UNIVERSIDADE D
COIMBRA

Mariana Dias Ribeiro

Relatório de Estágio e Monografia intitulada "Nanotechnology-based therapeutics strategies towards the topical treatment of hair loss" referente à Unidade Curricular "Estágio", sob orientação, da Dra. Cidália Roxo e do Professor Doutor António José Ribeiro e apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

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Setembro 2020

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Coimbra, 7 de setembro de 2020.

Mariana Dias Ribeiro

(Mariana Dias Ribeiro)

"Matar o sonho é matarmo-nos. É mutilar a nossa alma. O sonho é o que temos de realmente nosso, de impenetravelmente e inexpugnavelmente nosso.
"

Fernando Pessoa

Agradecimentos

Esta grande jornada está a chegar ao fim, uma grande etapa da minha vida, que está prestes a ser concluída, como tal, não posso deixar de fazer um agradecimento especial a quem contribui para este meu grande sonho.

Em primeiro lugar quero agradecer aos meus pais, pelo apoio, força e motivação, que sempre acreditaram em mim e se esforçaram incansavelmente para que este sonho se tornasse realidade. Obrigada por todo o vosso sacrifico! Adoro-vos!

Um segundo agradecimento é dirigido aos meus avós, obrigada por todo o apoio!

À minha amiga Patrícia Gomes pela sua lealdade e encorajamento… o que Coimbra uniu ninguém separa!

A todas as amizades que construi nestes 5 anos, afilhada e colegas de casa, ficarão para sempre no meu coração!

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À faculdade de Farmácia da Universidade de Coimbra, a todo o corpo docente, funcionários e colaboradores que constituem esta grande instituição.

Por fim a ti, Coimbra…

Obrigada!

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Parte I

Relatório de Estágio em Farmácia Comunitária

Farmácia São Sebastião

Lista de Abreviaturas

ANF – Associação Nacional de Farmácias

CIMPI – Centro de Informação do Medicamento de Preparação Individualizada

DCI – Denominação Comum Internacional

IMC – Índice de Massa Corporal

MICF – Mestrado Integrado em Ciências Farmacêuticas

MNSRM – Medicamentos Não Sujeitos a Receita Médica

MNSRM-EF – Medicamentos Não Sujeitos a Receita Médica de dispensa Exclusiva em Farmácia

MSRM – Medicamentos Sujeitos a Receita

SNS – Serviço Nacional de Saúde

SWOT – *Strengths* (Pontos Fortes), *Weaknesses* (Pontos Fracos), *Opportunities* (Oportunidades) e *Threats* (Ameaças)

1. Introdução

As Ciências Farmacêuticas representam uma área científica de grande relevo no campo das Ciências da Saúde. Decorrendo do facto de ser uma área muito abrangente e multidisciplinar as saídas profissionais relacionam-se com as atividades que se enquadram nas competências adquiridas podendo enveredar para qualquer uma destas áreas: farmácia comunitária e hospitalar, indústria farmacêutica, química e alimentar, análises clínicas, assuntos regulamentares relacionados com o medicamento e produtos de saúde, bem como outras ligadas ao doente, medicamento e saúde pública.

O Mestrado Integrado em Ciências Farmacêuticas (MICF) consagra uma formação de 5 anos, equivalente a 10 semestres, sendo que 9 desses semestres correspondem a uma formação teórica e prática a tempo integral, ministrado na Faculdade de Farmácia da Universidade de Coimbra. Culminando a formação teórica e prática seguem-se seis meses de estágio em Farmácia Comunitária ou em Farmácia Comunitária e em Farmácia Hospitalar ou outra área do medicamento, sendo indispensável e obrigatório que uma componente deste estágio seja realizada em Farmácia Comunitária.

Este estágio permite ter contacto direto com o que é a profissão de farmacêutico promovendo o desenvolvimento de competências necessárias para o exercício da profissão de forma independente, responsável e competente.

Levando em conta que a Farmácia Comunitária representa, na maioria das vezes, o primeiro e/ou último contacto dos utentes com os serviços de saúde é da responsabilidade e função do farmacêutico, como educador sanitário, aplicar todos os seus conhecimentos, ouvir, comunicar, informar e aconselhar os utentes, orientar ações no sentido de promover e educar para a saúde, e alertar para a utilização racional dos medicamentos. A mais valia da relação humana e da prestação de serviços na Farmácia Comunitária é obter a confiança por parte do doente/utente resultando numa melhor eficácia dos resultados.

Atualmente a Farmácia Comunitária, constituí um pilar imprescindível no sistema de saúde, uma vez que é considerada como indispensável pelo serviço que presta à sociedade desde os medicamentos aos produtos de bem-estar e conforto. Como tal, não deve ser entendida apenas como um estabelecimento que presta cuidados de saúde relacionados com o medicamento.

Por todas estas valências foi da minha preferência realizar o estágio apenas em Farmácia Comunitária, tendo o mesmo decorrido na Farmácia São Sebastião, em Coimbra. A Direção Técnica da Farmácia está a cargo da Dra. Ana Cristina Pimentel, tendo como equipa mais 3

farmacêuticos, de que fazem parte a Dra. Cidália Roxo, como farmacêutica substitua, o Dr. João Pinto e a Dra. Beatriz Póvoa.

2. Enquadramento: Farmácia São Sebastião

Foi no Bairro São Sebastião, na Rua António Jardim nº23, que a Farmácia São Sebastião teve início. Posteriormente e desde 2007, a Farmácia São Sebastião, manteve a sua atividade na Avenida Elísio de Moura permanecendo aí até 23 de maio de 2020.

No meu estágio tive a oportunidade de acompanhar a transferência de localização da Farmácia para a rua Vitorino Nemésio. A inauguração ocorreu a 25 de maio de 2020 e foi um privilégio ter estado presente neste célebre dia da história da Farmácia São Sebastião.

O motivo da alteração de localização da Farmácia teve por base o bem-estar do doente/utente, garantindo uma melhoria dos serviços prestados à comunidade. Tratando-se de uma localização mais central, com fácil acesso a qualquer ponto da cidade, permite servir um grupo mais diversificado de população. Contrapondo com a antiga, que prestava os seus cuidados essencialmente aos utentes que ali habitavam.

Esta alteração de localização permite, efetivamente, melhorar a acessibilidade da população aos medicamentos, a sua comodidade, bem como a viabilidade económica da farmácia, resultando num aumento dos serviços farmacêuticos de promoção da saúde e do bem-estar dos seus utentes. A localização mais "central" da Farmácia, permite ainda uma maximização da afluência de utentes ao longo do dia.

A Farmácia São Sebastião contempla um grupo de clientes que abrange todas as faixas etárias, desde pessoas com uma idade mais avançada, a jovens e crianças. Estagiar nesta farmácia deu-me a possibilidade de estar alerta para outra realidade que são os doentes toxicodependentes e indivíduos sem-abrigo e/ou emergência social, sendo que também estes constituíam presença neste estabelecimento, uma vez que, o "Farol" (Centro de Acolhimento de sem-abrigos e toxicodependentes), encontra-se situado em Tovim próximo da Farmácia.

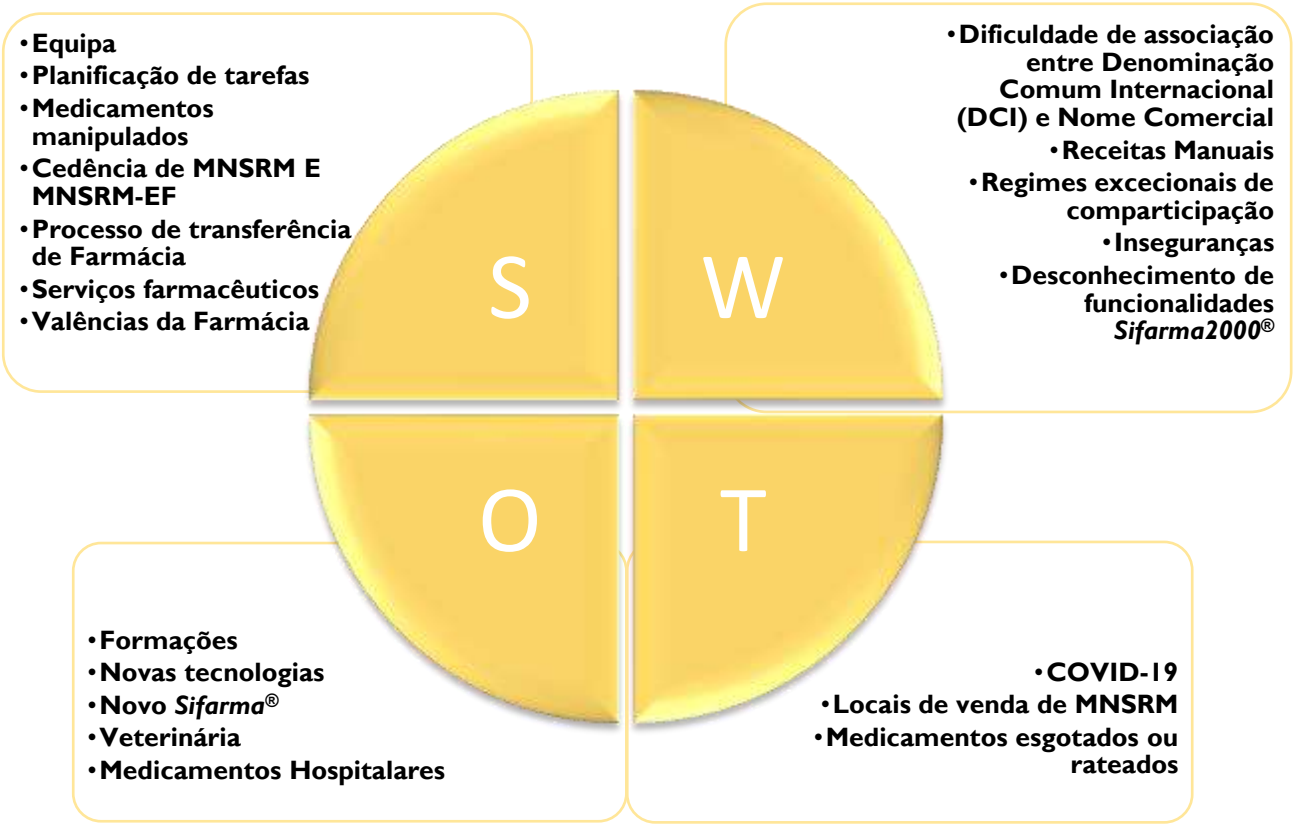
O horário de funcionamento da Farmácia é contínuo, não existindo pausa para almoço, exceto durante o estado de emergência em que o horário foi reduzido. A Farmácia está aberta ao público desde as 9h até às 20h, ininterruptamente em dias úteis. Aos sábados o horário sofre uma alteração para um período de tempo menor, funcionando desde as 9h até às 14h. Para além do habitual horário de funcionamento e sensivelmente 2 vezes por mês (inserida no regime de rotatividade das Farmácias de Coimbra) a Farmácia São Sebastião está de serviço 24h, desde o horário habitual de abertura até ao horário de encerramento do dia seguinte. Atualmente, devido ao contexto de pandemia em que nos encontramos, e após autorização pelo INFARMED, este serviço está reduzido a 1 vez por mês.

A Farmácia é composta por uma zona de acesso ao público, contendo 3 balcões de atendimento, gabinete de apoio ao utente; laboratório para a preparação de medicamentos manipulados; área de exposição de lineares (contendo produtos de dermofarmácia e cosmética; produtos de uso veterinário, suplementos, puericultura, nutrição infantil, etc.); zona de receção de encomendas; arrumação de medicamentos em gavetas; casa de banho; área de arrumação de medicamentos excedentes; sala de descanso e escritório.

3. Análise *SWOT*

De forma a avaliar o meu estágio com uma perspetiva própria e individualizada, irei apresentar uma análise *SWOT* (*Strenghts*, *Weaknesses*, *Opportunities* and *Threats*). Por conseguinte pretendo apresentar os pontos fortes, os pontos fracos, as oportunidades e as ameaças respeitantes ao culminar do meu estágio, fazendo um enquadramento com todos os conhecimentos adquiridos ao longo do meu percurso académico, exprimindo de que forma é que estes contribuíram para o meu desempenho enquanto profissional. Farei uma análise primária, sob a forma de perspetiva interna, dos pontos que considero como fortes e fracos e passarei para uma análise de carácter mais abrangente respeitante às oportunidades e ameaças que considerei relevantes enquanto estagiária.

Tabela 1. Análise *SWOT*



3.1. Pontos Fortes

Equipa

Para mim o sucesso enquanto profissional, advém não só dos conhecimentos teórico-práticos que fomos adquirindo ao longo destes 5 anos, mas também da forma como nos relacionamos com os que são os nossos colegas de trabalho. Considero como essencial promover o bom ambiente no local de trabalho, e saber lidar com as diferentes personalidades de forma a evitar conflitos. Ao longo destes meses, esforcei-me por me integrar na equipa tentando assimilar a cultura organizacional fortalecendo as relações interpessoais e nutrindo o bem-estar de todos. Durante esta experiência profissional vivenciei os valores e propósitos da Farmácia enquanto equipa de trabalho, de forma a colocar esses conhecimentos em prática no dia a dia.

Considero-me privilegiada pela maneira como fui acolhida pela equipa de profissionais da Farmácia, demonstrando sempre disponibilidade para me esclarecer dúvidas, para me ensinarem a cada dia o que é ser farmacêutico, a responsabilidade que é aconselhar a melhor terapêutica, específica para cada doente e para cada situação, a saber valorizar sempre a opinião do utente, dar orientações de forma a que a mensagem por nós transmitida seja compreendida, a preocupação em dar sempre um conforto e alento a quem deles necessite.

Foi muito gratificante sentir-me apoiada por toda a equipa, a forma como me encorajaram a ser autónoma e independente dando-me confiança para realizar as tarefas que me eram propostas. Tudo isto contribuiu de forma enriquecedora para a minha valorização pessoal e profissional.

Aponto também como vantagem o facto de a equipa ser constituída apenas por farmacêuticos permitindo-me ter diferentes perspetivas do percurso e das características peculiares na prática profissional de cada um deles. Na minha opinião ter uma equipa formada apenas por farmacêuticos constitui uma garantia na qualidade do aconselhamento e atendimento dos utentes pelas diversas valências que temos no nosso curso, constituindo um conjunto de características que individualizam e tornam autêntica toda equipa da Farmácia São Sebastião perante qualquer outra.

Em forma de conclusão posso afirmar que foi a união e o desejo de fazer mais e melhor por parte de todos que contribuíram para a minha positiva experiência em Farmácia Comunitária.

Planificação de tarefas

O meu estágio teve início a 6 de janeiro de 2020 e culminou a 17 de julho do mesmo ano.

A princípio exerci funções maioritariamente relacionadas com o contacto do medicamento em si, de maneira a familiarizar-me com os nomes comerciais e denominação internacional comum (DCI). Estas tarefas basearam-se na receção de encomendas e verificação do prazo de validade, de forma a retificar se era inferior ou superior à dos que se encontravam no stock da Farmácia, e atualizando o sistema informático sempre que um medicamento rececionado tinha prazo de validade inferior. Também aqui era analisada a margem aplicada aos medicamentos não sujeitos a receita médica (MNSRM), uma vez que são os únicos em que o Estado não tem interferência como agente pagador, dando oportunidade à Farmácia de definir o seu preço de venda ao público, tendo sempre em conta o custo e uma margem pré-definida. Aqui, conferia ainda se o número de embalagens rececionada estava de acordo com o que constava na nota de encomenda, verificava a integridade das embalagens e retificava ainda se tinham sido pedidas para um utente específico. Depois de realizada esta tarefa, dava lugar ao armazenamento dos produtos, onde medicamentos sujeitos a receita médica (MSRM) eram arrumados em gavetas, seguindo a ordem alfabética e forma farmacêutica, os restantes produtos eram acondicionados nos devidos lugares (prateleiras, gavetas do balcão e expositores).

O acondicionamento dos produtos era sempre baseado na regra de *First In, First Out*, dando lugar a que os produtos com menor prazo de validade fossem vendidos primeiro.

Observei tarefas tais como: avaliação crítica de prescrições médicas, a sua conferência, verificando, se cumprem as regras de prescrição previstas na lei e organização do receituário, tarefas que me permitiram tomar conhecimento quanto aos acordos entre a Associação Nacional de Farmácias (ANF) e as várias entidades responsáveis pela comparticipação. Pude ainda observar como era realizado o controlo e regularização de substância estupefacientes, psicotrópicos e benzodiazepinas e sua respetiva comunicação ao INFARMED.

