UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

NANOPARTÍCULAS CONTENDO PROPIONATO DE CLOBETASOL: PREPARAÇÃO, CARACTERIZAÇÃO E INCORPORAÇÃO EM HIDROGÉIS

DISSERTAÇÃO DE MESTRADO

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Santa Maria, RS, Brasil
2010

NANOPARTÍCULAS CONTENDO PROPIONATO DE CLOBETASOL: PREPARAÇÃO, CARACTERIZAÇÃO E INCORPORAÇÃO EM HIDROGÉIS

por

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Farmacêuticas, Área de Concentração em Controle e Avaliação de Insumos e Produtos Farmacêuticos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Ciências Farmacêuticas**

Orientador: Prof. Dr. Ruy Carlos Ruver Beck

Santa Maria, RS, Brasil

2010

Universidade Federal de Santa Maria
Centro de Ciências da Saúde
Programa de Pós-Graduação em Ciências Farmacêuticas

A Comissão Examinadora, abaixo assinada,
aprova a Dissertação de Mestrado

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elaborada por
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como requisito parcial para obtenção do grau de
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Santa Maria, 29 de março de 2010.

Aos meus pais e irmãs, pelo incentivo e apoio necessário para que eu chegasse até aqui.

AGRADECIMENTOS

À Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Ciências Farmacêuticas pelas oportunidades oferecidas.

Ao Departamento de Farmácia Industrial pela estrutura física oferecida.

Ao Prof. Dr. Ruy Carlos Ruver Beck, pela sua dedicada orientação, amizade, incentivo, conhecimentos compartilhados e exemplo profissional demonstrado.

Aos professores e funcionários do Departamento de Farmácia Industrial pela amizade e apoio.

Ao Corpo Docente do PPGCF, pelos conhecimentos transmitidos.

À Prof[a]. Dr[a]. Sílvia S. Guterres e Prof[a]. Dr[a] Adriana R. Pohlmann da UFRGS pela concessão das análises de tamanho de partícula, polidispersão e potencial zeta.

Aos bolsistas de iniciação científica e aos colegas de laboratório de Tecnologia Farmacêutica pelo auxílio, companheirismo e pelos bons momentos de convivência e descontração. Em especial a Karine, Michel, Roberta, Luana, Rossana, Marila, Aline, Juliana e Lucas. E ao Bítio por suas contribuições químicas.

À CAPES, órgão financiador da bolsa de estudos. À Rede de Nanocosméticos CNPq/MCT pelo apoio financeiro.

Ao CNPq (Programa PIBIC) e ao Programa FIPE Jr/UFSM pela concessão das bolsas de iniciação científica vinculadas a este trabalho.

RESUMO

Dissertação de Mestrado
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Universidade Federal de Santa Maria

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AUTORA: Márcia Camponogara Fontana
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Data e Local da Defesa: Santa Maria, 29 de março de 2010.

Este trabalho teve como principal objetivo o desenvolvimento de formulações nanoestruturadas contendo propionato de clobetasol. Inicialmente, foi validado um método cromatográfico para análise do propionato de clobetasol em suspensões de nanocápsulas poliméricas. As nanocápsulas e nanoesferas de poli(ε-caprolactona) (PCL) e nanoemulsões contendo propionato de clobetasol (0,5 mg/mL) foram preparadas pelo método da deposição interfacial do polímero pré-formado, nanoprecipitação e emulsificação espontânea, respectivamente. Foram avaliados teores de fármaco, eficiências de incorporação, pHs, diâmetros de partícula, índices de polidispersão, potenciais zeta, características morfológicas e estabilidade frente ao armazenamento das diferentes formulações. As nanocápsulas apresentaram maior estabilidade físico-química, seguida pelas nanoemulsões e nanoesferas. Na avaliação da liberação *in vitro* do propionato de clobetasol, as nanocápsulas apresentaram o maior controle na liberação do fármaco, seguindo um modelo biexponencial. O estudo da fotodegradação do propionato de clobetasol frente à luz UVA demonstrou a importância da presença do polímero e do óleo para o aumento da fotoestabilidade. Diante destes resultados, as nanocápsulas foram selecionadas para o estudo da influência do material polimérico sobre as características físico-químicas, estabilidade frente ao armazenamento, fotoestabilidade, perfil de liberação do fármaco e seu mecanismo de liberação. As nanocápsulas preparadas com poli(ácido lactídeo) (PLA) apresentaram uma maior estabilidade frente ao armazenamento em comparação com as nanocápsulas preparadas com poli(ácido lactídeo-co-glicolídeo) 50:50 e 85:15, embora sua estabilidade tenha sido inferior às nanocápsulas preparadas com PCL. O estudo da fotodegradação demonstrou a proteção do fármaco quando nanoencapsulado, independente do tipo de polímero empregado na sua preparação. A liberação *in vitro* demonstrou a liberação controlada do fármaco com transporte anômalo. Diante de todos esses resultados, as nanocápsulas preparadas com PCL foram selecionadas para o desenvolvimento de formas farmacêuticas semissólidas (hidrogéis). Formulações similares contendo nanoesferas e a nanoemulsão foram utilizadas para se avaliar a influência do polímero e do óleo sobre diferentes propriedades dos hidrogéis. Estas formas farmacêuticas foram avaliadas quanto ao teor de fármaco, pH, espalhabilidade, reologia e liberação *in vitro* do fármaco. Os hidrogéis apresentaram propriedades compatíveis com a aplicação tópica. A presença do fármaco nanoencapsulado nos hidrogéis proporcionou sua liberação controlada, principalmente para as formulações contendo as nanocápsulas. O perfil de liberação do fármaco a partir dos hidrogéis seguiu o modelo de Higuchi.

Palavras-chave: propionato de clobetasol; nanocápsulas; nanopartículas; hidrogel

ABSTRACT

Master Dissertation
Programa de Pós-Graduação em Ciências Farmacêuticas
Universidade Federal de Santa Maria

CLOBETASOL PROPIONATE-LOADED NANOPARTICLES: PREPARATION, CHARACTERIZATION AND INCORPORATION INTO HYDROGELS

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Place and Date of Defense: Santa Maria, March 29, 2010.

The aim of this work was the development of nanostructured formulations containing clobetasol propionate. Initially, it was validated a chromatographic method to assay clobetasol propionate in nanocapsule suspensions. Clobetasol propionate-loaded nanocapsules and nanospheres of poly(ε-caprolactone) (PCL) and nanoemulsion (0.5 mg mL^{-1}) were prepared by the interfacial deposition of preformed polymer method, nanoprecipitation and spontaneous emulsification, respectively. Formulations were characterized by means of drug content, encapsulation efficiency, pH, mean size, polydispersity index, zeta potential, morphology analysis, and stability under storage. The PCL nanocapsules showed the highest physicochemical stability, followed by the nanoemulsions and nanospheres. In the evaluation of *in vitro* release of clobetasol propionate, the nanocapsules showed a better control of drug release, according to the biexponential model. The photodegradation study of clobetasol propionate against UVA light showed the importance of the polymer and the oil in the nanoparticles to protect the drug from light. From these results, the nanocapsules were chosen for the study of the influence of the polymerid material on the physicochemical stability umder storage, photostability, release profile of the drug and its release mechanism. The nanocapsules prepared with poly(lactide) (PLA) showed a higher stability in comparison to the nanocapsules prepared with poly(lactide-co-glycolide) 50:50 and 85:15, although its stability was lower than nanocapsules prepared with PCL. Photodegradation studies demonstrated the pretection of the nanoencapsulated drug, regardless of the polymeric material of the nanocapsule's wall. The *in vitro* release study demonstrated the controlled release of the drug according to an anomalous transport. Due to these results, the nanocapsules prepared with PCL were selected for the development and preparation of hydrogels. Similar formulations containing nanospheres and nanoemulsion were used to evaluate the influence of polymer and oil on different properties of the hydrogels. These dosage forms were evaluated for drug content, pH, spreadability, rheology and in vitro drug release. All hydrogels presented properties compatible to the topical application. The presence of the drug-loaded nanoparticles in hydrogels led a slower drug release, especially for the formulation containing nanocapsules. The drug release profile was according to the Higuchi model.

Keywords: clobetasol propionate; nanocapsules; nanoparticles; hydrogel

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INTRODUÇÃO

INTRODUÇÃO

A nanotecnologia é um campo multidisciplinar em fase de desenvolvimento, que promete avanços significativos na engenharia e na medicina (EMERICH & THANOS, 2003). Na indústria farmacêutica, a nanotecnologia representa uma excelente estratégia para a expansão no mercado consumidor, resolvendo problemas como a vida de prateleira de alguns medicamentos, melhorando sua eficácia, segurança e aceitabilidade pelo paciente, além da possibilidade de vetorização de fármacos (SAHOO & LABHASETWAR, 2003).

Neste contexto, os sistemas coloidais nanoparticulados estão sendo estudados na área farmacêutica no desenvolvimento de sistemas carreadores de fármacos e ativos cosméticos, buscando-se uma maior eficácia, pelo aumento de sua especificidade e redução da sua toxicidade, podendo ser aplicados também em formulações dermatológicas, facilitando sua preparação devido ao seu tamanho submicrométrico (PERUGINI *et al.*, 2002; SCHAFFAZICK *et al.*, 2005; RAWAT *et al.*, 2006; ALVES *et al.*, 2007). Os nanossistemas já estão sendo introduzidos comercialmente, como nos cosméticos, protetores solares e nos produtos farmacêuticos (CROSERA *et al.*, 2009).

A baixa estabilidade e o transporte limitado de fármacos através do epitélio a partir dos medicamentos convencionais impedem muitas vezes que estes cheguem ao seu sítio de ação específico. Para contornar este problema, os nanossistemas apresentam vantagens proporcionando um controle temporal e/ou espacial na liberação do fármaco, aumentando a biodisponibilidade oral e ocular, protegendo o fármaco frente ao sistema biológico e também facilitando o seu transporte através das barreiras biológicas (ALONSO, 2004; VASIR *et al.*, 2005; RAWAT *et al.*, 2006).

Nanopartículas poliméricas são sistemas carreadores de fármacos que apresentam diâmetro inferior a 1 μm. Dependendo da sua composição e organização estrutural podem ser classificadas em nanocápsulas e nanoesferas, nas quais o fármaco pode ficar retido, adsorvido ou molecularmente disperso. A presença do óleo na nanocápsula forma uma estrutura vesicular, enquanto a sua ausência, nas nanoesferas, forma uma matriz polimérica. Da mesma forma, as nanoemulsões também têm sido estudadas como nanocarreadores de fármacos, embora sejam constituídas por gotículas submicrométrica não-poliméricas, apresentando a fase oleosa estabilizada por tensoativos (CALVO *et al.*, 1996; SCHAFFAZICK *et al.*, 2003; MARTINI *et al.*, 2007).

Diferentes métodos têm sido descritos na literatura para a preparação de nanocápsulas, nanoesferas e nanoemulsões. Independentemente do método de preparação, esses sistemas coloidais apresentam limitada estabilidade físico-química durante seu armazenamento, podendo apresentar precipitados, hidrólise do polímero e do óleo, além da contaminação microbiológica devido à grande quantidade de água presente nestes nanossistemas (SCHAFFAZICK *et al*., 2003; REDA & CARNEIRO, 2007). Como alternativa para contornar esta limitada instabilidade e também para o desenvolvimento de formas farmacêuticas finais, existem relatos na literatura demonstrando a eficiente e promissora incorporação destes sistemas coloidais nanoparticulados incorporados em formas farmacêuticas semissólidas para administração cutânea (ALVAREZ-ROMÁM *et al.*, 2001; MILÃO *et al.*, 2003; JIMÉNEZ *et al.*, 2004; ALVES *et al.*, 2005; LUENGO *et al.*, 2006; PAESE *et al.*, 2009; MARCHIORI *et al.*, 2010).

O estrato córneo representa um obstáculo para a liberação de muitos fármacos na terapêutica dermatológica. Frente a este obstáculo, o emprego da nanotecnologia apresenta algumas vantagens no desenvolvimento de produtos para administração cutânea, podendo modificar a farmacocinética e a biodistribuição de fármacos através da pele, além de minimizar seu efeito sistêmico (TING *et al.*, 2004; GUTERRES *et al.*, 2007). Os sistemas nanoestruturados apresentam uma grande área superficial, formando um filme sobre a pele e proporcionando uma liberação homogênea de fármacos lipofílicos quando aplicados na superfície cutânea (ALVAREZ-ROMÁN *et al.*, 2004). Além disso, outras vantagens têm sido demonstradas para a aplicação tópica desses sistemas, tais como: a retenção das nanopartículas sobre a pele (RAO e MURTHY, 2000) e no folículo piloso (LADERMANN *et al.*, 2007), o aumento da resporta terapêutica, a diminuição da inflamação e coceira na pele (KALARIYA *et al.*, 2005), o maior percentual de penetração do fármaco na epiderme e derme viável quando nanoencapsulado (ALVES *et al.*, 2007), além do baixo potencial de causar reações alérgicas (PAESE *et al.*, 2009) e a liberação mais lenta do fármaco (MARCHIORI *et al.*, 2010).

O propionato de clobetasol, o fármaco selecionado para esse estudo, é um glicocorticóide sintético dotado de potente ação anti-inflamatória e imunossupressora, que é administrado topicamente para o tratamento de dermatites que não respondem aos esteróides menos potentes (ANVISA, 2007; GOODMAN & GILMAN, 2007). Entretanto, apresenta vários efeitos colaterais e quando usado por um período prolongado pode ser absorvido através da pele para o sistema circulatório (ANVISA, 2007). Até o momento, não existem

estudos incorporando este fármaco em sistemas nanoestruturados poliméricos e nanoemulsões.

A partir do exposto, justifica-se o estudo inédito do desenvolvimento de nanopartículas poliméricas e nanoemulsões contendo propionato de clobetasol e sua posterior incorporação em uma forma farmacêutica semissólida. Este trabalho foi delineado com o propósito de se avaliar a obtenção de formulações estáveis contendo propionato de clobetasol associado a esses nanocarreadores, que apresentassem um maior controle no perfil de liberação do fármaco e a proteção frente a sua fotodegradação. A incorporação destes nanocarreadores a formas farmacêuticas semissólidas tem como propósito propiciar, além destas vantagens já citadas, a diminuição do contato imediato da quantidade total do fármaco diretamente com a pele, podendo levar a uma diminuição na irritação cutânea e o aumento na atividade terapêutica do fármaco.

OBJETIVO

1.1 Objetivo geral

Desenvolver formulações de base nanotecnológica contendo propionato de clobetasol (dispersões coloidais e hidrogéis), avaliando-se a influência do tipo de nanocarreador sobre as propriedades físico-químicas e sobre a liberação *in vitro* do fármaco a partir destas formulações.

1.2 Objetivos específicos

- Validar uma metodologia analítica por cromatografia líquida para quantificação do propionato de clobetasol em dispersões coloidais;

- Estudar a influência do tipo de sistema nanoestruturado (nanocápsulas, nanoesferas e nanoemulsões) sobre as características físico-químicas, estabilidade frente ao armazenamento, liberação *in vitro* e fotoestabilidade de nanopartículas contendo propionato de clobetasol;

- Avaliar o emprego de polímeros amorfos na preparação de nanocápsulas contendo propionato de clobetasol, estudando as propriedades físico-químicas, estabilidade frente ao armazenamento, liberação *in vitro* e fotoestabilidade do fármaco;

- Estudar o desenvolvimento de hidrogéis contendo as formulações coloidais selecionadas, considerando as propriedades adequadas para aplicação tópica destas formas farmacêuticas semissólidas (pH, espalhabilidade, comportamento reológico e liberação *in vitro* do fármaco).

REVISÃO DA LITERATURA

1.1 Nanociência e sistemas de liberação de fármacos

A nanociência é um ramo emergente e promissor da ciência que está associado a diversas áreas que trabalham com partículas submicrométricas. No campo farmacêutico está associada ao controle espacial ou temporal da liberação de fármacos ou ativos cosméticos. O desenvolvimento destes sistemas tem contribuído para controlar sua velocidade de liberação no organismo, modulando a permeação de substâncias através das barreiras fisiológicas, penetração na circulação e a chegada ao alvo terapêutico. Os carreadores nanoestruturados permitem direcionar o fármaco no organismo, evitando seu acúmulo em tecidos não-específicos, onde poderia apresentar toxicidade, aumentando sua concentração no local de ação e o seu índice terapêutico (RAWAT *et al.*, 2006). Esta alternativa à modificação química de moléculas ativas, através da sua associação a sistemas carreadores, permite a alteração das propriedades físico-químicas dos sistemas de administração sem modificar o mecanismo de ação do fármaco (SAHOO & LABHASETWAR, 2003).

Além disso, estudos demonstram outras vantagens da associação de fármacos a sistemas nanoestruturados, empregando diferentes vias de administração, como o desenvolvimento de sistemas que prolongam a liberação do fármaco, reduzem a irritação tecidual, aumentam a biodisponibilidade, protegem o fármaco frente a fatores intrínsecos e extrínsecos ao organismo e durante o armazenamento e aumentam o conforto do paciente devido a redução do número de administrações (BARRATT, 2000; SCHAFFAZICK *et al*., 2003; ALONSO, 2004; CRUZ *et al*., 2006; RAWAT *et al.*, 2006; ANTON *et al*., 2008; MARCHIORI *et al*., 2010).

Conforme comentado, um dos principais objetivos no desenvolvimento dos sistemas nanoestruturados de liberação de fármacos é o controle da disponibilidade do fármaco no local onde a atividade farmacológica é necessária (RAWAT *et al.*, 2006). Os tipos e o número de barreiras biológicas que o fármaco precisa atravessar para chegar ao seu local de ação dependem da via de administração da formulação, podendo ser o epitélio digestivo, o endotélio dos vasos sanguíneos, as mucosas ou a pele. O destino *in vivo* destes nanocarreadores depende dos processos de opsonização, das barreiras químicas e bioquímicas

a serem atravessadas, a ativação do sistema complento, além do tamanho e das propriedades de superfície das partículas (VAUTHIER & BOUCHEMAL, 2009).

A aplicação da nanotecnologia no desenvolvimento de sistemas de liberação de fármacos tem sido estudada empregando-se diferentes vias de administração, como a administração parenteral, oral, dérmica e ocular (ALONSO, 2004). As nanopartículas são reconhecidas como substâncias estranhas pelas proteínas plasmáticas e removidas da circulação pelo sistema fagocitário mononuclear, principalmente representado pelas células de Kupffer do fígado e macrófagos do baço e da medula óssea ou, ainda, estas podem atravessar o endotélio onde um órgão se encontra inflamado. Além disso, as nanopartículas podem apresentar um revestimento catiônico, que modifica a superfície da partícula diminuindo ou impedindo a deposição das proteínas plasmáticas e o seu reconhecimento pelos macrófagos. Assim, estas nanopartículas com superfície modificada permanecem por um maior tempo na circulação sanguínea, podendo chegar em outros sítios e proporcionando uma maior estabilidade e biodistribuição (SANTANDER-ORTEGA, 2007).

Entre os sistemas nanocarreadores mais estudados na área farmacêutica podem ser citados os lipossomas, as nanopartículas lipídicas, as nanoemulsões e as nanopartículas poliméricas (nanocápsulas e nanoesferas). Os lipossomas são estruturas constituídas principalmente por fosfolipídios organizados em bicamadas concêntricas, contendo um núcleo aquoso central, podendo encapsular fármacos de diferentes lipofilias, embora apresentem uma menor estabilidade quando comparada com as nanopartículas poliméricas (RAWAT *et al.*, 2006). As nanopartículas lipídicas são constituídas principalmente por lipídios, apresentando um núcleo hidrofóbico recoberto por fosfolipídios, podendo conter tanto um núcleo lipídico sólido como também um núcleo constituído por uma mistura de lipídios sólido e líquido. São amplamente estudadas na área dermatológica e cosmética e apresentam a vantagem da facilidade de transposição de escala através da homogeneização a alta pressão (MÜLLER *et al*., 2000). As nanocápsulas e as nanoesferas, conhecidas como nanopartículas poliméricas, são constituídas por um sistema reservatório contendo um núcleo oleoso cercado pela parede polimérica ou por uma matriz polimérica, respectivamente, permitindo um maior controle na liberação do fármaco. Fármacos altamente lipofílicos podem ser facilmente incorporados nestas nanopartículas, que apresentam vantagens com relação a estabilidade *in vivo* e também durante o armazenamento quando comparadas aos lipossomas (SCHAFFAZICK *et al*., 2003; RAWAT *et al.*, 2006). As nanoemulsões são formadas por gotículas de óleo estabilizadas por tensoativos e sua principal característica é a estabilidade das gotículas na dispersão, devido ao seu tamanho submicrométrico (CALVO *et al.*, 1996).

1.2 Nanopartículas poliméricas

As nanopartículas poliméricas, que apresentam diâmetro inferior a 1 µm, são classificadas em nanocápsulas e nanoesferas, conforme comentado anteriormente. As nanocápsulas apresentam um núcleo composto por óleo rodeado por um polímero e as nanoesferas são compostas apenas pela matriz polimérica (MORA-HUERTAS *et al.*, 2010). Por outro lado, a nanoemulsão é uma emulsão formada apenas pelo óleo e tensoativos (CALVO *et al.*, 1996). Considerando as formas de associação do fármaco nestes sistemas, este pode estar disperso, dissolvido no seu interior ou ainda pode ficar adsorvido na superfície externa da partícula (SCHAFFAZICK *et al*., 2003).

As nanocápsulas apresentam algumas vantagens em relação às nanoesferas, como a eficiência de encapsulação de fármacos lipofílicos, devido a presença do núcleo oleoso e o baixo teor de polímero. Ainda, a parede polimérica impede o contato direto do fármaco dissolvido no óleo com o tecido, reduzindo a irritação tecidual, ajuda a impedir a liberação imediata do fármaco e tem a função de proteger o fármaco contra a degradação causada pelo pH e pela luz (ANTON *et al*., 2008).

A caracterização físico-química das nanopartículas poliméricas pode ser considerada como uma tarefa que deve ser cuidadosamente planejada e realizada devido a natureza coloidal destes sistemas. Esta caracterização é constituída pela determinação da quantidade de fármaco associado às nanoestruturas e da eficiência de incorporação, avaliação da cinética de liberação do fármaco, da distribuição de tamanho de partícula, da distribuição de massa molar do polímero, pela determinação do potencial zeta e do pH, da análise morfológica e a avaliação da estabilidade em função do tempo de armazenamento, dentre outras (SCHAFFAZICK *et al*., 2003; MORA-HUERTAS *et al.*, 2010).

Existem diferentes métodos descritos na literatura para a preparação de nanopartículas poliméricas. Neste trabalho, foram empregados os métodos de deposição interfacial do polímero pré-formado e a nanoprecipitação, métodos patenteados por Fessi e colaboradores em 1988 para a preparação de nanocápsulas poliméricas e nanoesferas. Nestes métodos, uma fase orgânica miscível em água e contendo um tensoativo de baixo valor de EHL (equilíbrio hidrófilo/lipófilo) é injetada, sob agitação, sobre uma fase aquosa contendo um tensoativo de alto EHL. As nanopartículas são formadas espontaneamente pela rápida difusão do solvente e sua posterior evaporação. Para a preparação das nanocápsulas, é necessária a presença de um óleo e de um polímero na fase orgânica para constituição do seu núcleo oleoso e da parede

polimérica, respectivamete. No método da deposição interfacial do polímero pré-formado quando a fase orgânica entra em contato com a fase aquosa, o solvente orgânico difunde para a água, gerando uma turbulência interfacial (óleo/água) e o polímero precipita em volta das gotículas de óleo (FESSI *et al.*, 1988[a]). No método da nanoprecipitação ocorre o mesmo fenômeno, só que neste caso a fase orgânica não contêm o óleo, ocorrendo apenas a precipitação do polímero e a formação da estrutura matricial das nanoesferas (FESSI *et al.*, 1988[b]). Na preparação destes sistemas, o emprego de polímeros biodegradáveis é importante na área de liberação controlada de fármacos, devido a propriedade de serem metabolizados e reabsorvidos pelo organismo. Neste contexto, por serem aprovados pelo FDA para uso humano, os poliésteres de ácido lático [poli(ácido lactídeo) - PLA] e glicolídeo [poli(ácido glicolídeo) - PGA], seus copolímeros (PLGA) e também a poli(ε-caprolactona) - PCL são de grande interesse para o desenvolvimento de sistemas coloidais desta natureza (NAIR & LAURENCIN, 2007).

1.3 Poliésteres

A síntese de novos polímeros biodegradáveis tem proporcionado um grande avanço na área biomédica, como a engenharia dos tecidos, a medicina regenerativa, a terapia genética, a bionanotecnologia e os sistemas de liberação controlada de fármacos (JAIN, 2000; MIDDLETON & TIPTON, 2000; NAIR & LAURENCIN, 2007). Biodegradável é todo material que pode ser degradado por uma atividade biológica específica (SÖDERGARD & STOLT, 2002). Polímeros biodegradáveis são degradados *in vivo*, por hidrólise enzimática ou não enzimática das cadeias poliméricas ou pela combinação de ambos os processos, levando à erosão do polímero, sendo seus metabólitos eliminados pelas vias normais de excreção (JAIN, 2000; FAISANT *et al*., 2002). Os materiais poliméricos mais utilizados na preparação de nanopartículas poliméricas são os biodegradáveis, principalmente a PCL, o PLA e o PLGA, proporcionando a liberação controlada do fármaco, a sua fotoestabilidade e também protegendo-o da degradação química ou enzimática (MORA-HUERTAS *et al.*, 2010).

Importantes propriedades devem ser levadas em consideração na escolha de polímeros para o desenvolvimento de sistemas de liberação controlada de fármacos: devem ser biodegradáveis (degradados por organismos vivos), biocompatíveis (compatíveis com organismos vivos) e bioabsorvíveis (degradados no interior do corpo humano sem apresentar citotoxicidade) (KHOR *et al*., 2002; SINHA *et al*., 2004; SCHNELL *et al*., 2007). Além

disso, não devem provocar resposta inflamatória ou tóxica, devem ser capazes de serem metabolizados e eliminados do organismo, sendo que os respectivos produtos de degradação não devem ser tóxicos (CAO & WANG, 2009).

No caso dos polímeros naturais (colágeno, elastina, albumina, fibrina) a maioria sofre degradação enzimática *in vivo*, sendo que esta varia com o local onde ele se encontra, dependendo da disponibilidade e da concentração das enzimas. Os polímeros naturais possuem várias vantagens, como a bioatividade, a capacidade de apresentar receptor, a suscetibilidade de provocar degradação proteolítica e remodelação natural. Entretanto, apresenta algumas desvantagens, como a resposta imunogênica, a difícil purificação e a possibilidade de transmissão de doença. Em contrapartida, os polímeros sintéticos podem ser biologicamente inertes, apresentando propriedades uniformes entre os lotes e têm a vantagem de serem produzidos para aplicações específicas. Estes polímeros geralmente são biodegradados por hidrólise (JALIL & NIXON, 1990; NAIR & LAURENCIN, 2007).

Os poliésteres sintéticos biodegradáveis, como a poli(ε-caprolactona), o poli(ácido lactídeo), o poli(ácido glicolídeo) e o copolímero(ácido lactídeo-co-ácido glicolídeo) (Figura 1) são de grande interesse da área biomédica devido a excelente biocompatibilidade e biodegradabilidade. Estes polímeros são aprovados pela vigilância sanitária americana FDA para o uso em sistemas de liberação controlada (NAIR & LAURENCIN, 2007).

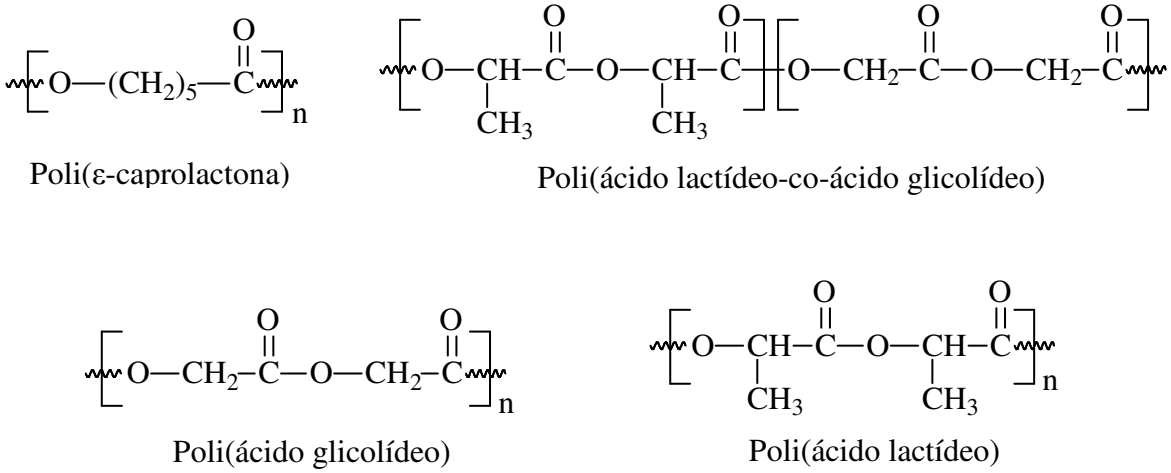

Figura 1 - Estrutura química dos poliésteres

A poli(ε-caprolactona) (PCL) é um polímero biodegradável, biocompatível e bioabsorvível. É um poliéster semicristalino, obtido por polimerização através da abertura do

anel da ε-caprolactona. A PCL é solúvel em solventes orgânicos, pode formar misturas miscíveis com vários polímeros, tem baixo ponto de fusão (59-64 °C) e temperatura de transição vítrea de - 60 °C. Sofre degradação hidrolítica, por apresentar ligações alifáticas, no entanto, sua velocidade de degradação é lenta (maior que 2 anos). Por sofrer uma degradação lenta, ter alta permeabilidade a muitos fármacos e por não ser tóxica, a PCL tem sido estudada na liberação controlada de fármacos e vacinas (MIDDLETON & TIPTON, 2000). Khor e colaboradores, em 2002, observaram o crescimento de células epiteliais em filmes de PCL, concluindo que este polímero pode ser utilizado no desenvolvimento de materiais suporte para regeneração da pele. Schnell e colaboradores, em 2007, produziram dois substratos poliméricos, um constituído por nanofibras de PCL e outro constituído por nanofibras de uma blenda de colágeno e PCL, obtendo uma boa adesão de células olfativas e do sistema nervoso em ambos os substratos.

