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UNIVERSIDADE D
COIMBRA

Melissa da Silva Gama

Relatório de Estágio e Monografia intitulada "Nanocarriers-based dermopharmaceutical formulations for atopic dermatitis" referentes à Unidade Curricular "Estágio", sob a orientação da Dra. Ana Maria Duarte Coelho da Cunha Martins Rico e do Professor Doutor Francisco José de Baptista Veiga, apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas

Setembro de 2020

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Coimbra, 28 de setembro de 2020.

Melissa da Silva Gama

(Melissa da Silva Gama)

"Sabes que o desenho do adeus
É fogo que nos queima devagar
E no lento cerrar dos olhos teus
Fica esperança de um dia aqui voltar

E levas em ti guardado
O choro de uma balada
Recordações de um passado
O bater da velha cabra

Capa negra de saudade
No momento da partida
Segredos desta cidade
Levo comigo pra vida"

Balada da Despedida 5º Ano Jurídico Coimbra – Fados de Coimbra

Agradecimentos

Finalizando o meu percurso académico, quero agradecer a todas as pessoas que contribuíram para que o mesmo se pudesse concretizar.

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Aos meus pais, pelo amor, por todo o apoio ao longo destes cinco anos e toda a confiança que sempre depositaram em mim. Um obrigado não chega, pois sem eles nada seria possível.

Ao meu irmão, por toda a amizade e companheirismo. Por saber que posso sempre contar com ele, para o que der e vier.

Ao meu avô, a minha estrela mais brilhante, que olha por mim todos os dias.

A toda a minha restante família, por fazerem parte da minha vida, em especial à minha tia Alexandra, pelos conselhos e por estar sempre presente em todos os momentos.

A todos os meus amigos, amigas e colegas de faculdade, por todos os momentos de amizade e alegria, por me fazerem tão feliz.

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A Coimbra, por me fazer sentir "em casa" desde o primeiro minuto, por todas as memórias, pelos melhores cinco anos da minha vida. Para sempre no meu coração.

Um enorme OBRIGADO a todos!

Índice

PARTE I

RELATÓRIO DE ESTÁGIO EM FARMÁCIA COMUNITÁRIA

Farmácia Central de Coimbra

Sob orientação da Dra. Ana Maria Duarte Coelho da Cunha Martins Rico

Abreviaturas

FC: Farmácia Comunitária

FFUC: Faculdade de Farmácia da Universidade de Coimbra

MICF: Mestrado Integrado em Ciências Farmacêuticas

MNSRM: Medicamentos não sujeitos a receita médica

PA: Princípio Ativo

PIM: Preparação Individualizada da Medicação

PUV: Produtos de Uso Veterinário

SWOT: *Strengths, Weaknesses, Opportunities, Threat*

1. Introdução

No âmbito da unidade curricular "Estágio Curricular" do 2° semestre do 5° ano do Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC), e com vista à obtenção do grau académico de Mestre em Ciências Farmacêuticas, realizei estágio na área de Farmácia Comunitária (FC). Este estágio é de caráter obrigatório e decorreu na Farmácia Central de Coimbra, do dia 6 de janeiro ao dia 7 de agosto de 2020, correspondendo a um total de 817,5 horas. Foi realizado sob a orientação da Dra. Ana Maria Rico, a diretora técnica da farmácia.

O estágio em FC permitiu-me consolidar e aplicar os conhecimentos teóricos adquiridos ao longo dos cinco anos do MICF, na prática profissional. Dentro do setor farmacêutico, a área da FC é a que possui maior visibilidade, não só por concentrar a maioria dos farmacêuticos a exercer, mas também pela ampla cobertura das farmácias a nível nacional e consequente proximidade à comunidade. Muitas vezes, a farmácia é o primeiro local ao qual os utentes se dirigem para esclarecer possíveis dúvidas com o seu estado de saúde ou com a terapêutica que estão a fazer, sendo também, o último contacto que têm imediatamente antes de começarem a terapêutica prescrita, podendo tirar dúvidas relativamente à posologia, condições de administração ou efeitos adversos da mesma. Desta forma, atualmente, o papel do farmacêutico comunitário já não se resume apenas à dispensa do medicamento, sendo considerado um profissional de saúde altamente qualificado, um especialista do medicamento e agente da saúde publica, fundamental tanto na cedência de medicamentos e prestação de serviços farmacêuticos como no aconselhamento ao doente e na promoção da literacia em saúde [1].

A Farmácia Central encontra-se em funcionamento desde 1830, sendo uma das farmácias mais antigas da cidade de Coimbra. A equipa é constituída por 4 colaboradores, dos quais 3 farmacêuticos e 1 técnica de farmácia, tratando-se de uma equipa dinâmica e coordenada. A manutenção do stock de medicamentos é efetuada por três fornecedores diários, havendo, pontualmente, compras diretamente ao laboratório. É possível encontrar uma variedade de serviços para além da cedência de medicamentos, tais como dispensa de dispositivos médicos, suplementos alimentares, produtos veterinários, produtos de dermocosmética, artigos de puericultura, produtos de saúde e bem-estar, serviços farmacêuticos como medição de parâmetros bioquímicos e preparação individualizada da medicação (PIM) e serviços de saúde, nomeadamente, consultas de nutrição e de podologia. No presente relatório irei apresentar uma análise SWOT (*Strengths, Weaknesses, Opportunities,*

Threats) que tem como finalidade avaliar os pontos fortes, os pontos fracos, as oportunidades e ameaças deste estágio.

2. Análise SWOT

A análise SWOT é uma ferramenta muito utilizada na avaliação de determinadas atividades, avaliando uma vertente mais interna através dos Pontos Fortes (*Strenghts*) e dos Pontos Fracos (*Weaknesses*) e uma vertente mais externa, através das Oportunidades (*Opportunities*) e das Ameaças (*Threats*) [2].

Aplicando no estágio que realizei na Farmácia Central, farei uma análise SWOT onde irei enumerar e, posteriormente, desenvolver, os pontos fortes, os pontos fracos, as oportunidades e as ameaças mais relevantes que surgiram no decorrer do mesmo.

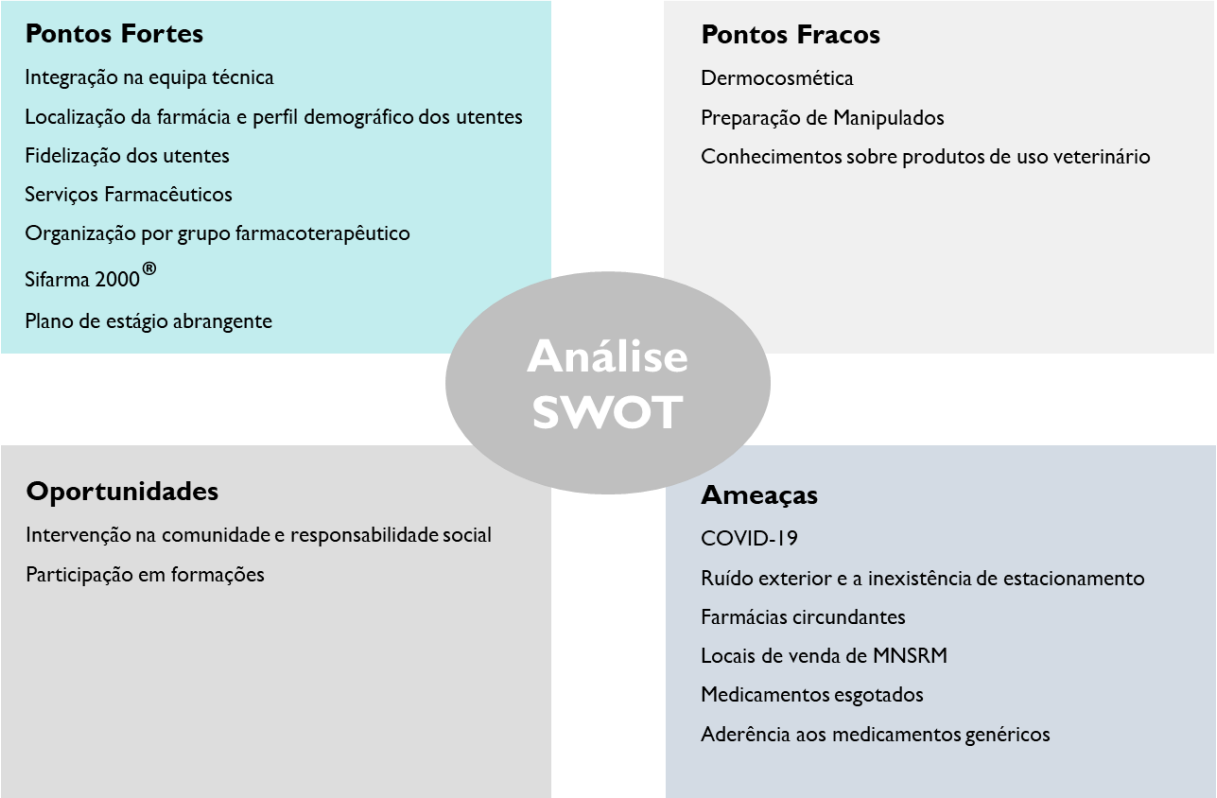

Figura.1. Análise SWOT do estágio realizado na Farmácia Central.

2.1. Pontos Fortes

2.1.1. Integração na equipa técnica

A Farmácia Central apresenta uma equipa competente, profissional, bem coordenada, focada no cliente e disposta a servir a comunidade. Durante todo o estágio tive oportunidade de observar e acompanhar de perto o dia a dia dessa equipa, sendo muito dinâmica e diversificada, com idades e experiências muito diferentes, o que foi vantajoso para o meu percurso enquanto estagiária, pois partilharam comigo imensos conhecimentos teóricos e também muitas experiências e saberes, o que me proporcionou uma vasta e enriquecedora aprendizagem. A simpatia e disponibilidade desta equipa em ensinar-me os procedimentos realizados na farmácia, auxiliar-me sempre que necessário e alertar-me para eventuais erros, foram cruciais no decorrer do estágio. A facilidade com que me integraram na equipa, o apoio e a confiança que depositaram em mim desde o início, refletiu-se muito no meu desempenho e permitiu-me adquirir uma enorme autonomia em todas as tarefas executadas na farmácia.

2.1.2. Localização da farmácia e perfil demográfico dos utentes

A Farmácia Central localiza-se na baixa de Coimbra, uma zona histórica da cidade. É uma zona com elevada densidade populacional, sendo frequentada por centenas de turistas durante todo o ano. Nas suas proximidades existe uma enorme diversidade de serviços, tais como instituições públicas e privadas de saúde, espaços comerciais e instituições bancárias, o que proporciona ao aumento da afluência de utentes. Também as paragens de autocarros e a praça de táxis em redor permitem que as pessoas que vivem noutras zonas da cidade se dirijam com alguma facilidade à farmácia. Desta forma, a população que frequenta a farmácia é bastante diversificada, o que me permitiu contactar com necessidades muito distintas e aprender a adaptar a minha linguagem a cada situação e pessoa. A faixa etária mais assídua corresponde à população mais idosa, que necessita de uma atenção especial, no sentido de serem pessoas mais frágeis e vulneráveis, que muitas vezes vivem sozinhas e procuram apoio e carinho nos farmacêuticos que confiam. É também muito comum encontrar utentes mais carenciados, com baixos níveis de literacia e toxiodependentes, sendo que estas são pessoas, por vezes, mais complicadas de lidar, de forma que é extremamente importante saber estar e comunicar com as mesmas, mantendo sempre uma postura profissional. Por outro lado, o facto desta farmácia ser frequentemente procurada por turistas, permitiu-me atender pessoas de outras nacionalidades e pôr em prática as minhas capacidades linguísticas, principalmente da língua inglesa. Estes têm necessidades bastante distintas dos utentes habituais, procurando, maioritariamente, o tratamento de situações agudas, através da dispensa de medicamentos

não sujeitos a receita médica (MNSRM) ou medicamentos não sujeitos a receita médica de dispensa exclusiva em farmácia e aconselhamento farmacêutico.

2.1.3. Fidelização dos utentes

Um utente satisfeito com os serviços prestados na farmácia é o ponto-chave para uma futura fidelização. Existe uma grande cooperação entre os colaboradores da farmácia, o que proporciona uma excelente prestação de serviços aos utentes e, consequentemente, leva à fidelização dos mesmos. Durante o estágio, percebi que existem muitos utentes fidelizados à farmácia, muitos deles desde há algumas décadas. Alguns chegam mesmo a procurar membros específicos da equipa, o que evidencia a sua satisfação e confiança no serviço prestado e a empatia criada com os mesmos. Em utentes fidelizados numa farmácia, torna-se mais fácil um atendimento personalizado e com elevada eficiência de resposta às suas necessidades, pois além de já os conhecermos, através da sua "ficha de cliente", conseguimos rapidamente auxiliar os mesmos relativamente à sua medicação e acompanhá-los ao longo da sua terapêutica. Estes utentes são geralmente idosos, portadores de múltiplas doenças crónicas e polimedicados, o que faz com que necessitem de um maior acompanhamento. Mais uma vez, o farmacêutico tem aqui um papel fundamental, na medida em que tem a capacidade de detetar muitos erros de medicação e interações medicamentosas que podem prejudicar os doentes e promover a adesão à terapêutica, melhorando a qualidade de vida dos mesmos. Neste sentido, para mim foi um ponto forte deste estágio, poder contactar com este tipo de utentes, chamados os "clientes habituais", dos quais alguns eu já tratava pelo nome e criei uma enorme empatia.

2.1.4. Serviços Farmacêuticos

A Farmácia Central, tal como a maioria das farmácias, dispõe de vários serviços farmacêuticos. Ao longo deste estágio tive oportunidade de realizar a medição de parâmetros bioquímicos tais como pressão arterial, glicémia, colesterol total e triglicerídeos. Os resultados destas medições eram registados num cartão que é entregue ao utente, o que permite sua monitorização e consequentemente cria oportunidade para realçar a importância da adesão à terapêutica e adoção de um estilo de vida saudável. Quando necessário, isto é, quando apresentados resultados muito diferentes dos normais, o farmacêutico tem o dever de reencaminhar o utente para o médico, de forma a lhe ser prestados todos os serviços de saúde que necessita. Logo nas primeiras semanas de estágio tive o caso de uma senhora idosa, cliente habitual da farmácia, que apresentava valores de pressão arterial elevadíssimos, sendo que a nossa atitude foi de aconselhar a ida ao médico e esperar que um membro da família a fosse buscar para a acompanhar ao hospital. Reconhecendo todo o cuidado que tivemos com a senhora, no dia seguinte foram à farmácia agradecer e informar-nos que estava tudo bem e

que teria sido apenas uma alteração consequente de algum stress emocional. Toda a equipa mostrou muita preocupação e disponibilidade para ajudar. É muito gratificante ver como pequenos gestos podem fazer a diferença na vida das pessoas. No decorrer do estágio pude também observar o farmacêutico na realização da PIM, apesar de não ser algo muito requisitado na farmácia. Além disso, tive a oportunidade de realizar PIM para um lar de idosos, o que me proporcionou muita autonomia a realizar este serviço (Figura 2). Poder observar e realizar estes serviços permitiu-me não só colocar em prática conhecimentos teóricos e adquirir novas competências, mas também compreender a importância dos mesmos para a população e o papel do farmacêutico na promoção da saúde e prevenção da doença. Nesta farmácia são realizadas consultas de nutrição com uma nutricionista, começando com um rastreio nutricional, gratuito, no qual os utentes fazem uma pequena avaliação nutricional. Posteriormente, se os utentes ficarem interessados em iniciar o plano terão, semanalmente, sessões com a nutricionista, de modo a efetuarem os vários passos que o plano inclui. Existem, também, consultas de podologia mensais realizadas por um podologista, nas quais os utentes podem receber diversos tratamentos.

Figura.2. Preparação Individualizada da Medicação.

2.1.5. Organização por grupo farmacoterapêutico

Ao contrário da maioria das farmácias, na Farmácia Central, a organização dos medicamentos não é feita por ordem alfabética, mas sim por grupo farmacoterapêutico (Figura 3). Pessoalmente, achei um ponto muito forte e muito vantajoso para a minha aprendizagem, na medida em que foi mais fácil associar cada medicamento à sua função. Nos primeiros dias do estágio estive mais voltada para a receção das encomendas e a sua arrumação, o que me ajudou a percecionar melhor onde se arrumava cada medicamento. No início foi mais complicado, tanto pelo facto de desconhecer certos princípios ativos (PAs), como alguns

nomes comerciais, mas com o decorrer do estágio acabou por ser muito benéfico, na medida em que ao fim de algum tempo, já conseguia fácil e rapidamente detetar para que era o medicamento só pelo PA ou nome comercial. Inicialmente, o atendimento ao público também foi um pouco mais difícil, pois ainda não tinha tanta destreza e, por vezes, demorava um pouco mais tempo a encontrar os medicamentos solicitados. Todos os colaboradores da farmácia foram fundamentais e ajudaram-me sempre a ultrapassar essa dificuldade. Além disso, colocaram ao nosso dispor um Prontuário Terapêutico, uma ferramenta essencial que no processo de pesquisa, de modo a facilitar a procura do medicamento em causa, assim como o Sifarma 2000®, que em caso de dúvida, nos dava a informação relativa à gaveta onde estaria. Já em situações de aconselhamento terapêutico, ajudou bastante, na medida em que me encaminhava diretamente para as gavetas que poderiam ter medicamentos que solucionassem o problema descrito.

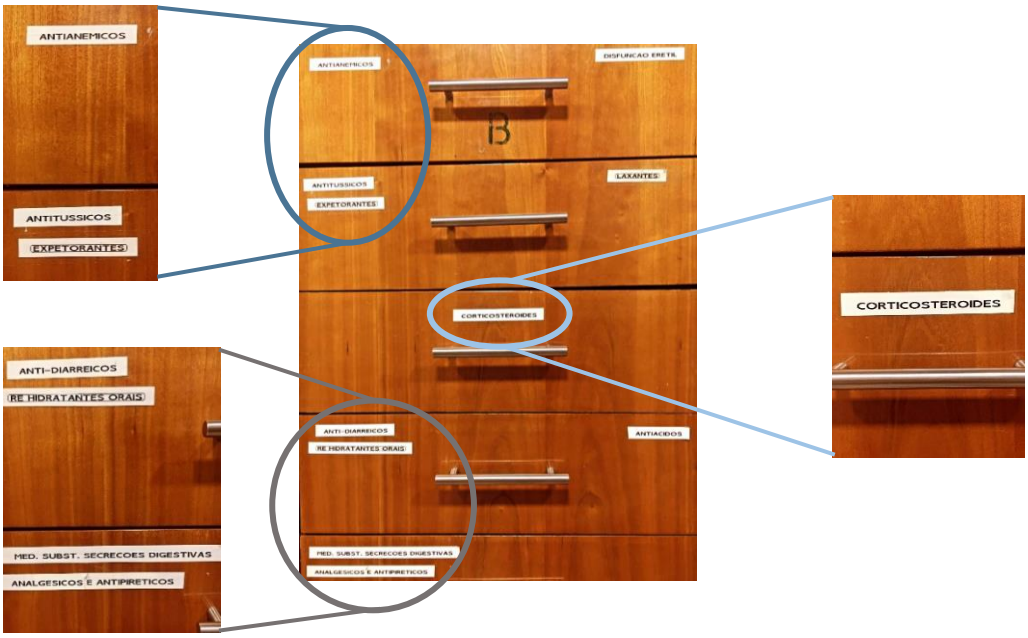

Figura.3. Organização dos medicamentos por grupo farmacoterapêutico.

2.1.6. Sifarma2000®

O *Sifarma2000®* é o *software* mais usado nas farmácias portuguesas. Foi desenvolvido "por e para farmacêuticos", tendo todas as ferramentas necessárias para auxiliar quer nos processos de gestão, quer no atendimento da farmácia comunitária. Isto permite ao farmacêutico estar mais focado no que é fundamental, o utente [3]. Na farmácia é usado este sistema informático, o que é um aspeto muito positivo para o meu estágio, na medida em que usá-lo diariamente me permitiu familiarizar com o mesmo e dominar as suas funcionalidades.