Depois executei tarefas onde já tinha um maior contacto com o público como a medição de parâmetros bioquímicos (pressão arterial, glicémia, colesterol total e triglicerídeos).

De seguida comecei a acompanhar os atendimentos que eram prestados. Esta função tinha como objetivo a observação dos passos fulcrais para um atendimento prestável. Neste ponto foi possível analisar as questões que eram pertinentes e essências para garantir o melhor aconselhamento, quais as recomendações a fazer, a devida posologia, alertar para possíveis efeitos secundários, interações e contraindicações.

Estas tarefas permitiram-me ter um maior domínio do sistema informático *Sifarma2000®*, uma vez que se torna essencial, para posteriormente ganhar independência e autonomia no atendimento.

Passado sensivelmente um mês comecei a realizar os atendimentos de forma autónoma, sempre com a supervisão de toda a equipa para qualquer dúvida que surgisse ou sempre que por qualquer eventualidade me sentisse mais insegura quanto ao que deveria aconselhar.

Medicamentos manipulados

Atualmente a percentagem de Farmácias que prepara e dispensa medicamentos manipulados é muito reduzida, como tal, considero como uma grande vantagem ter tido a oportunidade de estagiar numa Farmácia que mantém essa prática.

Segundo o Decreto-Lei n.º 95/2004 de 22 de Abril: "**Medicamento manipulado** é qualquer fórmula magistral ou preparado oficinal, preparado e dispensado sob a responsabilidade de um farmacêutico"; "**Fórmula magistral** é todo o medicamento preparado em farmácia de oficina ou nos serviços farmacêuticos hospitalares, segundo uma receita médica destinado a um determinado doente; "**Preparado oficinal** é todo o medicamento preparado segundo as indicações compendiais de uma farmacopeia ou de um formulário, nos locais referidos no ponto anterior, destinado a ser dispensado diretamente aos doentes assistidos por essa farmácia ou serviço." [1]

Compete ao farmacêutico garantir a preparação do medicamento manipulado de acordo com as Boas Práticas de Preparação de Medicamentos Manipulados, bem como, aprovar a prescrição do ponto de vista galénico e farmacoterapêutico, garantido sempre a sua qualidade. [1,2]

A preparação de manipulados destina-se fundamentalmente, a doentes individuais ou populações específicas uma vez que, em muitas destas situações a indústria não tem resposta adequada. Estas preparações têm como vantagens a dispensa de medicamentos na quantidade exata e adequada a cada doente; a garantia da qualidade dos medicamentos preparados, obedecendo à legislação em vigor e às normas de Boas Práticas na Preparação de Medicamentos Manipulados [3]; a associação de substâncias ativas que não estejam disponíveis na indústria e permite ainda a individualização das dosagens, personalizando-a para cada doente, contrariamente ao que acontece na indústria em que temos dosagens padronizadas.

A Farmácia São Sebastião dispõe de um laboratório com as condições adequadas à preparação de medicamentos manipulados. [4] Todas as preparações que são efetuadas possuem respetivamente, fichas de preparação (Anexo 1), o número de lote, as matérias primas utilizadas e o seu respetivo lote e o boletim de análise. É realizada também a

monitorização de todos os movimentos das matérias-primas (Anexo 2), os materiais utilizados, dados do prescritor, dados do utente, controlo de qualidade das preparações efetuadas, para além de informações tais como: os prazos de utilização, condições de conservação, rótulos e preço, de acordo com a legislação em vigor. [5]

Sempre que havia alguma dúvida em relação à preparação, formulação, acondicionamento ou prazo de utilização em relação à formulação dispensada contactava-se o Centro de Informação do Medicamento de Preparação Individualizada (CIMPI) de forma a obter apoio técnico-científico que permitisse esclarecer essas mesmas questões.

Nas Farmácias Comunitárias, o cálculo do preço de venda ao público dos medicamentos manipulados, é efetuado de acordo com os critérios definidos na Portaria n.º 769/2004, de 1 de julho, sendo que é calculado considerando 3 parcelas, das quais fazem parte: o valor dos honorários, cuja base é o fator (F), fator este que sofre alterações periódicas; o preço das matérias-primas e o preço dos materiais de embalagem. [5]

Foram realizados diversos tipos de preparações farmacêuticas desde cápsulas, pomadas, soluções e suspensões, o que me permitiu consolidar conhecimentos prático-laboratoriais de Farmácia galénica.

Cedência de MNSRM e MNSRM-EF

É na cedência de MNSRM que o farmacêutico pode ter um papel mais ativo enquanto profissional de saúde, uma vez que, ao contrário do que acontece com os MSRM, não é obrigatório o utente apresentar uma receita médica. Nesse sentido, acresce ao farmacêutico assumir um maior grau de responsabilidade para a avaliação, decisão e aconselhamento de um dado medicamento destinado a uma patologia menor.

Inserida na categoria de MNSRM temos a subcategoria de medicamentos não sujeitos a receita médica de dispensa exclusiva em farmácia (MNSRM-EF). A classificação de MNSRM-EF foi instituída em 2013 em Portugal, com a publicação do Decreto-Lei n.º 128/2013, de 5 de setembro, esta classificação permitiu que, para além de não ser exigido ao utente a receita médica, a dispensa do medicamento pelo farmacêutico está dependente da aplicação de protocolos de dispensa definidos pelo INFARMED. [6] Com a crescente publicidade e com a possibilidade da cedência de MNSRM fora das farmácias os utentes procuram cada vez mais soluções rápidas evitando ir frequentemente ao médico. A implementação desta subcategoria de MNSRM-EF, permitiu reforçar o papel do farmacêutico na intervenção farmacêutica e o papel das Farmácias no sistema de saúde, tendo como principal objetivo a promoção do uso racional do medicamento.

É crucial que o farmacêutico possua todas as informações necessárias sobre indicação farmacêutica em patologias menores. Nessa perspetiva, nos primeiros dias de estágio foi-me dada a oportunidade, no sentido de estar mais à vontade para quando tivesse lugar o atendimento ao público, de explorar os MNSRM, analisar diferenças entre as opções terapêuticas existentes na Farmácia tais como: o tipo de princípio ativo, a dose, forma farmacêutica, a frequência da administração e a duração do tratamento para que pudesse adequa-las a cada tipo de situação que surgisse.

Aquando da cedência de MNSRM foquei-me em manter um diálogo com o utente de forma a obter informação detalhada o suficiente sobre a patologia. Nesse sentido devem ser feitas certas questões chaves tais como: Há quanto tempo tem os sintomas? Apresenta outro tipo de sintoma? Onde ocorrem os sintomas? Como descreve os sintomas? Qual a frequência/intensidade dos sintomas? Posteriormente dava lugar à escolha do tratamento ou seleção do medicamento tendo sempre em consideração a preferência do utente ou aconselhava a referência ao médico quando fosse caso disso. Tive sempre a preocupação em aconselhar medidas não farmacológicas, por si só ou complementando com a ação farmacológica, como incentivar à mudança e/ou reforçar a adoção de hábitos de vida saudáveis. Por fim, cedia informações que permitissem, ao utente, melhorar a gestão da terapêutica tornando-o mais autónimo nesse sentido. Explicava a razão da seleção do medicamento, dava instruções de utilização e informava-o sobre possíveis efeitos secundários, interações e contraindicações.

Considero de extrema importância a exploração inicial dos MNSRM, na medida em que me ajudou a rever conhecimentos, a ter contacto com os nomes comerciais, e a esclarecer algumas dúvidas, permitindo-me ganhar mais confiança e segurança na fase de atendimento ao público.

Processo de transferência de Farmácia

Considero que foi uma ótima experiência ter vivenciado e presenciado o processo de transferência de uma farmácia. De toda a burocracia, trabalho, empenho e exigências inerentes a todo este processo. Foi também um colocar em "prática" conhecimentos adquiridos sobre legislação farmacêutica que fazem parte deste tipo de processos. Ressalto como uma oportunidade ter o privilégio de acompanhar todo o trabalho até ao concretizar do novo espaço, de organizar, e planear todos os pontos-chave para que desse lugar à inauguração da Farmácia. Assinalo ainda que o dia de inauguração me foi especialmente querido, por ter o *feedback* positivo das pessoas em relação ao novo espaço da Farmácia São Sebastião. Nesse

mesmo dia, como forma de boas vindas, foi oferecido a cada cliente uma flor e foi muito gratificante ver o carinho das pessoas pelo pequeno gesto.

Serviços farmacêuticos

Com o aumento da prevalência de doenças crónicas e tendendo ao facto de a população em Portugal estar cada vez mais envelhecida torna-se essencial o papel do farmacêutico no acompanhamento dos cuidados de saúde.

A Farmácia São Sebastião para além da cedência de medicamentos e outros produtos, oferece aos seus utentes a possibilidade da avaliação e controlo de parâmetros bioquímicos e fisiológicos dos quais fazem parte: a avaliação da pressão arterial, glicémia, triglicerídeos, colesterol total e índice de massa corporal (IMC). A Farmácia dispõe ainda de profissionais qualificados para a administração de injetáveis o que traz uma grande vantagem à população uma vez que a adesão por parte dos cidadãos à vacinação nas Farmácias tem vindo a aumentar, pelo menor tempo de espera e pela confiança que têm nos farmacêuticos. [7]

A realização destes serviços é essencial na medida em que é possível avaliar o estado de saúde do doente para além de que nos permite monitorizar a eficácia da terapêutica instituída, dando oportunidade ao farmacêutico de estabelecer um diálogo com o utente alertando-o para o uso racional do medicamento, da adoção de um estilo de vida saudável, dos benefícios da prática de exercício físico, da importância de ingerir água ao longo do dia, reduzir o consumo de sal e gorduras, restringir o consumo de álcool e tabaco bem como privilegiar a adoção de uma dieta variada e equilibrada.

Ao longo do meu estágio a realização destas funções deu-me oportunidade de adquirir proximidade com o doente permitindo um aconselhamento mais individualizado, sempre com a preocupação de consciencializar os utentes para a importância do controlo destes parâmetros estando ou não a ser medicados para uma dada patologia.

No meu entender, a prestação de serviços farmacêuticos, essenciais à saúde, é crucial quer de um ponto de vista preventivo quer terapêutico, permitindo igualmente a prevenção de *outcomes* negativos relativamente à medicação instituída.

Como forma de prevenção de infeções pelo vírus da imunodeficiência humana (VIH), Hepatite B e C a Farmácia São Sebastião disponibiliza, aos utilizadores de drogas injetáveis, um *kit* "Redução de riscos" com vista à distribuição do material esterilizado. Este kit contém uma seringa esterilizada, dois recipientes, um preservativo, um filtro, duas carteiras de ácido cítrico, uma ampola de água bidestilada, um toalhete desinfetante e um folheto informativo.

Valências da Farmácia

I- VALORMED

A VALORMED consiste numa sociedade sem fins lucrativos que tem por objetivo a gestão de resíduos conduzindo, de forma segura, a recolha e tratamento dos resíduos dos medicamentos, sujeitos ou não a receita médica, que já estejam fora do prazo de validade, que já não sejam utilizados ou até mesmo de embalagens vazias. [8] A Farmácia São Sebastião possui um contentor que serve de depósito a todos os resíduos que são entregues pelos utentes. Quando o contentor já não contém capacidade de armazenamento é selado para posteriormente ser recolhido pelos armazenistas. Com esta campanha associa-se a vantagem da preservação do ambiente e da saúde pública assegurando a eliminação dos medicamentos de uma forma segura. [9]

II - Cartão Saúda

A ANF, decorrente do programa "Farmácias Portuguesas" criou o cartão saúda, cujo propósito é a fidelização do utente à farmácia, uma vez que consiste na atribuição de pontos a todas as compras efetuadas, associando a possibilidade de todos os elementos da família poderem acumular pontos na mesma conta. A cada ano são emitidas duas edições (Primavera/Verão e Outono/Inverno) do catálogo do cartão saúda. A atribuição de pontos é feita segundo o tipo de compra, sendo que MSRM fornecem um ponto por dia enquanto que MNSRM, produtos de saúde e bem-estar e serviços farmacêuticos fornecem mais pontos. Depois há a possibilidade de trocar os pontos por vales, por produtos ou serviços da Farmácia, ou ainda trocar pontos por produtos ou serviços do catálogo.

III - Farmácia ABEM

O programa ABEM pertence à Associação Dignitude. Consiste numa rede solidária do medicamento, dando a oportunidade a pessoas com dificuldades económicas acederem aos MSRM gratuitamente. Cada beneficiário possui um cartão que deverá ser apresentado no ato da cedência dos medicamentos.

3.2. Pontos Fracos

Dificuldade de associação entre Denominação Comum Internacional (DCI) e Nome Comercial

Durante o meu percurso académico, nas aulas teóricas e teórico-práticas são-nos lecionados, essencialmente, os fármacos por DCI sendo raras as vezes em que abordamos o

nome dos medicamentos por nome de fantasia. A princípio senti algumas dificuldades em associar o nome da substância ativa com os nomes de fantasia.

Tal como já foi referido, atualmente, a prescrição de medicamentos por DCI é obrigatória, salvo raras exceções, contudo ainda existem muitos utentes que nos chegam à Farmácia que designam os medicamentos pelo seu nome comercial o que me causou por vezes uma certa insegurança por não saber identificar de imediato o medicamento em questão durante o atendimento. O contrário acontece com os utentes, uma vez que estando mais familiarizados com o nome de fantasia gera um pouco de confusão, levando-o por vezes a pensar que se tratam de medicamentos diferentes, devido a estas diferentes designações.

Nesse aspeto a receção de encomendas é uma ferramenta importante que nos permite ter maior contacto com ambas as designações, no entanto, só com algum tempo de experiência é que adquirimos mais prática, pelo constante contacto com os nomes de fantasia. Seria vantajoso, no meu entender, ter um maior contacto ao longo do curso com as designações de marcas comerciais tanto de MSRM como de MNSRM.

Receitas Manuais

Existem 3 tipos de apresentações de receita médica: a eletrónica materializada (em papel), a eletrónica desmaterializada (sem papel) e a manual. Segundo a legislação em vigor, para os 3 tipos de apresentação, a prescrição deve ser efetuada por DCI, salvo raras exceções que deverão ser corretamente justificadas, sendo elas: medicamentos com margem ou índice terapêutico estreito; intolerância ou reação adversa prévia ao medicamento; continuidade de tratamento superior a 28 dias. Nas duas primeiras é obrigatória a dispensa do medicamento com a denominação comercial prescrita. Na última exceção há a possibilidade da dispensa do um medicamento dentro do mesmo grupo homogéneo, desde que o P.V.P seja inferior à denominação comercial prescrita. [10]

Atualmente apenas é permitida a prescrição manual no caso de situações excecionais, tais como: falência informática; inadaptação fundamentada do prescritor, previamente confirmada e validada anualmente pela respetiva Ordem Profissional; prescrição no domicílio; até 40 receitas/mês. [10]

No caso concreto de receitas manuais e eletrónicas materializadas existem regras específicas quanto à prescrição de medicamentos. Podem ser prescritas até um máximo de 4 embalagens de medicamentos distintos. Por medicamento apenas podem ser prescritas 2 embalagens. No caso de medicamentos cuja apresentação seja sob a forma de embalagem unitária (contém apenas uma unidade de forma farmacêutica) podem ser prescritas 4 embalagens, no máximo, do mesmo medicamento. Existem também outros fatores a ter em

conta aquando da validação deste tipo de receitas manuais, nomeadamente se está corretamente datada e assinada; se contém vinheta identificativa do local de prescrição; vinheta identificativa do prescritor; se foi assinalada, com uma cruz, a exceção que justifica a utilização da receita manual; entre outras. [11]

Foi para mim um desafio contactar com as receitas manuais, sendo cada vez menos frequente a sua utilização, tinha alguma dificuldade sempre que me eram apresentadas na Farmácia, com este modelo de receita, é necessário reforçar a nossa atenção para a validação, pois existem fatores que a tornam distinta das receitas eletrónicas, atendendo ao facto de que por serem escritas manualmente poderão acarretar uma maior percentagem de erro, para além do que, ao contrário do que acontece com as receitas eletrónicas, a introdução do medicamento ou produto de saúde prescrito tem de ser efetuada manualmente no sistema informático, o que também só por si pode levar a erros na identificação do medicamento.

Este tipo de modelo de receita apresenta, portanto, algumas desvantagens no meu ponto de vista.