Devido a sua biocompatibilidade, a PCL tem sido estudada na engenharia de tecidos. Devido a sua degradação lenta, vários sistemas copoliméricos contendo PCL estão sendo estudados para melhorar as propriedades deste polímero. Copolímeros de ε-caprolactona com ácido láctico e com ácido glicólico apresentam uma maior velocidade de degradação. Os copolímeros de ε-caprolactona com ácido glicolídeo formam fibras menos rígidas, sendo utilizados em suturas (MIDDLETON & TIPTON, 2000).

Poli(ácido glicolídeo) (PGA) é um polímero biodegradável, altamente cristalino (45-55 %), com baixa solubilidade em solventes orgânicos. A temperatura de transição vítrea varia de 35-40 °C e seu ponto de fusão é 220-225 °C (MIDDLETON & TIPTON, 2000). O PGA é hidrolisado na porção éster, sua degradação ocorre entre 6-12 meses e pode ser excretado inalterado pelo rim, podendo ser quebrado em glicina e excretado na urina, ou pode ser convertido em gás carbônico e água, através do ciclo de Krebs (DECHY-CABARET *et al.*, 2004; MAURUS & KAEDING, 2004).

Por ter boa biodegradabilidade, o PGA tem sido estudado na engenharia de tecidos, ajudando a regenerar tecidos biológicos. Este apresenta excelente propriedade mecânica devido a sua alta cristalinidade, sendo estudado como dispositivo para fixação óssea. No entanto, a aplicação do PGA na área biomédica é limitada, devido a sua alta velocidade de degradação, por formar substâncias ácidas e ter baixa solubilidade. Para solucionar estas desvantagens, copolímeros contendo os monômeros deste polímero têm sido desenvolvidos (MIDDLETON & TIPTON, 2000).

O ácido lactídeo é uma molécula quiral, que existe em duas formas opticamente ativas, o $_{L}$-ácido lactídeo (isômero natural) e o $_{D}$-ácido lactídeo. A polimerização destes monômeros

forma o polímero $_{(D,L)}$-lactídeo. A polimerização dos monômeros ativos forma um polímero semicristalino e a mistura racêmica forma um polímero amorfo. Estes polímeros são solúveis em solventes orgânicos. Além disso, são degradados por hidrólise na ligação éster, liberando o ácido láctico que através do ciclo de Krebs é convertido em gás carbônico e água (MIDDLETON & TIPTON, 2000; DECHY-CABARET *et al.*, 2004; MAURUS & KAEDING, 2004).

O poli($_{L}$-ácido lactídeo) (PLLA) é um polímero altamente cristalino (37 %), consequentemente, é mais resistênte a hidrólise. Seu grau de cristalinidade depende do peso molecular e do processo de polimerização. Seu ponto de fusão é 175-178 °C e a temperatura de transição vítrea varia de 60-65 °C (MIDDLETON & TIPTON, 2000; MAURUS & KAEDING, 2004).

O PLLA é mais hidrofóbico que o PGA, absorve menos água e é degradado mais lentamente, tem boa resistência, sendo considerado um biomaterial ideal para aplicação ortopédica e em suturas. Quando seu peso molecular é alto, pode levar mais de 2 anos para ser totalmente reabsorvido *in vivo*. A velocidade de degradação depende do grau de cristalinidade do polímero e da porosidade da matriz (JAIN, 2000; MIDDLETON & TIPTON, 2000).

O poli($_{DL}$-ácido lactídeo) (PDLLA) é um polímero amorfo, sua temperatura de transição vítrea é de 55-60 °C e é menos resistente que o PLLA. É degradado mais rapidamente que o PLLA, podendo levar de 12 a 16 meses para ser degradado (MAURUS & KAEDING, 2004). Este polímero também tem sido estudado na liberação controlada de fármacos e na engenharia de tecidos. Alternativamente, vários copolímeros de $_{L}$-ácido lactídeo com ácido glicolídeo ou com $_{DL}$-ácido lactídeo tem sido estudados para o desenvolvimento de polímeros com melhores propriedades (MIDDLETON & TIPTON, 2000).

O copolímero poli(ácido lactídeo-co-ácido glicolídeo) (PLGA) é constituído de monômeros do ácido lactídeo (PLA) e ácido glicolídeo (PGA). O PLA é mais hidrofóbico que o PGA, consequentemente, o PLGA rico em PLA é menos hidrofílico, absorve menos água e degrada mais lentamente (MIDDLETON & TIPTON, 2000). Tanto os monômeros do $_{L}$-ácido lactídeo como o $_{DL}$-ácido lactídeo são utilizados para a co-polimerização. O poli($_{L}$-ácido lactídeo-co-ácido glicolídeo) é cristalino e o poli($_{DL}$-ácido lactídeo-co-ácido glicolídeo) é amorfo (JAIN *et al.*, 2000). O poli($_{DL}$-ácido lactídeo-co-ácido glicolídeo) 50:50 degrada em 1-2 meses, o 75:25 em 4-5 meses e o 85:15 em 5-6 meses. A temperatura de transição vítrea dos PLGAs é acima de 37 °C, por isso são sólidos a temperatura ambiente (JAIN *et al.*, 2000).

O PLGA sofre biodegradação hidrolítica (erosão em meio aquoso) pela quebra das ligações ésteres. Sua velocidade de degradação depende de vários parâmetros, como a relação de ácido lactídeo e glicolídeo, peso molecular e a forma e estrutura da matriz. Os ácidos láctico e glicólico são formados pela degradação do PLGA, e como já foi citado, entram no ciclo de Krebs para serem eliminados (O'HAGAN *et al*., 1998; JAIN *et al.*, 2000; FAISANT *et al*., 2002; DECHY-CABARET *et al.*, 2004). O PLGA foi aprovado pelo FDA para ser utilizado como um biomaterial, sendo biodegradável e biocompatível, e aplicado na área biomédica no desenvolvimento de sistemas de liberação controlada de fármacos e proteínas, na engenharia de tecidos e em fios de sutura. Vários veículos para liberação de fármacos e proteínas compostos de PLGA, como microesferas, microcápsulas, nanocápsulas, nanoesferas e nanofibras já foram desenvolvidos e estudados (JAIN *et al.*, 2000; FAISANT *et al*., 2002; TEIXEIRA *et al*., 2005).

1.4 Aplicação cutânea de sistemas nanoestruturados

A pele representa uma complexa barreira para a entrada e saída de substâncias, sendo composta pela epiderme, derme e hipoderme. A passagem de compostos através do estrato córneo, localizado na parte mais externa da epiderme, foi considerada por muito tempo o passo limitante para a absorção percutânea das substâncias ativas em níveis terapêuticos (GUTERRES *et al*., 2007). Entretanto, estudos recentes mostraram que o folículo piloso desempenha um importante papel na penetração das substâncias pela pele, principalmente quando associadas a nanoestruturas (LADERMANN *et al*., 2007).

As propriedades físico-químicas dos veículos e das substâncias ativas são consideradas as principais responsáveis pela distribuição do fármaco na pele (PARDEIKE *et al*., 2009). Para modular a absorção destas substâncias, é possível manipular as propriedades das formulações, como o coeficiente de partição do veículo, a difusão e a solubilidade do fármaco, além de suas propriedades físico-químicas, sua interação com a membrana celular e, também, sua farmacocinética (KALIA & GUY, 2001).

Com esse enfoque, os sistemas nanocarreadores de fármacos têm sido estudados para a aplicação cutânea, com o objetivo de controlar a liberação do fármaco na pele e melhorar sua eficácia (ALVES *et al.*, 2007). Estes sistemas apresentam um reduzido tamanho de partícula, podendo formar um filme sobre a pele, aumentando a hidratação e promovendo a permeação

de fármacos, além de proporcionar uma confortável aplicação sobre a pele. Além destas, outras vantagens também podem ser obtidas, como a proteção das substâncias frente à degradação química ou enzimática e a liberação prolongada dos fármacos, diminuindo sua frequência de aplicação (PERUGINI *et al*., 2002; BOUCHEMAL *et al.*, 2004).

Diferentes nanossistemas tem sido desenvolvidos para a aplicação tópica, como os lipossomas (RAO & MURTHY, 2000), as nanopartículas lipídicas (YUAN *et al.*, 2008), as nanocápsulas poliméricas (OURIQUE *et al.*, 2008), as nanoesferas (SHIM *et al.*, 2004) e as nanoemulsões (ALMEIDA *et al.*, 2009). No caso das nanopartículas poliméricas, estas têm sido estudadas para melhorar o tratamento de desordens cutâneas. Entretanto, a sua limitada estabilidade físico-química durante o armazenamento, estes sistemas estão sendo incorporados em formas farmacêuticas semissólidas, que também apresentam uma aplicação mais conveniente (MILÃO *et al.*, 2003; ALVES *et al.*, 2005; ALVES *et al.*, 2007; PAESE *et al.*, 2009; MARCHIORI *et al.*, 2010).

As formas farmacêuticas tópicas são baseadas, principalmente, em formulações de emulsões e géis, devido a maior capacidade de controlar a viscosidade destes sistemas, fornecendo as características apropriadas a aplicação cutânea. A escolha da matéria-prima influencia a reologia das formulações, que vai influenciar em todas as fases de sua produção, desde a mistura até o controle de qualidade (LIPPACHER *et al.*, 2001). Alguns estudos têm sido realizados com formulações semissólidas contendo nanopartículas poliméricas, utilizando diferentes polímeros para a formação do gel, como Pluronic F127 (MIYAZAKI *et al.*, 2003), Natrosol® 250 M (LUENGO *et al.*, 2006), Carbopol 940® (MILÃO *et al.*, 2003; ALVES *et al.*, 2005; ALVES *et al.*, 2007; PAESE *et al.*, 2009) e Carbopol Ultrez® 10 NF (MARCHIORI *et al.*, 2010).

Rao e Murthy (2000) prepararam um gel de hidroxipropilmetilcelulose com lipossomas contendo propionato de clobetasol, que resultou numa baixa absorção do fármaco através da pele de ratos, comprovado também pelo teste do branqueamento da pele humana, demonstando uma maior retenção deste fármaco nas camadas da pele, o que diminui o risco dos efeitos colaterais. Por outro lado, Miyazaki e colaboradores (2003) prepararam um gel com Pluronic F127 contendo indometacina associada a nanocápsulas. Neste caso foi demonstrada uma melhor permeação do fármaco através da pele a partir destas formulações em comparação com uma formulação contendo o fármaco livre, sugerindo a sua potencial utilização para o desenvolvimento de sistemas transdérmicos contendo indometacina.

Em 2003, Milão e colaboradores prepararam um gel hidrofílico de Carbopol 940® contendo diclofenaco associado a nanocápsulas (0,05 %). Estes géis apresentaram

comportamento não-Newtoniano com propriedades plásticas e as nanopartículas mostraram-se intactas no gel durante 3 meses de armazenamento, conforme observação através de microscopia eletrônica por criofratura.

Uma comparação das características reológicas de formulações de hidrogéis de Carbopol 940® contendo nimesulida associada a diferentes nanopartículas (nanocápsulas, nanoesferas e nanoemulsão) foi estudada por Alves e colaboradores (2005). Todos os hidrogéis apresentaram características pseudoplásticas, de acordo com o modelo de Ostwald e não foi detectado tixotropia em nenhuma formulação. A presença das nanoestruturas não demonstrou influência significativa nas propriedades físico-químicas dos géis desenvolvidos.

Para estudar a influência da nanoencapsulação sobre a penetração e permeação do ácido flufenâmico pela pele, Luengo e colaboradores (2006) prepararam nanopartículas com este fármaco e as incorporaram em gel de Natrosol® 250 M. Este estudo demonstrou que o transporte do fármaco para as camadas mais profundas da pele foi controlado a partir da formulação contendo nanopartículas em comparação com a formulação contendo o fármaco livre até 12 horas. Além disso, através de estudos morfológicos foi demonstrado que as nanopartículas distribuíram-se homogeneamente na superfície da pele e não foram detectadas dentro ou entre os corneócitos.

Em outro estudo de Alves e colaboradores (2007) foi avaliada a penetração e a distribuição da nimesulida após a aplicação tópica de hidrogéis contendo diferentes nanopartículas, em continuidade aos estudos relatados pelos mesmos autores em 2005. Os estudos foram realizados empregando pele humana e utilizando a técnica de "tape stripping" e o método das células de difusão de Franz. Neste estudo, a presença da nimesulida nas camadas viáveis da pele foi maior após a aplicação do hidrogel de Carbopol 940® contendo nimesulida associada às nanocápsulas, quando estes níveis foram comparados àqueles obtidos para os hidrogéis contendo nimesulida associada a nanoesferas ou nanoemulsão.

A eficiência do folículo piloso como reservatório para fármacos associados a nanoestruturas após aplicação tópica foi demonstrada por Ladermann e colaboradores, em 2007. Neste estudo, os autores mostraram a maior penetração de um marcador fluorescente no folículo piloso, após a aplicação seguida de massagem de uma formulação contendo este marcador associado a nanopartículas em comparação com uma formulação contendo o marcador na sua forma livre. Os autores demonstraram ainda a permanência do marcador no folículo piloso por até 10 dias após a administração da formulação contendo a sua forma nanoestruturada, enquanto este só pode ser detectado até o quarto dia após a administração da formulação contendo a sua forma livre.

Além destes estudos, o baixo potencial de alergenicidade de hidrogéis de Carbopol 940® contendo nanopartículas poliméricas foi recentemente relatado por Paese e colaboradores (2009). Através de um estudo de sensibilização em camundongos, os autores demonstraram o baixo potencial das nanocápsulas em causar reações alérgicas após o contato com a pele.

Em trabalho recente do nosso grupo de pesquisa, Marchiori e colaboradores (2010) demonstraram o aumento da atividade antiproliferativa da dexametasona associada a nanocápsulas poliméricas, sugerindo seu emprego no tratamento de desordens proliferativas da pele, como a psoríase. Assim, foram preparados hidrogéis de Carbopol Ultrez® 10 NF (1 %), contendo estas nanocápsulas, os quais apresentaram um maior controle da liberação *in vitro* do fármaco em comparação com uma formulação contendo o fármaco na sua forma livre.

Neste contexto, os trabalhos aqui abordados demonstram as potencialidades do emprego de formulações semissólidas de base nanotecnológica para o tratamento de diferentes desordens cutâneas. Conforme observado, os estudos da aplicação cutânea de formulações deste tipo são bem mais recentes que aqueles voltados a outras vias de administração, como a via oral, parenteral e oftálmica. Desta forma, existem ainda várias questões a serem respondidas e também vários fatores a serem estudados para que se possa cada vez mais melhorar a eficácia destes sistemas nanoestruturados para aplicação cutânea.

1.5 Propionato de clobetasol

O propionato de clobetasol, 9α-flúor-21-cloro-11β-hidróxi-16β-metilpregna-3,20-diona-1,4-dieno-17α-propionato (Figura 2), o fármaco selecionado neste estudo, é um glicocorticóide sintético potente, amplamente utilizado devido aos seus efeitos anti-inflamatório não-específico e imunossupressor, provocando vasoconstrição e diminuição na síntese de colágeno. É administrado topicamente para o tratamento de processos inflamatórios, como psoríase, eczemas recalcitrantes, lúpus eritematoso discóide, dermatite atópica grave, neurodermatite grave, líquen plano, dermatoses do couro cabeludo e outras dermatites que não respondem satisfatoriamente aos esteróides menos potentes (REEPMEYER *et al.*, 1998; GAGLIARDI *et al.*, 2002; ANVISA, 2007; GOODMAN & GILMAN, 2007). O propionato de clobetasol pode apresentar efeitos colaterais indesejáveis

após a sua aplicação tópica, tais como queimadura, irritação, coceira, inchaço das pálpebras, face ou lábios. Quando usado por um período prolongado, este fármaco pode ser absorvido através da pele para o sistema circulatório, causando mudanças na pele, como o afinamento, atrofia celular, aparecimento das veias, mudanças na cor ou quantidade de cabelo, podendo ocorrer também a supressão adrenal, retardo do crescimento em crianças, ganho de peso inexplicado, glaucoma e aparecimento de acne (FANG *et al.*, 1999; ANVISA, 2007).

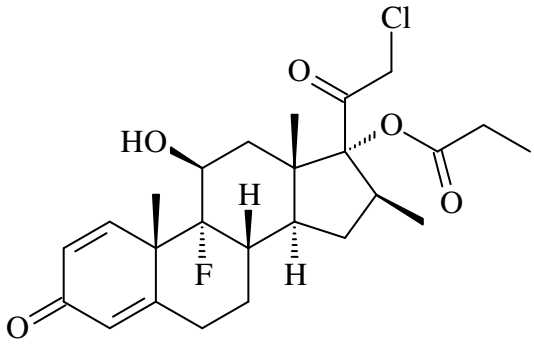

Figura 2. Estrutura química do propionato de clobetasol

Até o momento, a literatura não apresenta métodos de análise do propionato de clobetasol em nanocápsulas, nanoesferas e nanoemulsões e nem estudos de incorporação deste fármaco em nanopartículas poliméricas ou nanoemulsões. Da mesma forma, não existe nenhum relato a respeito do desenvolvimento de uma forma farmacêutica semissólida contendo propionato de clobetasol associado à nanocápsulas, nanoesferas e nanoemulsões. Entretanto, alguns trabalhos com enfoque na aplicação cutânea têm demonstrado a preparação de géis contendo o fármaco associado a lipossomas (RAO & MURTHY, 2000; CAPÓ *et al.*, 2004) ou nanopartículas lipídicas (HU *et al.*, 2002; HU *et al.*, 2005; KALARIYA *et al.*, 2005; HU *et al.*, 2006; YUAN *et al.*, 2008).

Rao e Murthy, em 2000, avaliaram a liberação intradérmica (em pele de ratos e humanos) de uma formulação tópica (gel de hidroxipropilmetilcelulose) contendo lipossomas de propionato de clobetasol. No estudo *in vitro*, os dados foram analisados para calcular a quantidade de fármaco absorvida e não absorvida pela pele e a relação entre estes dois parâmetros. No estudo *in vivo* a liberação do fármaco foi analisada pelo ensaio do clareamento da pele (vasoconstrição). Analisando os resultados, os pesquisadores concluíram que houve uma baixa absorção do propionato de clobetasol pela pele a partir do gel contendo

a forma lipossomal, com uma baixa concentração sanguínea do fármaco, indicando o acúmulo destas partículas sobre a pele.

Em 2002, Hu e colaboradores, prepararam nanopartículas de lipídio sólido contendo propionato de clobetasol como modelo de fármaco hidrofóbico. Estas foram preparadas através do método da difusão do solvente em um sistema aquoso ácido (pH 1,10). Após preparadas, as nanopartículas contendo o propionato de clobetasol foram separadas por centrifugação e liofilizadas. Os resultados deste estudo demonstraram um método inovador para a preparação de nanopartículas de lipídio sólido e exibindo uma rápida liberação inicial (3 horas) seguida de uma liberação gradual (4 dias) para fármacos lipofílicos.

Capó e colaboradores, em 2004, avaliaram a ação timolítica do clobetasol após aplicação de duas diferentes formulações de gel, uma contendo lipossomas de clobetasol e a outra contendo clobetasol na sua forma livre. Este estudo foi realizado em ratos adrenalectomizados e os resultados mostraram que a forma lipossomal do clobetasol apresentou uma potência 2,35 vezes maior quando comparado com a sua forma livre, com limite de confiança entre 91 e 109 %. Com isso, os pesquisadores concluíram que houve um aumento na atividade do clobetasol quando incorporado em lipossomas e que isto ajudaria a reduzir a dosagem deste fármaco, levando a diminuição dos efeitos adversos.

Em 2005, Hu e colaboradores prepararam carreadores lipídicos nanoestruturados de ácido esteárico contento propionato de clobetasol, com diferentes proporções de ácido oléico, pelo método da difusão do solvente em sistema aquoso. O tamanho de partícula e sua morfologia foram influenciados pela quantidade de lipídio líquido utilizado. O aumento na proporção de ácido oléico (até 30 %) demonstrou uma crescente incorporação de fármaco e tamanho das nanopartículas, e estas apresentaram forma esférica e superfície lisa. Os resultados demonstraram que os nanocarreadores lipídicos podem ser empregados como um carreador de fármaco com liberação inicial rápida seguida de um prolongado efeito, dependente da quantidade de ácido oléico utilizado, quando terapeuticamente desejado.

Kalariya e colaboradores, em 2005, prepararam nanopartículas de lipídio sólido, contendo propionato de clobetasol, pelo método da homogeneização à alta pressão e as incorporaram em um creme base. O perfil de liberação do fármaco a partir do creme contendo as nanopartículas foi comparado com uma formulação comercial através do método da difusão em célula de Franz. Foi realizado, também, um ensaio clínico com dezesseis pacientes apresentando eczema crônico. A permeação *in vitro* apresentou um fluxo de absorção do fármaco menor para o creme contendo o fármaco incorporado nas nanopartículas em comparação com o creme comercial. O creme nanoestruturado apresentou uma significativa

melhora na resposta terapêutica (redução de 1,9 vezes na inflamação e 1,2 vezes no prurido) quando comparado com o creme comercial.

Entretanto, embora alguns trabalhos na literatura tenham demonstrado alguns métodos de preparação e as potencialidades da nanoincorporação do propionato de clobetasol para controlar a liberação e melhorar as suas atividades farmacológicas após aplicação cutânea (RAO & MURTHY, 2000; HU *et al.*, 2002; CAPÓ *et al.*, 2004; HU *et al.*, 2005; KALARIYA *et al.*, 2005; HU *et al.*, 2006; YUAN *et al.*, 2008), não há relatos na literatura de trabalhos dedicados a otimização de sua incorporação em nanocápsulas poliméricas, nanoesferas e nanoemulsões e sua respectiva incorporação em formulações semissólidas.

CAPÍTULO 1: Desenvolvimento e validação de método analítico por CLAE para determinação de propionato de clobetasol em suspensões de nanocápsulas para aplicação tópica

CAPÍTULO 1: Desenvolvimento e validação de método analítico por CLAE para determinação de propionato de clobetasol em suspensões de nanocápsulas para aplicação tópica

1.1 Introdução

O emprego de métodos analíticos validados para a quantificação de fármacos é muito importante para o monitoramento das várias etapas do estudo de desenvolvimento de uma formulação, contemplando desde os estudos de pré-formulação até o controle de qualidade do produto farmacêutico acabado, além de apresentar relevância nos estudos clínicos. A validação deve garantir que o método atenda às exigências das aplicações analíticas, assegurando a confiabilidade dos resultados, a qual deve apresentar especificidade, linearidade, intervalo de concentração, precisão, exatidão, limite de detecção e quantificação (BRASIL, 2003). A Agência Nacional de Vigilância Sanitária (ANVISA), o *International Conference on Harmonization* (ICH) e o *Food and Drug Administration* (FDA) têm disponibilizado guias com diretrizes que devem ser adotadas na validação de um método analítico ou bioanalítico.

Alguns métodos de análise têm sido descritos na literatura para a quantificação do propionato de clobetasol em produtos cosméticos, como xampu, creme e loção. Estes métodos contemplam a cromatografia líquida (CL) com detecção na região ultravioleta e também acoplada a espectrofotômetro de massas (REEPMEYER *et al*., 1998; GAGLIARDI *et al*., 2000; GAGLIARDI *et al.*, 2002; MOSTAFA *et al.*, 2002). Além disto, recentemente foi publicado um método para a análise de vários glicocorticóides, entre eles o propionato de clobetasol, em amostras biológicas (músculo, fígado e rim de suínos), através da extração por fase reversa e análise por CL com detecção em espectrofotômetro de massas (SHAO *et al*., 2009). Entretanto, não há relatos na literatura de métodos de análise para quantificação do propionato de clobetasol em nanopartículas poliméricas.

Diante do exposto, o presente capítulo tem como objetivo descrever o desenvolvimento e a validação de uma metodologia analítica para a quantificação do propionato de clobetasol em nanopartículas através da cromatografia líquida.

PUBLICAÇÃO 1: Development and Validation of a Fast RP-HPLC Method for the Determination of Clobetasol Propionate in Topical Nanocapsule Suspensions

Artigo aceito para publicação no periódico Journal of Chromatographic Science

Development and Validation of a Fast RP-HPLC Method for the Determination of Clobetasol Propionate in Topical Nanocapsule Suspensions

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Abstract

A simple and rapid HPLC method is validated for the determination of clobetasol propionate in topical nanocapsule suspensions. The method is carried out on an RP-18 column with a mobile phase composed of methanol-water (80:20 v/v) and UV detection at 241 nm. The method validation yields good results with respect to linearity, specificity, precision, accuracy, and robustness. The calibration curve in the range of 5.0-40.0 μg mL^{-1} shows a correlation coefficient of 0.9999. Precision (intra-day and inter-day) is demonstrated by the relative standard deviation lower than 1.5 %. Accuracy is assessed by the recovery test of clobetasol propionate from sample matrixes (98.33 ± 0.88 %). In conclusion, the method is suitable to be applied to assay clobetasol propionate in topical formulations of polymeric nanocapsules, avoiding the use of a buffer solution in the mobile phase.

Keywords: clobetasol propionate, high performance liquid chromatography, nanocapsules, validation

Introduction

Clobetasol propionate (Figure 1) is a potent topical glucocorticosteroid with a molecular mass of 467.0 Da (1-3). It is a white or almost white, crystalline powder; practically insoluble in water; freely soluble in acetone and in dichloromethane; and sparingly soluble in ethanol (3). The administration of clobetasol propionate is widely used for the treatment of skin disorders such as atopic dermatitis, capillaris dermatitis and psoriasis (2, 4-6). It has been used in clinical practice because of its anti-inflammatory, antipruriginous and vasoconstrictor activities (6). Prolonged therapy with clobetasol propionate preparations may result in adverse effects like skin atrophy, cutaneous reactivity and suppression of the hypothalamic-pituitary-adrenal axis (4). Furthermore, the use of greasy and high residual topical formulations (creams and ointments) could reduce patient compliance in long-term therapies (7).

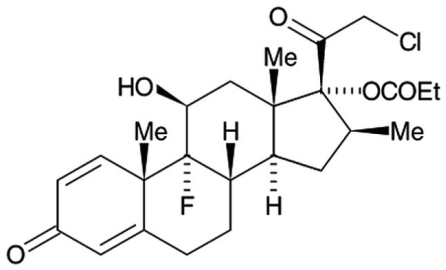

Figure 1. Chemical structure of clobetasol propionate

On the nanotechnology field some studies have been reported in the literature on the preparation of clobetasol propionate-loaded solid lipid nanoparticles and liposomes (8-10). However, no studies on the development of polymeric nanoparticles containing clobetasol propionate have been reported so far. This way, a novel pharmaceutical dosage form for topical administration of this drug consisting of clobetasol propionate-loaded nanocapsule suspensions is under development by our research group in order to reduce the irritation of the treated area and/or to allow its formulation in hydrophilic vehicles. Nanocapsules are polymeric nanoparticles composed of an oily core surrounded by a thin polymer wall, in which the drug could be dissolved in the oil core, dispersed within the particle or adsorbed at the interface particle/water (11). The small size of these carriers facilitates their formulation in dermatological products and enables comfortable application to the skin (12-13).

Some HPLC and spectrophotometric methods have been reported to assay clobetasol propionate in topical products (solutions, shampoos and creams), liposomes and solid lipid

nanoparticles (4, 9, 14-17). The United States Pharmacopoeia (17) presents an HPLC method to assay clobetasol propionate in topical solution. However, this method cannot be applied to nanocapsule formulations due to the use of a mobile phase (acetonitrile: 0.05 M phosphate buffer: methanol - 95:85:20 v/v) to dilute the samples. This sample preparation does not allow the release of clobetasol propionate encapsulated in the nanocapsules considering its high aqueous solvent concentration. In fact, literature does not show any validation of an HPLC method for quantitative determination of clobetasol propionate in nanocapsule suspensions. Thus, the aim of the present study was to develop and validate a simple and reliable HPLC method for clobetasol propionate assay in topical nanocapsule suspensions, avoiding the use of a buffer solution in the mobile phase.

Experimental

Materials and Reagents

Clobetasol propionate was obtained from Neo Química (Goiás, Brazil). HPLC grade acetonitrile and methanol were acquired from Tedia (São Paulo, Brazil). Poly-ε-caprolactone (PCL) and sorbitan monostearate (Span 60®) were purchased from Sigma-Aldrich (São Paulo, Brazil); caprilic/capric triglyceride mixture delivered from Brasquim (Porto Alegre, Brazil); polysorbate 80 (Tween 80®) was supplied by Henrifarma (São Paulo, Brazil); and acetone by Vetec (Rio de Janeiro, Brazil). All chemicals and solvents presented pharmaceutical grade and were used as received.