2.1.7. Plano de estágio abrangente

O estágio permitiu-me, de uma forma geral, aprender tudo o que se faz na farmácia. Inicialmente fiquei mais voltada para a receção de encomendas e armazenamento dos produtos farmacêuticos, onde foi realçada a importância da verificação de prazos de validade, margens e cálculo de preços. Isto permitiu o primeiro contacto com o programa informático Sifarma 2000® e com as embalagens dos medicamentos e a organização das mesmas na farmácia. É essencial saber como funciona a gestão da farmácia, de modo a que os medicamentos estejam disponíveis para satisfazer as necessidades dos utentes. Numa fase seguinte, comecei a acompanhar os atendimentos realizados pelos elementos da equipa e após algum tempo comecei o atendimento ao público, sob supervisão de um farmacêutico até possuir capacidade para realizar o atendimento e dispensa de medicamentos de forma autónoma. Além disto, pude realizar mais tarefas, como reposição de *stock*, controlo dos prazos de validade, reorganização da farmácia, prestação de serviços farmacêuticos, entre outros. Quanto à reorganização da farmácia, esta é muito importante a nível de produtos sazonais ou produtos integrados numa campanha especial, os quais têm de ter especial destaque. No controlo de prazos de validade é necessário retirar os produtos que terminem a validade no mês seguinte e marcar os produtos da farmácia cuja validade irá terminar até seis meses, de modo a que, caso necessário, se possam devolver. Tive também a oportunidade de observar o processo de faturação, que consiste na conferência e envio do receituário às entidades responsáveis no fim de cada mês, avaliando se as receitas médicas foram aviadas e os medicamentos dispensados sem erros e com segurança para o utente. É uma tarefa de extrema importância para a farmácia, pois basta um erro para poder prejudicar a farmácia no reembolso das comparticipações feitas pelo estado. O facto de ter tido a oportunidade de acompanhar esta atividade, permitiu-me ter um maior contacto com os diferentes organismos de comparticipação existentes e com os detalhes de cada receita médica.

2.2. Pontos Fracos

2.2.1. Dermocosmética

A Dermocosmética é uma área farmacêutica em crescente desenvolvimento, na medida em que, atualmente, cada vez mais há a preocupação das pessoas por uma imagem mais cuidada. Sendo uma farmácia mais pequena e com uma população-alvo mais restrita, que pouco procura produtos dermocosméticos, na Farmácia Central a aposta em Dermocosmética é muito limitada. Possui alguns produtos de diferentes marcas, mas apenas

a *Bioderma* tem a gama completa, uma vez que não se justifica maior diversidade. No entanto, nota-se o cuidado de que as existentes na farmácia são as mais requisitadas e de grande qualidade, satisfazendo as necessidades dos utentes. Há uma grande falha de várias marcas, nomeadamente as mais caras, visto que é crucial para a boa gestão da farmácia, adequar os preços dos produtos ao público-alvo. Apesar de ser evidente a razão desta falha, como estagiária é um dos pontos fracos deste estágio que posso referir, na medida em que para mim é uma área de grande interesse e em que me tenciono especializar futuramente, pelo que gostava de ter tido oportunidade de contactar com os vários tipos de gamas existentes e ter alguma experiência acrescida no que toca ao aconselhamento de produtos dermocosméticos.

2.2.2. Preparação de Manipulados

De acordo com o Decreto-Lei n.° 95/2004, de 22 de abril, um medicamento manipulado corresponde a "qualquer fórmula magistral ou preparado oficinal preparado e dispensado sob a responsabilidade de um farmacêutico" [4].

Nos dias de hoje, devido à grande evolução da indústria farmacêutica existe uma enorme variedade de formulações no mercado, o que se traduz num decréscimo na necessidade de preparação de medicamentos manipulados, pelo que é um serviço que deixou de existir em diversas farmácias. Dado as reduzidas dimensões e o facto de não reunir as condições exigidas, na farmácia central não se faz preparação de medicamentos manipulados. Na minha opinião é um ponto fraco, pois os medicamentos manipulados têm um papel importante em diversas patologias, em situações de ajuste de dose, principalmente em pediatria ou para obtenção de preparações que ainda não existam no mercado. Além disso, não me permitiu ter experiência nessa área nem colocar em prática os conhecimentos adquiridos na cadeira "Farmácia Galénica", o que seria importante, na medida em que poderei, muito provavelmente, ter de realizar a preparação de manipulados no meu futuro profissional.

2.2.3. Conhecimentos sobre produtos de uso veterinário (PUV)

Atualmente, quase todas as pessoas têm animais de estimação e é notória a sua preocupação e cuidado com os mesmos. Deste modo, a área da veterinária tem vindo a ganhar uma grande importância, sendo que está em crescente desenvolvimento. Neste sentido, torna-se fulcral que o farmacêutico tenha um grande domínio acerca de produtos de uso veterinário. Durante o estágio, uma das minhas grandes dificuldades foi a dispensa ou aconselhamento de PUV, propor soluções para as solicitações dos utentes e esclarecê-los quanto à forma correta de utilização destes produtos. É verdade que o plano curricular do MICF é muito completo e permite aos estudantes adquirir as competências necessárias em variadas áreas, contudo, no

que toca à área de PUV, considero os meus conhecimentos muito escassos. Apesar de no quinto ano existir a unidade curricular "Preparações de Uso Veterinário", o seu programa foca-se demasiado na farmacocinética e formas farmacêuticas, e não tanto no aconselhamento, o que no contexto real da farmácia comunitária se revela insuficiente, dado a diversidade de formulações existentes no mercado com distintas indicações e as várias especificações a ter em conta. Apesar de toda a equipa ter estado sempre disponível em auxiliar-me quando necessitei e isso me permitir adquirir algumas competências nesta área, a farmácia não possui muitos dos produtos que existem, pelo que sinto que ainda tenho muito a aprender para ser capaz de dominar o aconselhamento de PUV com a competência necessária a uma farmacêutica.

2.3. Oportunidades

2.3.1. Intervenção na comunidade e responsabilidade social

A área da farmácia comunitária é, sem dúvida, a que tem uma maior interação farmacêutico-doente, sendo uma atividade que exige bastante conhecimento, mas muito desafiante e gratificante. É com base nesta interação que o farmacêutico comunitário se diferencia e é, nos dias de hoje, considerado um agente da saúde pública. O seu papel não passa apenas pelo aconselhamento e cedência de medicamentos e produtos de saúde, sendo que cada vez mais as farmácias comunitárias estão empenhadas em disponibilizar serviços de saúde essenciais aos utentes, quer na vertente terapêutica como na vertente preventiva. Este estágio permitiu-me compreender a importância da farmácia na comunidade onde se insere, quer na promoção da saúde e prevenção da doença como na consciencialização para problemas sociais. Neste sentido, é de realçar a enorme importância que a farmácia teve desde o início do surto de coronavírus, na dispensa de máscaras e explicação detalhada da sua correta utilização, na dispensa de álcool gel, na educação da população acerca de possíveis medidas preventivas a adotar e no esclarecimento das constantes dúvidas dos utentes sobre esta infeção viral que se faz sentir em todo o mundo.

2.3.2. Participação em formações

No decorrer do estágio tive oportunidade de frequentar várias formações dirigidas por especialistas sobre diversos temas, nomeadamente, a palestra sobre "Nutrição clínica" onde nos foram expostos e explicados os produtos da *Fresubin*, a formação sobre o coronavírus, um dos temas mais preocupantes da atualidade, onde nos explicaram de uma forma geral a origem e progressão desta infeção viral, bem como medidas preventivas que, como

farmacêuticos, devemos transmitir aos nossos utentes. Também fui a uma apresentação da gama de produtos da "Bioativo", na qual explicaram os benefícios que os mesmos podem trazer numa determinada situação em específico e o seu correto aconselhamento. Para além destas, pude também assistir a algumas formações sobre MNSRM e outros produtos ministradas no espaço da farmácia por delegados de informação médica. Estas formações foram uma mais-valia, na medida em que abordaram temas de interesse e me permitiram não só consolidar conhecimentos adquiridos, mas também conhecer melhor certos produtos específicos, auxiliando no aconselhamento farmacêutico dos mesmos. É uma excelente forma de ampliar as nossas competências e conseguirmos responder mais eficientemente às necessidades dos utentes. Sendo que a área de saúde é uma área em contante evolução, como farmacêuticos, temos o dever de estar sempre o mais atualizados possível, de modo a proporcionar o melhor atendimento aos nossos utentes, sendo que estas formações são essenciais para conseguir essa atualização constante.

2.4. Ameaças

2.4.1. COVID-19

Esta pandemia mundial em que vivemos veio alterar a vida de todas as pessoas. Devido ao COVID-19, tive que interromper o estágio durante quase dois meses. Quando regressei, muitas modificações tinham sido realizadas. O uso obrigatório de máscara, distanciamento dos utentes ao balcão, desinfeção constante e a limitação do máximo de 2 pessoas dentro da farmácia, foram algumas das medidas tomadas de acordo com o plano de contingência do Centro de Informação do Medicamento (CEDIME) [5], para evitar a propagação do vírus. As pessoas evitavam sair de casa, e, nesse sentido, as entregas ao domicílio foram muito recorrentes e a afluência de utentes na farmácia diminui bastante.

2.4.2. Ruído exterior e a inexistência de estacionamento

Visto que a Farmácia Central se localiza numa rua muito movimentada e tem sempre as duas portas abertas, o ruído do tráfego é muito grande e faz-se sentir durante todo o dia. Esta situação é de facto muito incómoda, na medida em que dificulta a comunicação com o utente e temos de pedir, muitas vezes, para o mesmo repetir o que nos está a dizer. Desta forma, trata-se de uma ameaça ao normal funcionamento da farmácia, uma vez que o ambiente deve ser sempre o mais profissional e calmo possível, de modo a reunir as condições necessárias para um bom atendimento. Não existir estacionamento naquela zona da cidade é, sem dúvida, uma grande ameaça. Qualquer pessoa que viva noutro local da cidade tem de

estacionar bastante longe caso queira deslocar-se a esta farmácia o que, a maioria das vezes, não acontece e faz com que as pessoas prefiram deslocar-se a uma outra com maior acessibilidade. Assim sendo, os utentes que frequentam a farmácia, normalmente são pessoas da zona que se deslocam a pé ou algumas que andam de transportes públicos.

2.4.3. Farmácias circundantes

A Baixa de Coimbra tem inúmeras farmácias muito próximas umas das outras, sendo a Farmácia Central uma delas. Isto constitui uma enorme ameaça para a sua subsistência devido à grande oferta que existe. Apesar de ser uma situação inevitável, é necessário tentar contorná-la da melhor forma, de modo a que os utentes tenham preferência por esta farmácia. Isto pode ser feito através de diversas campanhas, através de serviços prestados pela farmácia e, principalmente através de um bom atendimento, que cative os utentes a regressar pela sua satisfação com a qualidade do serviço prestado.

2.4.4. Locais de venda de MNSRM

Atualmente, a existência de estabelecimentos autorizados a vender MNSRM tem vindo a aumentar. Além das parafarmácias, a maioria das grandes superfícies também já vende este tipo de medicamentos. É, sem dúvida, uma grande concorrência para as farmácias, principalmente, pelos medicamentos serem vendidos a preços bastante inferiores relativamente ás mesmas. Ao negociarem grandes volumes de compras, as grandes superfícies comerciais conseguem melhores condições e, consequentemente, melhores preços e mais apelativos. Além de ser uma ameaça a nível económico, é também uma ameaça a nível da saúde pública, na medida em que as pessoas que os vendem têm pouca formação no que toca ao aconselhamento de produtos farmacêuticos. No caso da Farmácia Central, é de salientar que tem nas suas proximidades vários locais de venda de MNSRM, como a Wells, Pingo Doce, Continente, entre outros. Desta forma, cabe à equipa da farmácia alertar os utentes para esta situação, mostrando-lhes as vantagens de se deslocarem à farmácia para serem aconselhados devidamente por profissionais qualificados e evitarem certos riscos, como interações medicamentosas e efeitos adversos, de forma a minimizar uma automedicação irresponsável.

2.4.5. Medicamentos esgotados

No decorrer deste estágio verifiquei que existem vários medicamentos de uso crónico que se encontram esgotados durante meses, o que provoca descontentamento entre os utentes e compromete a imagem da farmácia perante os mesmos, colocando em risco a adesão à terapêutica, dado que em diversas situações não é possível alterar o medicamento pois não existe nenhum similar disponível. Como exemplos, o Victan 2mg e o Serenal 50mg, que até à

data se encontram indisponíveis. Estas situações complicam os atendimentos, pois provocam insatisfação entre os utentes, pondo em causa a confiança dos mesmos no farmacêutico, acabando por "culpar" a própria farmácia sem perceber realmente o porquê do acontecimento. Neste sentido, é importante saber explicar de forma clara que o medicamento está esgotado a nível do fornecedor e que a farmácia nada pode fazer para alterar isso e também arranjar alternativas para que os utentes continuem a terapêutica.

2.4.6. Aderência aos medicamentos genéricos

O medicamento genérico é um medicamento com a mesma substância ativa, forma farmacêutica, dose e indicação terapêutica que o medicamento de marca que lhe serviu de referência. Ao longo do estágio, atendi muitos utentes que não sabiam o que era um medicamento genérico e outros que ainda não confiam nesses medicamentos relativamente à sua eficácia e/ou segurança. Nesse sentido, é fundamental esclarecer o utente acerca destas questões, o que nem sempre é uma tarefa fácil. Quando possível, o farmacêutico tem o dever de perguntar ao utente se prefere optar pelo medicamento de marca ou o genérico. Alguns utentes compreendem a nossa explicação e acabam por preferir o medicamento genérico, uma vez que tem um preço mais baixo. Contudo, há muitos utentes que ficam reticentes à mudança, ou por já estarem familiarizados com a imagem das embalagens e têm receio de trocar, ou simplesmente por não confiarem nos genéricos. Esta situação traz algumas complicações no atendimento, que temos de superar através de uma correta e esclarecedora comunicação.

3. Casos Clínicos

3.1. Caso I

Um rapaz com cerca de 30 anos dirige-se à farmácia e pede uma pomada para as lesões que apresentava na pele, essencialmente na face e mãos. Tendo em conta a localização e aspeto das lesões cutâneas, questionei se por acaso já lhe tinha sido diagnosticado algum tipo de distúrbio de pele, visto que parecia tratar-se de Dermatite Atópica. Nesse sentido, ele confirmou que se tratava dessa mesma doença cutânea e mostrou uma receita já dispensada, na qual lhe tinha sido prescrita uma pomada com corticosteróides. Assim sendo, cedi-lhe em venda suspensa, um creme que contém valerato de dexametasona, muito eficaz na redução da inflamação da pele. Ao dispensar o creme, expliquei que o mesmo deve ser usado num curto período de tempo, devendo depois suspender, e que só deve ser aplicado nas zonas afetadas 2-3 vezes por dia, ressaltando os efeitos secundários. Além disso, aconselhei algumas medidas não farmacológicas como a toma diária de banho, uso diário de emolientes, ingestão de muita àgua, evitar contacto com alergenos, de forma a manter ao máximo a fase de remissão e evitar crises. Como é um distúrbio que causa muito prurido, também referi que em dias que apresente comichão poderia tomar um anti-histamínico, de modo a evitar o agravamento das lesões.

3.2. Caso II

Uma senhora com cerca de 60 anos vai à farmácia para aviar uma receita. Estava muito pálida, com ar cansado. Nesse sentido, perguntei-lhe se estava tudo bem e precisava de alguma coisa, ao que me respondeu que realmente não se estava a sentir nada bem, com sensação de desmaio. Pedi-lhe que me acompanhasse, dei-lhe um copo de água e medi-lhe e a tensão arterial. Estava claramente com hipotensão, pelo que lhe dei um pouco de sal para colocar debaixo da língua. Enquanto aguardávamos melhorias, consegui conversar com a senhora para perceber a origem do problema, onde a mesma me confessou, muito emocionada, que tinha anemia e que não comia quase nada devido a *stress* e falta de apetite. Além de apoio moral para tentar aliviar a tristeza em que se encontrava, aconselhei o Ceregumil, um xarope que além de estimular o apetite, também funciona como suplemento alimentar. Medi-lhe de novo a tensão arterial e os valores já estavam dentro do normal. Por fim, expliquei-lhe a importância de uma dieta equilibrada, principalmente, em pessoas com anemia e sugeri-lhe que caso não melhorasse, marcasse uma consulta médica. Agradeceu imenso o cuidado e saiu da farmácia com ótimo aspeto, o que para mim foi extremamente gratificante.

4. Conclusão

A realização do estágio curricular em farmácia comunitária é uma experiência essencial que não só me permitiu consolidar e pôr em prática os conhecimentos adquiridos ao longo dos cinco anos de curso, como adquirir novas competências que só com a prática se conseguem.

Possibilitou o meu primeiro contacto com a realidade profissional, permitindo-me compreender a importância do papel que o farmacêutico comunitário desempenha na sociedade e terminar o curso mais bem preparada para entrar no mercado de trabalho, não só ao nível da dispensa de medicamentos, como também no relacionamento com os utentes. Foi uma aprendizagem gradual e contínua, que melhorou a minha destreza e autonomia. É fulcral sentirmo-nos confiantes, para que os utentes se sintam à vontade de nos questionar acerca de qualquer assunto e nós saibamos responder. Apesar de ser um trabalho que exige muito de nós, o afeto criado com os utentes e o desafio de melhorar a saúde e bem-estar da população é muito gratificante.

Todos os conhecimentos que adquiri serão uma mais valia para o meu futuro enquanto farmacêutica, sendo que a aquisição de conhecimentos não pode nem deve ficar por aqui. Foram meses de partilha de valores e memórias com a equipa técnica, as restantes estagiárias e os utentes. Resta-me agradecer a toda a equipa pelo apoio, dedicação e carinho ao longo deste estágio.

5. Referências Bibliográficas

1. Ordem dos Farmacêuticos. - **A Farmácia Comunitária**. [Acedido a 6 de fevereiro 2020]. Disponível em: https://www.ordemfarmaceuticos.pt/pt/areasprofissionais/farmacia-comunitaria/a-farmacia-comunitaria/

2. GÜREL, E. - SWOT Analysis: a theoretical review. The Journal of International Social Research. 10 (2017) 994-1006.

3. Glintt. O que fazemos. Ofertas. Software solutions. **Sifarma**. Disponível em: https://www.glintt.com/pt/o-que-fazemos/ofertas/SoftwareSolutions/Paginas/Sifarma.aspx

4. MINISTÉRIO DA SAÚDE - Decreto-Lei n.º 95/2004, de 22 de abril. Lisboa: Diário da República. Série I-A, pág. 2439-2441. [Acedido a 8 de fevereiro 2020]. Disponível em: https://dre.pt/application/conteudo/223251

5. Ordem dos Farmacêuticos. - Campanhas. Coronavírus. Planos de contingência. **Plano de Contingência COVID-19 – Farmácia**. [Acedido a 8 de agosto 2020]. Disponível em: https://www.ordemfarmaceuticos.pt/fotos/editor2/2019/WWW/campanhas/coronavirus/V5_Plano_de_Contingencia_03_04_2020.pdf

PARTE II

MONOGRAFIA

"Nanocarriers-based dermopharmaceutical formulations for atopic dermatitis"

Sob a orientação do Professor Doutor Francisco Veiga

Abbreviations

AA: arachidonic acid

AD: atopic dermatitis

AdCbl: adenosylcobalamin

BDP: beclomethasone dipropionate

BMD: betamethasone dipropionate

BMV: betamethasone valerate

CC: commercial cream

Chol: cholesterol

CHS: contact hypersensitivity

COX-2: cyclooxygenase-2

CP: clobetasol propionate

CsA: cyclosporin A

DES: desonide

DFV: diflucortolone valerate

DNCB: 1-chloro-2,4-dinitrobenzene

DNFB: 2,4-dinitrofluorobenzene

DPCP: diphenylcyclopropenone

DSPE: distearoyl-phosphatidylethanolamine

EE: entrapment efficiency

EL-LIP: elastic liposome

ER143: synthetic HNE inhibitor

FA: fluoresceinamine

GA: glycyrrhizic acid

HA: hyaluronic acid

HACaT cells: Immortalized Human Keratinocytes

HC: hydrocortisone

HET-CAM: hen's egg test on the chorioallantoic membrane

HG: hydrogel

HHSE: Human heat-separated epidermis

HNE: human neutrophil elastase

HPMC: hydroxypropylmethylcellulose

HST: hirsutenone

HT: hydroxytyrosol

IFN: interferon

IgE: immunoglobulin E

IL: interleukin

iNOS: nitric oxide synthase

LEC: lecithin

LIP: liposome

MCT: medium-chain triglycerides

MPB-PE: N-[4-(p-maleimidophenyl)butyryl]-phosphatidylethanolamine

MTX: methotrexate

NA: not available

NC: nanocarrier

NE: nanoemulsion

NIC: nicotinamide

NLC: nanostructured lipid carrier

NP: nanoparticle

OA: oleic oil

ORG: oregonin

OVA: ovalbumin

PC: phosphatidylcholine

PDC: prednicarbate

PDI: polydispersity index

PEG: polyethylene glycol

PG: propylene glycol

PGZ: pioglitazone

PIC: pimecrolimus

PLA: poly (lactic acid)

PLG: poly (lactide-co-glycerol)

PLGA: poly (lactic-co-glycolic acid)

PVA: polyvinyl alcohol

RT: room temperature

SA: stearyl amine

SC: stratum corneum

SCH: stratum corneum hydration

SDC: sodium deoxycholate

SLN: solid lipid nanoparticle

TAC: tacrolimus

Tat: Trans-activating transcriptional activator

TCI: topical calcineurin inhibitor

TEWL: transepidermal water loss

Th: helper T cell

TMA: trimellitic anhydride

TNCB: trinitrochlorobenzene

TPA: 12-o-tetradecanoylforbol 13-acetate

TXG: taxifolin glycoside

VE: vitamin E

ZP: zeta potential

Abstract

Atopic dermatitis (AD) is a chronic disease that affects the skin and it is characterized by highly itchy inflammation. The current treatment consists of a multi-stage approach and aims to establish persistent disease control, towards the improvement of the quality of life of the patients. Topical therapy is the basis of AD treatment, however, due to the difficulty of crossing the skin barrier, topical application of drugs remains a challenge. In fact, in addition to the low skin bioavailability, and limited accessibility to deeper skin of the drugs − due to difficulty in penetrating the epidermis −, used drugs are associated with serious adverse effects, which are responsible for safety and efficacy limitations, leading to a reduction in patients' compliance. Nanotechnology arises as an emerging approach for the treatment of AD, allowing for controlled release, targeted delivery, improved penetration and bioavailability of drugs assets, resulting into marked therapeutic efficacy and reduction of adverse effects. Although its promising outputs as a therapeutic alternative, additional studies are needed to recognize the toxicological characteristics, cost-benefit and long-term safety of nanocarriers. Advanced drug delivery systems, particularly nanoemulsions, liposomes, ethosomes, transfersomes, solid lipid nanoparticles, nanostructured lipid carriers, nanocrystals, polymeric nanoparticles, and polymeric micelles have been used for this purpose, and will be thoroughly addressed in this review as promising nanoformulations for the topical treatment of AD.

