of the powders was assayed to make sure that no drug losses occurred during the process. The drug content was close to the expected values (3.62 ± 0.09 mg/g and 4.68 ± 0.42 mg/g for CAR-NC-SD and CAR-HS-SD, respectively). CAR-NC-SD had a lower drug content due to their lower drug:raw materials ratio (m/m). Therefore, the powders were able to be used in the production of the tablets.

8.3.3. Preparation and characterization of sublingual tablets

The max force necessary to produce tablets with 6 mm of diameter was 4.7 ± 1.6 Kg and 6.3 ± 0.4 Kg for CAR-NC-T and CAR-HS-T, respectively. Consequently, CAR-NC-T was formed with a work of 3.2 ± 1.0 Kg.sec while CAR-HS-T needed a work of 6.1 ± 1.0 Kg.sec. Detailed properties of tablets are showed in Table 1. Mean weight and diameter were not affect the by presence of the nanocapsules. However, powders produced with nanoencapsulated CAR showed a better compaction behaviour than powders produced with its hydroalcoholic solution affording to tablets with lower thickness. This can be explained by the lower force and work necessary to obtain the tablets containing nanocapsules. Moreover, the drug content was lower for tablets prepared with the spray-drying powders containing nanocapsules due to the lower drug:raw material ratio (m/m) for this formulation, as earlier commented.

Figure 2 shows the SEM images of the tablets. Particles with nanometric size were observed on surface and in the cross-section (inner compartment) of tablets produced with powders containing nanocapsules. On the other hand, tablets produced with powders containing non-encapsulated drug showed their surface and inner compartment clear of spherical particles. These SEM images indicate that the compression process did not interfere in the nanocapsules structure confirming their integrity in tablets developed. However, in order to

confirm the integrity of the nanocapsules, in vitro drug release studies were carried out.

Table 1. Properties of tablets containing the carvedilol nanoencapsuled (CAR-NC-T) and carvedilol free (CAR-HS-T) [mean ± SD].

	Mean weight (mg)	**Mean diameter (mm)**	**Mean thickness (mm)**	**Drug content (mg/tablet)**
CAR-NC-T	44 ± 4[a]	6 ± 0[a]	2.7 ± 0.2[a]	0.164 ± 0.017[a]
CAR-HS-T	48 ± 2[a]	6 ± 0[a]	3.2 ± 0.2[b]	0.218 ± 0.009[b]

SD = standard deviation. Means, in column, with different letter are significant statistically different ($p < 0.05$).