Clobetasol propionate-loaded nanocapsule suspensions were prepared by interfacial deposition of preformed polymer method as described by Fessi and co-workers (18). The formulations were prepared with (0.5 mg mL^{-1}) and without clobetasol propionate.

Apparatus and Chromatographic Conditions

Two HPLC systems were used in this study, which was performed at room temperature (25 ± 1 °C). HPLC A was employed to carry out all the validation study. HPLC B was used in order to compare the results obtained by two different apparatus as an intermediate precision. HPLC A consisted of a Shimadzu LC-10A system (Kyoto, Japan) equipped with a model LC-10AD pump, an UV-VIS SPD-10A Module, an SLC-10A system controller and RP-18 Gemini column (250 mm x 4.60 mm, 5 µm particle size, 110Å pore diameter) and HPLC B consisted of a Shimadzu LC-20A system (Kyoto, Japan) equipped with a model LC-20AT pump, an SPD-M20A PDA detector, a CBM-20A system controller,

SIL-20A auto sampler and RP-18 Gemini (250 mm x 4.60 mm, 5 µm particle size, 110Å pore diameter). The mobile phase consisted of a methanol-water (80:20 v/v) at isocratic flow rate (1 mL min^{-1}) until 9.0 min of run. The injection volume was 20 µL. Detection was performed at 241 nm.

Sample Preparation

Nanocapsule suspensions used for the evaluation of all parameters were freshly prepared. 1.0 mL of nanocapsule suspensions was diluted with acetonitrile to a concentration of 20.0 µg mL^{-1}. The use of acetonitrile was necessary to dissolve the nanocapsules and to release the entire drug from the nanocapsules. The resulting solution was filtered through a 0.45 µm membrane and injected in the HPLC system (n = 3).

Standard Solution

Stock standard solution (0.5 mg mL^{-1}) was prepared by dissolving 25.0 mg clobetasol propionate in 50.0 mL of methanol. From this solution, a working standard of 20 µg mL^{-1} was prepared by using 5 mL of the stock standard solution in 25.0 mL of mobile phase. In addition, the stock standard solution was diluted, as necessary, with the mobile phase to give five standard solutions with different concentrations of clobetasol propionate (5.0, 10.0, 20.0, 30.0 and 40.0 µg mL^{-1}), which were used in the linearity study. All solutions were filtered (0.45 µm) before injected (n = 3) in the HPLC system.

Method Validation

Validation was carried out assessing the following parameters: linearity, range, specificity, precision, accuracy, and detection and quantification limits, according to the International Conference on Harmonization (ICH) guidelines (19).

Specificity

Specificity was evaluated by analyzing solutions containing all the components of the clobetasol propionate-loaded NC suspensions, except the drug (blank NC suspensions). The system response was examined for the presence of interference or overlaps with clobetasol propionate responses.

Linearity, limits of detection and quantification

Linearity was evaluated by the injection and analysis of five concentrations of standard solutions in clobetasol propionate concentrations of 5.0, 10.0, 20.0, 30.0 and 40.0 μg mL^{-1}, as described in the preparation of the standard solution. Three independent calibration curves were constructed and linearity was evaluated by the least-squares regression analysis. Limits of detection (LOD) and quantification (LOQ) were calculated directly from the calibration plot. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation of intercept and S is the slope of the calibration plot (19).

Precision

Repeatability (intra-day precision) was evaluated by measuring, in triplicate, six different samples at the same concentration (20.0 μg mL^{-1}) under the same experimental conditions and on the same day. Intermediate precision was calculated from results obtained by the analysis of samples with the same concentration (20.0 μg mL^{-1}) on three different days (inter-day precision) or using two different HPLC apparatus (HPLC A and HPLC B – inter-apparatus precision). Precision (repeatability and intermediate precision) was expressed as relative standard deviation – RSD (%).

Accuracy

Accuracy was evaluated assaying, in triplicate, samples of known concentrations (NC suspensions) spiked with three different concentrations of standard solution (5.0, 10.0 and 20.0 μg mL^{-1}) at three different levels (lower, medium and upper concentration), giving sample solutions with concentration of 15.0, 20.0 and 30.0 μg mL^{-1}. Recovery (%) was calculated from differences between the peak areas obtained for spiked and unspiked solutions.

Robustness

Robustness was evaluated by the deliberate variation of the mobile phase, flow rate, and wavelength. Sample solutions were evaluated for each variation of the method conditions.

Results and Discussion

HPLC has been widely studied in pharmaceutical analysis, including drug assay in products based on nanotechnology (20-21). Nanoparticle suspensions are complex matrixes

composed of at least polymer, oil, and surfactants. This way, the analytical method to assay drugs in these systems must be carefully developed and validated to demonstrate its suitability. In this work, chromatographic conditions were adjusted in order to obtain efficient routine analysis. Methanol was chosen instead of acetonitrile as the organic solvent to compose the mobile phase due to its lower cost. Three proportions of the mobile phase (methanol–water) were evaluated: 70:30 (v/v), 80:20 (v/v) and 90:10 (v/v), showing retention time for clobetasol propionate of 15.40, 6.75 and 4.13 min, respectively. All proportions of mobile phase showed adequate free from tailing peaks of clobetasol propionate. However, considering that our goal was to obtain a run time less than 10 min, the proportion 70:30 (v/v) was discarded. Between the proportions 80:20 (v/v) and 90:10 (v/v), the former presented a higher number of theoretical plates (N = 8710) compared to the latter (N = 7387), which led us to choose the proportion 80:20 (v/v) for the following studies. This choice was also reinforced by the relative higher retention time under these conditions aiming to use the same conditions, with previous validation, to carry out future drug release and in vitro skin permeation studies. In the next step, we evaluated the use of a stationary phase with a different length (RP-18 Gemini column, 150 mm x 4.60 mm, 5 µm particle size, 110Å pore diameter), which showed a retention time lower than 4 minutes and a lower number of theoretical plates (N = 3845). In order to test the influence of the pH of the mobile phase, we used ortophosphoric acid (20 % w/v) to adjust the apparent pH of the mobile phase to pH 3.0 and pH 5.0. The mobile phase without pH adjustment showed apparent pH 6.9. No influence of pH was observed on retention time, number of theoretical plates and asymmetry. The increase of the flow rate to 1.2 mL min^{-1} was also evaluated. Under these conditions, the retention time of clobetasol propionate was 5.65 min, which represented a low decrease compared to the use of a flow rate of 1.0 mL min^{-1}. This higher flow rate led to an increase in the HPLC system pressure (above 150 kgf), which could reduce the column life. We also evaluated the possible precipitation of the sample after its redispersion in mobile phase. No precipitation of any component of the formulations was observed because their low concentration in the sample as well as the high amount of organic solvent (methanol) in the mobile phase. In addition, no significant increase on the pressure of the HPLC system during the analyses was observed (even after more than 300 injections of sample solutions in the same column over 6 months) which could be related to the precipitation of some material at the front part of the column. Regarding the peak shape, it was not observed any significant change in the clobetasol propionate peak by using acetonitrile (sample solvent) instead of mobile phase (standard solution solvent), as can be visualized in Figure 2 (A and B). Thus, the

mobile phase composed of methanol and water in the proportion 80:20 (v/v) at a flow rate of 1.0 mL min^{-1} was considered reliable, suitable and adequate, with a retention time for clobetasol propionate of 6.75 min (Figure 2) and run time of 9 minutes. Compared to the USP method (17) to assay clobetasol propionate in topical solutions, the developed method avoids the use of a buffer solution in the mobile phase contributing for the lifetime increase of columns and other components of the chromatographic system.

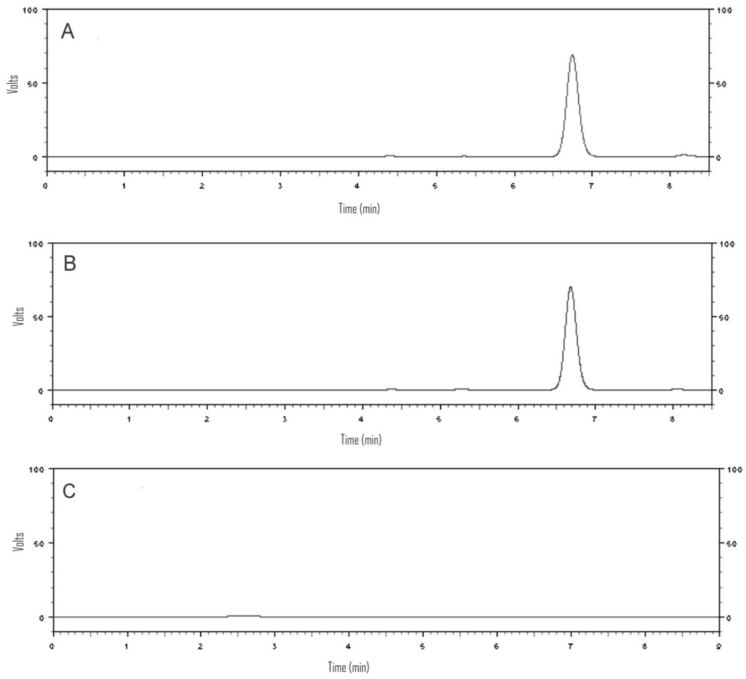

Figure 2. Chromatograms obtained from: A) clobetasol propionate reference substance (20 µg mL^{-1}); B) clobetasol propionate-loaded nanocapsule suspensions (20 µg mL^{-1}); C) unloaded nanocapsule formulations (placebo formulation).

Regarding the specificity evaluation, the chromatograms shown in Figure 2 demonstrate that the method is specific and no interference from the excipients was observed. In order to confirm this absence of interference, a peak-purity evaluation using the photodiode-array (PDA) was carried out. These analyses showed that no impurities and/or excipients were co-eluting with the clobetasol propionate peak.

Good linearity was observed in the 5.0-40.0 µg mL^{-1} range. The linear equation obtained by the least-square method was y = 38640.69x – 14382.43 and showed an adequate determination coefficient (r^2 = 0.9999). The validity of the assay was verified by analysis of variance. This revealed that the regression equation was linear ($F_{calculated}$ = 13655 > $F_{critical}$ = 4.96, P = 5 %) with no linearity deviation ($F_{calculated}$ = 0.43 < $F_{critical}$ = 3.71; P = 5 %). In

addition, the t-test of the y-intercept ($t_{calculated}$ = - 2.69, $p > 0.05$) showed that it did not differ significantly from zero. LOD and LOQ were 0.45 and 1.38 µg mL^{-1}, respectively.

Repeatability (intra-day precision) and intermediate precision (inter-day and inter-apparatus precision) are given in Table 1. All data are lower than the acceptance criterion of 2 %. Regarding the accuracy evaluation, good recoveries (97 – 100 %) were obtained (Table 2).

Table 1. Results from the repeatability (intra-day precision) and intermediate precision (inter-day and inter-apparatus precision) of the method

	Theoretical amount (µg mL^{-1})	Experimental amount (µg mL^{-1} ± SD)	% Recovered	RSD (%)
Intra-day (n = 6)	20.0	20.05 ± 0.33	100.23 ± 1.64	0.37
Inter-day				
Day 1 (n = 3)	20.0	19.85 ± 0.22	99.24 ± 1.09	1.10
Day 2 (n = 3)	20.0	19.87 ± 0.10	99.35 ± 0.49	0.49
Day 3 (n = 3)	20.0	19.93 ± 0.23	99.64 ± 1.17	1.17
Mean ± SD (n = 9)	20.0	19.88 ± 0.04	99.41 ± 0.21	0.21
Inter-apparatus				
HPLC A (n = 3)	20.0	20.17 ± 0.25	100.85 ± 1.20	1.22
HPLC B (n = 3)	20.0	19.93 ± 0.23	99.65 ± 1.15	1.15
Mean ± SD (n = 6)	20.0	20.04 ± 0.25	100.20 ± 1.25	1.25

Regarding the evaluation of robustness, the deliberate variation of the method conditions had no significant effect on assay data or on chromatographic performance, indicating the robustness of method. The results from robustness testing are presented in Table 3. With respect to the composition of HPLC mobile phase, no significant influence in % content of clobetasol propionate was found when changing the mobile phase composition to 75:25 (methanol:water) and also 85:15 and flow rates at 1.00 ± 0.10 mL min^{-1}. The effect of wavelength was studied by varying ± 4 nm. In order to demonstrate the applicability, clobetasol propionate-loaded nanocapsule suspensions were assayed (3 batches) using the

conditions described in this study. The determination of drug content in sample solutions showed results according to the theoretical value (0.500 ± 0.005 mg mL^{-1}; 0.515 ± 0.005 mg mL^{-1}; 0.510 ± 0.005 mg mL^{-1}). RSD values were lower than 2.0 % from triplicate analysis of each suspension, which indicates a precise analytical methodology.

Table 2. Results from accuracy determination of the method

Amount of clobetasol propionate			Recovery (%)	RSD (%)
Known sample	Added µg mL^{-1}	Found µg mL^{-1}		
10.0 ± 0.03	5	14.67 ± 0.06	97.81 ± 0.55	0.57
10.0 ± 0.03	10	19.87 ± 0.14	99.35 ± 0.76	0.77
10.0 ± 0.03	20	29.35 ± 0.05	97.83 ± 0.49	0.50

Table 3. Results from study of method robustness

Conditions		% clobetasol propionate	RSD (%)
Recommended conditions*		99.38	0.24
Mobile phase (methanol-water)			
	75:25	99.16	0.13
	85:15	99.21	0.72
λ (nm)			
	237	99.45	0.64
	245	99.88	0.75
Flow rate (mL min^{-1})			
	0.90	99.75	0.63
	1.10	99.39	0.41

* The recommended chromatographic conditions were: RP-18 Gemini column (250 mm x 4.60 mm, 5 µm particle size, 110Å pore diameter) with methanol–water 80:20 (v/v) as mobile phase at a flow rate of 1.0 mL min^{-1}, and UV detection at 241 nm.

Conclusions

A rapid, specific and reliable HPLC method has been developed and validated for the assay of clobetasol propionate in topical nanocapsule suspensions, which are complex

polymeric mixtures. The analytic methodology proposed is simple, precise, accurate and linear in the concentration range of 5.0-40.0 μg mL^{-1}. Furthermore, the method involves the use of a simple mobile phase without buffer solution and minimum sample preparation.

Acknowledgements. The authors thank the financial support of Rede Nanocosméticos/CNPq. M. O. Bastos thanks Programa FIPE Jr/UFSM for his fellowship.

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CAPÍTULO 2: Nanoencapsulação no controle da liberação e aumento da fotoestabilidade do propionato de clobetasol: influência do sistema nanoestruturado

CAPÍTULO 2: Nanoencapsulação no controle da liberação e aumento da fotoestabilidade do propionato de clobetasol: influência do sistema nanoestruturado

2.1 Introdução

A farmacologia dermatológica possibilita o acesso direto do fármaco à pele como um órgão-alvo para diagnóstico e tratamento. Os fármacos devem penetrar no interior do tecido para ter eficácia, mas muitas vezes eles chegam a corrente sanguínea causando efeitos colaterais indesejáveis (GOODMAN & GILMAN, 2007). O propionato de clobetasol é utilizado na terapêutica da psoríase e dermatite atópica e apresenta muitos efeitos colaterais, como a irritação da pele e a atrofia celular (FANG *et al.*, 1999). Estes efeitos indesejáveis poderiam ser minimizados através da incorporação do fármaco em um sistema nanoestruturado, proporcionando uma liberação homogênea e protegendo a pele do contato imediato com o fármaco (BOUCHEMAL *et al.*, 2004). Até o momento, a nanoprecipitação é o método mais utilizado na preparação de nanopartículas para aplicação cutânea, por ser um método simples, espontâneo, eficiente, reprodutível e apresentar uma alta capacidade de incorporação de fármacos. Neste método, a poli(ε-caprolactona) é o polímero semicristalino, biocompatível e biodegradável que tem sido mais empregado na preparação das nanopartículas poliméricas (ALVAREZ-ROMÁN *et al.*, 2001; MILÃO *et al.*, 2003; JIMÉNEZ *et al.*, 2004; ALVES *et al.*, 2007; OURIQUE *et al.*, 2008; ALMEIDA *et al.*, 2009). Ainda não existem trabalhos na literatura com a incorporação do propionato de clobetasol em nanopartículas poliméricas e nanoemulsões, apenas existem trabalhos com a preparação de nanopartículas lipídicas e lipossomas contendo este fármaco (RAO & MURTHY, 2000; HU *et al.*, 2002; CAPÓ *et al.*, 2004; HU *et al.*, 2005; KALARIYA *et al.*, 2005; HU *et al.*, 2006; YUAN *et al.*, 2008). A maior parte destes trabalhos relata o emprego do propionato de clobetasol como modelo de fármaco hidrofóbico. Portanto, este capítulo descreve o desenvolvimento e a caracterização de suspensões de nanocápsulas poliméricas e nanoemulsões contendo propionato de clobetasol, empregando a poli(ε-caprolactona) como polímero biodegradável. Além disso, neste capítulo é abordado o estudo da fotoestabilidade do fármaco frente à luz UVA e o estudo da liberação *in vitro* do fármaco.

PUBLICAÇÃO 2: Nanoencapsulation as a way to control the release and to increase the photostability of clobetasol propionate: influence of the nanostructured system

Artigo publicado no periódico Journal of Biomedical Nanotechnology

Nanoencapsulation as a way to control the release and to increase the photostability of clobetasol propionate: influence of the nanostructured system

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Abstract

The aim of this study was to prepare and to evaluate the physicochemical and *in vitro* drug release characteristics of different nanostructured systems containing clobetasol propionate (CP): CP-loaded polymeric nanoparticles (nanocapsules and nanospheres) and CP-loaded nanoemulsion. Physicochemical characteristics of the formulations were monitored up to 9 months after preparation by means of drug content, encapsulation efficiency, mean size, polydispersity index, pH, and zeta potential. *In vitro* drug release studies were carried out using the dialysis bag method. Photostability of CP-loaded nanoparticles was evaluated by their exposition to UVA radiation. All formulations presented nanometric mean size (140 - 220 nm), polydispersity index below 0.25, neutral pH values, negative zeta potential and encapsulation efficiency close to 100 %. All these parameters, except pH, remained unchangeable up to 9 months of storage at room temperature for CP-loaded nanocapsules. On the other hand, CP-loaded nanospheres and nanoemulsion showed an increase in their mean size, as well as in polydispersity index under storage (after 6 and 9 months, respectively). *In vitro* drug release studies showed a controlled release of CP from nanoparticles (nanocapsules > nanospheres > nanoemulsion) with a low burst release. Photostability of CP under UVA radiation was improved by its incorporation into nanoparticles (nanocapsules > nanoemulsions > nanospheres).

Key-Words: Clobetasol propionate, nanocapsules, nanoemulsions, nanoparticles, in vitro drug release, photostability.

Nanoencapsulation as a way to control the release and to increase the photostability of clobetasol propionate: influence of the nanostructured system

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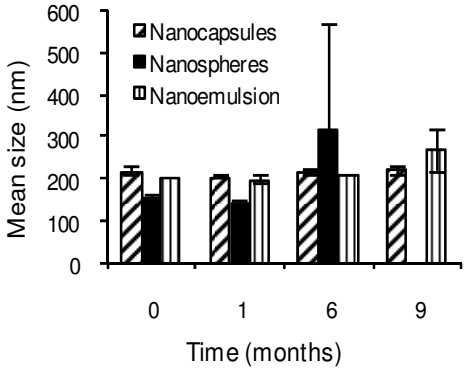
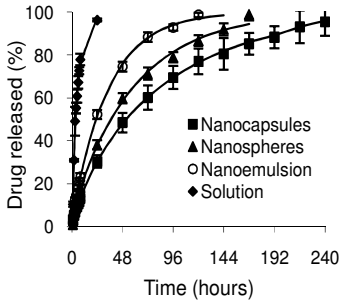




Formulations showed particle sizes in the submicrometric range. *In vitro* drug release studies demonstrated the role of the polymer to control the drug release from the nanostructures. Nanoencapsulation of clobetasol propionate allows improvement of its photostability against UVA radiation.

1. INTRODUCTION

Clobetasol propionate is the most potent available topical glucocorticoid.[1] Its clinical effectiveness in the treatment of psoriasis and atopic dermatitis is related to its vasoconstrictive, anti-inflammatory, immunosuppressive, and antiproliferative effects.[2] Prolonged therapy with clobetasol propionate preparations may result in adverse effects specific for topical therapy such as cutaneous reactivity, skin atrophy and suppression of the hypothalamic-pituitary-adrenal axis.[3] Even short-term treatments with clobetasol propionate applied once a day for 3 days can alter epidermal structure and function in humans.[4] From the formulation point of view, clobetasol propionate presents some drawbacks due to its low water solubility, leading to the development of hydroalcoholic solutions, ointments or creams, as well as its significant photodegradation under UV light exposition.[5]

Nanoparticle (submicronic system) is a generic term to refer to nanocapsules (NC) and nanospheres (NS), which are polymeric nanocarriers presenting vesicular and matricial structures, respectively.[6] Nanoemulsion, a non-polymeric nanocarrier, is a submicrometric emulsion.[7] These colloid systems are stabilized by surfactants at the interface particles/water, preventing particle agglomeration and/or drug leakage.[6,8] They present some advantages for topical application like a sustained release, a decrease in drug toxicity, and a high inclusion rate for lipophilic substances. Their small particle size provides an increase in the solubility of some drugs, close contact to the stratum corneum, as well as a formation of a film on the skin surface[9], which is desirable in some skin diseases such as atopic eczema.[10] Other advantages of these systems include protection of labile substances from chemical degradation induced by the UV light[11-14] and a decrease in cutaneous irritation.[15]

Microspheres[16], liposomes[17,18] and nanostructured solid lipid carriers[15,19-22] have already been proposed for clobetasol propionate targeting the skin. However, up to now no studies have been reported in the literature on the preparation of clobetasol propionate-loaded polymeric nanoparticles or clobetasol propionate-loaded nanoemulsions.

Rao and Murthy (2000) prepared a topical formulation containing clobetasol propionate-loaded liposomes for intra-dermal delivery (rat and human skin), which presented a low absorption into the blood stream indicating its main accumulation in the skin. Another work has showed an increase of the thymolitic activity in rats (2.35 fold) after clobetasol propionate-loaded liposome administration compared to its free form.[18] According to the authors, this increase could help to reduce the dosage of this drug followed by the decrease of the adverse effects. However, the development of liposomes has been limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the

presence of blood components and poor physicochemical stability under storage compared to the polymeric nanoparticles.[6,23]

Kalariya and co-workers (2005) incorporated clobetasol propionate-loaded solid lipid nanoparticles in a cream base to evaluate the *in vitro* drug permeation (Franz static diffusion cell across human cadaver skin) and its clinical performance in the treatment of chronic eczema patients compared to an equivalent marketed formulation. Clobetasol propionate-loaded solid lipid nanoparticles presented particle size of 177 nm and drug entrapment of 92.05 %. *In vitro* permeation studies revealed a lower mean flux value and higher skin uptake of clobetasol propionate from cream containing solid lipid nanoparticles compared to the marketed drug cream. This study demonstrated that the entrapment of clobetasol propionate in solid lipid nanoparticles significantly improved the therapeutic response in terms of reduction of inflammation and itching compared to the marketed cream.[15]

Based on these considerations, the aim of the present work was to develop new clobetasol propionate-loaded nanocarriers. The final goal was to improve its photostability against UVA light exposition, allowing the preparation of aqueous formulation of clobetasol propionate for topical treatments. In addition, the study aimed the control of drug release which can potentially reduce the immediate contact of the total applied drug to the skin. In this way, three nanocarriers presenting different structural organization (nanocapsules - NC, nanospheres - NS and nanoemulsion - NE) were compared in order to establish a rational comprehension of their behavior to select the best formulation. They were characterized by means of drug content, encapsulation efficiency, pH, mean size, polydispersity index, TEM, zeta potential and stability under storage. In addition, we investigated the in vitro drug release from the different nanostructured systems, as well as the photochemical stability of clobetasol propionate in the formulations under UVA radiation.

2. MATERIALS AND METHODS

2.1. Materials

Clobetasol propionate was a gift from Neo Quimica (Goiás, Brazil). Poly(ε-caprolactone) (PCL) and sorbitan monostearate (Span 60®) were purchased from Sigma-Aldrich (São Paulo, Brazil); caprylic/capric triglyceride mixture was delivered from Brasquim (Porto Alegre, Brazil); polysorbate 80 (Tween 80®) and polyethylene glycol 400 was supplied by Henrifarma (São Paulo, Brazil) and ALPHA Química (Porto Alegre, Brazil), respectively; dialysis bags (Spectra Por 7, 10 Kd, Spectrum Laboratories, USA) were purchased from

Bioagency (São Paulo, Brazil) and acetone from Vetec (Rio de Janeiro, Brazil). Clobetasol propionate commercial solution (Capillary solution, Merck) was purchased locally and claimed to contain 0.5 mg mL^{-1}. HPLC grade acetonitrile and methanol were acquired from Tedia (São Paulo, Brazil). All chemicals and solvents presented pharmaceutical or HPLC grade and were used as received.

2.2. Preparation of nanoparticles

Clobetasol propionate-loaded NC, NS and NE were prepared by interfacial deposition of preformed polymers[24], nanoprecipitation[25] and spontaneous emulsification[26] respectively, at a concentration of 0.5 mg mL^{-1}. For the preparation of NC, 25 mg of polymer (PCL), 191.5 mg of sorbitan monostearate, 0.82 mL caprylic/capric triglyceride mixture and 12.5 mg of clobetasol propionate were dissolved in 67 mL of acetone. This organic solution was added under moderate magnetic stirring into 134 mL of an aqueous phase containing 191.5 mg of polysorbate 80. Magnetic stirring was maintained for 10 min. Then, acetone was removed and the aqueous phase concentrated by evaporation (bath at 40 °C) under reduced pressure (0.07 bar) and the final formulation was adjusted to 25 mL. NS was prepared omitting the presence of the oily phase (CP-NS) and for the preparation of NE it was omitted the presence of the polymer (CP-NE). In order to study the influence of clobetasol propionate on the nanodispersions, blank NC, NS and NE formulations were prepared as described above omitting the addition of the drug (NC-B, NS-B and NE-B, respectively). All formulations were made in triplicate, stored at room temperature (25 ± 2 °C) and protected from light (amber glass flasks).

2.3. Characterization of nanoparticles

2.3.1. Determination of drug content and encapsulation efficiency

Total drug was determined after dissolution of nanoparticles (1 mL) in 25 mL of acetonitrile. Free clobetasol propionate (non-associated to nanostructures) was determined in the ultrafiltrate after separation of the nanoparticles by ultrafiltration/centrifugation technique (Ultrafree-MC 10,000 MW, Millipore) at 12,000 rpm during 5 min. Drug entrapped in the nanostructures was calculated by the difference between the total and the free drug concentrations, measured in the nanoparticles (total drug) and in the ultrafiltrate (nonentrapped drug), respectively. Encapsulation efficiency was determined by the quotient of the drug entrapped and total drug content. Clobetasol propionate was assayed by HPLC according to a method previously validated[27] that consisted of a Shimadzu LC-20A system

(Kyoto, Japan) equipped with a model LC-20AT pump, an SPD-M20A PDA detector, a CBM-20A system controller, SIL-20A auto sampler and an RP-18 Gemini column (250 mm x 4.60 mm, 5 µm particle size, 110Å pore diameter). The mobile phase consisted of a methanol-water (80:20 v/v) at isocratic flow rate (1 mL min^{-1}) until 9.0 min of run and UV detection at 241 nm. The injection volume was 20 µL.

2.3.2. pH measurements

pH values of nanoparticle formulations were determined directly in the dispersions using a calibrated potentiometer (MPA-210 Model, MS-Tecnopon, São Paulo, Brazil).

2.3.3. Particle size analysis, polydispersity indices and zeta potential

Particle sizes and polydispersity indices (n = 3) were estimated by photon correlation spectroscopy (PCS) after adequate dilution of an aliquot of the formulation in purified water (Zetasizer Nanoseries, Malvern Instruments, Worcestershire, UK). Zeta potentials were measured using the same instrument at 25 °C, after the dilution of the samples in 10 mM NaCl aqueous solution.

2.3.4. Morphological analyses

Morphological analyses were conducted at Centro de Microscopia (UFRGS, Brazil) by transmission electron microscopy (TEM; Jeol, JEM 1200 Exll, Japan) operating at 80 kV. Diluted suspensions and nanoemulsions were deposited on specimen grid (Formvar-Carbon support films, Electron Microscopy Sciences), negatively stained with uranyl acetate solution (2% w/v)[28] and observed at different magnifications.

2.4. Stability studies

The effect of storage time on the different nanoparticle formulations was monitored up to 9 months after preparation by means of drug content, encapsulation efficiency, pH, particle size, polydispersity index and zeta potential. Formulations were stored at room temperature (25 ± 2 °C) and protected from light (amber glass flasks with polypropylene closures).

2.5. *In vitro* drug release assay

In vitro drug release profiles from CP-NC, CP-NS and CP-NE were evaluated (n = 3) by the dialysis bag method, using water/Tween 80®/PEG 400 (60:0.5:40 v/v) pH 7.60 as medium, at 37°C.[19] The dialysis bag (Spectra Por 7, 10 Kd), containing 1 mL of the sample

(0.5 mg mL^{-1}), was put into a 250 mL erlenmeyer which contained 200 mL of dissolution medium under constant moderate stirring. 2 mL of the external medium was withdrawn from the system at predetermined time interval, replaced by an equal volume of fresh medium, and filtered through a 0.45 µm membrane. Clobetasol propionate was assayed in the samples by HPLC according to the method previously described. However, in order to allow a better drug detection, the injection volume was adjusted to 100 µL. This HPLC method was validated according to the following characteristics[29]: linearity (y = 190399.44x – 762.24, n = 3, r^2 = 0.9995), range (0.1 – 3.0 µg mL^{-1}), precision (repeatability: 0.06 - 0.40 %; intermediate precision: 0.19 - 0.86 %), and accuracy (98.15 - 101.79 %).