Keywords: Atopic dermatitis, nanocarrier, topical administration, skin inflammation, nanotechnology.

Resumo

A dermatite atópica (DA) é uma doença crónica que afeta a pele e é caracterizada por uma inflamação fortemente pruriginosa. O tratamento atual consiste numa abordagem por várias fases, e visa estabelecer o controlo persistente da doença, tendo em vista a melhoria da qualidade de vida dos doentes. A terapia tópica é a base do tratamento da DA, no entanto, devido à dificuldade de atravessar a barreira da pele, a aplicação tópica de fármacos continua a ser um desafio. Na verdade, além da baixa biodisponibilidade na pele, e do acesso limitado às camadas da pele mais profundas − devido à dificuldade de penetração na epiderme −, os fármacos usados estão associados a graves efeitos adversos, responsáveis por limitações de segurança e eficácia, levando à redução da adesão à terapêutica. A nanotecnologia surge como uma abordagem emergente para o tratamento da DA, permitindo uma libertação controlada, direcionada, e melhoria da penetração e da biodisponibilidade dos fármacos, resultando na melhoria da eficácia terapêutica, e na redução de efeitos adversos. Apesar dos resultados promissores como alternativas terapêuticas, são necessários estudos adicionais para reconhecer as características toxicológicas, o custo-benefício e a segurança a longo prazo dos nanotransportadores usados para este fim. Sistemas avançados de libertação de fármacos, particularmente nanoemulsões, lipossomas, etossomas, transfersomas, nanopartículas de lípidos sólidos, vetores lipídicos nanostruturados, nanocristais, nanopartículas poliméricas, e micelas poliméricas têm sido usados para este propósito e serão descritivamente abordados nesta revisão como nanoformulações promissoras para o tratamento tópico da DA.

Palavras-chave: Dermatite atópica, nanotransportador, administração tópica, inflamação da pele, nanotecnologia.

1. Introduction

Atopic dermatitis (AD), or atopic eczema, is a chronic inflammatory skin disease characterized by intense itching and recurrent eczematous lesions (AKHTAR, N. *et al.*, 2017, WEIDINGER, S. *et al.*, 2018, ZHUO, F.*et al.*, 2018). It is considered one of the most common skin diseases, which can be manifested at any time in life, but with a higher prevalence in children (AKHTAR, N. *et al.*, 2017, WEIDINGER, S. *et al.*, 2018). It is estimated that an individual will develop AD throughout life with probability of about 20%, particularly in developed countries (SOUTO, E.B. *et al.*, 2019, WEIDINGER, S. *et al.*, 2018). It seems to have a female preponderance, especially in adolescence or adulthood (SILVERBERG, J.I., 2020). Although the pathological process of this disease is not fully understood, it appears to result from the complex interaction between skin barrier dysfunction and immune dysregulation, displaying underlying genetic, environmental and infectious factors (AKHTAR, N. *et al.*, 2017, SILVERBERG, J.I., 2020, SOUTO, E.B. *et al.*, 2019). AD is characterized to be a highly heterogeneous disorder that presents several lesions morphologies, and a distribution pattern that varies according to patient age. In addition to skin lesions and pruritus, which are usually the most burdensome symptoms, patients with AD often suffer from xerosis, skin pain and sleep disorders, which cause a great loss in their quality of life. It is also closely associated with other atopic comorbidities, such as asthma and allergic rhinitis, and with non-atopic comorbidities, in particular mental disturbances, such as anxiety and depression (SILVERBERG, J.I., 2020, WEIDINGER, S. *et al.*, 2018). AD treatment approaches focus on repairing the skin barrier, controlling itching, reducing inflammation and preventing or reducing possible infections (AKHTAR, N. *et al.*, 2017). Bearing in mind the disease prevention, it is very important to adopt non-pharmacological measures, such as avoiding contact with causative allergens, daily skin care and regular bathing (AKHTAR, N. *et al.*, 2017, SOUTO, E.B. *et al.*, 2019, ZHUO, F. *et al.*, 2018). The daily application of moisturizers and emollients seems to slow the progression or even decrease the risk of developing AD in children, being also crucial to keep patients in remission and reducing the number of flares, which reduces ultimately the need for topical corticosteroids application (SOUTO, E.B. *et al.*, 2019, WEIDINGER, S. *et al.*, 2018). Conventional pharmacological therapy is based on the use of topical corticosteroids as the first line of treatment to reduce skin inflammation. However, these drugs evidence many adverse effects, such as skin atrophy, striae, telangiectasias, depigmentation and acne, being generally indicated for short-term treatment, with low recommendation in children (SOUTO, E.B. *et al.*, 2019, WEIDINGER, S. *et al.*, 2018, ZHUO, F. *et al.*, 2018). Topical calcineurin inhibitors (TCIs), pimecrolimus (PIC) and tacrolimus (TAC) are the anti-inflammatory options

that have been used as alternatives to the adverse effects of topical corticosteroids. These drugs evidence lower adverse effects, so they can be used for longer time-periods, are more suitable for children, and may be applied in anatomically sensitive sites where topical corticosteroids are contraindicated (WEIDINGER, S. *et al.*, 2018, ZHUO, F. *et al.*, 2018). TAC is insoluble in water, presenting difficulties to permeate the skin, a low bioavailability, as well as burning sensation and pruritus at the application site (POPLE, P.V. and SINGH, K.K., 2010). More recently, crisaborole, an anti-inflammatory of the phosphodiesterase E4 inhibitors class, has been approved in the galenic form of ointment as another alternative for the treatment of AD (SILVERBERG, J.I., 2020, WEIDINGER, S. *et al.*, 2018). Despite presenting some risks, phototherapy can be recommended as a short-term therapy. When topical treatments and phototherapy are not effective, or in the most severe cases, systemic immunosuppressants, such as cyclosporin A (CsA) and methotrexate (MTX), or systemic corticosteroids, may be considered (SOUTO, E.B. *et al.*, 2019, WEIDINGER, S. *et al.*, 2018). Biological drugs, in particular dupilumab, provide targeted therapy, that has recently shown to benefit AD treatment, not only in reducing inflammation, but also in relieving itching. These drugs are able to improve the response of patients undergoing topical corticosteroids and resolve cases of patients who are also resistant to systemic treatments (DAMIANI, G. *et al.*, 2019, WEIDINGER, S. *et al.*, 2018). Besides, itching is very commonly controlled by using first generation antihistamines, which are sedative and, therefore, contribute to improving the sleep of these patients. Where required, antibiotics are used to eradicate skin infections (SOUTO, E.B. *et al.*, 2019).

As aforementioned, despite the available diversity of current topical and systemic treatments, those are associated with undesirable side effects, which consequently reduce patient adherence to treatments (AKHTAR, N. *et al.*, 2017, SOUTO, E.B. *et al.*, 2019). The topical administration route appears as very advantageous in comparison to the systemic administration route, as it allows the direct application of the drug in the intended site of action, preventing the entry into the bloodstream, and thus reducing undesirable systemic effects. To achieve the desired therapeutic effect, topical formulations must be able to penetrate the skin and allow an effective concentration of the drug. Successful topical therapy depends on the permeability of the skin, the physicochemical characteristics of the drug, and the physicochemical properties of the carrier (ESPINOZA, L.C. *et al.*, 2019). Conventional topical formulations fail to provide skin drug targeting and controlled drug release, often resulting in undesirable systemic drug uptake (GUNGOR, S. and REZIGUE, M., 2017). Additionally, difficulties exist in the ability of drugs to cross the protective barrier of the skin

after topical application, which limits their accessibility to the deeper skin, and consequently leads to low bioavailability in the skin, compromising the therapeutic effectiveness (AKHTAR, N. *et al.*, 2017, SOUTO, E.B. *et al.*, 2019). The poor drug solubility and its undesired metabolism before it can act on the intended site may also be a problem (SOUTO, E.B. *et al.*, 2019). New advanced systems and processes for drug delivery appear to overcome some of these disadvantages (GUPTA, M. *et al.*, 2012). Nanotechnology has been receiving a notable recognition by investigators due to their promising advantages which have been well described including, but not exclusively, in terms of topical drug delivery (ZHUO, F. *et al.*, 2018). Nanocarriers (NCs) are materials with dimensions ranging from 1 to 1000 nm (SHAO, M.*et al.*, 2016), capable of high entrapment efficiency values, which altogether results in improved biopharmaceutical profiles (SOUTO, E.B. *et al.*, 2019, ZHUO, F. *et al.*, 2018). NCs present improvements in the level of drug solubility and skin permeability, thus increasing the penetration of drugs through the stratum corneum (SC), allowing greater bioavailability in the skin and therapeutic efficacy outcomes. In addition, less amount of drug is required due to targeted delivery possibility and dose control provided by the NCs, which limits the adverse effects associated with drugs and allows for a superior treatment safety profile (SOUTO, E.B. *et al.*, 2019). In this way, nanoformulations are presented as improved technological approaches over conventional topical formulations, and are considered very promising drug delivery carriers for topical administration of drugs deep in the epidermis, projecting better drug release profiles to achieve therapeutic goals (AKHTAR, N. *et al.*, 2017, SOUTO, E.B. *et al.*, 2019). Additionally, while the skin penetration of NCs may be limited in healthy skin, in situation of disease − where the skin barrier is disrupted, e.g., AD and psoriasis − drug delivery using nanoparticles (NPs) is advantageous. Several types of NPs have been proposed for topical administration of different drugs used in AD. Despite all the benefits demonstrated, it is extremely important to ensure the safety and effectiveness of NCs to allow the generation of potentially marketable products, so additional studies still are needed to recognize their toxicological characteristics and long-term safety profiles (SOUTO, E.B. *et al.*, 2019). The therapeutic superiority of NCs-based dermopharmaceutical formulations for the topical treatment of AD cutaneous lesions is discussed in the following sections.

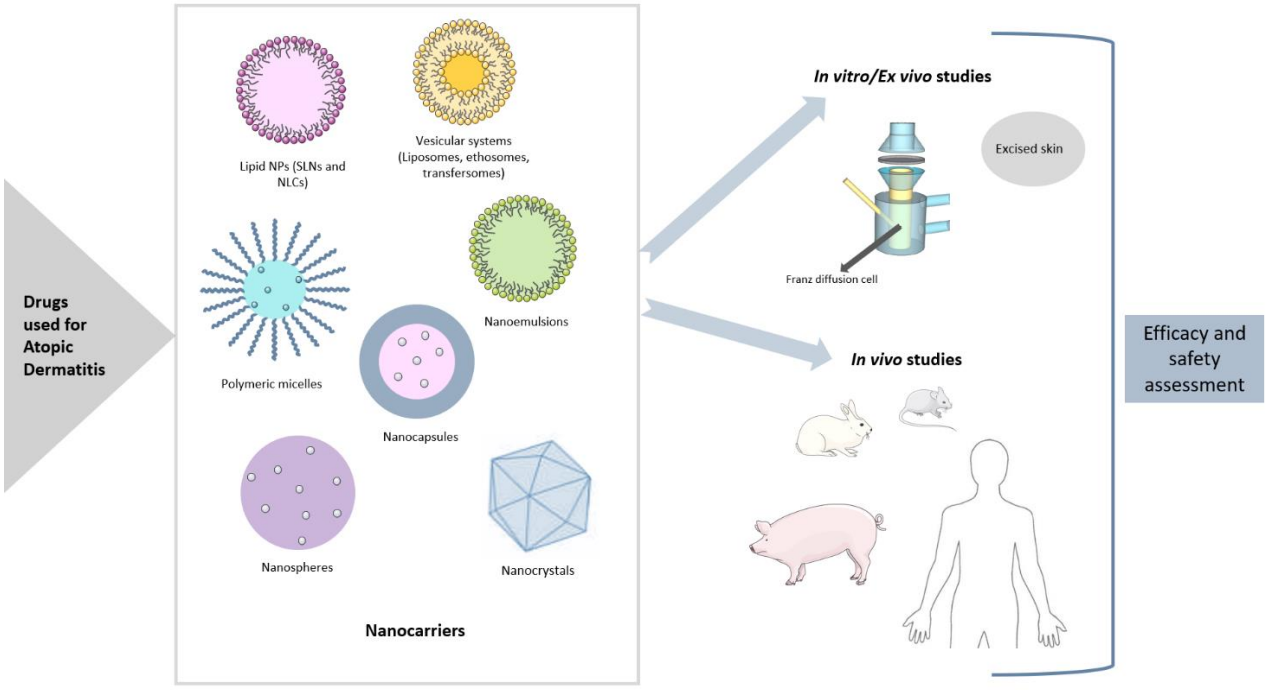

Fig. 1. Graphical abstract.

2. Nanocarriers-based dermopharmaceutical formulations for atopic dermatitis

Many studies have carried out to develop NCs for the topical delivery of drugs for AD treatment, including nanoemulsions (NEs), liposomes, ethosomes, tranfersomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), nanocrystals, polymeric NPs, polymeric micelles, etc. In addition to topical corticosteroids, TAC, CsA, and other innovative drugs have also been used to study the therapeutic efficiency of different NCs. Representative studies regarding NCs-based dermopharmaceutical formulations for atopic dermatitis will be summarized next (see Annex 2).

2.1. Lipid-based nanocarriers

NPs composed of lipids have several advantages over other NCs due to the similarity with the skin lipidic composition (SOUTO, E.B. *et al.*, 2019). Several types of lipid-based NCs have been studied as possible drug delivery vehicles for the local treatment of skin diseases such as AD, including NEs, vesicular systems (such as liposomes, ethosomes, transfersomes, among others), and lipid NPs (SLNs, and NLCs).

2.1.1. Nanoemulsions

NEs are stable colloidal nanocarriers widely used in the pharmaceutical and cosmetic industries that are characterized by a homogeneous appearance and low viscosity. Due to their oily and surfactant-based composition and the ability to provide improved solubility of lipophilic and hydrophilic drugs those structures can improve skin permeation (ESPINOZA, L.C. *et al.*, 2019). To formulate NEs, a high amount of surfactant is needed which may enhance the irritation potential, requiring the performance of skin irritation potential studies before application. With a particle size, normally ranging from 10-200 nm, these NCs supply medications that are poorly soluble in water to deeper layers of the skin, reducing adverse effects by decreasing the dose (ALAM, M.S. *et al.*, 2013). The characteristic positive charge of NEs allows a physical-chemical interaction with the negative charge of the SC corneocytes, resulting in an improved permeation, longer retention time, and greater bioavailability of drugs on the skin (SHAO, M. *et al.*, 2016). The preparation methods are easy and inexpensive, and those are considered a very auspicious drug delivery system (ESPINOZA, L.C. *et al.*, 2019).

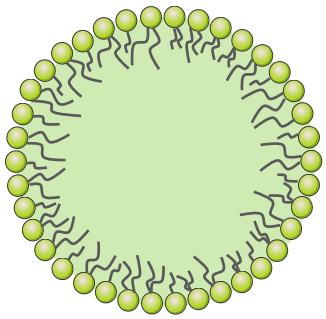

Fig. 2. Nanoemulsions structure.

Alam *et al.* (ALAM, M.S. *et al.*, 2013) developed NEs for the topical administration of clobetasol propionate (CP), a topical corticosteroid used to treat AD and psoriasis. The optimized formulation was subjected to anti-inflammatory activity and the irritation potential *in vivo* studies. CP-loaded NE showed greater inhibition of edema (84,15% up to 12h) compared to the marketed cream (Glevate®) and the placebo NE. The eucalyptus oil present in the NE structure also appeared to have anti-inflammatory activity. CP-loaded NE irritation studies did not reveal signs of irritation, despite the presence of a large quantity of surfactants. NEs was suggested by a promising vehicle for the topical delivery of CP in the treatment of inflammatory skin diseases.

Pioglitazone (PGZ), despite being used as an antidiabetic agent, evidenced recently a role in modulating the inflammatory response. Thus, it has been studied as a possible candidate for the treatment of inflammatory skin diseases. To improve the solubility of PGZ and its bioavailability in the skin, Espinoza *et al.* (ESPINOZA, L.C. *et al.*, 2019) proceeded to its incorporation in a NE. *In vitro* release studies revealed that PGZ-loaded NEs allow a controlled PGZ release. An *ex vivo* skin permeation study showed a high permeation flux of PGZ into the skin when incorporated into NEs, which may be due to Labrasol® capacity of improving the solubility of the drug. PGZ-loaded NEs favored the retention of PGZ in the skin, guaranteeing a prolonged local action, and minimizing adverse reactions, while reducing the frequency of application. The evaluation of the *in vivo* inflammatory efficacy on the skin showed that PGZ-loaded NE suppressed markedly the inflammatory cytokines IL-6, IL-1β and TNF-α levels, restored anti-inflammatory mRNA values, and reduced slightly the redness. Besides, histopathological analysis showed that treatment with PGZ-loaded NE improved the structural characteristics of SC, and decreased inflammatory cell infiltrates, and the thickness of the dermis. To confirm tolerance to PGZ-loaded NE, an *in vivo* study was carried out on twelve volunteers with healthy skin. Apart from decreasing the value of transepidermal water loss (TEWL), the formulation also increased the value of stratum corneum hydration (SCH), suggesting no skin surface damage, as well as hydration level of the SC improvement. A

tendency for TEWL and SCH values to return to baseline states was verified, pointing to the biocompatibility of the formulation. This study suggested the therapeutic potential of PGZ-loaded NE for the topical treatment of inflammatory dermatological conditions, such as AD.

2.1.2. Vesicular systems

Vesicular systems are one type of NCs that can be classified based on their main components into liposomes, ethosomes, transfersomes, niosomes, among others. Structurally, vesicles contain both an aqueous core and lipid bilayer allowing the encapsulation of both lipophilic and hydrophilic drugs (KAPOOR, B. *et al.*, 2019).