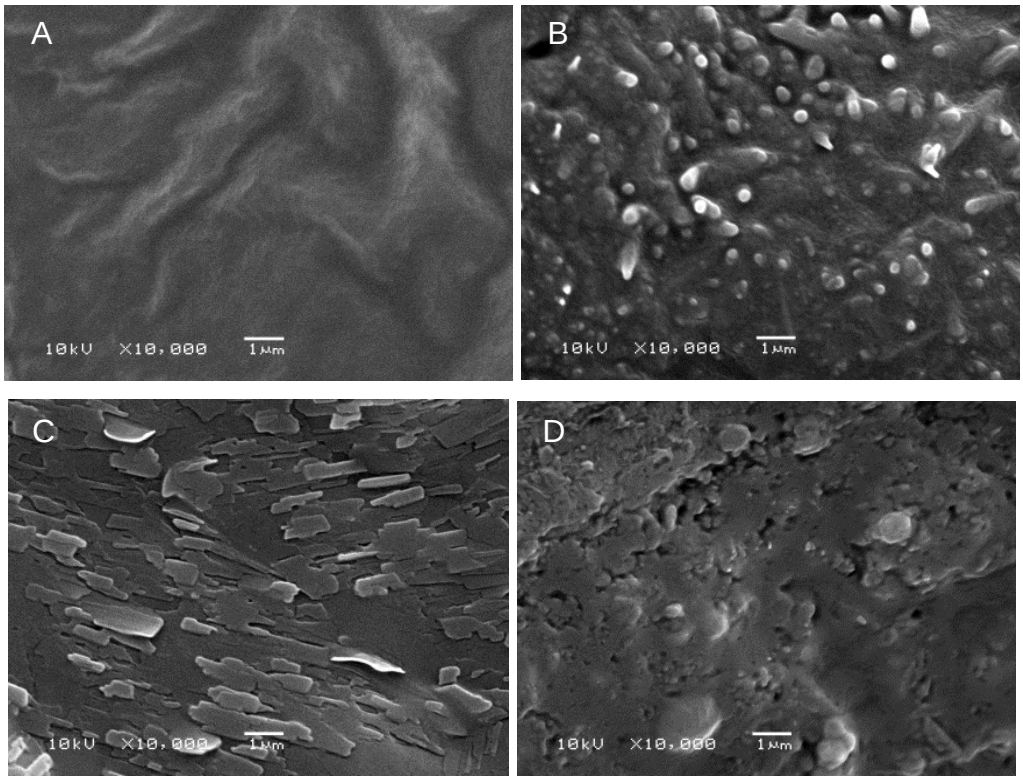

Figure 2. Scanning electron microscopy (SEM) images of cross-section (A) and surface (C) of tablets containing non-encapsulated (CAR-HS-T); cross-section (B) and surface (D) of tablets containing carvedilol-loaded nanocapsules (CAR-NC-T).

8.3.4. In vitro drug release from sublingual tablets

The tablets were dispersed into 1 mL of simulated saliva to mimetic the biological condition (PATEL et al., 2011). Drug release data are depicted in Figure 3. Nanoencapsulated carvedilol showed a slower release from tablets

compared to those tablets prepared with the non-encapsulated drug. These data corroborate with those previously reported for the drug release from carvedilol-loaded nanocapsule suspension and the diffusion of the non-encapsulated carvedilol solution (CHAVES et al., 2017a) as well as from the respective spray-dried powders (CHAVES et al., 2017b). The ability of nanocapsules to control the release of drugs has been reported and explained by their vesicular structure and supramolecular organization (JÄGER et al., 2009). In this scenario, the release data reported for the tablets confirm the conservation of the original structure of nanocapsules after the compression process, in agreement with the morphological analysis by SEM. Hence, the next step was carried out to evaluate the release of the nanocapsules from the tablets after their disintegration by DLS analyses.

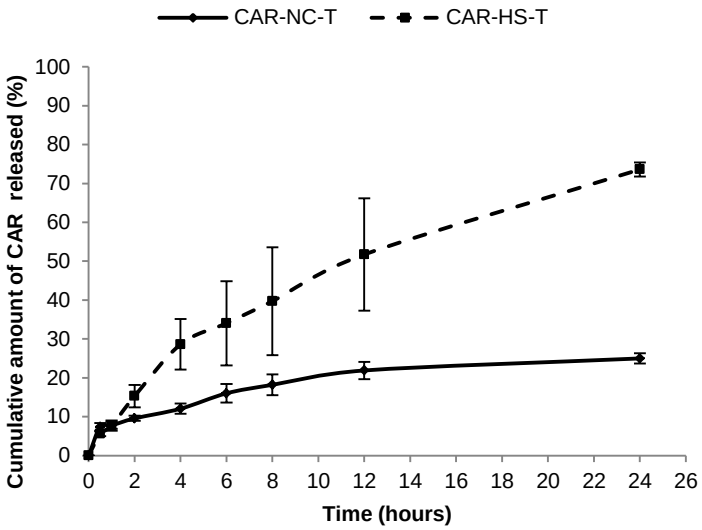

Figure 3. Drug release profiles from tablets containing non-encapsulated carvedilol (CAR-HS-T) and tablets containing carvedilol-loaded nanocapsules (CAR-NC-T).

8.3.5. In vitro nanocapsules release from sublingual tablets

Nanocapsules are the principal constituent of this novel sublingual tablet and their release is of extreme importance to allow their adherence to the sublingual cavity to prolong the drug residence time. Size distribution profiles by DLS after tablet disintegration in the artificial saliva are showed in Figure 4. Tablets produced with spray-dried nanocapsules suspensions exhibited a unimodal peak similar to the original suspension. On the other hand, tablets containing

non-encapsulated carvedilol showed a heterogeneous particle size distribution with modes lower than 180 nm. These particle size populations may be explained by the presence of undissolved drying adjuvants. These data support the hypothesis about the production of sublingual tablets releasing mucoadhesive nanoparticles and prolonging their drug release and residence time in the sublingual cavity.