Regimes excecionais de comparticipação

Os medicamentos são comparticipados, segundo determinados níveis de escalões (A,B,C ou D), que são estabelecidos de acordo com as indicações terapêuticas do medicamento, as entidades que o prescrevem bem como o consumo acrescido para doentes que sofram de determinadas patologias nomeadamente: Paramiloidose; Lúpus; Hemofilia; Hemoglobinopatias; Doença de Alzheimer; Psicose maníaco-depressiva; Doença inflamatória intestinal; Artrite reumatoide espondilite anquilosante; Dor oncológica moderada a forte; Dor crónica não oncológica moderada a forte; Procriação medicamente assistida; Psoríase; Ictiose.

É de notar que se exigem condições específicas quanto à prescrição para a aplicação destes mesmos regimes de comparticipação. É, portanto, imprescindível ter conhecimento das imposições legais respeitantes a estes sistemas, nesse sentido, nos primeiros dias de estágio foram-me fornecidas as ferramentas necessárias para que pudesse tomar conhecimento dos despachos legais e dos atuais sistemas de comparticipação.

Pelo facto de durante o meu percurso académico, não me terem alertado/sensibilizado para estas condições de prescrição específicas senti, inicialmente, alguma dificuldade em proceder à verificação das mesmas.

Inseguranças

O facto de o estágio curricular ser realizado apenas no 5º ano não nos permite ter ideia do que é a prática diária em Farmácia Comunitária e mesmo tendo já realizado 2 Estágios de

Verão, numa perspetiva mais observacional, o contacto direto com público aquando do atendimento ao balcão foi inicialmente desafiante, na medida em que senti certas inseguranças em transmitir informações que não fossem corretas ou pelo receio de não cumprir os procedimentos informáticos.

Também por vezes a impaciência dos utentes aumentava a minha insegurança e nervosismo, no sentido em que, fornecer todas as informações essenciais e proceder aos passos necessários para a cedência do medicamento ou de produtos de saúde no programa informático no menor tempo possível, era realmente desafiante.

Por ser estagiária, e não estando os utentes habituais da farmácia comigo familiarizados, senti por vezes uma certa resistência a serem atendidos por mim ou mesmo quando atendidos tinham receio em aceitar o meu aconselhamento.

É de notar que estas inseguranças e receios foram regredindo ao longo do meu estágio, pela prática diária, o que me permitiu consolidar e adquirir mais conhecimentos tornando-me mais confiante e ir ganhando também a confiança dos utentes.

Desconhecimento de funcionalidades *Sifarma2000*®

Este sistema informático, empregue em grande parte das Farmácias em Portugal, representa o instrumento de trabalho da Farmácia, é a partir dele que são realizados diferentes procedimentos tais como: pedido e gestão de encomendas, receção de encomendas, controlo de *stocks*, prazos de validade, controlo de estupefacientes, psicotrópicos e benzodiazepinas, gestão de clientes, emissão e regularização de devoluções, realizar a dispensa de produtos farmacêuticos e/ou outros produtos. O *Sifarma2000*® disponibiliza um conjunto de ferramentas que permite ter conhecimento da posologia, de contraindicações, interações, informação sobre princípios ativos e excipientes, possíveis reações adversas, precauções a tomar, entre outras informações disponíveis.

Inicialmente, por não estar muito familiarizada com o *Sifarma2000*® na componente de atendimento, a adaptação e o reconhecimento das várias funcionalidades do sistema informático foram um pouco difíceis para mim.

Detetada esta minha inexperiência com o sistema informático, senti que por vezes, a eficácia dos meus atendimentos era comprometida, não me permitindo avançar sozinha, sendo que várias vezes tinha mesmo que chamar um colega para me elucidar em relação ao programa informático, o que remetia para um maior tempo de espera e impaciência por parte do utente, causando-me um certo desconforto perante este.

3.3. Oportunidades

Formações

Como futura farmacêutica considero essencial que o conhecimento adquirido ao longo do nosso percurso académico não permaneça estático, nesse sentido, a participação em ações de formação permite tomar conhecimentos de novos temas, novas abordagens ou até mesmo novos produtos que tenham surgido até então. Tive a oportunidade de frequentar duas formações presenciais: uma relacionada com nutrição clínica, mais direcionada aos problemas de desnutrição e outra relacionada com a COVID-19. Ambas foram bastantes enriquecedoras, no sentido em que focaram problemas atualmente bastante relevantes. Devido à situação a que estamos a vivenciar e por todas as medidas preventivas a que estamos obrigados a cumprir tive também a oportunidade de assistir a formações relacionadas com Dermofarmácia e Cosmética por meio de plataforma virtual, que apesar da interação ser um pouco diferente, como por exemplo em termos de poder testar produtos, penso que é uma boa alternativa para nos mantermos informados em relação a temas atuais e certas novidades no mercado. Como estagiária tenho consciência de que as ações de formação que frequentei enriqueceram os meus conhecimentos.

Novas tecnologias

Com a evolução dos tempos, o impacto das redes sociais no nosso dia a dia tem seguido uma trajetória exponencial. Redes sociais tais como o *Facebook* e o *Instagram* são atualmente grandes ferramentas de *marketing* para a Farmácia. É a partir destas tecnologias que podemos chegar informação, de forma rápida, à população em geral, seja ela de produtos, de campanhas profissionais, de rastreios, de informações de cariz científico ou até mesmo informações sobre a própria Farmácia como o seu horário ou atividades por ela organizadas. Estas ferramentas permitem também de alguma forma estar em contacto de forma mais próxima com os utentes.

Novo *Sifarma*®

O Novo *Sifarma*® apresenta-se com uma imagem renovada que nos remete para um conceito de *smartphone*, com o intuito de aplicação em meios eletrónicos que vão além do "monitor fixo" a que estamos habituados. No meu estágio tive a oportunidade de trabalhar neste novo software que apesar de ainda ter algumas falhas enquanto programa informático, encontra-se em fase de implementação nas Farmácias a nível nacional, sendo que este novo programa informático irá muito em breve substituir o *Sifarma2000*®. Visto que este programa irá constituir, num futuro breve, o programa informático das Farmácias Portuguesas considero

o facto de ter tido contacto com o Novo *Sifarma*® permitiu-me adquirir algumas noções relevantes para quando iniciar a minha vida profissional como farmacêutica em Farmácia Comunitária.

Veterinária

Deparei-me que era habitual a procura de medicamentos e produtos de uso veterinário, por parte dos utentes, na Farmácia São Sebastião. Sabemos que hoje em dia a área relacionada com o "espaço animal" tem vindo a ter uma grande representação, uma vez que as preocupações em proporcionar a melhor vida possível, aos animais, têm sido crescentes. Por ser uma área em expansão, constitui uma grande oportunidade de marcado para as Farmácias. Apesar de termos uma unidade curricular relacionada com Preparações de Uso Veterinário senti, aquando o aconselhamento, algumas inseguranças. No entanto, por ser uma Farmácia com grande procura nesta área, permitiu-me estar mais familiarizada com este tipo de produtos dando-me oportunidade de ao longo do estágio ter mais confiança em relação ao aconselhamento e cedência. Saliento ainda que tive oportunidade de contactar pela primeira vez com receitas veterinárias.

Medicamentos Hospitalares

Atendendo à situação pandémica que atualmente vivenciamos foi autorizada, pelo Ministério da Saúde, a dispensa de medicamentos hospitalares, pelas Farmácias, a doentes em regime de ambulatório. Considero esta medida como uma oportunidade para a articulação entre farmacêuticos comunitários e hospitalares, apresentando a meu ver um serviço que permite oferecer aos doentes uma maior comodidade, assegurando a continuidade do seu tratamento e evitando riscos desnecessários nas deslocações até aos hospitais.

3.4. Ameaças

COVID-19

A chegada do novo coronavírus coincidiu exatamente com o período de duração do meu estágio. Foi um desafio para mim trabalhar durante este período inicial em que ainda não havia casos na Europa, mas o alarmismo já abrangia o mundo. Os utentes chegavam à Farmácia por vezes em aflição sem saber o que fazer, com medo do que iria acontecer, a esgotar *stocks* de medicamentos, máscaras e álcool gel… E nós como futuros profissionais de saúde temos o dever de saber ouvir, acalmar, reconfortar e explicar. É essencial, tal como em qualquer outro atendimento e ainda mais numa situação como esta, adotar uma atitude empática, fazer

uso de uma linguagem clara e simples, reforçando uma mensagem positiva. Aquando a chegada do vírus a Portugal o meu estágio foi interrompido, estando aproximadamente suspenso 2 meses, esse facto levou a que o conhecimento por mim adquirido fosse também interrompido de certa forma. Contudo tive a possibilidade de retomar sentindo-me mais confiante para iniciar a minha carreira profissional. Foi também nesse período de interrupção do estágio, que a Farmácia elaborou o seu próprio plano de contingência sendo que tive a oportunidade de o colocar em prática aquando o meu regresso. O facto de estar exposta a uma situação nova, inesperada e desafiadora como esta, permitiu-me ganhar mais confiança nas funções por mim exercidas.

Locais de venda MNSRM

Com a autorização da venda de MNSRM fora das Farmácias, locais devidamente autorizados pelo INFARMED a venderem este tipo de medicamentos, passa a constituir uma ameaça direta para as Farmácias, uma vez que estes locais permitem uma maior flexibilidade de preços, culminado com uma redução dos mesmos. Torna-se, portanto, uma opção mais favorável para os utentes, devido aos preços praticados, de aceder a estes locais de venda de MNSRM. Atendendo ao facto de que estes medicamentos não necessitam de receita médica há uma maior acessibilidade por parte dos utentes em adquiri-los, no entanto, apesar de se tratarem de medicamentos para patologias *minor* há sempre riscos associados à sua má utilização, pelo que é fundamental alertar o utente tanto para os benefícios como para os efeitos secundários, riscos ou certas precauções na sua utilização, ora é aqui que faz toda a diferença o aconselhamento ser efetuado por profissionais devidamente qualificados para tal. Além do mais para as farmácias tornam-se insustentável praticar este tipo de preços, pois ao diminuir as suas margens, de modo a ganhar uma certa vantagem competitiva, vai afetar negativamente a economia da Farmácia.

Medicamentos esgotados ou rateados

Com todas as alterações que o setor farmacêutico tem vindo a vivenciar obriga a que haja uma racionalização no abastecimento de medicamentos, sendo notória a dificuldade em disponibilizar aos utentes os medicamentos de que necessitam. Deparei-me muitas das vezes com este tipo de situação, em que atender ao pedido feito pelo utente era em muitos casos dificultada pelas existências de medicamentos esgotados ou rateados. Para os utentes era por vezes difícil de entender este tipo de situações, pondo em causa a credibilidade da Farmácia em relação à dispensa destes medicamentos. Do meu ponto de vista esta realidade constitui uma forte ameaça para as Farmácias.

4. Caso Prático

Contraceção oral de emergência: Utente do sexo feminino, com cerca de 20 anos, dirige-se à Farmácia São Sebastião referindo que pretendia a "pílula do dia seguinte". Foi-lhe questionado se a utente era diretamente a pessoa que iria utilizar a contraceção oral de emergência de modo a direcionar as questões necessárias. Questionou-se se a relação sexual tinha sido desprotegida ou se houve falha de algum método contracetivo, em que fase do seu ciclo menstrual se encontrava e se a relação sexual tinha ocorrido num período de tempo inferior a 72h. Foi-lhe questionada a eventual toma de medicamentos de forma a avaliar possíveis interações bem como a avaliação da inexistência de patologias do foro cardiovascular, oncológicas ou outras que não permitissem a cedência. Avaliada a situação e após identificado que a utente estava no período indicado para a toma da contraceção de emergência foi-lhe aconselhada a toma de Postinor® (levonorgestrel). Por último foram dadas algumas informações tais como explicar que o levonorgestrel atua na fase de pré-ovulação e explicar que a contraceção de emergência não tem uma eficácia de 100% na prevenção de uma gravidez. Relembrar a importância do início ou da retoma do método de contraceção e alertar para os efeitos secundários tais como cefaleia, vómitos, dores pélvicas, tonturas, náuseas, sensibilidade mamária para além de um possível atraso da menstruação de 1 ou 2 dias e que caso vomite 3h após a toma, deverá repetir o processo. Também foi prestado aconselhamento contracetivo referindo que a contraceção de emergência não protege contra doenças sexualmente transmitidas e relembrando a importância de utilizar um método de contraceção barreira para prevenção das mesmas.

5. Conclusão

Findando o meu estágio em Farmácia Comunitária, faço positivamente o meu balanço final em relação ao mesmo. Foi para mim uma experiência bastante enriquecedora e desafiante, que recordarei para sempre. Tendo terminado este período de estágio recordo a aprendizagem por mim feita até então e é com uma atitude positiva que revejo esta etapa tão importante do meu percurso académico, afirmo garantidamente que esta grande experiência me permitiu crescer enquanto pessoa e enquanto profissional.

O meu estágio permitiu-me comprovar que, realmente, o nosso papel enquanto profissionais de saúde tem uma enorme relevância para a população em geral, para a promoção da literacia em saúde, e do bem-estar dos utentes. É muito gratificante ver a confiança depositada pelos utentes no nosso trabalho, e isso reforçou, para mim, o papel do

farmacêutico comunitário, dando-me ainda mais motivação para agarrar a que será a minha futura profissão e desempenha-la com o máximo profissionalismo e exigência.

Em última instância, não posso deixar de agradecer mais uma vez a quem me permitiu vivenciar toda esta enorme experiência, à Farmácia São Sebastião e à sua respetiva equipa, por toda a sua empatia desde início, por todo o seu profissionalismo e empenho, levarei como exemplo tudo o que me ensinaram e tudo o que vivenciei durante estes meses.

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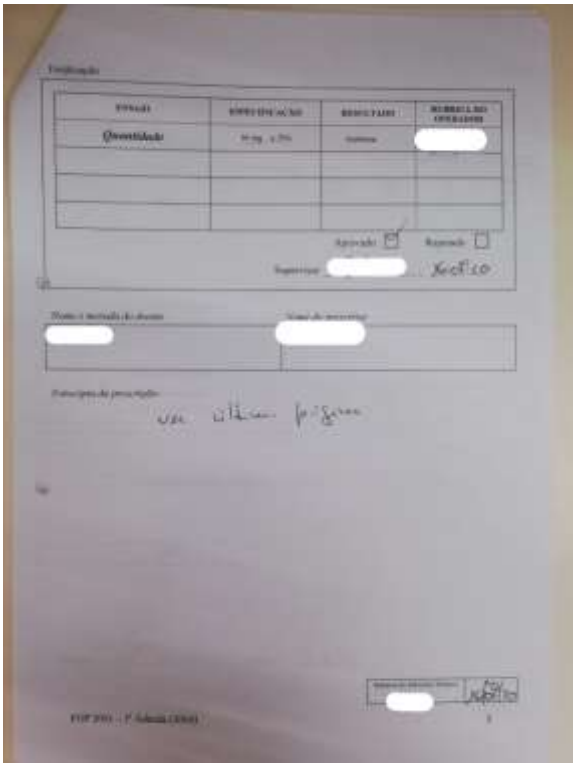
7. Anexos

Anexo 1. Ficha de preparação de medicamentos manipulados.

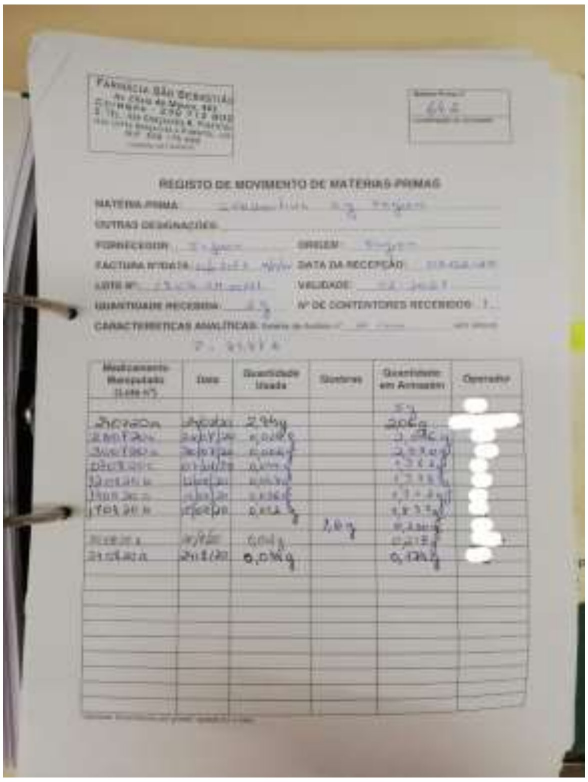

Farmácia São Sebastião

FICHA DE PREPARAÇÃO


16



Anexo 2. Ficha de registo de movimento das matérias primas.