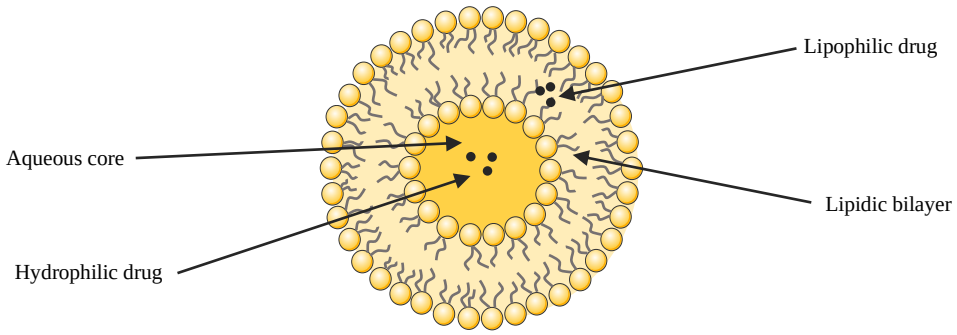

Fig.3. Representative scheme of a vesicular system, emphasizing the possibility of hydrophilic and lipophilic drugs entrapment.

2.1.2.1. Liposomes

Liposomes are drug delivery systems comprised of phospholipid bilayers similar to cell membranes. Those systems are spherical vesicles, formed by one or several concentric bilayers surrounding an aqueous phase. Cholesterol is another usual component of liposomes, which avoids phospholipid aggregation and increases the membrane fluidity. The combination of a hydrophobic phospholipid layer with a hydrophilic aqueous core is a great advantage, since liposomes can entrap water-soluble drugs in their core and low water-soluble ones in the membrane (GUPTA, M. *et al.*, 2012, TORCHILIN, V.P., 2005). The use of liposomes in medicine is vast and include cancer and gene therapy, stimulation of immune response and vaccination and treatment of infectious diseases, among others (LASIC, D.D. and PAPAHADJOPOULOS, D., 1998).

Liposomes are valuable drug delivery systems for topical drug delivery. The fact that they are similar to the epidermis concerning their lipid composition allows them to easily penetrate the epidermal barrier, more than conventional topical formulations. Thus, liposomes

topically applied to the skin tend to accumulate in the upper layers of the SC, where they function as a reservoir and provide a localized action (HUA, S., 2015, MARTO, J. *et al.*, 2016). Liposomes can also be used as transdermal drug delivery system. In fact, it is possible to enhance the skin permeation effect by changing the composition of liposomes, making them versatile drug delivery system (HUA, S., 2015). For example, a new generation of deformable or flexible vesicles, such as ethosomes and transfersomes (see following sections), has been developed to improve topical or transdermal drug delivery. Liposomes have been researched as possible nanocarriers of drugs for the treatment of dermatological diseases (e.g., acne, atopic dermatitis, psoriasis, vitiligo) and the results obtained have been confirming their therapeutic value (KAMRA, M. and DIWAN, A., 2017). Several drugs used in the treatment of AD have been encapsulated in liposomes, including antibiotics, corticosteroids, TAC, antihistamines, among others (SOUTO, E.B. *et al.*, 2019). Some of the more important studies will be summarized next.

Topical vitamin B12 has been used as a treatment for AD but its photosensitivity and low skin permeability limit its use. To try to overcome these limitations, Jung *et al.* (JUNG, S.H. *et al.*, 2011) prepared a liposomal hydrogel of adenosylcobalamin (AdCbl), a derivative of vitamin B12, which fared better than the AdCbl solution or cream, or liposomes alone, reducing dermatitis score of AD skin lesions, skin thickness, parakeratosis and total serum IgE levels.

Kang *et al.* (KANG, M.J. *et al.*, 2011) prepared elastic liposomes (EL-LIP) conjugated with a Tat-peptide for the topical administration of hirsutenone (HST), an immunomodulator used to treat skin lesions in AD. EL-LIP/Tat-peptide allowed a greater *in vitro* permeation flux of HST into the skin compared to conventional cream. Moreover, in addition to a significantly faster improvement in the severity index of skin lesions *in vivo*, beneficial effects related to immunity were demonstrated, such as more market decreased levels of cyclooxygenase-2 (COX-2), nitric oxide synthase (NOS), interleukin (IL)-4, IL-13, immunoglobulin E (IgE) and eosinophils. These results were in agreement with previous studies in which liposomal delivery systems were used for the delivery of taxifolin glycoside (TXG) (KANG, M.J. *et al.*, 2010) and oregonin (ORG) (KANG, M.J., 2010). Authors have found a notable improvement in skin hydration levels, a higher recovery in the integrity of the SC barrier, as well as a significant reduction in the TEWL. Additionally, liposomal delivery systems were able to efficiently normalize the levels of IL-4, IgE, and interferon (IFN)-γ in the blood (KANG, M.J. *et al.*, 2010).

These studies indicated that ORG, TXG, or HST incorporated in liposome-based formulations may constitute promising therapeutic alternatives for the topical treatment of AD.

Also, Eroglu *et al.* (EROGLU, I. *et al.*, 2016) prepared liposomes (LIP) loaded with betamethasone valerate (BMV) / diflucortolone valerate (DFV) and incorporated them into a chitosan gel, towards the obtainment of the appropriate viscosity and spreadability for an easy skin application. *Ex vivo* permeation studies showed that LIPs allowed longer drug retention and accumulation in SC and epidermis when compared to commercial cream. LIPs-incorporated gel allowed greater inhibition of rat paw edema, showing a higher anti-inflammatory activity than the commercial cream. LP-incorporated gel decreased TEWL and erythema, and a rapid recovery of the lesions of rats with DNFB-induced AD was found. In addition, histological analysis revealed that LIP-incorporated gel induced higher inhibition of edema, vasodilation, and lymphocyte infiltration, and reduced up to 50% the amount of mast cells infiltrated in the lesions. Skin irritation studies in healthy rats showed that, after applying the LP-incorporated gel, no significant differences were verified in the TEWL and erythema values. No morphological changes in the tissue were found, which confirms that this formulation does not induce an irritating effect on the skin. It should be noted that skin recovery was achieved with only 1/10 of the drug used in commercial creams, minimizing the side effects associated with corticosteroids, thereby rendering LP-incorporated gel a very promising and safe alternative for topical treatment of inflammatory skin diseases, as AD.

2.1.2.2. Ethosomes

Ethosomes are modified lipid vesicles, introduced in 2000, which are composed of a high concentration of ethanol - a permeation enhancer of several drugs (CARRERAS, J.J. *et al.*, 2020)- being characterized by a high deformability. As the remaining vesicular delivery systems, ethosomes provide an effective delivery of both lipophilic and hydrophilic drugs (EL-MENSHAWE, S.F. *et al.*, 2019, KAPOOR, B. *et al.*, 2019, LI, G. *et al.*, 2012b). Upon penetration, ethosomes break, and phospholipids are retained in the epidermis, while the trapped drug penetrates gradually. The use of ethosomes for drug dermal delivery has been studied for the treatment of various skin diseases, including psoriasis, AD, and alopecia. Also, binary ethosomes, which are composed of two alcohols (ethanol and propylene glycol) instead the use of just ethanol, are an interesting modification of ethosomes, endowed with higher stability and capacity to penetrate the deeper layers of the skin (KAPOOR, B. *et al.*, 2019).

Li *et al.* (LI, G. *et al.*, 2012a) incorporated TAC into ethosomes. The presence of ethanol affected the particle size of the NPs, and favored the encapsulation of TAC compared to

traditional liposomes. *In vitro* studies have shown a greater amount of TAC retained in the epidermis and dermis after topical application of the ethosomal formulation, revealing a deeper permeation capacity when compared to liposomes and Protopic® (0.1% TAC ointment). A further study performed by the same group (LI, G. *et al.*, 2012b) confirmed these results, and verified a more significant reduction in ear edema and in the number of mast cells infiltrated at the site of inflammatory reactions after the topical application of TAC-loaded ethosomes in AD-like skin lesions-bearing mice. Such data suggested the higher anti-inflammatory efficacy of the ethosomal formulation compared to liposomes and Protopic® (0.1% TAC ointment).

Vitamin B3, called nicotinamide (NIC), holds antioxidant and anti-inflammatory properties, and a recognized anti-AD effect (PAN, W. *et al.*, 2016, YU, K. *et al.*, 2018). El-Menshawe *et al.* (EL-MENSHAWE, S.F. *et al.*, 2019) developed thermosensitive ethosomal gels for topical application of NIC using stearyl amine (SA) as a positive charge inducer. The developed formulations were optimized until the ideal concentrations of ethanol, phosphatidylcholine and propylene glycol were found, in order to increase the NIC EE and the vesicle deformability. *In vitro* studies have shown that the increase in SA concentration led to a decrease in the skin release and flow of NIC, and resulted in a significant increase of the NIC amount retained in the skin layers. It was noteworthy that a smaller amount of Polyox™ resulted in drug skin retention. *In vivo* studies revealed that the topical application of two optimized NIC-loaded ethosomal gels in induced AD-bearing rats promoted a significant decrease in inflammation and reduced the maturation of corneocytes, presenting a superior anti-inflammatory activity when compared to Betaderm® (0.1% BMV cream), together with an immunosuppressive effect. This study was highly innovative, attending to the indication that NIC can be used as a new pharmacological approach for the topical treatment of AD, being its incorporation in vesicles beneficial to circumvent the high solubility in water, and thus improve its percutaneous delivery.

2.1.2.3. Transfersomes

Transfersomes were introduced in 1992, and consist in vesicles composed of phospholipids and an edge activator, usually a single chain surfactant, characterized by being highly elastic and ultra deformable (KAPOOR, B. *et al.*, 2019). Their high deformability gives them the ability to squeeze in order to be able of permeation through intercellular regions of SC (CHAUHAN, S. *et al.*, 2018).

Chauhan *et al.* (CHAUHAN, S. *et al.*, 2018) formulated a hydrogel containing glycyrrhizic acid (GA)-loaded transfersomes, to assess its anti-inflammatory activity towards

topical AD treatment. Besides the suitable viscosity and spreadability of the optimized formulation, it exhibited a controlled GA release profile. *Ex vivo* permeation studies depicted a lower GA permeation, confirming the ability to remain in the upper skin layers, and to achieve a high drug deposition. The formulated hydrogel was able to significantly reduce *in vivo* the scratching score and erythema score in mice. The hematological parameters obtained in the treatment group were closer to those obtained in the control group. Altogether, results suggested the effectiveness and the safety of the developed formulation against AD.

Carreras *et al.* (CARRERAS, J.J. *et al.*, 2020) developed ultraflexible lipid vesicles for the topical application of cyclosporine A (CsA), a drug with immunosuppressant properties used to treat AD and psoriasis. The ability of CsA to penetrate the skin was evaluated *in vitro*, using human epidermis in Franz diffusion cells, and it was concluded that conventional liposomes, transfersomes, and ethosomes effectively delivered CsA to the skin. The tranfersomes obtained by extrusion was the most effective when correlating the flux to the applied dose. Although *in vivo* efficacy and safety studies are needed, the authors designed CsA-loaded transfersomes for topical application as a promising alternative to the oral administration of CsA for the treatment of AD, minimizing the adverse effects associated with its systemic absorption.

2.1.3. Lipid nanoparticles

Lipid NPs, developed in the 90s decade of last century, are heterogeneous systems composed by an inner lipid phase and an external aqueous phase, stabilized by one or two surfactants. There are two types of lipid NPs for topical application. SLNs and NLCs (GARCÊS, A. *et al.*, 2018). Lipid NPs enclose several advantages, namely non-toxicity, enhancement of drug penetration up to the epidermis, thus reducing systemic absorption, and protection of drugs that are sensitive to light, oxidation and hydrolysis (MONTENEGRO, L. *et al.*, 2016, MULLER, R.H. *et al.*, 2002). Also, their production methods are easy and can be easily scaled-up (PURI, A. *et al.*, 2009). Both SLNs and NLCs are small-sized and, upon topical application, adhere to the lipid film of the SC and modulate the amount of drug that penetrates the skin. Additionally, lipid NPs have occlusive properties that lead to an increase in skin hydration (MONTENEGRO, L. *et al.*, 2016). One of the disadvantages of this type of drug delivery system is that they can only incorporate lipophilic compounds. In the case of hydrophilic drugs, a sufficient drug loading can only be achieved with very potent drugs that act at very low concentrations, e.g., β-interferon (MÜLLER, R.H. *et al.*, 2011). Lipid NPs have proven to be very advantageous for topical application. To use these benefits, it is necessary to ensure

sufficient encapsulation efficiency (EE), which can be achieved with a complex lipid matrix with high solubility potential for the selected asset (KOVAČEVIĆ, A.B. *et al.*, 2020).

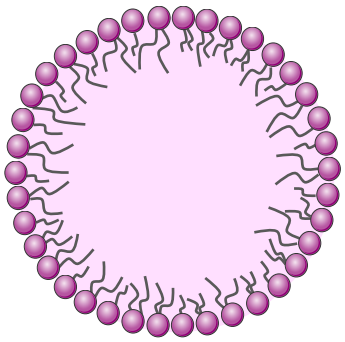

Fig.4. Lipid nanoparticles structure.

2.1.3.1. Solid lipid nanoparticles

SLNs were first developed in 1990, and are NPs that are totally composed of lipids that are solid at room temperature (GARCÊS, A. *et al.*, 2018, MÜLLER, R.H. *et al.*, 2011). These NPs have a solid lipid core stabilized by surfactants in an aqueous medium, where lipophilic drugs can be entrapped (MÜLLER, R.H. *et al.*, 2011, MURPHY, E.C. *et al.*, 2019). Because they are made of solid lipids only (e.g., tristearin), they form liquid crystals with no imperfections, thus exhibiting a limited space to host drug molecules. Additionally, the perfect lipid crystals often lead to drug expulsion (BUNJES, H. *et al.*, 1996).

Santos Maia *et al.* (SANTOS MAIA, C. *et al.*, 2002) performed *in vitro* studies using reconstructed epidermis and human abdominal skin to study the effect of incorporating prednicarbate, a glucocorticoid widely used in AD treatment, in SLNs. Results showed that the use of SLNs directed prednicarbate to the epidermis, thus reducing the undesired skin atrophy caused by the inhibition of fibroblasts, which is usual during long term treatment with glucocorticoids.

SLNs have also been used to improve skin penetration of CsA, as reported by Kim *et al.* (KIM, S.T. *et al.*, 2009). *Ex vivo* permeation studies using murine skin showed that the permeation efficiency of CsA-loaded SLNs was twice as high as that of a CsA-oil mixture. Furthermore, *in vivo* studies using a murine model of AD, showed a relief of AD symptoms related to a decrease in Th2-related cytokines IL-4 and IL-5.

Pople *et al.* (POPLE, P.V. and SINGH, K.K., 2010) developed SLNs to encapsulate TAC, as a way to decrease the side effects of this topical calcineurin inhibitor. *In vitro* and *ex vivo* studies showed a higher drug release, and pig ear skin penetration and accumulation when compared to the Protopic® (0.1% TAC ointment). *In vivo* studies in rats showed a significantly higher deep skin penetration for the TAC-loaded SLNs, as well as higher skin retention in the SC, epidermis and dermis. Additionally, *in vivo* studies using rabbits showed that the encapsulated TAC did not cause skin irritation unlike the marketed product. The same group reported a study of the *in vivo* assessment of the dermatopharmacokinetics, biodistribution and efficacy of the TAC-loaded SLNs (POPLE, P.V. and SINGH, K.K., 2012). In guinea pigs, the bioavailability of the NPs was higher than the commercial ointment, and in albino rats the skin penetration of the SLNs was also significantly higher. Further studies in albino rats also suggested a targeting potential for the SLNs, with localization at the target skin area and no systemic spreading. Additionally, in BALB/c mice, the suppression of inflammatory AD skin lesions was more efficient when the TAC-loaded SLNs were used, compared to the commercial ointment and the placebo.

Recently, Kang *et al.* (KANG, J.H. *et al.*, 2019) studied the potential application of thermosensitive SLNs for the delivery of TAC. The particles revealed a high drug loading efficiency. Both *ex vivo* studies using rat dorsal skin and *in vivo* studies in New Zealand white rabbits showed that the TAC-loaded SLNs penetrate to deeper layers of skin in comparison to the commercial TAC ointment. The highest TAC concentrations were found *in vivo* in the SC for both the ointment and the TAC-loaded SLNs, but the TAC from the ointment could only be detected up to 150 μm of skin depth, while TAC from the SLNs was present in high concentrations up to 300 μm and could still be detected at 500 μm, although faintly. Thus, the results suggested that these thermosensitive SLNs, whose thermodynamic properties are dictated by the surfactant, enhance drug delivery to the skin.

Betamethasone valerate (BMV), a potent topical corticosteroid widely used in the treatment of AD, must be used frequently, being responsible for the probability enhancement of side effects occurrence. Zhang *et al.* (ZHANG, J. and SMITH, E., 2011) developed and characterized SLNs to encapsulate BMV, a corticosteroid widely used for the treatment of AD. The percutaneous permeation of the different formulations, including BMV-loaded monostearin-based SLN, BMV-loaded beeswax-based SLN, BMV suspension and BMV-loaded commercial lotion, was performed *in vitro* using human skin. The BMV-loaded monostearin SLN showed the most promising results, particularly: notable controlled release properties, significantly higher drug reservoir effect in the epidermis, and lower dermal permeation rate,

especially contrasting with the commercial lotion. These results highlighted the strong potential of SLNs to specifically target BMV to the epidermis, while allowing minimal drug accumulation in the dermis, and consequently, in the systemic circulation, reducing the undesirable systemic side effects associated with the drug.

2.1.3.2. Nanostructured lipid carriers

NLCs are a second generation of lipid NPs developed in 1999, which, similarly to SLNs, are surfactant-stabilized, but that contain both solid and liquid lipids in their cores. This distorts the formation of perfect crystals and the imperfections, which allow more space for drug molecules to be entrapped (FANG, C.L. *et al.*, 2013, MÜLLER, R.H. *et al.*, 2011, PARDEIKE, J. *et al.*, 2009). Additionally, NLCs have a lower water content of the particle suspension than SLNs, and minimize the potential expulsion of drugs during storage (MEHNERT, W. and MÄDER, K., 2001).

Nagaich *et al.* (NAGAICH, U. and GULATI, N., 2018) developed, optimized and characterized BMV-loaded NLCs, ascertaining the possibility of using this formulation as a once-a-day dosing therapy for AD. *Ex vivo* studies using a BMV-loaded NLC-based gel and plain BMV-loaded gel applied on rat abdominal skin revealed that the BMV-loaded NLC-based gel had enhanced permeation compared to the BMV-loaded gel. *In vivo* studies using a carrageenan-induced hind paw albino rat model of AD showed a significant extended anti-inflammatory effect of the BMV-loaded NLC-based gel. Taken together, the results supported the efficiency of the BMV-loaded NLC formulation as a once-daily efficient therapy for AD.

Betamethasone dipropionate (BMD) is another topical corticosteroid used in AD treatment. Kong *et al.* (KONG, X. *et al.*, 2016) developed BMD-loaded NLCs-based ointment. In comparison with the BMD-loaded NLCs Carbopol® emulgel ointment, the BMD-loaded NLCs W/O ointment showed higher release and retention and less penetration into the skin, being considered the most appropriate for topical use. The tissue distribution test suggested that the distribution of BMD was mostly in the skin, with insignificant amount of drug detected in the blood of mice. The W/O ointment with BMD-loaded NLCs was applied to live mice, confirming the desirable drug retention in the skin. Moreover, no edema and erythema of the rabbit's skin within 72h was found after the skin irritation study. Results indicated that the BMD-loaded NLCs W/O ointment was effective and safe for the topical use, capable of improving skin retention, reducing adverse effects induced by systemic absorption, and reducing skin irritation.

Eiras *et al.* (EIRAS, F. *et al.*, 2017) prepared and characterized a vitamin E (VE)-loaded NLCs incorporated in a hydrogel (HG) an antioxidant compound with protective and moisturizing properties. VE-loaded NLCs incorporated in a HG showed adequate pharmaceutical properties, *in vitro* biocompatibility with immortalized human keratinocytes (HaCaT) cells, and no irritating potential assessed by the Hen's egg test on the chorioallantoic membrane (HET-CAM), being its use considered safe for skin application. This study emphasized the potential of vitamin E and NLC of improving skin hydration, in parallel to the use of VE-loaded NLCs incorporated in HG as a cosmetic (moisturizing) or dermatological technological strategy able to treat xerosis and other skin disorders, such as AD.