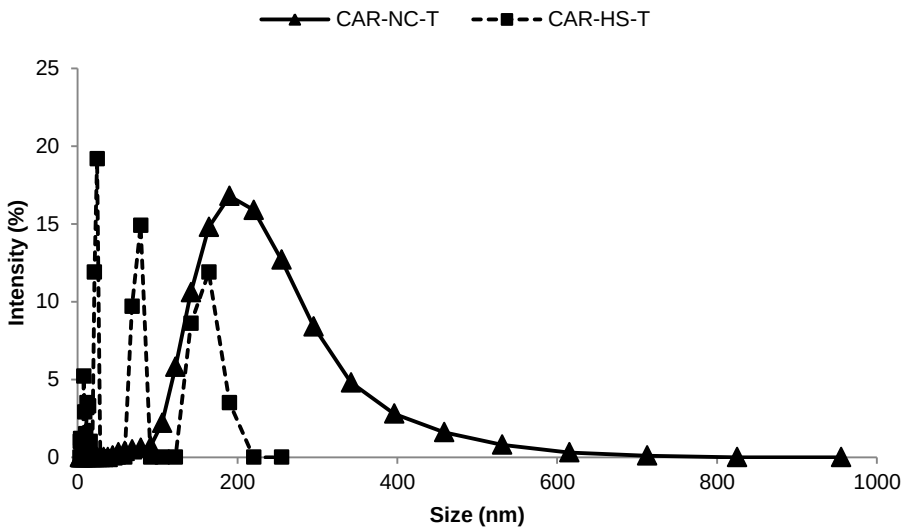

Figure 4. Size distribution of particles after tablets dissolution in artificial saliva.

8.3.6. Tablet disintegration in artificial saliva

Disintegration of the tablets was evaluated using 1 mL of artificial saliva, which corresponds to the amount available in the sublingual cavity, under biological conditions (PATEL et al., 2011). The tableting of spray-dried nanocapsules let to the production of tablets with a disintegration time of 24 min in artificial saliva affording 70% of drug released (Figure 5). Usually, faster disintegration is looked for sublingual administration, as the presence of a tablet in the oral cavity may interfere in speaking, eating and drinking (NARANG and SHARMA, 2011). However, disintegrating agents were not added to the formulations at this stage in order to avoid any additional interference in the studies. Therefore, future studies will be designed to accelerate the disintegration time of the tablets by the addition of disintegrating agents.

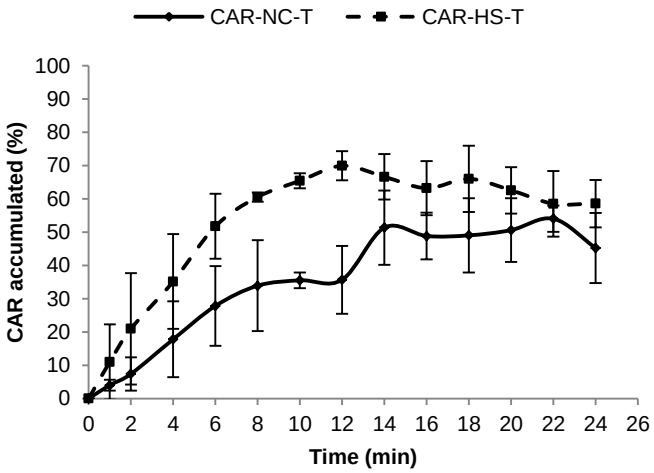

Figure 5. Time for tablets dissolution and drug concentration in artificial saliva.

8.4. CONCLUSION

This study developed tablets containing carvedilol-loaded nanocapsules using spray-dried powders as technological strategy. Furthermore, the original properties of nanocapsules were not altered by the compressing process. Therefore, the tablets produced represent a novel solid plataform containing a mucoadhesive nanotechnological system for sublingual administration of carvedilol.

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9. DISCUSSÃO GERAL

A degração pré-sistêmica, devido a processos de metabolização, é uma importante limitação sofrida por diversos fármacos que são administrados pela via oral. Suas biodisponibilidades podem ser melhoradas utilizando como via de administração a mucosa sublingual. Essa região possui um rico suprimento de vasos sanguíneos e os fármacos são absorvidos diretamente à corrente sanguínea sem degradações ou metabolizações prévias (GOSWAMI *et al.,* 2008). No entanto, o desenvolvimento de sistemas de entrega com características mucoadesivas é muito importante quando se utiliza a via sublingual para administração de fármacos. A boca está exposta a um constante fluxo de saliva, que pode remover parte do fármaco a ser absorvido (AL-GHANANEEM *et al.,* 2007; PATEL *et al.,* 2011).

