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Priscila Rosa

DESENVOLVIMENTO E AVALIAÇÃO BIOLÓGICA DE PREPARAÇÕES NANOESTRUTURADAS DE DESONIDA PARA USO TÓPICO

Santa Maria, RS
2018

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Tese apresentada ao Curso de Pós-Graduação em Ciências Farmacêuticas, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Doutor em Ciências Farmacêuticas**

Orientadora: Profª Dra. Andréa Inês Horn Adams
Coorientadora: Profª Dra. Cristiane de Bona da Silva

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Aprovado em 27 de agosto de 2018:

Andréa Inês Horn Adams, Dra. (UFSM)
(Presidente/Orientador)

Sandra Elisa Haas, Dra. (UNIPAMPA) - parecer

Clésio Soldateli Paim, Dr. (UNIPAMPA)

Leandro Machado de Carvalho, Dr. (UFSM)

Cristiane Franco Codevilla, Dra. (UFSM)

Santa Maria, RS
2018

DEDICATÓRIA

Dedico essa conquista à minha família.

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RESUMO

DESENVOLVIMENTO E AVALIAÇÃO BIOLÓGICA DE PREPARAÇÕES NANOESTRUTURADAS DE DESONIDA PARA USO TÓPICO

AUTORA: Priscila Rosa
ORIENTADORA: Andréa Inês Horn Adams
COORIENTADORA: Cristiane de Bona da Silva

Os corticoides tópicos são fármacos extensamente utilizados na dermatologia. Entretanto, a terapia com esses medicamentos apresenta algumas limitações relacionadas à duração do tratamento e à potência do fármaco, os quais podem favorecer o surgimento de efeitos indesejados. Os sistemas nanoparticulados vêm sendo considerados alternativas para o controle da liberação de fármacos, direcionamento específico e redução dos efeitos adversos. Neste contexto, este trabalho teve por objetivo preparar formulações semissólidas de base nanotecnológica contendo desonida, visando a obtenção de formulações aplicáveis ao tratamento de doenças dermatológicas. Para isso, foram desenvolvidas suspensões de nanocápsulas poliméricas empregando o polímero Eudragit ® RL 100, utilizando o óleo de açaí (OA) ou triglicerídeos de cadeia média (TCM) como núcleo oleoso e o fármaco na concentração de 0,25 mg mL^{-1}. As formulações contendo OA apresentaram tamanho médio de partícula em torno de 165 nm, e as com TCM, de aproximadamente 131 nm. Ambas apresentaram índice de polidispersão (PDI) < 0,2, pH ácido, teor de fármaco próximo ao valor teórico, eficiência de encapsulamento em torno de 81% e potencial zeta positivo. A exposição direta das suspensões de nanocápsulas às radiações UVA e UVC demonstrou que os sistemas coloidais foram eficazes na fotoproteção da desonida e que as formulações contendo OA apresentaram maior fotoestabilidade. O estudo de fototoxicidade *in vitro* indicou que na concentração de 0,5 mg mL^{-1} as formulações não apresentam potencial fototóxico em fibroblastos murinos e queratinócitos humanos, usando os ensaios de viabilidade celular MTT e NRU. Foram preparados hidrogéis nanoestruturados por meio da dispersão do polímero Amigel® *(sclerotium gum*) diretamente nas suspensões coloidais. Os semissólidos apresentaram pH compatível com a aplicação cutânea, teor de fármaco em torno de 0.25 mg g^{-1} e mantiveram as nanoestruturas. Quanto às características de fluxo, os hidrogéis apresentaram fluxo não Newtoniano, pseudoplástico, de acordo com o modelo de Ostwald. O estudo de permeação/penetração cutânea utilizando os hidrogéis nanoestruturados evidenciou que o fármaco atingiu o sítio de ação dos corticoides tópicos. A atividade biológica das preparações foi avaliada utilizando modelo de dermatite induzida por óleo de cróton e indicou que os hidrogéis nanoestruturados apresentaram efeito comparável com formulação contendo desonida disponível comercialmente.

Palavras-chave: Hidrogel. Óleo de açaí. Nanocápsulas poliméricas. Corticoide.

ABSTRACT

DEVELOPMENT AND BIOLOGICAL EVALUATION OF NANOSTRUCTURED PREPARATIONS CONTAINING DESONIDE FOR TOPICAL USE

AUTHOR: Priscila Rosa
ADVISOR: Andréa Inês Horn Adams
CO-ADVISOR: Cristiane de Bona da Silva

Topical corticosteroid are widely used in dermatology. However, the therapy with these drugs has some limitations related to the treatment duration and to drug potency, that may cause undesirable effects. Nanoparticulate systems have been considered alternatives for the drug control release, targeting to the specific site of action and reducing the adverse effects. In this context, the objective of this study was to prepare nanotechnologic-based semisolid formulations containing desonide, aiming to obtain applicable formulations for the treatment of dermatological diseases. Polymeric nanocapsule suspensions were prepared, employing the polymer Eudragit® RL 100, açai oil (AO) or medium chain triglycerides (MCT) as oily core and the drug at 0.25 mg mL^{-1}. Formulations containing AO presented mean particle size of 165 nm, and those prepared with MCT mean particle size around 131 nm. Both showed polidispersity index (PDI) < 0.2, acid pH, drug content near to the theoretical value, encapsulation efficiency of 81% and positive zeta potential. Direct exposure of the nanocapsule suspensions to UVA and UVC radiations showed that the colloidal systems were effective in protecting desonide from photodegradation and that the formulations containing açai oil presented greater photostability. *In vitro* phototoxicity study indicated that at concentration of 0.5 mg mL^{-1}, formulations did not present phototoxic potential in 3T3 murine fibroblasts and human keratinocytes using MTT and NRU cell viability assays. The nanostructured hydrogels were prepared by dispersing the Amigel® polymer (*sclerotium gum*) directly into the colloidal suspensions. Semisolids presented pH compatible with topical application, drug content around 0.25 mg mL^{-1}, and maintained the nanostructures. Regarding the flow properties, hydrogels presented non-Newtonian pseudoplastic flow, according to Ostwald model. The skin permeation/penetration study with the nanostructured hydrogels showed the drug reached the site of action of topical corticosteroids. Biological activity of the preparations was evaluated using a croton oil-induced dermatitis model and indicated that the nanostructured hydrogels had comparable effect to the commercially available formulation of desonide.

Keywords: Hydrogel. Açai oil. Polymeric nanocapsules. Corticosteroid.

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LISTA DE ABREVIATURAS E SIGLAS

CAS	Chemical Abstract Service
CT	Corticoides tópicos
DCB	Denominação comum brasileira
EGR	Elemento de resposta aos glicocorticoides
ICH	International Conference on Harmonisation
IL	Interleucina
NF kB	Fator nuclear Kb
OA	Óleo de açaí
PCL	Poly-ε-caprolactona
PLA	Ácido poliláctico
PLGA	Poli(ácido lático co-ácido-glicólico)
TCM	Triglicerídeos de cadeia média
TNF	Fator de necrose tumoral

SUMÁRIO

INTRODUÇÃO

1. INTRODUÇÃO

Os corticosteroides tópicos são fármacos extensamente utilizados em dermatologia, principalmente no tratamento de doenças inflamatórias, como dermatite atópica, psoríase, dermatite de contato ou manifestações tópicas de doenças auto-imunes (GUICHARD et al., 2015). Sua eficácia clínica está relacionada às propriedades vasoconstritoras, efeito antiproliferativo, anti-inflamatório e imunossupressor (GRAU, 2006; HUGHES; RUSTIN, 1997). Para a escolha do medicamento a ser utilizado são considerados fatores como o tipo de lesão a ser tratada, potência do corticosteroide e o veículo em que este está incorporado (GADBOIS; ARESMAN, 2017).

A desonida é um corticosteroide não fluorado utilizado no tratamento de dermatoses sensíveis aos corticoides desde a década de 1970. Quanto à sua potência, é classificada como corticoide de baixa potência nas formulações de creme e loção (HORN et al., 2010; PARISER, 2009) ou de potência moderada, em pomada e creme (HENGGE et al., 2006; ROOS et al., 2004). Geralmente, os glicorticoides de baixa e média potência são usados no tratamento de lesões inflamatórias em áreas sensíveis, como a face, pálpebras e áreas intertriginosas (GADBOIS; ARESMAN, 2017; HENGGE et al., 2006), e em pacientes pediátricos e idosos, devido a maior área superficial nos pediátricos e fragilidade da pele em pacientes idosos (HENGGE et al., 2006). Além disso, em muitos casos, os corticoides de baixa potência são preferidos, uma vez que os efeitos adversos cutâneos parecem ser menos graves do que os provocados pelos de alta potência, mesmo quando esses últimos são utilizados em concentrações mais baixas (NICHOLS, 2004).

Os corticosteroides são fármacos suscetíveis à hidrólise e fotólise (ALBINI, FASANI, 1998). Estudos avaliando a fotoestabilidade da desonida indicaram sua suscetibilidade à fotodegradação frente às radiações UVA, UVB e UVC. Iqbal, Husain e Gupta (2006) constataram a fotoinstabilidade do fármaco solubilizado em solventes orgânicos após exposição às radiações UVB e UVC. Santa e colaboradores (2013) verificaram a fotoinstabilidade da desonida em loção capilar disponível comercialmente frente à radiação UVA (352 nm). Após 15 h de exposição direta à radiação, houve decaimento de, aproximadamente, 60% no teor do fármaco. Estudos semelhantes, realizados com as formulações loção cremosa e gel creme, também evidenciaram a fotodegradação da desonida. Os autores verificaram decaimento de, aproximadamente, 50% no teor do fármaco após 15 e 24 h de irradiação direta (UVA, 352 nm), na loção cremosa e gel creme, respectivamente (BRAGA et al., 2013; BRAGA, 2013).

Tendo em vista a fotoinstabilidade do fármaco, trabalhos realizados por nosso grupo de pesquisa buscaram estratégias para melhorar a fotoestabilidade da desonida em formulações de uso tópico. Rosa e colaboradores (2014) relataram o uso da benzofenona-3 em baixas concentrações como estabilizante em formulação de solução capilar e gel creme. A adição da benzofenona-3 foi capaz de estabilizar o fármaco na solução capilar, uma vez que após o período de exposição de 15 h, o teor remanescente de fármaco foi de 48,9% na formulação sem benzofenona e 98,61% na formulação com benzofenona a 0,3%.. Já para a formulação gel creme a concentração de 0,1% do filtro UV foi eficaz na fotoestabilização da desonida, sendo que após 48 h de exposição à radiação UVA o teor residual de fármaco foi 95,70% em comparação a 22,53% obtida para a formulação comercial (ROSA et al., 2015).

Considerando a terapia com corticoides tópicos, a utilização desses medicamentos por períodos prolongados pode favorecer o surgimento de efeitos locais indesejados, tais como atrofia da pele, hipopigmentação e aumento da perda de água transepidermal. Em doenças como a dermatite atópica, a barreira epitelial sofre alterações, o que pode favorecer a absorção sistêmica desses fármacos e, dessa forma, acarretar no surgimento de efeitos adversos sistêmicos. Outra limitação relacionada à terapia tópica com corticoides é a baixa liberação do fármaco no sítio de ação. Nesse sentido, faz-se necessário o desenvolvimento de novas terapias medicamentosas ou formulações mais eficazes, que promovam a entrega do fármaco diretamente no local de ação (LIN et al., 2018).

A fim de melhorar a biodisponibilidade, modular a penetração dérmica de substâncias, aumentar o tempo de meia vida (GUTERRES; ALVES; POHLMANN, 2007) e a estabilidade de fármacos frente à hidrólise (FRANK et al., 2015; MAZZARINO et al., 2010) e oxidação (CORADINE et al., 2014), novas estratégias, como os sistemas nanoparticulados, vêm sendo estudadas. A associação de fármacos a nanocarreadores vem sendo citada também como alternativa promissora para o aumento da fotoestabilidade de fármacos sensíveis à radiação (FERREIRA et al., 2016; OURIQUE et al., 2008; SAVIAN et al., 2015; WEBER et al., 2015). Nesse sentido, a incorporação da desonida a nanocarreadores poderia promover o aumento da fotoestabilidade do fármaco, em relação às formulações convencionais. Além disso, sistemas nanocarreadores de fármacos, como dispersão aquosa ou associados a formas farmacêuticas têm sido citados como alternativa a fim de minimizar efeitos adversos de corticoides por promover a liberação do fármaco de maneira controlada (FONTANA et al., 2009; MATHES et al., 2016) e, simultaneamente, diminuir a taxa de penetração nas camadas cutâneas, contribuindo para o melhor efeito anti-inflamatório (de ANDRADE et al., 2015; FONTANA et al., 2011; MELERO et al., 2014).

Trabalho recente relata o desenvolvimento e estudo de liberação *in vitro* da desonida a partir de nanocápsulas poliméricas preparadas com os polímeros Eudragit S100, Eudragit L100, e poly-*ε*-caprolactona (PCL) (ANTONOW et al., 2016). Entretanto, estudos sobre o efeito da nanoencapsulação na fotoestabilidade do fármaco e o impacto sobre a permeação cutânea e atividade biológica desses sistemas ainda não estão descritos.

Tendo em vista as vantagens proporcionadas pelos sistemas nanoestruturados, os problemas enfrentados na terapêutica dos corticoides e a fotoinstabilidade da desonida em formulações disponíveis comercialmente, neste trabalho foram desenvolvidas e avaliadas biologicamente formulações de base nanotecnológica contendo desonida, voltados à administração tópica, com o objetivo de avaliar o efeito propiciado pelo nanoencapsulamento na estabilidade, na penetração/permeação cutânea e na atividade anti-inflamatória de formulações tópicas de desonida.

O trabalho está estruturado na forma de dois artigos científicos, a serem submetidos. No capítulo 1 é descrita a preparação de nanocápsulas poliméricas contendo desonida, com núcleo oleoso contendo óleo de açaí ou triglicerídeos de cadeia média. O capítulo 2 trata do desenvolvimento e avaliação biológica de hidrogel de base nanotecnológica, contendo as nanoestruturas preparadas previamente.

OBJETIVOS

2 OBJETIVOS

2.1 OBJETIVO GERAL

Este trabalho teve por objetivo desenvolver preparações semissólidas de base nanotecnológica contendo desonida e avaliar sua atividade biológica, visando a obtenção de formulações voltadas ao tratamento de doenças dermatológicas.

2.2 OBJETIVOS ESPECÍFICOS

- Preparar suspensões de nanocápsulas poliméricas contendo desonida pelo método da deposição interfacial do polímero pré-formado, usando o Eudragit® RL 100 como polímero e o óleo de açaí como núcleo oleoso;

- Avaliar as características físico-químicas das suspensões de nanocápsulas quanto ao teor de fármaco, eficiência de encapsulamento, diâmetro médio de partículas, índice de polidispersão, pH e potencial zeta;

- Avaliar a estabilidade das formulações desenvolvidas em temperatura ambiente;

- Verificar o perfil de fotodegradação da desonida nanoencapsulada frente às radiações UVA e UVC;

- Estudar o perfil de liberação *in vitro* do fármaco a partir dos sistemas desenvolvidos;

- Avaliar a fototoxicidade *in vitro* da desonida quando associada ou não às nanoestruturas;

- Desenvolver formulações semissólidas do tipo hidrogel a partir dos sistemas nanoparticulados propostos;

- Avaliar a permeação/penetração cutânea *in vitro* da desonida a partir dos hidrogéis desenvolvidos;

- Avaliar a atividade anti-inflamatória das formulações semissólidas utilizando modelo de dermatite atópica *in vivo.*

REVISÃO DA LITERATURA

3. REVISÃO DA LITERATURA

3.1 CORTICOIDES TÓPICOS: CARACTERÍSTICAS, ASPECTOS FARMACOLÓGICOS E TERAPÊUTICOS

Com a síntese da hidrocortisona, na década de 1950, os esteroides tópicos foram reconhecidos como agentes eficazes no tratamento de doenças de pele. Após a síntese de outros glicocorticoides e o desenvolvimento de veículos adequados, esses fármacos tornaram-se a base da terapia de diversas doenças inflamatórias cutâneas (WYATT; SUTTER; DRAKE, 2004).

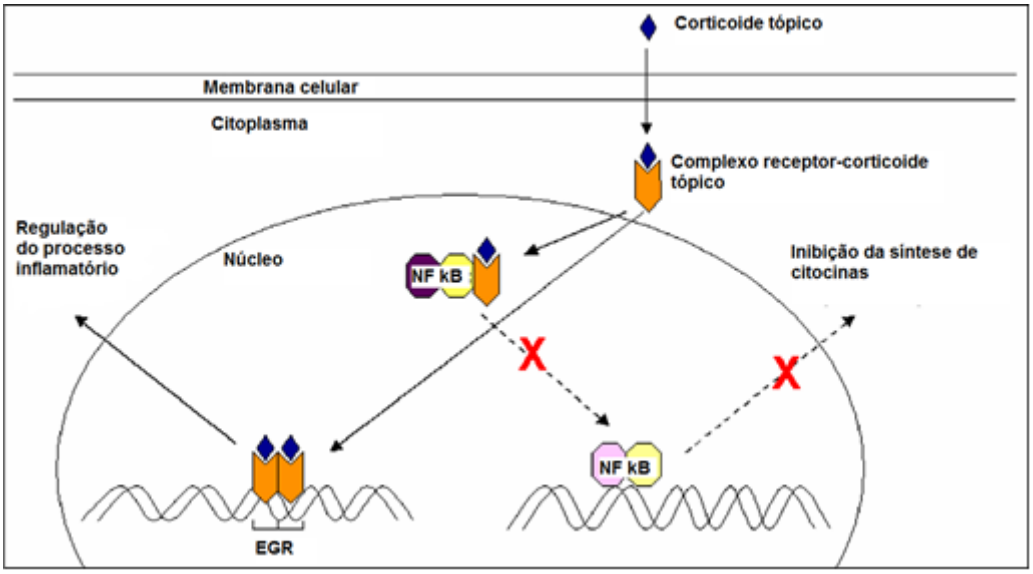
Os corticoides tópicos (CT) são usados no tratamento de eczemas, como a dermatite atópica e seborreica devido à sua ação anti-inflamatória, vasoconstritora, antipruriginosa e efeito antiproliferativo (CAHTART; THEOS, 2011). A dermatite atópica é uma doença cutânea inflamatória, crônica e reincidente que afeta até um quinto da população nos países desenvolvidos. Em geral, ocorre principalmente na infância, acometendo em torno de 10 a 20% das crianças, sendo também altamente prevalente em adultos (AUBERT-WASTIAUX et al., 2011; WEIDINGER; NOVAK, 2016). Esses fármacos são a base do tratamento da dermatite atópica e, para o controle da doença, muitas vezes, são utilizados durante meses ou anos. Embora causem efeitos colaterais, sua eficácia e segurança, quando utilizados de forma adequada, estão bem estabelecidas (AUBERT-WASTIAUX et al., 2011). A absorção dos CT está relacionada a diversos fatores, incluindo o tipo de veículo utilizado e a espessura e grau de inflamação da pele no local de aplicação (GELBARD; HEBERT, 2009). Para que possam exercer sua ação, os CT devem sofrer o processo de absorção percutânea. Esse é um processo complexo que depende das características físico-químicas do fármaco e veículo e das condições fisiológicas da pele, e que envolve os seguintes passos:

- Liberação do fármaco a partir da formulação;
- Penetração e permeação/difusão através do estrato córneo;
- Particionamento a partir do estrato córneo na epiderme viável e derme;
- Ligação aos receptores dos glicocorticoides na epiderme viável/derme.

As células alvo dos CT são os queratinócitos e fibroblastos, na epiderme e derme, onde estão localizados seus receptores. Os efeitos anti-inflamatório e imunossupressor parecem ser mediados pelos genes responsivos aos corticosteroides. Assim, o glicocorticóide se liga a seu receptor, que, dentro do núcleo, liga-se a regiões específicas que regulam o

processo inflamatório, como o fator nuclear Kb (NF kB) e o elemento de resposta aos glicocorticoides (EGR) (Figura 1). Além deste efeito direto, os CT também inibem a liberação de algumas interleucinas (IL-1; IL-2; IL-6), interferon e fator de necrose tumoral e a proliferação de células T. Nos fibroblastos, a IL-1 é responsável pela proliferação e indução da colagenase, e a IL-6 pela síntese, o que controla a espessura da pele. A inibição da IL-1 nos queratinócitos tem efeito antiinflamatório, enquanto que a mesma inibição, nos fibroblastos, tem efeito atrófico e antiproliferativo. O efeito vasoconstritor dos corticoides tópicos favorece a atividade antiinflamatória, diminuindo o eritema da área afetada; no entanto, este mecanismo de ação ainda não está completamente elucidado (GRAU, 2006; WIEDERSBERG; LEOPOLD; GUY, 2007).

Figura 1- Representação esquemática do mecanismo de ação dos corticoides tópicos. Fator nuclear Kb (NF kB), elemento de resposta aos glicocorticoides (EGR)

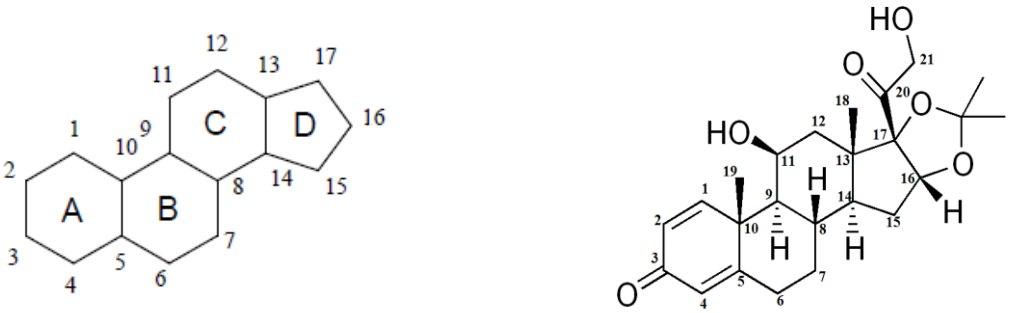


Fonte: (WIEDERSBERG; LEOPOLD; GUY, 2007)

Quanto à estrutura química, a presença do grupamento hidroxila livre no carbono 11 é essencial para que haja atividade tópica. A redução da atividade mineralocorticóide e aumento da atividade glicocorticóide podem ser adquiridas através da introdução de ligação dupla em C1 e substituição em C16 (Figura 2). A lipofilicidade e duração do efeito dos corticoides podem ser aumentadas através da adição de halogênio na posição seis ou nove. Além disso, a lipofilicidade e resistência ao metabolismo podem ser aumentadas através da acetilação ou esterificação no anel D. Com relação à farmacocinética, os corticoides tópicos apresentam

farmacocinética semelhante a dos corticosteroides administrados sistemicamente. Ligam-se às proteínas plasmáticas em diferentes graus, são metabolizados no fígado e excretados pelos rins, sendo que alguns corticoides e seus metabólitos são excretados pela bile. Corticosteroides que contêm grupos 17-hidroxil substituídos, como a desonida, são resistentes ao metabolismo local na pele. Dessa forma, aplicações repetidas podem provocar efeito cumulativo, o qual pode prolongar o efeito terapêutico, aumentar as reações adversas e aumentar a absorção sistêmica (WIEDERSBERG; LEOPOLD; GUY, 2007).

Figura 2- Estrutura química do ciclo básico dos corticoides (A) e estrutura química da desonida (B).



(A) (B)

Fonte: autor

3.2 DESONIDA

A desonida designada quimicamente por (11β, 16α)-11,21-diidroxi-16,17-[(1-metiletilideno)bis(oxi)]-pregna-1,4-dieno-3,20-diona, é um corticoide não halogenado (WIDESBERG; LEOPOLD; GUY 2007), considerado de baixa potência (classe VI), de acordo com a classificação da Organização Mundial da Saúde (OMS). É utilizada no tratamento de dermatoses de gravidade baixa à moderada e está registrada sob o número 638-94-8 no Chemical Abstract Service (CAS). Sua denominação comum brasileira (DCB) é desonida.

Algumas características físico-químicas da desonida são descritas abaixo:

- Fórmula química: $C_{24}H_{32}O_6$
- Massa molecular: 416,51 g/mol
- Ponto de fusão: 263-266 ºC para substância purificada a partir de acetato de etila e 274-275 ºC a partir de metanol

- Log P: 2,565 ± 0,628 a 25ºC
- pKa: 12,87 ± 0,10
- Solubilidade em água pH 7,00 a 25°C: 0,035 g/L

Conforme mencionado, as características químicas da desonida proporcionam efeito cumulativo na pele, prolongando o efeito terapêutico, aumentando a absorção sistêmica e por consequência a possibilidade de reações adversas (WIEDERSBERG; LEOPOLD; GUY, 2007).

No Brasil, a desonida encontra-se disponível sob as formas de loção cremosa, creme e pomada dermatológica (0,05%), gel creme (0,05%) e em associação com o antibiótico sulfato de gentamicina, na forma de gel creme (0,05%). Em outros países tem apresentação nas formas gel, loção, creme, pomada, e espuma, na concentração de 0,05% (FDA, 2018).

Corticoides tópicos de baixa potência, como a desonida, são usados principalmente em áreas mais sensíveis, como face, região genital e axilas, sendo utilizados, também, no tratamento de bebês e crianças (HORN et al., 2010). Em 2009, a desonida foi citada como o corticosteroide de baixa potência mais prescrito por dermatologistas nos EUA (GELBARD; HEBERT, 2009).

3.3 SISTEMAS NANOESTRUTURADOS COMO CARREADORES DE FÁRMACOS

Os sistemas nanoestruturados são sistemas coloidais, que apresentam dimensões de até 1 μm e diferem entre si de acordo com a composição e organização estrutural (MORA-HUERTAS; FESSI; ELAISSARI, 2010). Os nanocarreadores apresentam vantagens como promover o controle da liberação da substância ativa, melhorar a farmacocinética e a biodistribuição dos ativos terapêuticos, aumentar a estabilidade, além da vetorização do princípio ativo no local de ação, minimizando a toxicidade e a ocorrência de efeitos adversos. Devido a essas vantagens, têm atraído grande interesse no campo farmacêutico (GUTERRES; ALVES; POHLMANN, 2007; MORA-HUERTAS; FESSI; ELAISSARI, 2010; SCHAFFAZICK et al., 2003).

Na área farmacêutica, os nanocarreadores de maior relevância são os lipossomas, as nanopartículas lipídicas, as nanopartículas poliméricas (nanocápsulas e nanoesferas) e as nanoemulsões (GUTERRES; ALVES; POHLMANN, 2007).