MTX is a drug widely for the systemic treatment of inflammatory skin diseases, thanks to its immunosuppressive and anti-inflammatory activities. However, when administered topically, it exhibits low skin bioavailability. In this sense, Ferreira *et al.* (FERREIRA, M. *et al.*, 2016) formulated MTX-loaded NLCs to assess the potential of NLCs to improve the bioavailability and dermal penetration of MTX. Cetyl palmitate and Miglyol®812 combined with polysorbate 80 allowed a high drug EE, produced NCs with adequate particle size or topical administration (< 500 nm), increased the MTX solubility and favored its skin permeation. MTX-loaded NLCs proved to be less toxic to human keratinocytes and fibroblasts than the free drug, indicating biocompatibility. *In vitro studies* revealed a pattern of biphasic release with rapid initial release, followed by a sustained release, which guarantees the presence of the drug for a longer period, decreasing the frequency of application. The apical-to-basolateral flux of encapsulated MTX increased significantly (up to 2 times) compared to the free MTX suspension. NLCs are thereby presented as an interesting approach to the topical therapy for skin diseases, such as AD, able to improve the bioavailability, safety and effectiveness of low skin bioavailability drugs, as MTX.

2.2. Nanocrystals

Nanocrystals are nanostructures composed only by the drug and a stabilizer (namely non-ionic polymers or ionic surfactants), without the presence of a matrix, and with an increased particle size, ranging from 100 to 1000 nm. By increasing the dissolution rate and saturated solubility of poorly soluble drugs, nanocrystals provide significant improvements in the concentration gradient between the formulation and the skin, resulting in higher drug skin penetration and bioavailability outputs (ASSEM, M. *et al.*, 2019b). The larger surface promotes greater adhesion, with a consequent increase in the retention time at the skin (SANTOS, A.C.

et al., 2019). Moreover, nanocrystals are also able of accumulating in the skin appendages (ASSEM, M. *et al.*, 2019b).

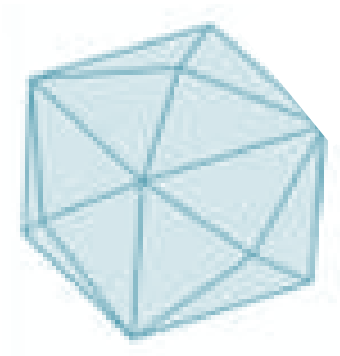

Fig. 5. Nanocrystals structure.

Assem *et al.* (ASSEM, M. *et al.*, 2019b) developed nanocrystals of beclomethasone dipropionate (BDP), a corticosteroid with notable anti-inflammatory activity used in the topical treatment of AD. The optimized formula was subjected to *ex vivo* permeation and drug deposition studies through mouse skin, and was compared to a Beclozone® (0.25% BDP cream). BDP-loaded nanocrystals showed not only a higher deposition of BDP on the skin, but also a lower flux, and, therefore, lower systemic exposed drug, compared to Beclozone® (0.25% BDP cream). Nanocrystals may act efficiently for the topical targeted delivery of low soluble drugs, increasing the skin bioavailability, and reducing systemic side effects associated with the conventional treatment.

2.3. Polymeric nanoparticles

Among the various existing nanocarriers, polymeric NPs have been recognized as the most potential drug delivery systems for topical delivery (PANDEY, M. *et al.*, 2019, ZHUO, F. *et al.*, 2018). Polymeric NPs are made of biocompatible and biodegradable polymers (EROGLU, I. *et al.*, 2016), with surface charge, and a particle size of 200-300 nm. Their high potential for the topical administration of drugs is due to the high EE, the controlled drug release patterns obtainment, together with the prevention of enzymatic drug degradation. An efficient triggered drug release may be achieved in response to the presence or absence of enzymes, pH changes, and other physiological stimuli. The possibility of targeted delivery makes possible the drug delivery to the site of action, minimizing undesirable off-target effects and toxicity (PANDEY, M. *et al.*, 2019, ZHUO, F. *et al.*, 2018). Also, polymeric NPs can act as drugs reservoirs, controlling the permeation in the deeper layers of the skin (BOISGARD, A.S. *et al.*, 2017).

Possibility of accumulation in hair follicles has also been reported (OZCAN, I. *et al.*, 2013). Altogether, these features allow for an increased drug skin bioavailability, rendering these nanostructures as very encouraging drug delivery strategies for skin delivery.

According to preparation methods and formulation components, polymeric NPs may be nanospheres or nanocapsules, in which the active ingredients are dispersed in the polymeric matrix or entrapped in the core, respectively. Several natural or synthetic polymers can be applied in the formulation of polymeric NPs, being chitosan a widely used natural biopolymer. In addition to the biocompatibility and biodegradability ascertained to chitosan, it encloses remarkable advantages for skin delivery attending to its healing and antimicrobial properties (SANTOS, A.C. *et al.*, 2019). Poly(lactide-co-glycolide) (PLGA) is another polymer, which has attracted considerable attention due to its properties, such as biocompatibility and biodegradability, drug protection from degradation, ability of sustained release, and the possibility of targeting to specific tissues (OZCAN, I. *et al.*, 2013).

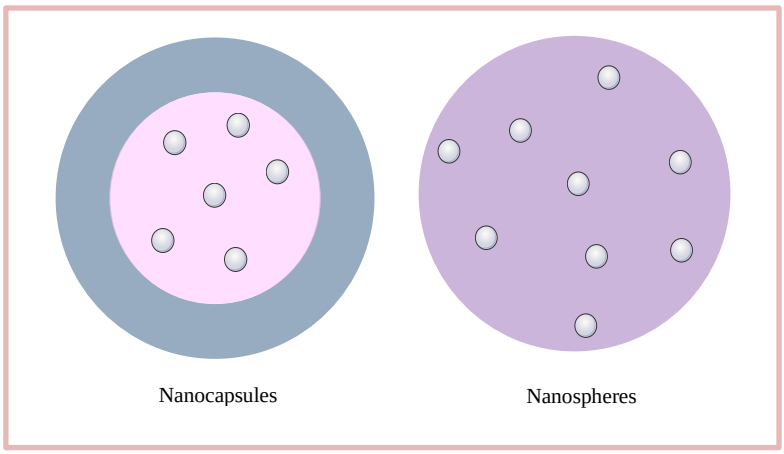

Fig.6. Polymeric nanoparticles structure.

Pan *et al.* (PAN, W. *et al.*, 2016) developed HA and cholesterol (Chol)-based NPs towards the obtainment of an amphiphilic conjugate for the encapsulation of TAC. In order to overcome the difficulty of skin penetration of TAC, NIC at 20% (w/v) was added as a hydrotropic solution to increase the solubility of TAC. *In vitro* permeation studies indicated that free NIC or unloaded HA/Chol-based NPs increased the deposition and permeation of TAC through the skin compared to the commercial ointment (Protopic® 0.1% TAC), while the encapsulation of NIC into HA/Chol-based NPs demonstrated significant improved synergistic effects. A synergistic improvement in the percutaneous TAC delivery by NIC/HA/Chol-based NPs was also demonstrated *in vivo*. HA/Chol-based NPs evidenced a

promoting effect on cell uptake using HaCaT cells. When C6 was incorporated into NIC/HA/Chol-based NPs, fluorescence was similar to unloaded HA/Chol-based NPs, which indicated that cell uptake was mainly attributed to the polymeric matrix of HA/Chol-based NPs. Thereby, despite the need of additional studies, the addition of NIC to HA-Chol-NPs may be a promising new strategy for the skin delivery of TAC towards the treatment of inflammatory skin diseases.

Poly (lactic acid) (PLA)- based NPs are biocompatible and biodegradable NCs, endowed with a strong safety profile record for human use. PLA-based NPs demonstrate a profile of controlled and sustained release of medications in the epidermis, together with a preferential accumulation in hair follicles after topical application. In this sense, Boisgard *et al.* (BOISGARD, A.S. *et al.*, 2017) developed innovative topical semi-solid formulations of fluorescent PLA-based NPs as anti-inflammatory drug delivery systems for the local treatment of inflammatory skin diseases, including AD and psoriasis. Unlike PLA-based NPs suspensions, innovative semi-solid formulations allowed easy skin application. The investigations carried out in terms of the spreadability, rheological behavior and conservation of the PLA-based NPs structure led to the selection of two semi-solid formulations (Avicel and Viscarin). *In vivo* studies carried out in mice for 8 consecutive days showed that neither of the two formulations induced skin irritation. *Ex vivo* penetration and permeation studies using VitroPharma diffusion cells demonstrated that the permeation of the encapsulated fluorophore through mouse skin explants decreased with semi-solid formulations with PLA-based NPs compared to PLA-based NPs suspensions, while the penetration profile was not significantly different. This reveals a great potential of these formulations containing PLA-based NPs for topical and targeted SC delivery directed, with minimal systemic absorption levels.

Zabihi *et al.* (ZABIHI, F. *et al.*, 2018) presented NPs of poly (lactide-co-glycerol) (PLG) to topically deliver TAC. No significant cytotoxicity was found in primary human fibroblasts and keratinocytes, thus confirming the high biocompatibility of the synthesized PLG polymer. Moreover, the incubation of PLG with skin lysates has confirmed its biodegradability, reducing the risk of toxicity and irritation after topical administration. Cutaneous absorption studies suggested that PLG-based NPs increased the levels of TAC in SC, epidermis, and dermis, compared to the Protopic® (0.03% TAC cream). To evaluate the anti-inflammatory activity, an *in vitro* study was carried out, using a model of inflammatory skin disease, deficient in filaggrin and containing TCD4 + cells, characterized by high levels of TSLP and IL-2. Moreover, *in vitro* studies confirmed the similar ability of TAC-loaded PLG-based NPs to suppress IL-2 expression in relation to the Protopic® (0.03% TAC cream), together with a non-expected

markedly decreased of TSLP after topical application of PLG-based NPs, compared to cream. PLG-based NPs has proven to be highly functional, water-soluble, with high load capacity, strong drug delivery skin efficiency, biodegradable, and biocompatible. The low toxicity and high anti-inflammatory efficacy highlighted the capabilities of this promising system to improve the topical delivery of TAC towards the treatment of inflammatory skin diseases, in which AD is included.

Try *et al.* (TRY, C. *et al.*, 2016) developed polymeric NPs with a particle size of 70 nm and 300 nm, which were studied regarding cutaneous distribution in mice and pigs with oxazolone-induced AD. PLGA was the polymer used and it was linked to fluoresceinamine to ensure the detection of NPs in the skin. None of the NPs penetrated healthy skin. The penetration of lower sized-NPs into the inflamed skin was greater than the higher-sized, suggesting that the penetration mechanism is dependent on the particle size of the polymeric NPs. This meant that smaller polymeric NPs (< 100 nm) can penetrate and accumulate in the viable epidermis of the inflamed skin, while larger NPs remain mostly on the surface of the epidermis. Moreover, a regression of the inflammation process was verified after the application of polymeric NPs, namely by a decrease in clinical signs of AD, such as erythema and edema. The ability to specifically penetrate inflamed skin combined with minimal accumulation in healthy skin reduces side effects related to drugs exposure on healthy skin typical of conventional formulations, thereby confirming the high potential of PLGA- NPs for the selective drug delivery in the treatment of inflammatory skin diseases, including AD.

To circumvent the systemic side effects of CsA, Badihi *et al.* (BADIHI, A. *et al.*, 2020) developed a topical anhydrous formulation of CsA-loaded PLGA-based NPs. *In vitro* studies using mouse activated splenocytes have shown that encapsulated CsA was biologically active, by inhibiting cell proliferation and interleukin (IL)-2 secretion. *Ex vivo* studies have shown that PLGA-based NPs increase the CsA penetration into the different layers, being the drug concentration in the dermis lower than in the epidermis of porcine ear skin. *Ex vivo* efficacy has also been demonstrated in a culture of human skin organs, particularly by a significantly reduction of pro-inflammatory cytokines secretion. To confirm the general activity of CsA loaded in PLGA-based NPs, the anti-inflammatory response *in vivo* was studied, and a higher efficacy in terms of preservation of the integrity of the skin, prevention of skin thickening, serum levels of IFN-γ, OVA-IgE and IL-4 reduction, and a significant anti-inflammatory response were exhibited, leading to an ear swelling decline of 30%. CsA-loaded PLGA-based NPs seemed to reach the epidermis and dermis in therapeutic concentrations, which makes

those nanostructures as promising topical drug delivery systems as an alternative to the systemic CsA administration, with minimized side effects.

Ozcan *et al.* (OZCAN, I. *et al.*, 2013) encapsulated BMV in PLGA-based NPs or lecithin (LEC)/chitosan-based NPs, to compare the effect of the two compositions in the performance of the NPs. Developed NPs were incorporated into a chitosan gel to obtain a viscosity suitable for easy application to the skin. *Ex vivo* permeation studies have shown that both NPs chitosan gel formulations have increased the epidermal accumulation of BMV compared to Betnovate® (0.1% BMV cream) which is traduced in a high advantage considering the epidermis as the target site of the topical corticosteroid treatment. In comparison with PLGA-based NPs, LEC/chitosan-based NPs retained a greater amount of BMV in the epidermis, being able to prolong the residence time in the skin and suppress transdermal delivery, minimizing the risk of systemic toxicity. The effectiveness of both formulations was evaluated *in vivo*, using the carrageenan-induced edema model in the rat's paw. The PLGA-based NPs chitosan gel formulation inhibited the formation of paw edema, with no significant difference in relation to the cream. On the other hand, the LEC/chitosan-based NPs chitosan gel formulation showed greater anti-inflammatory activity when compared to the cream, even containing only 1/10 of the BMV concentration. Moreover, LEC/chitosan-based NPs exhibited a skin whitening ability identical to the commercial cream. The TEWL measurements confirmed no change was encountered in the integrity of the skin after application of the NPs chitosan gel formulation. Thus, although both NPs are effective in delivering BMV to the skin, LEC/chitosan-based NPs are more advantageous drug delivery systems for the effective and safe treatment of inflammatory skin diseases, as AD.

Hussain *et al.* (HUSSAIN, Z. *et al.*, 2013) developed chitosan-based NPs for the topical co-administration of hydrocortisone (HC) and hydroxytyrosol (HT), and, in order to improve skin contact time and skin hydration, NPs were incorporated into a cream (W/O). *Ex vivo* permeation studies revealed that NPs were able of promoting a well-controlled HC permeation flow reduction, which may impact in the minimization of systemic adverse effects. Chitosan-based NPs also exhibited a greater accumulation of HC and HT in the epidermis and dermis, evidencing potential to facilitate localized drug administration, and to reduce the risk-benefit rates of corticosteroids. *In vivo* clinical studies, revealed that mice treated with chitosan-based NPs controlled more effectively the TEWL, intensity of erythema, dermatitis index and skin thickness. Such behavior was attributed to the synergistic effects of HC and HT to promote SC regeneration, and to decrease the inflammatory cascades underlying the

pathogenesis of AD. Chitosan may also has facilitated the reduction of TEWL, contributing to wound healing.

The hydrophilic nature of chitosan constitutes a limitation regarding the encapsulation of hydrophobic drugs. Similarly, to the previously discussed work of Pan *et al.* (PAN, W. *et al.*, 2016), in order to overcome the high hydrophobicity of TAC and increase its payload on chitosan based-NPs, Yu *et al.* (YU, K. *et al.*, 2018) added the hydrotropic agent NIC to form TAC-loaded NIC/chitosan-based NPs. NIC significantly increased the EE% of the TAC. *In vitro* and *in vivo* skin permeation studies have shown that NIC in combination with chitosan-based NPs synergistically improved the permeation and retention of TAC in the skin compared to Protopic® (0.1% TAC ointment). TAC-loaded NIC/chitosan-based NPs (0.03%, 0.05%, or 0.1%, w/v) markedly decreased the dermatitis score, ear thickness, and spleen index, compared to Protopic (0.1%, w/v). In addition, histopathological analysis showed that the treatment with TAC-loaded NIC/chitosan-based NPs decreased further the epidermal thickness and the number of infiltrated mast cells compared with Protopic (0.1% TAC ointment). Finally, the application of TAC-loaded NIC/chitosan-based NPs significantly decreased the total serum IgE levels, when compared to Protopic. NIC seems to play a dual role in the TAC-loaded NIC/chitosan-based NPs system, since, in addition to improving percutaneous permeation of TAC it also works as an adjuvant therapy in the relief of cutaneous symptoms of AD. In this way, NIC/chitosan-based NPs, with only 1/3 of the dose of TAC used in Protopic (0.1% TAC ointment), achieved superior anti-inflammatory and immunosuppressive effects, and a remarkable improvement in skin lesions. There by, NIC/chitosan-based NPs are able of reducing the required dose of TAC, and thus minimize the adverse effects, maintaining a good therapeutic efficacy, ultimately constituting a promising TAC-loaded drug delivery system for the effective topical treatment of AD.

Zhuo *et al.* (ZHUO, F. *et al.*, 2018) developed TAC-loaded HA/chitosan-based NPs for topical administration. The coating of the TAC-loaded chitosan-based NPs with HA resulted in a controlled release TAC *in vitro* profile. *Ex vivo* permeation studies showed that the flux of TAC from the HA/chitosan-based NPs was lower compared to the chitosan-based NPs, which was explained by the mucoadhesive properties of the HA, which provoke the NPs adherence to the appendages of the skin, and promote drug release in a controlled manner. Such behavior is beneficial since it is able of reducing the topical application frequency of the medication. Moreover, HA/chitosan-based NPs showed a superior amount of TAC retained in the epidermis and dermis, depicting the targeting ability, as a clear sign of biopharmaceutical efficiency. *In vivo*, HA/chitosan-based NPs promoted a higher reduction of TEWL, erythema

intensity, and dermatitis score, thus revealing superior anti-AD efficacy compared to chitosan-based NPs. Besides, HA/chitosan-based NPs promoted a decrease in the infiltration of inflammatory cells in the dermis and the degree of acanthosis, together with the capacity of restoration of the integrity of the skin.

Pandey *et al.* (PANDEY, M. *et al.*, 2019) developed BMV-loaded chitosan-based NPs. To further improve BMV targeting efficiency and enhance its therapeutic efficacy, BMV-loaded chitosan-based NPs were coated with hyaluronic acid (HA). The results showed that BMV-loaded HA/chitosan-based NPs exhibited adequate physicochemical characteristics and were considered physically stable. *In vitro* release studies revealed that BMV-loaded HA/chitosan-based NPs exhibited a more sustained and controlled drug release pattern in relation to the uncoated counterpart, which meets the requirements necessary to prolong the BMV retention time, improving therapeutic efficacy, and reducing the need for frequent topical applications. The nanoencapsulation of BMV into chitosan-based NPs improved the efficiency of BMV *ex vivo* permeation. However, due to the mucoadhesive features of HA, BMV-loaded HA/chitosan-based NPs exhibited a comparatively superior drug retention in the epidermis and dermis. This study suggests that BMV-loaded HA/chitosan-based NPs may be a promising NC for the targeted BMV topical delivery with improved anti-AD efficacy.

Barbosa *et al.* (BARBOSA, A.I. *et al.*, 2019) developed fucoidan/chitosan-based NPs for topical administration of MTX. The optimized formulation (proportion of 5:1 of fucoidan/chitosan ratio) was biocompatible with human fibroblasts and keratinocytes, and was able to significantly inhibit the production of pro-inflammatory cytokines from activated human monocytes, suggesting that MTX-loaded fucoidan/chitosan-based NPs can act as strong inflammation inhibitors. *In vitro* skin permeation studies have shown that MTX-loaded fucoidan/chitosan-based NPs evidence higher permeation capabilities in pig's ear skin compared to free MTX. The composition of the NPs affected the flow rate of MTX, pointing to a fucoidan interaction with the skin, capable of modulating the skin permeability of the NPs. In conclusion, fucoidan/chitosan-based NPs exert a potent anti-inflammatory effect and increase skin permeation, and are considered safe formulations, with potential application for the topical administration of MTX in the treatment of skin-related inflammatory diseases, as AD.