REGISTO DE MOVIMENTO DE MATÉRIAS-PRIMAS

MATÉRIA-PRIMA

OUTRAS DESIGNAÇÕES

FORNECEDOR — ORIGEM

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CARACTERÍSTICAS ANALÍTICAS

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Parte II

Monografia

"*Nanotechnology-based therapeutics strategies towards the topical treatment of hair loss*"

Abstract

From a pharmacotherapy standpoint, topical delivery of 5 alpha-reductase inhibitors and vasodilators are considered an interesting option for the treatment of Androgenetic alopecia (AGA). Their targeting to skin cells and other structures involved in AGA may decrease therapeutic dose and overtake the secondary effects associated with systemic delivery effects but local irritation mainly due to conventional formulations solvents, like ethanol and propylene glycol remains a challenge to overcome. Submicron formulations have revealed remarkable effects on skin permeation by drugs to circumvent several diseases and most particular in the treatment of AGA.

This review focus on nanotechnology-based therapeutic strategies for skin delivery of 5 alpha reductase inhibitors: Finasteride and Dutasteride as well as vasodilators like Minoxidil either alone or in combination. We critically discuss the most common nanoformulations, their production and physicochemical characterization and both *in vitro* and *in vivo* tests performed within the scope of AGA treatment.

Keywords: 5 alpha reductase inhibitors; androgenic alopecia; follicular targeting; nanoparticle; nanotechnology; therapeutics; topical drug delivery; vasodilator.

Resumo

A administração tópica de inibidores da 5 alfa-redutase e de vasodilatadores constitui uma estratégia farmacológica interessante no tratamento da Alopecia androgenética (AGA). A sua administração vetorizada para células da pele e outras estruturas envolvidas na AGA pode diminuir a dose terapêutica e dessa forma, ultrapassar os efeitos secundários associados aos efeitos da administração sistémica, mas a irritação local, principalmente devida à presença de co solventes como o etanol e propileno glicol em formulações convencionais, continua a ser um desafio a superar. As formulações baseadas em nanotecnologias revelaram efeitos notáveis na permeação cutânea, por medicamentos, para contornar várias doenças e mais particularmente no tratamento da AGA.

Esta revisão centra-se nas estratégias terapêuticas baseadas na nanotecnologia para a administração cutânea de inibidores da 5 alfa-redutase: Finasterida e Dutasterida, bem como vasodilatadores como Minoxidil, quer isoladamente quer em combinação. Discutimos criticamente as nanoformulações mais comuns, a sua produção e caracterização físico-química e os testes *in vitro* e *in vivo* realizados no âmbito do tratamento da AGA.

Palavras-chave: inibidores da 5 alfa redutase; alopecia androgenética; alvo folicular; nanopartículas; nanotecnologia; terapêuticas; libertação tópica de fármaco; vasodilatador.

List of Abbreviations

AGA - Androgenic alopecia

AgNP - Silver nanoparticle

AKT2 - ATP-sensitive potassium channel opener gene code

AuNP - Gold nanoparticle

CD - Cyclodextrin

CSO-SA - Chitosan oligomer stearic acid

DHT - Dihydrotestosterone

DPC - Dermal papilla cells

EL - Cremophor® EL

EMA - European Medicines Agency

FDA - Food and Drug Administration

FNS - Finasteride

FPHL - Female pattern hair loss

GL - Glycerol

HA-PLGA - Hyaluronate–poly(lactide-co-glycolide)

HBL - Hydrophile-lipophile balance

HP - Hydroxypropyl

IGF-1 - Insulin-like growth factor-1

ISO - International Organization for Standardization

KATP - ATP- sensitive potassium channel

LCN - Liquid crystalline nanoparticles

MNX - Minoxidil

MO - Monoolein

MS - Molar substitution degrees

NE - Nanoemulsion

NLC - Nanostructured lipid carriers

NTF - Nano-transferosomal

OA - Oleic acid

OECD - Organization for Economic Cooperation and Development

PAA - Poly (acrylic acid)

PDGF - Platelet-derived growth factor

PEG - Poly (ethylene glycol)

PG - Propylene glycol

PLGA - Poly (lactide-co-glycolide)

PS - Poly styrene

PSU - Pilosebaceous unit

RH - Cremophor® RH 40

SC - Stratum corneum

SLN - Solid lipid nanoparticle

SPC - Soya phosphatidylcholine

TiO_2 - Titanium dioxide

VEGF - Vascular endothelial growth factor

ZnO - Zinc oxide

Figure and Table Index

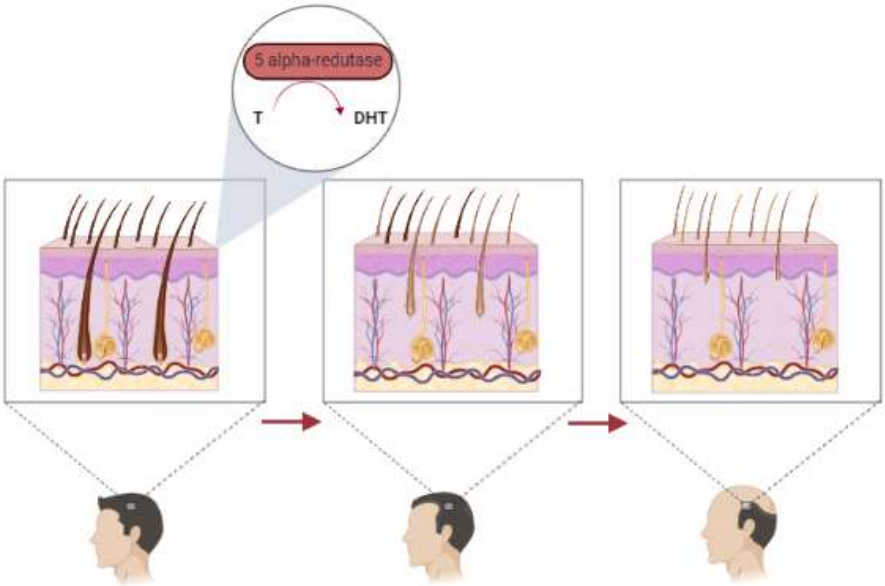
Annex Index

1. Introduction

Androgenetic alopecia (AGA), more commonly known as hair loss, is a consequence of hair growth cycling change as well as of follicular miniaturization (BAS *et al.*, 2015). In the course of this process, anagen phase shortens while the telogen phase is extended, occurring a transition of terminal to vellus follicles while the hair becomes finer and less pigmented (BAS *et al.*, 2015, KANTI *et al.*, 2018). The hair loss is common in male and female, and among Caucasians it can affect 70% of men and 42% of women (EPSTEIN *et al.*, 2019). In this pathology, variable patterns of hair loss are found in both sexes (BAS *et al.*, 2015). Despite being a benign pathology, this disease leads to psychological and social consequences, with an effect on the quality of life of an individual (BAS *et al.*, 2015).

So far, Food and Drug Administration (FDA) has approved two drugs for the treatment of AGA: topical Minoxidil (MNX), a vasodilator, approved for men and female pattern hair loss (FPHL) and oral Finasteride (FNS), a selective inhibitor for type 2 of 5 alpha-reductase. FNS may also be used off-label in women (EPSTEIN *et al.*, 2019). Both treatment options have shown important side effects often pointed out as the most important causes regarding patients lack of adherence to these treatments. Among the side effects attributed to topical administration of MNX stand out dermatitis, erythema, itching, dandruff, burning/stinging, weight gain, headache, respiratory infections and systemic effects such as arrhythmias, dizziness and low blood pressure (EPSTEIN *et al.*, 2019). In the same way, the oral administration of FNS has been also showing undesirable effects among which, erectile dysfunction, reduced libido, gynecomastia, variations in mood and ejaculation disorder. Besides, it may delay the diagnosis of prostate cancer (EPSTEIN *et al.*, 2019, KANTI *et al.*, 2018).

The 5 alpha-reductase is an enzyme that converts testosterone to dihydrotestosterone (DHT), event considered as determinant in AGA (BREITKOPF *et al.*, 2013, EPSTEIN *et al.*, 2019). The 5 alpha-reductase enzyme has two forms (type 1 and type 2) (EPSTEIN *et al.*, 2019). According to some researchers, individuals who do not have 5 alpha-reductase do not develop the disease (EPSTEIN *et al.*, 2019). Elevated DHT levels in the scalp are found in persons with AGA (BREITKOPF *et al.*, 2013) and it is believed that DHT interacts with the androgen receptor, activating signaling pathways, resulting in the extended telogen phases with damage in hair follicles (BREITKOPF *et al.*, 2013, EPSTEIN *et al.*, 2019) as it is illustrated in Figure 1.



(I) (II) (III)

Figure 1. Miniaturization of the hair follicle in the pathology of Androgenic alopecia. During Androgenetic alopecia, there are several distinct stages, in which at an early stage we have healthy, strong and resistant hair (I), but with the presence of high levels of dihydrotestosterone in the follicle begins to lose pigmentation, density and diameter, becoming progressively shorter (II), culminating in hair loss (III). T- Testosterone; DHT- Dihydrotestosterone.

Dutasteride (DTS), a nonselective 5 alpha-reductase inhibitor, is more potent than FNS in blocking of 5 alpha-reductase with a decline of the serum DHT degree of approximately 90% (EPSTEIN *et al.*, 2019, KANTI *et al.*, 2018). While not FDA approved for treatment of AGA, it is an alternative option to FNS (YORK *et al.*, 2020). However, in the same way as FNS, side effects associated with sexual dysfunction following its oral administration have been reported (CHOI *et al.*, 2016, VAÑÓ-GALVÁN *et al.*, 2019).

Thus, the adherence of patients to aforementioned therapies has been decreasing due to adverse effects particularly the ones associated with skin irritation and sexual dysfunction. Taking into account that both types of drugs must be administered to the end in order to obtain positive results its discontinuation leads to losses of all the advantages they may have caused (ANDY *et al.*, 2019, EPSTEIN *et al.*, 2019).

Drugs delivered by topical route may offer advantages compared to their oral intake, including a lower total daily dose to achieve therapeutic effect, site-specific drug delivery and eventually less drug side effects can be achieved (STANOS and GALLUZZI, 2013).

Topical delivery of 5 alpha-reductase inhibitors such as FNS (FARAH *et al.*, 2020) can reach therapeutic levels with reduced systemic absorption. A recent single-center and double-blind study compared the efficacy of topical 3% MNX solution either alone or in combination

with 0.25% FNS solution for treatment of postmenopausal FPHL patients (SUCHONWANIT *et al.*, 2019). The combined treatment showed best results with regard of increased hair diameter and increased hair density (IAMSUMANG *et al.*, 2020).

Combination therapy using drugs with different mechanisms of action can be therefore an option to decrease secondary effects in commonly AGA used drugs.

Recent developments in pharmacology and nanotechnology have strengthened their attention for nanoparticles-mediated combination therapy towards a synergistic therapeutic outcome (SHRESTHA *et al.*, 2019). Besides, as new drug delivery systems (LEE *et al.*, 2018) they have been used in the treatment of skin diseases (CHEN *et al.*, 2019) , due to their potential regarding a reduction of side effects and better controlled release of drugs (LADEMANN *et al.*, 2013).

This review aims to find out relevant nanoparticles that have been investigated for treatment in AGA, while topical carriers of MNX, FNS and DTS as illustrated in Figure 2. A systematic and critical overview of nanoencapsulated drugs *in vitro* and *in vivo* studies performed in the scope of treatment AGA the main objective as well as toxicity concerns and regulatory requirements towards their appropriate development for market introduction authorization.

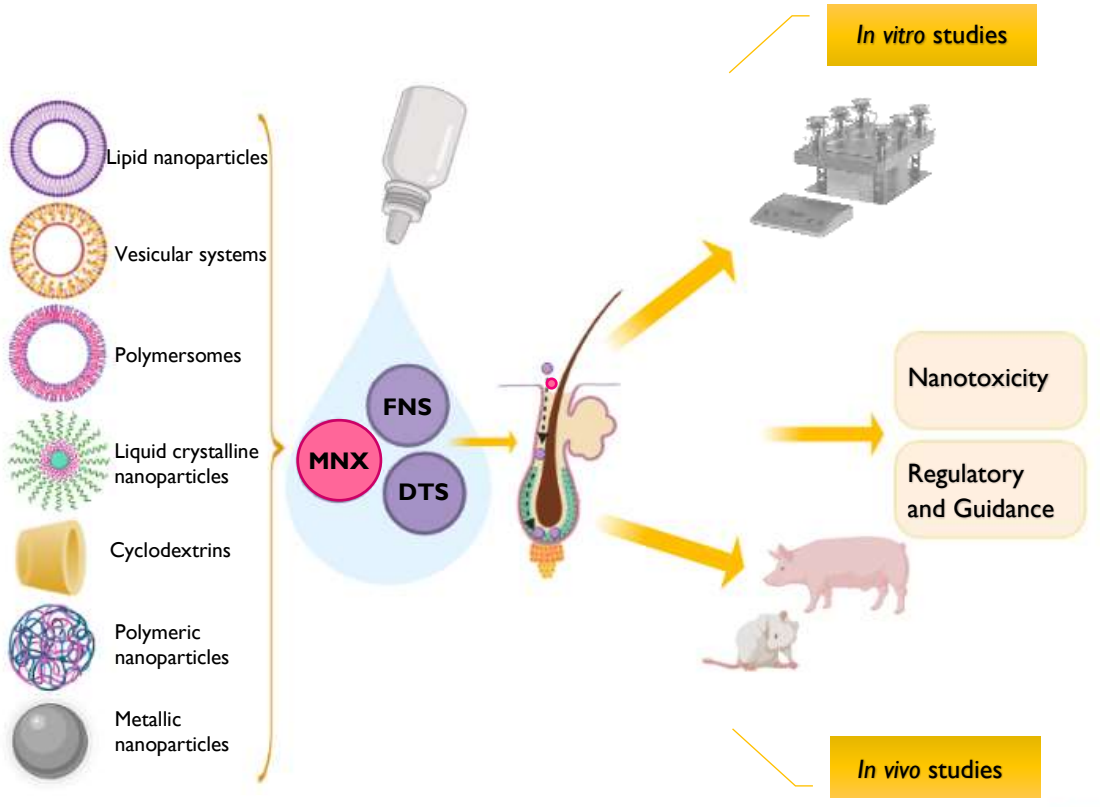


Figure 2. Schematic summary of the nanoparticles studied so far for Minoxidil (MNX), Finasteride (FNS) and Dutasteride (DTS) as topical administration systems for the treatment of Androgenetic alopecia.

2. Therapeutics for the treatment of hair loss by topical route

2.1. Formulation requirements

The skin has an impermeability function, preventing the passage of foreign agents in the body, for this reason the administration of particles to the desired target is a major challenge (CARTER *et al.*, 2019, IQBAL and BABOOTA, 2018). However, it demonstrates to be an alternative path for drug administration given the multiple benefits such as 1) non-painful route 2) good compliance by the user 3) improvement in drug delivery and 4) localized drug deposition (BOLZINGER *et al.*, 2012).

There are different drugs penetration pathways across the stratum corneum (SC): follicular (through hair shaft), intercellular (across intercellular lipid matrix) and intracellular (across the corneocytes and consequently passing by intercellular lipid matrix) routes.

The direction of topically applied drugs into annexes cutaneous, SC and across the skin has played an important role in the treatment of skin diseases (GRICE *et al.*, 2010). Drugs have to overtake the outermost skin to penetrate into or across the skin (BLUME-PEYTAVI *et al.*, 2010). Properties such as drug physicochemical characteristics (e.g. lipophilicity, molecular weight and structure) and the amount of topically applied formulations are critical parameters for the level of penetration through the SC.

Drug penetration across skin appendages (the follicular route) has gained increasing importance (BOLZINGER *et al.*, 2012). The follicular pathway may perform a critical role in administration drugs on the scalp by the large number of follicles in this place (CARTER *et al.*, 2019). The follicular route has emerged as an alternative route for formulations that cross hair follicle greatly efficiently. The hair follicles pass-through layers skin, where the SC is less dense, enclosures a close network of capillaries and presents diverse targets of interest for treatment of AGA (BOLZINGER *et al.*, 2012).