The diffusion of free clobetasol propionate across the dialysis bag was evaluated and used as control. In this case, we prepared an ethanol solution of clobetasol propionate (CP-ES) at the same concentration (0.5 mg mL^{-1}).

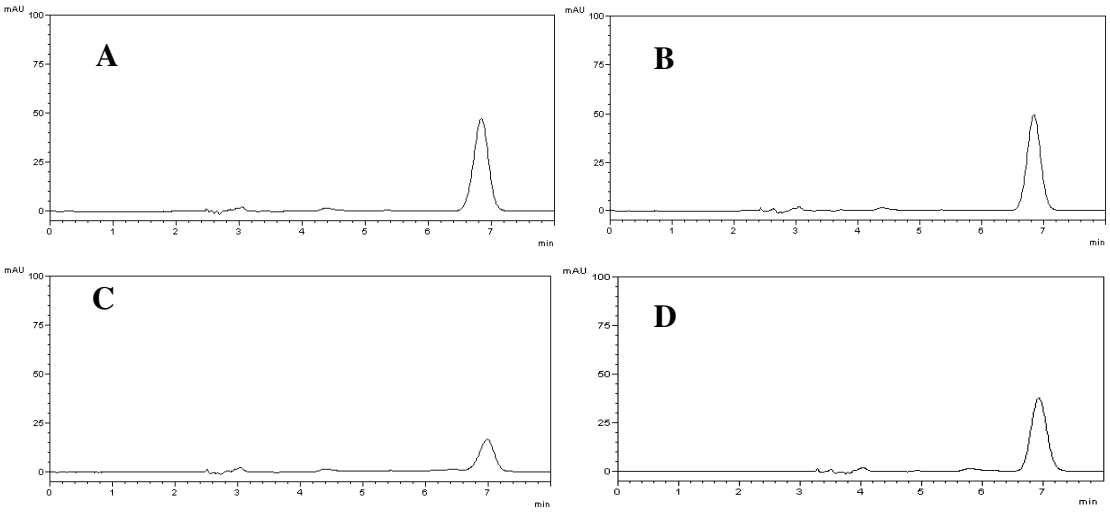
In order to obtain a better understanding of the influence of the type of nanoparticle structure on the drug release behavior of the nanoparticles, a mathematical modeling (MicroMath® Scientist® for Windows™) was used to analyze the drug release profiles[30]. Monoexponential ($C = C_0\ e^{-kt}$) and biexponential ($C = a\ e^{-k1t} + b\ e^{-k2t}$) models were used to evaluate the drug release profiles. The release rate constants are k, k_1 and k_2 and the initial concentration of drug are C_0, a and b. The selection of the model that best fitted the release profiles was based on the best correlation coefficient, the best model selection criteria (MSC), both provided by the software, and the best graphic adjustment.

2.6. Photodegradation studies

Photodegradation experiments were carried out using a UVA artificial lamp (Fluorescent blacklight blue lamps, 30 W, Ecolume). Clobetasol propionate-loaded nanoparticles (2 mL in a 5 mm quartz cuvette perfectly stoppered) were exposed to UVA radiation for 24 hours at a fixed distance of 16 cm (n = 3). As control, we evaluated the photostability of a clobetasol propionate ethanol solution (n = 3). After the appropriate exposure interval, 200 µL of samples was withdrawn, transferred into a 5 mL calibrated flask, diluted to volume with acetonitrile and filtered (0.45 µm membrane filter). Clobetasol propionate was assayed by HPLC according to a method previously validated, as described in 2.3.1. No additional peaks were observed in the chromatogram of samples irradiated by UVA light (Figure 1) and clobetasol propionate peak purity was close to 100 % for samples of all time points, as showed by the peak purity evaluation using the photodiode-array (PDA).

Results were expressed as percentage of clobetasol propionate degraded. The experiment was conducted until obtaining more than 50 % of photodegradation. In order to discard the hypothesis of thermal degradation, similar experiments were carried out with all formulations and solution covered by aluminum foil (protected from UV light). In addition, a clobetasol propionate commercial solution – CP-CS (Capillary solution, Merck) was also submitted to this photodegradation study. During irradiation the UVA-intensity was monitored and kept at 1.0 ± 0.1 mW/cm² at the sample level (UV-400 Model, ICEL Manaus, Brazil). The order of clobetasol propionate degradation in all samples was calculated using the graphic method.[31] Zero, first and second order graphs were drawn by plotting clobetasol propionate remaining (µg mL^{-1}) versus time, ln [clobetasol propionate remaining (µg mL^{-1})] versus time and 1/[clobetasol propionate remaining (µg mL^{-1})] versus time. The correlation coefficient of each graph was calculated. The plot with the best linearity was considered as the best description of the reaction order.

Figure 1. Chromatograms obtained in the photodegradation study for: (A) and (C) clobetasol propionate ethanol solution, at time 0 and after 8 h of UVA exhibition, respectively; (B) and (D) clobetasol propionate-loaded nanocapsules, at time 0 and after 8 h of UVA exhibition, respectively.



2.7. Statistical analysis

All formulations were prepared and analyzed in triplicate. Results are expressed as mean ± SD (standard deviation). One-way analysis of variance (ANOVA) was employed for comparison of the experimental data. Post-hoc multiple comparisons were done by Tukey's

test or Holm-Sidak test for significance at p–values ≤ 0.05. All analyses were run using the SigmaStat Statistical Program (Version 3.0, Jandel Scientific, USA).

3. RESULTS AND DISCUSSION

3.1. Preparation and characterization of nanostructured systems

After preparation, all formulations presented a macroscopic homogeneous appearance, like a milky bluish opalescent liquid. Physicochemical characteristics of the formulations after the preparation are presented in Table 1.

Table 1. Physicochemical characteristics of clobetasol propionate-loaded nanocapsules (CP-NC), clobetasol propionate-loaded nanospheres (CP-NS), clobetasol propionate-loaded nanoemulsions (CP-NE), unloaded nanocapsules (NC-B), unloaded nanospheres (NS-B) and unloaded nanoemulsion (NE-B)

Formulation	Drug content (mg mL^{-1})	Encapsulation efficiency (%)	pH	Particle size (nm)	PDI*	Zeta potential (mV)
CP-NC	0.51 ± 0.01	99.84 ± 0.06	$7.25 \pm 0.07^{a,b}$	$218 \pm 11^{a,b}$	0.16 ± 0.05^{a}	-7.25 ± 1.20^{a}
NC-B	-	-	7.12 ± 0.06^{b}	$213 \pm 05^{a,b}$	0.15 ± 0.01^{a}	-8.69 ± 1.01^{a}
CP-NS	0.46 ± 0.01	99.90 ± 0.01	$7.20 \pm 0.03^{a,b}$	152 ± 08^{d}	0.16 ± 0.04^{a}	-6.94 ± 0.72^{a}
NS-B	-	-	7.09 ± 0.06^{b}	148 ± 02^{d}	0.12 ± 0.03^{a}	-7.93 ± 0.91^{a}
CP-NE	0.50 ± 0.01	99.91 ± 0.01	7.33 ± 0.05^{a}	$204 \pm 03^{b,c}$	0.22 ± 0.03^{a}	-7.91 ± 0.81^{a}
NE-B	-	-	7.33 ± 0.05^{a}	196 ± 03^{c}	0.16 ± 0.02^{a}	-7.60 ± 0.58^{a}

Means, in column, with the same letter are not significantly different (p ≤ 0.05, ANOVA).

* PDI: polydispersity index

The formulations had drug content close to their theoretical value (0.5 mg mL^{-1}). Regarding the encapsulation efficiency, similar results were obtained for all formulations (higher than 99 %), independently of the nanostructure type (nanocapsules, nanospheres, nanoemulsion). Formulations showed neutral pH, particle sizes in the submicrometric range (140 - 220 nm), polydispersity index below 0.25 and negative zeta potential (mean values between -6.9 and -8.7 mV). Statistical analysis of the results presented in Table 1 did not show any influence of the presence of the drug on the physicochemical characteristics, being the results similar between loaded and unloaded formulations. The type of nanostructure influenced the mean particle size (NC > NE > NS). In the presence of the oily phase the

particles tend to present a larger mean size, which can be explained by the viscosity of the oil, which influenced the droplet formation during the addition of the organic to the aqueous phase.[6] On the other hand, the type of nanostructures did not influence the polydispersity index and the zeta potential, being in agreement with a previous report for indomethacin ethyl ester-loaded nanocapsules, nanospheres and nanoemulsion.[30] Similar zeta potential values are a consequence of the particle/emulsion coating with polysorbate 80. So, the physical colloidal stability of those nanocarriers is due to the steric effect of that surfactant at the interface particle/water. The negative values (~ -8 mV) are a consequence of the negative surface density of charge due to the presence of oxygen atoms in the molecules. Morphological analysis by transmission electron microscopy carried out for the clobetasol propionate-loaded nanoparticles showed homogeneous spherical-shaped particles (Figure 2), whose diameters were in agreement with those determined by PCS.

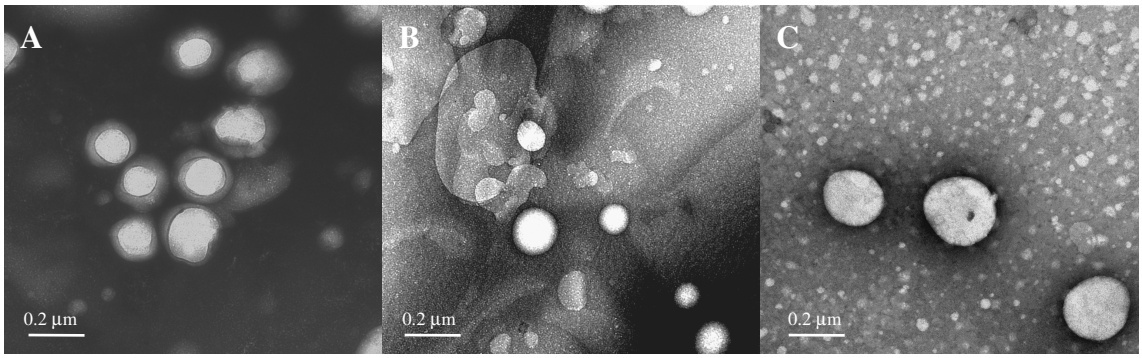


Figure 2. Transmission electron microscopy images of (A) clobetasol propionate-loaded nanocapsules, (B) clobetasol propionate-loaded nanospheres or (C) clobetasol propionate-loaded nanoemulsion [bar = 200 nm (100,000x)].

3.2. Stability studies

All formulations kept their milky white opalescent characteristic during the storage time. Results of mean size, polydispersity index and zeta potential of the formulations monitored for 9 months are presented in Figure 3. Regarding the mean sizes (200 - 230 nm), the polydispersity indexes (from 0.10 to 0.25), and the zeta potential values (from -6 to -14 mV), clobetasol-propionate-loaded NC could be considered stable for 9 months. On the other hand, NS and NE formulations showed an increase in the mean size, as well as in the polydispersity index after 6 and 9 months, respectively, showing their lower stability compared to the NC formulations. At these time periods, an increase of the standard deviation in these parameters (mean size and polydispersity index) were also observed. Due to these results, physicochemical characterization was not carried out for CP-NS after 9 months of

storage. Similar results were observed for the blank formulations (data not showed) demonstrating that the presence of clobetasol propionate did not alter those parameters during the stability studies. As can be seen in Figure 3C, for all formulations the zeta potential remained negative during the storage time, presenting a slight increase (in module) which could be viewed as a positive aspect in terms of physical stability. The interfacial triglyceride hydrolysis furnishes free fatty acids, which contributed to a decrease in the pH values, as well as caused a decrease in the zeta potential values due to the augmentation of the negative charges at the interface. The interaction of water and oil at the nanoscale was previously demonstrated.[32] High zeta potential values (in module) suggest low probability of particle aggregation.[6]

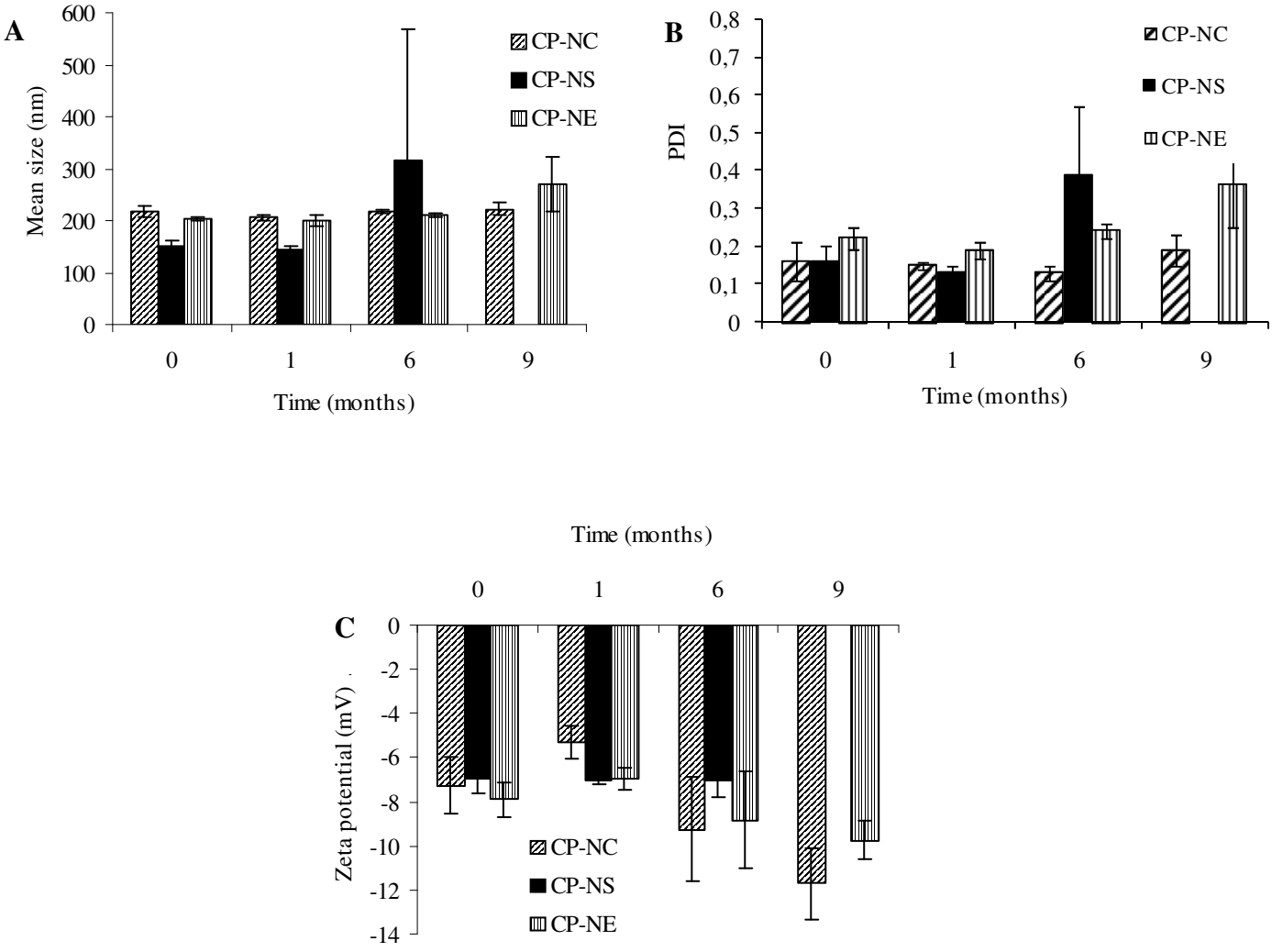


Figure 3. Physicochemical characteristics of clobetasol propionate-loaded nanocapsules (CP-NC), clobetasol propionate-loaded nanospheres (CP-NS) and clobetasol propionate-loaded nanoemulsion (CP-NE) during the storage time (room temperature and protected from light). A: mean size; B: polydispersity index (PDI) and C: zeta potential.

On the other hand, all formulations presented a decrease in values of pH after 1 month of storage up to 9 months (Figure 4). The decrease in the pH values could be explained by the hydrolysis of the triglyceride chains and the respective increase of the free fatty acid content, by the polymer chains relaxation, which exposes a higher number of terminal carboxylic groups or by the polymer degradation.[6,7,33-36] Regarding the drug content and the encapsulation efficiency, the formulations did not present any significant alteration during the storage time compared to the values determined after the preparation (Table 2). Peak-purity evaluation of chromatograms showed that no degraded products were co-eluting with the peak of clobetasol propionate during all stability study. Drug content and encapsulation efficiency were not determined for CP-NS and CP-NE after 6 and 9 months, respectively, due to their physical instability (mean particle size and polydispersity index) observed at this time point as discussed before.

Considering all results together, we can observe the role of the oily phase to maintain the stability of the colloidal dispersions (in terms of mean size and polydispersity index), in which CP-NC and CP-NE presented a higher stability on storage in relation to CP-NS. In addition, the presence of the polymer around the oily core (NC) prevented the agglomeration/coalescence of the vesicles ($p < 0.05$).

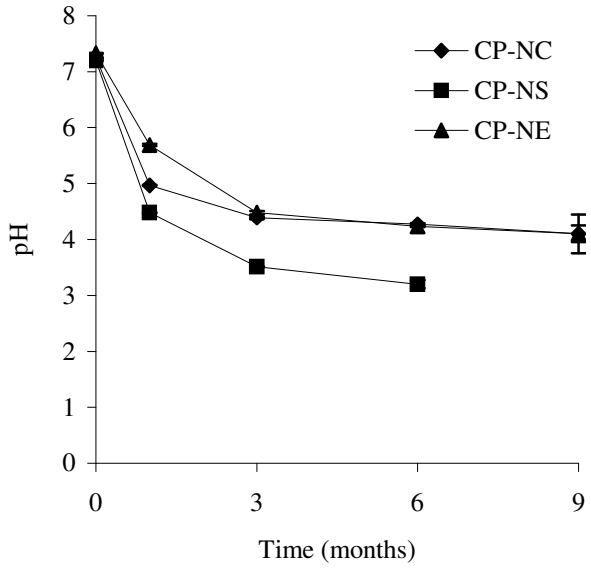


Figure 4. pH of clobetasol propionate-loaded nanocapsules (CP-NC), clobetasol propionate-loaded nanospheres (CP-NS) and clobetasol propionate-loaded nanoemulsion (CP-NE) during the storage time (room temperature and protected from light).

Table 2. Drug content and encapsulation efficiency of clobetasol propionate-loaded colloidal systems (CP-NC, CP-NS and CP-NE) during the storage time (room temperature and protected from light).

Time (months)	Formulation	Drug content (mg mL^{-1})	Encapsulation efficiency (%)
1	CP-NC	0.51 ± 0.01	99.93 ± 0.00
	CP-NS	0.44 ± 0.01	99.90 ± 0.01
	CP-NE	0.49 ± 0.02	99.90 ± 0.01
3	CP-NC	0.53 ± 0.01	99.89 ± 0.03
	CP-NS	0.47 ± 0.01	99.88 ± 0.04
	CP-NE	0.47 ± 0.03	99.87 ± 0.01
6	CP-NC	0.52 ± 0.02	99.93 ± 0.00
	CP-NS	---	---
	CP-NE	0.53 ± 0.01	99.86 ± 0.06
9	CP-NC	0.52 ± 0.04	99.70 ± 0.00
	CP-NS	---	---
	CP-NE	---	---

--- not determined

3.3. *In vitro* release assay

In vitro drug release studies were carried out to compare the ability of the three different nanostructures (NC, NS and NE) to control the release of clobetasol propionate. The drug release from polymeric nanostructured systems depends on the drug desorption from their surfaces, drug diffusion from the polymeric matrix (nanospheres) or through the polymeric wall (nanocapsules), polymer erosion or the combined process of diffusion and erosion.[6,23] In our study, the dialysis bag method was chosen due to its application for drug release studies where the release occurs over a long period (several days) from submicron-sized carriers. Results can reflect the release profile indicating the burst and sustained phases.[23,37]

Figure 5 shows the *in vitro* clobetasol propionate release profiles from the different nanostructured dispersions. As a control, the clobetasol propionate solution (CP-ES) was also assayed, showing the fastest diffusion through the dialysis bag. Comparing the drug release profiles from the different nanostructures, we can observe that the type of system influenced

this release. Clobetasol-loaded nanocapsules (CP-NC) sustained the release of clobetasol propionate for 240 hours (10 days) reaching mean release values above 95 % only after this period. On the other hand, clobetasol-loaded nanospheres (CP-NS) and clobetasol-loaded nanoemulsion (CP-NE) showed similar drug release (above 95 %) after 168 hours (7 days) and 120 hours (5 days), respectively.

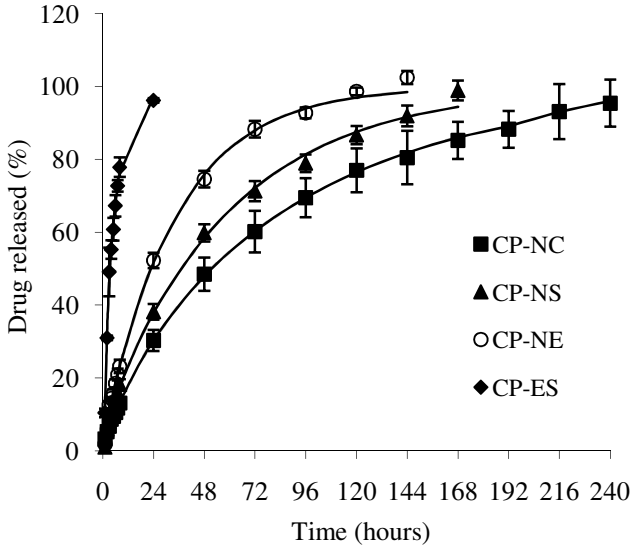


Figure 5. In vitro CP release profile from nanocarriers (CP-NC, CP-NS, CP-NE) and from ethanol solution (CP-ES) using dialysis bag method (n=3). The lines correspond to the fitting to the biexponential equation.

The release profiles were modeled using the monoexponential and biexponential equations. According to the values of the correlation coefficients and the model selection criteria (MSC), the best fitting was the biexponential equation for all formulations, as well as for CP-ES (Table 3). This result is in agreement with previous reports using different hydrophobic drugs[30,38-40], and with a previous study in which the *in vitro* drug release of CP-loaded nanostructured lipid carriers indicated a biphasic pattern. The profile in this case showed a burst release at the initial stage that was followed by a sustained phase with a constant rate.[19-22]

Table 3. Observed rate constants, correlation coefficients and MSC obtained by fitting of clobetasol propionate release from free clobetasol propionate (ethanol solution – CP-ES) and from different nanocarriers (CP-NC, CP-NS, CP-NE)

	CP-ES	CP-NC	CP-NS	CP-NE
Monoexponential				
k (h^{-1})	0.1912 ± 0.0155	0.0130 ± 0.0023	0.0187 ± 0.0016	0.0312 ± 0.0023
r (range)	0.9900 ± 0.0042	0.9981 ± 0.0013	0.9981 ± 0.0001	0.9987 ± 0.0005
MSC (range)	3.7132 ± 0.4318	4.7312 ± 0.7204	4.8244 ± 0.2344	5.6428 ± 0.3168
Biexponential				
k_1 (h^{-1})	0.6849 ± 0.1340	0.1087 ± 0.0586	0.1729 ± 0.0761	1.0247 ± 0.2150
k_2 (h^{-1})	0.1325 ± 0.0150	0.0115 ± 0.0032	0.0163 ± 0.0019	0.0289 ± 0.0021
a (mg mL^{-1})	0.5918 ± 0.1470	0.0936 ± 0.0911	0.1209 ± 0.0222	0.1281 ± 0.0078
b (mg mL^{-1})	0.6844 ± 0.0919	0.8899 ± 0.0929	0.8927 ± 0.0351	0.9656 ± 0.0054
r (range)	0.9970 ± 0.0015	0.9988 ± 0.0011	0.9990 ± 0.0004	0.9993 ± 0.0003
MSC (range)	4.3319 ± 0.6449	5.9591 ± 1.1946	5.6497 ± 0.3498	5.9288 ± 0.4707

The rate constants observed in our study for the burst phases (k_1) were 0.6849 ± 0.1340 h^{-1} (CP-ES), 0.1087 ± 0.0586 h^{-1} (CP-NC), 0.1729 ± 0.0761 h^{-1} (CP-NS) and 1.0247 ± 0.2150 h^{-1} (CP-NE) and the observed rate constants for the sustained phase (k_2) were 0.1325 ± 0.0150 h^{-1} (CP-SE), 0.0115 ± 0.0032 h^{-1} (CP-NC), 0.0163 ± 0.0019 h^{-1} (CP-NS) and 0.0289 ± 0.0021 h^{-1} (CP-NE). The initial concentrations of clobetasol propionate which contributed to the burst phases (a) for the nanoparticle formulations ranged between 0.09 and 0.13 mg mL^{-1} while the initial concentrations that contributed to the sustained phases (b) varied between 0.88 and 0.97 mg mL^{-1}. Those values showed that the drug was entrapped within the nanocarriers, around 90 %, and that only about 10 % was superficially adsorbed or free independently of the nanostructure. The kinetic experiments demonstrated that NC, NS and NE presented different behavior, even though the drug is likely entrapped within the nanostructures (CP-NC, CP-NS, CP-NE). In this case, the presence of the polymer (NS), the oil (NE) or both polymer and oil (NC) explains the differences in clobetasol propionate release rates. The slower drug release from nanocapsule formulation can be explained due to the high hydrophobicity and crystallinity of the poly(ε-caprolactone)[41], as well as the high lipophilicity of the drug, preventing its diffusion from CP-NC to the medium. Although CP-

NS presented a lower physicochemical stability compared to CP-NE, the *in vitro* drug release studies clearly demonstrated the influence of the polymer to control the release of clobetasol propionate from the nanoparticles. Those differences could be more evidenced by calculating the release half-life for each formulation. The release was faster for CP-NE (burst phase $t_{1/2}$ of 0.68 h and sustained phase $t_{1/2}$ of 24.00 h) compared to CP-NS (burst $t_{1/2}$ of 4.01 h and sustained phase $t_{1/2}$ of 42.51 h) and CP-NC (burst $t_{1/2}$ of 6.37 h and sustained phase $t_{1/2}$ of 60.26 h), which can be explained by the physicochemical characteristics of the polymer and the drug, as commented above. On the other hand, CP-ES showed values of 1.01 h for the burst $t_{1/2}$ and 5.23 h for the sustained phase $t_{1/2}$. Previous studies did not report differences of hydrophobic drug release profiles comparing NC and NE.[38,40,42] This similarity of behavior led those authors to suggest that the polymer coating of nanocapsules had no role in the release process, being the partition coefficient between the oily core and the aqueous medium the main factor governing the release process. However, a recent work[43] showed the influence of the polymeric wall on the drug permeability comparing poly(ε-caprolactone) nanocapsules to nanoemulsion. Those results support our findings concenrning the differences of drug release from CP-NC and CP-NE. Another factor besides clobetasol propionate partition coefficient between the oil and the aqueous medium influenced the drug release from the nanocapsule. The existence of an interaction between the drug and the polymer of the nanocapsules[40,44] could also be suggested. In addition, our results showed different drug release rates from NC (vesicular) and NS (matricial). Those results are in agreement with previous reports showing a better drug release control of polymeric nanoparticles containing an oily core.[30]

3.4. Photodegradation studies

Figure 6 shows the clobetasol propionate photodegradation plots obtained for the different formulations (NC, NS and NE), as well as for the free drug solutions (CP-ES and CP-CS)

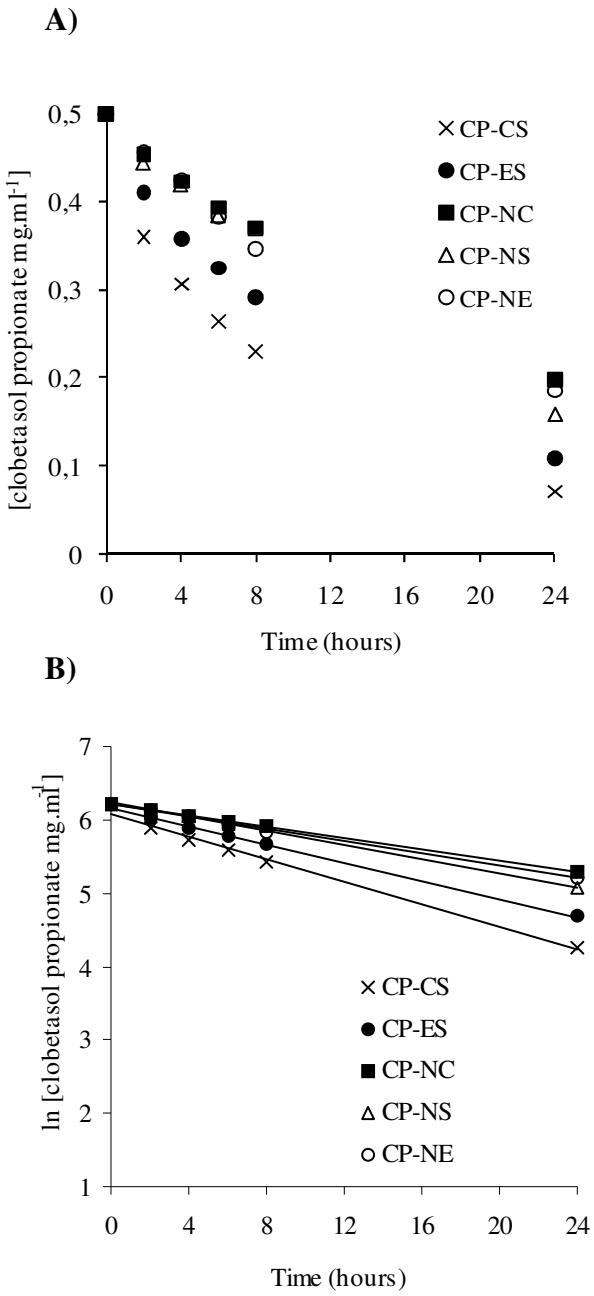


Figure 6. Photodegradation plots of free CP (ethanol solution – CP-ES, commercial solution – CP-CS) and CP-loaded NC, NS and NE exposed to UV light for 24 hours (n=3). A: concentration of clobetasol propionate remaining versus time; B: ln concentration of clobetasol propionate remaining versus time.