As nanopartículas poliméricas podem ser definidas como partículas coloidais sólidas e incluem as nanoesferas e as nanocápsulas. Uma de suas características principais é o tamanho,

que em geral varia de 100 a 500 nm (ANTON; BENOIT; SAULNIER, 2008; MORA-HUERTAS; FESSI; ELAISSARI, 2010).

Quanto a sua composição e estrutura, as nanopartículas poliméricas podem ser distinguidas em nanocápsulas e nanoesferas. As primeiras podem ser consideradas como sistemas vesiculares, consistindo, geralmente, em um núcleo oleoso circundado por uma parede polimérica. Nessas estruturas, o fármaco pode estar dissolvido ou disperso no núcleo e/ou adsorvido ao material polimérico. Já as nanoesferas não apresentam óleo em sua composição e são consideradas sistemas matriciais, nos quais o fármaco pode estar retido e/ou adsorvido à matriz polimérica (MORA-HUERTAS; FESSI; ELAISSARI, 2010; SCHAFFAZICK et al., 2003; SINGH; LILLARD, 2009;). Além destas, após modificações em sua composição, foram desenvolvidas nanocápsulas com núcleo hidrofóbico composto de uma dispersão de triglicerídeos de cadeia média e monoesterato de sorbitano, envolvido por uma parede polimérica composta de poli-ε-caprolactona, denominadas de nanocápsulas de núcleo lipídico (LNC) (JAGER et al., 2009; VENTURINI et al., 2011).

Para o desenvolvimento de nanopartículas poliméricas, a escolha do polímero deve ser baseada nas características de biocompatibilidade/biodegradabilidade do polímero, da via de administração pretendida, além do método utilizado no preparo e da aplicação (ANTON; BENOIT; SAULNIER, 2008). O polímero deve ser não tóxico, biocompatível, preferencialmente biodegradável, ou que seja facilmente eliminado pelo organismo (VAUTHIER; BOUCHEMAL, 2009). Esse ainda pode ser de origem natural ou sintética. Por serem materiais não tóxicos e apresentarem propriedades adequadas à formação da parede polimérica, os polímeros naturais têm demonstrado grande potencial no desenvolvimento de nanocápsulas poliméricas. Dentre os polímeros naturais comumente utilizados estão a quitosana, o alginato e a gelatina. Por sua vez, os polímeros sintéticos apresentam alta pureza e boa reprodutibilidade, além disso, podem promover a liberação sustentada da substância ativa durante vários dias (RONG et al., 2011). Na preparação de nanocápsulas, os polímeros sintéticos mais frequentemente utilizados são a poli-ε-caprolactona (PCL), o ácido poliláctico (PLA) e o poli(ácido lático co-ácido-glicólico) (PLGA) (FRANK et al., 2015). O polímero Eudragit® RL 100, utilizado neste trabalho, é um copolímero do polietilacrilato, metilmetacrilato e cloro-trimetil-amonioetil-metacrilato e contém de 8,8 a 12% de grupamentos amônio quaternário, que conferem carga positiva ao polímero (DAS, SURESH, DESMUKH, 2010; KATARA; MAJUMDAR, 2013). É insolúvel em pH fisiológico, com capacidade de inchamento limitada, e, devido à sua carga positiva, poderia interagir com fármacos aniônicos e a mucina (KATARA; MAJUMDAR, 2013). Quanto aos

nanocarreadores contendo o polímero, trabalhos descrevem a utilização do Eudragit® RL100 na preparação de nanopartículas contendo aceclofenaco (KATARA; MAJUMDAR, 2013), acetazolamida (VERMA et al., 2013) e anfotericina B (DAS, SURESH, DESMUKH, 2010) para administração ocular, e atazanavir (SINGH; PAI, 2016), para administração oral, entre outros.

Estudos realizados com nanocápsulas poliméricas têm relacionado a parede polimérica à capacidade de controlar a liberação da substância ativa (FRANK et al., 2015; FONTANA et al., 2009; HARTER, 2013; SANTOS et al., 2013;) e de proteger substâncias da fotodegradação, como verificado para a tretinoína (OURIQUE et al., 2008), clobetasol (FONTANA et al., 2009) ditranol (SAVIAN et al., 2015) e dipropionato de betametasona (WEBER et al., 2015), por exemplo.

No desenvolvimento de nanocápsulas, diferentes tipos de óleos são utilizados, e a incorporação de substâncias ativas deve considerar fatores como a lipofilia da substância, sua solubilidade no óleo e compatibilidade com o polímero empregado. Dentre eles, os triglicerídeos de cadeia média (TCM) são amplamente usados devido a sua capacidade de solubilizar diversos tipos de fármacos e serem biocompatíveis (MORA-HUERTAS; FESSI; ELAISSARI, 2010).

Nesse sentido, a utilização de diferentes óleos vegetais nesses sistemas vem despertando o interesse dos pesquisadores, uma vez que, agregada à função estrutural de núcleo oleoso, esses poderão exercer algum efeito sinérgico, de acordo com a função pretendida. Como exemplos, a maior fotoestabilidade relatada para nanocápsulas contendo tioconazol e óleo de café verde como núcleo oleoso, em comparação a nanocápsulas do fármaco preparadas com TCM (HARTER, 2013) e o aumento da fotoestabilidade do indol-3-carbinol (IC3) associado a nanocápsulas contendo óleo de rosa mosqueta (GEHRCKE et al., 2017).

Dentre as vias de administração para as quais nanocápsulas poliméricas vêm sendo desenvolvidas destacam-se as vias parenteral, tópica, oral e oftálmica (GUTERRES; ALVES; POHLMANN, 2007).

Considerando a via tópica, tem-se buscado novos sistemas de liberação de fármacos, mais específicos ao sítio de ação, a fim de otimizar o efeito terapêutico e minimizar efeitos adversos. Nesse contexto, as nanopartículas poliméricas têm se destacado devido ao tamanho nanométrico, carga superficial, alta eficiência de encapsulamento e a possibilidade de obter nanopartículas biocompatíveis e biodegradáveis, dependendo do polímero utilizado (SHAO et al., 2016).

Dentre os fatores que afetam a liberação de um fármaco após administração tópica estão o peso molecular e lipofilicidade da substância, o tipo de formulação, a presença de promotores de absorção e as condições físicas do estrato córneo. Os sistemas nanoestruturados podem modular a difusão do fármaco na pele, modificando sua atividade e/ou sua partição. Como consequência, podem alterar a farmacocinética e biodistribuição através da pele (GUTERRES; ALVES; POHLMANN, 2007). Considerando a barreira físico-química proporcionada pelo estrato córneo, estudos têm demonstrado a eficiência dos nanocarreadores em melhorar a absorção percutânea de fármacos, sem danificar o estrato córneo, liberando a substância ativa no seu sítio de ação (SHAO et al., 2016). A penetração e o transporte desses sistemas através da pele parecem ser mediados principalmente pela composição química da formulação, encapsulação, tamanho das nanopartículas e viscosidade da formulação. Considerando que, em geral, os sistemas nanoestruturados apresentam baixa viscosidade, a incorporação desses sistemas em formulações semissólidas modifica sua propriedades reológicas, promovendo aplicação tópica adequada, além de proporcionar maior estabilidade, uma vez que os sistemas nanoestruturados podem ter sua estabilidade limitada em meio aquoso (GUTERRES; ALVES; POHLMANN, 2007).

3.4 ÓLEO DE AÇAÍ

O fruto do açaí (*Euterpe oleracea*) é proveniente da palmeira de açaí, árvore nativa da região Amazônica, pertencente à família *Arecaceae*. As palmeiras de *Euterpe oleracea* podem alcançar altura superior a 25 m e produzem frutos com diâmetro de 1,0 a 1,5 cm de coloração roxa escura, colhidos principalmente entre os meses de julho e dezembro. Esses possuem apenas uma semente, equivalente a 80% do volume total do fruto, que é coberta por camadas fibrosas e um revestimento oleoso (FACHAVO et al., 2011; PACHECO-PALENCIA et al., 2008). O fruto do açaí é usado popularmente no tratamento da anemia, diarreia, dor, inflamação, hepatites e doenças renais. Além disso, vem sendo utilizado como alimento funcional devido aos seus benefícios nutricionais (MARQUES et al., 2016).

Estudos têm demonstrado que o fruto do açaí é rico em polifenóis bioativos, dos quais se destacam as antocianinas e os flavonóides. O óleo proveniente da espécie *Euterpe oleracea* contém diversos compostos fenólicos, sendo o ácido vanílico um de seus componentes majoritários, além de ácidos graxos como o ácido oleico, ácido palmítico e ácido palmitoleico (FAVACHO et al., 2011). Outro estudo, realizado por Marques e colaboradores (2016), confirmou os compostos descritos por Favacho e colaboradores e demonstrou a presença do

ácido gama-linolênico, ácido linoleico, ácido cinâmico, ácido cafeico, ácido ferúlico e quercetina como componentes do óleo de açaí (OA).

Devido à presença dos diversos compostos bioativos, o fruto do açaí tem atraído atenção para uso como alimento funcional, em dermocosméticos e como nutracêutico (FAVACHO et al., 2011). Nesse sentido, vários autores têm estudado as atividades biológicas desses compostos bioativos nas diferentes partes do fruto do açaí. Estudo realizado por Pacheco-Palencia e colaboradores (2008) avaliou a atividade antiproliferativa de extratos da polpa e do óleo do fruto do açaí em células de adenocarcinoma de cólon, e verificou inibição de até 90,7% da proliferação celular. Favacho e colaboradores (2011) avaliaram a atividade anti-inflamatória e efeito antinociceptivo do OA. Os autores atribuíram a atividade anti-inflamatória observada à composição do óleo, que, em termos de ácidos graxos, é muito semelhante à de outros óleos com atividade anti-inflamatória, como os óleos de oliva e de peixe. A genotoxicidade *in vivo* do OA foi estudada por Marques e colaboradores (2016) em leucócitos periféricos, fígado, medula óssea e células testiculares de ratos, por meio do ensaio do cometa e teste do micronúcleo. Os autores constataram que o OA não apresentou efeito genotóxico significativo, nas condições testadas. Quanto à utilização do óleo de açaí associado a nanopartículas, Valério e colaboradores (2013), descreveram a obtenção de nanopartículas de poliuretano contendo óleo de açaí pelo método de miniemulsão polimerização. Posteriormente, os mesmos autores relataram a preparação de nanopartículas de poliuretano utilizando diisocianato de isoforona e PCL, incorporando óleo de açaí e crodamol a esses nanocarreadores (VALÉRIO et al., 2014). Em trabalho recente, Monge-Fuentes e colaboradores (2017) descreveram a obtenção de nanoemulsões contendo óleo de açaí as quais, quando utilizadas em terapia fotodinâmica para o tratamento de melanoma não metastático, apresentaram propriedades de redução tumoral.

Os ácidos graxos têm sido considerados fundamentais na resposta inflamatória, uma vez que são fontes de diversos mediadores lipídicos (AMAGAI et al., 2015). Os ácidos graxos participam do processo da sinalização do cálcio, proteína C reativa, ativação da fosfolipase C, produção do inositol 1-4-5 trifosfato (IP3) e diacilglicerol. Além disso, são precursores de lipídios mediadores do processo inflamatório como ácido araquidônico, prostaglandinas, leucotrienos e tromboxanos. Alguns estudos têm indicado que o efeito anti-inflamatório dos ácidos graxos também pode estar relacionado à diminuição nos níveis de interleucina 1α (IL-1), interleucina 6 (IL-6), interleucina 1β (IL-1 β) e fator de necrose tumoral (TNF) (FAVACHO et al., 2011).

A pele possui, em sua composição, ácidos graxos que contribuem para a formação e manutenção da barreira epitelial. Esses também participam da manutenção da membrana e estrutura das células epiteliais (KENDALL; NICOLAOU, 2013; KENDALL et al., 2015). Em doenças cutâneas como a dermatite atópica e a psoríase, a barreira epitelial é afetada (FEINGOLD, 2014; GUPTA, 2015) e há aumento na secreção de citocinas inflamatórias como o TNF, IL-1, IL-6, IL-8, IL-10 e interferon gama (FEINGOLD, 2014). No tratamento da dermatite atópica, a utilização de medicamentos e terapias que buscam a restauração e manutenção da barreira epitelial podem ser associadas. Nesse sentido, terapias dermatológicas complementares, como o uso de medicamentos homeopáticos e óleos de origem vegetal e animal também vendo utilizadas no tratamento de desordens cutâneas (LANDIS et al., 2014).

3.5 FOTOESTABILIDADE

A fotodegradação tem sido relatada para um grande número de substâncias e normalmente segue mecanismos complexos. A perda da potência de um fármaco é a principal consequência da fotodecomposição, resultando em uma substância que pode ser terapeuticamente inativa. No entanto, degradações menos severas também podem originar produtos de degradação capazes de causar efeitos tóxicos. Dessa forma, a avaliação da fotoestabilidade torna-se parte importante do desenvolvimento dos fármacos e formas farmacêuticas (TØNESSEN, 2004).

As consequências práticas da fotoinstabilidade nem sempre serão as mesmas em todos os casos. Como exemplo, pode-se citar o nifedipino, fármaco fotossensível que é decomposto pela luz em apenas alguns minutos de exposição, enquanto outros fármacos, também considerados fotossensíveis, requerem um tempo superior para sofrer alguma decomposição. Assim, o conhecimento sobre a fotoestabilidade dos fármacos e produtos farmacêuticos tem importância na escolha da embalagem adequada, rotulagem, precauções que devem ser tomadas durante a manipulação do produto e no caso da existência de produtos de degradação, orientar sobre possíveis efeitos tóxicos (TØNNESEN, 2008).

O espectro de absorção de radiação de um fármaco pode indicar a faixa de comprimento de onda na qual o fármaco é suscetível à fotodegradação (MOORE, 2004). Grande parte dos fármacos e excipientes absorve radiação na faixa ultravioleta ou visível, tendo, assim, potencial de ser fotorreativo *in vitro* ou *in vivo* (TØNNESEN, 2008). Fármacos cujo máximo de absorção ocorre em comprimentos de onda superiores a 330 nm merecem atenção especial, visto que essa radiação tem a capacidade de penetrar na pele. Sendo assim, a

reação entre a radiação luminosa e os fármacos pode gerar produtos tóxicos e, consequentemente, promover fotossensibilização ou fototoxicidade (ALBINI; FASANI, 1998; ONOUE; TSUDA, 2006). Além disso, a administração de fármacos fotorreativos seguida de exposição à radiação pode ter por consequência o aparecimento de efeitos adversos (ONOUE; TSUDA, 2006; TØNESSEN, 2004). Esses incluem as reações fototóxicas, que se manifestam nas áreas expostas à radiação, e as reações foto-alérgicas, que são reações imunes e podem se manifestar em tecidos expostos ou não à radiação (ICH, 2013; TØNESSEN, 2008). A fotossensibilidade é prevista para classes terapêuticas como os antidepressivos, antihistamínicos, antihipertensivos, antimicrobianos, antineoplásicos, hipoglicemiantes, diuréticos, anti-inflamatórios não esteroidais e antipsicóticos. Geralmente, as substâncias químicas fotossensibilizantes apresentam configuração planar, tricíclica ou policíclica e peso molecular menor que 500 Da (TØNESSEN, 2008). Além disso, substâncias que absorvem radiação na faixa de 290 a 700 nm e se distribuem por tecidos expostos à radiação, como a pele, podem causar fototoxicidade e/ou fotoalergia (ICH, 2013). Em alguns casos, os fármacos podem não sofrer fotodegradação durante exposição à fonte de radiação, entretanto podem atuar como fonte de radicais livres ou formar metabólitos fototóxicos, *in vivo* (TØNESSEN, 2008).

O amplo uso de medicamentos, a grande exposição à luz de fontes artificiais, como lâmpadas, e à radiação solar, podem estar associados ao aumento do número de efeitos adversos induzidos pela radiação (TØNESSEN, 2008). Estudo publicado recentemente indicou que, em torno de 28% dos produtos de uso tópico que constam na USP têm a indicação de acondicionamento em embalagem que proteja da luz. Entretanto, apesar da recomendação durante o acondicionamento, as informações sobre precauções a serem tomadas durante o manuseio e aplicação são praticamente inexistentes. Além disso, dependendo da forma farmacêutica em que o fármaco se encontra, pode haver indicações distintas quanto à fotoproteção (BAERTSCHI et al., 2015).

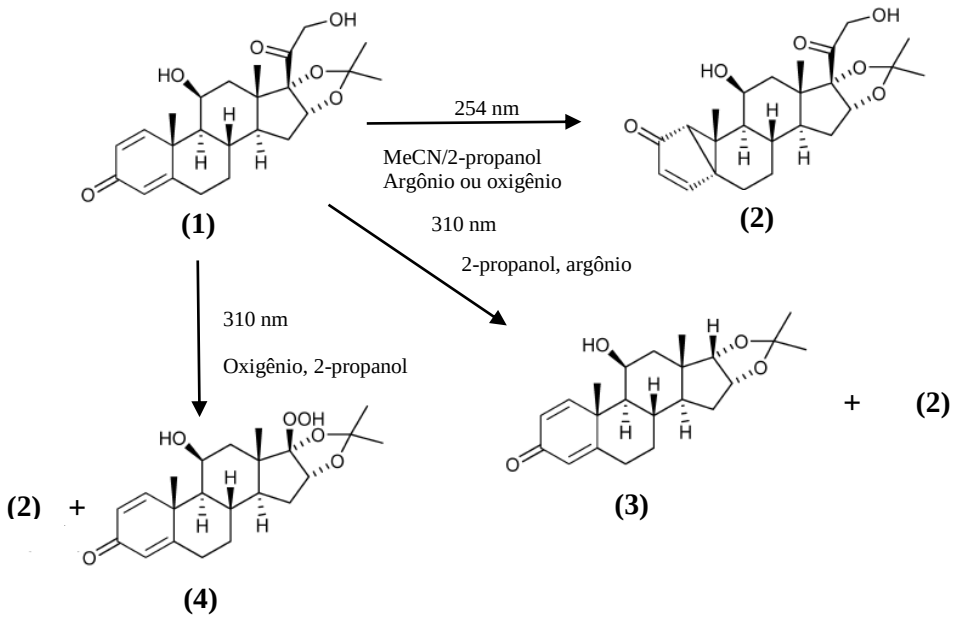
Neste sentido, torna-se importante que os estudos de fotoestabilidade sejam conduzidos de forma a simular as condições de uso do medicamento, principalmente para medicamentos aplicados topicamente, fármacos ou metabólitos que se acumulem em tecidos facilmente expostos à luz, fármacos fotolábeis e que possuam grupos funcionais capazes de induzir efeitos fototóxicos e fármacos administrados em altas doses, que podem ter efeito cumulativo (TØNESSEN, 2004). Ainda nesse contexto, estabelecer uma relação entre a fotoestabilidade e comprimento de onda a fim de verificar em que condições de irradiação o fármaco será mais suscetível à degradação, também pode ser relevante. Após a aplicação

tópica, para que haja interação entre o fármaco e a radiação, é necessário que fótons sejam absorvidos pelo fármaco, pela formulação ou pela pele (BAERTSCHI et al., 2015). A pele possui substâncias como alguns lipídios, proteínas, pigmentos, como a melanina, e o DNA que podem atuar como cromóforos endógenos e absorver radiação (BAERTSCHI et al., 2015; YOUNG et al., 2016).

As reações induzidas pela radiação ultravioleta e visível iniciam pela excitação das moléculas do fármaco, do estado fundamental para o estado reativo excitado, através da absorção de fótons de determinados comprimentos de onda (ALBINI; FASANI, 2004; MOORE, 2004). Durante este estágio, que tem duração de nanosegundos, as moléculas excitadas podem interagir com outras moléculas provocando modificações químicas (MOORE, 2004).

Em relação à fotoquímica dos corticoides, Ricci e colaboradores (2003) abordaram os principais mecanismos de fotodegradação de alguns destes fármacos. Os corticoides apresentam dois grupamentos cromóforos separados espacialmente, a cetona conjugada no anel A, e a cetona isolada no C_{20} (Figura 2). O estudo da fotoquímica da prednisolona (corticoide não fluorado) solubilizada em acetonitrila e em atmosfera de argônio indicou que após irradiação em 254 nm, 310 nm e 360 nm, a principal reação envolvida foi o rearranjo da cetona conjugada no anel A (RICCI et al., 2003). Iqbal, Husain e Gupta (2006) avaliaram a fotoquímica da desonida solubilizada em acetonitrila e propanol em atmosferas de argônio e oxigênio após exposição à radiação UVC (254 nm) e UVB (310 nm). Quando irradiada em 254 nm, os autores observaram a formação do mesmo produto de degradação, independentemente do solvente ou da atmosfera utilizada, o qual foi formado através do rearranjo da cetona conjugada no anel A. Já em 310 nm, a saturação e o solvente influenciaram os produtos de degradação obtidos. Nas mesmas condições estudadas por Ricci e colaboradores houve a formação de mistura de produtos complexos. Em solução de propanol e atmosferas de argônio e oxigênio, formaram-se hidroperóxido e um produto obtido através da quebra das ligações entre C_{17} e C_{20} (IQBAL; HUSAIN; GUPTA, 2006). A figura 3 apresenta os principais produtos de degradação obtidos no estudo da fotoquímica da desonida.

Figura 3- Principais produtos de degradação obtidos no estudo de fotoquímica da desonida



(1) Desonida (11β, 16α)-11,21-diidroxi-16,17-[(1-metiletilideno)bis(oxi)]-pregna-1,4-dieno-3,20-diona);
(2) 11 β,21-diidroxi-16α,17α-(1-metiletilidenodioxi)-1,5-ciclopregna-3-eno-2,20-diona;
(3) 11β-hidroxi-16α,17α-(1-metiletilidenodioxi)androsta-1,4-dieno-3ona;
(4) 17β-hidroperoxi-11b-hidroxi-16α,17α-(1 metiletilidenodioxi)androsta-1,4-dieno-3-ona.
Fonte: (IQBAL; HUSAIN; GUPTA, 2006)

Como forma de evitar que ocorram reações de fotodegradação em preparações sensíveis à luz, podem ser adotadas algumas estratégias. Muitos medicamentos são protegidos da luz utilizando-se embalagens não transparentes e proteção à luz durante as etapas de manipulação e armazenamento. Entretanto, o fármaco continuará fotoreativo quando exposto à radiação. Assim, a fotoestabilização da formulação deve ser adotada, quando possível. Nesse sentido, a adição de substâncias capazes de absorver radiação UV, de antioxidantes e o uso de atmosfera inerte são algumas alternativas para a fotoestabilização de fármacos em formas farmacêuticas (TØNESSEN, 2004).

Outra estratégia que vem sendo relatada é a associação de fármacos fotoinstáveis a nanocarreadores. De acordo com alguns estudos, a nanoencapsulação de substâncias ativas tem se mostrado uma alternativa promissora para a melhora da fotoestabilidade de fármacos suscetíveis à fotodegradação. Ourique e colaboradores (2008) verificaram a maior fotoestabilidade da tretinoína associada a nanocápsulas poliméricas de PCL. Fontana e colaboradores (2009) evidenciaram maior fotoestabilidade do clobetasol nanoencapsulado,

quando comparado à solução do fármaco livre e solução capilar comercial. No mesmo contexto, Savian e colaboradores (2015) também verificaram o aumento da fotoestabilidade do ditranol associado a nanocápsulas poliméricas. Outro estudo, conduzido por Weber e colaboradores (2015) evidenciou a diminuição da fotodegradação do dipropionato de betametasona quando nanoencapsulado. Nesse sentido, a parede polimérica, presente nesses sistemas, poderia restringir o contato do fármaco com o meio externo. Além disso, devido à cristalinidade do polímero utilizado nesses estudos (PCL), esse poderia refletir e dispersar a radiação absorvida (OURIQUE et al., 2008; WEBER et al., 2015). Com relação a outros sistemas nanoestruturados, trabalho recente evidenciou a maior fotoestabilidade do cetoprofeno associado à nanoemulsões contendo óleo de romã, relacionando esse efeito aos polifenóis e estrogênios presentes no óleo e ao sistema coloidal, que poderia dispersar a radiação incidente (FERREIRA et al., 2016).

CAPÍTULO 1: Desonide-loaded nanocapsules: physicochemical characterization, photostability study and evaluation of the *in vitro* phototoxic potential

CAPÍTULO 1: Desonide-loaded nanocapsules: physicochemical characterization, photostability study and evaluation of the *in vitro* phototoxic potential

1.1 Apresentação

Os corticoides tópicos são fármacos extensamente utilizados no tratamento de doenças dermatológicas, como dermatite atópica, psoríase, dermatite de contato e algumas doenças autoimunes (AUBERT-WASTIAUX et al., 2011; BELTRANI; BARSANTI; BIELORY, 2005; GUICHARD et al., 2015). Na pele, esses fármacos possuem como células alvo os queratinócitos e fibroblastos situados na epiderme e derme. Entretanto, os mesmos mecanismos de ação responsáveis pelo efeito terapêutico podem provocar o surgimento de efeitos indesejados, como a atrofia cutânea (GUICHARD et al., 2015; HENGGE, 2006; WIEDERSBERG; LEOPOLG; GUY, 2007). A desonida é um corticoide tópico não halogenado de baixa potência (WHO, 2017) comumente prescrita para o tratamento de desordens dermatológicas em pacientes idosos e pediátricos e utilização em áreas sensíveis do corpo, como a face (GELBARD, HEBERT, 2009). Estudos recentes reportam a fotoinstabilidade da desonida em preparações farmacêuticas produzidas industrialmente após exposição direta à radiação UVA (BRAGA et al., 2014; BRAGA, 2013; SANTA et al, 2013;).

A associação de fármacos fotoinstáveis a nanocápsulas tem sido citada como alternativa promissora para o aumento da fotoestabilidade de fármacos fotossensíveis. Dessa forma, o presente capítulo, redigido na forma de artigo científico, aborda o desenvolvimento de nanocápsulas catiônicas contendo óleo de açaí ou triglicerídeos de cadeia média e desonida. As preparações foram avaliadas quanto às suas características físico-químicas, perfil de liberação *in vitro*, estabilidade frente ao armazenamento em temperatura ambiente e fotoestabilidade após exposição às radiações UVA e UVC. Além disso, o efeito fototóxico e o potencial irritante *in vitro* das formulações foi avaliado.

Desonide-loaded nanocapsules: physicochemical characterization, photostability study and evaluation of the *in vitro* phototoxic potential

Priscila Rosa[1], Mariane Lago Friedrich[2], Juliana dos Santos[1], Daniele Rubert Nogueira Librelotto[1], Cristiane de Bona da Silva[2], Andréa Inês Horn Adams[2]

[1] Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal de Santa Maria, Santa Maria, Brazil.