2.2 Conventional formulations

The 5% and 2% solution are the two conventional formulations by MNX (EPSTEIN *et al.*, 2019). MNX of chemical structure 2,4 diamino-6 piperdinipyimidine-3-oxide is a derivative of pyrimidine and its mechanism of action on hair growth is not yet fully understood (GHONEMY *et al.*, 2019). Even so, MNX action is likely to be related to their ability to the opening of potassium channels, facilitating the oxygenation and the circulation of a greater number of nutrients by increasing blood flow (EPSTEIN *et al.*, 2019). These events trigger an increase in hair growth as well as an increase in vascular endothelial growth factor (VEGF), inhibition of the androgen route, and regulation of the prostaglandin (D2/E2) signaling. Overall,

a change in the hair cycle occurs in which a reduction in the telogen (inactive) phase of hair loss takes place concomitantly with the prolongation of the anagen phase (active growth) allowing thicker hair follicles to form (EPSTEIN *et al.*, 2019, GHONEMY *et al.*, 2019).

Adverse effects have been demonstrated to be more frequent with higher MNX concentration mainly due to a higher amount of propylene glycol (PG) or ethanol in 5% solution (EPSTEIN *et al.*, 2019) both of them acting as MNX solubilizing agents (LOPEDOTA *et al.*, 2015). Conventional formulations of MNX are usually applied twice a day and therefore an alcohol contact time with skin is higher and long enough to promote local dehydrating-related adverse effects (GRICE *et al.*, 2010, HERRMANN *et al.*, 2017, LOPEDOTA *et al.*, 2018).

The conventional administration of FNS consists of 1mg oral daily intake (EPSTEIN *et al.*, 2019). FNS is a synthetic azosteroid (4-aza-3-oxosteroid), firstly approved for benign prostatic hyperplasia treatment (ROQUE *et al.*, 2017). FNS is a selective inhibitor for type 2 of 5 alpha-reductase, thus preventing the transformation of testosterone into DHT, reducing the action of androgens on hair growth (BREITKOPF *et al.*, 2013).

However, FNS low aqueous solubility affects their transport process across the skin (AHMED and AL-ABD, 2018, LIMA *et al.*, 2018) thus hampering its use for the treatment of AGA. Its solubilization it's made through the use of cosolvents such as ethanol and PG.

Oral administration of DTS 0.5 mg per day has shown efficacy towards prevention or treatment of AGA (CHOI *et al.*, 2016, KANTI *et al.*, 2018) and usually it is used when no clinical improvement with FNS is found (JUNG *et al.*, 2014). There has been evidence that DTS is stronger than FNS in suppression type 2 (5 alpha-reductase) and besides it has shown a suppressing type 1 (5 alpha-reductase) higher effect when compared to FNS (JUNG *et al.*, 2014). However, its side effects associated with sexual dysfunction are likely to be greater when compared with FNS (JUNG *et al.*, 2014).

DTS is an extremely lipophilic molecule that presents poor aqueous solubility, lower than MNX and FNS, making difficult its incorporation in conventional topical formulations without the use of cosolvents, so the presence of a viscous or oily residue on the scalp may result in the rejection of the application of this drug because of its undesirable appearance on the hair (USHIROBIRA *et al.*, 2020).

3. Nanotechnology-based formulations for the topical treatment of hair loss

Nanotechnology emerges, consequently, as an alternative to improve patient compliance, providing effective, safe and better control of, drug release, while reducing the secondary effects of the conventional formulations (NISKA *et al.*, 2018). Besides, nanoparticles can be stored in the follicles and stay there for a long time. After stored in specific target

structures nanoparticles may release the drug and, therefore, exercise their effects as illustrated in Figure 3.

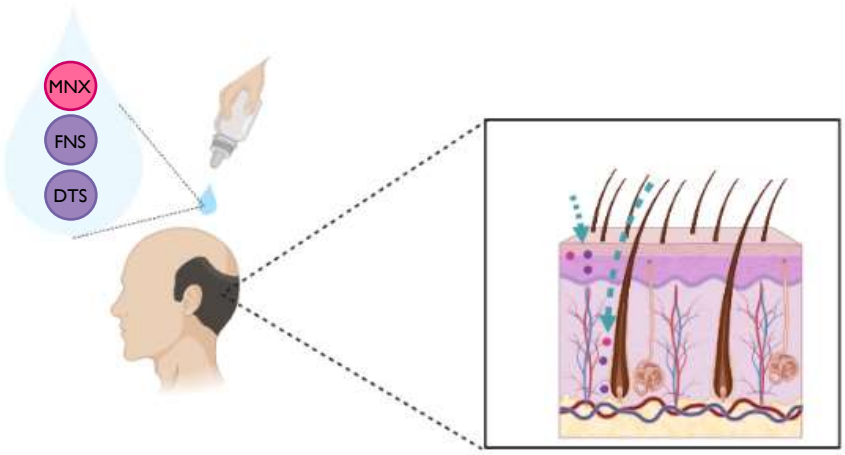


Figure 3. Drug release from nanoparticles across stratum corneum or follicular route. Topical application of nanotechnology-based formulations containing as drug: Minoxidil (MNX), Finasteride (FNS) or Dutasteride (DTS), and their release according to the follicular route or through the stratum corneum.

There have been several delivery systems based on nanotechnology developed specifically for the topical treatment of AGA. These nanotechnology-based delivery systems have been produced using several materials such as polymers, lipids, metals, etc. The following subsections describe the wide range of nanoparticles explored in management of AGA among which lipid nanoparticles, vesicular systems, polymeric nanoparticles, polymersomes, liquid crystalline nanoparticles, cyclodextrins and inorganic nanoparticles such as metallic nanoparticles.

3.1. Lipid nanoparticles

Lipid nanoparticles emerged approximate thirty years ago and have shown an increasing development in the pharmaceutical and cosmetic sector (MULLER *et al.*, 2007). They have properties suitable for the topical application of drugs among which, ability to transport drugs either hydrophobic and hydrophilic, more economical than mostly of other delivery nanosystems as well as the control and targeting drug release through the nanoparticle (KAKADIA and CONWAY, 2015). Most investigations related to lipid nanoparticles are concentrated in the cosmetic field, because its small size favors the penetration of drugs in the SC (SCHROETER *et al.*, 2010).

Lipid nanoparticles are an established strategy for therapeutic applications allowing entrapment as both lipophilic drugs a hydrophilic, enhancing the stability of pharmaceuticals

and regulate and targeting drug release. They have been considered a promising strategy as a drugs delivery system in AGA treatment for its potential for better-monitored release kinetics (DOKTOROVOVA *et al.*, 2016). They can be classified into two major groups: Solid lipid nanoparticles (SLN) constituted of a mixture of solid lipids, and Nanostructured lipid carriers (NLC). NLC are the second generation of lipid particles with improved properties, due to an organization lower level of lipid core (DOKTOROVOVA *et al.*, 2016). The composition of NLC are based in a mixture of solid lipid with liquid lipid (KAKADIA and CONWAY, 2015). This difference in the composition of the NLC creates various imperfections in matrix, allowing better accommodation of the drug (JAISWAL *et al.*, 2016). The SC constitutes a hydrophobic layer and lipophilic molecules can penetrate across the layer easily because of development of a film on the skin surface, with occlusion effect, enhancing skin permeation (KAKADIA and CONWAY, 2015).

3.2. Vesicular nanoparticles

Vesicular transporters have shown great potential for topically administered drugs in the treatment of AGA (PEREIRA *et al.*, 2018, SINGLA and SACHDEVA, 2015). They can improve the transport of drugs through the skin and decrease side effects by conventional formulations often associated with drug systemic absorption. They are often claimed as non-toxic and non-irritating systems capable of carrying hydrophilic and lipophilic drugs (NOUNOU *et al.*, 2008). Vesicular transporters are nanoparticles composed of lipids in which the drug is trapped inside an aqueous core and included in the lamellar membrane, depending on the physicochemical characteristics (MBAH *et al.*, 2014). Given the fact that they are lipophilic particles, and due to their nanosize, these nanocarriers more easily permeate the SC (JAIN *et al.*, 2017).

3.2.1 Niosomes

Niosomes or non-ionic surfactant vesicles are constituted by surfactant molecules (as poly (oxyethylene alkyl ethers)) and cholesterol (HUA, 2015). They are similar to liposomes with enhanced stability and low production cost (IQBAL and BABOOTA, 2018). The incorporation of surfactants, besides making them more stable can increase niosomes skin permeation because they have the capability of distortion the structure of the SC becoming the later looser and more permeable thus promoting the uptake, and fusion of niosomes with SC (HUA, 2015, IQBAL and BABOOTA, 2018). Initially formulated for cosmetic industry they have been used for drug delivery because of their advantages associated with reduced skin irritation, enhanced skin penetration and drug accumulation in the deep layers of the skin

(IQBAL and BABOOTA, 2018, MOHAMED *et al.*, 2008). These effects on skin have been described as niosomes size dependent (IQBAL and BABOOTA, 2018).

3.2.2 Ethosomes

Ethosomes are composed of phospholipids, water and high concentration of alcohol (20–50% of ethanol and 15% of isopropyl alcohol or PG) (HUA, 2015, MOTA *et al.*, 2017) and they have shown an improved skin delivery of encapsulated drugs (HUA, 2015). The high alcohol concentration results in malleable and very fluid vesicles that can trigger the drug into deeper layers of the skin. The alcohol content can also modify the structure of the SC to easily penetrate across the skin (TOUITOU *et al.*, 2000, TOUITOU *et al.*, 2000). Ethosomes are a potential system to deliver both hydrophilic and lipophilic drugs into the skin, with several advantages among which easier production, high stability, great encapsulation efficiency, and low risk profile (TOUITOU *et al.*, 2000, ZHU *et al.*, 2013).

3.2.3 Transfersomes

Transfersomes are deformable vesicles used for drug delivery to the skin (BORGHETI-CARDOSO *et al.*, 2020) constituted by an aqueous core surrounded phospholipids (as phosphatidylcholine) and surfactants (BENSON, 2006, RAJAN *et al.*, 2011). The surfactant works as an edge activator that confers flexibility proprieties on the vesicles thus destabilizing lipid bilayers and deforming the structure of vesicle (BENSON, 2006). In contrast to liposomes considered too large cross SC pores transferosomes sizing up to 500 nm have the capacity to squeeze across the SC pores (RAJAN *et al.*, 2011). Transfersomes can disturb intercellular lipid structure and penetrate deeper into the layers of the skin under the influence of the osmotic gradient (HUA, 2015). Transferosomes are biocompatible and biodegradable vesicles, that can prevent metabolic degradation of the drug. Moreover, they provide slowly and gradual drug delivery and they can accommodate as much hydrophobic as hydrophilic drugs (RAJAN *et al.*, 2011).

3.3. Polymersomes

Polymersomes (artificial polymeric vesicles) have similar structures with liposomes, constituted of an aqueous core surrounded by a polymeric membranes made of amphiphilic polymers (BORGHETI-CARDOSO *et al.*, 2020) and for this reason, they belong to the nanocapsule group. They are self-assembled polymers, with shells composed of amphiphilic block copolymer, engineered with an appropriate hydrophilic/hydrophobic equality. Among the advantages of polymersomes stands out their increase stability, mechanical strength, good

chemical versatility, as well as their capacity to encapsulate both hydrophobic and hydrophilic drugs. As a result, polymersomes are a great opportunity to formulate effective therapeutic systems for controlled drug release. (IYISAN and LANDFESTER, 2019, JINFENG *et al.*, 2012).

3.4. Liquid Crystalline Nanoparticles

Liquid Crystalline Nanoparticles (LCN) are represented by a highly twisted lipid bilayer and two water channels which do not cross and, they have multiples advantages such as high solubilization of both hydrophobic, and hydrophilic drugs, biocompatibility, biodegradability, improved stability and easy formulation (RIZWAN *et al.*, 2013, YAGHMUR and GLATTER, 2009). The enhancement in skin drug delivery associated with LCN is mainly attributed to the bioadhesive properties of their biological membrane-mimicking structure and the permeation-enhancing effect of some ingredients used for their formulation, particularly glyceryl monooleate (FREAG *et al.*, 2019). Skin penetration of FNS by monoolein (MO)-based LCN appears to be optimized upon incorporation of a surfactant such as poloxamer® P407 (a block copolymer) (BOYD *et al.*, 2009).

3.5. Cyclodextrins

Cyclodextrins (CD) are resulting cyclic oligosaccharides by enzymatic digestion of starch with compounds a lipophilic central cavity and hydrophilic outer surface (FIREMAN *et al.*, 2011, JANSOOK *et al.*, 2018). CD are consisting of D-glucopyranose units and there are three different CD developed for pharmaceutical products, being them: α, β, and γ with six, seven, and eight D-glucose units, respectively. CD protect the drug to enzymatic degradation and can improve drug permeability and drug bioavailability, such as enhance aqueous solubility of MNX and enhance stability of FNS (JANSOOK *et al.*, 2018). Their cavity is adequate to may load a great number of drugs able to form drug-CD inclusion complexes that can improve topical drug delivery to target hair follicles and pores on the skin (LOFTSSON, 2014).

3.6. Polymeric Nanoparticles

Polymeric nanoparticles are defined as solid, submicron-sized drug carriers that may or may not biodegradable and the term polymeric nanoparticle is a collective name for both nanospheres and nanocapsules (LOPES *et al.*, 2017). Polymeric nanoparticles can be prepared from biodegradable polymers (e.g. poly (acrylamide), poly (styrene) (PS), poly (methyl methacrylate) and poly (acrylates) or non-biodegradable which can still be divided into natural polymers (such as chitosan, alginate, gelatin and albumin) and synthetics (such as poly (ε-caprolactone), poly (ethylene-glycol) (PEG), poly (lactide-co-glycolide) (PLGA)and poly

(lactide) (REIS *et al.*, 2017). Its use for the topical administration of drugs has played an important role, with focus on targeted delivery to the follicles, supplying the drug in a sustained way for a long time (IQBAL and BABOOTA, 2018, MOTA *et al.*, 2017).

3.7. Metallic Nanoparticles

Metallic nanoparticles have multiple applications, including in the field of topical application. Metals and metallic oxides have been used, for their healing abilities, sun protection, treatment of infections caused by fungi or bacteria (NISKA *et al.*, 2018). They have played a major role in treatment of skin diseases with regard to the targeted delivery of drugs (NAAHIDI *et al.*, 2013, NISKA *et al.*, 2018). Among the most used metallic nanoparticle, we find titanium dioxide (TiO2), gold nanoparticles (AuNPs), zinc oxide (ZnO) nanoparticles and silver nanoparticles (AgNPs) (GOYAL *et al.*, 2016, NISKA *et al.*, 2018).

4. Physicochemical characterization

Depending on their physicochemical properties such as size and surface charge, nanotechnology-based formulations may be able to across intact into the skin or upon their degradation on skin surface release of incorporated drugs into the skin can occur.

Here, are presented the latest advances in nanotechnology for application of MNX, FNS, and DTS for the treatment of AGA demonstrating their composition, method of preparation, particle size, zeta potential, encapsulation efficiency and stability as discriminated in Annex 1.

MNX-NLC and FNS-NLC prepared by ultrasonication method (GOMES *et al.*, 2014) with a size around 200 nm were considered as appropriate for topical administration (GOMES *et al.*, 2014). Upon a 28-day storage stability assay, MNX NLC showed physical stability with no major changes in particle size, and zeta potential. The slight decrease of encapsulation efficiency during storage by MNX-nanoparticles was attributed to MNX higher hydrophilic character when compared with FNS thus remaining inside NLC during storage (GOMES *et al.*, 2014).

MNX-loaded gel NLC were prepared by ultrasonication (UPRIT *et al.*, 2013) in order to optimize formulation parameters such as concentration of liquid lipids (oleic acid) and solid lipids (tristearin). A ratio tristearin/oleic acid (2:1) led to NLC with higher encapsulation efficiency, low particle size as well as physical stability based on zeta potential and encapsulation values (UPRIT *et al.*, 2013).

DTS-NLC were coated with chitosan oligomer stearic acid (CSO-SA) to increase DTS delivery to the hair follicle (NOOR *et al.*, 2017). The stearic-acid increased the lipophilicity of chitosan and stearic acid-chitosan derivative was confirmed by ^{1}H NMR and FTIR studies. DTS-

NLC showed a high encapsulation efficiency around 98%, and a drug loading around 3.5%. Addition of CSO-SA to DTS-NLCs gave, NLC a high positive charge.