The photodegradation profiles of the free clobetasol propionate solutions (CP-ES and CP-CS) and of the nanostructured systems (CP-NC and CP-NE or CP-NS) fit a first order kinetic. The drug entrapment in the nanostructured systems clearly reduced the photodegradation rate compared to the free drug solutions. Clobetasol propionate solution or

drug-loaded formulations protected from light showed more than 95 % of intact drug after 24 h of irradiation. The result refuted any thermal degradation and confirmed the UVA photodegradation of the drug. Free clobetasol propionate (CP-ES and CP-CS) showed half-life times of 10.99 ± 0.89 and 8.95 ± 0.15 hours, respectively, whereas CP-loaded nanoparticles showed higher clobetasol propionate half-life times between 14 and 18 hours (1.7 to 2 fold higher than in solutions, ANOVA, $p \leq 0.05$) (Table 4). Comparing the different nanostructured systems, this improvement was higher for CP-NC (18.13 ± 0.30 h^{-1}) than for CP-NE and CP-NS (16.79 ± 0.54 h^{-1} and 14.61 ± 0.52 h, respectively) (ANOVA, $p \leq 0.05$). The results show the importance given by the presence of the polymer (PCL) and the oil to prevent the photodegradation of clobetasol propionate. The presence of both (polymer plus oil) led to the best protection against UVA light, as showed by the NC. This better protection presented by nanocapsules could be partially attributed to the polymer, which has the ability of reflecting and scattering UV radiation.[11,13,41,45]

Table 4. Photodegradation study of free CP (ethanol solution – CP-ES and commercial solution – CP-CS) and CP-loaded NC, NS and NE exposed to UV light for 24 hours (n=3)

Formulation	k (min^{-1})	$t_{1/2}$ (hours)*	r^2
CP-NC	0.0382 ± 0.0006^{d}	18.13 ± 0.30^{a}	0.9992
CP-NS	0.0477 ± 0.0017^{c}	14.61 ± 0.52^{c}	0.9927
CP-NE	0.0413 ± 0.0013^{d}	16.79 ± 0.54^{b}	0.9982
CP-ES	0.0633 ± 0.0052^{b}	10.99 ± 0.89^{d}	0.9909
CP-CS	0.0774 ± 0.0013^{a}	8.95 ± 0.15^{e}	0.9890

* $t_{1/2}$ calculated according to the equation related to first kinetic of reaction.

Means, in column, with the same letter are not significant different ($p \leq 0.05$, ANOVA)

4. CONCLUSION

Clobetasol propionate-loaded nanocapsules, nanospheres and nanoemulsion were successfully prepared. However, clobetasol propionate-loaded nanocapsules presented a higher physicochemical stability under storage at room temperature compared to nanospheres and nanoemulsion. *In vitro* drug release studies demonstrated the role of the polymer to control the drug release from the nanostructures. The mathematical modeling showed a biexponential drug release profile from all formulations, which presented low burst release phase, showing a high entrapment of the drug within the nanocarriers (close to 90 %),

independently of the nanostructure. In addition, clobetasol propionate-loaded nanoparticles improved clobetasol propionate photostability, being the polymeric nanocapsules more efficient than the polymeric nanospheres and nanoemulsion. Finally, nanoencapsulation of clobetasol propionate (mainly in nanocapsules) allows the control of its release rate and the improvement of its photostability against UVA radiation. The results showed that those nanostructured systems are a potential alternative to the preparation of aqueous topical clobetasol propionate delivery systems to treat skin diseases.

Acknowledgements

The authors thank the financial support of Rede Nanocosméticos/CNPq, CNPq-Brazil and CAPES-Brazil. K. Coradini thanks CNPq (PIBIC/CNPq-Brazil) and Programa FIPE Jr/UFSM for her fellowship. M. C. Fontana thanks CAPES-Brazil for her fellowship.

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CAPÍTULO 3: Nanocápsulas preparadas a partir de poliésteres amorfos: efeito sobre as características físico-químicas, liberação do fármaco e fotoestabilidade

CAPÍTULO 3: Nanocápsulas preparadas a partir de poliésteres amorfos: efeito nas características físico-químicas, liberação do fármaco e fotoestabilidade

3.1 Introdução

No capítulo anterior, foi demonstrado o desenvolvimento de nanopartículas poliméricas e nanoemulsões contendo propionato de clobetasol, com características físico-químicas adequadas e demonstrando a proteção do fármaco frente à luz UVA. Neste estudo, as nanocápsulas apresentaram a maior estabilidade frente ao armazenamento e o melhor controle da liberação *in vitro* do fármaco.

Na continuidade do trabalho, neste capítulo, foram preparadas nanocápsulas poliméricas a partir de diferentes polímeros amorfos com diferentes características hidrofílicas. Analisando-se a influência do material polimérico (PLA, PLGA 50:50 e PLGA 85:15) sobre as características físico-químicas, a estabilidade frente ao armazenamento, a fotoestabilidade e o perfil de liberação *in vitro* do fármaco. Além disso, o mecanismo de liberação do fármaco a partir das nanocápsulas poliméricas foi determinado.

PUBLICAÇÃO 3: Nanocapsules prepared from amorphous polyesters: effect on the physicochemical characteristics, drug release, and photostability

Artigo publicado no periódico Journal of Nanoscience and Nanotechnology

Nanocapsules prepared from amorphous polyesters: effect on the physicochemical characteristics, drug release and photostability

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ABSTRACT

The influence of the polymeric amorphous materials on the physicochemical and drug release properties of drug-loaded nanocapsules as well as their role on the protection of the entrapped drug against the degradation induced by UV radiation was evaluated. Nanocapsules were prepared by interfacial deposition of preformed polymer (PLA, PLGA 50:50 and PLGA 85:15) using clobetasol propionate as the drug model. *In vitro* drug release was evaluated by the dialysis bag method. Photochemical stability was studied under UVA radiation. After preparation, all formulations presented nanometric mean size (180 – 200 nm), polydispersity index below 0.20, acid pH, negative zeta potential, and encapsulation efficiency close to 100 %. Clobetasol propionate-loaded PLGA nanocapsules presented a lower physicochemical stability, showing a high drug leakage during 3 months of storage. *In vitro* studies showed biphasic drug release from all nanocapsules (according to an anomalous transport) and no influence of the hydrophilic characteristics of the amorphous polymeric material on the release rate. The photostability of clobetasol propionate under UVA radiation was improved by its incorporation into PLA and PLGA nanocapsules showing that besides semi-crystalline polymers, amorphous polymers could also efficiently protect nanoencapsulated drugs against UV radiation.

Keywords: Clobetasol propionate, nanocapsules, in vitro drug release, photostability, PLA, PLGA.

GRAPHICAL ABSTRACT

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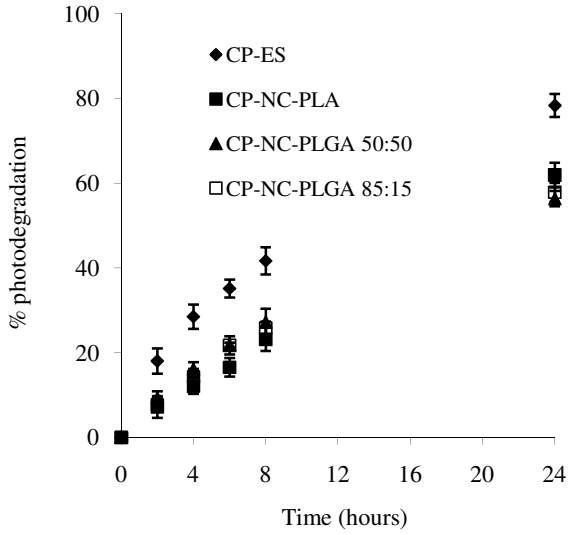

1. INTRODUCTION

Nanocapsules are submicronic polymeric colloidal systems characterized by a lipophilic core surrounded by a polymeric layer, in which drugs are dissolved in the oil and/or dispersed within the particle.[1,2] Additionally, the drug can be adsorbed at the interface particle/water.[3] Different methods are described in the literature to obtain polymeric nanoparticles. From a general point of view, these methods are based on *in situ* polymerization or precipitation of preformed polymers.[4] To date, the nanoprecipitation method has been the most commonly used to formulate polymeric nanoparticles intended for cutaneous applications.[5-9] The advantage of this method is the spontaneous, simple, efficient and reproducible formation of small particles exhibiting a high drug loading capacity.[7]

These nanometric systems have a great surface area, which renders them highly satisfactory properties for the application of lipophilic substances promoting a homogeneous drug release.[10] Additionally, their small sizes facilitate their formulation in dermatological products and enable comfortable application to the skin.[11] Nanocapsules present some advantages for topical application due to their potential to modify the activity of drugs, delay and control of the drug release, as well as the increase of the drug adhesivity or its time of permanence in the skin.[12] Other advantages of these systems include protection of labile substances from chemical degradation induced by UV light[13] and a decrease in cutaneous irritation.[14]

Several studies have been devoted to the evaluation of the *in vitro* drug release profile from nanocapsules lately.[15-20] According to the literature, nanocapsules are vesicular nanocarriers whose experimental data suggest that the polymeric wall is an important factor for drug release kinetics.[20] Differences in *in vitro* drug release and structural organization at molecular level (among nanocapsules and other nanocarriers) were previously reported by Cruz and co-workers.[18] In this work, the authors showed a similar behavior in the *in vitro* drug release from nanocapsules, nanospheres and nanoemulsion when the drug was likely adsorbed on the nanocarriers. On the other hand, when the drug was likely to be entrapped within the nanocarriers, the drug release from nanocapsules, nanospheres and nanoemulsion presented different behaviors, although all of them fitted the biexponential model. In this last situation, the presence of the polymer and the oil in nanocapsules led to a slower drug release in the burst release phase as well as in the sustained release phase.

In another work, Polleto and co-workers[20] reported that the release of indomethacin ethyl ester from poly(ε-caprolactone) nanocapsules was affected by the polymer concentration in the original nanocapsule formulations. It is known that the higher the polymer

concentration, the lower the relative permeability of drugs. The results of this work showed that the polymeric material acts as a diffusional barrier. In addition, as the number of particles was not affected, the diffusion was suggested as the main drug release factor. The modeling of these results showed a good fit to the Fick's first law.

Our group has recently reported the preparation of clobetasol propionate-loaded nanocarriers (nanocapsules, nanospheres and nanoemulsions). The development of a novel pharmaceutical dosage form for topical administration of clobetasol propionate based on nanotechnology has been designed to reduce the irritation of the treated area and/or to allow its formulation in hydrophilic vehicles.[21] All drug-loaded nanocarriers showed adequate physicochemical characteristics, a control of the drug release and a protection of this drug against UVA radiation. However, clobetasol propionate-loaded nanocapsules presented a better physicochemical stability (9 months) and a better control of the *in vitro* drug release. Regarding the mathematical modeling, the drug release data from nanocapsules fitted the biexponential model, presenting a low burst release phase, which indicates a high entrapment of the drug within the nanocarriers (close to 90 %).

In this previous work by Fontana and co-workers[21] poly(ε-caprolactone), a semi-crystalline biodegradable aliphatic polyester, was used as the nanocapsule polymeric wall. Biodegradable polymers are of large interest because they make possible *in vivo* biodegradation and subsequent removal from the body.[22] However, for the preparation of polymeric nanocapsules there are other biodegradable linear aliphatic polyesters that are attractive and widely used, as the ones derived from lactic acid (PLA), glycolic acid (PGA), and their copolymers (PLGA).[23,24]

Polyesters are thermoplastic polymers with hydrolytically labile aliphatic ester linkages in their backbone.[25] The biodegradation of polymeric biomaterials involves cleavage of hydrolytically or enzymatically sensitive bonds in the polymer leading to polymer erosion.[26] On the formulating of a drug delivery device, the understanding of the physical, chemical, and biological properties of the polymer is very helpful. In addition, the semi-crystallinity of the polymeric wall in nanocapsules is sometimes related to their ability to prevent the degradation of entrapped drugs against ultraviolet radiation.[7,11,13,21,27]

Among the variety of known biodegradable polyesters, poly(glycolide) – PGA - (Fig. 1) can be considered one of the first biodegradable synthetic polymers investigated for biomedical applications.[25] It is a highly crystalline polymer (45-55 % crystallinity) and therefore exhibits a high tensile modulus with very low solubility in organic solvents.[28] Glycolide monomer is synthesized from the dimerization of glycolic acid. The ring opening

polymerization of glycolide (a cyclic lactone) yields high-molecular-weight materials with about 1-3 % residual monomer (Fig. 1).[29] However, its biomedical application is limited due to its high rate of degradation, acidic degradation products and low solubility. This way, several copolymers containing glycolide units have been developed to overcome the inherent disadvantages of poly(glycolide).[25]

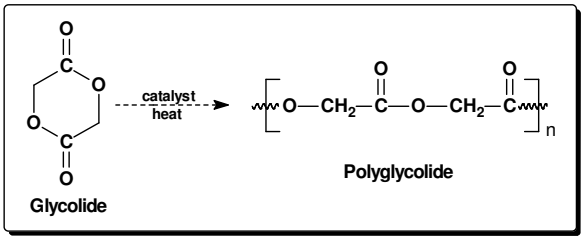

Figure 1. Synthesis of poly(glycolide) (PGA).

The polyester poly(lactide) - PLA - exists in an optically active stereoregular form ($_{L}$-PLA) and in an optically inactive racemic form ($_{D,L}$-PLA). $_{L}$-lactide is the naturally occurring isomer and $_{D,L}$-lactide is the synthetic blend of $_{D}$-lactide and $_{L}$-lactide. The polymerization of lactide is similar to that of glycolide (Fig. 2).[29] Poly($_{L}$-lactide) is also a crystalline polymer (~37% crystallinity) and a slow degrading polymer compared to poly(glycolide).[29] Poly($_{D,L}$-lactide) is an amorphous polymer due to the random distribution of $_{L}$- and $_{D}$-lactide units. Due to its amorphous nature, the polymer shows much lower strength (~1.9 GPa) compared to poly($_{L}$-lactide). Being a low strength polymer with faster degradation rate compared to poly($_{L}$-lactide), it is a preferred candidate for developing drug delivery vehicles.[25]

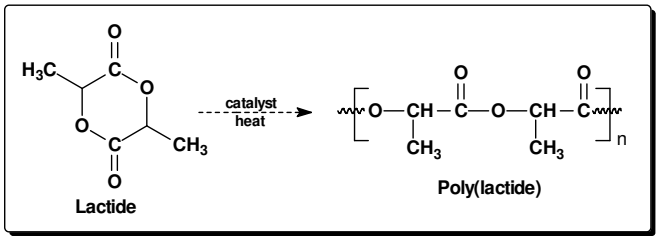

Figure 2. Synthesis of poly(lactide) (PLA).

Using poly(glycolide) and poly(lactide) properties as base materials, it is possible to copolymerize the two monomers to extend the range of homopolymer properties (Fig. 3). Copolymers of glycolide with both $_{L}$-lactide and $_{D,L}$-lactide have been developed for both device and drug delivery applications.[29] Lactic acid is more hydrophobic than glycolic acid

and hence lactide-rich PLGA copolymers are less hydrophilic, absorb less water, and subsequently degrade more slowly.[30,31] Crystallinity of the PLGA copolymer is dependent on the type and the molar ratio of the individual monomer components (lactide and glycolide) in the copolymer chain. PLGA polymers containing 50:50 ratio of lactic and glycolic acids are hydrolyzed much faster than those containing higher proportion of either of the monomers. PLGA copolymers prepared from $_{L}$-PLA and PGA are crystalline copolymers while those from $_{D,L}$-PLA and PGA are amorphous in nature.[32]

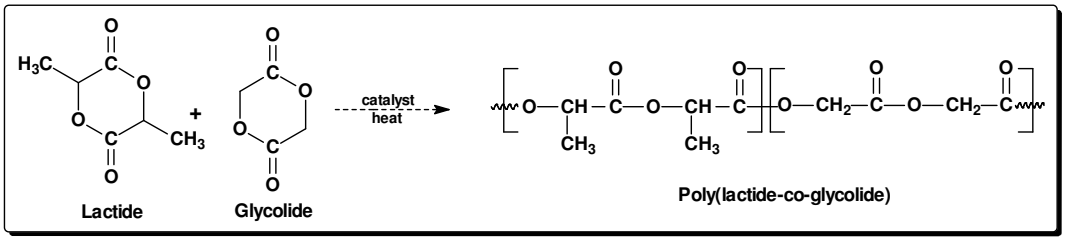

Figure 3. Synthesis of poly(lactide-*co*-glycolide) (PLGA).

Taking all these considerations together, the aim of this work was to evaluate the potential of amorphous polyesters presenting different hydrophilic characteristics (PLA, PLGA 50:50 and PLGA 85:15) to prevent the UV photodegradation and to control the release of an entrapped drug in nanocapsules. Clobetasol propionate was chosen as a model drug due to its high entrapment within the nanocapsules,[21] which allows a better understanding of the role of the polymer on the drug release and the prevention against UV radiation. In addition, the influence of these different polymeric walls (PLA, PLGA 50:50 and PLGA 85:15) on the physicochemical characteristics of the nanocapsules, on the mechanism of drug release as well as on the stability under storage was evaluated.

2. MATERIALS AND METHODS

2.1 Materials

Clobetasol propionate was a gift from Neo Quimica (Goiás, Brazil). Poly($_{D,L}$-lactide) (PLA), poly(lactide-*co*-glycolide) (PLGA 50:50 and PLGA 85:15) and sorbitan monostearate (Span 60®) were purchased from Sigma-Aldrich (São Paulo, Brazil); caprylic/capric triglyceride mixture was delivered from Brasquim (Porto Alegre, Brazil); polysorbate 80 (Tween 80®) and polyethylenoglicol 400 was supplied by Henrifarma (São Paulo, Brazil) and Alpha Química (Porto Alegre, Brazil), respectively; dialysis bags (Spectra Por 7, 10 Kd, Spectrum Laboratories, USA) were purchased from Bioagency (São Paulo, Brazil) and

acetone from Vetec (Rio de Janeiro, Brazil). HPLC grade acetonitrile and methanol were acquired from Tedia (São Paulo, Brazil). All chemicals and solvents presented pharmaceutical or HPLC grade and were used as received.

2.2 Preparation of nanocapsules

Nanocapsule suspensions (NC) were prepared (n = 3) by the interfacial deposition of the preformed polymer method.[33] 25 mg of polymer (PLA, PLGA 50:50 or PLGA 85:15), 191.5 mg of sorbitan monostearate, 0.82 mL caprylic/capric triglyceride mixture and 12.5 mg of clobetasol propionate were dissolved in 67 mL of acetone. This organic solution was added into 134 mL of an aqueous phase containing 191.5 mg of polysorbate 80 under moderate magnetic stirring. Magnetic stirring was maintained for 10 min. Then, acetone was removed and the aqueous phase concentrated by evaporation at 40° C under reduced pressure (25 mL) obtaining a final concentration of 0.5 mg mL^{-1} of clobetasol propionate (CP-NC-PLA, CP-NC-PLGA 50:50 and CP-NC-PLGA 85:15, respectively). In order to study the influence of the presence of clobetasol propionate on the NC characteristics, blank NC formulations were prepared as described above omitting the addition of the drug (B-NC-PLA, B-NC-PLGA 50:50 and B-NC-PLGA 85:15, respectively). All formulations were made in triplicate and stored at room temperature and protected from light.

2.3 Characterization of nanocapsules

After preparation, nanocapsule suspensions were characterized by means of drug content, incorporation efficiency, pH, mean size, polydispersity index, and zeta potential. These physicochemical characteristics were also evaluated three months after preparation. Formulations were stored at room temperature and protected from light.

2.3.1 Determination of drug content and encapsulation efficiency

Total drug was determined after dissolution of nanocapsules (1 mL) in 25 mL of acetonitrile. Free clobetasol propionate was determined in the ultrafiltrate after separation of the nanocapsules by the ultrafiltration/centrifugation technique (Microcon 10,000 MW, Millipore). The drug entrapped in the nanostructures was calculated by the difference between the total and the free drug concentrations, measured in the nanocapsules and in the ultrafiltrate, respectively. Encapsulation efficiency was determined by the quotient of the drug entrapped and total drug content. Clobetasol propionate was assayed by HPLC according to a method previously validated.[34]

2.3.2 pH measurements

pH values of nanocapsule formulations were determined directly in the dispersions using a calibrated potentiometer (MPA-210 Model, MS-Tecnopon, São Paulo, Brazil).

2.3.3 Particle size, polydispersity indices and zeta potential analysis

Particle sizes and polydispersity indices (n = 3) were estimated by photon correlation spectroscopy (PCS) after adequate dilution of an aliquot of the formulation in purified water (Zetasizer Nanoseries, Malvern Instruments, Worcestershire, UK). Zeta potentials were measured using the same instrument at 25 °C, after dilution of the samples in 10 mmol L^{-1} NaCl aqueous solution.

2.4 In vitro release assay

In vitro drug release profiles from CP-NC-PLA, CP-NC-PLGA 50:50 and CP-NC-PLGA 85:15 were evaluated (n = 3) by the dialysis bag method, using water/Tween 80®/PEG 400 (60:0.5:40 v/v) pH 7.60 as medium, at 37°C.[21,35] The dialysis bag (Spectra Por 7, 10 Kd), containing 1 mL of the sample (0.5 mg mL^{-1}), was put into a 400 mL erlenmeyer containing 200 mL of dissolution medium under constant moderate stirring. 2 mL of the external medium was withdrawn from the system at predetermined time interval, replaced by an equal volume of fresh medium, and filtered through a 0.45 µm membrane. Clobetasol propionate was assayed in the samples by HPLC according to a method previously validated.[21,34]

In order to obtain a better understanding about the influence of the type of the polymeric material on the clobetasol propionate release behavior from nanocapsules, the mathematical modeling (MicroMath® Scientist® for Windows™) was used to analyze the drug release profiles.[18] Monoexponential ($C = C_0\, e^{-kt}$) and biexponential ($C = a\, e^{-k_1 t} + b\, e^{-k_2 t}$) models were used to evaluate the drug release profiles.[19] The release rate constants are k, k_1 and k_2 and the initial concentrations of the drug are C_0, a and b. In addition, in order to determine the clobetasol propionate release mechanism, 60 % of the initial fraction of clobetasol propionate release was fitted to the Korsmeyer-Peppas model ($f_t = at^n$), where f_t is the fraction of clobetasol propionate released at time t (min), a (min^{-1}) is a constant which incorporates structural and geometric characteristics of the carrier, and n is the exponent which indicates the mechanism of release. The selection of the model, which best fitted the release profiles, was based on the best correlation coefficient, the best model selection criteria (MSC) (both provided by the software), and the best graphic adjustment.

2.5 Photodegradation studies

Photodegradation experiments were carried out using a UVA artificial lamp (Fluorescent blacklight blue lamps, 30 W, Ecolume). Clobetasol propionate-loaded nanocapsules (2 mL in a 5 mm quartz cuvette perfectly stoppered) were exposed to UVA radiation for 24 hours at a fixed distance of 16 cm (n = 3). As a control we evaluated the photostability of a clobetasol propionate ethanolic solution (n = 3). After the appropriate exposure interval, 200 µL of the samples was withdrawn, transferred into a 5 mL calibrated flask, diluted to volume with acetonitrile and filtered (0.45 µm membrane filter). Clobetasol propionate was assayed by HPLC according to a method previously validated.[34] No additional peaks were observed in the chromatogram of samples irradiated by UVA light and clobetasol propionate peak purity was close to 100 % for samples of all time points, as showed by the peak purity evaluation using the photodiode-array (PDA). The results were expressed as percentage of clobetasol propionate degraded. The experiment was conducted until obtaining more than 50 % of photodegradation. The hypothesis of thermal degradation could be discarded as previously reported.[21] During irradiation, the UVA-intensity was monitored and kept at 1.0 ± 0.1 mW/cm² at the sample levels.

2.6 Statistical analysis

All formulations were prepared and analyzed in triplicate. Results are expressed as mean ± SD (standard deviation). One-way and two-way analysis of variance (ANOVA) was employed for comparison of the experimental data. Post-hoc multiple comparisons were done by Tukey's test for significance at p–values ≤ 0.05. All analyses were run using the SigmaStat Statistical Program (Version 3.0, Jandel Scientific, USA).

3. RESULTS AND DISCUSSION

3.1 Preparation and physicochemical characterization of nanoparticles

Clobetasol propionate-loaded nanocapsules were prepared using amorphous polyesters presenting different hydrophilic characteristics. All formulations presented a macroscopic homogeneous appearance, like a milky bluish opalescent liquid, regardless of the type of the polymer. Their physicochemical characteristics after preparation are presented in Tables 1 and 2.

Table 1. Drug content, encapsulation efficiency and pH of clobetasol propionate-loaded NC and respective blank formulations prepared with PLA, PLGA 50:50 and PLGA 85:15 (after preparation)

Formulation	Drug content (mg mL^{-1})	Encapsulation Efficiency (%)	pH
CP-NC-PLA	0.45 ± 0.01	99.47 ± 0.09	3.38 ± 0.03
B-NC-PLA	-	-	3.41 ± 0.05
CP-NC-PLGA 50:50	0.47 ± 0.01	99.44 ± 0.00	3.48 ± 0.11
B-NC-PLGA 50:50	-	-	3.83 ± 0.11
CP-NC-PLGA 85:15	0.45 ± 0.01	99.90 ± 0.04	3.51 ± 0.02
B-NC-PLGA 85:15	-	-	3.47 ± 0.02

Table 2. Particle size, polydispersity index and zeta potencial of clobetasol propionate-loaded NC and respective blank formulations prepared with PLA, PLGA 50:50 and PLGA 85:15 (after preparation)

Formulation	Particle size (nm)	Polydispersity index	Zeta potential (mV)
CP-NC-PLA	182 ± 04	0.10 ± 0.01	- 7.74 ± 0.80
B-NC-PLA	183 ± 03	0.09 ± 0.01	- 7.46 ± 0.26
CP-NC-PLGA 50:50	174 ± 03	0.13 ± 0.02	- 8.04 ± 1.12
B-NC-PLGA 50:50	184 ± 04	0.16 ± 0.01	- 8.41 ± 0.33
CP-NC-PLGA 85:15	196 ± 03	0.12 ± 0.03	- 9.37 ± 2.28
B-NC-PLGA 85:15	191 ± 02	0.14 ± 0.02	- 7.67 ± 0.64

As can be seen, all formulations presented drug content close to their theoretical value (0.45-0.47 mg mL^{-1}) and similar encapsulation efficiency (higher than 99 %). Formulations presented submicrometric particle sizes in the range of 170 to 200 nm, low polydispersity index (below 0.20), negative zeta potential, and acid pH values. Comparing the results from clobetasol propionate-loaded nanocapsules to their respective unloaded-nanocapsules (blank formulations), no influence of the presence of the drug on the physicochemical characteristics for all formulations could be observed.

Regarding the use of different polymeric materials to prepare these formulations, the results showed that the different hydrophilic characteristics of the amorphous polymer used in this work did not influence the physicochemical characteristics of the clobetasol propionate-loaded nanocapsules. The physicochemical characteristics of these formulations were similar to those presented by clobetasol propionate-loaded nanocapsules prepared with a semi-crystalline polymer [poly(ε-caprolactone) - PCL], which presents a more hydrophobic characteristic.[21] However, a high difference in pH values could be observed. Formulations prepared with PLA and PLGA presented a lower pH acid compared to those prepared with PCL.[21] These differences may be explained by the residual presence of free acids in the NC suspensions prepared with PLA and PLGA.[29,36]

In order to evaluate the effects of the polymeric material on the physicochemical properties of formulations under storage, we stored them at room temperature and protected from light. Tables 3 and 4 show the characteristics of formulations after 3 months of storage under these conditions. According to these results, a decrease in the drug content could be observed for PLGA nanocapsule formulations (ANOVA, $p \leq 0.05$). On the other hand, PLA formulation did not show significantly lower drug content compared to the value obtained after preparation (ANOVA, $p > 0.05$). These decreases suggest a drug leakage (surface desorption and/or diffusion of the drug to the aqueous medium followed by crystallization) as previously observed for dexamethasone-loaded nanospheres and nanocapsules.[37,38] The different behavior between the polymeric materials (PLGA and PLA) may be attributed to the lower hydrophilic characteristic and subsequent slower degradation rate presented by PLA.[30,31]

Despite this decrease in the drug content, the mean particle size presented by all formulations (ranged from 170 to 210 nm after preparation and under storage) suggests a good stability of formulations in relation to particle aggregation or adhesion phenomena. This suggestion can be reinforced by the low values of polydispersity index (lower than 0.20), which also kept unchangeable during the storage time.