[2] Departamento de Farmácia Industrial, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, Brazil.

Abstract: This study aimed to develop Eudragit® RL 100 nanocapsules loaded with desonide (DES), using açai oil (AO) or medium chain triglycerides (MCT) as oil core. Pre-formulation study showed that AO and MCT are suitable to prepare the nanocapsules. The nanocapsules presented mean particle size around 165 e 131 nm for those prepared with AO and MCT, respectively; polydispersity index values < 0.20, positive zeta potential values, drug content close to the theoretical value (0.25 mg mL-1), and DES encapsulation efficiency around 81%, regardless of the oil core (AO or MCT). Considering the photoinstability reported to DES, photodegradation studies were performed. The UVA (365 nm) and UVC (254 nm) photodegradation studies revealed less degradation of DES when associated to the nanocapsules containing AO in comparison with those with MCT. The *in vitro* release study showed a biphasic release profile for both nanocapsule suspensions: an initial burst effect followed by a prolonged DES release. In addition, the formulations were considered non-phototoxic at 0.5 mg mL^{-1} when tested on 3T3 murine fibroblasts and HaCaT human keratinocytes using the MTT and NRU viability assays. In conclusion, the nanocapsule formulations developed in this study might be considered promising for therapeutic applications.

Keywords: nanoparticles, açai oil, photostability, phototoxic activity

1. Introduction

Corticosteroids are drugs commonly used in dermatology, mainly for the treatment of inflammatory skin diseases, such as atopic dermatitis, psoriasis, contact dermatitis and some auto-immune topical disorders (AUBERT-WASTIAUX et al., 2011; BELTRANI; BARSANTI; BIELORY, 2005; GUICHARD et al., 2015). The anti-inflammatory activity of topical glucocorticoids (TG) has an immediate effect, inducing vasoconstriction decreasing the edema and erythema and effects mediated by regulation of glucocorticoid receptor (BELTRANI; BARSANTI; BIELORY, 2005; GUICHARD et al., 2015; WIEDERSBERG; LEOPOLG; GUY, 2007). In the skin, the TG target cells are the keratinocytes and fibroblasts within the epidermis and dermis. However, the same mechanisms of action responsible for the therapeutic effect can cause adverse effects as skin atrophy, resulting from the antiproliferative effect on fibroblasts (GUICHARD et al., 2015; HENGGE, 2006; WIEDERSBERG; LEOPOLG; GUY, 2007).

Desonide (DES) is a synthetic non-fluorinated corticosteroid classified as TG low potency (class VI) according to World Health Organization (WHO, 2017). It is commonly prescribed for the treatment of atopic dermatitis in sensitive areas as the face and intertriginous regions, in elderly and children. DES has been used for over 30 years for the treatment of steroid-responsive dermatoses and has already been considered the low potency corticosteroid most prescribed by dermatologists in the USA (GELBARD, HEBERT, 2009). Recently, some studies reported the desonide photoinstability in commercially available formulations after direct exposure to UVA radiation (BRAGA et al., 2014; BRAGA, 2013; SANTA et al., 2013).

The ability of nanocapsules to protect substances from chemical and photodegradation has been well demonstrated by previous studies (GEHRCKE et al. 2017; GEHRCKE, 2018; OURIQUE et al. 2008; SANTOS et al., 2013). Polymeric nanocapsules (NCs) are vesicular systems composed by a polymeric wall surrounding a core, generally oily (MORA-HUERTAS; FESSI; ELAISSARI, 2010). Regarding the oily core, medium chain triglycerides (MCT) are generally used as the oil for the preparation of nanocapsules. However, in the last years, vegetable oils have been introduced as the oily core because they could provide protection for the encapsulated substance and improve the drug pharmacological effect (GEHRCKE, 2018; SANTOS et al., 2014).

In this context, studies with the açai oil (AO), indicated the antiproliferative activity from fruit pulp extracts and oil from açai fruit pulp in colon adenocarcinoma cells (PACHECO-PALENCIA, 2008). Favacho and co-workers (2011) attributed the AO anti-inflammatory effect to its composition, which is similar to other oils with the same activity, such as olive oil and fish oil. The AO genotoxic effect was evaluated by an *in vivo*study in peripheral leukocytes, liver, bone marrow and testicular cells of rats, through the comet assay and micronucleus test. In these conditions, OA did not present a significant genotoxic effect (MARQUES et al., 2016).

Concerning the use of desonide in nanostructured systems, Antonow and co-workers (2016) described its incorporation into nanocapsules. The authors reported the development of desonide-loaded nanocapsules using Eudragit® S100, Eudragit® L100 and poly(ε-caprolactone) as polymers and MCT as oily core. However, there are no studies about the desonide photostability associated to nanocarriers or the preparation of nanocapsules containing desonide associatedto açai oil as the oily core.

Thus, this study aimed to develop cationic desonide-loaded nanocapsules using Eudragit® RL 100, a biocompatible polymer. The formulations were prepared using MCT and AO as the oily core in order to evaluate the influence of the oil in nanocapsules characteristics and photostability. Moreover, the *in vitro* irritant and phototoxic effects of formulations were evaluated.

2. Material and methods

2.1 Materials

Desonide (purity of 97.42%) was obtained from Fagron (São Paulo, Brazil). Polysorbate 80 and Span 80® (sorbitan monooleate) were bought from Sigma Aldrich (São Paulo, Brazil). Medium chain triglyceride was acquired from Delaware (Porto Alegre, Brazil) and açai oil (AO) was kindly donated by Beraca (Ananindeua, Brazil). Eudragit RL 100 was purchased from Evonik (Essen, Germany). Neutral red (NR) solution 3.3 g/L, 2,5-diphenyl-3,-(4,5-dimethyl-2-thiazolyl) tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), phosphate buffered saline (PBS) solution, Earles balanced salt solution (EBSS), fetal bovine serum (FBS) and trypsin-EDTA solution (0.5 g porcine trypsin and 0.2 g EDTA.4Na/L of Hanks′ Balanced Salt Solution) were obtained from Sigma-Aldrich (São Paulo, Brazil). Dulbecco's Modified Eagle's Medium (DMEM), supplemented with L-glutamine (584 mg/l) and antibiotic/antimicotic (50 mg/mL gentamicin sulfate and 2 mg/L amphotericin B) were

purchased from Vitrocell (Campinas, Brazil). Methanol and acetonitrile HPLC grade were obtained from Sigma Aldrich (São Paulo, Brazil). All other solvents and reagents were of analytical grade and used as received.

2.2 Açai oil fatty acids profile

Açai oil was saponified in methanolic KOH solution and then esterified in methanolic H_2SO_4 solution (HARTMANN; LAGO, 1973). Methylated fatty acids were analyzed using an Agilent Technologies gas chromatograph (HP 6890N) equipped with a capillary column (DB-23 60 m x 0.25 mm x 0.25 µm) and flame ionization detector. The temperature of the injector port was set at 250°C and the carrier gas was nitrogen (0.6 mL min^{-1}). After injection (1 µL, split ratio 50:1), the oven temperature was hold at 150°C for 1 min, then it was increased to 240°C at 4°C/min and hold at this temperature for 12 min. Standard fatty acid methyl esters (37-component FAME Mix from Sigma, Saint Louis, USA) were run under the same conditions and the subsequent retention times were used to identify the fatty acids. Fatty acids were expressed as percentage of the total ones identified.

2.3 Pre-formulation studies

2.3.1 Dissolution/swelling of polymer films

Eudragit® RL 100 (EUD) films were prepared dissolving 1.0 g of the polymer in acetone followed by the solvent evaporation at room temperature. Portions of the films (around 25 mg) were accurately weighed and kept in contact with AO and MCT, in a quantity enough to cover the films. On the days 5, 7, 15 and 30 the films were removed, in sequence carefully dried with absorbent paper and accurately weighed. The difference between the initial and final weight was calculated.

2.3.2 Determination of desonide solubility in the oils

To evaluate the desonide solubility in MCT and AO, an excessive amount of DES was dispersed in 1.0 mL of each oil (n=3). The samples were magnetically stirred over 60 min and then centrifuged at 10,000 rpm during 10 min. An aliquot of the supernatant was diluted in methanol and the DES content was determined through a previously validated HPLC method.

2.4 Preparation of nanocapsule suspensions

Nanocapsules suspensions were prepared by the interfacial deposition of preformed polymer method described by Fessi and co-workers (FESSI et al., 1989). Briefly, an organic phase containing the polymer (0.25 g), Span 80® (0.1925 g), AO at 2.5% (DES-NC_{AO}) or MCT at 2.5% (DES-NC_{MCT}), desonide (0.0025 g) and acetone (67.5 mL for DES-NC_{AO} and 135 mL for DES-NC_{MCT}), were kept at 40 ºC under moderate magnetic stirring for 60 minutes. This organic phase was injected, under magnetic stirring at room temperature,into an aqueous phase (135 mL) containing polysorbate 80 (0.1925 g). The mixture was homogenized during 10 minutes and then the acetone and part of water were eliminated by evaporation under reduced pressure to achieve 25 mL of formulation corresponding to 0.25 mg mL^{-1} of desonide. For comparative purposes, formulations without the drug (B-NC_{AO} and B-NC_{MCT}) were also prepared. Additional nanocarriers (nanoemulsions and nanospheres) were prepared for further photostability evaluation. Nanoemulsions (DES-NE_{AO} and DES-NE_{MCT}) were prepared by the spontaneous emulsification solvent diffusion method using the same aqueous and organic composition of the nanocapsule suspensions, except the polymer. DES-nanospheres (DES-NS), that do not contain oil in their composition, were prepared following the same procedures employed in the preparation of nanocapsule suspensions. All formulations were prepared in triplicate and stored in amber glass containers at room temperature (22 ± 2).

2.5 Desonide quantitative assay

The quantitative analyses were performed by HPLC using a Shimadzu (Kyoto, Japan) LC system equipped with a LC 20AT pump, SPD M20A detector, a CBM 20A system controller, DGU degasser and SIL 20AHT autosampler. A mobile phase composed by a mixture of methanol, acetonitrile and ultrapure water adjusted to pH 4.0 with ortophosphoric acid 18% (60:10:30, v:v:v) was eluted at flow rate of 1.0 $mL.min^{-1}$. The chromatographic separation was achieved using a Phenomenex Luna® (Torrance, USA) RP-C_{18} column (150 x 4.6 mm; 5 μm) coupled to a C_{18} guard column. Detection was performed at 244 nm and the injection volume was 20 μL. The analytical method was validated for the assay of desonide in formulations according to the ICH guidelines (ICH, 2005). The method was specific, linear in the range of 5-100 μg mL^{-1} ($y = 45212x + 2310$; $r = 0.9999$), precise, in levels of repeatability (RSD of 2.0% to DES-NC_{AO} and 1.32% to DES-NC_{TCM}) and intermediate precision (RSD of 1.67% and 1.61% to DES-NC_{Ao} and DES-NC_{MCT}, respectively) and accurate (recovery of 100.83% to DES-NC_{OA} and 100.63% to DES-NC_{MCT}).

Desonide content in NCs was determined by the dilution of 1.0 mL of each sample in 5 mL of methanol followed by sonication for 10 min. Then, the volume was filled up with the same solvent to 10 mL, providing DES theoretical concentration of 25 µg mL^{-1}. The samples were filtered through a 0.45 µm nylon membrane filter.

2.6 Characterization of nanocapsule suspensions

The presence of micrometric population of particles, size distribution and SPAN were evaluated by laser diffraction using the refraction index of Eudragit® RL 100 (1.38). The formulations were dispersed in distilled water directly in the equipment sampling apparatus (Mastersizer 3000, Malvern Instruments, UK). The refractive index of Eudragit RL 100 (1.38) was used. Mean particle sizes and polydispersity indexes (PDI) were determined by photon correlation spectroscopy (PCS) (Zetasizer, Malvern Instruments, UK) after samples dilution in ultrapure water (1:500). Zeta potential was determined by electrophoretic mobility (Zetasizer, Malvern Instruments, UK) after the dilution of samples (1:500) in 10 mM NaCl and pH was directly evaluated in the suspensions using a calibrated potentiometer (Denver Instruments).

Desonide total content in nanostructures was determined by the analyses of samples at 25 µg mL^{-1}, prepared as described in the section 2.5. The encapsulation efficiency (EE) was evaluated by ultrafiltration/centrifugation technique in which an aliquot of 300 µL of the formulations was placed in a 10 000 MW centrifugal device (Amicon®, Millipore, USA) and centrifuged during 10 min at 10 000 rpm, to separate the free drug from the nanostructures. The ultrafiltrate, which contains the free drug, was analyzed by the analytical method described in the section 2.5. The EE (%) was calculated as the difference between total and free concentrations of desonide determined in the nanostructures and in the ultrafiltrate, respectively.

2.7 Stability study

2.7.1 Stability at room temperature

The effect of storage time in the stability of formulations was monitored during 30 days. Formulations were stored at room temperature in amber glass containers (22 ºC ± 2 ºC)

and evaluated regarding desonide content, pH, zeta potential, mean diameter, polydispersity index and encapsulation efficiency.

2.7.2 Photostability study

For this purpose, 1.0 mL of a methanolic drug solution (0.25 mg mL $^{-1}$), DES-NC_{AO} (0.25mg mL $^{-1}$) and DES-NC_{MCT} (0.25mg mL $^{-1}$) were disposed into UV transparent plastic cuvettes, placed at a fixed distance and exposed to UV radiation, inside a chamber with mirrored surfaces. The samples were exposed to UVA (365 nm, Ecolume, 30 W) and UVC (254 nm, Philips, 30 W) radiation during 36 h and 90 min, respectively. At predetermined intervals the exposition was finished and samples were analyzed for desonide content, according procedure described on the item 2.5. In order to discard thermal degradation, samples protected from the light (dark controls) were simultaneously exposed to UV radiation and analyzed. The experiment was performed in triplicate.

The degradation kinetics of DES against UVC radiation was determined according to zero order ($C = C_0$-kt), first order ($lnC = lnC_0$- kt) and second order ($1/C = 1/C_0 + kt$) equations. The best correlation coefficient (r) indicated the reaction order and the parameters of t_{90} and t_{50} were estimated, according to the given model.

2.8 *In vitro* release study

Desonide *in vitro* release from NCs was studied by the dialysis bag diffusion technique. For this, 2 mL of samples, DES-methanolic solution, (0.25 mg mL^{-1}) or NCs (DES-NC_{AO} and DES-NC_{MCT}, 0.25mg mL^{-1}), were placed in the dialysis bag (10000 Da, Spectra Pore),which were immersed in 70 mL of sodium acetate buffer pH 4.5 containing 0.5% of polysorbate 80 and maintained under constant moderate stirring at 37 °C. The release medium was chosen in order to obtain sink conditions. At predetermined intervals (0.5, 1, 2, 4, 6, 8 and 10 hours), aliquots of 1.0 mL of the release medium were withdrawn and replaced by the same volume of fresh medium. The amount of drug released was determined by HPLC, using the analytical conditions previously described (Section 2.5).

The mathematical modeling was performed using the Scientist 2.0 software (MicroMath, USA) by fitting the data to mono ($\ln C = \ln C_0 - kt$) and bi-exponential equations ($C = Ae^{-\alpha t} + Be^{-\beta t}$). The equation that better describes DES release was selected by the highest model selection criteria (MSC) value and the correlation coefficient (r). The drug release mechanism was evaluated by fitting the data to Korsmeyer-Peppas model ($ft = at^n$).

2.9 Phototoxicity test

The phototoxicity assay was carried out as described in OECD Guideline for Testing Chemicals 432, with some modifications. Cell lines 3T3 and HaCaT were used as *in vitro* models to predict cutaneous phototoxicity. Two 96-well plates per cell line were seeded with cells (1 x 10^5 cells/mL for 3T3 and 8.5 x 10^4 cells/mL for HaCaT) in 100 µL of DMEM medium supplemented with 10% FBS (v/v) and incubated at 37°C, 5% CO_2 for 24 h to allow the formation of a monolayer. Then, the cells were treated with DES, DES-NC_{AO}, B-NC_{AO}, DES-NC_{MCT} and B-NC_{MCT} at 0.5 µg mL^{-1} in Earles Balanced Salt Solution (EBSS) and pre-incubated at 37 °C and 5% of CO_2 for 1 h. One plate of each cell line was exposed to a UVA dose irradiation of 1.5 J/cm^2, whereas the other plate was wrapped in aluminum foil and then exposed to the same condition. UVA irradiation was performed using a HPA 400/S (UV-Bravo, Germany) UVA lamp, with a spectral range of 300-400 nm. After irradiation, the treatment was replaced by complete medium and the plates were incubated again, under the same experimental conditions, to complete 24 h of incubation. The cell viability obtained with each sample at 0.5 µg mL^{-1} in both cell lines was determined by MTT and NRU assays. The phototoxic potentials were estimated comparing the cell viabilities obtained in the presence and absence of UVA radiation. Chlorpromazine (CPZ) at 5.0 µg mL^{-1}, a known phototoxic chemical, was employed as positive control.

2.10 Evaluation of irritant potential of nanocapsules

In order to evaluate the irritant potential of the formulations prepared, the Hen's Egg Test Chorioallantoic Membrane (HET-CAM) method was employed. The formulations analyzed were DES-NC_{AO}, B-NC_{AO}, DES-NC_{MCT}, B-NC_{MCT} and non-associated drug (dispersed in polissorbate 80 and water). The positive control was NaOH 0.1 M and the negative control was 0.9% NaCl. For the test, fertile chicken eggs on 10^{th} day of incubation were used. Initially, the eggshell around the air chamber was removed and the inner membrane was moisten with 0.9% NaCl. Then, the membrane was carefully removed and 0.3 mL of formulations were applied directly onto the chorioallantoic membrane (CAM) (n=4/formulation). After 20 s, the formulations were removed using 0.9% NaCl and CAM was monitored during 300 s. The time for the appearance of each reaction was monitored and recorded, in seconds. The endpoints observed were vasoconstriction, hemorrhage and coagulation. The irritation score (IS) was calculated according to Eq 1.

$$IS = 5\,x\frac{(301 - h)}{300} + 7\,x\frac{(301 - v)}{300} + 9\,x\frac{(301 - c)}{300}$$

where, h = hemorrhage time; v = vasoconstriction time, and c = coagulation time.

According to the IS values, the formulations were classified as non-irritant(0–0.9), slightly (1–4.9), moderate (5–8.9) and severe irritant (9–21).

2.11 Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA), post hoc Tukey's test and Student's t test and at a significance level of 5%.

3. Results

3.1 Fatty acid profile of açai oil

Considering that several factors can affect the composition of vegetable oils, we developed chromatographic analyses to evaluate the quali/quantitative composition of the açai oil used in this study (Table 1). The most predominant fatty acids found were oleic, palmitic and linoleic, whereas palmitoleic and stearic acids were present at lower levels. The quali-quantitative composition of the fatty acids present in the AO evaluated is in agreement with the composition of *Euterpe oleracea* fruit oil reported (CIR, 2011; NASCIMENTO et al., 2007; PACHECO-PALENCIA, 2008).

Table 1. Fatty acid composition of açai oil

Fatty acid	% of total fatty acids identified
C16:0 (palmitic acid)	21.78
C16:1n7 (palmitoleic acid)	3.26
C18:0 (stearic acid)	2.17
C18:1n9 (oleic acid)	57.42
C18:1n7 (7-octadecenoic acid)	3.24
C18:2n6 (linoleic acid)	11.08
C18:3n3 (alpha-linolenic acid)	0.59

Other fatty acids	< 0.5

3.2 Pre-formulation studies

In order to verify a possible interaction between polymer and the oils (AO and MCT), Eudragit® RL100 films were kept submersed in the oils for 30 days. The initial mass ofthe polymers films in AO was 28.3± 5.0 mg and after 30 days in contact with oil, the film mass was 27.9 ± 4.9 mg. To the polymer films immersed in MCT, the initial mass was 25.78 ± 2.9 mg and the final mass was 25.16 ±3.7 mg. Statistical analysis (Student's *t test*) indicated that there was no significant change in the both EUD films weight, showing that these oils are nonsolvents for this polymer ($p > 0.05$).

Regarding DES solubility, the results indicated solubility of approximately 291 µg mL^{-1}in AO and 792 µg mL^{-1} in MCT, which were considered adequate to prepare the nanocapsules suspensions in the experimental conditions used in this study.

3.3 Preparation and characterization

After preparation, NC suspensions containing AO presented a slightly yellowish milky appearance and NCs prepared with MCT were white milky in appearance. Both NCs showed opalescent bluish reflection resulting from Brownian motion of the colloidal structures. The analysis of NC suspensions by laser diffractometry demonstrated that 90% of the particles were lower than 429 ± 4 nm and 413 ± 2 nm for DES-NC_{AO} and DES-NC_{MCT}, respectively. Volume-weighed mean diameter (D4,3) were 284 ± 3 nm for DES-NC_{AO} and 262 ± 4 nm for DES-NC_{MCT}, with SPAN values < 2.0 for both formulations.

The formulations presented size in the nanometric range, PDI values <0.2, positive zeta potential and acidic pH. Table 2 presents the physicochemical characteristics of formulations. Moreover, DES content was close to theoretical value (0.25 mg/mL) and EE was around 82% for both formulations.

Table 2. Physicochemical characteristics of nanocapsule suspensions

Analysis	**B-NC_{AO}**	**DES-NC_{AO}**	**B-NC_{TCM}**	**DES-NC_{TCM}**
Mean diameter (nm)	163 ± 3	165 ± 2	135 ± 3	131 ± 2

Polidispersity index	0.13 ± 0.01	0.12 ± 0.03	0.15 ± 0.02	0.15 ± 0.02
Zeta potential	+14.9 ± 1.25	+13.8 ± 0.28	+9.3 ± 1.01	+6.9± 0.67
pH	5.71 ± 0.06	5.00 ± 0.09	4.98 ± 0.17	4.38 ± 0.13
DES content (mg mL^{-1})	---	0.259 ± 0.004	---	0.262 ± 0.009
EE (%)	---	81.77 ± 1.82	---	81.64 ± 0.39

3.4 Stability studies

3.4.1 Stability at room temperature

The prepared nanocapsules did not shown any changes in appearance during the storage period. Regarding the particle size (Fig 1a), we verified that formulations maintained the initial characteristics ($p>0.05$) as well as the polidispersity index which did not shown any significant difference ($p>0.05$) at the end of storage. With respect to pH (Fig 1b), after 30 days, no differences were observed in relation to the initial values ($p>0.05$), for both formulations. Concerning the zeta potential (Fig 1c), DES-NC$_{AO}$ did not present any alteration in this parameter ($p>0.05$), considering the initial and final values. However, a significant increase ($p<0.05$) in zeta potential was observed to DES-NC$_{MCT}$ from the seventh day of storage.

Desonide content was also evaluated. The results showed that at the end of 30 days, the drug content was within the most common specification (90-110%) for drug products. DES content was 98.23 ± 1.06% for DES-NC$_{AO}$ and 94.88 ± 1.78 for DES-NC$_{MCT}$. Determining the encapsulation efficiency indicated no decay ($p>0.05$) in the encapsulated amount in relation to the initial time, for both NCs.

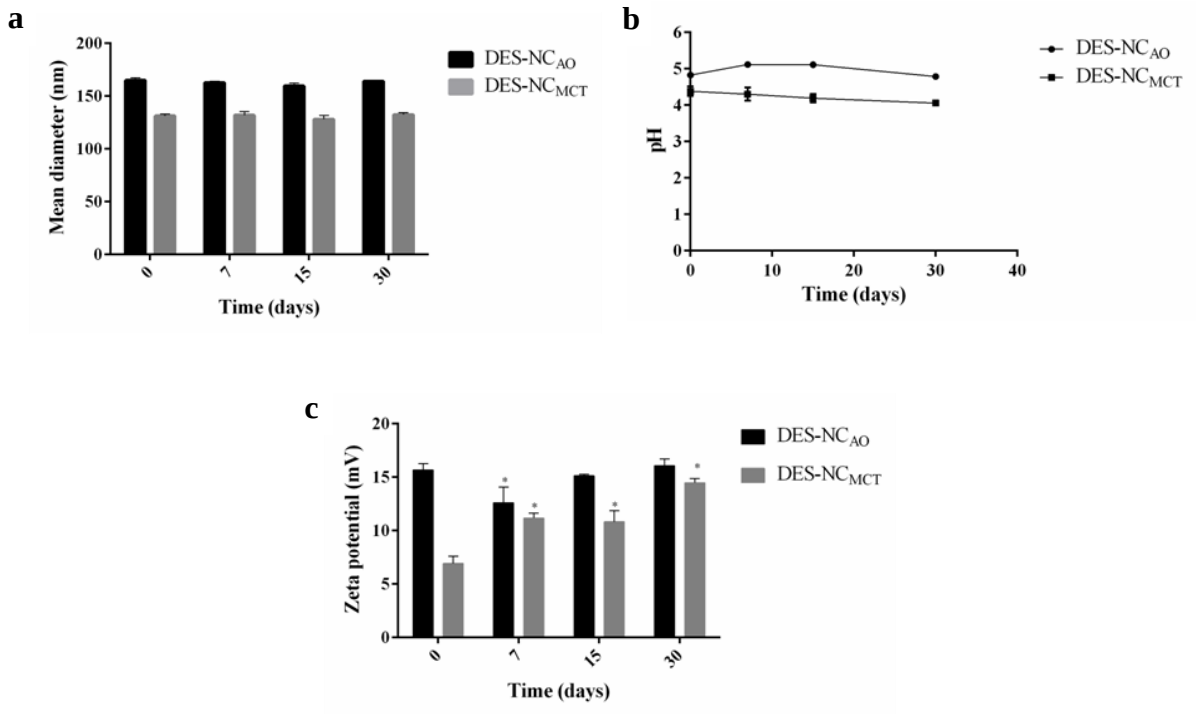


Fig. 1. Mean diameter (a), pH (b) and zeta potential (c) of DES-NC$_{AO}$ and DES-NC$_{MCT}$, during stability study. *Significant difference compared to zero time.

3.4.2 Photostability study

Both NCs reduced DES photodegradation, regarding that the remaining contents after 36 h of UVA exposure were 68.51% and 56.39%, for DES-NC$_{AO}$ and DES-NC$_{MCT}$, respectively. In the same conditions, a solution of DES in methanol (DES-MS) presented a residual content of 35.92% (Fig 2). The protection effect against UVA radiation was higher with AO NCs than with MCT NCs ($p<0.05$). The assay of DES-MS, DES-NC$_{AO}$ and DES-NC$_{MCT}$ submitted to the same procedure, but protected from light showed DES content around 99%, indicating that degradation occurs without the influence of the temperature.