Patients with AD or psoriasis show an excessive amount of human neutrophil elastase (HNE) due to an imbalance between this proteolytic enzyme and its endogenous inhibitors, which leads to severe tissue damage. In this context, Marto *et al.* (MARTO, J. *et al.*, 2018)

associated a promising new synthetic HNE inhibitor (ER143) with starch-based nanocapsules, leading to the development of an optimized formula with adequate pharmaceutical attributes. ER143-loaded starch-based nanocapsules showed a controlled drug release profile, and high permeation and drug retention in pig skin in *in vitro* conditions, which was found to be extremely beneficial attending to the location of the HNE receptors in the dermis. The study of the anti-inflammatory activity *in vivo* showed that erythema and edema were attenuated by 98% after local application on mice ear, revealing a superior pharmacological performance when compared to a commercial lotion (0.1% hydrocortisone butyrate). Despite the low anti-inflammatory activity of the ER143 solution, ER143 improves its anti-inflammatory effects when encapsulated in the starch-based nanocapsules, which proves the synergistic action of starch in the anti-inflammatory activity of ER143. In general, all these studies have shown that the encapsulation of ER143 in starch-based nanocapsules can be a useful and promising approach for the treatment of inflammatory skin diseases.

Rosa *et al.* (ROSA, P. *et al.*, 2019) developed Eudragit® RL 100-based nanocapsules for the incorporation of desonide (DES), a low-power topical corticosteroid used in the treatment of AD. Two types of nanocapsules were prepared, by using medium-chain triglycerides (MCT) or açai oil as the oil core. Both formulations were found to be physically and chemically stable at room temperature. Açai oil-based nanocapsules showed a greater effect against UV-A and UV-C radiation, emphasizing that the type of oil impacts on the photostability of DES. As topical corticosteroids act on the skin at the level of fibroblasts and keratinocytes, the phototoxicity of the formulations was tested on murine T3 fibroblasts and human HaCaT keratinocytes, and no phototoxicity was found. Besides, all formulations were classified as mildly irritating. *In vitro* release studies have shown that DES-loaded nanocapsules showed a biphasic release profile, characterized by an initial rapid release, followed by a prolonged release. Given the advantages presented, the suspensions of DES-loaded nanocapsules developed where be considered promising for the topical treatment of inflammatory skin diseases, enclosing AD.

2.4. Polymeric micelles

In recent years, polymeric micelles have emerged as promising NCs, due to their amphiphilic constitution. Polymeric micelles are composed of a hydrophobic inner core stabilized by a hydrophilic outer face, resulting from the combination of amphiphilic co-polymers in concentrations above their critical micellar concentration. Polymeric micelles

exhibit a particle size that ranges between 10 nm and 100 nm. The common application of polymeric micelles in the pharmaceutical field focus the enhancement of the bioavailability of orally or intravenously-administered poorly soluble drugs. Additionally, polymeric micelles can also be utilized for topical administration, as a viable technological strategy to increase the drug solubility, and to enhance its penetration in the SC, and deposition into the dermis (ASSEM, M.*et al.*, 2019a).

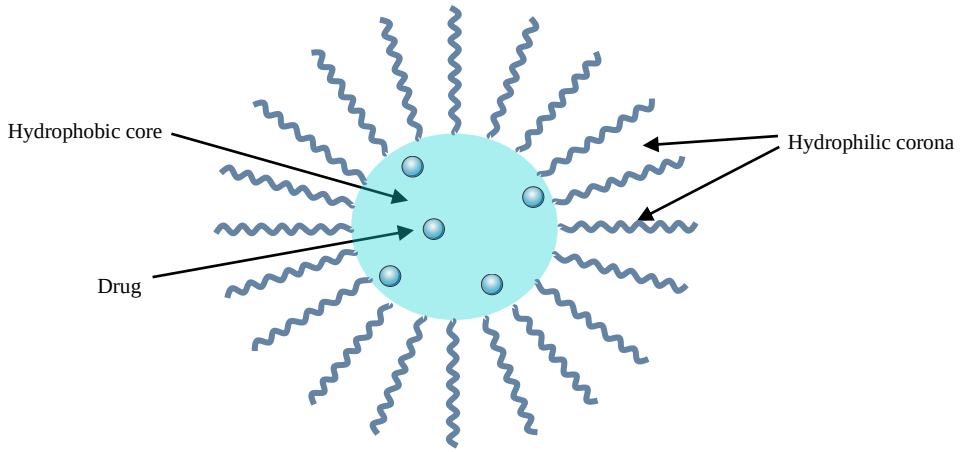

Fig.7. Polymeric micelles structure.

Assem *et al.* (ASSEM, M. *et al.*, 2019a) developed polymeric micelles for the dermal administration of BDP. The ideal PM formula was incorporated into a hydroxypropylmethylcellulose (HPMC) hydrogel, and was subjected to *ex vivo* permeation and drug deposition studies, using newborn CD-1 mice skin, in order to compare with Beclozone® (0.25% BDP cream). BDP-loaded polymeric micelles incorporated in HPMC-based hydrogel achieved a specific delivery to the skin, evidencing a higher amount of BDP retained in the skin, and minimum systemic levels. Such behavior will possibly result in lower side effects and superior patient compliance. An *in vivo* histopathological study using Wistar rats was performed to assess the efficiency of healing. BDP-loaded polymeric micelles HPMC hydrogel allowed tissue regeneration in a shorter period (6 days) compared to the commercial cream (12 days), representing a very advantageous strategy for faster and efficacious treatment of skin lesions, and ultimately for improving the quality of life of patients with AD.

3. Safety of nanocarriers-based dermatological formulations for atopic dermatitis: regulatory and toxicological aspects

The use of nanotechnology in products for the topical treatment of AD is recognized as a promising strategy due to the unique physical and chemical characteristics exhibited by NPs. However, some toxicological aspects still need to be considered. Although very advantageous, NCs also have potential risks related to the small particle size presented, that may allow them to stronger penetrate the skin, and possibly reach the systemic circulation (NASIR, A., 2010, SOUTO, E.B. *et al.*, 2019). In fact, the nanometric dimension is responsible for an increase in the surface area of contact, enhancing the interaction between the NC and biological systems, and thus to be more reactive (GUPTA, M. *et al.*, 2012, NEL, A. *et al.*, 2006). Thus, it is expected that nanocarriers may hold higher potential for toxicity, with consequent harmful effects *in vivo* (SOUTO, E.B. *et al.*, 2019). Intensive research is necessary to reach out the real capability of penetrating the skin achieved by the NCs, how long those systems remain, and which problems they can cause. Particles of 7000 nm of particle size or smaller can penetrate damaged skin, as is the case of what happens in AD (NASIR, A., 2010). Therefore, the *in vitro* and *in vivo* studies developed must be carried out with damaged skin, since it is more permeable and the pharmacokinetic and pharmacodynamic profiles of the NCs may be different than when applied to healthy skin (WANG, S. *et al.*, 2014). Ilves *et al.* (ILVES, M., 2014) developed a study in which ZnO NPs have been applied to injured skin in a prototype of AD. As predicted, ZnO NPs can penetrate deep into the skin layers, and stimulate the production of IgE antibodies systemically, evidencing the propensity that formulations containing these NPs may promote allergic effects on the skin, while suppressing inflammatory activities. Hirai *et al.* (HIRAI, T. *et al.*, 2015) demonstrated that amorphous silica NPs (SNPs) penetrate the skin and promote various harmful biological effects, representing a risk factor to aggravate any immune skin disease. The reduced particle size of SNPs induced the production of TSLP and IL-18, which has led to the systemic Th2 response and the worsening of skin lesions. However, it was concluded that the adequate alteration of the physical-chemical characteristics of the SNPs, namely the particle size, is critical in the development of safe NPs. Histological investigation and measurement of ear thickness did not show the worsening of AD-type skin lesions after SNPs cutaneous application. It was described that NPs did not directly affect the allergen-specific immune response. However, when present in agglomerates adsorbed by allergens, SNPs have led to a low rate of IgG/IgE ratio, an essential risk factor for atopic human allergies. Thus, it has been suggested that, by reducing interactions between NCs and allergens, the safety of SNPs may be increased. In addition to the change of physical

and chemical characteristics, the application of coatings in NCs surfaces to try to reduce their toxic effects is also being performed (PRAJITHA, N. *et al.*, 2019). Another aspect to be aware of is that NCs usually have a large amount of surfactants, which can induce adverse effects, such as erythema, edema, irritation or toxicity (SANTOS, A.C. *et al.*, 2020). Besides, the studies developed usually assess the toxicity in short term, so it is worth highlighting the importance of assessing the toxicity of NCs in the long term, in order to predict the chronic exposure of formulations based on nanotechnology (DUNCAN, R. and GASPAR, R., 2011). Given the increased development of nanoformulations, regulatory organizations, such as the European Medicines Agency (EMA) or the Food and Drug Administration (FDA), are concerned with developing guidelines related to toxicity assessment. Currently, there are only industry standards for assessing toxicity in nanotechnology-based formulations, which were developed by the International Organization for Standardization (IOS) in association with the Organization for Economic Cooperation and Development (OECD) (SANTOS, A.C. *et al.*, 2020). According to IOS, a "nanomaterial" is a material with an external dimension at the nanoscale or having an internal or surface structure at the nanoscale with a particle size in the range of 1 to 100 nm. However, not all regulatory organizations agreed with this definition, and other definitions have been developed (SANTOS, A.C. *et al.*, 2020). Also, there is still the question of deciding whether nanotechnology-based formulations for drug delivery are considered medical products or devices (KELLY, B., 2010). Therefore, an appropriate regulatory framework is necessary, namely the development of specific guidelines and regulations regarding the manufacture and evaluation of pharmacokinetic, pharmacodynamic and toxicological profiles, to ensure the safety and efficiency of nanotechnology-based formulations and allow approval to place on the market (HOFMANN-AMTENBRINK, M. *et al.*, 2014, KELLY, B., 2010).

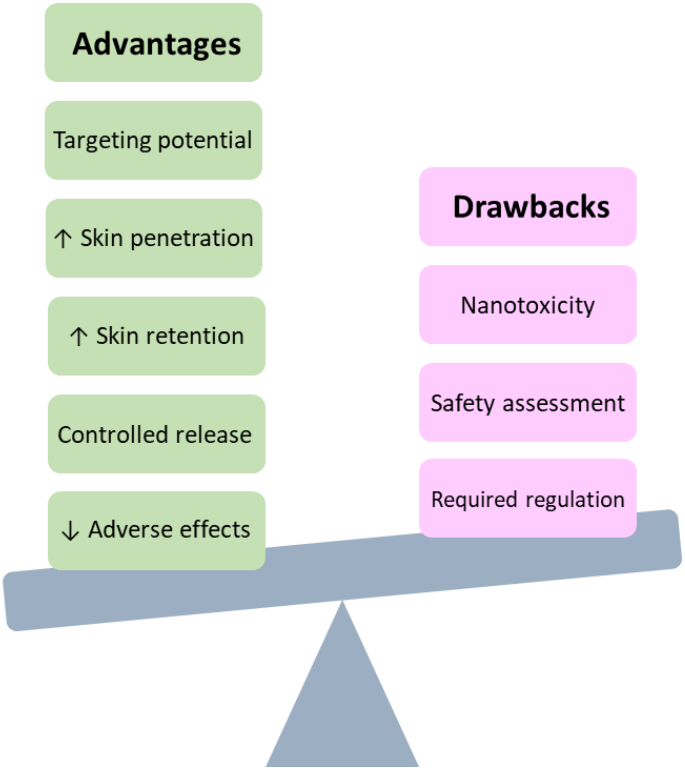

Fig.8. Major advantages and drawbacks associated to nanocarriers-based dermopharmaceutical formulations for atopic dermatitis.

4. Conclusions and future prospects

AD is one of the most prevalent inflammatory skin disorders, with a very complex pathophysiology which is not fully understood. The mechanisms underlying the disease is a must to define therapeutic targets and allow the development of an effective treatment. In fact, non-pharmacological approaches are well accepted for the treatment of mild to moderate AD, however, chronic AD cases require pharmacological treatment, which, apart from the recognized limited efficacy, is associated with many adverse effects, leading to decreased treatment tolerability by patients and therapeutic failure. In this sense, new and advanced targeted therapies are demanded. Nanotechnology assumes a pivotal role as a reliable technological tool for the development of new drug delivery systems, mediated by the use of drug-loaded nanocarriers, i.e., structures sized at the nanoscale where the drug is encapsulated. In the context of the topical treatment of AD, several nanocarriers, particularly nanoemulsions, liposomes, ethosomes, transfersomes, SLNs, NLCs, nanocrystals, polymeric NPs, and polymeric micelles have been investigated. Nanocarriers enable controlled and targeted drug delivery, and favor the skin retention at the target site, improving the skin permeation and bioavailability, while avoiding off-target effects. Such behavior enables the

achievement of the therapeutic superiority over conventional therapies, by aiming to effectively control the severity and progression of AD. Several studies have already been carried out demonstrating the therapeutic efficacy of NCs in the management and progression prevention of AD its, much still needs to be investigated and accomplished for the generation of marketable products. In addition to the lack of data related to the characterization of nanoformulations, such as stability tests, another major gap is the lack of data regarding the safety profile. Establishing a safety profile is extremely important, and requires international guidance and regulation, which is still scarce in this matter, making market approval and clinical translation a difficult path. Moreover, additional studies are needed to assess the therapeutic potential of NCs in clinically relevant and specific models of disease. It is expected that new nanotechnology-based methodologies will revolutionize aspects of clinical dermatology, and soon will offer NC-based products for the efficient treatment of AD prone to be translated to the clinic.

5. References

AKHTAR, NIDA; VERMA, ANURAG; PATHAK, KAMLA - Exploring preclinical and clinical effectiveness of nanoformulations in the treatment of atopic dermatitis: Safety aspects and patent reviews. *Bulletin of Faculty of Pharmacy, Cairo University*. Vol. 55. n.° 1 (2017). p. 1-10.

ALAM, MD SARFARAZ; ALI, MD SAJID; ALAM, NAWAZISH; SIDDIQUI, MASOOM RAZA; SHAMIM, MD; SAFHI, M. M. - In vivo study of clobetasol propionate loaded nanoemulsion for topical application in psoriasis and atopic dermatitis. *Drug Invention Today*. Vol. 5. n.° 1 (2013). p. 8-12.

ASSEM, M.; KHOWESSAH, O. M.; GHORAB, D. - Optimization and Evaluation of Beclomethasone Dipropionate Micelles Incorporated into Biocompatible Hydrogel Using a Sub-Chronic Dermatitis Animal Model. *AAPS PharmSciTech*. Vol. 20. n.° 4 (2019a). p. 152.

ASSEM, MENNA; KHOWESSAH, OMNEYA MOHAMMED; GHORAB, DALIA - Nano-crystallization as a tool for the enhancement of beclomethasone dipropionate dermal deposition: Formulation, in vitro characterization and ex vivo study. *Journal of Drug Delivery Science and Technology*. Vol. 54. (2019b). p. 101318.

BADIHI, A.; FRUSIC-ZLOTKIN, M.; SOROKA, Y.; BENHAMRON, S.; TZUR, T.; NASSAR, T.; BENITA, S. - Topical nano-encapsulated cyclosporine formulation for atopic dermatitis treatment. *Nanomedicine*. Vol. 24. (2020). p. 102140.

BARBOSA, A. I.; COSTA LIMA, S. A.; REIS, S. - Development of methotrexate loaded fucoidan/chitosan nanoparticles with anti-inflammatory potential and enhanced skin permeation. *Int J Biol Macromol*. Vol. 124. (2019). p. 1115-1122.

BOISGARD, A. S.; LAMRAYAH, M.; DZIKOWSKI, M.; SALMON, D.; KIRILOV, P.; PRIMARD, C.; PIROT, F.; FROMY, B.; VERRIER, B. - Innovative drug vehicle for local treatment of inflammatory skin diseases: Ex vivo and in vivo screening of five topical formulations containing poly(lactic acid) (PLA) nanoparticles. *Eur J Pharm Biopharm*. Vol. 116. (2017). p. 51-60.

BUNJES, H.; WESTESEN, K.; KOCH, M.H.J. - Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. *Int J Pharm*. Vol. 129. n.° 1 (1996). p. 159-173.

CARRERAS, J. J.; TAPIA-RAMIREZ, W. E.; SALA, A.; GUILLOT, A. J.; GARRIGUES, T. M.; MELERO, A. - Ultraflexible lipid vesicles allow topical absorption of cyclosporin A. *Drug Deliv Transl Res*. Vol. 10. n.° 2 (2020). p. 486-497.

CHAUHAN, SWATI; GULATI, NEHA; NAGAICH, UPENDRA - Fabrication and evaluation of ultra deformable vesicles for atopic dermatitis as topical delivery. *International Journal of Polymeric Materials and Polymeric Biomaterials*. Vol. 68. n.° 5 (2018). p. 266-277.

DAMIANI, G.; EGGENHOFFNER, R.; PIGATTO, P. D. M.; BRAGAZZI, N. L. - Nanotechnology meets atopic dermatitis: Current solutions, challenges and future prospects. Insights and implications from a systematic review of the literature. *Bioact Mater*. Vol. 4. (2019). p. 380-386.

DUNCAN, R.; GASPAR, R. - Nanomedicine(s) under the microscope. *Mol Pharm*. Vol. 8. n.° 6 (2011). p. 2101-41.

EIRAS, F.; AMARAL, M. H.; SILVA, R.; MARTINS, E.; LOBO, J. M. S.; SILVA, A. C. - Characterization and biocompatibility evaluation of cutaneous formulations containing lipid nanoparticles. *Int J Pharm*. Vol. 519. n.° 1-2 (2017). p. 373-380.

EL-MENSHAWE, SHAHIRA F.; SAYED, OSSAMA M.; ABOU-TALEB, HEBA A.; EL TELLAWY, NADA - Skin permeation enhancement of nicotinamide through using fluidization and deformability of positively charged ethosomal vesicles: A new approach for treatment of atopic eczema. *Journal of Drug Delivery Science and Technology*. Vol. 52. (2019). p. 687-701.

EROGLU, I.; AZIZOGLU, E.; OZYAZICI, M.; NENNI, M.; GURER ORHAN, H.; OZBAL, S.; TEKMEN, I.; ERTAM, I.; UNAL, I.; OZER, O. - Effective topical delivery systems for corticosteroids: dermatological and histological evaluations. *Drug Deliv*. Vol. 23. n.° 5 (2016). p. 1502-13.

ESPINOZA, L. C.; SILVA-ABREU, M.; CALPENA, A. C.; RODRIGUEZ-LAGUNAS, M. J.; FABREGA, M. J.; GARDUNO-RAMIREZ, M. L.; CLARES, B. - Nanoemulsion strategy of pioglitazone for the treatment of skin inflammatory diseases. *Nanomedicine*. Vol. 19. (2019). p. 115-125.

FANG, C. L.; AL-SUWAYEH, S. A.; FANG, J. Y. - Nanostructured lipid carriers (NLCs) for drug delivery and targeting. *Recent Pat Nanotechnol*. Vol. 7. n.° 1 (2013). p. 41-55.

FERREIRA, M.; SILVA, E.; BARREIROS, L.; SEGUNDO, M. A.; COSTA LIMA, S. A.; REIS, S. - Methotrexate loaded lipid nanoparticles for topical management of skin-related

diseases: Design, characterization and skin permeation potential. *Int J Pharm*. Vol. 512. n.° 1 (2016). p. 14-21.

GARCÊS, A.; AMARAL, M. H.; SOUSA LOBO, J. M.; SILVA, A. C. - Formulations based on solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for cutaneous use: A review. *Eur J Pharm Sci*. Vol. 112. (2018). p. 159-167.

GUNGOR, S.; REZIGUE, M. - Nanocarriers mediated topical drug delivery for psoriasis treatment. *Curr Drug Metab*. Vol. 18. n.° 5 (2017). p. 454-468.

GUPTA, M.; AGRAWAL, U.; VYAS, S. P. - Nanocarrier-based topical drug delivery for the treatment of skin diseases. *Expert Opin Drug Deliv*. Vol. 9. n.° 7 (2012). p. 783-804.