On the other hand, pH values showed a slightly decrease for all formulations, except for CP-NC-PLA. This decline is common in polymeric nanocapsules prepared with polyester and may be explained either by the polymeric chain relaxation, which exposes a higher number of terminal carboxylic groups, or by the polymer degradation[1] leading to the release of free lactic acid or free glycolic acid to the aqueous medium. The latter hypothesis is in agreement with our previous explanation for the decrease in drug content presented only by PLGA formulations, which presented more accentuated decline in their pH values.

Table 3. Drug content and pH of clobetasol propionate-loaded and unloaded nanocapsules prepared with PLA, PLGA 50:50 and PLGA 85:15, after the storage time (3 months)

Formulation	Drug content (mg mL^{-1})	pH
CP-NC-PLA	0.42 ± 0.02	3.36 ± 0.19
B-NC-PLA	-	3.15 ± 0.02
CP-NC-PLGA 50:50	0.37 ± 0.09	2.95 ± 0.03
B-NC-PLGA 50:50	-	3.14 ± 0.27
CP-NC-PLGA 85:15	0.36 ± 0.03	3.28 ± 0.07
B-NC-PLGA 85:15	-	3.11 ± 0.08

Table 4. Particle size, polydispersity index and zeta potencial of clobetasol propionate-loaded and unloaded nanocapsules prepared with PLA, PLGA 50:50 and PLGA 85:15, after the storage time (3 months)

Formulation	Particle size (nm)	Polydispersity index	Zeta potential (mV)
CP-NC-PLA	188 ± 02	0.10 ± 0.00	- 10.35 ± 0.50
B-NC-PLA	188 ± 02	0.09 ± 0.03	- 10.57 ± 1.33
CP-NC-PLGA 50:50	178 ± 06	0.14 ± 0.02	- 10.05 ± 1.54
B-NC-PLGA 50:50	183 ± 04	0.16 ± 0.01	- 9.87 ± 2.27
CP-NC-PLGA 85:15	201 ± 03	0.14 ± 0.02	- 9.81 ± 1.78
B-NC-PLGA 85:15	202 ± 07	0.18 ± 0.00	- 11.13 ± 3.75

Regarding zeta potential, the values remained negative during the storage time for all samples, presenting a slight increase (in module) that could be viewed as a positive aspect in terms of the physical stability.

Comparing the physicochemical characteristics of PLA and PLGA clobetasol propionate-loaded nanocapsules, no significant differences could be observed in terms of mean size, pH, polydispersity index and zeta potential, showing no influence of the type of the hydrophilic characteristics of the polymeric material on these properties. In addition, no differences in these characteristics could be observed between PLA and PLGA formulations and PCL clobetasol propionate-loaded nanocapsules,[21] except for pH. On the other hand, the

physicochemical stability of PLGA formulations, mainly in terms of drug content, was lower compared to PLA nanocapsules as well as compared to similar PCL nanocapsules.[21]

3.2 In vitro drug release assay

In vitro drug release studies were carried out to verify the influence of the hydrophilic characteristics of the polymeric material of formulations (CP-NC-PLA, CP-NC-PLGA 50:50, CP-NC-PLGA 85:15) on the release of nanoencapsulated clobetasol propionate. The release rate of the drug from polymeric nanoparticles is one of the most important parameters to be evaluated.[39] Drugs formulated in polymeric devices could be released by drug desorption, by diffusion through the polymer barrier, by erosion of the polymer material or by a combination of these factors.[1,40] Polymer properties related to the drug release rate and degradation pattern are quite dependent on the solubility, morphology, and stability, which in turn can be related to the structure of the polymer.[39]

In vitro release curves of the three formulations as well as of the drug ethanolic solution (free drug) are shown in Fig. 4. As can be observed, nanocapsule formulations presented a biphasic drug release pattern, representing a burst release at the initial stage followed by a sustained release at a constant rate. Regardless of the polymer property, the release of clobetasol propionate was slower for nanocapsule formulations compared to the free drug solution (CP-ES), since for this solution it depends only on the diffusion of the drug through the dialysis bag. Clobetasol-loaded nanocapsules (CP-NC-PLA, CP-NC-PLGA 50:50, CP-NC-PLGA 85:15) sustained the release of clobetasol propionate for 192 hours (8 days) reaching mean release values above 100 % only after this period. Comparing the drug release profiles from nanocapsules prepared with polymers presenting different hydrophilic characteristics, it could be observed that this characteristic did not present a high influence on the drug release. On the other hand, comparing these results with previous findings for clobetasol propionate release from PCL nanocapsules, a slower drug release from this formulation could be observed. In this study, PCL nanocapsules sustained the release of clobetasol propionate for 240 hours (10 days) reaching mean release values above 95 % only after this period. Besides its higher lipophilic characteristic, PCL has a semi-crystalline structure, which means it presents an arrangement more uniform of the molecules.[25] In this case, the drug finds a larger resistance to the diffusion through the polymer matrix, taking a slower liberation of the drug through this polymer matrix, different from that obtained for PLA and PLGA nanocapsules. These polymers (PLA and PLGA) have an amorphous

structure, providing a faster drug release from nanocapsules, due to larger easiness of diffusion of the drug through the capsule wall.

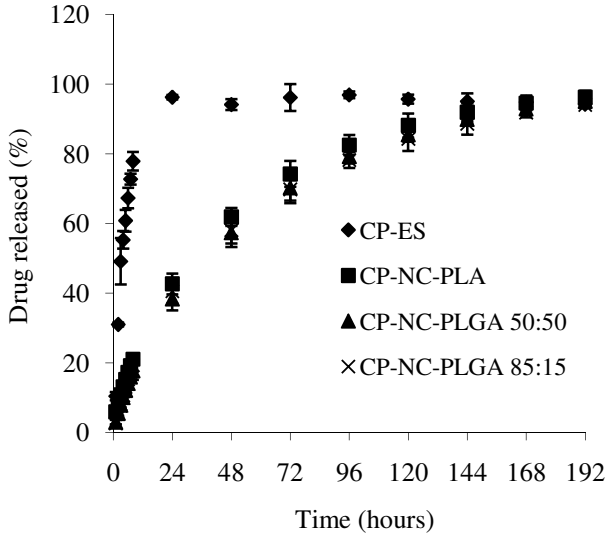

Figure 4. *In vitro* release profile of clobetasol propionate from ethanolic solution (CP-ES) and from nanocapsules (CP-NC-PLA, CP-NC-PLGA 50:50, CP-NC-PLGA 85:15) using dialysis bag method, n=3.

The mathematical modeling was used to analyze the drug release profiles. Each release profile (CP-NC-PLA, CP-NC-PLGA 50:50, CP-NC-PLGA 85:15) was modeled using monoexponential and biexponential equations. As can be observed in Table 5, according to the values of the correlation coefficients and the model selection criteria (MSC), the best fitting was the biexponential equation for all formulations. This result is in accordance with the clobetasol propionate release from PCL nanocapsules[21] as well as other reports in the literature for hydrophobic drugs.[18,41,42]

The rate constants for the burst (k_1) and the sustained phases (k_2) among formulations were not statistically different (ANOVA, $p > 0.05$). No differences were found when compared to PCL formulations either.[21] Regarding the initial concentrations of clobetasol propionate for burst phases (*a*) and sustained phases (*b*), they showed that all formulations presented similar coefficients (ANOVA, $p > 0.05$). Moreover, around 80 - 90 % of the drug is entrapped within the nanocarriers and only about 10 - 20 % is superficially adsorbed, regardless of the polymer used.[18] These results are similar to those obtained for PCL nanocapsules.[21] On the other hand, it is important to point out that PLA and PLGA

formulations presented slower release rates compared to the clobetasol propionate solution (CP-ES).

Table 5. Observed rate constants, correlation coefficients and MSC obtained by fitting of clobetasol propionate release from free clobetasol propionate (ethanolic solution – CP-ES) and from nanocapsules (CP-NC-PLA, CP-NC-PLGA 50:50, CP-NC-PLGA 85:15)

	CP-ES	CP-NC-PLA	CP-NC-PLGA 50:50	CP-NC-PLGA 85:15
Monoexponential				
k (h^{-1})	0.1912 ± 0.0155	0.0208 ± 0.0018	0.0177 ± 0.0014	0.0179 ± 0.0019
r (range)	0.9900 ± 0.0042	0.9963 ± 0.0004	0.9958 ± 0.0015	0.9963 ± 0.0009
MSC (range)	3.7132 ± 0.4318	3.8660 ± 0.1149	4.2837 ± 0.4654	4.0301 ± 0.2275
Biexponential				
k_1 (h^{-1})	0.6849 ± 0.1340	0.1060 ± 0.0510[a]	0.1284 ± 0.0376[a]	0.1111 ± 0.0313[a]
k_2 (h^{-1})	0.1325 ± 0.0150	0.0160 ± 0.0019[a]	0.0149 ± 0.0015[a]	0.0135 ± 0.0012[a]
a (mg mL^{-1})	0.5918 ± 0.1470	0.1565 ± 0.0493[a]	0.1307 ± 0.0839[a]	0.2106 ± 0.0724[a]
b (mg mL^{-1})	0.6844 ± 0.0919	0.8121 ± 0.0572[a]	0.8662 ± 0.0811[a]	0.7953 ± 0.0737[a]
r (range)	0.9970 ± 0.0015	0.9978 ± 0.0003	0.9972 ± 0.0009	0.9984 ± 0.0007
MSC (range)	4.3319 ± 0.6449	4.8971 ± 0.1157	4.6743 ± 0.3562	5.3248 ± 0.5308

Means, in line, with the same letter are not significant different ($p \leq 0.05$, ANOVA)

In order to determine if the mechanism of clobetasol propionate release was different for formulations prepared with the different polymers, the semi-empiral Korsmeyer-Peppas model was used. For all formulations the n values varied from 0.48 to 0.53 (Table 6). For spherical particles, $0.43 < n < 0.85$ indicates that the release mechanism is an anomalous transport, in which the mass transfer occurs due to the drug Fickian diffusion and the relaxation of the polymer chains.[43] Thus, the mechanism of the clobetasol propionate release from nanocapsules was the same (anomalous transport) regardless of the type of the polymer.

Furthermore, the application of the Korsmeyer-Peppas model to the analysis of the release data of clobetasol propionate from nanocapsules prepared with poly(ε-caprolactone) previously reported by our group, led to a n value of 0.53 ± 0.01. This way, an anomalous transport for nanocapsules prepared with a polymer presenting higher hydrophobia and a semi-crystalline nature was also demonstrated. From these results, it could be observed that

clobetasol propionate diffuses through the polymeric wall as well as the aqueous phase interacts with the particles during the drug release leading to the relaxation of the polymeric wall, as previously demonstrated.[44] In addition, according to Poletto and co-workers,[20] since the quantitative polymer concentration was the same in all formulations (10 mg mL^{-1}) and they presented similar particle size distribution, it could be inferred that the capsule wall of PLA and PLGA nanocapsules present the same permeability to the drug, regardless of the polymer properties.

Table 6. Korsmeyer-Peppas release exponent (*n*), model selection criteria (MSC) and correlation coefficient (r) by fitting the clobetasol propionate release from different nanocapsule formulations (CP-NC-PLA, CP-NC-PLGA 50:50, CP-NC-PLGA 85:15) (n=3)

Formulation	*n*	MSC	r
CP-NC-PLA	0.48 ± 0.01	3.97 ± 0.25	0.9930 ± 0.0017
CP-NC-PLGA 50:50	0.53 ± 0.02	4.12 ± 0.46	0.9938 ± 0.0027
CP-NC-PLGA 85:15	0.51 ± 0.02	3.96 ± 0.41	0.9929 ± 0.0026

3.3 Photodegradation studies

Clobetasol propionate presents a significant photodegradation under UV light exposition,[45] which is an important drawback to the efficient development of adequate topical formulations. In our previous work,[21] we showed a significant improvement of clobetasol propionate photodegradation by its incorporation in PCL nanocapsules. This ability is as a general rule attributed to the semi-crystalline characteristic of PCL.[7,11,13] In order to obtain a better understanding about the protection of drugs against UV radiation by means of nanoencapsulation, we studied the photodegradation of clobetasol propionate-loaded nanocapsules prepared with amorphous polymers (PLA and PLGA). Figure 5 shows the clobetasol propionate photodegradation profile from these different nanocapsules in comparison to an ethanolic drug solution in the same concentration (CP-ES).

As showed in Figure 5, the incorporation of clobetasol propionate in different nanocapsules led to a similar decrease on its photodegradation rate when compared to the ethanolic solution (CP-ES). The photodegradation profile of clobetasol propionate was according to a zero or first order for the PLA nanocapsules or ethanolic solution and PLGA

nanocapsules, respectively. The calculated kinetic order and half-lives ($t_{1/2}$) of each clobetasol propionate-loaded nanocapsule formulation are shown in Table 7.

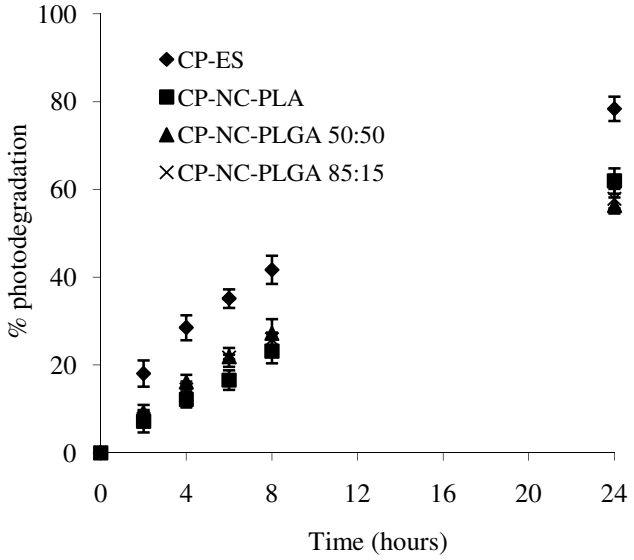

Figure 5. Percentual of photodegradation of free clobetasol propionate solution - ethanolic solution (CP-ES) and clobetasol propionate-loaded nanocapsules (CP-NC-PLA, CP-NC-PLGA 50:50, CP-NC-PLGA 85:15), n=3.

Table 7. Photodegradation of free clobetasol propionate solution and clobetasol propionate-loaded nanocapsules (CP-NC-PLA, CP-NC-PLGA 50:50, CP-NC-PLGA 85:15) exposed to UV light for 24 hours (n=3)

Formulation	Kinect order	$t_{1/2}$ (hours)[a]	r
CP-ES	First	10.99 ± 0.89[a]	0.9954
CP-NC-PLA	Zero	19.75 ± 1.08[b]	0.9989
CP-NC-PLGA 50:50	First	21.09 ± 1.22[b]	0.9985
CP-NC-PLGA 85:15	First	19.41 ± 1.07[b]	0.9994

[a] $t_{1/2}$ calculated according to the equation related to kinetic of reaction.

Means, in column, with the same letter are not significant different ($p \leq 0.05$, ANOVA)

The drug entrapment in all nanostructured systems clearly reduced the photodegradation rate compared to the free drug solution. In addition, as PLA and PLGA

formulations showed similar clobetasol propionate half-life against UVA radiation, it can be observed that nanocapsules prepared with polymer presenting different hydrophilic characteristics (PLA or PLGA) present similar photoprotection of clobetasol propionate against UVA radiation. Furthermore, half-lives presented by these formulations were similar to the ones showed for nanocapsules prepared with a semi-crystalline polymer (PCL).[21] Taking into account that PLA and PLGA are amorphous polymers, this comparison allows us to suggest that if the drug is mainly entrapped within the nanocapsules, the semi-crystalline property of a polymer did not influence the protection of the drug against UV radiation, as sometimes suggested.[7] This way, the protection presented by nanocapsules should be partially attributed due to the presence of the polymer, regardless of its amorphous or semi-crystalline state and/or to the nanometric size of the carrier, what makes it able to reflect the UV light protecting the drug.

4. CONCLUSION

PLA and PLGA (50:50 and 85:15) clobetasol propionate-loaded nanocapsules were successfully prepared, presenting adequate physicochemical characteristics. Although no influence of the hydrophilic characteristics of the polymer was observed on the physicochemical properties of nanocapsules, the ones prepared with the most hydrophobic polymer (PLA) showed a higher physicochemical stability. All formulations are able to control the drug release from nanocapsules, regardless of the polymeric material of the nanocapsule wall, according to a biexponential model, showing low burst release. Considering the Korsmeyer-Peppas model, the anomalous transport was determined as the mechanism of clobetasol release, regardless of the type of the polymer. In addition, the nanoencapsulation of clobetasol propionate in PLA and PLGA nanocapsules allowed to improve its photostability against UVA radiation, showing that nanocapsule wall consisted of amorphous polymers are also able to protect entrapped drugs from UV radiation.

Acknowledgements

K. Coradini thanks CNPq and Programa FIPE Jr/UFSM for her scholarship. M. C. Fontana thanks CAPES/Brazil for her fellowship. The authors thank the financial support of Rede Nanocosméticos/CNPq and CNPq/Brazil.

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CAPÍTULO 4: Hidrogel contendo nanopartículas de propionato de clobetasol para tratamento dermatológico: desenvolvimento, caracterização reológica e estudo da liberação *in vitro* do fármaco

CAPÍTULO 4: Hidrogel contendo nanopartículas de propionato de clobetasol para tratamento dermatológico: desenvolvimento, caracterização reológica e estudo da liberação *in vitro* do fármaco

4.1 Introdução

Até o presente momento, foi apresentada neste trabalho a possibilidade de emprego da suspensão de nanopartículas poliméricas e nanoemulsões contendo propionato de clobetasol para a aplicação tópica, sendo que as nanocápsulas preparadas com o polímero poli(ε-caprolactona) apresentaram a maior estabilidade frente ao armazenamento e o maior controle da liberação do fármaco.

Diante desses resultados, nesta etapa do trabalho foi avaliada a incorporação destas nanocápsulas em uma forma farmacêutica semissólida (hidrogel) e utilizando formulações similares contendo o fármaco associado a nanoesferas e a nanoemulsão, como comparativo para análise da influência da presença da poli(ε-caprolactona) e/ou do óleo nas nanopartículas. Neste sentido, este capítulo apresenta a preparação e a análise reológica dos hidrogéis, além os estudos de liberação *in vitro* do propionato de clobetasol a partir destas formulações. É importante ressaltar que até o momento não há relatos na literatura da preparação de hidrogéis contendo propionato de clobetasol associado a nanocápsulas, nanoesferas e nanoemulsão.

PUBLICAÇÃO 4: Hydrogel containing clobetasol propionate-loaded nanoparticles for dermatological treatments: development, rheological characterization and *in vitro* drug release study

Artigo a ser submetido ao periódico Drug Development and Industrial Pharmacy

Hydrogel containing clobetasol propionate-loaded nanoparticles for dermatological treatments: development, rheological characterization and *in vitro* drug release study

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ABSTRACT

Clobetasol propionate (CP) is clinically used in dermatology for the treatment of skin disorders such as atopic dermatitis and psoriasis. In the present work we evaluated the influence of CP-loaded polymeric nanoparticles on the physicochemical and rheological properties of Carbopol Ultrez® 10 NF hydrogels as well as on its *in vitro* drug release profile. Hydrogels containing CP-loaded nanocapsules and CP-loaded nanospheres (HG-CP-NC and HG-CP-NS, respectively) were prepared as well as hydrogels containing CP-loaded nanoemulsions (HG-CP-NE), in order to study the influence of the polymeric material on these properties. Nanoparticles presented encapsulation efficiency close to 100 %, nanometric mean size (140-220 nm), and low polydispersity index (< 0.25). Hydrogels were obtained using a bioadhesive polymer (Carbopol Ultrez® 10 NF) and characterized according to the following characteristics: drug content, pH, spreadability, viscosity and *in vitro* drug release. All hydrogels presented adequate pH values (5.50 - 6.50) and drug content (0.05 %) as well as spreadability factors between 2.50 and 3.90 $mm^2.g^{-1}$. Rheograms exhibited a non-Newtonian behavior for all formulations presenting pseudoplastic characteristics and thixotropy. *In vitro* studies showed a controlled release of CP from hydrogels containing the nanoencapsulated drug following the Higuchi's model (HG-CP-NC: 1.03 ± 0.11 $\mu g.cm^{-2}.h$, HG-CP-NS: 1.13 ± 0.12 $\mu g.cm^{-2}.h$, HG-CP-NE: 1.65 ± 0.19 $\mu g.cm^{-2}.h$) compared to the hydrogels containing the free drug (HG-CP: 2.79 ± 0.22 $\mu g.cm^{-2}.h$). The best release control was obtained for semisolid formulations containing CP-loaded nanocapsules and CP-loaded nanospheres.

KEYWORDS: clobetasol propionate, hydrogels, *in vitro* release, nanoparticles.

1. INTRODUCTION

Topical corticosteroids have been widely used to treat skin diseases. Their clinical effectiveness in the treatment of psoriasis and atopic dermatitis is related to their vasoconstrictive, anti-inflammatory, immunosuppressive and antiproliferative effects (Senyigit et al., *in press*). However, the use of topical glucocorticoids, after systemic and topical administration, is limited due to their adverse effects, such as skin atrophy, steroid acne, hypopigmentation, and allergic contact dermatitis, (Hengge, et al., 2006; Zöller et al., 2008). Currently, researches have been focusing mainly on the development of strategies to improve the benefit-risk ratio of glucocorticosteroids (Capó et al., 2004; Kalariya et al., 2005; Marchiori et al., 2010). With the goal of decreasing the adverse effects, nanotechnology collaborates through the reduced particle size of its nanosystems, improving the absorption and therapeutic concentration of the drug in the target tissue, allowing reproducible and long-term release of the drug at the target site, reducing the frequency of drug administration, and improving its pharmacokinetics (Sahoo et al., 2007). Clobetasol propionate (CP) is a super-high-potency dihalogenated corticosteroid used for the treatment of skin disorders such as atopic dermatitis and psoriasis (Gordon et al., 1998; Wiedersberg et al., 2008).

Polymeric nanocapsules are vesicular nanocarrier systems in which an oily phase is confined in a cavity surrounded by a thin polymeric membrane (Vauthier & Bouchemal, 2009). On the other hand, polymeric nanospheres are formed by a polymeric matrix (Schaffazick et al., 2003). The preparation of semisolid formulations containing polymeric nanostructured systems for cutaneous application have been studied lately aiming to control the release of some active substances, to improve their photostability (Milão et al., 2003; Jiménez et al., 2004; Alves et al., 2005; Alves et al., 2007; Paese et al., 2009; Marchiori et al., 2010), and to promote the drug penetration in the stratum corneum (Alves et al., 2007). Moreover, the small particle size of the nanocarriers ensures close contact with the stratum corneum (Guterres et al., 2007).

In 2003, Milão and co-workers showed that hydrophilic gels (Carbopol 940®) containing diclofenac-loaded nanocapsules present non-Newtonian behaviors with plastic properties. Intact nanostructures in gels were observed by freeze-fracture electronic mycroscopy after 3 months of storage at room temperature. Jiménez and co-workers (2004) demonstrated that the skin accumulation of octyl methoxycinnamate after the application of an oil-in-water emulsion containing its free form was significantly lower in comparison to the formulation containing octyl methoxycinnamate-loaded nanocapsules. This study demonstrated that the inclusion of octyl methoxycinnamate-loaded nanocapsules in sunscreen

formulations decreases its skin accumulation since the *in vitro* octyl methoxycinnamate release mechanism was governed by its high lipophilicity and by the hydrophobicity and crystallinity of the polymeric material.

A comparison among Carbopol 940® hydrogels containing different nimesulide-loaded nanoparticles (nanocapsules, nanospheres and nanoemulsion) was reported by Alves and co-workers (2005). All formulations presented pseudoplastic characteristics according to the Ostwald's model and no thixotropic phenomenon was detected, regardless of the type of nanoparticles. In the following study, the authors (Alves et al., 2007), studied the human skin penetration and skin distribution of nimesulide after the topical application of these formulations, using the tape stripping technique and Franz-type diffusion cells. Hydrogels (HG) containing nimesulide-loaded nanocapsules presented a higher penetration of nimesulide in the deeper skin in comparison to the formulations containing nimesulide-loaded nanospheres or nanoemulsion. The efficiency of hair follicles to act as a drug reservoir after the topical application of a formulation containing nanoparticles was demonstrated by Ladermann and co-workers, in 2007. In this study they showed a deeper penetration of a dye in hair follicles after the application (with massage) of a formulation containing dye-loaded nanoparticles in comparison to the formulation containing the dye in the non-particle form. The differential stripping showed that nanoparticles were stored in the hair follicles up to 10 days, while the non-particle form could be detected only up to 4 days. In addition, Paese and co-workers (2009) showed that the presence of nanocapsules in hydrogels did not produce contact sensitization in mice, demonstrating their low potential to cause contact allergic reactions.

Regarding studies conducted to develop semisolids containing CP-loaded nanoparticles, Rao and Murthy (2000) reported a lower absorption of this drug into the blood stream after the topical application of HPMC gels containing CP-loaded lipossomes compared to the same formulation containing the free drug. This result was explained by the higher drug accumulation in the skin, according to the *in vitro* CP diffusion studies across rat skin membranes. Furthermore, Capo and co-workers (2004) showed that the liposomal CP formulation presented a potency 2.35 times higher compared to the formulation containing its free form. This increase could help to reduce the dosage of the drug, leading to a decrease of its adverse effects.

Kalariya and co-workers (2005) demonstrated a lower mean flux value of CP from a cream containing the drug-loaded solid lipid nanoparticles compared to a marketed cream

containing the free drug. In addition, the nanostructured cream presented a better therapeutic response (1.9 fold for inflammation and 1.2 fold for itching) than the marketed formulation.

Recently we reported a study which developed new CP-loaded nanocarriers (nanocapsules, nanospheres and nanoemulsion) as an alternative to its topical administration (Fontana et al., 2009). All drug-loaded nanocarriers showed a controlled drug release and a protection of this drug against UVA radiation. The polymer, poly(ε-caprolactone) (PCL), and the oil demonstrated an important influence on the drug release profile. Furthermore, PCL nanocapsules showed a better control of drug released compared to other polyesters (Fontana et al., 2010a).

Regarding the studies on the development of semisolids containing CP-loaded nanoparticles, neither reports on the study of such formulations containing CP-loaded polymeric nanoparticles nor comparisons between the feasible to control the *in vitro* drug release from hydrogels similarly to liquid colloidal suspensions have been reported. In the present work we evaluated the influence of CP-loaded polymeric nanoparticles on the physicochemical and rheological properties of Carbopol Ultrez® 10 NF hydrogels prepared only with aqueous solvents as well as on their *in vitro* drug release profiles. These formulations could promote a controlled drug release with a consequent decrease in the immediate contact of the total applied drug to the skin, as well as prevent the cutaneous irritation. In order to study the influence of the polymeric material on these characteristics, hydrogels containing CP-loaded nanoemulsion were also prepared and characterized.

2. MATERIALS AND METHODS

2.1. Materials

CP was a gift from Neo Quimica (Goiás, Brazil). PCL and sorbitan monostearate (Span 60®) were acquired from Sigma-Aldrich (São Paulo, Brazil). Caprylic/capric triglyceride mixture and imidazolidinyl urea were delivered from Brasquim (Porto Alegre, Brazil) and Alpha Quimica (São Paulo, Brazil), respectively; polysorbate 80 (Tween 80®) and polyethylene glycol 400 were supplied by Henrifarma (São Paulo, Brazil). Acetate cellulose membranes (0.45 μm pore size) were acquired from Millipore (São Paulo, Brazil). Carbopol Ultrez® 10 NF and triethanolamine were acquired from DEG (São Paulo, Brazil). Clobetasol propionate commercial gel (Clob-X 0.05 %, batch 2880038, Galderma) was purchased locally. HPLC grade methanol was acquired from Tedia (São Paulo, Brazil). Ethanol and acetone were acquired from Impex (São Paulo, Brazil). All chemicals and solvents were used as received.

2.2. Preparation of nanocapsules (NC), nanospheres (NS) and nanoemulsion (NE)

CP-loaded nanocapsules (CP-NC), nanospheres (CP-NS) and nanoemulsion (CP-NE) were prepared by interfacial deposition of preformed polymer (Fessi et al., 1988a), nanoprecipitation (Fessi et al., 1988b) and spontaneous emulsification (Martini et al., 2007), respectively, at a drug concentration of 0.5 mg mL^{-1}, according to the quali-quantitative formula described by Fontana and co-workers (2009). Briefly, an organic solution containing the drug, the medium chain triglyceride mixture, sorbitan monostearate, PCL and acetone was added under moderate magnetic stirring to an aqueous solution containing polysorbate 80. The magnetic stirring was maintained for 10 minutes. Then, acetone was removed and the aqueous phase concentrated by evaporation (bath at 40 °C) under reduced pressure. The final formulation was adjusted to 25 mL. Blank NC, NS and NE formulations (B-NC, B-NS, B-NE) were prepared as control, omitting the presence of the drug (Fontana et al., 2009). All formulations were prepared protected from light and kept in the dark during all the time.

2.3. Physicochemical characterization of nanoparticle dispersions

After preparation, nanoparticle formulations were characterized based on the following parameters: drug content, encapsulation efficiency, pH, particle size, polydispersity index and zeta potential, according to the methodology described in our previous report (Fontana et al., 2009).