MNX NLC produced by hot high-pressure homogenization (HPH) incorporating stearic acid (as solid lipid) and oleic acid (as liquid lipid), increased the solubility of MNX (WANG *et al.*, 2017). The lipid higher solubility of MNX did allow its accommodation in higher amount, so drug loading was increased and the stability of nanoparticles was enhanced as well. Particle size decreased as the oleic acid (OA) amount increased probably due to NLC different viscosities by varying concentration of both stearic acid and oleic acid. Higher oleic acid content led a decreasing effect on viscosity inside NLC thus reducing the surface tension and a lower size and smoother surface particles were produced. NLC dispersion presented nanometric sized particles with high encapsulation efficiency of MNX as well as a, higher stability compared to SLN.

ALJUFFALI *et al.* (2014) developed lipid nanocarriers designated squarticles for treating hair loss. In this process two sebum-derived lipids were used to prepare MNX nanocarriers: NLC and nanoemulsions (NE). The squarticles were prepared by ultrasonication and the size of NLC squarticles was around 177 nm while NE of squarticles showed a mean size estimated at 193 nm. The zeta potential values were quite similar, with no significant difference between them, -54 mV and -57 mV for NLC and NE, respectively, which reveals a promising capacity for nanoparticle stability. The squarticles showed good MNX encapsulation efficiency for NLC (63%) and NE (64%) (ALJUFFALI *et al.*, 2014).

Antibodies-based MNX-loaded squarticles with the purpose of targeting dermal papilla cells (DPCs) were prepared and addition of the platelet-derived growth factor (PDGF) antibody to the squarticles led to a decrease in particle size (195 nm) whereas no change was found in the zeta potential, remaining anionic (-46 mV), due to deoxycholic acid. The squarticles revealed an encapsulation efficiency of MNX around 50% (ALJUFFALI *et al.*, 2015).

MNX-loaded niosomes were prepared with different surfactants by ethanol injection method (MALI *et al.*, 2013). Among formulation parameters studies, cholesterol concentration increased the MNX encapsulation efficiency although larger vesicles, around 470 nm, were obtained. Among niosomes drawbacks, stands out a low shelf life because of instability and drug leakage (VADLAMUDI DR and M.SEVUKARAJAN, 2012). Moreover, sorbitan monostearate (Span® 60) was the surfactant allowing the higher encapsulation efficiency, better stability of niosomes upon storage, and lowest mean particle size (MALI *et al.*, 2013).

FNS-loaded ethosomes prepared by film dispersing were studied for formulation impact on vesicular physicochemical properties (RAO *et al.*, 2008). Increasing ethanol concentration increased encapsulation efficiency of FNS and decreased ethosomes mean size,

lowest size was around 92 nm. According to authors, this effect was due to ethosomes higher capability to condense lipid vesicles promoting a switch of vesicles zeta potential from positive to negative values (RAO *et al.*, 2008).

FNS-loaded ethosomes were also prepared by ultrasonication towards the FNS targeting to the pilosebaceous unit (PSU) (WILSON *et al.*, 2018). Physicochemical characterization results revealed a major influence of ethanol concentration and soya phosphatidylcholine (SPC) on ethosomes size and FNS encapsulation efficiency. PSU targeting by nanoparticle was higher for nanoparticles sizing within 100-300 nm. The most stable ethosomes, prepared with the highest concentration of ethanol and lowest amount of SPC, presented a mean size around 111 nm and an encapsulation efficiency reaching 80% (WILSON *et al.*, 2018).

FNS polymersomes based on PS and poly (acrylic acid) (PAA) were prepared by co-solvent self-assembly (CAON *et al.*, 2014). Upon chitosan coating polymersomes negative potential zeta value changed to positive value and among coated FNS carriers, the one coated with oligo-chitosan showed the lowest mean particle size (CAON *et al.*, 2014).

FNS-loaded LCN prepared by ultrasonication revealed to be most stable in the presence of poloxamer® 407 (MADHESWARAN *et al.*, 2013). Nanoparticle average size was 154-189 nm and formulation additives showed insignificant influence on particle size excluding the increasing effect provided by OA. Encapsulation efficiency was more than 98%. The role of surfactant was found to be relevant rather for stability regardless their concentration (MADHESWARAN *et al.*, 2013).

The role of two polyoxyethylated nonionic surfactants, cremophor® EL (EL) and RH 40 (RH) on FNS-loaded LCN formulations capability to deliver FNS by skin was evaluated (MADHESWARAN *et al.*, 2014). Optimal FNS-loaded LCN were prepared by ultrasonication with surfactant concentration in the range of 0.5-2.5% and 1.5-4.0% for RH and EL, respectively. FNS-loaded LCN mean particle, prepared by varying concentrations of RH and EL was in the range of 159-209 and 154-243 nm, respectively. LCN size was dependent on RH and EL concentration. RH and EL showed a more pronounced effect on LCN zeta potential when compared to poloxamer® 407 (MADHESWARAN *et al.*, 2014).

Chitosan coated FNS and DTS loaded LCN were prepared by ultrasonication method (MADHESWARAN *et al.*, 2017). The particle size and zeta potential were around 245 nm and -19 mV, respectively, for uncoated LCN. Chitosan-coated LCN showed a size and zeta potential values around 300 nm and -30 mV, respectively (MADHESWARAN *et al.*, 2017).

FNS-Nano-transferosomal (NTF) gel formulations prepared for FNS topical application (AHMED and RIZQ, 2018) presented a particle size value between 171.0 and 299.6 nm and encapsulation efficiency values between 69.7% and 93.1%. The lower vesicle size of NTF was

attributed mainly to the low hydrophile-lipophile balance (HLB) of the surfactant. Therefore, low water uptake into vesicle core took place thus reducing surface energy associated with high hydrophobicity of edge activator thus allowing the formation of small sizing vesicles. The high encapsulation efficiency was related to FNS low aqueous solubility.

The efficacy of topical transfersomes containing both MNX and caffeine to promote hair growth was studied by RAMEZANI *et al.* (2018). Transfersomes were prepared through the thin film hydration method and surfactants other than phospholipids, polysorbate 80 and polysorbate 20 increased permeation of MNX. The encapsulation efficiency was between 13.62 and 48.82% and a negative effect of polysorbate 20 on lipid bilayer formation and its stability was observed. This effect was explained by phosphatidylcholine ionization in acidic medium thus increasing water solubility of MNX. Accordingly, charges similarity between the polysorbates, with focus on polysorbate 20, and remaining membrane ingredients was considered relevant with regard to transfersome formation and stability.

LIMA *et al.* (2018) executed a pre-formulation study using FNS, in order to formulate a topical matrix system for the treatment of AGA. Hydrophilic polymer hydroxypropylcellulose, increased the capacity of the FNS to form inclusion complexes with hydroxypropyl-cyclodextrin (HP-CD) (LIMA *et al.*, 2018).

Methyl-β-CD/MNX inclusion complexes with calcium alginate as a gelling agent were formulated as an aqueous topical treatment of AGA (LOPEDOTA *et al.*, 2015).

Alginate-based hydrogels CD with hydroxypropyl-β-cyclodextrin (HP-β-CD) having 0.65 and 0.85 molar substitution degree (MS) were studied for MNX topical administration and HP-β-CD MS 0.65 was the formulation ablest to improve MNX aqueous solubility (LOPEDOTA *et al.*, 2018). HP-β-CD forms an excellent inclusion complex with MNX increasing aqueous drug solubility comparing to β-CD.

Nanoparticles made of PLGA and loaded with FNS were prepared by solvent evaporation (ROQUE *et al.*, 2017). Nanoparticles with a size around 300 nm and negative zeta potential were able to reach the skin and hair follicles. A high FNS encapsulation efficiency was achieved suggesting a good relationship between the lipophilic drug and PLGA.

Chitosan-coated DTS-loaded nanocapsules were prepared by interfacial deposition technique of performed polymers to increase FNS targeted delivery to the hair follicle (USHIROBIRA *et al.*, 2020). Nanocapsules showed a high encapsulation efficiency, above 94% and were stable up to 90 days. Chitosan coating increased nanoparticle size from 199 nm to 225 nm and zeta potential from -13.6 mV to +40.2 mV (USHIROBIRA *et al.*, 2020).

Chitosan-coated MNX nanoparticles were also prepared by inotropic gelation with the goal of targeting MNX to the outermost skin layers, and, specially, to hair follicles (MATOS *et al.*, 2015). Monomodal distributed MNX nanoparticle with a mean size of 236 nm and a positive zeta potential were obtained and highest MNX encapsulation efficiency, around 73%, was obtained by using a polymer:drug ratio equal to 1:1 (w/w).

MNX PLGA nanoparticles showed a mean diameter in the range 120-140 nm, which was, considered as size-suitable for delivery to hair follicles (TAKEUCHI *et al.*, 2018).

Hyaluronate-PLGA nanoparticles (HA-PLGA) were also prepared by the same technique for improving the delivery efficiency of MNX in AGA treatment (JEONG *et al.*, 2019). MNX-loaded nanoparticles showed a mean size around 159 nm whilst HA-PLGA/MNX nanoparticles showed a mean size around 243 nm. HA-nanoparticles showed a negative zeta potential in the range -43.2 to -0.4 mV due to their conjugation with HA.

MNX-loaded metallic nanoparticles using zirconia beads were prepared by bead milling (NAGAI *et al.*, 2019). MNX nanoparticles mean size and zeta potential were within 90-300 nm and -9.9 mV, respectively. For the design of nanotechnology-based formulations, stability it is most relevance. Stability study, revealed no agglutination of nanoparticles for 2 weeks but particle size increased over following 2 weeks, with a mean size of 294 nm.

5. *In vitro* and *in vivo* characterization studies and results of nanotechnology-based formulations associated with treatment of Androgenetic alopecia

The development of a successful nanotechnology-based topical formulations requires a full and depth understanding of both nanoparticles impact on skin and their transport pathways.

It is thus necessary to perform *in vitro* skin permeation and penetration experiments for assessment of performance of these formulations as well as to evaluate skin damage those formulations may cause (HERRMANN *et al.*, 2017).

Nanotechnology-based formulations for topical application of MNX, FNS, and DTS were critically analyzed with regard *in vitro* and *in vivo* studies for treatment of AGA as illustrated in Annexes 2,3 and 4.

It must be emphasized that comparative analysis of results among formulations discriminated throughout the several annexes has limitations. In fact, experimental conditions set-up by authors were not the same and in some experimental researches no analysis of results was made due to either extremely different experimental conditions or lack of data regarding relevant factors having impact on main assays such as amount of drug or equivalent, release medium composition, among others.

MNX and FNS once encapsulated in NLC revealed a different drug release pattern: a sustained and prolonged effect of NLC on MNX release was observed whereas no FNS was released from NLC after a 24h assay. In the skin penetration assays, both FNS-NLC and MNX-NLC revealed reduced levels of penetration (GOMES *et al.*, 2014).

MNX-loaded gel NLC formulated with an optimal ratio of tristearin/oleic acid showed an MNX biphasic release pattern, a burst release was followed by a sustained and constant release (UPRIT *et al.*, 2013).

DTS-NLC coated with chitosan showed reduced DTS release rate over 12h assay compared to uncoated DTS-NLC. In the *in vitro* permeation studies, the amount of DTS in the skin was higher with both uncoated and coated DTS-NLC formulations when compared to other nanoparticle formulations. Nanoparticles sizing between 200 and 250 nm showed high potential to penetrate hair follicles. Chitosan bioadhesive properties and its polycationic form increased DTS uptake by opposite charged hair follicles (FRANK *et al.*, 2020). Both uncoated and coated DTS-NLC were pointed out as suitable approach for topical delivery of DTS. These permeation effects provided by chitosan coating could be due to higher interaction between polysaccharide positive opposite charge and skin or even due to its bioadhesive properties, however, in order to prove these permeation results it would be necessary to perform additional experiments (e.g. *in vivo* studies). Cytotoxicity study revealed that both uncoated and coated DTS-NLC, enhanced approximately 20-fold the maximum non-toxic concentration. By their mechanism of targeting delivery, an occlusion effect in the skin is expected thus improving skin hydration (NOOR *et al.*, 2017).

MNX release from NLC made of stearic acid and oleic acid as solid lipid and liquid lipid, respectively, was faster in comparison to SLN due to MNX easier diffusion from liquid lipid with regard to solid liquid. A higher skin permeation of MNX was observed for NLC and a skin permeation enhancer role was found for OA. The same formulation improved MNX retention in the skin allowing the reduction of adverse reactions. Furthermore, compared with a conventional formulation for the topical delivery of MNX, a barely perceptible skin irritation was found for MNX-NLC (WANG *et al.*, 2017).

In vitro skin studies performed with MNX squarticles revealed that both NLC and NE enhanced skin uptake of MNX (ALJUFFALI *et al.*, 2014). A further increase of MNX deposition on skin was possible with NLC. Squarticles lipid structure led to the formation of a thin film over the skin surface, providing hydration and occlusion effects thus increasing MNX absorption. The squarticles displayed a lower flux of MNX through the skin compared with the control solution, which resulted in higher skin MNX accumulation therefore reducing vasodilator systemic absorption. MNX NLC showed the highest release rate of MNX when

compared with both MNX-NE and MNX control solution. All MNX formulations (NLC, NE and control) revealed a burst release within first 8h with more than 85% of MNX release compared to that from 24h cumulative release (ALJUFFALI *et al.*, 2014). A major role played by VEGF in the hair cycle as well as its presence in hair bulbs allows angiogenesis to start (ALJUFFALI *et al.*, 2014). All MNX-loaded formulations increased VEGF expression in dermal papilla cells, but a more pronounced effect was observed for NLC. Skin toxicity by squarticles was first evaluated *in vitro* by using papilla cells. The NE showed cell viability around 100% indicating that NE were nontoxic to dermal papilla cells within concentrations tested. On the other hand, NLC at the highest concentration showed a dermal survival rate around 76%. *In vivo* skin irritation test, revealed a despicable irritation by squarticles whilst both NLC and NE demonstrated no signs of erythema. The findings indicate that MNX-loaded squarticles showed an improvement both in skin and follicular uptake (ALJUFFALI *et al.*, 2014).

Nanoparticulate inclusion decreased MNX release, particularly nanocarriers containing antibody. MNX release from squarticles exhibited a biphasic pattern with a burst release occurring during the first 8h and slower drug release. MNX release from PDGF-squarticles was assigned to a zero-order kinetics. *In vitro* skin studies demonstrated that MNX-loaded squarticles functionalized with PDGF increased MNX skin deposition and accumulation in the mouse and porcine skin, respectively. The cyclical process of the hair follicle is regulated by hair follicle stem cells, whose activation and subsequent differentiation is mediated by mesenchymal cells. The DPCs are base-located hair follicle mesenchymal cells responsible for the development and growth of the hair (CALABRESE, 2020). The PDGF receptor is express in DPCs and its role as a therapeutic target for MNX has been widely studied (SIVARAM *et al.*, 2018). *In vivo* studies showed MNX PDGF squarticles accumulation in epidermis, hair follicles and other deep layers of the skin thus supporting a skin reservoir effect rather than MNX systemic spread. MNX PDGF squarticles showed the highest uptake for hair follicles in relation to a control solution and unloaded squarticles. A moderate proliferation of DPCs and expression of VEGF was obtained, which was considered as suitable to improve the treatment of AGA (ALJUFFALI *et al.*, 2015).

Niosomal gel (surfactant/cholesterol ratio of 1:2) increased skin permeation and deposition of MNX compared to control MNX gel. A similar effect on MNX skin permeation and accumulation was also observed for higher-cholesterol content formulations, significant in MNX skin accumulation. Furthermore, increasing cholesterol concentration in the niosome formulation, skin retention of MNX was improved (MALI *et al.*, 2013).

Ethosomes revealed also an enhancing effect on skin accumulation of FNS in contrast to other formulations (liposomes and controls) as the later showed an accumulation of FNS

preferentially in the deep skin layers. Ethosomes also revealed the maximum percutaneous flux of FNS and obtained values were described as beneficial for AGA treatment (RAO *et al.*, 2008). Another study by same authors analyzed the performance of the ethosomes in the distribution of FNS through layers of skin and results showed that after 12h, the distribution of FNS in epidermis to dermis was 15.4% versus 46.0%, respectively, for ethosomes, while liposomes and hydroethanolic solution results were 47.9% versus 19.4% and 48.5% versus 22.8%, respectively. FNS accumulation exhibited reverse distribution profile which was considered as promising for FNS towards its effects at target site in a most efficient way (RAO *et al.*, 2015).