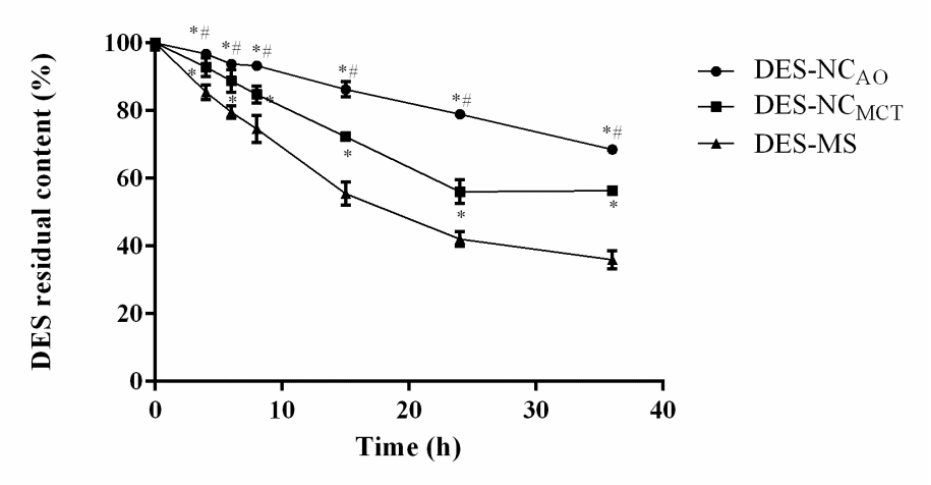


Fig. 2. Desonide residual content from nanocapsules and from solution, after UVA radiation exposure. *Significant difference between desonide as a solution in methanol (DES-MS) and nanocapsules suspensions (DES-NC$_{AO}$ and DES-NC$_{MCT}$); #significant difference between DES-NC$_{AO}$ and DES-NC$_{MCT}$.

In order to verify the polymer effect and the influence of the oil used to compose the core of NCs in the photostability of DES, nanospheres (NS) and nanoemulsions (NE) were also prepared. The results indicated that all the nanostructures were able to protect DES from UVA degradation (Table 3) since drug residual content after 36 h of UVA irradiation was greater in the nanostructures than in solution ($p<0.05$). Considering the nanostructures, it can be observed that DES-NC$_{MCT}$, DES-NE$_{MCT}$ and DES-NS, and did not differ on DES residual content ($p>0.05$) at the end of UVA exposure time (36 h), presenting drug remaining content of 56.39%, 54.41% and 54.39%, respectively. At the same time, DES-NC$_{AO}$ and DES-NE$_{AO}$ presented similar effect ($p>0.05$) on DES photoprotection. Desonide residual content was 68.51% in DES-NC$_{AO}$ and 64.60% in DES-NE$_{AO}$. These results suggest that the polymeric wall had no influence in protecting drug from photodegradation. The nanocarries containing açai oil in their composition (NC and NE) showed higher effect against UVA radiation than the other structures ($p<0.05$), indicating that the oil used to compose NE and NCs core influenced DES photostability.

Table 3. Desonide residual content (%) from nanostructures and non-associated drug observed in the UVA (365 nm) photostability study.

	DES residual content (%) from nanostructures and solution (mean ± SD)					
Time (h)	**DES-MS**	**DES-NC$_{AO}$**	**DES-NC$_{MCT}$**	**DES-NE$_{AO}$**	**DES-NE$_{MCT}$**	**DES-NS**

0	100± 1.20	100±0.81	100±0.79	100±0.90	100±0.85	100±0.98
4	85.42±2.20^{*a}	96.79±0.72^{*b}	92.85±2.81^{*c}	95.44±0.89$^{*b,\ c}$	93.56±0.67^{*b,c}	93.07±1.49^{*c}
6	79.57±1.90^{*a}	93.83±0.96^{*b}	88.76±3.39^{*c}	94.93±1.20^{*b}	90.91±1.05^{*b,c}	90.94±1.52^{*b,c}
8	74.63±4.03^{*a}	93.31±0.70^{*b}	84.81±2.49^{*c}	91.98±1.41^{*b}	86.61±1.07^{*c}	87.91±0.84^{*c}
15	55.46±3.43^{*a}	86.26±2.26^{*b}	72.26±1.13^{*c}	84.62±2.65^{*b}	75.77±3.19^{*c}	81.60±2.01^{*b}
24	42.05±2.20^{*a}	78.96±1.26^{*b}	56.04±3.49^{*c}	76.66±1.91^{*b}	67.38±1.56^{*d}	71.35±2.02^{*d}
36	35.92±2.72^{*a}	68.51±1.30^{*b}	56.39±1.39^{*c}	64.60±1.92^{*b}	54.41±0.19^{*c}	54.39±1.06^{*c}

Note: DES-MS: desonide dissolved in methanol, at 0.25 mg ml^{-1}; DES-NE$_{AO}$: DES-loaded nanoemulsion containing 2.5% of AO; DES-NE$_{MCT}$: DES-loaded nanoemulsion containing 2.5% of MCT; DES-NS: DES-loaded nanosphere. *Significant difference against zero time, in the same column Means followed by the same letter in each line do not differ (Tukey *post-hoc* test, α=0.05).

NCs showed a more prominent photoprotective effect against UVC radiation. After 90 min of exposure, DES content was 44.11% for DES-NC$_{AO}$ and 34.33% for DES-NC$_{MCT}$, whereas the residual content of DES in methanol was 37.90% after 15 min of UVC irradiation (Fig 3). In this condition, the degradation kinetics of DES was estimated. For DES-NC$_{AO}$ and DES-NC$_{MCT}$, the kinetic degradation followed first order, whereas DES-MS followed zero order (Table 4).

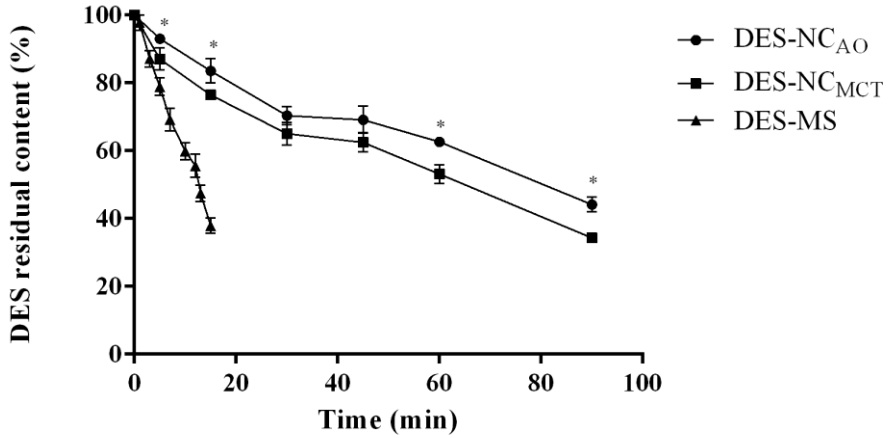


Fig. 3. Desonide remaining content from nanocapsules and desonide dissolved in methanol, at 0.25 mg mL^{-1} after UVC irradiation. Asterisks denote significant difference between NCs formulations.

Table 4. Parameters obtained in the UVC degradation kinetics study

Formulation	Reaction order	t_{90} (min)	t_{50}(min)
DES-NC_{AO}	First	13.2	86.6
DES-NC_{MCT}	First	10.6	69.3
DES-MS	Zero	2.4	12.1

3.5 *In vitro* release study

Concerning *in vitro* DES release,NCs prepared with AO or MCT released 98.74 ± 0.05% and 96.58 ± 0.41%, respectively after 10 hours of experiment (Fig 4). On the other hand, DES-MS was totally released in 4 hours (98.81 ± 1.42%). Data were fitted to zero, mono and biexponential equations. The release profile of both NCs is better described by the biexponential equation, since higher values of MSC and correlation coefficient were obtained to this model. Regarding DES-MS, the results showed a good fit to zero order model. The mathematical modeling using Korsmeyer-Peppas model indicated correlation coefficients higher than 0.99 and release exponent (n) about 0.61, suggesting anomalous case as release mechanism for both NCs.

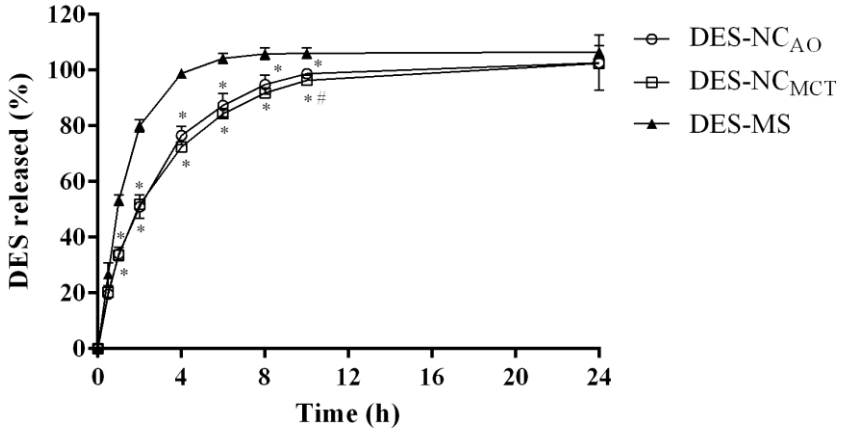


Fig. 4. DES release profile from DES-NC_{AO}, DES-NC_{MCT} and methanolic solution. * significant difference between methanolic solution and NCs; # significant difference between DES-NC_{AO} and DES-NC_{MCT}.

3.6 Phototoxicity test

The phototoxicity of unloaded DES and DES-loaded NCs was tested on 3T3 murine fibroblasts and HaCaT human keratinocytes using the MTT and NRU viability assays. The measurement of the phototoxic potential is obtained from the comparison between the

response from non-irradiated and irradiated plates, in each treatment. Additionally, the data from non-irradiated plates provide the cytotoxicity effect of the treatment.

To both cell lines, no phototoxic effect was observed with all the treatments evaluated, since no significant effect was observed in the irradiated plates in comparison to those non-irradiated, for the same treatment ($p>0.05$) (Fig 5).

About the cytotoxicity, all NC formulations induced higher cell damage than free desonide, particularly in the HaCaT cells, as determined by the MTT assay ($p<0.05$). These results suggest a cytotoxic effect of these systems.

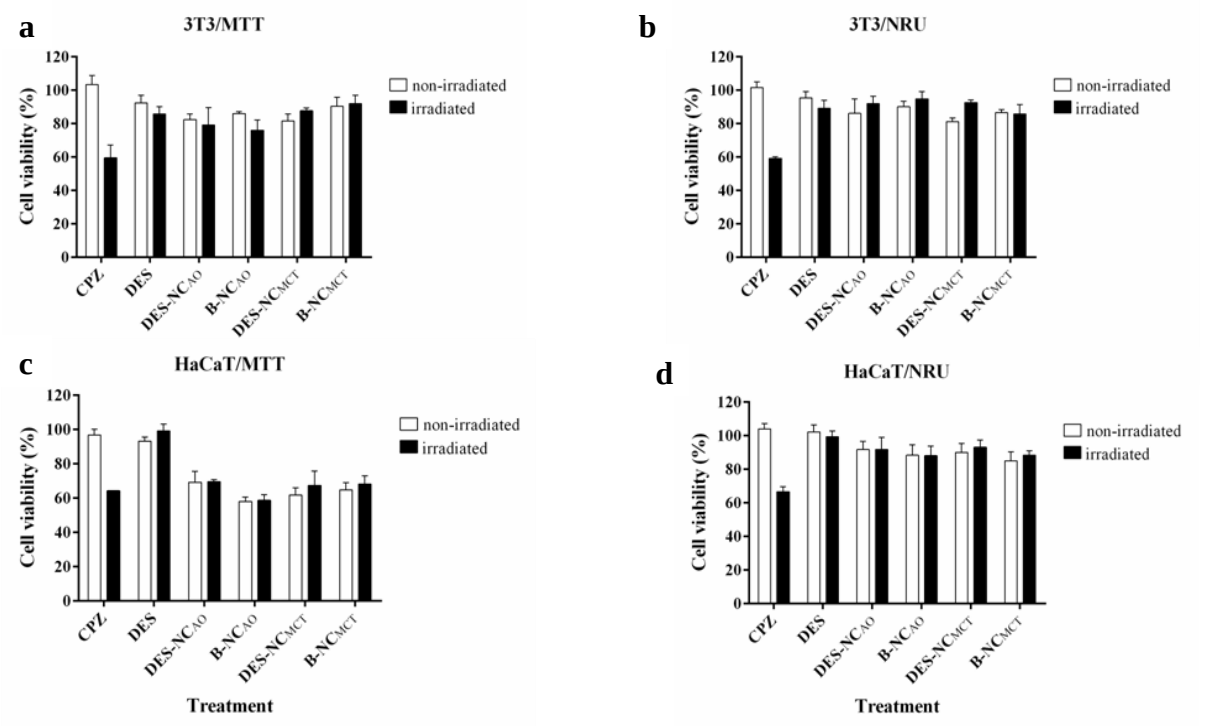


Fig. 5. Phototoxicity of free DES and NCs formulations at concentration of 0.5 mg mL^{-1}, expressed as cell viability (%) on 3T3 and HaCat cells line, measured by NRU and MTT assays. White bars = non-irradiated cells and black bars = UVA 1.5 J/cm^2 irradiated cells. Data are expressed as mean ± SEM.

3.7 Irritant potential of formulations

The irritant potential of the prepared nanocapsulesand DES in the free form were evaluated by HET-CAM method. The positive control tested (0.1 M NaOH) was classified as severe irritant, which is in agreement to literature (ICCVAM, 2006; VINARDEL, 1994). As

expected, for negative control (0.9% NaCl), no irritant reactions were observed. With respect to the DES-NCs, regardless the type of oil used to compose the core of NCs, the irritant scores (IS), 3.80 ± 0.22 for DES-NC_{AO} and 3.78 ± 0.30 for DESNC_{MCT}, indicated the formulations were slightly irritant.Blank nanocapsules(IS of 3.74 ± 0.02 to B-NC_{AO} and 3.84 ± 0.32 to B-NC_{MCT}) and non-encapsulate DES (IS of 2.96 ± 0.42) were also classified as slightly irritant.

4. Discussion

Considering that nanocapsules are core-shell structures, the appropriate choice of the polymer and oil used to compose the core is essential for the maintenance of this structures (MORA-HUERTAS; FESSI; ELAISSARI, 2010; MORA-HUERTAS et al., 2012). Therefore, the choice of the oil should consider drug solubility, toxicity and the compatibility with the polymer. In this context, capric/caprylic triglycerides are commonly used as the oil core of these systems for solubilizing a wide range of active substances, and to be compatible with different polymers (MORA-HUERTAS; FESSI; ELAISSARI, 2010). The polymer selection must consider the biodegradability/biocompatibility characteristics, the intended route of administration and the method used in the preparations and application (ANTON; BENOIT; SAULNIER, 2008). Our results indicated that AO is a suitable material for the development of Eudragit® RL100 NCs, since dissolution/swelling of polymer filmswas not observed. Furthermore, the DES solubility in AO indicated that the oil is suitable for the preparation of NCs.

In sequence, NCs were prepared by the deposition of preformed polymer, a commonly method used to prepare these nanostructures (MORA-HUERTAS; FESSI; ELAISSARI, 2010). Initial tests were performed using AO at 3.0%; however, after 24h formulations presented creaming. Thus, the percentage of oil was reduced to 2.5% and more stable and homogenous systems were obtained. The results showed that DES-NC_{AO} presented larger particle size in comparison to DES-NC_{MCT}. Similar results were observed by Mora-Huertas and co-workers (2012), whose reported higher size of nanocapsules prepared with almond oil and corn oil, in comparison to the ones prepared with MCT. This effect was attributed to the high quantities of mono- and di-long-chain unsaturated fatty acids present in those oils. The AO used in our study presented high quantities of mono- and di-long-chain unsaturated fatty acids (around 75%), which may explain the larger particle size obtained to these formulations.

In relation to zeta potential, all formulations presented positive values (+6.9 to +14.9). NC_{AO} showed higher potential zeta values than those observed to NC_{MCT} ($p<0.05$). This

parameter reflects the particle surface charge and is related to the chemical nature of the polymer, the stabilizers present in the formulation and the pH of medium (BHATTACHARJEE, 2016; MORA-HUERTAS; FESSI; ELAISSARI, 2010). Eudragit® RL 100 is a copolymer of polyethylacrylate, methylmethacrylate and chloro-trimethylammonioethyl methacrylate, and contains 8.8 to 12% of quaternary ammonium groups, which confer positive charge to the polymer (DAS, SURESH, DESMUKH, 2010; KATARA; MAJUMDAR, 2013). The PDI values were less than 0.20, indicating a homogeneous distribution of size, regardless the oil type ($p>0.05$).

Taking into account the photoinstability of desonide formulations (BRAGA et al., 2013; SANTA et al., 2013) and the advantages of NCs, which can improve drugs photostability, we carried out a comparative photodegradation study. To perform the evaluation, aliquots of NCs and desonide solution at the same drug concentration were exposed to UVA and UVC radiation. Our findings showed that the association of DES with nanostructures had a positive effect over the drug photostability. The results indicated that both formulations significantly reduced the DES degradation. Aiming to elucidate the mechanisms involved in the photoprotection conferred by the NCs, nanoemulsions (NEs) containing AO or MCT and nanospheres (NS) were prepared and submitted to the same UVA irradiation conditions.

Regarding to the structure, nanospheres do not present oil in their composition and can be considered matrix systems (MORA-HUERTAS; FESSI; ELAISSARI, 2010; SCHAFFAZICK et al., 2003; SINGH; LILLARD, 2009). Nanoemulsions are considered kinetically stable systems due to their smaller particle size compared to conventional emulsion and have been associated with the improvement of drug stability (FERREIRA et al., 2015, 2016). The results showed that after 36h of UVA irradiation, formulations containing AO in their composition proved to be more photostable regardless of their structure (NE or NC). The residual drug content from the other nanostructures prepared (DES-NS, DES-NE_{MCT} and DES-NC_{MCT}) was significant lower, regardless of being matrix, core shell or nanoemulsion systems. In this context, it can be suggested the minimal influence of the polymeric wall in protecting DES from degradation, in the NCs developed. Moreover, the oil type used to prepare the formulations influenced the protection. These findings are in agreement with the results obtained in the photostability evaluation of the other nanostructures prepared (DES-NS, DES-NE_{MCT} and DES-NC_{MCT}). These nanostructures presented similar drug residual content regardless of being matrix, core shell or nanoemulsion systems.

A complementary photostability study, employing UVC radiation (254 nm) was performed. Although most of this radiation is filtered by the ozone layer, the evaluation of the chemical and biological effects of UVC radiation has received attention, both for a better understanding of the photochemistry involved in these degradation reactions and for the knowledge of the specific damage it may cause (MOORE, 2004).Considering that UVC is the most energetic ultraviolet radiation and, thus, photodegradation reactions occur more quickly, the use of this radiation source becomes interesting, since it exposes the sample to a more drastic degradation condition. The results showed that also in this condition, the colloidal systems were able to improve DES photostability. The nanoencapsulation increased 5.5 and 4.4 times the $t_{90}$of desonide in DES-NC_{AO} and DES-NC_{MCT}, respectively, demonstrating that the oil used to compose the NCs core influenced the photostability of desonide also in this condition. Recently, Gehrcke and co-workers (2017) reported the improvement of indole-3-carbinol (IC3) photostability associated to nanocapsules containing rose-hip oil or MCT as oily core. The authors found that the oil type used to prepare the NCs influenced this protection.

Regarding the *in vitro* release profiles, the mathematical modeling indicated that both NCs formulations best fitted to the biexponential model. In this case, the release occurs at two different steps. The biphasic release profile presents the advantage of an initial rapid drug release, followed by a prolonged release, which may maintain the drug effects for a longer time. The burst effect observed is probably related to the encapsulation efficiency of NCs, which was around 80%. The mechanisms of release were analyzed by Power Law model. According to it, for spherical drug release systems the limits for the release exponent are the following: $n = 0.43$ indicates Fickian diffusion; $0.43 < n < 0.85$ corresponds to anomalous transport and $n \geq 0.85$ implies in case II transport (KORSMEYER et al 1983). From the results obtained, both formulations showed release mechanism according to anomalous transport, indicating the polymer chain relaxation followed by the diffusion of desonide.

Over the past few years, several *in vitro* test methods have been suggested as alternatives to *in vivo* assays. HET-CAM represents a suitable method to evaluate eye irritation and also the tissue irritation for dermally applied substances (GOEBEL; NEUBERT; WOLHRAB, 2011). The CAM has a large blood supply and due to its vascular mucosa, similar to human eye, irritating effects become visible and endpoints like vasoconstriction, hemorrhage and coagulation can be observed (ICCVAM, 2006; FANGUEIRO et al.,2016; GOEBEL;NEUBERT; WOLHRAB 2011). In this study, we evaluated the irritant potential of DES in the free form and nanoencapsulated. Results indicated the slightly irritant potential of

all the formulations. Similar findings were observed by Weber and co-workers (2015) in the assessment of irritation of betamethasone dipropionate lipid-core nanocapsules.

It is well known that UV and visible radiation can induce the photochemical activation of certain chemicals (LYNCH; WILCOX, 2011). The phototoxic reactions induced by drugs are caused after the skin exposure to photoreactive drugs, triggered by UVA and UVB radiation (ONOUE et al., 2010; ZUBA et al., 2016).Phototoxicity studies are generally performed on products that are exposed to solar radiation (VINARDELL, 2015). In this context, the *in vitro* 3T3 NRU phototoxicity test is a validated alternative methodology for the evaluation of phototoxic risk of pharmaceutical and cosmetic formulations (BACCARIN et al., 2013; OECD, 2004). In this study, we chose HaCaT and 3T3 as model cell lines to evaluate the cutaneous phototoxic potential of DES in both free and nanoencapsulated forms, using the NRU and MTT viability assays. The results obtained suggest that the treatments, at the concentration tested, are non-phototoxic. The decrease of the cell viability related to NCs, observed with HaCaT/MTT assay, suggests a cytotoxic effect of the nanostructured systems, which could be related to the polymer and/or the presence of surfactants in these formulations. Similar effect was reported by Contri et al. (2016) and Mendes et al. (2015). This cytotoxicity was not detected by the NRU assay in the same cell line, which might be attributed to the different sensitivity of each viability endpoint. Nogueira and co-workers (2013) also described different sensitivity of HaCaT and 3T3 cells, using both MTT and NRU assays, to detect the phototoxic potential of nanovesicles.

5. Conclusions

In this study we showed the feasibility of the development of desonide-loaded nanocapsules with açai oil as oily core. The nanoencapsulation enhanced desonide photostability against UVA and UVC radiations and reduced the release rate of drug. Besides, all formulations showed to be non-phototoxic in the concentration tested.

Considering the results and the advantages of association of DES to nanocarriers, the nanocapsules developed can be considered promising for inclusion into topical formulations, aiming adequate cutaneous administration.

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References

ANTON, N., BENOIT, J-P.; SAULNIER, P. Design and production of nanoparticles formulated from nano-emulsion templates—A review. **Journal of Controlled Release**, v. 128, p.185–199, 2008.

AUBERT-WASTIAUX, H. et al. Topical corticosteroid phobia in atopic dermatitis: a study of its nature, origins and frequency. **British Journal of Dermatology**,v.165,p.808-14, 2011.

BACCARIN, T. et al. Photoprotection by *Punica granatum* seed oil nanoemulsion entrapping polyphenol-rich ethyl acetate fraction against UVB-induced DNA damage in human keratinocyte (HaCaT) cell line. **Journal of Photochemistry & Photobiology, B: Biology**, v. 153, p.127–136, 2015.

BELTRANI, V.S.; BARSANTI, F.A.; BIELORY,L. Effects of Glucocorticosteroids on the Skin and Eye. **Immunology and Allergy Clinics of North America**, v. 25, p.557– 580, 2005.

BHATTACHARJEE, S. DLS and zeta potential – What they are and what they are not? **Journal of Controlled Release**, v. 235, p. 337–351, 2016.

BRAGA, M.P et al. Assay and Photodegradation Kinetics of Desonide Lotion by an LC-UV Stability-Indicating Method. **Journal of Liquid Chromatography & Related Technologies**, v. 37, 1968-84, 2014.

BRAGA, M.P. Desonida: validação de métodos analíticos e estudo de fotoestabilidade de formulações, 2013. 121 f. **Dissertação** (Mestrado em Ciências Farmacêuticas) - Universidade Federal de Santa Maria, Santa Maria, 2013.

CIR. Cosmetic Ingredient Review. **Plant-Derived Fatty Acid Oils as Used in Cosmetics**. p. 32, 2011. Washington, DC.

CONTRI, R.V. et al. Skin penetration and dermal tolerability of acrylic nanocapsules: Influence of the surface charge and a chitosan gel used as vehicle**. International Journal of Pharmaceutics**, v. 507, p. 12–20, 2016.

SANTA, F. et al. Development and validation of a simple stability-indicating LC-method and UVA photostability study of desonide hair lotion. **Current Analytical Chemistry**, v.9, p. 659-667, 2013.

DAS S., SURESH, P.K., DESMUKH, R. Design of Eudragit RL 100 nanoparticles by nanoprecipitation method for ocular drug delivery. **Nanomedicine**, v. 6, n. 2. p. 318-23, 2010.

FANGUEIRO, J.F. et al. Biopharmaceutical evaluation of epigallocatechin gallate-loaded cationic lipid nanoparticles (EGCG-LNs): In vivo, in vitro and ex vivo studies. **International Journal of Pharmaceutics**, v.502, p. 161–169, 2016.

FAVACHO, A.S.H. et al. Anti-inflammatory and antinociceptive activities of *Euterpe oleracea* oil**. Revista Brasileira de Farmacognosia**, v.21,n. 1,p.105-114, 2011.

FERREIRA, L.M. et al. Ketoprofen-loaded pomegranate seed oil nanoemulsion stabilized bypullulan: Selective antiglioma formulation for intravenous administration. **Colloids and Surfaces B: Biointerfaces,** v.130, p. 272-277, 2015.

FERREIRA, L.M. Pomegranate seed oil nanoemulsions improve the photostability and in vivo antinociceptive effect of a non-steroidal anti-inflammatorydrug. **Colloids and Surfaces B: Biointerfaces**, v, 144, p.214–221, 2016.