Drug content (mg mL^{-1}) was determined after dissolution of nanoparticles in acetonitrile. Encapsulation efficiency was determined by the quotient of the drug entrapped and the total drug content by ultrafiltration/centrifugation technique (Microcon 10,000 MW, Millipore). CP was assayed by liquid chromatography according to a method previously validated (Fontana et al., 2010b). pH values were determined directly in the dispersion using a calibrated potentiometer (MPA-210 Model, MS-Tecnopon, São Paulo, Brazil).

Particle sizes and polydispersity indices were estimated by photon correlation spectroscopy (PCS) after adequate dilution of an aliquot of the suspension in purified water (Zetasizer Nanoseries, Malvern Instruments, Worcestershire, UK). Zeta potentials were measured using the same instrument at 25 °C, after the dilution of the samples in 10 mM NaCl aqueous solution.

2.4. Preparation of hydrogels

Hydrogels were prepared with a mortar and pestle. Carbopol Ultrez® 10 NF (acrylic acid polymer) was used for the preparation of the hydrogels due to its widely use in

pharmaceutical formulations and its fast redispersion in water (Fresno Contreras et al., 2001), according to the quali-quantitative formula described in Table 1. Briefly, Carbopol Ultrez® 10 NF was dispersed using CP-loaded nanoparticle formulations (NC, NS or NE) resulting in a concentration of 0.05 % of the drug. This dispersion was neutralized with triethanolamine to obtain an adequate semisolid formulation for skin application. Imidazolidinyl urea was added as preservative. Using the same method, formulations containing blank nanoparticles and a control hydrogel (without any nanoparticle formulation) were prepared. In addition, a hydrogel containing free CP was prepared. In this case, water and an etanolic solution containing the drug was used instead of nanoparticles during the dispersion of the polymer. The formulations were called HG-CP-NC, HG-CP-NS and HG-CP-NE for hydrogels containing CP-loaded nanocapsules, nanospheres and nanoemulsion, respectively; HG-B-NC, HG-B-NS and HG-B-NE for the respective hydrogels containing blank nanoparticles; HG-CP for hydrogel containing free CP, HG-B (without drug - placebo hydrogel 1) and HG (without drug and ethanol - placebo hydrogel 2) for control hydrogels. All formulations were prepared in triplicate.

Table 1 – Quali-quantitative composition of hydrogels.

Component	HG-CP-NC, NS or NE	HG-B-NC, NS or NE	HG	HG-CP	HG-B
Carbopol Ultrez® 10 NF	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
Imidazolidinyl urea	0.6 g	0.6 g	0.6 g	0.6 g	0.6 g
Triethanolamine	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g
Clobetasol propionate	-	-	0.05 g	0.05 g	-
CP-NC, CP-NS or CP-NE	ad 100 g	-	-	-	-
B-NC, B-NS or B-NE	-	ad 100 g	-	-	-
Ethanol	-	-	-	ad 50 g	ad 50 g
Ultra pure water	-	-	ad 100 g	ad 50 g	ad 50 g

2.5. Physicochemical characterization of hydrogels

Hydrogels were characterized according to the following characteristics: drug content, pH, spreadability, rheological behavior and *in vitro* drug release. In order to establish an adequate comparison, a commercial hydrogel (HG-C) was characterized in paralel (CP commercial gel - Clob-X 0.05 %, batch 2880038, Galderma).

2.5.1. Assay of CP in hydrogels

The content of CP in hydrogels was assayed by liquid chromatography (LC). Approximately 1.0 g of each formulation was placed in a 25 mL volumetric flask. Methanol was added and the flask was maintained under moderate stirring by ultrasound for 30 minutes. This sample was centrifuged, filtered through a paper filter (Quantitative filter, JP41 – 28 µm) and through a 0.45 µm nylon membrane before LC analysis. The chromatographic system was previously validated and consisted of a Gemini RP-18 column (250 x 4.60 mm, 5 µm particle size, 110 Å pore diameter, Phenomenex, Torrance, USA) and a Shimadzu LC-20A system, (LC-20AT pump, SPD-M20A photodiode-array (PDA) detector, CBM-20A system controller, SIL-20A auto sampler, Shimadzu, Tokyo, Japan). The mobile phase at a flow rate of 1.0 mL min^{-1} consisted of methanol-water (80:20 v/v). The volume injected was 20 µL and the drug was detected at 241 nm. The method was linear (r^2 = 0.9995, y = 39220.89x – 9236.51) in the range of 5.0 – 40.0 µg mL^{-1}, precise (repeatability: 0.05 – 0.25 %; intermediate precision: 0.29 – 0.96 %), accurate (100.12 %) and specific. The specificity was tested in presence of the excipients of the formulations (nanoparticles and hydrogels) and demonstrated that these components did not alter the CP assay. Peak-purity evaluation using the PDA detector showed that no impurities and/or excipients were co-eluting with the CP peak.

2.5.2. Determination of pH

pH values of hydrogels (n = 3) were determined in the dispersion of an aliquot of the formulation in ultrapure water (10 %, w/v) using a calibrated potentiometer (MPA-210 Model, MS-Tecnopon, São Paulo, Brazil).

2.5.3. Evaluation of rheological properties of hydrogels

The rheological study was carried out at 25 ± 1 °C using a rotational viscosimeter (LVDV II+ Pro model, Brookfield, USA) spindle SC4-25 with a small sample adaptador. The data obtained were analyzed with the Rheocalc software (V3.1-1 version, Brookfield, USA).

The shear stress ramp was applied for 1200 s, and 20 different points were recorded, using a shear rate interval of 0.05 s^{-1}. The aim of this characterization was to evaluate the influence of different CP-loaded nanoparticles on the rheological properties of hydrogels. The rheograms were analysed using different flow models: Binghan ($\tau = \tau + \eta\gamma$), Casson ($\tau = \tau_o^{0.5} + \eta^{0.5}\gamma^{0.5}$), Ostwald ($\tau = \kappa\gamma^{0.5}$) and Herschel-Bulkley ($\tau = \tau_o + \kappa\gamma^{0.5}$), where τ is the shear stress, τ_o is the yield stress, η is the viscosity, n is the index of flow, κ is the index of consistency and γ is the shear rate (Kim et al., 2003).

2.5.4. Determination of the spreadability

The evaluation of spreadability was performed at room temperature (25 ± 1 °C) and using the parallel plate method with weights previously described by Borghetti and Knorst (2006). The sample was introduced in a central hole (1 cm) of a mold glass plate. The mold plate was carefully removed and the sample was pressed subsequently with glass plates of known weights, with intervals of 1 min between each plate. Spreading areas reached by samples between each addition of a glass plate were measured in millimeters in vertical and the horizontal axes. Results were expressed in terms of the spreading area as a function of the applied mass according to the following equation (Eq. 1) and represent the mean of three determinations:

$$S_i = \frac{d^2 \cdot \pi}{4} \qquad (1)$$

in which S_i is the spreading area (mm^2) after the application of a determined mass i (g), and d is the mean diameter (mm) reached by each sample. The spreading area was plotted against the plate weights to obtain the spreading profiles.

The spreadability factor (S_f) was also calculated and represents the spread a formulation is able to expand on a smooth horizontal surface when a gram of weight is added on it, under the conditions described in the methodology above. The following equation (Eq. 2) is used to calculate the spreadability factor (Milan et al., 2007):

$$S_f = \frac{A}{W} \qquad (2)$$

in which S_f (mm^2.g) is the spreadability factor resulting from the ratio between (A) the maximum spread area (mm^2) after the addition of the sequence of weights used in the experiment and (W) the total weight added (g).

2.6. *In vitro* drug release assay

In vitro release of CP from HG-CP-NC, HG-CP-NS, HG-CP-NE and HG-CP was studied using vertical Franz diffusion cells at 37 ± 0.5 °C (n = 6). This study aimed to evaluate if the nanoencapsulation of CP could impact in some modification on its release behavior from hydrogels. Two independent experiments (n = 3) were carried out for each formulation. Acetate cellulose membrane (0.45 μm pore size) was fitted between donor and receptor compartment. The diffusion area was 2.14 cm^2 and the receptor chamber volume was 6.0 mL. The receptor medium consisted of water/Tween 80®/PEG 400 (60:0.5:40 v/v) (Hu et al., 2002; Fontana et al., 2009; Fontana et al., 2010a) and was continuously stirred. One gram of hydrogel containing 500 μg of CP (infinite dose) was evenly spread on the membrane surface. Half a milliliter of the receptor medium was taken at predetermined time intervals of 2, 3, 4, 5, 6, 7 and 8 h and replaced by an equal volume of fresh medium. The amount of CP released was determined by LC, according to a method previously validated for *in vitro* drug release studies (Fontana et al., 2009).

The Higuchi's model ($C = k.t^{0.5}$) was used to evaluate the influence of the type of the nanoparticle structure in the hydrogel on the drug release profiles. C is the cumulative amount of drug released at time t and k is a constant reflecting the design variables of the system related to the diffusion area, diffusion coefficient and drug's solubility in the system (Siepmann & Peppas, 2001; Quintanar-Guerrero et al., 2008). The mathematical modeling was performed using the software MicroMath® Scientist® for Windows™.

2.7. Statistical analysis

All formulations were prepared and analyzed in triplicate. Results are expressed as mean ± SD (standard deviation). One-way analysis of variance (ANOVA) was used to compare the experimental data. Post-hoc multiple comparisons were done by Tukey's test for significance at p-values ≤ 0.05. All analyses were run using the SigmaStat Statistical Program (Version 3.0, Jandel Scientific, USA).

3. RESULTS AND DISCUSSION

3.1. Characterization of nanoparticles

CP-NC, CP-NS and CP-NE presented a macroscopic homogeneous appearance, drug content close to their theorical value (0.5 mg mL^{-1}), and encapsulation efficiency close to 100 %. All formulations presented neutral pH values, nanometric mean size (140-220 nm),

low polydispersity index (< 0.25) and negative zeta potential. These results are in accordance with our previous study for CP-loaded nanoparticles (Fontana et al., 2009).

3.2. Characterization of hydrogels

The use of CP-loaded nanoparticles to prepare the hydrogels allowed preparing these hydrophilic semisolid dosage forms without the use of non-aqueous cosolvents. Marketed formulations currently available are prepared using ethanol as cosolvent. Carbopol Ultrez® 10 NF hydrogels were prepared using CP-NC, CP-NS and CP-NE (0.5 mg mL^{-1}) freeshly prepared (HG-CP-NC, HG-CP-NS, HG-CP-NE, respectively). For comparison purposes, hydrogels containing free CP (HG-CP), blank nanoparticles (HG-B-NC, HG-B-NS, HG-B-NE) and placebo formulations (HG-B and HG) were also prepared. All hydrogels showed white color, glossy and homogeneous aspect and satisfactory organoleptic characteristics regardless of the type of the nanostructure. On the other hand, HG-B and HG as well a HG-CP presented a transparent and homogeneous aspect.

Table 2 shows characteristics of hydrogels containing drug-loaded nanoparticles, the hydrogel containing the free drug (HG-CP) as well as placebo hydrogels (HG-B, HG). All hydrogels presented drug content close to their theorical value (0.50 mg g^{-1}) and pH values around 6.0, which is adequate for the topical application (Alves et al., 2007). Formulations prepared with blank nanoparticles presented similar pH values compared to those containing the drug (5.83 ± 0.16, 5.70 ± 0.09 and 5.92 ± 0.14 for HG-B-NC, HG-B-NS and HG-B-NE, respectively). No influence of the nanoparticles nor the presence of the drug was observed on this parameter ($p > 0.05$). However, statistical analyses showed lower pH values for all formulation compared to the commercial formulation (7.20 ± 0.05).

The evaluation of rheological properties of semisolid forms has a fundamental importance, since it serves as a support in predicting the effects of the formulation and processing characteristics of the product and can evaluate their quality and stability (Borghetti and Knorst, 2006). The rheological properties of these formulations are therefore closely related to filling and removal of packaging, to their spreadability and adherence to the skin, to their acceptability by the patient, and to the physical stability of the product (Borghetti and Knorst, 2006).

Table 2 – Physicochemical characteristics of hydrogels (mean ± standard deviation, n = 3).

Formulation	Drug content (mg mL^{-1})	pH
HG-CP-NC	0.518 ± 0.006	$5.97 \pm 0.19^{a,b}$
HG-CP-NS	0.512 ± 0.014	$5.85 \pm 0.09^{a,b}$
HG-CP-NE	0.519 ± 0.009	5.70 ± 0.14^{a}
HG	-	6.16 ± 0.06^{b}
HG-CP	0.509 ± 0.011	6.11 ± 0.08^{b}
HG-B	-	5.67 ± 0.02^{a}

Means, in column, with the same letter are not statistically different (Anova, TuKey test, $p \leq 0.05$)

Figure 1 shows the reograms of formulations containing CP (Figure 1A) and placebo formulations (Figure 1B) obtained by plotting the applied shear rate as a function of the shear stress. As can be see in rheograms, the formulations showed non-Newtonian behavior with pseudoplastic properties, in which the viscosity decreases with increasing the shear rate (Kim et al., 2003), as better observed in Figure 2 (A and B). In addition, all formulations presented thixotropy. This phenomenon indicates the dismantling of the three-dimensional structure of the system and has a special interest in the technology of semisolid, making it more fluid when submitted to external pressure and therefore spreading more easily in the applied region (Chhabra and Richardson, 2008). Alves and co-workers (2005) also observed the non-Newtonian behavior and the pseudoplastic characteristic of gels uninfluenced by the presence of nanocapsules, nanospheres and nanoemulsion. However, the thixotropic behavior was not observed by these authors probably due to the lower concentration of the gel-forming polymer used by them.

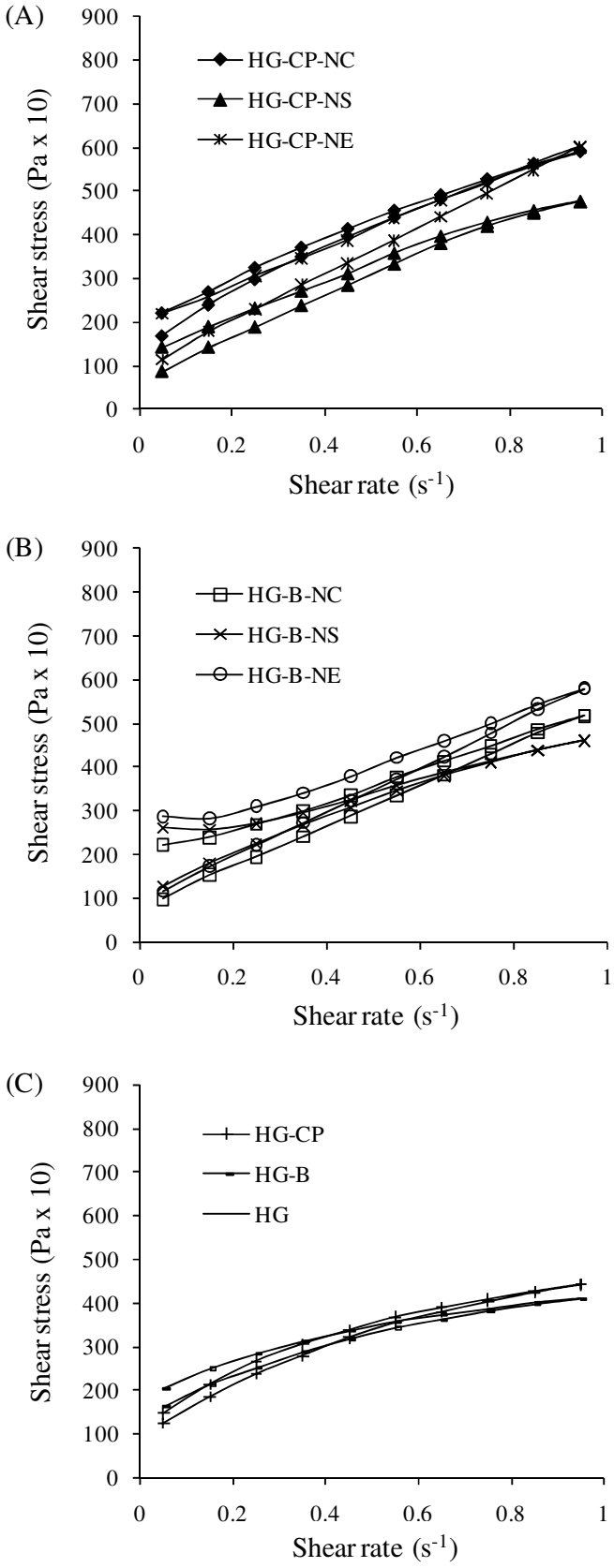

Figure 1 – Rheograms of hydrogels (n = 3): (A) hydrogels containing CP-loaded nanoparticles; (B) hydrogels containing blank nanoparticles; (C) hydrogels containing free CP and placebo formulations.

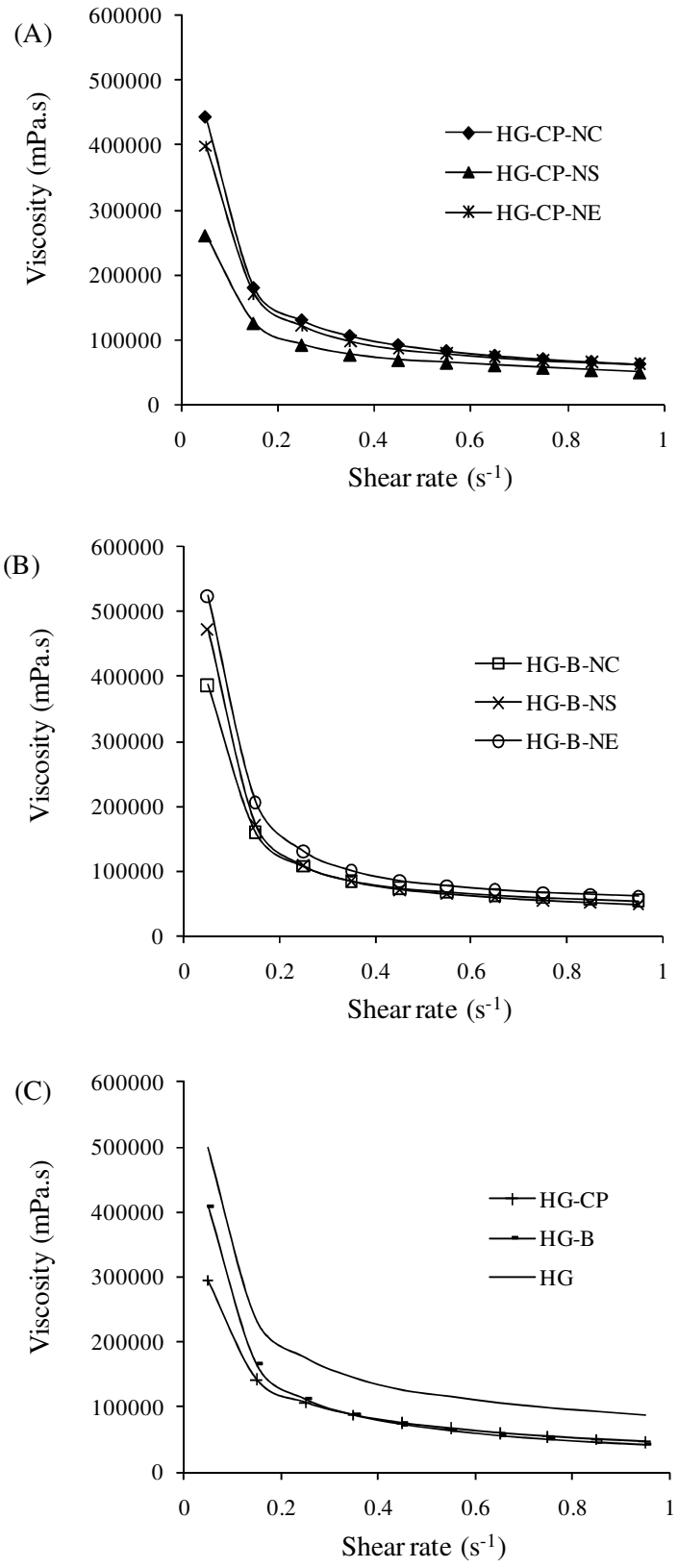

Figure 2 – Graphic representation of viscosity (mPa.s) of hydrogels in relation to the shear rate (s^{-1}) (n = 3): (A) hydrogels containing CP-loaded nanoparticles; (B) hydrogels containing blank nanoparticles; (C) hydrogels containing free CP and placebo formulations.

There are several models which may be used to establish the flow index (*n*) in different now-Newtonian systems, such as Binghan, Casson, Ostwald, and Herschel-Bulkley models. The rheograms obtained for all formulations fitted better to the Herschel-Bulkley's model, showing regression coefficients higher than 0.99 (Table 3).

Table 3 - Regression coefficient (r^2) for various flow models in shear rate-shear stress curve.

Formulation	Binghan	Casson	Ostwald	Herschel-Bulkley
HG-CP-NC	0.966 ± 0.028	0.982 ± 0.002	0.942 ± 0.037	**0.998 ± 0.001**
HG-CP-NS	0.963 ± 0.001	0.990 ± 0.001	0.949 ± 0.001	**0.998 ± 0.001**
HG-CP-NE	0.987 ± 0.03	0.988 ± 0.002	0.935 ± 0.00	**1.000 ± 0.001**
HG	0.955 ± 0.001	0.992 ± 0.002	0.966 ± 0.001	**0.999 ± 0.001**
HG-CP	0.880 ± 0.006	0.961 ± 0.002	0.958 ± 0.006	**0.993 ± 0.003**
HG-B	0.955 ± 0.023	0.989 ± 0.008	0.991 ± 0.012	**1.000 ± 0.005**

For topical anti-inflammatory formulations, the consistency of the samples is specially an important feature, due to the fact that it must be applied to the skin in thin layers (Martinez et al., 2007). The yield stress (YS), flow indices (*n*) and consistency (κ) were determined for all formulations after preparation and the results are shown in Table 4. According to the selected model, almost all flow indices were lower than 1 indicating a pseudoplastic behavior. However, the formulation containing the drug-loaded nanoemulsion (HG-CP-NE) showed a flow index close to 1 with a YS of 8.66 ± 0.84 (Pa), demonstrating a tendency to a plastic behavior (Chhabra and Richardson, 2008). No significant diference was observed between the formulations containing the drug-loaded nanosystem and their respective placebo formulation (n = 0.99 ± 0.13, 0.71 ± 0.19 and 1.00 ± 0.06 for HG-B-NC, HG-B-NS and HG-B-NE, respectively) and control formulation (HG), showing no influence of the presence of the drug on this parameter (ANOVA, $p > 0.05$). In addition, HG-CP prepared with a hydroalcoholic solution and its respective control formulation (HG-B) also presented flow indices below 1 as well as the commercial product (n = 0.35 ± 0.27), indicating their pseudoplastic behaviors. Pseudoplastic and plastic behaviors were previously reported for Carbopol® hydrogels containing polymeric nanoparticles and nanoemulsions. Alves and co-workers (2005) and

Paese and co-workers (2009) showed a pseudoplastic behavior of hydrogels containing nimesulide-loaded nanoparticles and benzophenone-loaded nanocapsules, whose rheograms fit better to the Ostwald model. Milão and co-workers (2003) reported a plastic behavior for hydrogels containing diclofenac-loaded nanocapsules, with a good fitting to the Casson's model. These different findings could be related to the differences in the type of the polymer (Carbopol 940 x Carbopol Ultrez NF 10) and its concentration in the formulation (0.2 % x 0.5 %).

Regarding the consistency index all hydrogels containing drug-loaded nanoparticles as well as free CP showed similar values (ANOVA, $p > 0.05$). No difference was observed in relation to their placebo formulations (κ = 46940 ± 5718 mPa.s, 41485 ± 6403 mPa.s and 51419 ± 3237 mPa.s for HG-B-NC, HG-B-NS and HG-B-NE, respectively). In addition, all formulations presented lower consistency indices in comparison to the commercial formulation (187534 ± 53690 mPa.s).

The pseudoplastic behavior is characterized by the existence of a YS which must be exceeded before the fluid deforms or flows (Chhabra and Richardson, 2008). According to our results (Table 4) small yields were required to the formulations start to flow and no statistical diferences were observed between the formulations containing the different drug-loaded nanoparticles (ANOVA, $p > 0.05$).

Table 4 - Flow index (n), consistency index (κ), yield stress (YS) and spreadability factor (S_f) of the hydrogels.

Formulation	n	κ (mPa.s)	YS (Pa)	S_f ($mm^2.g^{-1}$)
HG-CP-NC	$0.81 \pm 0.39^{a,b}$	53479 ± 10400^{a}	5.32 ± 3.67^{a}	3.87 ± 0.34^{a}
HG-CP-NS	$0.80 \pm 0.00^{a,b}$	44642 ± 2832^{a}	3.41 ± 0.46^{a}	3.27 ± 0.03^{a}
HG-CP-NE	0.96 ± 0.03^{a}	53732 ± 5111^{a}	8.66 ± 0.84^{a}	2.64 ± 0.18^{b}
HG	$0.63 \pm 0.09^{a,b}$	71830 ± 7531^{a}	13.20 ± 10.50^{a}	3.13 ± 0.37^{b}
HG-CP	0.49 ± 0.05^{b}	44463 ± 3330^{a}	1.52 ± 1.35^{a}	3.69 ± 0.07^{a}
HG-B	0.41 ± 0.06^{b}	35667 ± 2741^{a}	6.60 ± 6.76^{a}	3.53 ± 0.25^{a}

Means, in column, with the same letter are not statistically different (Anova, TuKey test, $p \leq 0.05$).

In addition, no significant diference was observed between the formulations containing the drug-loaded nanosystem and their respective placebo formulation (YS = 7.49 ± 1.83 Pa, 6.69 ± 1.99 Pa and 9.22 ± 1.21 Pa for HG-B-NC, HG-B-NS and HG-B-NE, respectively). In a similar way, no significant diference was observed between the formulation containing the free drug (HG-CP) and its respective control formulation (HG-B). These results demonstrate that the presence of the drug does not show any influence on this parameter (ANOVA, $p > 0.05$). Furthermore, commercial hydrogel showed a similar YS (YS = 4.46 ± 14.26 Pa) compared to all formulations (ANOVA, $p > 0.05$).

Spreadability is another important characteristic of hydrogels to be evaluated during the development studies, being responsible for the correct dosage transfer to the target site and the easily application on the substrate (Garg et al., 2002). The results of the spreadability of hydrogels in function of the added weight are shown in Figure 3. The graphic representation of spreadability shows that the incorporation of the nanoparticles in the semisolid did not modify their profiles compared to HG-CP and HG-B. In addition, hydrogels containing polymeric nanoparticles (NC or NS) showed a tendency to a better spreadability profile (higher spread area per weight) compared to the hydrogel containing NE, regardless of the presence of the drug (Figure 3A and 3B).

The spreadability factor (Table 4) was calculated to analyze the differences among the formulations. Regarding the formulations containing drug-loaded nanoparticles (HG-CP-NC and HG-CP-NS) showed the higher spreadability factor (ANOVA, $p \leq 0.05$). This value was similar to those obtained for HG-CP and HG-B. The lowest spreadability factor was showed by the HG-CP-NE. In addition, similar results were obtained for formulations containing blank nanoparticles compared to their respective drug-loaded formulations (S_f = 3.48 ± 0.36 $mm^2.g^{-1}$, 3.52 ± 0.03 $mm^2.g^{-1}$ and 2.91 ± 0.15 $mm^2.g^{-1}$ for HG-B-NC, HG-B-NS and HG-B-NE, respectively). These results are in accordance with the spreadability behavior observed in Figure 3. However, HG-C showed a higher spreadability factor (S_f = 5.12 ± 0.31 $mm^2.g^{-1}$) ($p \leq 0.05$) compared to all other hydrogels. The higher spreadability of HG-C could be explained by the presence of some excipients in its composition, such as alantoin and poly(ethyleneglycol). Alves and co-workers (2005) evaluated the spreadability of hydrogels using the same method and did not detect any significant difference between formulations containing nanoparticles (NC, NS and NE) and the gel control (Carbopol 940® at 0.2 % w/w).