FNS release pattern showed a biphasic release pattern with almost 25% of FNS release occurring in the initial phase. Comparatively sustained pattern FNS release from ethosomes was observed up to 8h. FNS-loaded ethosomes containing permeation enhancers were evaluated for *in vitro* skin permeation studies performed with rat skin and scalp skin from human cadaver and results showed an increased skin permeation by FNS but systemic drug reaching levels could not be excluded. Despite the promising results of the FNS-loaded ethosomes as great option to improve permeation to and through the skin, the use of permeation enhancers was not considered a valid option by authors (WILSON *et al.*, 2018).

Polymersomes revealed an increasing effect on the residence time of FNS in the skin while avoiding its passage across the dermis, thus avoiding replication of side effects associated with its oral administration. Upon chitosan-coating, polymersomes interaction with external skin layers due to the positive charge of chitosan, much more favorable to establish carriers interactions with negative charge of phospholipids facilitating entry through the membrane (CAON *et al.*, 2014).

FNS-loaded LCN prepared by ultrasonication were studied for the effect of solvent and surfactant on FNS permeation and retention behavior across the skin (MADHESWARAN *et al.*, 2013). No effect of surfactant concentration on FNS release patterns could be find. *In vitro* study results showed that, as the concentration of MO increased a slower diffusion of FNS from lipid bilayer occurred. Upon addition of glycerol (GL), PG and PEG an increased FNS release was obtained which was explained by the reduced hydrophobic environment inside the nanoparticles, created by these additives. Skin permeation and retention of FNS results were consistent with the ones from FNS release study showing that the highest permeation and retention of FNS in the skin was obtained with formulation containing GL, PG and PEG (MADHESWARAN *et al.*, 2013).

The role of two polyoxyethylated nonionic surfactants, cremophor® EL and RH 40, (designated as EL and RH, respectively) on FNS-loaded LCN properties showed that LCN

produced with RH led to an FNS biphasic release profile with a higher rate of FNS release within first 6h followed by a slow rate release. On the other hand, LCN produced with EL or poloxamer® 407 revealed a direct effect of surfactant concentration on cumulative release percentage of FNS. The skin permeation study showed that increasing surfactant concentration a faster skin FNS permeation rate is obtained. The impact study of RH and EL concentrations on the dose of FNS retained in the, skin revealed that a low surfactant concentration leads to an increasing retention time phenomenon. Thus, the surfactant concentration performed a critical role in release pattern of FNS from LCN (MADHESWARAN *et al.*, 2014).

Chitosan coated FNS and DTS loaded LCN prepared by ultrasonication (MADHESWARAN *et al.*, 2017) provided DTS a slower release profile than that of FNS due to higher lipophilic characteristics of DTS. Chitosan coating improved skin permeation of both FNS and DTS compared with LCN uncoated and control solution, making proof that chitosan positive charge and LCN unique structure promote the interaction of Chitosan-LNC with the skin and consequently improves the skin permeation of both drugs. Chitosan-LCN and LCN increased the epidermis and dermis FNS and DTS retention in relation to control solution and the *in vivo* mice skin retention studies showed that Chitosan-LCN enhanced skin permeation of FNS and DTS compared with LCN and control solution. FNS and DTS were, distributed homogenously through the skin layers and in the cellular uptake assay chitosan-LCN enhanced uptake by cells because of its positive surface charge favoring their keratinocytes internalization (MADHESWARAN *et al.*, 2017).

LCN presents appealing properties for a topical delivery system such as permeation enhancer, increased skin retention, controlled release, nontoxic and potential to encapsulate hydrophobic drugs like FNS and DTS (MADHESWARAN *et al.*, 2013, MADHESWARAN *et al.*, 2014).

NTF gel formulations exhibited an enhancing effect on rat skin permeation of FNS when compared with control. These results were consistent with uniform fluorescence intensity studies performed in rat skin following NTF-dying with rhodamine. FNS-NTF gel formulations ultra-deformable structure as well the inclusion of edge activator and the large surface area of FNS-NTF facilitated their delivery and permeation across the skin layers. The improved delivery of FNS from NTF gel formulations indicate that FNS-NTF is a potential alternative of FNS delivery likely to decrease secondary effects of FNS therapy by oral route (AHMED and RIZQ, 2018).

MNX release from transfersomes formulations revealed that first order kinetics best fitted for the release behavior of both MNX and caffeine. The efficacy of topical transfersome

containing both MNX and caffeine, in promote hair growth was studied in wistar rats by RAMEZANI *et al.* (2018). Transfersome effectively promoted hair growth as well as enhanced the hair weight over a 30-day topical administration. It was observed a high efficiency by transfersome for drug delivery to follicles (RAMEZANI *et al.*, 2018).

A gel formulation using the methyl-β-CD/MNX inclusion complex with calcium alginate as a gelling agent forming a fully aqueous formulation for topical treatment of AGA, improved the MNX skin permeation with a significant skin retention of MNX (LOPEDOTA *et al.*, 2015).

The hydrogel HP-β-CD/MNX inclusion complexes in addition to modulating the MNX release profile, reveal that it could improve the penetration, bioavailability, accumulation, and stability of MNX (LOPEDOTA *et al.*, 2018). Furthermore, the gel HP-β-CD/MNX showed highest efficacy in improved hair growth, increasing the hair length, the number and diameter of the follicles in relation to the MNX ethanolic solution. In the gene expression analysis, the gel HP-β-CD/MNX exhibits a better performance in target gene expression, in particular, enhanced expression of the ATP-sensitive potassium channel opener gene code (AKT2) revealing being able to potentiate the ATP-sensitive potassium channel (KATP) signaling pathway, and regulating hair growth. A down-regulation of the androgen receptor expression as well as the higher tolerability profile of the gel HP-β-CD/MNX were observed in relation to ethanolic solution of MNX (TRICARICO *et al.*, 2018).

FNS-loaded PLGA nanoparticles developed by solvent evaporation (ROQUE *et al.*, 2017) showed also a prolonged effect on FNS release and results suggested an increased FNS residence time in the skin. PLGA revealed a non-toxic profile upon a toxicity test on a *Saccharomyces cerevisiae* model and *in vivo* human safety testing by excipients showed that formulation excipients were safe towards skin. Further studies included efficacy and safety regarding FNS-loaded formulations. Overall, PLGA nanoparticles proved to be useful and safe for the topical administration of FNS in the treatment of AGA (ROQUE *et al.*, 2017).

Chitosan-coated DTS-loaded nanocapsules intended for targeted DTS delivery to the hair follicle revealed (USHIROBIRA *et al.*, 2020) DTS accumulation in the follicles, and upon massaging an increased effect on DTS accumulation was observed. Chitosan coating provided a slow and controlled DTS release from nanoparticles which decreased total of therapeutic dose and consequently side effects occurrence since a small amount of drug remains trapped in the skin (USHIROBIRA *et al.*, 2020).

Chitosan-coated MNX-loaded nanoparticles prepared by inotropic gelation with the goal to target the MNX to the outermost skin layers, and, specially, into hair follicles (MATOS *et al.*, 2015) revealed a sustained effect on MNX release, prolonging pharmacological effect and

consequently reducing the number of daily administrations. Furthermore, Chitosan nanoparticles showed a trend for its accumulation in the hair follicles maintaining high MNX concentrations there (MATOS *et al.*, 2015).

MNX-loaded PLGA nanoparticles prepared by solvent evaporation (TAKEUCHI *et al.*, 2018) when submitted to *in vitro* permeation testing revealed an increasing effect on MNX amount delivered to hair follicles as well as a controlled released of the vasodilator drug. Thus, MNX-loaded PLGA was considered to be adequate as an effective treatment for AGA (TAKEUCHI *et al.*, 2018).

HA-PLGA nanoparticles prepared by solvent evaporation for improving the delivery efficiency of MNX in AGA treatment (JEONG *et al.*, 2019) displayed a controlled release of MNX from PLGA nanoparticles and an increasing effect on skin MNX permeation as well as a high efficiency delivery of the vasodilator into hair follicles. Moreover, in cell viability test using fibroblast cells, no cytotoxicity effect was found into the cells (JEONG *et al.*, 2019).

MNX-loaded metallic nanoparticles using zirconia beads prepared by bead millings showed an enhancing effect on MNX delivery into the hair follicles as well as a significant increase in hair growth in C57BL/6 mice compared with conventional formulations. The MNX levels in the skin were lower than conventional formulations and no erythema or inflammation signs were observed on the skin. In addition, no MNX was detected in the plasma whilst high levels of MNX were found in the hair bulbs, thus suggesting a better treatment efficiency. Moreover, MNX-loaded metallic nanoparticles significantly increased the levels of VEGF protein, which acts as a promoter of hair growth, triggering vasculogenesis, and angiogenesis and insulin-like growth factor-1 (IGF-1) thus, activating hair root cells and suppressing catagen and telogen phases (NAGAI *et al.*, 2019).

6. Toxicity issues

Topical drug administration was developed as an alternative in the administration of drugs whose traditional routes of administration are subject to side risks, proving to be a beneficial approach with positive and improved results in the treatment of AGA (IQBAL and BABOOTA, 2018). Topical administration based on nanoparticles, it seems to be both safer and more effective in relation to conventional formulations, however, its evaluation regarding the safety profile it is a must task to be achieved (NASIR, 2010).

Nanostructures are particles whose size is around one hundred nanometers, so there is the possibility of obtaining undesirable results due to nanoscale size which seems to be associated with an increase in toxicity, raising doubts related to nanoparticles harmfulness (MARQUART *et al.*, 2020, NASIR, 2010, NEL *et al.*, 2006).

So far, what is known is that there is a relationship between the particle size, its surface area and its toxicity profile (NEL *et al.*, 2006) as it can be seen in Figure 4.

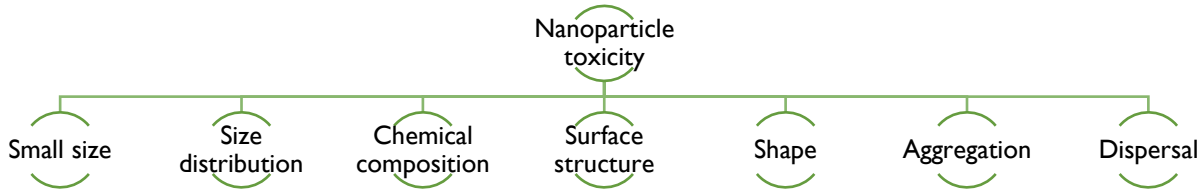


Figure 4. Nanoparticle settings that determine toxicity.

Even though there is evidence that irrespective of nanomaterial used, most nanoparticles (especially the ones larger than 20 nm) are not likely to penetrate healthy skin viable tissues and the transappendageal pathway can be the dominant route of their entry into skin (CROSERA *et al.*, 2009, RAMEZANLI *et al.*, 2017).

Still, its nano-size allows the creation of a larger surface area, favoring the interaction between nanoparticles and biological systems. There are, therefore, still many uncertainties related to the risks of nanoparticles in the human organism, being essential the evaluation of their consequences for health, knowing the life cycle of nanoparticles, the human exposure pathways as well as the performance of nanoparticles on body. Unfortunately, there are few studies *in vivo*, which leads to uncertainties in the safety of these particles in humans (ANTONIO *et al.*, 2014). It is essential to deliver guidelines and testing procedures that guarantee the safe manufacture and use of nanoparticles on the market in the near future (GUPTA *et al.*, 2012).

Although incredible in terms of physicochemical properties, the characteristics of nanoparticles raise concerns regarding side effects on biological systems (NEL *et al.*, 2006). As technological development advances, there are raised expectations towards *in vivo* and *in vitro* testing thereby allowing to improve available data and consequently a refined safety profile to the use of nanoparticle will be available as soon as possible (ANTONIO *et al.*, 2014, MARQUART *et al.*, 2020).

The International Organization for Standardization (ISO) in collaboration with the Organization for Economic Cooperation and Development (OECD) developed guidelines to be used in the industry sector thus appearing the ISO/TS-13830:2013 and the ISO-19007:2018 with a view to perform full toxicity tests for nanoparticle. At the end of 2017, the FDA published guidance aimed at the industry highlighting the need and the importance of safety profile for nanoparticle use even if as been already their use found safe.

In order to a conclusion, it is worth highlighting that insistence that set up good *in vivo* and *in vitro* toxicity models that measure the toxicological profile of nanoparticles should be further developed, having as its main focus on the safety of patients.

7. Regulatory affairs

There is currently an immediate need to address adequate and establish adequate regulatory guidelines to promote market authorization of nanoparticles, ensuring their safety and efficacy (PATRAVALE *et al.*, 2012).

Despite the high potential in the therapeutic market, both physicochemical and biological properties of nanoparticles are often characterized under no regulated standard procedures. There is still a lack of consensus among scientists about definitions and standards results in unbalanced regulation of nanotechnology-based systems (HOFMANN-AMTENBRINK *et al.*, 2014, PATRAVALE *et al.*, 2012).

The FDA and the European Medicines Agency (EMA) are the main regulatory institutions essential to the regulation of nanotechnology-based formulations (BEER, 2016). The EMA has already reviewed several marketing authorization applications for nanoparticles whose assessment was based on pre-existing regulatory principles, yet there is an urgent need to adapt the current regulatory framework for these new systems (OOMEN *et al.*, 2018).

A distinction has been between medicines and medical devices on the basis of EU-DIRECTIVE-2001/83/EC for medicinal products and EU-DIRECTIVE-93/42/EEC for medical devices, the classification is made according to the mode of action of nanoparticle.

The definition of nanoparticles is one of the key elements to adapt their regulation. ISO has proposed as a definition of nanoparticles any material with an external or surface dimension in the nano range (1 nm to 100 nm). However, there is still no consensus among regulators on a standard definition of nanoparticles, each adopting its own definition (BEER, 2016).

We can therefore conclude that the regulation of nanoparticles is in the process of development. There is a need to clarify the classification and characterization of nanoparticles as well as to address clinical and non-clinical issues and their safety. These entire aspects become essential both for ensuring the safety of the patient for the pharmaceutical industry in order to facilitate nanoparticle their market introduction.

8. Conclusion and Future perspectives

MNX, FNS and DTS are drugs being used in AGA topical therapeutics through the conversion of hair follicles into the anagen phase. Conventional formulations consisting of

skin permeation enhancers such as PG and ethanol are associated with local adverse effects, hampering patient tolerability to AGA treatment and ultimately results in therapeutic low outcomes. Nanotechnology-based formulations are gaining prominence as valid alternative therapeutic strategies delivered by skin route, including AGA by potentiating both skin and follicular delivery of active ingredients such as MNX, FNS and DTS while decreasing side effects of conventional formulations, thus providing treatment outcomes and patient compliance. Furthermore, an increasing effect on bioavailability, hair follicle accumulation together with skin permeation are claimed by MNX, FNS and DTS upon delivery by nanoparticles.

Although there is no suggestion as to the type of nanoparticles that can offer a better skin transport, and because we have formulations prepared by different methods and tested by different methods, lipid nanoparticles, are among the most suitable formulations due to the lipid character of these systems. Hydrophobic-hydrophobic interaction between the sebum and the nanoparticle, allows drugs to be transported directly to the hair follicle, increasing their bioavailability, thereby allowing achieving a lower effect therapeutic dose.

Nanotechnology-based drug delivery systems have been shown to be a beneficial alternative for topical application of lipophilic pharmaceuticals such as FNS and DTS, with the possibility of including the drug internally, and to be delivered in an aqueous formulation.

Regarding MNX and FNS release, the encapsulation of both drugs in nanotechnology-based systems provides a sustained, controlled and targeted delivery, thereby promoting MNX and FNS delivery optimization.

The results obtained through the association of the 2 anti-alopecia drugs, MNX and FNS, incorporated in NLC, demonstrated a prolonged release profile and low levels of skin penetration, characteristics that demonstrate that this new formulation may constitute an optimal therapeutic alternative for AGA.

Nevertheless, there is still missing standard and comprehensive characterization techniques of nanotechnology-based formulations, such as physicochemical characterization and stability assays, a constraint whose impacts have been decreased but still requiring attention if regulatory rules are to be met. Moreover, data associated with in vitro and in vivo testing, with focus on in vitro skin permeation assays, can hardly be compared among formulations due to a lack of standardization in experimental conditions.

Nanotechnology-based formulations have been tested mostly *in vitro* still in the very near future evidence collected by *in vitro* assays are expected to confirmed in vivo experiments, thus reinforcing the reliability of *in vitro*/*in vivo* correlations.