GEHRCKE, M et al. Enhanced photostability, radical scavenging and antitumor activity of indole-3-carbinol-loaded rose hip oil nanocapsules. **Materials Science and Engineering C**, v.74, p. 279–286, 2017.
GEHRCKE, M et al. Nanocapsules improve indole-3-carbinol photostability and prolong its antinociceptive action in acute pain animal models. **European Journal of Pharmaceutical Sciences,** v. 111, p. 133-141, 2018.

GELBARD, C. M., HEBERT, A. A. Desonide hydrogel: advances in vehicle technology. **Expert Review of Dermathology**, v. 4, n. 1, p. 23-27, 2009.

GOEBEL, A.S.B., NEUBERT, R.H.H., WOLHRAB,J. Dermal targeting of tacrolimus using colloidal carrier systems. **International Journal of Pharmaceutics**, v. 404, p. 159-168, 2011.

GUICHARD, A. et al. Effects of topical corticosteroids on cell proliferation, cell cycle progression and apoptosis: In vitro comparison on HaCaT. **International Journal of Pharmaceutics**, v. 479, p. 422-9, 2015.

HARTMAN, L., LAGO, R.C.A. Rapid preparation of fatty acids methyl esters. **Laboratory Practice, London**, v.22, p.475-476, 1973.

HENGGE, U. R., et al. Adverse effects of topical glucocorticosteroids. **Journal of American Academy Dermatology**, v. 54, n. 1, p. 1-15, 2006.

HORN, E.J. et al. Topical corticosteroids In psoriasis: strategies for improving safety. **European Academy of Dermatology and Venereology**, v. 24, p. 119-24, 2010.

ICCVAM-Recommended Test Method Protocol: **Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) Test Method**, 2006.

KATARA, R.; MAJUMDAR, K.D. Eudragit RL 100-based nanoparticulate system of aceclofenac for ocular delivery. **Colloids and Surfaces B: Biointerfaces**, v. 103, p.455–462, 2013.

KORSMEYER, R.W., Gurny,R., E. Doelker, Buri, P., Peppas, N.A. **International Journal of Pharmaceutics**, v. 15, n. 25,1983.

LYNCH, A.M., WILCOX, P. Review of the performance of the 3T3 NRU in vitro phototoxicity assay in the pharmaceutical industry**. Experimental and Toxicologic Pathology** v. 63 p. 209–214, 2011.

MARQUES, S.E. et al. Evaluation of the genotoxicity of *Euterpe oleraceae* Mart. (Arecaceae) fruit oil (açaí), in mammalian cells in vivo. **Food and Chemical Toxicology**, v. 93, p.13-19, 2016.

MENDES, L. P. Biodegradable nanoparticles designed for drug delivery: The number of nanoparticles impacts on cytotoxicity. **Toxicology in Vitro**, v. 29, p. 1268–74, 2015.

MOORE, D. E. Photophysical and photochimical aspects of drug stability. In: TØNESSEN, H.H. **Photostability of drugs and drug formulations**. 2nd. ed. Boca Raton: CRC Press LLC, 2004.

MORA-HUERTAS, C. E.; FESSI, H.; ELAISSARI, A. Polymer-based nanocapsules for drug delivery. **International Journal of Pharmaceutics**, v. 385, p. 113-142, 2010.

MORA-HUERTAS, C.E. et al. Nanocapsules prepared via nanoprecipitation and emulsification–diffusion methods: Comparative study. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 80, p. 235-239, 2012.

NASCIMENTO, R.J.S. et al. Composição em ácidos graxos do óleo da polpa de açaí extraído com enzimas e hexano. **Rev. Bras. Frutic., Jaboticabal** - SP, v. 30, n. 2, p. 498-502, 2008.

NOGUEIRA, D. R. et al. New cationic nanovesicular systems containing lysine-based surfactants for topical administration: Toxicity assessment using representative skin cell lines. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 83, p. 33–43, 2013.

ONOUE, S. et al. High-Throughput Screening System for Identifying Phototoxic Potential of Drug Candidates Based on Derivatives of Reactive Oxygen Metabolites. **Pharmaceutical Research**, v. 27, p.1610–1619, 2010.

OECD GUIDELINE FOR TESTING OF CHEMICALS 432. **In Vitro 3T3 NRU phototoxicity test**, 2004.

OURIQUE, A.F. et al. Tretinoin-loaded nanocapsules: Preparation, physicochemical characterization, and photostability study. **International Journal of Pharmaceutics**, v. 352, p.1-4, 2008.

PACHECO-PALENCIA, A. L.et al. Absorption and Biological Activity of Phytochemical-Rich Extracts from Açai (Euterpeoleracea Mart.) Pulp and Oil in Vitro. **Journal of Agricultural and Food Chemistry**, v. 56, n. 10, p. 3593-3600, 2008.

ROSA, P. et al. In vitro and in vivo evaluation of a desonide gel-cream photostabilized with benzophenone-3. **Drug Development and Industrial Pharmacy**, v.42,n.1,p.19-27, 2015.

ROSA, P. et al. Investigation of the stabilizing effects of antioxidants and benzophenone-3 on desonide photostability. **Journal of The American Association of Pharmaceutical Scientists,** v. 15, n.5. p. 1155-62, 2014.

SANTOS, S.S. et al. Clotrimazole-loaded Eudragit® RS100 nanocapsules: Preparation, characterization and in vitro evaluation of antifungal activity against Candida species. **Materials Science and Engineering C**, v. 33,p. 1389-94, 2013.

SANTOS, S.S.S. Formulation and in vitro evaluation of coconut oil-core cationicnanocapsules intended for vaginal delivery of clotrimazole. **Colloids and Surfaces B:Biointerfaces** v. 116,p. 270-276, 2014.

SAVIAN, A.L. et al. Dithranol-loaded lipid-core nanocapsules improve the photostability and reduce the in vitro irritation potential of this drug. **Materials Science and Engineering C**, v. 46, p. 69-76, 2015.

SCHAFFAZICK, R. S.; GUTERRES, S.S.; FREITAS, L.L.; POHLMANN, A.R. Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração e fármacos. **Química Nova**, v 26, n 5, p 726-737, 2003.

SINGH, R.; LILLARD, W.J. Nanoparticle-based targeted drug delivery. **Experimental and Molecular Pathology**, v. 86, p.215–223, 2009.

VINARDELL, M.P. The use of non-animal alternatives in the safety evaluations of cosmetics ingredients by the Scientific Committee on Consumer Safety (SCCS). **Regulatory Toxicology and Pharmacology**, v. 71, n. 2, p. 198-204, 2015.

VINARDELL, M.P., M., p. Macián,M. Alternative methods in the study of the safety of cosmetics. **Toxicology in Vitro**, v. 8, p. 467-470, 1994.

WEBER, J. et al. Association of Borage Oil and Betamethasone Dipropionate in Lipid-Core Nanocapsules: Characterization, Photostability and In Vitro Irritation Test. **Journal of Nanoscience and Nanotechnology**, v.. 15, 1–9, 2015.

WIEDERSBERG, S., LEOPOLD, C.S., GUY, R.H. Bioavailability and bioequivalence of topical glucocorticoids. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 68, p. 453-466, 2008.

ZUBA, E.B. et al. Drug-induced Photosensitivity. **Acta Dermatovenerologica Croatica**, v. 24, p. 55-64, 2016.

WHO. World Health Organization. Classification of topical corticosteroids. Disponível em: http://apps.who.int/medicinedocs/en/d/Jh2918e/32.html#Jh2918e.32.1. Acesso em set 2018.

CAPÍTULO 2: Hydrogel containing desonide-loaded açai oil based nanocapsules: *in vitro* and *in vivo* evaluation

CAPÍTULO 2: Hydrogel containing desonide-loaded açai oil based nanocapsules: *in vitro* and *in vivo* evaluation

2.1 Apresentação

O uso tópico de corticoides está relacionado com efeitos adversos locais e sistêmicos (BELTRANI; BARSANTI; BIELORY, 2005; HENGGE et al., 2006). Nesse sentido, sistemas de liberação de fármacos tais como os lipossomas e as nanopartículas lipídicas e poliméricas têm sido estudados visando a otimização terapêutica e minimização dos efeitos adversos associados à terapia com corticoides tópicos.

A fim de obter preparações com características reológicas mais adequadas à aplicação tópica, estudos têm relatado a incorporação das suspensões de nanocápsulas poliméricas em veículos semissólidos como géis e emulsões. Assim, o presente capítulo foi redigido na forma de artigo científico e aborda o desenvolvimento de hidrogéis de base nanotecnológica, preparados a partir das suspensões de nanocápsulas contendo desonida, descritas no Capítulo 1 deste trabalho. Foram preparados hidrogéis, utilizando o polissacarídeo natural Amigel® como polímero formador de gel, como forma farmacêutica para a administração da desonida associada às nanocápsulas poliméricas. As formulações semissólidas foram avaliadas quanto às suas características físico-químicas, estabilidade frente ao armazenamento em temperatura ambiente, perfil de distribuição do fármaco nas camadas epiteliais e bioadesão. A atividade biológica das preparações foi estudada utilizando modelo de inflamação cutânea induzida por óleo de cróton, em camundongos.

Hydrogel containing desonide-loaded açai oil based nanocapsules: *in vitro* and *in vivo* evaluation

Priscila Rosa[1], Mariane Lago Friedrich[2], Juliana dos Santos[1], Natháli Pegoraro[3], Camila Camponogara[3], Sara Marchesan Oliveira[3], Cristiane de Bona da Silva[2], Andréa Inês Horn Adams[2]

[1] Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal de Santa Maria, Santa Maria, Brazil.

[2] Departamento de Farmácia Industrial, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, Brazil

[3] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, Universidade Federal de Santa Maria, Santa Maria, Brazil

Abstract

This study aimed the development of topical formulations (hydrogels) containing desonide-loaded açai oil based nanocapsules by addition of sclerotium gum (2.5%) to the suspensions and its evaluation as an alternative to the treatment of dermatological disorders. For this purpose, an animal model of acute skin inflammation induced by croton oil was employed. Drug delivery systems such as polymeric nanocapsules have been used as strategies to optimize the therapy and minimize the side effects of topical corticoids. The hydrogels presented pH compatible with topical application, drug content above 90% and mean diameter in the nanometric range, after redispersion in water. The semisolids showed a suitable spreadability, non-Newtonian flow with pseudoplastic behavior, and stability under room conditions during 30 days. Through an *in vitro* skin permeation/penetration study it was demonstrated higher amount of desonide retained in the epidermis, site of action of the glucocorticoids, from the hydrogels containing desonide. Hydrogels containing desonide-loaded açai oil based nanocapsules showed to be more effective in reduce mice ear edema compared to the other formulations evaluated. The semisolid formulations based on desonide nanocapsules presented biological effect comparable to the commercially available formulation, which presents the double drug concentration and then may be considered a promising approach to treat dermatological disorders.

Keywords: ear edema; corticosteroids; sclerotium gum; açai oil; semisolid formulation

1. Introduction

Topical glucocorticoids (TG) are among the most common drugs prescribed by dermatologists (ŞENYIĞIT et al., 2009) for the treatment of psoriasis and atopic dermatitis (AUBERT-WASTIAUX et al., 2011). The clinical efficacy of these drugs is related to their anti-inflammatory, immunosuppressive, vasoconstrictive and anti-proliferative effects (GRAU, 2006; ŞENYIĞIT et al., 2009). Desonide is a low potency synthetic non-fluorinated glucocorticoid used for the treatment of sensitive areas such as face and intertriginous region and in elderly and children (GELBARD, HEBERT, 2009; HORN et al., 2009). Despite the clinical efficacy in therapy of inflammatory diseases of this class of drugs, TG can induce local and systemic side effects. Skin reactivity, telangiectasia, atrophy and hypopigmentation are common local side effects induced by these compounds. Among the systemic side effects induced by the TG are endocrine effects, such as Cushing syndrome, metabolic events such as hyperglycemia and adrenocortical suppression and disturbs on electrolyte balance, which can cause edema and hypertension (BELTRANI; BARSANTI; BIELORY, 2005; HENGGE et al., 2006).

Drug delivery systems such as liposomes (ELSAYED et al., 2007), solid lipid nanoparticles (MAIA; MEHNERT; SCHÄFER-CORTING, 2000) and polymeric nanoparticles (BEBER et al., 2014, 2016; DE ANDRADE et al., 2015a; FONTANA et al., 2011a; MARCHIORI et al., 2010) have been used as strategies to optimize the therapy and minimize the side effects of TG. One approach to reduce the systemic adverse effects of TG is to enhance the drug permeability, aiming to reduce the topically applied dose (ŞENYĞIT et al., 2009). Considering the drug delivery systems, polymeric nanocapsules can be considered vesicular systems composed by a core, generally oily, surrounded by a polymeric wall (MORA-HUERTAS; FESSI; ELAISSARI, 2010; SCHAFFAZICK et al., 2003). Concerning the oily core of polymeric nanocapsules, vegetable oils have been used, since they demonstrated to improve the pharmacological drug effect and provided protection for the encapsulate substance (SANTOS et al., 2014; GEHRCKE, 2018). In this scenario, studies with açai oil have indicated antiproliferative activity in colon adenocarcinoma cells (PACHECO-PALENCIA, 2008), anti-inflammatory effect (FAVACHO et al., 2011) and absence of genotoxic effect in peripheral leukocytes, liver, bone marrow and testicular cells of rats (MARQUES et al., 2016).

Polymeric nanocapsules have been studied for cutaneous application of glucocorticoids such as clobetasol propionate (DE ANDRADE et al., 2015a), dexamethasone (BEBER et al., 2014, 2016) and bethametasone dipropionate (WEBER et al., 2015).

Desonide-loaded nanocapsules prepared using poly-(ε-caprolactone), Eudragit® S100 and Eudragit® L100 as polymeric wall have been proposed to topical administration of this drug (ANTONOW et al., 2016); however, there is no evaluation about the influence of nanoencapsulation on the drug permeation/penetration profile.

Semisolid formulations containing polymeric nanocapsules are often based on emulsions and gels. Their rheological properties can be modulated, providing appropriate characteristics for cutaneous application (GUTERRES; ALVES; POHLMANN, 2007). In this context, the incorporation of nanocapsule suspensions in semisolid vehicles has been reported using gel forming polymer such as Carbopol Ultrez ® 10 NF (BEBER et al., 2016; DE ANDRADE et al., 2015b; FONTANA et al., 2011b; OURIQUE et al., 2011), gellan gum (MARCHIORI et al., 2017; PEGORARO et al., 2017) and Pemulen® TR1 (DE LIMA et al., 2017). Hydrogels are formed by a three-dimensional network of hydrophilic crosslinked polymers. They have broad biomedical application in the administration of drugs, artificial organs and mobilization of enzymes due to their great water content and elasticity, which turn them similar to natural tissues and therefore biocompatible (KRISHNA RAO et al., 2006; ŞÖLENER, 2008). Microbial polysaccharides gums are carbohydrate polymers produced by a wide variety of microorganisms and have been emerging in pharmaceutical industry due to its characteristics such as biocompatibility, biodegradability, chemical and mechanical resistance, bioadhesiveness and gelling power (MANJANNA; SHIVAKUMAR; PRAMODKUMAR, 2009). Scleroglucan produced by the *Sclerotium rolfsii* fungus is a neutral polysaccharide, stable in a wide range of pH values and temperatures (COVIELLO et al., 2016; FIUME et al., 2016).

Therefore, this study aimed to develop a hydrogel based on Scleroglucan, a gel forming polymer, as a final dosage form for DES-loaded Eudragit ® RL 100 nanocapsules containing açai oil as oily core. These novel semisolid formulations were characterized regarding their physicochemical properties, permeation/penetration skin profile and bioadhesive potential. Furthermore, the anti-inflammatory effect of nanostructured formulations in a model of croton oil-induced acute skin inflammation in mice was investigated.

2. Material and methods

2.1 Material

Desonide (DES) (97.42%) was purchased from Fagron (São Paulo, Brazil). The açai oil (AO) was donated by Beraca Ingredientes Naturais SA (Ananindeua, Brazil). Medium chain tryglicerides (MCT) were bought from Delaware (Porto Alegre, Brazil). Polysorbate 80, sorbitan monooleate, methanol and acetonitrile were acquired from Sigma Aldrich (St Louis, USA). Eudragit RL 100® was bought from Evonik (Essen, Germany). Amigel® (Sclerotium gum) was purchased from PharmaSpecial (Itapevi, Brazil). Imidazolidinyl ureia was acquired from All Chemistry (São Paulo, Brazil). Commercial gel cream (Adinos®, lot 1709428 Aché, Garulhos, Brazil) was acquired locally. All other chemicals presented pharmaceutical grade and were used as received. For the *in vivo* evaluation, ketamine (Dopalen®) and xylazine (Anasedan®) were acquired from Ceva (Paulínia, Brazil). Croton oil was acquired from Sigma Chemical Co. (St. Louis, USA). Hematoxylin-eosin and paraffin were acquired from Merck (Darmstadt, Germany). Acetone, formaldehyde, ethanol and acetic acid were purchased from Vetec (Rio de Janeiro, Brazil).

2.2 Preparation of nanocapsule suspensions

DES-loaded nanocapsules (DES-NC) were prepared following the interfacial deposition of preformed polymer method (FESSI et al., 1989). An organic phase constituted by Eudragit RL® 100 (0.25 g), sorbitan monooleate (0.1925 g), MCT or AO (625 µL), DES (6.25 mg) and acetone (135 mL for DES-NC_{AO} and 67.5 mL for DES-NC_{MCT}) was kept under moderate magnetic stirring at 40º C, during an hour. This phase was injected into an aqueous dispersion (135 mL) containing polysorbate 80 (0.1925 g) and was maintained under moderate magnetic stirring for 10 min. In sequence, acetone and part of the water were removed by evaporation at 40 ºC under reduced pressure to obtain 25 mL of final volume and 0.25 mg mL^{-1} of DES.

2.3 Preparation of semisolid formulations

Hydrogels (HGs) were prepared by adding the preservative (imidazolidinyl ureia at 0.6%) directly to the nanocapsule suspensions, under moderate stirring. In sequence, sclerotium gum (2.5 %) was added to the dispersions, followed by moderate magnetic stirring during 20 min. The obtained hydrogels were denominated HG-$DESNC_{AO}$ and HG-$DESNC_{MCT}$. For the HG containing free DES (HG-DES), the drug was previously dispersed in polysorbate 80 (0.77%), then water (q.s 25 g) and preservative (0.6%) were added to the dispersion. After homogenization, sclerotium gum (2.5%) was incorporated into the dispersion and this was maintained under moderate magnetic stirring for 20 min. DES content

in all HGs was 0.25 mg g^{-1} (0.025%). The vehicle was prepared following the same methodology, dispersing imidazolidinyl urea (0.6%) and sclerotium gum (2.5%) in water and polysorbate 80 (0.77%). The blank formulations (HG-NC_{AO} and HG-NC_{MCT}) used in the *in vivo* study were prepared following the same procedure employed for the nanostructured hydrogels, however, the nanocapsule suspensions did not contain DES. All formulations were prepared in triplicate.

2.4 Hydrogels characterization

On the first 48h after preparation, HGs were characterized regarding macroscopic characteristics such as appearance and color, pH, mean particle diameter, viscosity, spreadability and drug content. The pH values were measured in HG aqueous dispersion (10%, w/v) using a calibrated potenciometer (Model UB-10, Denver Instrument, USA). The nanocapsules mean particle size was evaluated by photon correlation spectroscopy (PCS) technique using ZetaSizer Nano Series (Malvern, UK) by dispersing an aliquot of the samples in ultrapure water (1:500, w:v). Evaluation of rheological properties was conducted at 25 ± 2º C employing a rotational viscosimeter DV II + Pro Model (Brookfield, USA) using a SC4-25 spindle and a small sample adapter. The shear stress ramp was applied during 60 s and samples were submitted to a range of speed between 0.132 and 22 s^{-1}. In order to establish the rheological behavior, data was analyzed according to different flow models: Bingham ($\tau = \tau_0 + \eta\gamma$), Casson ($\tau = \tau_0{}^{0.5} n + \eta^{0.5} \gamma^{0.5}$), Power Law ($\tau = \kappa \gamma^n$) and Herschell-Bulkley ($\tau = \tau_0 + \kappa \gamma^n$), where τ is the shear stress, τ_0 is yield stress, η is the viscosity, n is the flow index, κ is the consistency index and γ is the shear rate (KIM et al., 2003).

The spreadability of formulations was evaluated trough the parallel plate method, according to the methodology described by Rigo and co-workers (2012). The sample was placed in a central hole of a mold glass plate, positioned on a scanner surface. The mold glass was carefully removed and the sample was pressed by glass plates of known weight, which were added in intervals of 1 min, totaling 20 plates. The images were captured and the spread areas were calculated using the ImageJ software (Version 1.49q, National Institutes of Health, USA). The spreadability factor (S_f) represents the formulation ability to spread on a horizontal surface when a gram of weight is added on it and was calculated using the following equation:

$$S_f = A/W$$

Where S_f is the spreadability factor (mm^2 g^{-1}); A is the maximum spread area (mm^2) and W is the total weight added (g).

The analysis of DES content in semisolids formulations was performed by HPLC. An aliquot of 1.0 g of each formulation was accurately weighted and 10 mL of methanol were added. The dispersion was kept under stirring during 10 min and then it was quantitatively transferred to a 20 mL volumetric flask. The volume was filled up with methanol and the dispersion was centrifuged under 4000 rpm during 10 minutes. The supernatant was filtered through a 0.45 µm membrane and injected into the HPLC system. Chromatographic system consisted of a Shimadzu (Kyoto, Japan) LC system equipped with a LC 20-AT pump, SPD M-20A diode array detector, a CBM 20-A system controller, DGU degasser and SIL 20-AHT autosampler. The mobile phase was a mixture of methanol, acetonitrile and ultrapure water adjusted to pH 4.0 with ortophosphoric acid 18% (60:10:30, v:v:v) eluted at 1.0 mL min^{-1}. An RP-18 column (150 x 4.6 mm, 5µm), coupled to a RP-18 guard column was employed. The injection volume was 20 µL and the detection was at 244 nm. The analytical method proved to be specific, without excipient interference, linear in the range of 5-100 µg mL^{-1} and precise in levels of repeatability and intermediate precision (RSD < 2.0%).

2.5 Stability study

To assess the formulations stability under storage, HGs were packed in plastic double wall pots and maintained at room temperature (22 ± 2 ºC) during 30 days. In predetermined intervals (7, 15 and 30 days), formulations were analyzed according to their macroscopic characteristics (such as appearance and color), pH, mean diameter, rheological properties, spreadability and desonide content. Drug peak purity was monitored by diode array detector to ensure the specificity of the analytical method.

***2.6 In vitro* release study**

The DES *in vitro* release from HG containing NCs or non-associated drug and commercial gel cream was studied using vertical Franz diffusion cells, at 32 ± 0.5 ºC (n=6). The diffusion area was 3.14 cm^2. A dialysis membrane (10000 Da, Spectra Pore) was fitted between donor and receptor compartment. The receptor medium, composed by sodium acetate buffer pH 4.5 and 0.5% of polysorbate 80, was constantly, moderately stirred (400 rpm) and contemplated the sink condition. A formulation amount corresponding to 500 µg of DES (infinite dose) was spread on the membrane surface. At predetermined time intervals (30 min, 1, 2, 4, 6 and 8h), 0.5 mL of the receptor medium was withdrawn and replaced by an equal volume of fresh medium. DES released was determined by HPLC, following the previously described conditions. To evaluate the drug release profiles, the Higuchi´s model ($C = kt^{0.5}$)

was used. *C* indicates the amount of drug released at the time *t*, and *k* is a constant, related to diffusion area, diffusion coefficient and drug solubility in the system (FLYNN et al., 1999; HAUCK et al., 2007; SHAH et al., 1991).

2.7 In vitro skin penetration and permeation assay

Skin samples were obtained from the abdominal area of female pigs donated from a local slaughterhouse. The visible hair from the skin surface was cut and adipose tissue was carefully removed. Pieces of skin were cut into circles, wrapped in aluminum foil and stored at -18 ºC until the time of use. The study was carried out using vertical Franz diffusion cells and sodium acetate buffer pH 4.5 containing 0.5% of polysorbate 80 was employed as release medium, under the same conditions used in the *in vitro* release study. The skin pieces were placed between the donor and receptor chamber with a diffusion area of 3.14 cm^2. The dermal side was in contact with the receptor medium and quantities of hydrogels (HG-$DESNC_{AO}$, HG-$DESNC_{MCT}$, HG-DES and commercial gel cream) corresponding to 500 µg of DES were applied to the skin surface (n = 6/formulation). After 8 h, the excess of formulation was removed with cotton and the amount of DES in each skin layer was determined. Stratum corneum (SC), epidermis (EP) and dermis (DE) were analyzed. Aliquots of the receptor medium were collected and assayed for DES content.

SC was removed by tape stripping using 18 successive tapes (Scotch tape 3M, USA). Epidermis and dermis were separated by heating the skin in a water bath (60 ºC) during 45 s. DES was extracted from each skin layer with acetonitrile (6.0 mL for SC, 1.0 mL for EP and 3.0 mL for DE), followed by vortex mixing during 2 min and sonication (15 min). DES was assayed by HPLC using the same analytical conditions previously described. The method was linear in the range of 0.5 – 50 µg mL^{-1} ($y = 43570x - 2754$; $r = 0.9999$) and the mean percentage recovery from skin was 100.84 %. Furthermore, the method was precise regarding repeatability (RSD of 1.94 %, n = 6) and intermediate precision (RSD of 2.86 %, n = 6).

2.8 Bioadhesion measurements

The hydrogels (HG-$DESNC_{AO}$, HG-DES-NC_{MCT}, Vehicle and commercial gel-cream) were subjected to bioadhesion measurements using a tensile stress tester (TA.XTplus Texture Analyzer; Stable Microsystem, UK). An amount of 0.2 g of each formulation was applied on the surface of a probe and porcine skin was used as membrane. Each piece of skin (n = 6) was put in contact with the sample using a force of 50 mN during 180 s. After the contact, the

probe was removed by a constant rate of 10 mm s^{-1}. The parameters of force (mN), distance (mm) and work of adhesion (mN mm^{-1}) were determined.

2.9 Antiedematogenic effect

2.9.1 Animals

Male *Swiss* mice weighing about 25-30 g were employed. Animals were kept under controlled temperature (22 ± 2°C) and on a 12 h light-dark cycle, and they were fed with standard laboratory chow and water *ad libitum*. At least 1 h before performing the experiments, animals were acclimatized to the experimental room. Experiments were carried out between 08:00 a.m. and 5:00 p.m. All experiments were performed according to current ethical guidelines for care of laboratory animals (ZIMMERMANN, 1983) and all procedures were approved by the Animal Use Ethics Committee of the Federal University of Santa Maria (Process number 3758220817). The number of animals and amount of irritant agent employed were the minimum necessary to demonstrate consistent effects of the treatments (n= 5-6 animals/group). When possible, both mice ears were employed to minimize the number of animals used. All experiments were conducted blindly and carried out by a single experimenter.