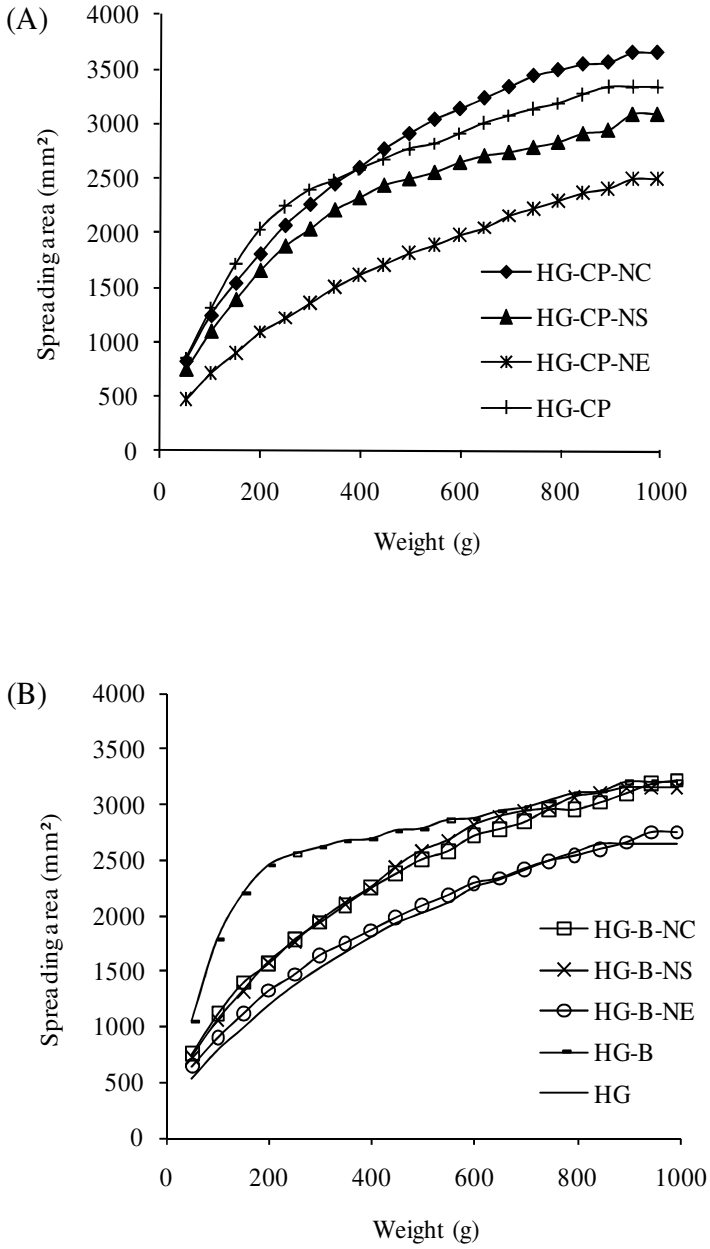

Figure 3 - Graphic representation of spreadability of hydrogels (n = 3): (A) hydrogels containing CP; (B) blank hydrogels (placebo hidrogels).

3.3. In vitro CP release from hydrogels

In vitro release studies from hydrogels containing CP-loaded nanoparticles (HG-CP-NC, HG-CP-NS and HG-CP-NE) and hydrogel containing free clobetasol propionate (HG-

CP) were carried out using vertical diffusion Franz cells. Figure 4 shows the *in vitro* CP release profiles from the different hydrogels. HG-CP showed a higher amount of CP released after 8 h compared to the formulations containing the drug-loaded nanoparticles. Among these formulations, HG-CP-NC and HG-CP-NS presented a lower amount of drug released per cm^2 after 8 h compared to HG-CP-NE (ANOVA, $p \leq 0.05$).

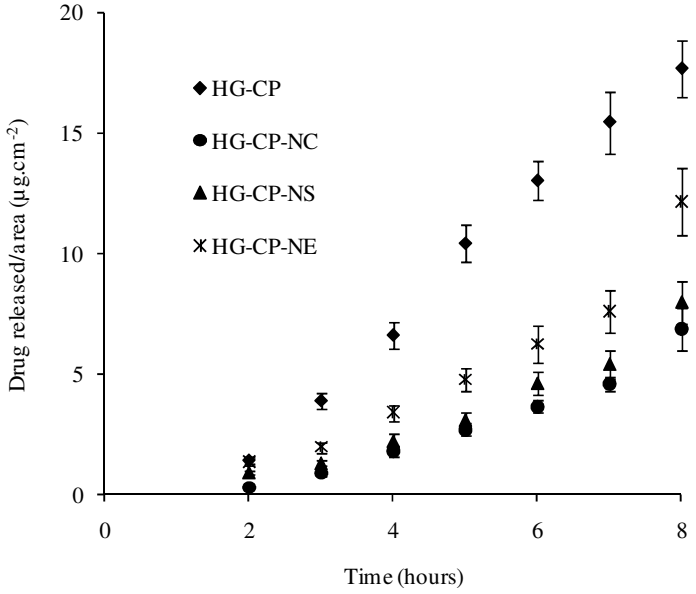

Figure 4 - Release profile of clobetasol propionate from hydrogel containing CP-loaded NC, NS, NE and hydrogel containing free CP using vertical Franz diffusion cells (n=6).

Drug flux of each formulation was determined by the slope of the curve obtained by plotting the amount of CP released per cm^2 against the square root of time (Flynn et al., 1999). Hydrogels containing the nanoencapsulated drug showed a lower release rate of CP (HG-CP-NC: 1.03 ± 0.11 $\mu g.cm^{-2}.h$, HG-CP-NS: 1.13 ± 0.12 $\mu g.cm^{-2}.h$, HG-CP-NE: 1.65 ± 0.19 $\mu g.cm^{-2}.h$) compared to the hydrogels containing the free drug (HG-CP: 2.79 ± 0.22 $\mu g.cm^{-2}.h$) (ANOVA, $p \leq 0.05$). Regarding the formulations containing the nanostructured systems, HG-CP-NC and HG-CP-NS presented a similar release rate ($p > 0.05$) and a higher release rate compared to the HG-CP-NE ($p \leq 0.05$). In addition, the results were modeled according to the Higuchi's model presenting a good correlation coefficient and model selection criteria. Higuchi constants (*k*) are shown in Table 5. Considering the results from the *in vitro* drug release studies, it can be demonstrated the importance given by the presence of the polymer to control the *in vitro* release of CP from hydrogels. The influence of the viscosity can be refuted. All drug-loaded nanoparticle hydrogels presented similar consistency indices

(Table 4), as previously discussed. The importance of the polymer was previously reported for the respective nanoparticle liquid dispersions (Fontana et al., 2009), although a statistical difference could be observed between the CP release from NC and NS suspensions in this case. The absence of this difference in the release rate from hydrogels could be explained by the influence of the higher viscosity of such formulations.

Table 5 - Observed rate constants (*k*), correlation coefficients (*r*) and MSC obtained by fitting the clobetasol propionate release from free clobetasol propionate (HG-CP) and from different nanocarriers (HG-CP-NC, HG-CP-NS, HG-CP-NE) according to the Higuchi's square root model.

Higuchi model	HG-CP	HG-CP-NC	HG-CP-NS	HG-CP-NE
k (h^{-1})	4.822 ± 0.308^{a}	1.497 ± 0.122^{c}	1.800 ± 0.144^{c}	2.657 ± 0.258^{b}
r (range)	0.994 ± 0.003	0.959 ± 0.017	0.936 ± 0.011	0.932 ± 0.026
MSC (range)	0.692 ± 0.047	0.430 ± 0.096	0.442 ± 0.063	0.466 ± 0.080

Means, in line, with the same letter are not statistically different (Anova, TuKey test, $p \leq 0.05$).

4. CONCLUSION

Hydrogels containing CP-loaded nanoparticles were prepared without using non-aqueous cosolvents, showing adequate drug content, pH compatible to the topical application and pseudoplastic behavior. *In vitro* drug release studies showed a controlled CP release following the Higuchi's model from hydrogels containing the nanoencapsulated drug compared to the hydrogels containing the free drug. The best release control was obtained for semisolid formulations containing CP-NC and CP-NS, as observed for their respective colloidal suspensions, showing the influence of the presence of the polymer in obtaining this behavior. These formulations could be suggested as a new nanomedicine for the topical treatment of psoriasis and atopic dermatitis.

Acknowlegdements: M. C. F. thanks CAPES/Brasil for her scholarship. R. R. H. thanks Programa FIPE Jr/UFSM for her fellowship. The authors thank to Rede Nanocosméticos CNPq/MCT for the financial support.

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DISCUSSÃO GERAL

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Os sistemas carreadores de substâncias ativas, como as nanoemulsões e as nanopartículas poliméricas, têm sido muito estudados nas últimas décadas. Diversos métodos de preparação, utilizando diferentes polímeros, óleos e tensoativos têm sido empregados para a obteção desses nanossistemas (SOPPIMATH *et al.*, 2001; SCHAFFAZICK *et al.*, 2005; CRUZ *et al.*, 2006; FRIEDRICH *et al.*, 2008; OURIQUE *et al.*, 2008; ALMEIDA *et al.*, 2009). Recentemente, estes sistemas de liberação controlada de fármacos têm sido incorporados em hidrogéis, com o objetivo de aumentar a estabilidade destas suspensões coloidais e obter uma forma farmacêutica com consistência e propriedades reológicas adequadas para aplicação tópica (MILÃO *et al.*, 2003; ALVES *et al.*, 2005; MARCHIORI *et al.*, 2010).

Considerando as atividades anti-inflamatórias e imunossupressoras do propionato de clobetasol e os seus efeitos colaterias após aplicação tópica (FANG *et al.*, 1999), este estudo visou a possibilidade de se obter uma liberação mais lenta do fármaco a partir da sua nanoencapsulação, podendo diminuir a sua irritação cutânea após a aplicação tópica e uma melhora na fotoestabilidade do fármaco. A partir disso, foi estudado o comportamento de diferentes formulações de base nanotecnológica (dispersões coloidais e semissólidos) contendo propionato de clobetasol, avaliando-se, principalmente, o efeito do tipo de nanocarreador (nanocápsulas, nanoesferas ou nanoemulsões) e do material polimérico sobre as diferentes propriedades das formulações.

Com o objetivo de verificar a possibilidade de incorporação do propionato de clobetasol nas nanopartículas foram preparadas preliminarmente nanocápsulas contendo este fármaco, através do método da deposição interfacial do polímero pré-formado (FESSI *et al*., 1988a). Neste teste foi avaliado o emprego do propionato de clobetasol em duas diferentes concentrações (0,50 e 1,00 mg/mL). A formulação que continha o propionato de clobetasol na concentração de 1,00 mg/mL foi descartada por apresentar precipitado 15 dias após a preparação.

Após a escolha da concentração adequada de fármaco, foi desenvolvido e validado um método através da cromatografia líquida, para a determinação do propionato de clobetasol em suspensões de nanocápsulas poliméricas (Capítulo 1). Embora a literatura científica já apresentasse alguns métodos analíticos para quantificação deste fármaco em formulações

farmacêuticas (REEPMEYER *et al*., 1998; GAGLIARDI *et al*., 2000; GAGLIARDI *et al.*, 2002; MOSTAFA *et al.*, 2002), ainda não havia sido descrito nenhum método que pudesse ser aplicado a matrizes complexas, como em suspensões de nanocápsulas poliméricas. Além disso, buscamos um método que não utilizasse tampão na consituição da fase móvel, de maneira a prolongar a vida útil do equipamento e da coluna cromatográfica e de permitir a extração do fármaco a partir das formulações. O método demonstrou boa especificidade com relação aos demais excipientes da formulação, sendo preciso (DPR < 1,5 %), exato (98,33 %), robusto e linear na faixa de concentração de 5 a 40 µg/mL. Na análise da precisão, foi avaliado o emprego de dois sistemas cromatográficos distintos, que demonstraram respostas analíticas similares.

Após o desenvolvimento analítico, no Capítulo 2, foram desenvolvidas e caracterizadas nanoemulsões e suspensões de nanocápsulas e nanoesferas poliméricas contendo propionato de clobetasol (0,50 mg/mL), empregando a poli(ε-caprolactona) como polímero biodegradável. As formulações foram caracterizadas físico-quimicamente em relação ao teor de propionato de clobetasol, eficiência de incorporação do fármaco nas nanopartículas, pH, diâmetro médio de partícula, índice de polidispersão, potencial zeta e estabilidade (MORA-HUERTAS *et al.*, 2010). No estudo da estabilidade, as formulações foram avaliadas imediatamente após a preparação e após 3, 6 e 9 meses de armazenamento. Todas as formulações apresentaram um aspecto macroscópico homogêneo e leitoso. O efeito Tyndall (reflexo azulado) foi observado para todas as formulações preparadas. Este reflexo azulado pode ser explicado pelo tamanho nanométrico e pelo movimento Browniano das nanopartículas (SCHAFFAZICK *et al.*, 2003). Através da microscopia eletrônica de transmissão pode-se observar o formato esférico homogêneo das nanopartículas.

Após o preparo, as formulações apresentaram teor de fármaco próximo ao teórico, eficiência de incorporação próximo a 100 %, pH na faixa neutra, potencial zeta negativo, índice de polidispersão entre 0,12 e 0,22 e diâmetro médio de partícula na faixa nanométrica. As nanoesferas apresentaram diâmetro médio estatisticamente menor que as nanocápsulas e nanoemulsões, explicado pela ausência de óleo em sua estrutura. Na comparação dos resultados obtidos entre as formulações contendo o propionato de clobetasol com aqueles obtidos a partir das respectivas formulações brancas não foi possível observar a influência da presença do fármaco sobre as características físico-químicas dos sistemas. Os resultados de diâmetros médios, índices de polidispersão e potenciais zeta apresentados pelas formulações coloidais estiveram de acordo com os valores normalmente encontrados para as nanopartículas (FRIEDRICH *et al*., 2008; OURIQUE *et al*., 2008; ALMEIDA *et al*., 2009).

Com relação à estabilidade das formulações em função do tempo de armazenamento, pode-se observar o aumento do potencial zeta (em módulo) para todas as formulações, contribuindo com a estabilidade dos nanossistemas (SCHAFFAZICK *et al.*, 2003). Na análise do teor de fármaco e na eficiência de incorporação as formulações não sofreram alterações durante o armazenamento. No entanto, todas as nanopartículas apresentaram uma dimuição do pH após 1 mês de armazenamento, que pode ser explicada pelo relaxamento das cadeias da poli(ε-caprolactona) expondo seus grupamentos ácidos, pela degradação deste polímero ou ainda pela hidrólise dos triglicerídeos presentes no óleo utilizado nas nanocápsulas e nanoemulsões (ALMEIDA *et al*., 2009). As nanocápsulas permaneceram estáveis por 9 meses. Por outro lado, as nanoemulsões apresentaram um aumento do índice de polidispersão no 9º mês e as nanoesferas apresentaram um aumento no tamanho de partícula e também no índice de polidispersão no 6º mês, resultados que podem ser explicados pela aglomeração e coalescência das nanopartículas (SCHAFFAZICK *et al.*, 2003).

A análise da liberação *in vitro* do propionato de clobetasol a partir das diferentes nanopartículas foi realizada através do método dos sacos de diálise. O tipo de nanopartícula influenciou a liberação do propionato de clobetasol. As nanocápsulas apresentaram um maior controle de liberação, sendo necessários 10 dias para atingir um percentual superior a 95 % de liberação do fármaco. Essa propriedade de controle da liberação demonstrada pelas nanocápsulas foi seguida pelas nanoesferas (7 dias) e nanoemulsões (5 dias). Este resultado é particularmente interessante se comparado ao comportatmento da solução etanólica do propionato de clobetasol, de onde o fármaco difundiu totalmente para o meio de liberação em 24 horas. De acordo com a modelagem matemática, todas as nanopartículas apresentarm uma liberação inicial rápida seguida de uma liberação lenta do fármaco, seguindo o modelo biexponencial. Desta forma, foi demonstrado que 90 % do propionato de clobetasol encontra-se retido no interior da nanopartícula e que apenas 10 % está adsorvido na sua superfície, independente do tipo de nanopartícula. Esta liberação controlada pode ser explicada pela presença do óleo, pela alta lipofilia do fármaco e também pela presença da poli(ε-caprolactona), um polímero altamente cristalino e hidrofóbico (CRUZ *et al.*, 2006).

O estudo da fotodegradação do propionato de clobetasol frente à luz UVA demonstrou que tanto o óleo como a poli(ε-caprolactona) presente nas nanopartículas são importantes na fotoproteção do fármaco (OURIQUE *et al*., 2008). As nanopartículas (nanocápsulas, nanoesferas e nanoemulsão) reduziram a fotodegradação do propionato de clobetasol quando comparada com a solução capilar comercial e com a solução etanólica contendo o fármaco livre. As nanocápsulas apresentaram maior tempo de meia-vida (18,16 horas), seguidas pela

nanoemulsão (16,79 horas) e nanoesferas (14,61 horas). Por outro lado, a meia-vida da solução capilar comercial e da solução etanólica foi de 10,99 horas e 9,06 horas, respectivamente.

Diante desses resultados, as nanocápsulas foram selecionadas para a etapa seguinte (Capítulo 3), onde foram preparadas nanocápsulas com 3 diferentes poliésteres amorfos com diferente hidrofilia, o poli($_{DL}$-ácido lactídeo), o poli(ácido lactídeo-co-ácido glicolídeo) 50:50 e o poli(ácido lactídeo-co-ácido glicolídeo) 85:15, utilizando o propionato de clobetasol na concentração de 0,5 mg/mL.

Independente do tipo de polímero amorfo utilizado, todas as formulações apresentaram aparência macroscópica homogênea, teor de fármaco próximo ao teórico, eficiência de incorporação próxima a 100 %, pH ácido, tamanho de partícula na faixa nanométrica, baixo índice de polidispersão e potencial zeta negativo. Comparando os resultados entre a formulação de nanocápsulas contendo propionato de clobetasol com a formulação de nanocápsulas sem o fármaco novamente não foi possível observar influência da presença do fármaco sobre as características físico-químicas avaliadas. Os diâmetros médios e os índices de polidispersão apresentados pelas suspensões de nanocápsulas estiveram de acordo com valores normalmente encontrados para nanocápsulas preparadas pelo método da deposição interfacial de polímeros pré-formados (FRIEDRICH *et al*., 2008).

No estudo da estabilidade frente ao armazenamento, as nanocápsulas preparadas com PLA foram estáveis por 3 meses, enquanto que aquelas preparadas com PLGA (50:50 e 85:15) apresentaram diminuição no teor do propionato de clobetasol após 3 meses de armazenamento. Os tamanhos de partícula e os índices de polidispersão não sofreram alterações durante o armazenamento. Por outro lado, o potencial zeta sofreu um pequeno aumento em módulo em todas as formulações. As nanocápsulas preparadas com PLGA apresentaram uma pequena diminuição do pH, provavelmente devido a liberação de ácido láctico e glicólico livres no meio. A menor estabilidade destas formulações em relação às formulações preparadas com poli(ε-caprolactona) pode ser explicada pela maior velocidade de degradação do PLA e PLGA, conforme comentado na revisão da literatura (JAIN *et al.*, 2000; MAURUS & KAEDING, 2004).

No estudo da liberação *in vitro* do propionato de clobetasol a partir das nanocápsulas preparadas com polímeros de diferentes hidrofobias foi observado que, independente do tipo de poliéster utilizado, a liberação do fármaco foi controlada, tendo 100 % do fármaco sido liberado apenas após 8 dias de experimento. De acordo com a modelagem matemática, a liberação do fármaco a partir destas formulações também apresentou um comportamento

biexponencial, com uma liberação inicial lenta seguida de uma liberação prolongada (CRUZ *et al.*, 2006), sugerindo que apenas 10-20 % do fármaco se encontra adsorvido na superfície externa da nanocápsula e 80-90 % está retido em seu interior, de maneira similar às formulações com PCL.

Comparando os resultados obtidos nos estudos de liberação *in vitro* para as formulações de nanocápsulas preparadas com PCL (Capítulo 2) e as nanocápsulas preparadas com os polímeros amorfos (Capítulo 3), pode-se verificar que o emprego da PCL como parede polimérica, por ser um polímero com estrutura semicristalina, ter um arranjo mais organizado das cadeias poliméricas e por ser mais hidrofóbico (MIDDLETON & TIPTON, 2000), levou a uma liberação mais prolongada do propionato de clobetasol.

Ainda, através da Lei das Potências foi possível estudar o mecanismo pelo qual o propionato de clobetasol é liberado das nanocápsulas poliméricas (KORSMEYER *et al.*, 1983). Neste estudo tanto as nanocápsulas preparadas com PCL, quanto as preparadas com os outros poliésteres, apresentaram uma liberação com comportamento anômalo, demonstrando que o fármaco é liberado tanto por difusão através do polímero como também entre as cadeias poliméricas após seu relaxamento (SOPPIMATH *et al.*, 2001).

O estudo da fotoproteção do propionato de clobetasol associado à nanocápsulas poliméricas preparadas com PLA e PLGA demonstrou que estes polímeros amorfos também protegem o fármaco da fotodegradação e que não houve diferença significativa entre eles. Estes resultados foram similares aos obtidos com as nanocápsulas preparadas com o polímero semicristalino (PCL). Desta forma, concluiu-se que a fotoproteção do fármaco não é proporcionada pela estrutura do polímero, mas sim, pela presença do polímero (amorfo ou semicristalino) e pelo tamanho reduzido das partículas da suspensões coloidais, que refletem a luz incidente sobre a formulação protegendo o fármaco.

Com o objetivo de preparar uma formulação semissólida contendo propionato de clobetasol evitando-se o emprego de um cossolvente não-aquoso, que pudesse ser uma alternativa viável para o tratamento de dermatites que não respondem aos corticóides menos potentes e com a possibilidade de diminuir os efeitos colaterais deste fármaco, as suspensões de nanocápsulas preparadas com PCL, que apresentaram a maior estabilidade frente ao armazenamento e o melhor controle da liberação do fármaco, além de uma significativa fotoproteção do mesmo, foram selecionadas para a preparação de géis hidrofílicos empregando Carbopol Ultrez® 10 NF (Capítulo 4). Formulações similares foram preparadas a partir de nanoesferas e nanoemulsões com o objetivo de se estudar a influência da presença da poli(ε-caprolactona) e do óleo nas nanopartículas. Estes hidrogéis foram caracterizados

através do teor de fármaco, pH, espalhabilidade e viscosidade. Para se avaliar a liberação *in vitro* do propionato de clobetasol a partir dos semissólidos foi empregado o método de difusão em células de Franz (MARCHIORI *et al.*, 2010).

Os hidrogéis contendo as diferentes nanoestruturas apresentaram um aspecto homogêneo, brilhoso e coloração branca, enquanto que os hidrogéis sem nanopartículas se apresentaram incolores (Figura 1). Todas as formulações demonstraram teor de fármaco próximo ao teórico e pH compatível com a administração tópica (5,5 - 6,5). Com relação aos aspectos reológicos, todos hidrogéis apresentaram um comportamento não-Newtoniano e com propriedades pseudoplásticas, indicando que a viscosidade dos semissólidos diminui com o aumento da taxa de cisalhamento, como também foi demonstrado nos estudos de Alves e colaboradores (2005) e Paese e colaboradores (2009). Além disto, todas as formulações seguiram o modelo de Herschel-Bulkley e apresentaram tixotropia. De acordo com este modelo, os índices de consistência e os índices de fluxo foram similares para todas as formulações, exceto para o hidrogel comercial que apresentou um índice de consistência estatisticamente superior ($p \leq 0,05$).

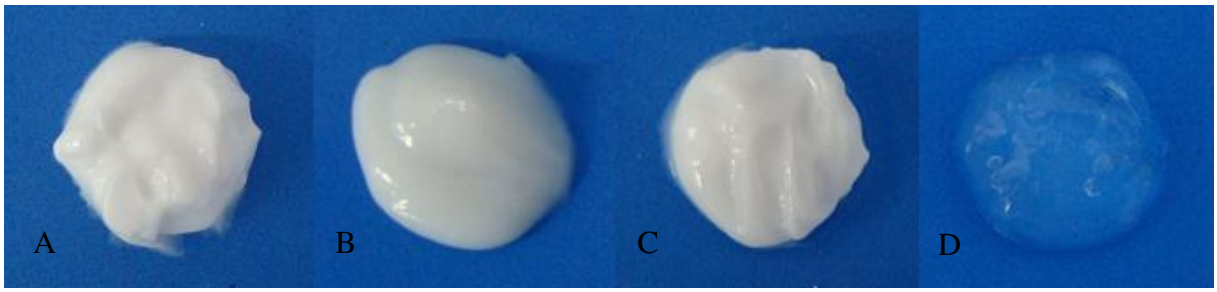

Figura 1 - Hidrogéis contendo nanocápsulas, nanoesferas e nanoemulsão de propionato de clobetasol (A, B e C, respectivamente) e hidrogel contendo propionato de clobetasol livre (D).

Para se comparar a espalhabilidade dos diferentes hidrogéis foi calculado o fator de espalhabilidade. A presença das nanoestruturas não influenciou a espalhabilidade do hidrogel, como também foi observado por Alves e colaboradores em 2005. Os hidrogéis contendo a nanoemulsão apresentaram uma menor espalhabilidade comparada àqueles contendo nanocápsulas e nanoesferas. No entanto, o hidrogel atualmente disponível no mercado apresentou espalhabilidade superior. Apesar disto, independente do tipo de nanopartícula presente nos hidrogéis e considerando-se os resultados da análise reológica, do pH, do teor de fármaco e da espalhabilidade, pode-se concluir que todos os hidrogéis preparados estão adequados para a administração tópica, evitando-se o emprego de cossolventes não-aquosos.

O estudo da liberação *in vitro* do propionato de clobetasol a partir dos hidrogéis contendo as diferentes nanopartículas demonstrou um controle da liberação do propionato de clobetasol quando esses resultados são comparados àqueles obtidos para o hidrogel contendo o fármaco livre. Esta liberação ocorreu de acordo com o modelo de Higuchi (MARCHIORI *et al.*, 2010), embora não tenha sido observada uma diferença significativa entre os hidrogéis contendo nanocápsulas e nanoesferas. No entanto, o hidrogel contendo a nanoemulsão apresentou um menor controle de liberação do fármaco. Estes resultados demonstram a importância da presença do polímero (PCL) nas estruturas para a obtenção de um controle da liberação do propionato de clobetasol a partir dos hidrogéis.

A partir dos resultados obtidos foi demonstrada a possibilidade de preparação de nanocápsulas, nanoesferas e nanoemulsão contendo propionato de clobetasol e sua incorporação em hidrogéis sem a presença de solventes orgânicos, mostrando-se como uma alternativa viável para o tratamento de doenças cutâneas, como a psoríase e a dermatite atópica. Desta forma, este estudo demonstrou a viabilidade tecnológica de se obter diferentes formulações nanoestruturadas contendo propionato de clobetasol, incluindo-se as suspensões coloidais, nanoemulsões e as formas farmacêuticas semissólidas. A influência de fatores como o tipo de nanopartícula e o tipo de material polimérico sobre diferentes propriedades dos sitemas foi investigada. Considerando-se o conjunto dos resultados pode-se sugerir a formulação de nanocápsulas preparada com PCL e sua respectiva formulação semissólida como aquelas mais promissoras para estudos posteriores. Essa sugestão está baseada no principal objetivo inicial do trabalho que era de desenvolver uma formulação de base nanotecnológica como alternativa viável para o tratamento de afecções cutâneas, evitando o contato imediato da pele com a dose total de fármaco aplicada.

Como perspectivas para a continuidade deste trabalho pode-se citar o estudo da permeação cutânea do propionato de clobetasol a partir da(s) formulação(ões) desenvolvida(s) e estudos para se avaliar a atividade farmacológica do fármaco após aplicação tópica da(s) formulação(ões). Com relação a esse último aspecto, já se encontra em andamento a avaliação da atividade dos hidrogéis contendo propionato de clobetasol associado a nanocápsulas ou propionato de clobetasol livre no tratamento da dermatite de contato induzida por sulfato de níquel, em modelo animal.

CONCLUSÕES

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- No presente trabalho foi desenvolvido um método cromatográfico para o doseamento do propionato de clobetasol em suspensões de nanocápsulas, permitindo uma análise rápida e simples;

- Foi possível preparar nanocápsulas, nanoesferas e nanoemulsões contendo propionato de clobetasol (0,50 mg/mL) apresentando alta eficiência de incorporação e tamanho nanométrico de partículas, sendo que a suspensão de nanocápsulas apresentou a maior estabilidade frente ao armazenamento;

- A liberação *in vitro* ressaltou o importante papel da PCL no controle da liberação do fármaco a partir das nanoestruturas demonstrando um comportamento biexponencial;

- As nanopartículas aumentaram a fotoestabilidade do propionato de clobetasol frente à luz UVA, sendo que as nanocápsulas foram mais eficientes do que as nanoesferas e nanoemulsões nessa proteção;

- As nanocápsulas preparadas com PLA e PLGA (50:50 e 85:15) contendo propionato de clobetasol (0,5 mg/mL) apresentaram adequadas características físico-químicas e alta eficiência de incorporação, sendo que as formulações preparadas com o polímero mais hidrofóbico (PLA) apresentaram maior estabilidade frente ao armazenamento;

- Independente do material polimérico, todas as formulações demonstraram o controle da liberação do fármaco a partir das nanocápsulas de acordo com o modelo biexponencial;

- Considerando a Lei das Potências, o mecanismo de liberação do fármaco ocorreu através do transporte anômalo, independente do tipo de polímero utilizado;

- A nanoencapsulação do propionato de clobetasol nas nanocápsulas permitiu o aumento da fotoestabilidade frente à radiação UVA, demonstrando a capacidade dos polímeros amorfos protegerem o fármaco;

- O maior controle de liberação do propionato de clobetasol a partir das nanocápsulas e a maior estabilidade frente ao armazenamento foram observados para aquelas formulações preparadas empregando a PCL como parede polimérica;

- Hidrogéis de Carbopol Ultrez® 10 NF contendo propionato de clobetasol (0,05 %) nanoencapsulado foram preparados sem o uso de cossolventes não-aquosos e apresentando comportamento pseudoplástico. O estudo da liberação *in vitro* do fármaco a partir dos hidrogéis demonstrou o controle da liberação do fármaco comparado com o hidrogel contendo o fármaco livre. O maior controle da liberação foi demonstrado pelas formulações semissólidas contendo o fármaco incorporado em nanocápsulas e nanoesferas, conforme observado nos estudos de liberação a partir das respectivas suspensões coloidais, demonstrando a influência da presença da PCL;

- A suspensão de nanocápsulas preparada com PCL e sua respectiva formulação semissólida são formulações de base nanotecnológica representando uma alternativa viável para o tratamento de afecções cutâneas, evitando o contato imediato da pele com a dose total de fármaco aplicada.

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