HIRAI, T.; YOSHIOKA, Y.; TAKAHASHI, H.; ICHIHASHI, K.; UDAKA, A.; MORI, T.; NISHIJIMA, N.; YOSHIDA, T.; NAGANO, K.; KAMADA, H.; TSUNODA, S.; TAKAGI, T.; ISHII, K. J.; NABESHI, H.; YOSHIKAWA, T.; HIGASHISAKA, K.; TSUTSUMI, Y. - Cutaneous exposure to agglomerates of silica nanoparticles and allergen results in IgE-biased immune response and increased sensitivity to anaphylaxis in mice. *Part Fibre Toxicol*. Vol. 12. (2015). p. 16.

HOFMANN-AMTENBRINK, M.; HOFMANN, H.; HOOL, A.; ROUBERT, F. - Nanotechnology in medicine: European research and its implications. *Swiss Med Wkly*. Vol. 144. (2014). p. w14044.

HUA, S. - Lipid-based nano-delivery systems for skin delivery of drugs and bioactives. Vol. 6. n.° 219 (2015).

HUSSAIN, Z.; KATAS, H.; MOHD AMIN, M. C.; KUMOLOSASI, E.; BUANG, F.; SAHUDIN, S. - Self-assembled polymeric nanoparticles for percutaneous co-delivery of hydrocortisone/hydroxytyrosol: an ex vivo and in vivo study using an NC/Nga mouse model. *Int J Pharm*. Vol. 444. n.° 1-2 (2013). p. 109-19.

ILVES, MARIT - Topically applied ZnO nanoparticles suppress allergen induced skin inflammation but induce vigorous IgE production in the atopic dermatitis mouse model. *Particle and Fibre Toxicology*. Vol. 11:38. (2014).

JUNG, S. H.; CHO, Y. S.; JUN, S. S.; KOO, J. S.; CHEON, H. G.; SHIN, B. C. - Topical application of liposomal cobalamin hydrogel for atopic dermatitis therapy. *Pharmazie*. Vol. 66. n.° 6 (2011). p. 430-5.

KAMRA, M.; DIWAN, A. - Liposomes in dermatological diseases. *JAPTRonline*. Vol. 5. n.° 2 (2017).

KANG, J. H.; CHON, J.; KIM, Y. I.; LEE, H. J.; OH, D. W.; LEE, H. G.; HAN, C. S.; KIM, D. W.; PARK, C. W. - Preparation and evaluation of tacrolimus-loaded thermosensitive solid lipid nanoparticles for improved dermal distribution. *Int J Nanomedicine*. Vol. 14. (2019). p. 5381-5396.

KANG, M. J.; EUM, J. Y.; JEONG, M. S.; PARK, S. H.; MOON, K. Y.; KANG, M. H.; KIM, M. S.; CHOI, S. E.; LEE, M. W.; LEE, D. I.; BANG, H.; LEE, C. S.; JOO, S. S.; LI, K.; LEE, M. K.; SEO, S. J.; CHOI, Y. W. - Tat peptide-admixed elastic liposomal formulation of hirsutenone for the treatment of atopic dermatitis in NC/Nga mice. *Int J Nanomedicine*. Vol. 6. (2011). p. 2459-67.

KANG, M. J.; EUM, J. Y.; PARK, S. H.; KANG, M. H.; PARK, K. H.; CHOI, S. E.; LEE, M. W.; KANG, K. H.; OH, C. H.; CHOI, Y. W. - Pep-1 peptide-conjugated elastic liposomal formulation of taxifolin glycoside for the treatment of atopic dermatitis in NC/Nga mice. *Int J Pharm*. Vol. 402. n.° 1-2 (2010). p. 198-204.

KANG, MYUNG JOO - Facilitated Skin Permeation of Oregonin by Elastic Liposomal Formulations and Suppression of Atopic Dermatitis in NC/Nga Mice. *Pharmaceutical Society of Japan*. Vol. 33 No.1. (2010). p. 100-106.

KAPOOR, B.; GUPTA, R.; GULATI, M.; SINGH, S. K.; KHURSHEED, R.; GUPTA, M. - The Why, Where, Who, How, and What of the vesicular delivery systems. *Adv Colloid Interface Sci*. Vol. 271. (2019). p. 101985.

KELLY, BRIAN - Nanomedicines: regulatory challenges and risks ahead. (2010).

KIM, S. T.; JANG, D. J.; KIM, J. H.; PARK, J. Y.; LIM, J. S.; LEE, S. Y.; LEE, K. M.; LIM, S. J.; KIM, C. K. - Topical administration of cyclosporin A in a solid lipid nanoparticle formulation. *Pharmazie*. Vol. 64. n.° 8 (2009). p. 510-4.

KONG, XIN; ZHAO, YUAN; QUAN, PENG; FANG, LIANG - Development of a topical ointment of betamethasone dipropionate loaded nanostructured lipid carrier. *Asian Journal of Pharmaceutical Sciences*. Vol. 11. n.° 2 (2016). p. 248-254.

KOVAČEVIĆ, ANĐELKA B.; MÜLLER, RAINER H.; KECK, CORNELIA M. - Formulation development of lipid nanoparticles: Improved lipid screening and development of tacrolimus loaded nanostructured lipid carriers (NLC). *International Journal of Pharmaceutics*. Vol. 576. (2020). p. 118918.

LASIC, D.D.; PAPAHADJOPOULOS, D. - Medical Applications of Liposomes. Amsterdam: Elsevier Science, 1998. ISBN: 9780444829177.

LI, G.; FAN, C.; LI, X.; FAN, Y.; WANG, X.; LI, M.; LIU, Y. - Preparation and in vitro evaluation of tacrolimus-loaded ethosomes. *ScientificWorldJournal*. Vol. 2012. (2012a). p. 874053.

LI, G.; FAN, Y.; FAN, C.; LI, X.; WANG, X.; LI, M.; LIU, Y. - Tacrolimus-loaded ethosomes: physicochemical characterization and in vivo evaluation. *Eur J Pharm Biopharm*. Vol. 82. n.° 1 (2012b). p. 49-57.

MARTO, J.; RUIVO, E.; LUCAS, S. D.; GONCALVES, L. M.; SIMOES, S.; GOUVEIA, L. F.; FELIX, R.; MOREIRA, R.; RIBEIRO, H. M.; ALMEIDA, A. J. - Starch nanocapsules containing a novel neutrophil elastase inhibitor with improved pharmaceutical performance. *Eur J Pharm Biopharm*. Vol. 127. (2018). p. 1-11.

MARTO, J.; VITOR, C.; GUERREIRO, A.; SEVERINO, C.; ELEUTERIO, C.; ASCENSO, A.; SIMOES, S. - Ethosomes for enhanced skin delivery of griseofulvin. *Colloids Surf B Biointerfaces*. Vol. 146. (2016). p. 616-23.

MEHNERT, W.; MÄDER, K. - Solid lipid nanoparticles: Production, characterization and applications. *Adv Drug Deliv Rev*. Vol. 47. n.° 2 (2001). p. 165-196.

MONTENEGRO, L.; LAI, F.; OFFERTA, A.; SARPIETRO, M.G.; MICICCHÈ, L.; MACCIONI, A.M.; VALENTI, D.; FADDA, A.M. - From nanoemulsions to nanostructured lipid carriers: A relevant development in dermal delivery of drugs and cosmetics. *J Drug Deliv Sci Technol*. Vol. 32. (2016). p. 100-112.

MULLER, R. H.; RADTKE, M.; WISSING, S. A. - Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv Drug Deliv Rev*. Vol. 54 Suppl 1. (2002). p. S131-55.

MÜLLER, R. H.; SHEGOKAR, R.; KECK, C. M. - 20 years of lipid nanoparticles (SLN & NLC): Present state of development & industrial applications. *Current Drug Discovery Technologies*. Vol. 8. n.° 3 (2011). p. 207-227.

MURPHY, E.C.; SCHAFFTER, S.W.; FRIEDMAN, A.J. - Nanotechnology for psoriasis therapy. *Curr Derm Rep*. Vol. 8. (2019). p. 14.

NAGAICH, U.; GULATI, N. - Preclinical Assessment of Steroidal Nanostructured Lipid Carriers Based Gels for Atopic Dermatitis: Optimization and Product Development. *Curr Drug Deliv*. Vol. 15. n.° 5 (2018). p. 641-651.

NASIR, A. - Nanotechnology and dermatology: part II--risks of nanotechnology. *Clin Dermatol*. Vol. 28. n.° 5 (2010). p. 581-8.

NEL, A.; XIA, T.; MADLER, L.; LI, N. - Toxic potential of materials at the nanolevel. *Science*. Vol. 311. n.° 5761 (2006). p. 622-7.

OZCAN, I.; AZIZOGLU, E.; SENYIGIT, T.; OZYAZICI, M.; OZER, O. - Comparison of PLGA and lecithin/chitosan nanoparticles for dermal targeting of betamethasone valerate. *J Drug Target*. Vol. 21. n.° 6 (2013). p. 542-50.

PAN, W.; QIN, M.; ZHANG, G.; LONG, Y.; RUAN, W.; PAN, J.; WU, Z.; WAN, T.; WU, C.; XU, Y. - Combination of hydrotropic nicotinamide with nanoparticles for enhancing tacrolimus percutaneous delivery. *Int J Nanomedicine*. Vol. 11. (2016). p. 4037-50.

PANDEY, M.; CHOUDHURY, H.; GUNASEGARAN, T. A. P.; NATHAN, S. S.; MD, S.; GORAIN, B.; TRIPATHY, M.; HUSSAIN, Z. - Hyaluronic acid-modified betamethasone encapsulated polymeric nanoparticles: fabrication, characterisation, in vitro release kinetics, and dermal targeting. *Drug Deliv Transl Res*. Vol. 9. n.° 2 (2019). p. 520-533.

PARDEIKE, J.; HOMMOSS, A.; MULLER, R. H. - Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *Int J Pharm*. Vol. 366. n.° 1-2 (2009). p. 170-84.

POPLE, P. V.; SINGH, K. K. - Targeting tacrolimus to deeper layers of skin with improved safety for treatment of atopic dermatitis. *Int J Pharm*. Vol. 398. n.° 1-2 (2010). p. 165-78.

POPLE, P. V.; SINGH, K. K. - Targeting tacrolimus to deeper layers of skin with improved safety for treatment of atopic dermatitis-Part II: in vivo assessment of dermatopharmacokinetics, biodistribution and efficacy. *Int J Pharm*. Vol. 434. n.° 1-2 (2012). p. 70-9.

PRAJITHA, N.; ATHIRA, S. S.; MOHANAN, P. V. - Bio-interactions and risks of engineered nanoparticles. *Environ Res*. Vol. 172. (2019). p. 98-108.

PURI, A.; LOOMIS, K.; SMITH, B.; LEE, J. H.; YAVLOVICH, A.; HELDMAN, E.; BLUMENTHAL, R. - Lipid-based nanoparticles as pharmaceutical drug carriers: from concepts to clinic. *Crit Rev Ther Drug Carrier Syst*. Vol. 26. n.° 6 (2009). p. 523-80.

ROSA, P.; FRIEDRICH, M. L.; DOS SANTOS, J.; LIBRELOTTO, D. R. N.; MAURER, L. H.; EMANUELLI, T.; DA SILVA, C. B.; ADAMS, A. I. H. - Desonide nanoencapsulation with acai oil as oil core: Physicochemical characterization, photostability study and in vitro phototoxicity evaluation. *J Photochem Photobiol B*. Vol. 199. (2019). p. 111606.

SANTOS, A. C.; MORAIS, F.; SIMOES, A.; PEREIRA, I.; SEQUEIRA, J. A. D.; PEREIRA-SILVA, M.; VEIGA, F.; RIBEIRO, A. - Nanotechnology for the development of new cosmetic formulations. *Expert Opin Drug Deliv*. Vol. 16. n.° 4 (2019). p. 313-330.

SANTOS, ANA CLÁUDIA; PEREIRA-SILVA, MIGUEL; GUERRA, CATARINA; COSTA, DIANA; PEIXOTO, DIANA; PEREIRA, IRINA; PITA, INÊS; RIBEIRO, ANTÓNIO J.; VEIGA, FRANCISCO - Topical Minoxidil-Loaded Nanotechnology Strategies for Alopecia. *Cosmetics*. Vol. 7. n.° 2 (2020). p. 21.

SANTOS MAIA, C.; MEHNERT, W.; SCHALLER, M.; KORTING, H. C.; GYSLER, A.; HABERLAND, A.; SCHAFER-KORTING, M. - Drug targeting by solid lipid nanoparticles for dermal use. *J Drug Target*. Vol. 10. n.° 6 (2002). p. 489-95.

SHAO, M.; HUSSAIN, Z.; THU, H. E.; KHAN, S.; KATAS, H.; AHMED, T. A.; TRIPATHY, M.; LENG, J.; QIN, H. L.; BUKHARI, S. N. A. - Drug nanocarrier, the future of atopic diseases: Advanced drug delivery systems and smart management of disease. *Colloids Surf B Biointerfaces*. Vol. 147. (2016). p. 475-491.

SILVERBERG, J. I. - Atopic Dermatitis in Adults. *Med Clin North Am*. Vol. 104. n.° 1 (2020). p. 157-176.

SOUTO, E. B.; DIAS-FERREIRA, J.; OLIVEIRA, J.; SANCHEZ-LOPEZ, E.; LOPEZ-MACHADO, A.; ESPINA, M.; GARCIA, M. L.; SOUTO, S. B.; MARTINS-GOMES, C.; SILVA, A. M. - Trends in Atopic Dermatitis-From Standard Pharmacotherapy to Novel Drug Delivery Systems. *Int J Mol Sci*. Vol. 20. n.° 22 (2019).

TORCHILIN, V. P. - Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov*. Vol. 4. n.° 2 (2005). p. 145-60.

TRY, C.; MOULARI, B.; BEDUNEAU, A.; FANTINI, O.; PIN, D.; PELLEQUER, Y.; LAMPRECHT, A. - Size dependent skin penetration of nanoparticles in murine and porcine dermatitis models. *Eur J Pharm Biopharm*. Vol. 100. (2016). p. 101-8.

WANG, S.; SU, R.; NIE, S.; SUN, M.; ZHANG, J.; WU, D.; MOUSTAID-MOUSSA, N. - Application of nanotechnology in improving bioavailability and bioactivity of diet-derived phytochemicals. *J Nutr Biochem*. Vol. 25. n.° 4 (2014). p. 363-76.

WEIDINGER, S.; BECK, L. A.; BIEBER, T.; KABASHIMA, K.; IRVINE, A. D. - Atopic dermatitis. *Nat Rev Dis Primers*. Vol. 4. n.° 1 (2018). p. 1.

YU, K.; WANG, Y.; WAN, T.; ZHAI, Y.; CAO, S.; RUAN, W.; WU, C.; XU, Y. - Tacrolimus nanoparticles based on chitosan combined with nicotinamide: enhancing percutaneous

delivery and treatment efficacy for atopic dermatitis and reducing dose. *Int J Nanomedicine*. Vol. 13. (2018). p. 129-142.

ZABIHI, F.; GRAFF, P.; SCHUMACHER, F.; KLEUSER, B.; HEDTRICH, S.; HAAG, R. - Synthesis of poly(lactide-co-glycerol) as a biodegradable and biocompatible polymer with high loading capacity for dermal drug delivery. *Nanoscale*. Vol. 10. n.° 35 (2018). p. 16848-16856.

ZHANG, J.; SMITH, E. - Percutaneous permeation of betamethasone 17-valerate incorporated in lipid nanoparticles. *J Pharm Sci*. Vol. 100. n.° 3 (2011). p. 896-903.

ZHUO, F.; ABOUREHAB, M. A. S.; HUSSAIN, Z. - Hyaluronic acid decorated tacrolimus-loaded nanoparticles: Efficient approach to maximize dermal targeting and anti-dermatitis efficacy. *Carbohydr Polym*. Vol. 197. (2018). p. 478-489.

6. Annexes

Annex 1. Nanotechnology-based formulations applied to topical administration of drugs for the treatment of atopic dermatitis.

Nanotechnology based-formulations	Main composition	Drug	Particle size (nm)	ZP (mV)	PDI	EE (%)	Stability	Ref.
Nanoemulsions	Eucalyptus oil Tween 20 Ethanol	CP	NA	NA	NA	NA	NA	(ALAM, M.S. *et al.*, 2013)
	Castor oil Labrasol® Transcutol® Propylene glycol	PGZ	182 ± 1.36	+12,37 ± 1,64	0,352 ± 0,04	NA	Physically stable (60 days/ 20°C and 40°C)	(ESPINOZA, L.C. *et al.*, 2019)
Liposomes	PC Chol	AdCbl	106.4 ± 2.2	0.3 ± 0.2	NA	NA	NA	(JUNG, S.H.*et al.*, 2011)
	Phospholipon 90G Chol	BMV	223.7 ± 1.4	-27.4 ± 0.5	0.485 ± 0.02	46.3 ± 0.5	Good stability (3 months/25°C)	(EROGLU, I. *et al.*, 2016)
		DFV	331.9 ± 5.7	-29.4 ± 0.6	0.543 ± 0.03	39.5 ± 1.2		
	PC Tween 80 Chol Tat peptide	HST	130 -150	-10	NA	> 70	NA	(KANG, M.J. *et al.*, 2011)
	PC Tween 80 Tat peptide	ORG	130.6 ± 3.4	-9.3 ± 2.6	0.132	55.2 ± 6.2	NA	(KANG, M.J. *et al.*, 2010)
	PC Tween 80 MPB-PE Pep-1 peptide	TXG	131.2 ± 1.9	+26.7 ± 3.6	0.12 ± 0.04	29.5 ± 1.8	NA	(KANG, M.J., 2010)
Transfersomes	1-α PC Sod. Deoxycholate Ethanol Chol	GA	56.94	−4.76 −36.4 **hydrogel incorporated	1.00	85.73 ± 0.56	stable (2 months) **	(CHAUHAN, S. *et al.*, 2018)
	Phospholipon® 90G Chol Tween 20 D-limonene	CsA	139 ± 3	−6 ± 2	0.238	58.3 ± 6.1	NA	(CARRERAS, J.J. *et al.*, 2020)

Ethosomes	Lipoid S 100 Ethanol	TAC	85 ±13	+4.8	0.298 ± 0.024	78.7 ± 3.3	Physically stable (3 months/4°C)	(LI, G. *et al.*, 2012a)
	Ethanol PG Lipoid S 100	TAC	103.7 ± 0.9	NA	0.26 ± 0.03	79.5 ± 3.1	NA	(LI, G. *et al.*, 2012b)
	Ethanol PC PG SA	NIC	480 ± 12.18 488 ± 10.25	+25 ± 1.14 +58.1 ± 6.22	NA	NA	NA	(EL-MENSHAWE, S.F. *et al.*, 2019)
Solid lipid nanoparticles	Compritol Poloxamer 188	PDC	144	NA	0.339	NA	Physically stable	(SANTOS MAIA, C. *et al.*, 2002)
	Ticaprin Egg-PC Tween 80 DSPE-PEG t-butyl alcohol	CsA	73	-16	NA	90.80 ± 0.36	NA	(KIM, S.T.*et al.*, 2009)
	Glyceryl trimyristate Tween 80 Sorbitan monooleate	TAC	75.9	NA	0.141	93.92	NA	(POPLE, P.V. and SINGH, K.K., 2010)
	Glyceryl trimyristate Sorbitan monooleate	TAC	NA	NA	NA	NA	NA	(POPLE, P.V. and SINGH, K.K., 2012)
	Coco glyceride Soybean lecithin Brij® 93 Brij® 58 Poloxamer 188 PEG-40 stearate PEG-1000	TAC	152.9 ± 4.09	NA	0.25 ± 0.03	88.9 ± 0.7	NA	(KANG, J.H. *et al.*, 2019)
	Monostearin	BMV	135.7 ± 15.1	-71.2 ± 1.4	0 - 0.3	90.58-93.75	NA	(ZHANG, J. and SMITH, E., 2011)