2.9.2 Croton oil-induced ear edema model

Acute ear edema was induced by a single topical application of the croton oil irritant (1 mg/ear/20 µL diluted in acetone) on the right ear of the animal (DE BRUM et al., 2016; PIANA et al., 2016).

2.9.3 Topical treatments

Fifteen milligrams of the developed semisolid formulations were applied on mice ear immediately after croton oil application, in order to evaluate their topic anti-inflammatory effect on ear edema croton oil-induced. *Swiss* mice were divided into seven groups with animals each: naïve (no treatment); croton oil; croton oil + hydrogel containing açai oil based nanocapsules (HG-NC_{OA}); croton oil + hydrogel containing desonide-loaded açai oil based nanocapsules (HG-$DESNC_{OA}$); croton oil + hydrogel containing medium chain triglycerides based nanocapsules (HG-NC_{TCM}); croton oil + hydrogel containing desonide-loaded medium

chain triglycerides based nanocapsules (HG-DESNC$_{TCM}$); croton oil + commercial gel cream containing desonide (0.50 mg g^{-1}) (positive control).

2.9.4 Ear edema measurements

Ear thickness was measured before (basal measure) and after induction of the inflammatory response using a digital micrometer (Digimess, Brazil). Mice were previously anesthetized with ketamine/xylazine (90 mg kg^{-1} + 30 mg kg^{-1}, respectively); after, micrometer was applied near the tip of the ear, just distal to the cartilaginous ridges. Croton oil-induced ear edema was characterized by an increase in ear thickness at 6 h after its application (maximum edematogenic effect). Thus, the ear thickness was measured before and 6 h after topical application of the croton oil or croton oil plus treatments. Only a single investigator performed the measures in order to reduce the variation between analyses.

2.9.5 Histological analysis

Mice were euthanized 6 h after croton oil application or croton oil plus treatments, ears were collected and fixed in a mixture of 80% ethanol, 40% formaldehyde and acetic acid (16:2:1) (Alfac solution) in order to conduct the histological analysis. Ear samples were embedded in paraffin, sectioned at 5 μm and finally stained with hematoxylin-eosin. For qualitative light microscopic analysis of the inflammatory cells infiltration response, a representative area was selected and the cellular inflammatory response was analyzed employing 20 x objective lens (OLIVEIRA et al., 2014). Only one investigator conducted this analysis to reduce the source of bias.

2.10 Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test at a significance level of 5%. Results of *in vivo* evaluation are presented as the mean + standard error of the mean (SEM), which are reported as geometric means plus their respective 95% confidence limits. The maximum inhibitory effect (E_{max}) was calculated in relation to the control groups response. Statistical analysis was assessed by ANOVA followed by post hoc Newman-Keuls' post-tests. All tests were carried out using GraphPad 6.0 Software (USA).

3. Results and discussion

3.1 Hydrogels characterization

In this work, desonide-loaded nanocapsules containing AO or MCT as oil core were used for the development of hydrogels. DES-NC_{AO} and DES-NC_{MCT} showed satisfactory characteristics, with nanometric size (165 ± 2 nm and 131 ± 2 nm, respectively), and low polidispersity index (< 0.20 for both formulations), indicating the system homogeneity; slightly acid pH (5.00 ± 0.09 for DES-NC_{AO} and 4.38 ± 0.13 for DES-NC_{MCT}) and zeta potential of +13.8 ± 0.28 for DES-NC_{AO} and +6.9 ± 0.67 for DES-NC_{MCT}.

Scleroglucan is a non-ionic fungal polysaccharide and it was chosen to develop this study because it has a good stability profile and useful properties to pharmaceutical products (MANJANA; SHIVAKUMAR; PRAMODKUMAR, 2009). After preparation, hydrogels showed homogeneous and bright aspect. HG-$DESNC_{AO}$ presented slightly yellowish color whereas HG-$DESNC_{MCT}$ was white in color. The hydrogel containing DES non-encapsulated and vehicle had transparent appearance. Table 1 shows the physico-chemical characteristics of the prepared hydrogels. The hydrogels prepared from nanocapsules suspensions showed lower pH values ($p<0.05$) than the non-nanostructured ones. This may be due to acidic pH of the colloidal suspensions. Formulations were considered compatible with cutaneous administration since pH skin ranges from 4.0 to 6.3, depending on the area (SCHREML et al., 2010). PCS technique was used to evaluate the nanocapsules mean diameter in the hydrogels after their aqueous redispersion. In other studies, PCS analysis was used to verify the presence of the nanostructures after aqueous redispersion of gel prepared with natural gel-forming polymer, such as gellan gum and pullulan (PEGORARO et al., 2017; MARCHIORI et al., 2017; DE LIMA et al., 2017). Results indicated that HG-$DESNC_{AO}$ and HG-$DESNC_{MCT}$ presented particles on the nanometric range, similar to the original colloidal suspensions, indicating the nanostructures were maintained after incorporation into semisolid. Compared to nanostructured formulations, vehicle and HG-DES showed higher values ($p<0.05$) of mean diameter and PDI. Regarding DES content, the obtained values were close to 100%, indicating that minimal loss occurred during the preparation of the semisolids.

Table 1. pH values, mean diameter, spreadability factor (Sf), drug content and PDI of semisolid formulations

Formulation	pH	Mean diameter	Sf ($mm^2\ g^{-1}$)	Drug content (%)	PDI

		(nm)			
HG-DESNC$_{OA}$	5.89 ± 0.07	140 ± 3	4.34 ± 0.28	101.54 ± 0.47	0.18 ± 0.01
HG-DESNC$_{MCT}$	5.95 ± 0.09	142 ± 2	4.94 ± 0.31	91.49 ± 1.05	0.25 ± 0.09
HG-DES	7.04 ± 0.02	791 ± 125	3.83 ± 1.20	99.80 ± 1.57	0.73 ± 0.01
Vehicle	7.12 ± 0.06	869 ± 60	3.34 ± 0.37	----	0.67 ± 0.05

The semisolid formulations are usually studied regarding their rheological and spreading characteristics. With respect to rheological properties, the evaluation of flow characteristics are useful to assess the quality and stability of semisolid formulations. Moreover, the flow properties can influence the pharmaceutical development process, such as filling, mixing and removal from the container before the application (LEE; MOTURI; LEE, 2009). The hydrogels rheograms were obtained by graphical representation of shear rate versus shear stress (Fig 1). The formulations showed non-Newtonian flow, since a non-linear relation between shear rate and shear stress was observed; additionally, they did not show thixotropy. The rheograms also indicated a pseudoplastic behavior, which better fit to Power Law (Ostwald) model, presenting correlation coefficients >0.99. Vinarta and co-workers (2013) found similar rheological properties to scleroglucan solutions. In the pseudoplastic flow, with increasing shear rate, a decline in viscosity is observed (LEE; MOTURI; LEE, 2009). Considering the topical administration, this rheological behavior is interesting, since in this type of flow when an external force is applied the formulation become less viscous (CHHABRA; RICHARDSON, 2008).

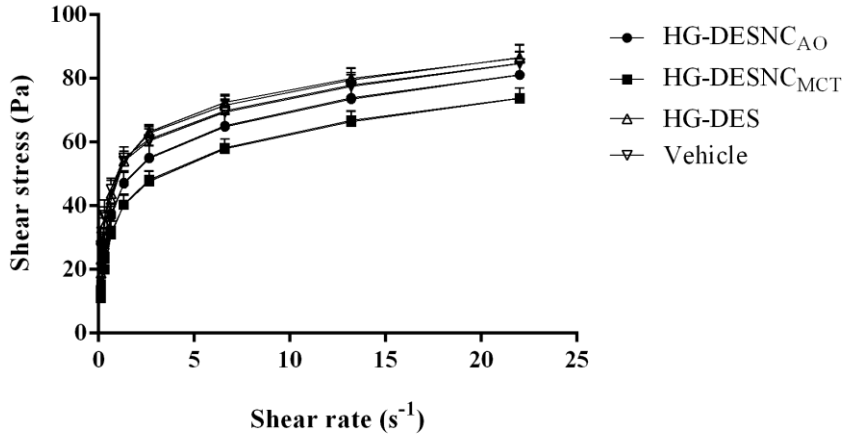

Fig. 1. Rheological behavior of hydrogels containing nanoencapsulated DES (HG-DESNC$_{AO}$ and HG-DESNC$_{MCT}$), the free compound (HG-DES) and the vehicle.

Concerning the spreadability, it can be defined by as the expansion of a semisolid formulation on a surface after a specified time and is closely related to the formulation application in site of action (BORGHETTI; KNORST, 2006). The easiness to spread a formulation on a substrate is an important factor that may impact in topical therapy efficacy (GUPTA; GARG, 2002). Fig 2 shows the spreadability profile of the hydrogels. Spreadability factors of the developed hydrogels were between 3.34 ± 0.37 and 4.94 ± 0.31 mm^2 g^{-1}. Greater spreadability factors ($p<0.05$) were obtained with nanostructured hydrogels, indicating that for the same applied force a larger area is achieved with these formulations.

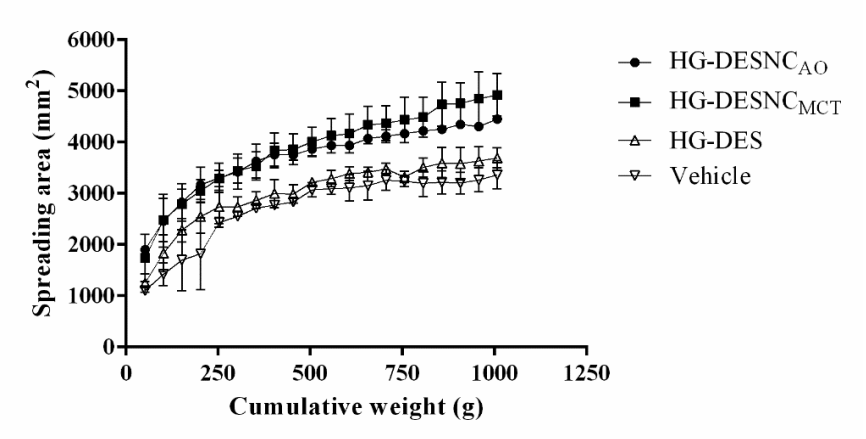

Fig 2. Hydrogels spreadability profiles.

3.2 Stability study

In relation to 30-day the stability study, macroscopic evaluation showed no changes in appearance and color. Regarding the DES content, in HG-DESNC$_{AO}$ it remained approximately 100 % (103.4 ± 0.43%) after 30 days of storage. In the HG-DESNC$_{MCT}$ the DES content was 92.50 ± 0.53 % after the same storage period, indicating a decay around 8% on its content. The hydrogel containing the non-encapsulated drug showed drug content of 84.23 ± 2.43 % after 15 days of storage, indicating the drug protection provided by the polymeric wall. These results indicated that the nanostructured hydrogels remained within the most common shelf life specification, which indicates the loss of 10% of the content as the maximum acceptable (CARSTENSEN, RHODES, 2007). Evaluation of pH showed that

nanostructured formulations maintained their initial values, during the 30-day storage ($p>0.05$). Regarding mean particle size, both nanostructured formulations remained at the nanoscale range during the storage. For HG-DESNC$_{AO}$ it was not observed significant changes compared to the initial particle size and PDI values ($p>0.05$). For HG-DESNC$_{MCT}$ an increase in the mean particle size was evidenced ($p<0.05$); however, the dispersion of the system (PDI) remained unchanged ($p>0.05$). Viscosity of hydrogels was also evaluated. With respect to HG-DESNC$_{AO}$, this parameter did not suffer alteration ($p>0.05$) during the period of evaluation. HG-DESNC$_{MCT}$ presented an increase in viscosity, which may be probably related to the loss of water evidenced by a decrease in gel weight. In relation to the spreadability factors, this parameter remained unchanged ($p>0.05$) for HG-DESNC$_{AO}$, as for the HG-DESNC$_{MCT}$ a decrease in spreadability factor ($p<0.05$) was observed from de 7th day, which was maintained until the 30-day storage. These set of results suggest that HG-DESNC$_{AO}$ has superior stability profile than HG-DESNC$_{MCT}$.

3.3 *In vitro* release study

The DES release from HG containing NC or free compound was studied using vertical Franz diffusion cells. Many authors have performed the *in vitro* release with Franz cells as a tool to characterize semisolids formulations containing nanoparticles (BEBER et al., 2016; DE LIMA et al., 2017; FONTANA et al., 2011; MARCHIORI et al., 2017). The results indicated that the release profiles follow Higuchi´s model, since the cumulative amount of DES released was linear and directly proportional to the square root of time (FLYNN et al., 1999; HAUCK et al., 2007; SHAH et al., 1991). Drug release was determined by the slope of the curve obtained in the mathematical modeling. HG-DESNC$_{AO}$ presented higher release rate than the other prepared hydrogels ($p<0.05$), and thus, a higher DES amount reached the receptor medium when it was associated to this formulation. After 8h of experiment, the amount of DES released from HG-DESNC$_{AO}$ was around 48.7 $\mu g/cm^2\ h^{½}$, while for HG-DESNC$_{MCT}$ was 41.1 $\mu g/cm^2\ h^{½}$. With the conventional formulations, HG-DES and commercial formulation, the drug released after 8 h was 40.2 and 40.1 $\mu g/cm^2\ h^{½}$, respectively. Similar results were found by Marchiori and co-workers (2017), which described that a higher amount of silibinin reached the receptor phase when it was associated with nanocapsules.

3.4 *In vitro* skin permeation and penetration study

In order to determine the drug localization and to quantify the DES delivered to each skin layer after the applying of hydrogels, an *in vitro* study was performed using vertical Franz diffusion cells and porcine skin as biological membrane. DES was analyzed in the *stratum corneum*, according to the tape stripping technique, and in the subjacent layers, epidermis and dermis, using skin extraction techniques. The receptor compartment was analyzed to determine the amount of DES permeated. Fig. 3 shows the amount of DES retained in the different skin layers. All the preparations showed higher levels of DES retained in stratum corneum than in the other skin layers. In addition, DES was not detected in the receptor compartment. Comparing the developed hydrogels (HG-DESNC$_{AO}$, HG-DESNC$_{MCT}$ and HG-DES) with the commercial gel cream, the results indicated that higher amount of DES penetrated the stratum corneum from HG-DESNC$_{MCT}$ ($p<0.05$). In epidermis, occurred higher penetration of DES from the developed hydrogels in comparison to commercial gel cream ($p<0.05$). Regarding to dermis, both nanostructured hydrogels promoted a lower DES retention compared to the non-nanostructured formulations ($p<0.05$).

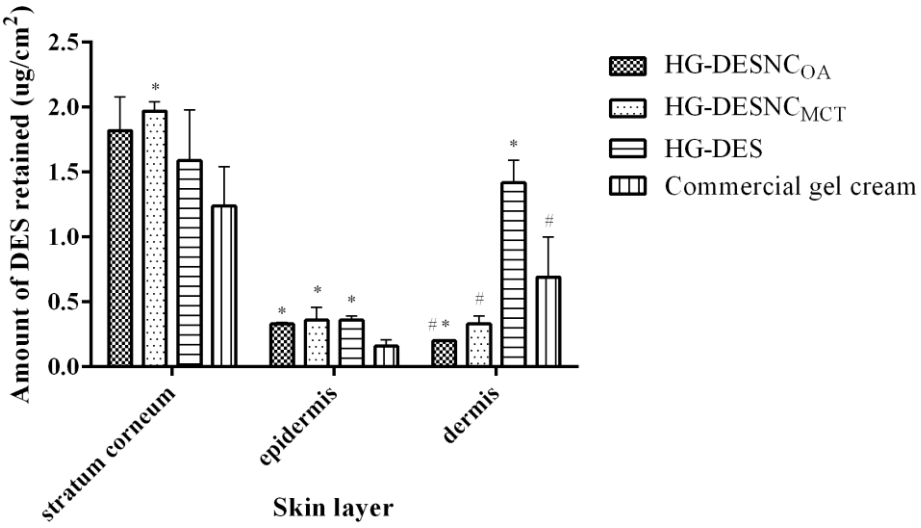

Fig. 3. Amount of DES retained in the *stratum corneum*, epidermis and dermis after 8 hours from hydrogels containing desonide-loaded nanocapsules (HG-DESNC$_{AO}$ and HG-DESNC$_{MCT}$), non-encapsulated drug (HG-DES) and commercial gel cream. The results are expressed as mean ± standard deviation (n=6). *shows significant difference compared to the commercial gel cream; # shows significant difference compared to the HG-DES (One-way ANOVA followed by Tukey's *post-hoc* test, $p<0.05$).

The human skin acts as a physical barrier, protecting against environment penetration of chemicals and microorganisms (JUNGERSTED et al., 2008; PAM et al., 2016). The

stratum corneum is the outermost layer and is mainly responsible for the maintenance of the skin barrier function. It is composed of anucleated dead epidermal cells, filled with keratin filaments and embedded in a lipid matrix (PAM et al., 2016). Due to its composition, it represents the principal limit to drug penetration (SALA et al., 2018). To achieve their therapeutic effect, TG need to overcome this barrier in order to reach their sites of action, the epidermis and dermis, where the fibroblasts and keratinocytes presenting glucocorticoid receptor are located (WIEDERSBERG; LEOPOLD; GUY, 2008).

The present *in vitro* skin penetration/permeation study showed that the developed formulations presented significantly higher amount of DES in the epidermis, site of action of TG, when compared to the commercial formulation. It also can be observed that the conventional formulations (HG-DES and commercial formulation) provided higher amounts of DES retained in the dermis, which may indicate higher levels of drug available for systemic absorption, since the dermis is vascularized. Considering the systemic and topical adverse effects of TG, over the years, research has focused on strategies to increase the safety of TG treatments, including special vehicles, new application schedules and new agents (ŞENYIĞIT et al., 2009). In this scenario, the polymeric nanocapsules has been cited as a suitable alternative for skin local drug delivery, since they may accumulate in hair follicules and allow the drug release to diffuse to the inner skin layers (BEBER et al., 2014, 2016). Considering that no DES was found in the receptor medium, and the low content of the drug retained in the dermis from the nanostructured formulations, it could be suggested that the polymeric nanocapsules decrease the risk of systemic absorption.

3.5 Bioadhesion measurements

In vitro adhesion of the formulations to the porcine skin was studied by means of bioadhesion experiments using a tensile stress tester. Nanostructured hydrogels (HG-DESNC$_{AO}$ and HG-DESNC$_{MCT}$), vehicle and the commercial gel cream were evaluated. For the tensile stress measurements, the force, displacement distance and work needed to detach the hydrogels from the biological membrane were determined. The comparative results of mucoadhesion of formulations, where the work values represent the bioadhesive properties of each formulation (CHAVES et al., 2018; FRANK et al., 2015, 2014; SANDRI et al., 2004), indicated that there was no statistical differences between the semisolid formulations ($p>0.05$), suggesting that the developed hydrogels and the commercial formulation were similar.

3.6 Antiedematogenic effect

The antiedematogenic activity of the nanostructured hydrogels in an acute contact dermatitis model induced by croton oil was assessed. Croton oil contains 12-O-tetradecanoylphorbol-13-acetate (TPA) and other phorbol esters as main irritant agents (SARAIVA et al., 2011). Its application triggers an inflammatory response characterized by edema, leading to increased vascular permeability, cell infiltration and proliferation, production of arachidonic acid metabolites, cytokines and other proinflammatory mediators (PATRICK; BURKHALTER; MAIBACH, 1997; RAO et al., 1993). An immediate effect of the TG is to induce vasoconstriction, which decreases the tissue edema, erythema and heat (BELTRANI; BARSANTI; BIELORY, 2005). Thus, our aim was to assess the antiedematogenic effect of the nanostructured hydrogels in a model of croton oil induced skin inflammation, since it mimics several human skin diseases, such as psoriasis and atopic dermatitis (PIETROVSKI et al., 2011; SATO et al., 2004).

A single topical application of croton oil on ear induced an increase of the ear thickness with an E_{max} of 126 ± 0.009 µm 6 h after the induction (Fig 4). The topical treatment with commercial gel cream, used as positive control, and with the developed semisolids effectively decreased the ear thickness compared to the croton oil group.

HG-DESNC$_{AO}$ and HG-DESNC$_{MCT}$ reduced the ear edema croton oil-induced, with an I_{max} of 74±8% and 41±4%, respectively. The commercial cream formulation containing desonide (GC-C) decreased the ear edema croton oil-induced in 63±4%.

Comparing both nanostructured hydrogels, HG-DESNC$_{AO}$ was more effective in reducing the ear thickness than HG-DESNC$_{MCT}$. Moreover, it can be observed a combined effect of DES and AO, since the hydrogel formulation containing desonide-loaded açai oil based nanocapsules significantly reduced the croton oil induced ear edema when compared to the HG-DESNC$_{MCT}$. These are interesting founds since the use of emollients have been cited as alternative to reduce the need and enhance the response of topical corticoids (ARCHER, 2017). Considering that the AO used to prepare the nanocapsule suspensions presented the oleic, palmitic and linoleic acids as the most predominant fatty acids, these fatty acids could contribute to the maintenance of the epithelial barrier.

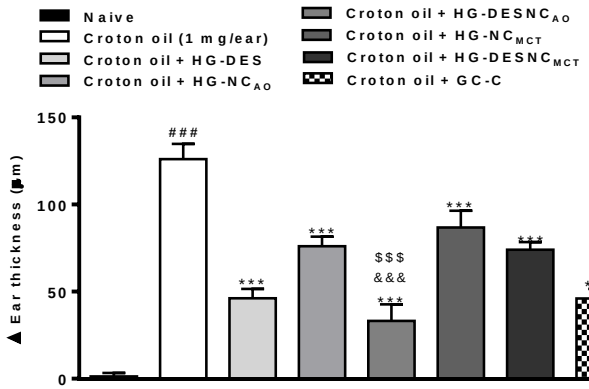

Fig. 4. Antiedematogenic effect of semisolid formulations on croton oil-induced skin inflammation in mice. Each bar represents the mean + SEM (n = 6–8); $^{###}p < 0.001$ shows significant difference when compared to the naïve group. $***p < 0.001$ shows significant difference when compared to the croton oil group. $^{\&\&\&}p < 0.001$ shows significant difference when compared with croton oil + HG-NC$_{AO}$ group; $^{\$\$\$}p < 0.001$ shows significant difference when compared to croton oil + HG-DESNC$_{MCT}$ group. One-way ANOVA followed by post hoc Newman-Keuls test.

To confirm the inflammatory cells infiltration, we carried out histological analysis on the ear tissue. We observed that the application of croton oil promoted an intense inflammatory cell infiltration (97 ± 6 polymorphonuclear cells per field) when compared to the naïve group (21 ± 3 polymorphonuclear cells per field). The topical treatment with semisolids containing DES-loaded nanocapsules (HG-DESNC$_{AO}$ and HG-DESNC$_{MCT}$) and the commercial gel cream decreased the cell infiltration compared to the croton oil group (Fig 5). Comparing the nanostructured hydrogels, the treatment with HG-DESNC$_{MCT}$ showed fewer polymorphonuclear cells per field (54 ± 8) than with HG-DESNC$_{AO}$ (74 ± 3 polymorphonuclear cells per field). As expected, the commercial cream also decreased the inflammatory cells infiltration (45±4 polymorphonuclear cells per field).

It is important to emphasize that the present work demonstrated the promising biological effect of the nanotechnology-based hydrogels, since their antiedematogenic effect was comparable to the commercially available formulation, which presents the double drug concentration. This is an interesting result and corroborate those obtained in the *in vitro* skin permeation/penetration study, which indicated higher amounts of DES retained in epidermis, site of action of the glucocorticoids, provided by the nanostructured hydrogels. Moreover, the DES retained in the dermis from the nanotechnology-based hydrogels was lower compared to the hydrogel containing the free-DES and the commercial formulation, which may indicate

lower amount of drug reaching bloodstream and, therefore, fewer side effects with an equivalent biological effect.

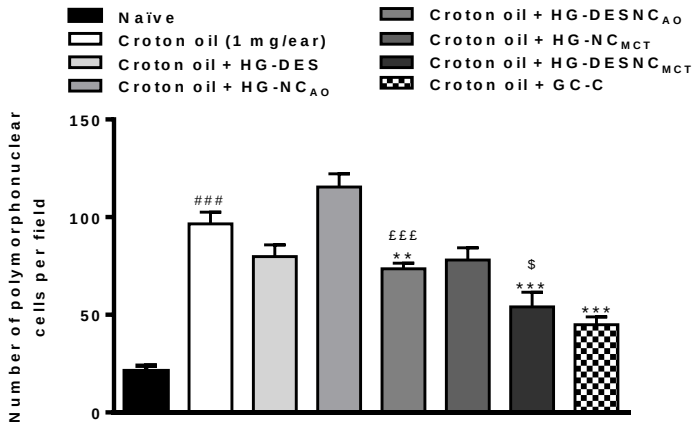

Fig. 5. Effect of the semisolid formulations topically applied to inflammatory cells infiltration croton oil-induced. Each bar represents the mean + SEM (n = 5–6); $^{###}p<0.001$ shows significant difference when compared to the naïve group. $^{**}p<0.01$ and $^{***}p<0.001$ show significant difference when compared to the croton oil group. $^{£££}p<0.001$ shows significant difference when compared to the HG-NC$_{AO}$ and $^{\$}p<0.05$ shows the significant difference when compared to croton oil + HG-DESNC$_{AO}$ group. One-way ANOVA followed by post hoc Newman-Keuls test.

4.Conclusion

In this study, we developed for the first time semisolid formulations based on desonide-loaded nanocapsules by the addition of a natural exopolyssacharide as polymer-gel forming. The hydrogels presented satisfactory physicochemical characteristics: pH compatible with topical application, adequate drug content and pseudoplastic behaviour. The investigation of the antiedematogenic effect in an animal model of croton oil induced skin inflammation demonstrated the promising biological effect of the semisolids formulations of desonide associated to the nanocarriers. Furthermore, the *in vivo* experiments showed a combined effect of desonide and açai oil, presenting advantages when compared to the

conventional formulation, allowing the use of a lower drug dosage to obtain similar biological effects.