Nesse sentido, este trabalho estudou o uso das nanocápsulas no desenvolvimento de sistemas carreadores de fármacos com características mucoadesivas. As nanocápsulas são estruturas formadas por uma parede polimérica envolvendo um núcleo oleoso e os polímeros são os materiais mais utilizados no desenvolvimento de sistemas com propriedades adesivas (ANDREWS *et al.*, 2009). Além disso, a estruturação de materiais em partículas nanométricas permite um aumento da área superficial, que pode resultar na intensificação das propriedades desses componentes (SOSNIK *et al.*, 2014).

Sendo assim, a primeira etapa deste estudo envolveu a análise das propriedades adesivas de três polímeros, Eudragit® RS100, Eudragit® S100 e PCL, quando estruturados em nanocápsulas. Os resultados demonstraram que a nanoestruturação promoveu um aumento na força de atração entre os polímeros e a superfície adesiva, para os três materiais estudados. Além disso, o veículo em que as nanocápsulas estavam veiculadas, suspensão, hidrogel ou pó, influenciou na performance adesiva dos sistemas. Hidrogéis, devido a sua maior viscosidade, estão sendo estudados com uma alternativa para melhorar a adesividade de formulações (CONTRI et al., 2014) e a viabilidade dessa estratégia foi observada nesse estudo. Hidrogéis de hidroxietilcelulose contendo nanocápsulas foram mais adesivos que seus respectivos pós e suspensões. Por outro lado, as suspensões de nanocápsulas e os pós desenvolvidos não apresentaram diferenças entre si em relação a capacidade mucoadesiva, demonstrando que o processo de secagem das suspensões pela

técnica de aspersão não interferiu na propriedade adesiva das nanopartículas, podendo essa técnica ser utilizada na secagem de nanocápsulas mucoadesivas sem prejuizo das suas propriedades adesivas.

A mucosa sublingual possui características aniônicas devido a presença de moléculas de mucina em sua composição (BANSIL *et al.,* 2006; KHUTORYANSKIY, 2011). Desse modo, a carga de superfície das nanocápsulas pode interferir na interação dos sistemas com as superfícies adesivas. Nanocápsulas formadas por Eudragit® RS100, que apresentaram carga de superfície positiva, devido as características catiônicas desse polímero (PIGNATELLO et al., 2002), foram capazes de interagir de uma maneira mais eficiente com as mucosas vaginal e bucal e com os discos de mucina, em comparação com as nanocápsulas aniônicas formadas por Eudragit® S100 e PCL. Essas duas últimas, por sua vez, interagiram com as superfícies adesivas com a mesma intensidade, uma vez que possuem cargas de superfície semelhantes.

Nessa parte do estudo foi também avaliado o comportamento das superfícies adesivas em refletir a real adesividade das formulações. Para isso, foram utilizados como superfície mucoadesiva, mucosas sublingual e vaginal extraídas de porcos, além de discos de mucina. A mucina é uma glicoproteína presente no muco, que confere as características adesivas à essa membrana (BANSIL *et al.,* 2006; KHUTORYANSKIY, 2011). Ela está comercialmente disponível e é muito utilizada como mebrana adesiva nos estudos *in vitro* de mucoadesão, uma vez que, é mais fácil de ser obtida que mucosa animal ou humana. Os resultados demonstraram que a intensidade das interações foi significativamente superior para os discos de mucina que para as mucosas de porco. No entanto, as diferenças observadas entre as nanocápsulas catiônicas e aniônicas, e entre os veículos em que estas estão veiculadas (suspensão, hidrogel ou pó), foram as mesmas para mucosas suínas ou discos de mucina. Ou seja, discos de mucina podem ser utilizados para comparação de formulações e nos estudos de pré-formulações, enquanto, mucosas de porco são mais adequadas para fazer correlações com situações *in vivo*.