Nanostructured lipid carriers	Precirol ATO 5 OA 2.0% Tween 80 (W/O ointment incorporated)	BMD	169.1 ± 0.5	-23.4	0.195 ± 0.013	85	NA	(KONG, X. *et al.*, 2016)
	Cetyl palmitate Miglyol Polysorbate 80	MTX	485 ± 5	-24 ± 1	0.26	83 ± 1	Physically and chemically stable (3 months/ 25°C)	(FERREIRA, M. *et al.*, 2016)
	Precirol ATO 5 Tween 80 Cetrimide	VE	< 105 (smaller population); < 328 (larger population)	NA	NA	NA	Stable (7 months/20±1°C) - Organoleptic, pH, rheological measurements, texture and particle size unaltered	(EIRAS, F. *et al.*, 2017)
Nanocrystals	Pluronic F127 SDC HPMC	BDP	622 ± 21	-18 ± 0.01	0.3 ± 0.01	NA	NA	(ASSEM, M. *et al.*, 2019b)
Polymeric micelles	Pluronic F127 Pluronic L-121	BDP	120.4 ± 17.39	-16.25 ± 0.77	0.33 ± 0.05	79.40 ± 1.54	Stable (3 months); No significant difference of EE% and particle size	(ASSEM, M. *et al.*, 2019a)
Polymeric nanoparticles	Chitosan	HC, HT	NA	NA	NA	NA	NA	(HUSSAIN, Z. *et al.*, 2013)
	FA-PLGA 5%PVA	-	66 ± 0.3	-0.8 ± 0.2	0.14 ± 0.01	NA	NA	(TRY, C. *et al.*, 2016)
	Lecithin/Chitosan	BMV	274.6 ± 14	+40.8 ± 2.80	0.241 ± 0.02	88.12 ± 0.5	Physically and chemically stable (3months/25°C)	(OZCAN, I. *et al.*, 2013)
	PLGA		280.9 ± 1.7	-5.62 ± 0.28	0.171 ± 0.02	84.62 ± 0.6		
	Fucoidan Chitosan	MTX	354 ± 8	-36 ± 2	0.18 ± 0.04	79.8 ± 0.2	Stable (2-4 weeks/4°C)	(BARBOSA, A.I. *et al.*, 2019)
	Chitosan, HA	BMV	< 300 ± 28	+58 ± 8	NA	86 ± 5.6	NA	(PANDEY, M. *et al.*, 2019)
	Starch Miglyol	ER143	175 - 487	+31.3	NA	78.6	NA	

	Tween®80 Cetrimide Ethanol							(MARTO, J. *et al.*, 2018)
	PLA	Encapsulated fluorophore	227	-58.0	0.023	87.95	NA	(BOISGARD, A.S. *et al.*, 2017)
	PLGA (oleic:labrafil formulation)	CsA	162.0 ± 0.75	−36.0 ± 0.6	0.061 ± 0.02	92.15	NA	(BADIHI, A. *et al.*, 2020)
	HA Chol 20%NIC	TAC	208.0 ± 2.2	-41.9 ± 3.0	0.224 ± 0.031	79.2 ± 7.2	NA	(PAN, W. *et al.*, 2016)
	Chitosan 20%NIC	TAC	110.2 ± 7.9	19.7 ± 0.8	0.20 ± 0.02	92.2 ± 2.2	NA	(YU, K. *et al.*, 2018)
	PLG	TAC	200 - 300	NA	1,4	NA	NA	(ZABIHI, F. *et al.*, 2018)
	Chitosan HA PVA	TAC	223 ± 12	+49 ± 3.94	NA	53.39 ± 4.87	NA	(ZHUO, F. *et al.*, 2018)
	Açai oil	DES	165 ± 2	+13.8 ± 0.3	0.12 ± 0.03	81.8 ± 1.8	Physically and chemically stable (30 days/RT); Photostable	(ROSA, P. *et al.*, 2019)

Abbreviations: AdCbl, adenosylcobalamin; BDP, beclomethasone dipropionate; BMD, betamethasone dipropionate; BMV, betamethasone valerate; CsA, cyclosporine A; Chol, cholesterol; CP, clobetasol propionate; DES, desonide; DFV, diflucortolone valerate; EE, entrapment efficiency; ER143; synthetic human neutrophil elastase inhibitor; FA, fluoresceinamine; GA, Glycyrrhizic acid; HA, hyaluronic acid; HC, hydrocortisone; HPMC, Hydroxypropyl methyl cellulose; HST, hisrsutenone; HT, hydroxytyrosol; MPB-PE, N-[4-(p-maleimidophenyl)butyryl]-phosphatidylethanolamine; MTX, methotrexate; NA, not available; NIC, nicotinamide; OA, oleic oil; ORG, oregonin; PC, phosphatidylcholine; PDC, prednicarbate; PDI, polydispersity index; PEG, polyethylene glycol; PG, propylene glycol; PGZ, pioglitazone; PLA, poly (lactic acid); PLG poly (lactide-co-glycerol); PLGA poly (lactic-co-glycolic acid); PVA, polyvinyl alcohol; RT, room temperature; SA, stearyl amine; SDC, Sodium deoxycholate; Tat, Trans-activating transcriptional activator; TXG, taxifolin glycoside; TAC, tacrolimus; VE, vitamin E; ZP, zeta potential.

Annex 2. *In vitro* and *in vivo* results overview of nanotechnology-based formulations for topical administration of drugs for the treatment of atopic dermatitis.

			***In vitro/Ex vivo* studies**		***In vivo* studies**			
Nanotechnology based-formulations	**Main composition**	**Drug**	**Model**	**Output**	**Animal Model**	**Efficacy**	**Safety**	**Ref.**
Transfersomes	1-α PC Sod. Deoxycholate Ethanol Chol	GA	Franz diffusion cell; Franz diffusion cell (rat abdominal skin)	*In vitro* release: controlled release profile (89.8% up to 24 h) *Ex vivo* permeation: ↓permeation (only 5,8% upto 24h)	DNCB- induced AD BALB/c mice	- ↑ decrease in the scratching and erythema score - Hematological parameters normalized	NA	(CHAUHAN, S. *et al.*, 2018)
	Phospholipon® 90G Chol Tween 20 D-limonene	CsA	Franz diffusion cell (HHSE)	*In vitro* absorption: ↑ Flux/dose	NA	NA	NA	(CARRERAS, J.J. *et al.*, 2020)
Liposomes	PC Chol	AdCbl	Franz diffusion cells (excised mouse skin)	*In vitro* penetration: ↑17x skin penetration	DNCB-induced AD NC/Nga mice	↓hypertrophy and hyperkeratosis in the epidermis	NA	(JUNG, S.H.*et al.*, 2011)
	Phospholipon 90G Chol	BMV	Franz diffusion cells (rat abdominal skin)	*Ex vivo* permeation: ↑ SC and epidermis retention	Carrageenan-induced rat paw edema	↑ paw edema inhibition	NA	(EROGLU, I. *et al.*, 2016)
					DNFB-induced AD male albino Wistar rats	↓TEWL and erythema	NA	
					Healthy animals	NA	Skin irritation test: - No significant TEWL value changes - No erythema - No morphological tissue changes	
	PC Tween 80 Chol Tat peptide	HST	Franz diffusion cell (depilated mouse skin)	*In vitro* permeation: ↑ Flux	DPCP-induced AD NC/Nga mice	- Lesions recovery: rapid (2 weeks) - COX-2, iNOS, IL-4, IL-13, IgE, and eosinophils levels: ↑suppression	NA	(KANG, M.J. *et al.*, 2011)
	PC Tween 80 Tat peptide	ORG	Franz diffusion cell	*In vitro* permeation: ↑ Flux	DPCP-induced AD NC/Nga mice	- Rapid and profound improvement of skin lesions	NA	(KANG, M.J., 2010)

			(depilated mouse skin)			- COX-2, iNOS, IL-4, IgE, and eosinophils levels: ↑ suppression		
	PC Tween 80 MPB-PE Pep-1 peptide	TXG	Franz diffusion cell (depilated mouse skin)	*In vitro* permeation: ↑ delivery of TXG into the skin	TNCB -induced AD NC/Nga mice	- ↑ reduction in TEWL values - Rapid and complete recovery in skin barrier function - Normalized the IL-4, IgE, and IFN- levels	NA	(KANG, M.J. *et al.*, 2010)
Ethosomes	Lipoid S 100 Ethanol	TAC	Franz diffusion cell (male SD rats skin)	In vitro permeation: ↑ permeation vs. traditional liposomes and Protopic® ointment	NA	NA	NA	(LI, G. *et al.*, 2012a)
	Ethanol PG Lipoid S 100	TAC	Franz diffusion cell (male Wistar rats skin)	*In vitro*: ↑ permeation vs. traditional liposomes and Protopic® ointment	DNFB-induced AD BALB/c mice	- ear edema: ↑ reduction - number of mast cells infiltrated: ↓	NA	(LI, G. *et al.*, 2012b)
	Ethanol PC PG SA	NIC	Franz diffusion cells (hairless rat skin)	*In vitro* permeation: - ↓ release and flux - ↑ skin retention	TNCB-induced eczema male Wistar rats	- ↓ inflammation signs - ↓ IgE levels	NA	(EL-MENSHAWE, S.F. *et al.*, 2019)
Nanostructured lipid carriers	Precirol ATO 5 OA 2.0% Tween® 80 (W/O ointment incorporated)	BMD	Franz diffusion cell (rabbit abdominal skin);	*In vitro* permeation and release: - ↑ release and skin retention - ↓ skin permeation	Rabbits	NA	Skin irritation: ↓ edema and erythema score	(KONG, X. *et al.*, 2016)
			Abdomen KM mice skin	Tissue distribution: - ↑ skin retention - ↓ BMD in the blood				
	Cetyl palmitate Miglyol Polysorbate 80	MTX	Dialysis bag diffusion	*In vitro* release: sustained release profile	NA	NA	NA	(FERREIRA, M. *et al.*, 2016)

			Fibroblasts and HaCa T cells	↓ toxicity (biocompatible)				
	Precirol ATO 5 Tween 80 Cetrimide	VE	HaCa T cells	*In vitro* biocompatibility studies (MTT reduction and NR uptake assays): -biocompatible	NA	NA	NA	(EIRAS, F. *et al.*, 2017)
			Fertilized chicken eggs	*In vitro* irritant potential study (HET-CAM assay): Score 0 (non-irritant)				
Nanoemulsions	Eucalyptus oil Tween 20 Ethanol	CP	NA	NA	Nickel-induced contact dermatitis Wistar rats	Edema: ↑ inhibition (84,15% up to 12h)	Skin irritation score (14 days): 1,21±1,66 non-irritant	(ALAM, M.S. *et al.*, 2013)
	Castor oil Labrasol® Transcutol® Propylene glycol	PGZ	Franz diffusion cells	*In vitro* release: controlled release	AA-induced ear inflammation male BALB/c mice TPA-induced ear inflammation adult male CD-1 mice healthy women	- Inflammatory cytokines levels: ↑suppression - Restoration of anti-inflammatory mRNA values - Redness reduction - ↓inflammatory cell infiltrates - ↓thickness of the dermis	- ↓TEWL - ↑SCH -biocompatible	(ESPINOZA, L.C. *et al.*, 2019)
			Franz diffusion cells (healthy women skin)	*Ex vivo* permeation: ↑Flux ↑ skin retention				
Solid lipid nanoparticles	Compritol Poloxamer 188	PDC	Franz diffusion cells (human skin)	*In vitro:* targeting to the epidermis	NA	NA	NA	(SANTOS MAIA, C. *et al.*, 2002)
	Ticaprin Egg-PC Tween 80 DSPE-PEG t-butyl alcohol	CsA	Franz type vertical diffusion cells (mouse skin)	*In vitro* viable skin permeation: ↑ 2-fold vs. CsA-oil mixture	OVA-induced AD mouse	↓ IL-4 and IL-5 levels	NA	(KIM, S.T.*et al.*, 2009)
	Glyceryl trimyristate Tween 80	TAC	Franz static diffusion cell	*In vitro* release: biphasic release pattern with initial burst release followed by sustained release	Albino rats	- similar occlusiveness and skin hydratation, - ↑ skin retention and penetration	NA	

	Sorbitan monooleate		Pig ear skin	*Ex vivo*: ↑skin penetration and accumulation	Rabbits	NA	No skin irritation	(POPLE, P.V. and SINGH, K.K., 2010)
	Glyceryl trimyristate Sorbitan monooleate	TAC	NA	NA	guinea pigs	↑ 3.02-fold skin bioavailability	Non-toxic	(POPLE, P.V. and SINGH, K.K., 2012)
					albino Wistar rats	↑ epidermis and dermis penetration targeting potential (target dendritic-immune-cells)		
					DNFB-induced AD BALB/c mice	↑ skin lesions suppression		
	Coco glyceride Soybean lecithin Brij® 93 Brij® 58 Poloxamer 188 PEG-40 stearate PEG-1000	TAC	Franz diffusion cells (excised rat dorsal skin)	*Ex vivo* penetration: deeper skin penetration vs. Protopic®	New Zealand white rabbits	Deeper skin penetration	- No skin irritation - Erythema score:1.2	(KANG, J.H. *et al.*, 2019)
	Monostearin	BMV	Franz diffusion cells (human thigh skin)	- controlled release - ↑ drug reservoir in the epidermis -↓permeation for the dermis	NA	NA	NA	(ZHANG, J. and SMITH, E., 2011)
Nanocrystals	Pluronic F127 SDC HPMC	BDP	Wistar rats skin	*Ex vivo* permeation and deposition: - ↓flux - ↑accumulation in the skin layers	NA	NA	NA	(ASSEM, M. *et al.*, 2019b)
Polymeric micelles	Pluronic F127 Pluronic L-121 (HPMC hydrogel incorporated)	BDP	Newborn CD-1 mice skin	*Ex vivo* permeation and deposition: - ↓amount of drug permeated - ↑skin retention	Adult male Wistar rats	Almost complete skin healing earlier (6 days) vs. commercial cream (12 days)	NA	(ASSEM, M. *et al.*, 2019a)
	Chitosan	HC, HT	Franz diffusion cells (full-thickness NC/Nga mouse skin)	*Ex vivo* permeation: - ↓flux - ↑epidermis and dermis retention	DNFB-induced AD-like skin lesions eight-week-old NC/Nga mice	↓ TEWL, intensity of erythema, dermatitis index and skin thickness	NA	(HUSSAIN, Z. *et al.*, 2013)
	FA	-	NA	NA	- Oxazolone-induced AD pig	- ↑ inflamed skin penetration	NA	

Polymeric nanoparticles	PLGA 5%PVA				- Oxazolone-induced AD-like skin lesions male swiss mice	- ↓ edema and erythema score		(TRY, C. *et al.*, 2016)
	PLGA	BMV	Franz diffusion cells (rat abdominal skin)	*Ex vivo* permeation: ↑epidermis retention vs. CC	Carrageenan-induced male albino Wistar rat paw edema	- Identical decrease paw thickness vs. CC; - ↓ decreased skin-blanching vs.CC	no significant difference TEWL values before and after application	(OZCAN, I. *et al.*, 2013)
	Lecithin Chitosan	BMV		*Ex vivo* permeation: ↑2x epidermis retention vs. CC		- ↑decrease paw thickness vs. CC; - Identical decrease skin-blanching vs. CC		
	Fucoidan Chitosan	MTX	Franz diffusion cells (pig ear skin)	*In vitro* permeation: ↑ MTX permeated	NA	NA	NA	(BARBOSA, A.I. *et al.*, 2019)
	HA Chitosan	BMV	Dynamic dialysis membrane; Franz diffusion cells (Wistar albino rats skin)	*In vitro*: Controlled release *Ex vivo*: ↑epidermis and dermis retention	NA	NA	NA	(PANDEY, M. *et al.*, 2019)
	Starch Miglyol Tween®80 Cetrimide Ethanol	ER143	Franz diffusion cells (newborn pig skin)	*In vitro*: ↑ skin permeation and retention	Croton oil-induced ear inflammation BALB/c mice	↑ erythema inhibition and identical edema inhibition vs. commercial lotion	NA	(MARTO, J. *et al.*, 2018)
	PLA (Avicel and Viscarin based formulations)	Encapsulated fluorophore	VitroPharma diffusion cells (mouse skin explants)	Ex vivo: - Identical skin penetration - ↓ skin permeation vs. PLA NPs suspension	TMA-induced AD like lesions male BALB/cByJ mice	NA	No skin irritation (8 days)	(BOISGARD, A.S. *et al.*, 2017)
	PLGA (oleic:labrafil formulation)	CsA	Activated mouse splenocytes	*In vitro:* ↑ inhibition cell proliferation and IL-2 secretion	- OVA-induced AD BALB/c mice - DNFB-induced CHS mice	- Preserved the integrity of the skin - Prevented skin thickening - ↓ serum levels of IFN-γ, OVA-IgE and IL-4	NA	(BADIHI, A. *et al.*, 2020)
			Pig ear skin	*Ex vivo*: ↑ penetration into the different skin layers				

		Human skin organ culture	*Ex vivo*: ↓ secretion of pro-inflammatory cytokines		- ↓ 30%ear swelling vs. other groups		
HA Chol 20%NIC	TAC	Franz diffusion cells (rat skin)	*In vitro:* ↑ skin retention and permeation	NA	NA	NA	(PAN, W. *et al.*, 2016)
Chitosan 20%NIC	TAC	Franz diffusion cells (abdominal rat skin)	*In vitro* permeation: - ↑~2-fold skin permeation - ↑skin retention (vs. Protopic)	DNCB-induced AD BABL/c mice	↓ dermatitis score, ear thickness spleen index, epidermal thickness, number of infiltrated mast cells and serum total IgE levels	NA	(YU, K. *et al.*, 2018)
PLG	TAC	Franz diffusion cells (excised human skin)	- ↑ SC, epidermis and dermis penetration - Identical inhibition of IL-2 expression vs. commercial cream - ↑ inhibition of TSLP expression vs. commercial cream	NA	NA	NA	(ZABIHI, F. *et al.*, 2018)
Chitosan HA PVA	TAC	Franz diffusion cells (NC/Nga mouse skin)	*In vitro:* sustained release profile *Ex vivo:* ↓ permeation flux ↑ retention in dermis and epidermis	DNFB-induced AD eight-week-old NC/Nga mice	- TEWL, erythema intensity and dermatitis index: ↑ reduction - Minimal dermis infiltration of inflammatory cells - ↓ acanthosis - Restoration of the skin integrity	NA	(ZHUO, F. *et al.*, 2018)
Açai oil	DES	Dialysis bag diffusion	*In vitro* release study: biphasic release profile	NA	NA	NA	(ROSA, P. *et al.*, 2019)
		MTT and NRU viability assays (murine fibroblasts and HaCaT human keratinocytes)	Phototoxicity test: non-phototoxic				
		HET-CAM	Irritant potential evaluation: slightly irritant				

Abbreviations: AA, arachidonic acid; AdCbl, adenosylcobalamin; AD, atopic dermatitis; BDP, beclomethasone dipropionate; BMD, betamethasone dipropionate; BMV, betamethasone valerate; CC, commercial cream; Chol, cholesterol; CHS, contact hypersensitivity; COX-2, cyclooxygenase-2; CP, clobetasol propionate; CsA, cyclosporine A; DES, desonide; DFV, diflucortolone valerate; DNCB,1-cholro-2,4-dinitrobenzene; DNFB, 1-Fluoro-2,4-dinitrobenzene; DPCP, diphenylcyclopropenone; DSPE, distearoyl-phosphatidylethanolamine; ER143; synthetic human neutrophil elastase inhibitor; FA, fluoresceinamine; GA, Glycyrrhizic acid; HA, hyaluronic acid; HACaT cells, Immortalized Human Keratinocytes; HC, hydrocortisone; HET-CAM- hen's egg test on the chorioallantoic membrane; HHSE, Human heat-separated epidermis; HPMC, Hydroxypropyl methyl cellulose; HST, hisrsutenone; HT, hydroxytyrosol; IgE, immunoglobulin E; IL, interleukin; IFN, interferon; MPB-PE, N-[4-(p-maleimidophenyl)butyryl]-phosphatidylethanolamine; MTX, methotrexate; NA, not available; NIC, nicotinamide; iNOS, nitric oxide synthase; OA, oleic oil; ORG, oregonin; OVA, ovalbumin; PC, phosphatidylcholine; PDC, prednicarbate; PEG polyethylene glycol; PG, propylene glycol; PGZ, pioglitazone; PLA poly (lactic acid); PLG poly (lactide-co-glycerol); PLGA poly (lactic-co-glycolic acid); PVA, polyvinyl alcohol; SA, stearyl amine; SC, stratum corneum; SCH, stratum corneum hydration; SDC, Sodium deoxycholate; TAC, tacrolimus; Tat, Trans-activating transcriptional activator; TEWL, transepidermal water loss; TNCB, trinitrochlorobenzene; TPA, 12-o-tetradecanoylphorbol 13-acetate; TXG, taxifolin glycoside; TMA, trimellitic anhydride; VE, vitamin E.