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References

ARCHER, C. B. Atopic dermatitis. **Medicine (United Kingdom)**, [s. l.], v. 45, n. 6, p. 379–382, 2017. Disponível em: <http://dx.doi.org/10.1016/j.mpmed.2017.03.001>

AUBERT-WASTIAUX, H. et al. Topical corticosteroid phobia in atopic dermatitis: A study of its nature, origins and frequency. **British Journal of Dermatology**, [s. l.], v. 165, n. 4, p. 808–814, 2011.

BEBER, T. C. et al. Submicron polymeric particles prepared by vibrational spray-drying: Semisolid formulation and skin penetration/permeation studies. **European Journal of Pharmaceutics and Biopharmaceutics**, [s. l.], v. 88, n. 3, p. 602–613, 2014. Disponível em: <http://dx.doi.org/10.1016/j.ejpb.2014.07.008>

BEBER, T. C. et al. Cationic Polymeric Nanocapsules as a Strategy to Target Dexamethasone to Viable Epidermis: Skin Penetration and Permeation Studies. **Journal of Nanoscience and Nanotechnology**, [s. l.], v. 16, n. 2, p. 1331–1338, 2016. Disponível em: <http://www.ingentaconnect.com/content/10.1166/jnn.2016.11670>

BELTRANI, V. S.; BARSANTI, F. A.; BIELORY, L. Effects of glucocorticosteroids on the skin and eye. **Immunology and Allergy Clinics of North America**, [s. l.], v. 25, n. 3, p. 557–580, 2005.

BORGHETTI, G. S.; KNORST, M. T. Desenvolvimento e avaliação da estabilidade física de loções O/A contendo filtros solares. **Revista Brasileira de Ciencias Farmaceuticas/Brazilian Journal of Pharmaceutical Sciences**, [s. l.], v. 42, n. 4, p. 531–537, 2006. Disponível em: <http://www.scopus.com/inward/record.url?eid=2-s2.0-33947631476&partnerID=tZOtx3y1>

CHAVES, P. D. S. et al. Mucoadhesive Properties of Eudragit®RS100, Eudragit®S100, and Poly(ε-caprolactone) Nanocapsules: Influence of the Vehicle and the Mucosal Surface. **AAPS PharmSciTech**, [s. l.], 2018. Disponível em: <http://link.springer.com/10.1208/s12249-018-0968-5>

COVIELLO, T. et al. Influence of borate amount on the swelling and rheological properties of the Scleroglucan/borax system. **Journal of Applied Polymer Science**, [s. l.], v. 133, n. 3, p.

1–10, 2016.

DE ANDRADE, D. F. et al. Nanoencapsulation of Clobetasol Propionate Decreases Its Penetration to Skin Layers Without Changing Its Relative Skin Distribution. **Journal of nanoscience and nanotechnology**, [s. l.], v. 15, n. 1, p. 875–879, 2015. a. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/26328452>

DE ANDRADE, D. F. et al. Nanoencapsulation of Clobetasol Propionate Decreases Its Penetration to Skin Layers Without Changing Its Relative Skin Distribution. **Journal of Nanoscience and Nanotechnology**, [s. l.], 2015. b.

DE LIMA, J. A. et al. Novel Pemulen/Pullulan blended hydrogel containing clotrimazole-loaded cationic nanocapsules: Evaluation of mucoadhesion and vaginal permeation. **Materials Science and Engineering C**, [s. l.], v. 79, p. 886–893, 2017.

FIUME, M. M. et al. Safety Assessment of Microbial Polysaccharide Gums as Used in Cosmetics. **International Journal of Toxicology**, [s. l.], v. 35, n. Supplement 1, p. 5S–49S, 2016.

FLYNN, G. L. et al. **Assessment of value and applications of in vitro testing of topical dermatological drug products**, 1999.

FONTANA, M. C. et al. Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate. **European Journal of Pharmaceutics and Biopharmaceutics**, [s. l.], v. 79, n. 2, p. 241–249, 2011. a. Disponível em: <http://dx.doi.org/10.1016/j.ejpb.2011.05.002>

FONTANA, M. C. et al. Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate. **European Journal of Pharmaceutics and Biopharmaceutics**, [s. l.], v. 79, n. 2, p. 241–249, 2011. b. Disponível em: <http://dx.doi.org/10.1016/j.ejpb.2011.05.002>

FRANK, L. a. et al. Improving drug biological effects by encapsulation into polymeric nanocapsules. **Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology**, [s. l.], v. 7, n. 5, p. 623–639, 2015.

FRANK, L. A. et al. Chitosan gel containing polymeric nanocapsules: A new formulation for vaginal drug delivery. **International Journal of Nanomedicine**, [s. l.], v. 9, n. 1, p. 3151–3161, 2014.

GRAU, P. S. Corticoides tópicos. Actualización. **Medicina Cutanea Ibero-Latino-Americana**, [s. l.], v. 34, n. 1, p. 33–38, 2006.

GUTERRES, S. S.; ALVES, M. P.; POHLMANN, A. R. Polymeric nanoparticles, nanospheres and nanocapsules, for cutaneous applications. **Drug target insights**, [s. l.], v. 2, n. April, p. 147–157, 2007.

HAUCK, W. W. et al. Reliability and reproducibility of vertical diffusion cells for determining release rates from semisolid dosage forms. **Pharmaceutical Research**, [s. l.], v. 24, n. 11, p. 2018–2024, 2007.

HENGGE, U. R. et al. Adverse effects of topical glucocorticosteroids. **Journal of the American Academy of Dermatology**, [s. l.], v. 54, n. 1, p. 1–18, 2006.

LEE, C. H.; MOTURI, V.; LEE, Y. Thixotropic property in pharmaceutical formulations. **Journal of Controlled Release**, [s. l.], v. 136, n. 2, p. 88–98, 2009. Disponível em: <http://dx.doi.org/10.1016/j.jconrel.2009.02.013>

MARCHIORI, M. C. L. et al. Hydrogel containing silibinin-loaded pomegranate oil based nanocapsules exhibits anti-inflammatory effects on skin damage UVB radiation-induced in mice. **Journal of Photochemistry and Photobiology B: Biology**, [s. l.], v. 170, p. 25–32, 2017. Disponível em: <http://dx.doi.org/10.1016/j.jphotobiol.2017.03.015>

MARCHIORI, M. L. et al. Hydrogel containing dexamethasone-loaded nanocapsules for cutaneous administration: preparation, characterization, and in vitro drug release study. **Drug development and industrial pharmacy**, [s. l.], v. 36, n. 8, p. 962–971, 2010.

MARQUES, E. S. et al. Evaluation of the genotoxicity of Euterpe oleraceae Mart. (Arecaceae) fruit oil (açaí), in mammalian cells in vivo. **Food and Chemical Toxicology**, [s. l.], v. 93, p. 13–19, 2016. Disponível em: <http://dx.doi.org/10.1016/j.fct.2016.04.018>

MORA-HUERTAS, C. E.; FESSI, H.; ELAISSARI, A. Polymer-based nanocapsules for drug delivery. **International Journal of Pharmaceutics**, [s. l.], v. 385, n. 1–2, p. 113–142, 2010.

OURIQUE, A. F. et al. Improved photostability and reduced skin permeation of tretinoin: Development of a semisolid nanomedicine. **European Journal of Pharmaceutics and Biopharmaceutics**, [s. l.], v. 79, n. 1, p. 95–101, 2011. Disponível em: <http://dx.doi.org/10.1016/j.ejpb.2011.03.008>

PEGORARO, N. S. et al. Nanoencapsulation of coenzyme Q10 and vitamin E acetate protects against UVB radiation-induced skin injury in mice. **Colloids and Surfaces B: Biointerfaces**, [s. l.], v. 150, p. 32–40, 2017. Disponível em <http://dx.doi.org/10.1016/j.colsurfb.2016.11.013>

SALA, M. et al. Lipid nanocarriers as skin drug delivery systems: Properties, mechanisms of skin interactions and medical applications. **International Journal of Pharmaceutics**, [s. l.], v. 535, n. 1–2, p. 1–17, 2018. Disponível em: <https://doi.org/10.1016/j.ijpharm.2017.10.046>

SARAIVA, R. A. et al. Topical anti-inflammatory effect of Caryocar coriaceum Wittm. (Caryocaraceae) fruit pulp fixed oil on mice ear edema induced by different irritant agents. **Journal of Ethnopharmacology**, [s. l.], v. 136, n. 3, p. 504–510, 2011.

ŞENYIĞIT, T. et al. Different approaches for improving skin accumulation of topical corticosteroids. **International Journal of Pharmaceutics**, [s. l.], v. 380, n. 1–2, p. 155–160, 2009.

SHAH, V. P. et al. **In Vitro Release of Hydrocortisone from Topical Preparations and Automated Procedure**, 1991.

WEBER, J. et al. Association of Borage Oil and Betamethasone. [s. l.], v. 15, n. xx, p. 1–9, 2015.

WIEDERSBERG, S.; LEOPOLD, C. S.; GUY, R. H. Bioavailability and bioequivalence of topical glucocorticoids. **European Journal of Pharmaceutics and Biopharmaceutics**, [s. l.], v. 68, n. 3, p. 453–466, 2008.

DISCUSSÃO

6. DISCUSSÃO

Como principal barreira física ao ambiente externo, a pele exerce papel fundamental na defesa do organismo quando submetido a agressões, invasão de micro-organismos e outros agentes nocivos. Em alguns casos, a reação imunológica inadequada pode implicar na patogênese de doenças inflamatórias de pele, como a psoríase e a dermatite atópica (SARAIVA et al., 2011). Como terapia farmacológica geralmente são utilizados os CT. O tratamento com esses fármacos, no entanto, está associado ao desenvolvimento de reações indesejadas, como a atrofia da pele, telangectasias e estrias, no local de aplicação, e, também, efeitos decorrentes de sua absorção sistêmica (BELTRANI; BARSANTI; BIELORY, 2005; EROGLU et al., 2014; HENGGE et al., 2006). Contrariando o efeito sistêmico, decorrente da absorção dos CT, outro desafio relacionado ao tratamento com esses medicamentos é a baixa liberação do fármaco no sítio de ação desses fármacos (LIN et al., 2018).

Em relação à dermatite atópica, o tratamento visa a restauração da função da barreira cutânea e/ou reduzir a inflamação da pele. Dessa forma, as terapias tópicas desempenham papel fundamental no tratamento dos sintomas clínicos. Atualmente, o tratamento envolve o uso de anti-inflamatórios, como os CT, a fim de normalizar a diferenciação e reduzir a hiperproliferação epidérmica (LIN et al., 2018) e, de forma complementar, a aplicação de emolientes, visando a recuperação da barreira epitelial (LIN et al., 2018; NG; LIEW; ANG, 2015).

O uso de nanocarreadores para a administração dos CT tem sido citado como alternativa promissora para contornar as limitações da terapia com esses fármacos. Por possuírem tamanho nanométrico poderiam facilitar a penetração cutânea, promover maior retenção do fármaco no local de ação, prevenir sua degradação, além de evitar a absorção sistêmica (BEBER et al., 2014; DE ANDRADE et al., 2015; LIN et al., 2018;). Tendo em vista suas potencialidades farmacológicas, os óleos vegetais têm sido amplamente utilizados na composição do núcleo oleoso de nanocápsulas poliméricas, buscando vantagens como o sinergismo terapêutico, propriedades antioxidantes, dentre outras. (GEHRCKE et al., 2016; FERREIRA et al., 2016; MARCHIORI et al., 2017; SANTOS et al., 2014). Visando a obtenção de sistemas mais estáveis, adequados à aplicação cutânea, capazes de vetorizar o fármaco para seu local de ação, buscando minimizar efeitos adversos e melhorar seu efeito farmacológico, este trabalho propôs o desenvolvimento de nanocápsulas poliméricas contendo desonida e óleo de açaí como núcleo oleoso. Ainda, desenvolver formulações semissólidas a

partir das suspensões coloidais, com características adequadas para a aplicação pretendida e avaliar sua atividade biológica.

Considerando-se a via de administração do fármaco, a possibilidade de efeito conjunto proporcionado pelo núcleo oleoso e o ineditismo da formulação, testes iniciais foram realizados empregando a poli-ε-caprolactona (PCL) (MM=70.000 – 90.000) como polímero e o óleo de açaí como núcleo oleoso. Formulações contendo o óleo em diferentes concentrações (3,3%, 2,5% e 2,0%) foram preparadas e como tensoativos de baixo EHL foram testados o monoestearato e o monooleato de sorbitano. As formulações preparadas com o tensoativo monoestearato de sorbitano (EHL= 4,7) apresentaram precipitação durante o processo de evaporação do solvente. Assim, optou-se por utilizar o monooleato de sorbitano (EHL= 4,3) para a preparação das suspensões. Variações nas quantidades de tensoativo, de acetona e de óleo foram realizadas, a fim de preparar formulações com tamanho exclusivamente nanométrico e estáveis. No entanto, independente das quantidades empregadas, as suspensões apresentavam instabilidade físico-química, evidenciada por precipitação, nas primeiras 24 h após a preparação, inviabilizando a sua utilização.

A partir desses resultados, optou-se por preparar nanocápsulas contendo desonida utilizando-se outro polímero. O Eudragit RL® 100 é um co-polímero do polietilacrilato, metilmetacrilato e cloro-trimetil-amonioetil-metacrilato, o qual, por apresentar carga positiva, poderia favorecer a adesão das nanopartículas na pele, carregada negativamente. Assim, os testes preliminares foram realizados empregando o polímero Eudragit® RL100 e TCM como núcleo oleoso, na concentração de 3,3% (NC_{TCM}).

Macroscopicamente, as NC_{TCM} apresentaram-se com aspecto homogêneo, opalescentes, com reflexo azulado (Efeito Tyndal), relacionado ao movimento Browniano das partículas, característico dos sistemas coloidais. Entretanto, a análise das formulações, por difração a laser, demonstrou a presença de partículas micrométricas, com valores de SPAN próximos a 2,0 e diâmetro médio em torno de 400 nm. Considerando que o tamanho das partículas e a homogeneidade do sistema são influenciados pelas concentrações das matérias-primas na fase orgânica (JORNADA et al., 2012), as quantidades de dois componentes da fase orgânica (óleo e acetona) foram variadas em relação à formulação inicial. Para avaliar o efeito das variações, determinaram-se os valores de SPAN e diâmetro médio das suspensões obtidas.

Inicialmente alterou-se a quantidade de TCM para 2,5%. Com a diminuição do óleo, houve um decréscimo no tamanho das partículas (D4,3 em torno de 262 nm) e maior homogeneidade do sistema foi observada (SPAN em torno de 1,0). Esse resultado sugere relação diretamente proporcional entre a quantidade de óleo e o tamanho de partícula.

Quantidades maiores de óleo levariam ao aumento da viscosidade da fase orgânica, dificultando a difusão do solvente e propiciando a formação de partículas maiores (FERREIRA et al., 2016). Com a obtenção de resultados satisfatórios, não foram realizadas novas modificações sendo, então, a formulação contendo TCM a 2,5% selecionada para caracterização e ensaios subsequentes.

Na preparação das nanocápsulas poliméricas contendo OA, testes iniciais foram realizados utilizando o óleo na concentração de 2,5%. As suspensões coloidais apresentaram aspecto homogêneo, leitoso, levemente amarelado e efeito Tyndal. A análise por difração a laser indicou a distribuição unimodal das partículas, na escala manométrica, com valores de SPAN em torno de 1,3 e tamanho médio de partícula (D4,3) em torno de 312 nm. Buscando a obtenção de sistemas mais homogêneos, manteve-se a concentração de OA e dobrou-se a quantidade de acetona na fase orgânica. A análise granulométrica indicou a obtenção de partículas menores (284 ± 3 nm) e um sistema mais homogêneo (SPAN <2.0). Estudo realizado por Contri e colaboradores (2016) relatou a obtenção de gotículas menores de óleo na fase orgânica quando o óleo era reduzido e a acetona aumentada. De acordo com os autores, as gotículas menores, na fase orgânica, levariam à formação de partículas de diâmetro menor, aumentando a homogeneidade do sistema (CONTRI et al., 2016). Dessa forma, para a preparação das suspensões coloidais contendo OA como núcleo oleoso, utilizou-se o dobro da quantidade de acetona empregada na preparação das suspensões de nanocápsulas contendo TCM.

Tendo em vista as atividades biológicas relatadas para o óleo de açaí, buscou-se avaliar o perfil de ácidos graxos do óleo utilizado na preparação das formulações. O perfil obtido foi semelhante ao descrito na literatura, sendo os componentes majoritários o ácido oleico, palmítico e linoleico. Os ácidos graxos têm sido considerados fundamentais nas respostas inflamatórias, uma vez que são fonte de vários mediadores lipídicos (AMAGAI et al., 2015; HUBLER; KENNEDY, 2016). Assim, a associação do óleo de açaí à desonida nas formulações propostas mostrava-se promissora. As suspensões de nanocápsulas contendo desonida e OA ou TCM apresentaram características físico-químicas adequadas e eficiência de encapsulamento em torno de 82%. A avaliação da estabilidade dessas formulações, em temperatura ambiente, demonstrou que, em geral, os sistemas mantiveram suas características iniciais.

Em relação à fotoestabilidade, os corticoides são considerados fármacos com potencial fotorreativo (ALBINI; FASANI, 1998; CAFFIERI et al., 2008). A fotodegradação da hidrocortisona, em solução e em formulação disponível comercialmente, foi relatada após

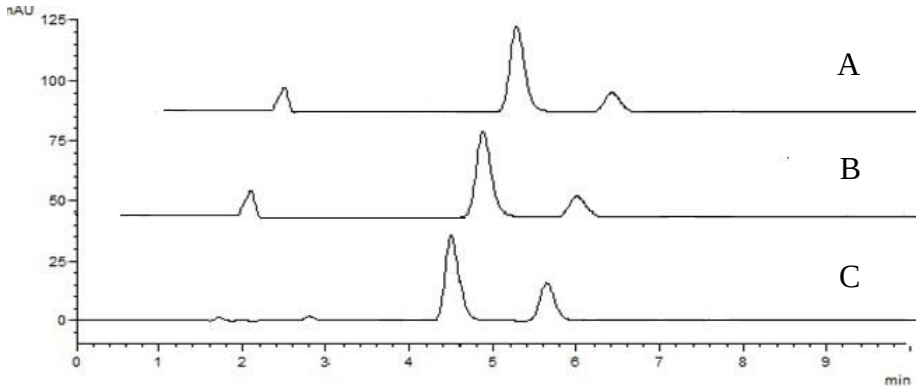
exposição à radiação UVB (CAFFIERI, 2008). Já a fluocinolona acetonida e a flumetasona mostraram fotoinstabilidade frente às radiações UVA e UVB, ambas em meio aquoso e em solventes orgânicos (MIOLO et al., 2011). A avaliação da fotoestabilidade da desonida nas formas farmacêuticas loção capilar, loção cremosa e gel creme demonstrou significativa diminuição no teor do fármaco, nas diferentes preparações após exposição direta à radiação UVA (BRAGA et al., 2013; BRAGA, 2013), enquanto trabalhos recentes demonstraram a adição do filtro UV benzofenona-3 em baixas concentrações como estabilizante nas formulações solução capilar e gel creme (ROSA et al., 2014, 2015).

Considerando que a nanoencapsulação tem se mostrado uma ferramenta para o aumento da fotoestabilidade dos fármacos, buscou-se avaliar a fotoestabilidade das suspensões coloidais contendo desonida preparadas neste trabalho. Verificou-se que as formulações contendo o fármaco nanoencapsulado apresentaram teor de fármaco remanescente superior ao observado para a desonida solubilizada em metanol. Observou-se, ainda, que para o mesmo período de exposição direta à radiação UVA (36 h) as suspensões que continham desonida e OA apresentaram teor residual superior àquelas que continham o fármaco e TCM ($p < 0.05$). A fim de elucidar o efeito do polímero e a influência do óleo que compõe o núcleo oleoso, foram preparadas nanoesferas e nanoemulsões, as quais foram expostas às mesmas condições de irradiação. Foi observado que as nanoemulsões contendo OA são mais fotoestáveis. Esse resultado não foi influenciado pela eficiência de encapsulamento (EE), visto que a EE de ambas foi de 70%. Com relação às nanoesferas, que não contêm óleo na composição, a EE foi de 77%. Dessa forma, evidenciou-se que a fotoestabilidade das formulações contendo AO foi superior ($p<0,05$) às que continham TCM e às nanoesferas. Esse conjunto de resultados sugere que o principal fator envolvido na fotoproteção da desonida associada às nanocápsulas foi o tipo de óleo utilizado.

A fotodegradação da desonida também foi avaliada frente à radiação UVC (254 nm). Embora a maior parte dessa radiação seja filtrada pela camada de ozônio, recentemente, a avaliação dos efeitos químicos e biológicos da radiação UVC tem recebido cada vez mais atenção, tanto para um melhor entendimento da fotoquímica envolvida nessas reações de degradação, quanto para o conhecimento dos danos específicos que essa pode provocar (MOORE, 2004). Considerando que a UVC é a radiação ultravioleta mais energética e, dessa forma, as reações de fotodegradação ocorrem mais rapidamente, o emprego dessa fonte de radiação torna-se interessante, uma vez que expõe a amostra a uma condição mais drástica de degradação. Nessa condição, a proteção do fármaco, conferida pela nanoencapsulação torna-se mais evidente, visto que após 15 minutos o teor residual de desonida solubilizada em

metanol foi de 37,9%, enquanto que, no mesmo tempo, as DES-NC_{OA} e DES-NC_{TCM} apresentaram teor residual de 83,55% e 76,48%, respectivamente. A degradação do fármaco, tanto em solução quanto nas nanocápsulas, resultou na formação de um pico adicional com tempo de retenção próximo de seis minutos (Figura 4).

Figura 4- Cromatogramas obtidos na fotólise da desonida nas DES-NC_{OA} (A), DES-NC_{TCM} (B) após 90 min de irradiação e solubilizada em metanol (C) após 15 min de exposição à radiação UVC.



Fonte: autor

Com relação à segurança das suspensões coloidais, buscou-se avaliar o grau de irritação das formulações utilizando o teste HET-CAM, o qual é aceito em muitos países para a avaliação de irritantes fortes e moderados e vem sendo utilizado em substituição ao teste de Draize (CONTRI et al., 2016; ICCVAM, 2006). Verificou-se que as formulações provocaram alterações semelhantes na membrana corioalantoide, indicando que os componentes das nanoestruturas não alteraram o grau de irritação do fármaco. Ainda nesse sentido, considerando a fotoinstabilidade relatada para os corticoides, investigou-se a fototoxicidade da desonida associada e não associada às nanocápsulas e das nanoestruturas sem o fármaco, por meio de ensaio *in vitro* utilizando fibroblastos (3T3) e queratinócitos (HaCaT) como linhagens celulares e MTT e NRU como ensaios para a determinação da viabilidade celular. Tendo em vista que as reações fototóxicas podem ser causadas após a exposição da pele a fármacos fotorreativos, sendo desencadeada pelas radiações UVA e UVB (ONOUE et al., 2010), a investigação da fototoxicidade da desonida torna-se relevante. Os resultados observados indicaram que, nas condições utilizadas, o fármaco não encapsulado e as

formulações nanoestruturadas não apresentaram fototoxicidade. Esse conjunto de dados foi apresentado no **Capítulo 1**.

Tendo em vista a administração tópica, buscou-se desenvolver formulação semissólida adequada à administração cutânea, cujos resultados foram descritos no **Capítulo 2.** Os escleroglucanos, polissacarídeos de origem natural, possuem propriedades físico-químicas vantajosas, incluindo a estabilidade em ampla faixa de pH. Além disso, alguns produtos contendo polissacarídeos naturais são indicados para o uso em crianças, aplicação em mucosas e regiões sensíveis, como os olhos (FIUME et al. 2016,), tornando o polímero escolhido ainda mais atrativo para o desenvolvimento das formulações nanoestruturadas.

Neste trabalho, foram preparados hidrogéis pela incorporação de Amigel® (*sclerotium gum)* às suspensões de nanocápsulas e, para fins comparativos, formulações contendo o fármaco não encapsulado. Essas preparações apresentaram características físico-químicas adequadas para a aplicação pretendida, sendo mantidas as nanoestruturas. O estudo do comportamento reológico demonstrou que os hidrogéis possuem fluxo do tipo pseudoplástico, interessante para a aplicação tópica, uma vez que quando aplicada força externa, o sistema se torna menos viscoso, facilitando a administração. Ainda relacionado à aplicação das formulações no local de ação, a avaliação da espalhabilidade mostrou resultados que corroboraram os obtidos na determinação da viscosidade aparente dos hidrogéis, indicando que as preparações nanoestruturadas necessitam da aplicação de menor força para espalhar-se em área maior.

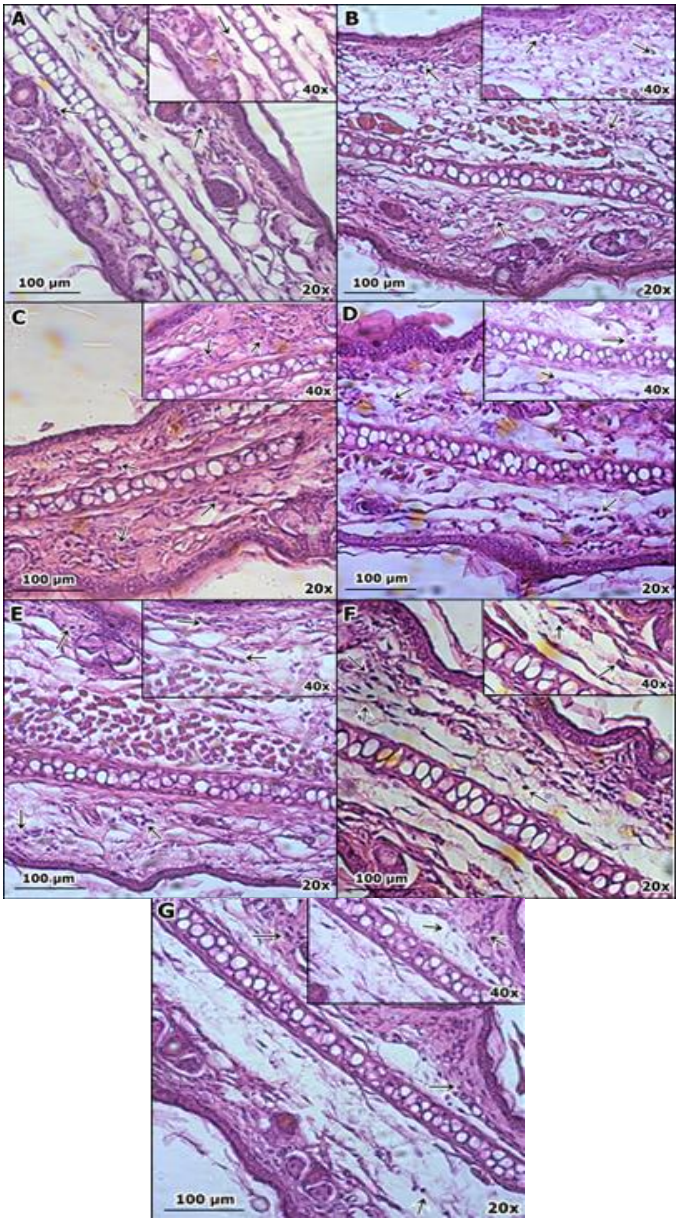
Considerando a terapia tópica, os corticoides possuem como sítio de ação os queratinócitos e fibroblastos, presentes na epiderme e derme. Dessa forma, é necessário que os fármacos atinjam essas camadas para que possam ligar-se aos seus receptores (WIEDERSBERG; LEOPOLD; GUY, 2007). A avaliação do perfil de distribuição da desonida nas camadas cutâneas a partir das formulações de base nanotecnológica, realizada por meio de ensaio *in vitro*, demonstrou a habilidade do fármaco em atingir o local de ação dos corticoides. Com relação às camadas epiteliais, verificou-se menor quantidade de fármaco retida na derme a partir dos hidrogéis nanoestruturados, em comparação com as formulações convencionais. Extrapolando essa observação para o ambiente biológico e considerando a vascularização da derme, esse resultado poderia indicar menor quantidade do corticoide disponível para absorção e possível efeito sistêmico.