Comprovada a potencialidade das nanocápsulas como sistemas mucoadesivos, essas partículas foram então estudadas como sistemas carreadores de carvedilol para administração sublingual. Carvedilol é uma

fármaco com multipla ação cardiovascular que, quando administração pela via oral, sofre um extenso metabolismo de primeira passagem no fígado, o qual torna sua biodisponibilidade extremamente limitada (25-35%) (DANDAN *et al.,* 2012). E, por causa disso, este fármaco é um importante candidato a ser administrado pela via sublingual. Além disso, suas características lipofílicas (ABREU *et al.*, 2000) permitem a sua encapsulação nessse tipo de sistema.

O carvedilol foi encapsulado em nanocápsulas formadas por Eudragit® RS100 e PCL, compreendendo sistemas de características catiônicas e aniônicas, respectivamente. A carga de superfície influenciou na capacidade das nanocápsulas de interagir com moléculas de mucina, confirmando o melhor desempenho adesivo das partículas catiônicas. No entanto, ambas as nanopartículas foram capazes de reter uma quantidade maior de fármaco na superfície da mucosa sublingual que uma solução do fármaco, na presença de um fluxo salivar mimetizado. Tal efeito, proporcionou a permeação, através da mucosa sublingual, de uma quantidade maior de fármaco a partir das nanoestruturas, já que estas promoveram uma disponibilidade maior de fármaco para ser absorvido, que a solução.

Adicionalmente, o carvedilol nanoencapsulado foi capaz de permear tanto através de mucosa sublingual de porco, quanto através de uma monocama de células de epitélio oral, viabilizando o seu uso através da via sublingual de administração. Essa permeação não provocou nenhum efeito deletério a integridade da membrana celular, uma vez que, as formulações não foram citotóxicas às células. Nesta etapa foi verificado, também, que a monocama formada por células SCC4, extraídas de carcinoma de língua, pode ser utilizada como um modelo alternativo de mucosa sublingual em ensaios de transporte de fármacos. Os resultados demonstraram que a concentração de carvedilol permeado através da monocamada celular teve uma correlação linear com a concentração permeada através da mucosa sublingual de porco.

Os comprimidos são a principal forma farmacêutica utilizada para administração de fármacos pela via sublingual e podem ser produzidos empregando a técnica de compressão de pós (RAWAS-QALAJI *et al.,* 2006). Sendo assim, a próxima etapa deste estudo foi desenvolver e caracterizar materiais pulverulentos a partir das suspensões de nanocápsulas, pela técnica de secagem por aspersão, uma vez que a viabilidade da secagem de

nanocápsulas mucoadesivas por essa técnica já havia sido investigada. Os pós produzidos com a mistura lactose/polivinipirrolidona como adjuvantes de secagem, apresentaram características adequadas e a recuperação do tamanho nanométrico das partículas originais, após redispersão aquosa dos pós, foi observada. Essas partículas não apresentaram prejuízos em relação a sua estrutura macromolecular e propriedades iniciais, após processo de secagem.

Os pós contendo apenas adjuvantes de secagem apresentaram interação com a mucosa, que potencializou a capacidade mucoadesiva dos pós formados por nanocápsulas de Eudragit® RS100. No entanto, para os pós contendo nanocápsulas, o trabalho necessário para romper essa ligação foi equivalente ao pó formado somente por adjuvantes. Além disso, nanocápsulas secas redispersas em água continuaram interagindo com as moléculas de mucina, sendo essa interação mais intensa para as nanocápsulas catiônicas, que para as aniônicas, corroborando com os resultados anteriores. Essa partículas foram capazes, ainda, de promover a retenção do fármaco sobre a mucosa sublingual, na presença de fluxo salivar mimetizado, e tal efeito promoveu a permeação de maiores quantidades de fármaco para o meio receptor que a solução do fármaco, como observado para as suspensões. Sendo assim, os resultados evidenciaram, novamente, que o processo de secagem não altera as favoráveis propriedades das nanocápsulas poliméricas, como carreadores mucoadesivos para administração de carvedilol pela via sublingual, sendo as nanopartículas catiônicas as mais promissoras. Ou seja, nanocápsulas secas, obtidas pela técnica de aspersão, podem ser utilizadas no desenvolvimento de comprimidos para administração sublingual de carvedilol, como um sistema nanotecnógico mucoadesivo.

Portanto, na etapa final deste estudo, comprimidos sublinguais contendo carvedilol nanoencapsulado foram produzidos a partir dos pós desenvolvidos. Partículas de tamanho nanométrico foram observadas no interior e na superfície dos comprimidos e no meio salivar após desintegração dos comprimidos. O processo de compressão não alterou a estrutura molecular das nanocápsulas, uma vez que, o perfil de controle de liberação do fármaco, semelhante as suspensões originais, foi mantido. A produção de comprimidos, utilizando pós formados por componentes hidrossolúveis (lactose e

polivinipirrolidona) deu origem a uma forma farmacêutica que se desintegrou totalmente em saliva artificial. No entanto, o tempo de desintegração foi um pouco superior ao que normalmente é utilizado para administração pela via sublingual. Sendo assim, estudos futuros envolvendo a adição de agentes que facilitem a desintegração do comprimido podem ser realizados, afim de garantir um comprimido mais adequado para administração por essa via. Apesar disso, esses comprimidos representam plataformas sólidas inovadores, contendo um sistema nanotecnológico com propriedades mucoadesivas para a administração sublingual de carvedilol. Esse é o primeiro estudo que descreve o desenvolvimento de comprimidos a partir de nanocápsulas secas por aspersão.

Finalmente, é importante salientar, que a seção de revisão do tema dessa tese traz uma extensa revisão dos estudos que abordaram a aplicação de nanopartículas poliméricas no tratamento e prevenção de doenças orais, publicados nos últimos oito anos. Essa revisão é de grande importância para demonstrar as potencialidades das nanopartículas poliméricas como sistemas carreadores de fármacos ou como sistemas nanoestruturados de polímeros com atividade biológica, como a quitosana por exemplo, para aplicação na cavidade oral. As abordagens estudadas demonstram a versatilidade desses sistemas e os resultados promissores que a aplicação destes sistemas na área odontológica tem demonstrado.

10. CONCLUSÕES

Sistemas poliméricos, com características mucoadesivas promissoras, podem ser obtidos a partir da estruturação de polímeros em nanocápsulas. A melhor performance mucoadesiva, quando nanoestruturado, foi observada para os polímeros Eudragit® RS100, Eudragit® S100 e PCL. Além disso, a propriedade mucoadesiva de nanocápsulas pode ser potencializada a partir da sua veiculacão em hidrogéis ou, até mesmo, não alterada quando secas por processo de aspersão;

Discos de mucina podem ser utilizados, como superficies mucoadesivas alternativas, em estudos preliminares, enquanto que, mucosas suínas são ideias para mimetizar condições *in vivo* de adesão;

Nanocápsulas catiônicas, formadas por Eudragit® RS100, interagem melhor com superfícies adesivas, comparadas a partículas aniônicas, formadas por Eudragit® S100 ou PCL;

O carvedilol foi capaz de permear tanto através de mucosa sublingual de porco, quanto através de membrana celular de células de epitélio oral (SCC4), sendo que os perfis de permeação foram influenciados pela sua nanoencapsulação, que controlou a velocidade de permeação do fármaco em função do tempo;

A permeação do fármaco através da monocama celular não alterou a integridade da membrana;

Monocamadas de células SCC4 podem ser utilizada como um modelo alternativo de membrana sublingual, considerando que o perfil de permeação de carvedilol através dessa camada teve uma corelação linear com o perfil de permeação através de mucosa sublingual de porco;

A nanoencapsulação do carvedilol foi essencial para aumentar a quantidade de fármaco em contato com a mucosa sublingual e, assim, promover um aumento na quantidade de fármaco permeado, na presença de um fluxo salivar mimetizado;

A conversão das suspensões de nanocápsulas em materiais pulverulentos, a partir da técnica de secagem por aspersão, não interferiu na estrutura molecular e nas propriedades mucoadesivas das nanocápsulas;

Comprimidos sublinguais inovadores, contendo carvedilol encapsulado, foram produzidos a partir das nanocápsulas secas por aspersão e utilizando o

processo de compressão direta, o qual não interferiu na estrutura supramolecular original das nanopartículas;

Este estudo desenvolveu um sistema sólido nanotécnológico inédito para administração de carvedilol através da via sublingual, caracterizado como nanomedicamento com propriedades mucoadesivas.

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