A fim de demonstrar o efeito biológico dos hidrogéis de base nanotecnológica, utilizou-se modelo *in vivo* de inflamação cutânea aguda induzida por óleo de cróton, sendo avaliado o edema provocado na orelha de camundongos após a aplicação dos tratamentos.

Essa avaliação demonstrou a atividade antiedematogênica dessas formulações, as quais provocaram a redução do edema induzido pelo agente irritante. Por meio do estudo *in vivo* também foi possível observar o efeito conjunto promovido pelo OA e a desonida nanoencapsulados, uma vez que, em associação promoveram maior redução no edema em comparação com as demais formulações estudadas. A análise histológica indicou que os hidrogéis nanoestruturados reduziram a infiltração de células inflamatórias (Figura 5). Vale ressaltar que a formulação semissólida disponível comercialmente possui o dobro da concentração de desonida em comparação aos hidrogéis preparados a partir das suspensões de nanocápsulas desenvolvidas neste trabalho.

O conjunto de resultados obtidos neste trabalho evidenciou o desenvolvimento de hidrogéis de base nanotecnológica adequados à aplicação tópica. Os estudos de fotodegradação demonstraram a associação da desonida às nanocápsulas poliméricas como alternativa eficaz para o aumento da fotoestabilidade do fármaco, e que esse resultado é influenciado pelo tipo de óleo utilizado para compor o núcleo oleoso das nanocápsulas. Por sua vez, os semissólidos preparados apresentaram efeito antiedematogênico em modelo *in vivo* de dermatite atópica, podendo ser considerados uma alternativa para o tratamento de doenças cutâneas.

Figura 5- Efeito das formulações semissólidas aplicadas topicamente na infiltração de células inflamatórias induzida pelo óleo de cróton.



Análise histológica (A-G; hematoxilina-eosina 200x and 400x) e número de polimorfonucleares por campo (H) do tecido das orelhas dos camundongos após 6 h da aplicação do óleo de cróton ou óleo de cróton e tratamentos. A: naïve; B: óleo de cróton; C: óleo de cróton + HG-NC_{OA}; D: óleo de cróton + HG-$DESNC_{OA}$; E: óleo de cróton + HG-NC_{TCM}; F: óleo de cróton + HG-$DESNC_{TCM}$; G: óleo de cróton + gel creme comercial. As setas indicam a presença de células inflamatórias.
Fonte: autor

CONCLUSÕES

7. CONCLUSÕES

- Utilizando o método de deposição interfacial do polímero pré-formado foi possível preparar nanocápsulas poliméricas com o polímero Eudragit® RL 100, contendo desonida e triglicerídeos de cadeia média ou óleo de açaí como núcleo oleoso;
- As suspensões de nanocápsulas contendo desonida apresentaram características físico-químicas adequadas (tamanho nanométrico, baixo índice de polidispersão, potencial zeta positivo) e compatíveis com outros sistemas nanoparticulados relatados na literatura;
- A nanoencapsulação da desonida proporcionou o aumento da fotoestabilidade do fármaco frente às radiações UVA e UVC;
- As suspensões de nanocápsulas contendo desonida e óleo de açaí apresentaram fotoestabilidade superior àquelas contendo TCM;
- O estudo de liberação *in vitro* do fármaco a partir das nanopartículas demonstrou o controle da liberação da desonida, segundo modelo biexponencial e transporte anômalo;
- A avaliação da fototoxicidade *in vitro*, em fibroblastos murinos e queratinócitos humanos, utilizando os ensaios de viabilidade celular MTT e NRU demonstrou que as suspensões de nanocápsulas e o fármaco livre não possuem potencial fototóxico, nas condições estudadas;
- Os hidrogéis de base nanotecnólogica, preparados a partir da dispersão do polímero Amigel® nas suspensões de nanocápsulas apresentaram pH compatível com a aplicação tópica, teor de fármaco adequado e comportamento de fluxo pseudoplástico;
- A avaliação da estabilidade das formulações, em temperatura ambiente, demonstrou que os hidrogéis preparados a partir das suspensões de nanocápsulas contendo desonida e óleo de açaí apresentaram estabilidade superior às demais formulações preparadas;
- O estudo da permeação/penetração cutânea *in vitro* indicou que as formulações nanoestruturadas proporcionaram maior quantidade de desonida na epiderme, local de ação dos corticoides tópicos;
- A avaliação da atividade anti-edematogênica em modelo *in vivo* evidenciou a atividade biológica dos hidrogéis nanoestruturados e o efeito combinado da desonida e do óleo de açaí;
- As formulações semissólidas nanoestruturadas desenvolvidas neste trabalho apresentaram características físico-químicas satisfatórias para a aplicação tópica;

REFERÊNCIAS

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ALBINI, A; FASANI, E. **Drugs: Photochemistry and Photostability**. Cambridge: The Royal Society of Chemistry, 1998.

ALBINI, A; FASANI, E. Rationalizing the protochemistry of drugs. In: TØNESSEN, H.H. **Photostability of drugs and drug formulations**. 2nd ed. Boca Raton: CRC Press LLC, 2004.

AMAGAI, Y.et al. Dihomo-g-linolenic acid prevents the development of atopic dermatitis through prostaglandin D1 production in NC/Tnd mice. **Journal of Dermatological Science**, v.79, p.30–37, 2015.

ANTON, N., BENOIT, J-P.; SAULNIER, P. Design and production of nanoparticles formulated from nano-emulsion templates—A review. **Journal of Controlled Release**, v. 128, p.185–199, 2008.

ANTONOW, B.M. et al. Development and Physicochemical Characterization of Desonide-Loaded Nanocapsule Suspensions. **Advances in Materials Science and Engineering**, p. 1-12, 2016.

AUBERT-WASTIAUX, H. et al. Topical corticosteroid phobia in atopic dermatitis: a study of its nature, origins and frequency. **British Journal of Dermatology**,v.165,p.808-14, 2011.

BAERTSCHI, S.W. et al. Implications of In-Use Photostability: Proposed Guidance for Photostability Testing and Labeling to Support the Administration of Photosensitive Pharmaceutical Products, Part 2: Topical Drug Product. **Journal of Pharmaceutical Sciences**, v.104, p. 2688-2701, 2015.

BEBER, T.C. et al. Cationic Polymeric Nanocapsules as a Strategy to Target Dexamethasone to Viable Epidermis: Skin Penetration and Permeation Studies. **Journal of Nanoscience and Nanotechnology**, v. 16, p. 1331-8, 2016.

BELTRANI, V.S.; BARSANTI, F.A.; BIELORY, L. Effects of glucocorticoids on the skin and eye. **Immunology and allergy clinic of North America**, v. 25, p. 557-80, 2005.

BHATTACHARJEE, S. DLS and zeta potential – What they are and what they are not? **Journal of Controlled Release**, v. 235, p. 337–351, 2016.

BRAGA, M.P et al. Assay and Photodegradation Kinetics of Desonide Lotion by an LC-UV Stability-Indicating Method. **Journal of Liquid Chromatography & Related Technologies**, 2013

BRAGA, M.P. Desonida: validação de métodos analíticos e estudo de fotoestabilidade de formulações, 2013. 121 f. **Dissertação (Mestrado em Ciências Farmacêuticas)** - Universidade Federal de Santa Maria, Santa Maria, 2013.

CAFFIERI, S. et al. UVB photolysis of hydrocortisone 21-acetate. **Journal of Pharmaceutical and Biomedical Analysis**, v.47, p. 771-77, 2008.

CHASSOT, J.M. Desenvolvimento de nanocápsulas poliméricas para liberação pulmonar do dipropionato de beclometasona. **Dissertação (Mestrado em Ciências Farmacêuticas)** - Universidade Federal de Santa Maria, Santa Maria, 2013.

CONTRI, R.V. et al. Vegetable oils as core of cationic polymeric nanocapsules: influence on the physicochemical properties. **Journal of Experimental Nanoscience**, v. 12, p.1-12, 2012.

CONTRI, R.V.; et al. Nanoencapsulation of Rose-Hip Oil Prevents Oil Oxidation and Allows Obtainment of Gel and Film Topical Formulations. **AAPS PharmSciTech**, v. 17, n 4, p. 863-71, 2016.

CORADINE, K. et al. Co-encapsulation of resveratrol and curcumin in lipid-core nanocapsules improves their in vitro antioxidant effects. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 88, p. 178–185, 2014.

CORADINI, K. et al. Co-encapsulation of resveratrol and curcumin in lipid-core nanocapsules improves their in vitro antioxidant effects. European Journal of

SANTA, F. et al. Development and validation of a simple stability-indicating LC-method and UVA photostability study of desonide hair lotion. **Current Analytical Chemistry**, v.9, p. 659-667, 2013.

DAS S., SURESH, P.K., DESMUKH, R. Design of Eudragit RL 100 nanoparticles by nanoprecipitation method for ocular drug delivery. **Nanomedicine**, v. 6, n. 2. p. 318-23, 2010.

DE ANDRADE D.F., et al. Nanoencapsulation of Clobetasol Propionate Decreases Its Penetration to Skin Layers Without Changing Its Relative Skin Distribution. **Journal of Nanoscience and Nanotechnology**, v. 15, p. 875-9, 2015.

EROGLU, I. et al. Effective topical delivery systems for corticosteroids: dermatological and histological evaluations. **Drug delivery**, v. 23, n. 5, p. 1502-13, 2016.

FAVACHO, A.S.H. et al. Anti-infl ammatory and antinociceptive activities of Euterpe oleracea oil. **Revista Brasileira de Farmacognosia**, v.21,n. 1,p.105-114, 2011.

FDA. United States Food and Drug Administration. 2010. OTC Active Ingredients. **FDA. United States Food and Drug Administration**. Disponível em < http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm> Acesso em: 04/08/18.

FEINGOLD, R. K. The Adverse Effect of IFN Gamma on Stratum Corneum Structure and Function in Psoriasis and Atopic Dermatitis. **Journal of Investigative Dermatology**, v. 134, p.597-600, 2014.

FERREIRA, L.M. Pomegranate seed oil nanoemulsions improve the photostability andin vivo antinociceptive effect of a non-steroidal anti-inflammatorydrug. **Colloids and Surfaces B: Biointerfaces**, v, 144, p.214–221, 2016.

FIUME, M. M. et al. Safety Assessment of Microbial Polysaccharide Gums as Used in Cosmetics. **International Journal of Toxicology**, v. 35, n. 1_suppl, p. 5S–49S, 2016.

FONTANA, M. C. et al. Nanoencapsulation as a way to control the release and to increase the photostability of clobetasol propionate: influence of the nanostructured system. **Journal of Biomedical Nanotechnology**, v. 5, p. 254–263, 2009.

FONTANA, M.C. et al. Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 79, p. 241-9, 2011.

FRANK, L. A. et al. Improving drug biological effects by encapsulation into polymeric nanocapsules. **Nanomedicine and Nanobiotechnology**, v. 7, p. 623-39, 2015.

FRANK, L.A. et al. Chitosan gel containing polymeric nanocapsules: a new formulation for vaginal drug delivery. **International Journal of Nanomedicine**, v.9, p. 3151-61, 2014.

GEHRCKE, M. et al. Enhanced photostability, radical scavenging and antitumor activity of indole-3-carbinol-loaded rose hip oil nanocapsules. **Materials Science and Engineering C**, v. 74, p. 279-86, 2017.

GELBARD, C. M., HEBERT, A. A. Desonide hydrogel: advances in vehicle technology. **Expert Review of Dermathology**., v. 4, n. 1, p. 23-27, 2009.

GRAU, P. S. Corticoides tópicos. Actualización. **Medicina Cutánea Ibero Latino Americana**, v. 34, n. 1, p. 33-38, 2006.

GUICHARD, A. et al. Effects of topical corticosteroids on cell proliferation, cell cycle progression and apoptosis: In vitro comparison on HaCaT. **International Journal of Pharmaceutics**, v. 479, p. 422-9, 2015.

GUPTA, D. Atopic dermatitis A Common Pediatric Condition and Its Evolution in Adulthood. **Medical Clinics of North America**, v. 99, p.1269–1285, 2015.

GUTERRES, S.S.; ALVES, M.P.; POHLMANN, A.R. Polymeric nanoparticles, nanospheres and nanocapsules, for cutaneous applications. **Drug Target Insights**, v 2, p 147-157, 2007.

HARTER, A. Desenvolvimento de nanoemulsões e nanocápsulas poliméricas contendo tioconazol e incorporação em hidrogéis. 2013. **Dissertação (Mestrado em Ciências Farmacêuticas)** – Universidade Federal de Santa Maria, Santa Maria, 2013.

HENGGE, U. R., et al. Adverse effects of topical glucocorticosteroids. **Journal of American Academy Dermatology**, v. 54, n. 1, p. 1-15, 2006.

HORN, E.J. et al. Topical corticosteroids In psoriasis: strategies for improving safety. **European Academy of Dermatology and Venereology**, v. 24, p. 119-24, 2010.

HUBLER, M. J.; KENNEDY, A. J. Role of lipids in the metabolism and activation of immune cells. **Journal of Nutritional Biochemistry**, [s. l.], v. 34, p. 1–7, 2016. Disponível em: <http://dx.doi.org/10.1016/j.jnutbio.2015.11.002>

HUGHES, J.; RUSTIN, M. Corticosteroids. **Clinics in Dermatology**, v. 15, p. 715-721, 1997.

ICCVAM-**Recommended Test Method Protocol: Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) Test Method**, 2006.

ICH. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. **ICH Harmonised Tripartite Guideline Photosafety Evaluation of Pharmaceuticals**, S10, 2013.

IQBAL, J; HUSAIN, A.; GUPTA, A. Photochemistry of desonide, a non-fluorinated steroidal anti-inflamatory drug. **Chemical & Pharmaceutical Bulletin**, v. 54, n. 6, p. 836-838, 2006.

JAGER, E. et al. Sustained Release from Lipid-Core Nanocapsules by Varying the Core Viscosity and the Particle Surface Area. **Journal of Biomedical Nanotechnology**, v. 5, n. 1, p.130-40, 2009.

JORNADA, D. S. Lipid-core nanocapsules: mechanism of self-assembly, control of size and loading capacity. **Soft Matter**, v. 8, p. 6646-55, 2012.

KATARA, R.; MAJUMDAR, K.D. Eudragit RL 100-based nanoparticulate system of aceclofenac for ocular delivery. **Colloids and Surfaces B: Biointerfaces**, v. 103, p.455–462, 2013.

KENDALL, A. C.; NICOLAOU, A. Bioactive lipid mediators in skin inflammation and immunity. **Progress in Lipid Research**, v. 52, p. 141–164, 2013.

KENDALL, C.A. et al. Distribution of Bioactive Lipid Mediators in Human Skin. **Journal of Investigative Dermatology**, v. 135, p. 1510–20, 2015.

KENDALL, C.A.; NICOLAOU, A. Bioactive lipid mediators in skin inflammation and immunity. **Progress in Lipid Research**, V. 52, P. 141-64, 2013.

LACHMAN, L.; DeLUCA,P.; AKERS, A. J. Testes de estabilidade e fundamentos de cinética química. In: LACHMAN, L.; LIEBERMAN, H. A.; KANIG, J. L. **Teoria e prática na indústria farmacêutica**, v.2. Lisboa: Fundação Calouste Gulbenkian, 2001.

LANDIS, T.E. et al. Complementary and Alternative Medicine Use in Dermatology in the United States. **The Journal of Alternative and complementary**, v. 20, n. 5,p.392-8, 2014.

LIN, Y. K. The Use of Therapeutic Nanoparticulate Systems for Treating Atopic Dermatitis. **Current Nanoscience**, v. 14, p. 3-16, 2018.

MARCHIORI, M. C. L. et al. Hydrogel containing silibinin-loaded pomegranate oil based nanocapsules exhibits anti-inflammatory effects on skin damage UVB radiation-induced in mice. **Journal of Photochemistry and Photobiology B: Biology**, v. 170, p. 25–32, 2017.

MARQUES, S.E. et al. Evaluation of the genotoxicity of Euterpe oleraceae Mart. (Arecaceae) fruit oil (açaí), in mammalian cells in vivo**. Food and Chemical Toxicology**, v. 93, p.13-19, 2016.

MATHES, C. et al. Nanocarriers for optimizing the balance between interfollicular permeation and follicular uptake of topically applied clobetasol to minimize adverse effects. **Journal of Controlled Release** v. 223, p. 207-14, 2016.

MAZZARINO, L. et al. Curcumin-loaded polymeric and lipid nanocapsules: preparation, characterization and chemical stability evaluation. **Latin American Journal of Pharmacy**,v. 29, n. 6, p. 933-40, 2010.

MELERO, A. et al. Nanoencapsulation in lipid-core nanocapsules controls mometasone furoate skin permeability rate and its penetration to the deeper skin layers. **Skin Pharmacology and Physiology**, v. 27, p. 217-28, 2014.

MONGE-FUENTES, V. et al. Photodynamic therapymediated by acai oil (Euterpe oleracea Martius) in nanoemulsion: A potential treatment for melanoma. **Journal of Photochemistry & Photobiology, B: Biology**, v. 166, p. 301–310, 2017.
.
MOORE, D. E. Photophysical and photochimical aspects of drug stability. In: TØNESSEN, H.H. **Photostability of drugs and drug formulations**. 2nd. ed. Boca Raton:CRC Press LLC, 2004.

MORA-HUERTAS, C. E.; FESSI, H.; ELAISSARI, A. Polymer-based nanocapsules for drug delivery. **International Journal of Pharmaceutics**, v. 385, p. 113-142, 2010.

NG, J.P.X.; LIEW, H.M.; ANG, S. B. Use of emollients in atopic dermatitis. **Journal of European Academy of Dermatology and Venereology**, v. 29, p. 854-57, 2015.

NICHOLS, W. K. Hormônios e antagonistas hormonais. In: GENNARO, A. R. **A ciência e a prática da farmácia**. 20. ed. Rio de Janeiro: Guanabara Koogan, 2004. cap. 77, p. 1421.

OECD. Organization for Economic Cooperation and Development (OECD) (2004) **OECD Guideline for Testing of Chemicals, 432, In Vitro 3T3 NRU Phototoxicity Test**, OECD, Paris.

ONOUE, S. et al. High-Throughput Screening System for Identifying Phototoxic Potential of Drug Candidates Based on Derivatives of Reactive Oxygen Metabolites. **Pharmaceutical Research**, v. 27, p.1610–1619, 2010.

ONOUE, S.; TSUDA, Y. Analytical Studies on the Prediction of Photosensitive/Phototoxic Potential of Pharmaceutical Substances. **Pharmaceutical Research**, v. 23, n.1, p. 156-164, 2006.

OURIQUE, A.F. et al. Tretinoin-loaded nanocapsules: Preparation, physicochemical characterization, and photostability study. **International Journal of Pharmaceutics**, v.352,p.1-4, 2008.

PACHECO-PALENCIA, A. L.; DUNCAN, E.C.; TALCOTT, T.S. Phytochemical composition and thermal stability of two commercial açai species, Euterpe oleracea and Euterpe precatoria. **Food Chemistry**,v. 115, 1199–1205, 2009.

PACHECO-PALENCIA, A. L.et al. Absorption and Biological Activity of Phytochemical-Rich Extracts from Açai (Euterpe oleracea Mart.) Pulp and Oil in Vitro. **Journal of Agricultural and Food Chemistry**, v. 56, n. 10, p. 3593-3600, 2008.

PARISER, D. Topical corticosteroids and topical calcineurin inhibitors in the treatment of atopic dermatitis: focus on percutaneous absorption. **American Journal of Therapeutics**, v. 16, p. 264-273, 2009.

RICCI, et al. General patterns in the Photochemistry of Pregna-1,4-dien-3,20-diones. **The Journal of Organic Chemistry**, v. 68, p. 4361-4366, 2003.

RONG, X. et al. Applications of Polymeric Nanocapsules in Field of Drug Delivery Systems. **Current Drug Discovery Technologies**, v. 8, p. 173-87, 2011.

ROOS, T. C. et al. Recents advances in treatment strategies for atopic dermatitis. **Drugs**, v. 64, n 23, p. 2639-2666, 2004.

ROSA, P. et al. In vitro and in vivo evaluation of a desonide gel-cream photostabilized with benzophenone-3. **Drug Development and Industrial Pharmacy**, v.42,n.1,p.19-27, 2015.

ROSA, P. et al. Investigation of the stabilizing effects of antioxidants and benzophenone-3 on desonide photostability. **Journal of The American Association of Pharmaceutical Scientists** v. 15, n.5. p. 1155-62, 2014.

SANTOS, S.S. et al. Clotrimazole-loaded Eudragit® RS100 nanocapsules: Preparation, characterization and in vitro evaluation of antifungal activity against Candida species. **Materials Science and Engineering C**, v. 33,p. 1389-94, 2013.

SANTOS, S.S.S. Formulation and in vitro evaluation of coconut oil-core cationicnanocapsules intended for vaginal delivery of clotrimazole. **Colloids and Surfaces B:Biointerfaces,** v. 116, p. 270-76, 2014.

SARAIVA, R.A.et al. 2011. Topical anti-inflammatory effect of Caryocarcoriaceum Wittm. (Caryocaraceae) fruit pulp fixed oil on mice ear edema induced by different irritant agents. **Journal of Ethnopharmacology**, v. 136, p. 504-10, 2011.

SAVIAN, A.L., et al. Dithranol-loaded lipid-core nanocapsules improve the photostability and reduce the in vitro irritation potential of this drug. **Materials Science and Engeneering C**, v. 46, p. 69-76, 2015.

SCHAFFAZICK, R. S.; GUTERRES, S.S.; FREITAS, L.L.; POHLMANN, A.R. Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração e fármacos. **Química Nova**, v 26, n 5, p 726-737, 2003.

SCI FINDER WEB - CAS **Chemical Abstracts Service**. Disponível em: < https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf>. Acesso em: jun 2018.

SHAO, M. et al. Drug nanocarrier, the future of atopic diseases: Advanced drug delivery systems and smart management of disease. **Colloids and Surfaces B: Biointerfaces**, v.147, p.475–491, 2016.

SINGH, R.; LILLARD, W.J. Nanoparticle-based targeted drug delivery. **Experimental and Molecular Pathology**,v. 86, p.215–223, 2009.

SINGH, G.; PAI, R.S. Atazanavir-loaded Eudragit RL 100 nanoparticles to improve oral bioavailability: optimization and *in vitro/in vivo* appraisal. **Drug Delivery**, v. 23, n. 2, p. 532-539, 2016.

TØNNESEN, H. H. Photoreactivity of drugs. In: **Solar Radiation and Human Health**. Oslo: The Norwegian Academy of Science and Letters, 2008.

TØNNESEN, H. H. Photostability testing of drugs and drug formulations-Why and How? In: TONESSEN, H. H. **Photostability of drugs and drug formulations**. 2.nd ed. Boca Raton: CRC Press LLC, 2004.

VALÉRIO, A.; ARAÚJO, H.H. P; SAYER, C. Preparation of Poly(Urethane-urea) Nanoparticles Containing Açaí Oil by Miniemulsion Polymerization. **Polímeros**, v. 23, n. 4, p. 451-455, 2013.

VALÉRIO, A. et al. Degradable polyurethane nanoparticles containing vegetable oils. **European Journal of Lipid Science and Technology**, v. 116, p. 24-30, 2014.

VAUTHIER, C.; BOUCHEMAL, K. Methods for the preparation and manufacture of polymeric nanoparticles. **Pharmaceutical Research**, v 26, n 5, 2009.

VENTURINI, C. G. et al. Formulation of lipid-core nanocapsules. **Colloids and Surfaces A: Physicochem. Eng. Aspects**, v. 375, p. 200–208, 2011.

VERMA, P. et al. Development, in vitro and in vivo characterization of Eudragit RL 100 nanoparticles for improved ocular bioavailability of acetazolamide. **Drug Delivery**, v. 20, n.7, p. 269-276, 2013.

WEBER, J. et al. Association of Borage Oil and Betamethasone Dipropionate in Lipid-Core Nanocapsules: Characterization, Photostability and In Vitro Irritation Test. **Journal of Nanoscience and Nanotechnology**, v. 16, n.2, p.1354-62, 2015.

WEIDINGER, S. NOVAK, N. Atopic dermatitis. **The Lancet**, v. 387, n.10023, p. 1109-22, 2016.

WIEDERSBERG, S., LEOPOLD, C.S., GUY, R.H. Bioavailability and bioequivalence of topical glucocorticoids. **European Journal of Pharmaceutics and Biopharmaceutics**, v. 68, p. 453-466, 2008.

WYATT, E. L.; SUTTER, S. H.; DRAKE, L. A. Farmacologia dermatológica. In: GOODMAN, L. S. **Goodman & Gilman: As bases farmacológicas da terapêutica**. Seção XIV: Dermatologia. 10. ed. Rio de Janeiro: McGraw-Hill, 2004. cap. 65, p. 1351-1352.

YOUNG, A.R.; CLAVEAU, J.; ROSSI, A.N. Ultraviolet radiation and the skin: Photobiology and sunscreen photoprotection. **Journal of American Academy of Dermatology**, 2016.