Due to their size correlated physicochemical properties and often resulting biological effects, nanotechnology-based formulations can require also a safety profile upon international guidance and regulation. However, those guidelines are still scarce, hampering nanotechnology-based products' clinical translation and market approval. It is imperative that international authorities such as EMA and FDA develop guidance to provide enlightenment towards a correct manufacturing processes as well as toxicity assessments, particularly concerning the long-term exposure to nanotechnology-based products, and, particularly, to topical nanoformulations intended to treat AGA.

Nanotechnology-based formulations applied for topical administration of MNX, FNS and DTS were compared with regard drugs release profile from nanoparticles, skin permeation, targeting potential and toxicity concerns all of them within the scope of AGA treatment, as shown in Table 1.

Table 1. Characteristics of nanotechnology-based formulations as delivery systems for MNX, FNS and DTS, with regard to encapsulated drugs' release profile, skin penetration, follicular targeting and toxicity.

Formulation	Release	Skin penetration	Follicular targeting	Toxicity
NLC	-Prolonged release of MNX (GOMES *et al.,* 2014) (WANG *et al.,* 2017); -MNX burst release followed by sustained release (UPRIT *et al.,* 2013); -DST-NLC coated with modified chitosan indicated slow release over the first 12h (NOOR *et al.,* 2017); -Faster release of MNX by NLC (WANG *et al.*, 2017).	-Low levels of penetration with nanoparticles loaded with MNX and FNS (GOMES *et al.*, 2014); -DST-NLC coated with CSO-SA and uncoated promoted skin permeation of DTS (NOOR *et al.,* 2017); -Pronounced permeation of MNX-NLC (WANG *et al.*, 2017).	-NA.	-Cytoxicity maximum non-toxic concentration was increased by DTS-NLC (NOOR *et al.*, 2017); -No erythema after administration of MNX-NLC (WANG *et al.*, 2017).
Squarticles	-Fast release of MNX in the first 8h followed by plateau (ALJUFFALI *et al.,* 2014); -Prolonged release of MNX (ALJUFFALI *et al.,* 2015).	-Squarticles reduced MNX skin penetration; improved MNX deposition and accumulation into skin (ALJUFFALI *et al.,* 2014); -Enhanced MNX skin deposition and accumulation and wide distribution of MNX loaded nanoparticles in all layer's skin (ALJUFFALI *et al.,* 2015).	-Increased MNX follicular uptake and large accumulation of MNX-loaded squarticles in hair follicles (ALJUFFALI *et al.,* 2014); -Wide diffusion of MNX loaded nanoparticles in hair follicles and increased MNX follicular uptake (ALJUFFALI *et al.,* 2015).	-NE and NLC were nontoxic (ALJUFFALI *et al.,* 2014).
Niosomes	-NA.	-Enhancement of MNX skin accumulation (MALI *et al.*, 2013).	-NA.	-NA.
Ethosomes	-Burst phase followed by sustained release of FNS for about 8h (WILSON *et al.,* 2018).	-Highest FNS skin accumulation and permeation (RAO *et al.,* 2008; RAO *et al.,* 2015); - Higher FNS skin permeation (WILSON *et al.,* 2018).	-NA.	-NA.
Chitosan-decorated polymersomes	-Greatest control over the FNS release profile (CAON *et al.*, 2014).	-Greater FNS retention in the skin (CAON *et al.*, 2014).	-NA.	-NA.
LCN	-The release of FNS from LCN were dependent on the amount of additives and MO (MADHESWARAN *et al.,* 2013); -Faster release of FNS within first 6h followed by a slow rate release (MADHESWARAN *et al.,* 2014); -Controlled release of FNS and DTS from LCN and CHI-LCN (MADHESWARAN *et al.,* 2017).	-The permeation and retention of LCN loaded with FNS were dependent on the amount of additives and MO (MADHESWARAN *et al.,* 2013). -Higher surfactant concentrations increased skin permeation and lower surfactant concentrations increased skin retention (MADHESWARAN *et al.,* 2014); -Higher cumulative FNS/DTS permeation, retention and distribution in the skin (MADHESWARAN *et al.,* 2017).	-NA	-NA.
NTF gel formulation	-FNS sustained release (AHMED *et al.*, 2018).	-Enhanced the amount of FNS skin penetration and increase FNS accumulation into skin layers (AHMED *et al.*, 2018).	-NA.	-NA.
Transfersomes	-MNX release kinetics was first order (RAMEZANI *et al.*, 2018).	-NA.	-Higher efficacy of MNX delivery to follicles (RAMEZANI *et al.*, 2018).	-NA.

Cyclodextrin	-Methyl-β-CD containing gel increased MNX release (LOPEDOTA *et al*., 2015); -CD-containing gel MNX release rate (LOPEDOTA *et al.,* 2018); -HP-β-CD enhanced the dissolution rate of MNX (TRICARIO *et al*., 2018).	-Increased retention and accumulation of MNX (LOPEDOTA *et al*., 2015); -Increased MNX accumulation and permeation in the skin (LOPEDOTA *et al.,* 2018).	-Improved hair length, hair growth and bulb diameter (TRICARIO *et al*., 2018).	-NA.
Polymeric nanoparticles	-Prolonged release of FNS (ROQUE et al., 2017); -Controlled release of DTS from chitosan coated nanoparticles (USHIROBIRA *et al*., 2020); -MNX sustained release (MATOS *et al*., 2015); -MNX controlled release (TAKEUTCHI *et al*., 2018); -MNX controlled release with HA-PLGA nanoparticles (JEONG *et al*., 2019).	-Low levels of FNS skin penetration (ROQUE *et al*., 2017); -Enhanced DTS skin permeation (USHIROBIRA *et al*., 2020); -Higher MNX skin permeation (JEONG *et al*., 2019).	-Increased hair follicles targeting; uncoated nanoparticles favored DTS penetration into the hair follicles (USHIROBIRA *et al*., 2020); -Higher MNX accumulation into the follicles; increased MNX penetration into hair follicles; MNX release targeted to the hair follicles (MATOS *et al*., 2015); -Improved MNX delivery into hair follicles (TAKEUTCHI *et al*., 2018); - Showed ability to MNX delivery to hair follicle (JEONG *et al*., 2019).	-No signs of erythema or adverse reactions (ROQUE et al., 2017); -No cytotoxicity (JEONG *et al*., 2019).
Metalic nanoparticles	-NA.	-Lower MNX levels in the skin (NAGAI *et al.,* 2019).	-Increased MNX content in hair bulbs (NAGAI *et al.,* 2019).	-NA.

Abbreviations: CHI-LCN: Chitosan-coated liquid crystalline nanoparticle; CSO-SA: Chitosan oligomer stearic acid; DTS: Dutasteride; FNS: Finasteride; HA-PLGA: Hyaluronate–poly (lactide-co-glycolide); HP-β-CD: Hydroxypropyl-β-cyclodextrin; LCN: Liquid crystalline nanoparticle; MNX: Minoxidil; MO: Monoolein; NA: Not available; NE: Nanoemulsions; NLC: Nanostructured lipid carrier; NTF: Nano-transferosomal PLGA: Poly (lactide-co-glycolide); SLN: Solid lipid nanoparticle.

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10. Annexes

Annex 1. Physicochemical characterization of nanoparticles applied to topical administration of Minoxidil (MNX), Finasteride (FNS) and Dutasteride (DTS) in Androgenetic alopecia (AGA) treatment.

Nanoparticle	Drug	Composition	Preparation Method	PS (nm)*	ZP (mV)*	EE (%)*	Stability**** (study duration/ temperature)	Ref.
NLC	MNX	Cetyl palmitate; oleic acid; polysorbate 60	Melt-dispersion Ultrasonication	c.a. 180.0	c.a. -33.0	31.9	(28 days/NA) Slight decrease of EE	GOMES *et al.*, 2014
				c.a. 190.0	c.a. -37.0	22.2		
	FNS	Cetyl palmitate; oleic acid; polysorbate 60; preciol® ATO 5; miglyol® 812		c.a. 210.0	c.a. -25.0	76.8	(28 days/NA) PS, EE, and ZP unaltered	
				c.a. 240.0	c.a. -25.0	87.8		
	MNX	Oleic acid; tristearin; soya lecithin; polysorbate 80; pluronic® F-68	Melt-dispersion Ultrasonication	280.4**	-42.4**	86.1**	(NA/NA) Physical stability based on ZP and EE	UPTRI *et al.*, 2013
	DTS	**CSO-SA:** NLC coated with 5% stearic acid-chitosan	Melt-dispersion Ultrasonication	220.1**	+25.8**	97.8**	(60 days/4-8°C and 25°C) PS stable at 4-8°C but aggregation occurred at 25°C	NOOR *et al.*, 2017
		NLC: Phosal® 53 MC; lutrol® micro 68		187.6**	- 18.3**	97.8**		
	MNX	Oleic acid; stearic acid; polysorbate 80; sorbitan monooleate	Hot HPH	281.4**	-32.9**	92.5**	(3 months/4°C and 25°C) PS and EE unaltered	WANG *et al.*, 2017
Squarticles ***	MNX	**NLC:** Squalene; glyceryl palmitostearate; hydrogenated soy phosphatidylcholine; water; pluronic® F-68	HSH Ultrasonication	177.0	-54.0	63.3	NA	ALJUFFALI *et al.*, 2014
		NE: Squalene: hydrogenated soy phosphatidylcholine; pluronic® F-68		193.0	-56.6	63.5		
	MNX	**NLC:** Squalene; hexadecyl palmitate;hydrogenated soy phosphatidylcholine; deoxycholic acid; pluronic® F-68	HSH Ultrasonication	236.0	-43.8	54.4	NA	ALJUFFALI *et al.*, 2015
				194.5	-45.5	49.2		
Niosomes	MNX	Sorbitan monostearate/cholesterol	Ethanol injection	470.0**	NA	34.7**	(90 days/4°C) 40% loss of MNX and decreased size of niosome	MALI *et al.*, 2013
Ethosomes	FNS	**Ethosomes:** Soybean phospholipid; ethanol	Film dispersing	92.0	-9.0	78,1	NA	RAO *et al.*, 2008; RAO *et al.*, 2015
		Liposomes: Soybean phospholipid		129.0	8.9	59.7		
	FNS	FB without permeation enhancers: Soy lecithin; ethanol; propylene glycol	Ultrasonication	107.8**	-3.6**	84.9**	(12 months/ (3-5°C and 25°C) EE, decreased over time; PS remained unchanged at 3/5°C but increased at 25°C;	WILSON *et al.*, 2018
		FB; oleic acid		143.6	-11.8	86.3		
		FB; thymol		110.7	-2.1	80.1		
		FB; isopropyl myristate		126.3	-8.3	88.4		
Chitosan-decorated polymersomes	FNS	Oligochitosan; poly (styrene-block-acrylic acid)	Co-solvent self-assembly	183.0**	+33.7	NA	NA	CAON *et al.*, 2014
		37 KDa chitosan; poly (styrene-block-acrylic acid)		363.0**				

LCN	FNS	FB without additives: Monoolein; poloxamer® 407	Ultrasonication	153.8 – 188.8	NA	99.2 – 99.4	NA	MADHESWARAN *et al.*, 2013
		FB; glycerol (20% w/w)		190.4		99.7		
		FB; oleic acid (10% w/w)		232.6		99.4		
		FB; propylene glycol (20% w/w)		160.9		99.6		
		FB; polyethylene glycol 400 (15% w/w)		161.9		98.9		
	FNS	Cremophor® RH 40 (0.5 – 2.5% w/w); monoolein	Ultrasonication	159.2 – 208.6	-22.1 – -24.6	99.2 – 99.4	NA	MADHESWARAN *et al.*, 2014
		Cremophor® EL (1.5 – 4.0% w/w); monoolein		153.7 – 243.0	−22.0 - −23.9	98.9 – 99.2		
		Poloxamer® 407; monoolein		167.0	-21.5	99.6		
	FNS	**LCN:** Monoolein; poloxamer® 407	Ultrasonication	244.9	-19.2	98.1	NA	MADHESWARAN *et al.*, 2017
	DTS					99.3		
	FNS	**CHI-LCN:** Chitosan; monoolein; poloxamer® 407		306.1**	+29.6**	99.0**		
	DTS					99.1**		
NTF gel formulation	FNS	Phospholipon® 90G; sorbitan tristearate	Modified thin film hydration	299.6	NA	69.7	NA	AHMED *et al.*, 2018
				171.0		89.4		
				197.4		93.1		
Transfersomes	MNX	Soy phosphatidylcholine; polysorbate 80; polysorbate 20; caffeine	Thin film hydration	NA	NA	13.6 – 48.8	(28 days/25°C) Stable based on vesicle counting: polysorbate 20 caused perturbation of the transfersome stability.	RAMEZANI *et al.*, 2018
Cyclodextrin	FNS	2-HP-β-CD; hydroxypropylcellulose	NA	NA	NA	NA	NA	LIMA *et al.*, 2018
	MNX	Methyl-β-CD	Freeze-drying	NA	NA	NA	(48h/25°C) Stable: phase-solubility studies;	LOPEDOTA *et al.*, 2015
Cyclodextrin (alginate-based hydrogel)	MNX	HP-β-CD (0.65 and 0.85 molar substitution degree)	NA	NA	NA	NA	(3 months/25°C) Stable (appearance, pH, viscosity, and drug content) size	LOPEDOTA *et al.*, 2018
	MNX	HP-β-CD (0.65 molar substitution degree)	Physical misture Freeze-drying Kneading Spray-drying	NA	NA	NA	NA	TRICARIO *et al.*, 2018
Polymeric nanoparticles	FNS	PLGA	Emulsification /solvent diffusion	316.5	–5.7	79,5	NA	ROQUE *et al.*, 2017
	DTS	Chitosan	Interfacial deposition technique of preformed polymers	224.9** – 326.5**	+ 40.2** – + 42.9**	76.3** – 94.7**	(90 days/8°C and 25°C) Stable;	USHIROBIRA *et al.*, 2020
		Poly (ε-caprolactone)		182.4 – 220.8	-13.6 – -23.7	96.7 – 98.4		
	MNX	Chitosan	Ion gelation	235.5**	+38.6**	73.0**	NA	MATOS *et al.*, 2015

	MNX	PLGA	Solvent evaporation	118.9**	-2.3**	NA	NA	TAKEUCHI *et al.*, 2018
	MNX	HA-PLGA	Solvent evaporation	243.0	-0.4	55.5	NA	JEONG *et al.*, 2019
		PLGA		159.0	-43.2	40.7		
Metalic nanoparticles	MNX	Zirconia beads; methylcellulose; mannitol	Bead milling	90.0 – 300.0	-9.9	NA	(4 weeks/NA) From 2 weeks on there was an increase in the nanoparticle size.	NAGAI *et al.*, 2019

* Values were presented as mean results without standard deviation
** Data of optimal formulation according to authors
*** Squarticles as a Lipid Nanocarrier
**** Stability data based on particle size, zeta potential or encapsulation efficiency according to authors' disclosure data

Abbreviations: CD: Cyclodextrin CHI-LCN: Chitosan-coated liquid crystalline nanoparticle; CSO-SA: Chitosan oligomer stearic acid; DTS: Dutasteride; EE: Encapsulation efficiency; FB: Formulation base; FNS: Finasteride; HA-PLGA: Hyaluronate–poly (lactide-co-glycolide); HPH: High-pressure homogenization; HSH: High speed-homogenization; LCN: Liquid crystalline nanoparticle; MNX: Minoxidil; NA: Not available; NE: Nanoemulsion; NLC: Nanostructured lipid carrier; NTF: Nano-transferosomal; PLGA: Poly (lactide-co-glycolide); PS: Particle size; ZP: Zeta potential.

Annex 2. *In vitro* release studies of nanoparticles applied to topical administration of MNX, FNS and DTS in AGA treatment.

		***In vitro* study**				
Nanoparticle	**Drug**	**Study objective**	**Model**	**Experimental conditions (Drug dose;medium release;drug assay;time study**	**Output**	**Ref.**
NLC	MNX	IVRT	Celulose membrane (3.5 KDa)	-NA; PBS (pH 7.4/37°C/100 rpm); spectrophotometry; 24h.	- MNX sustained and prolonged release.	GOMES *et al.*, 2014
	FNS	IVRT	Celulose membrane (3.5 KDa)	-NA; PBS (pH 7.4/37°C/100 rpm); spectrophotometry; 24h.	- FNS was not released.	
	MNX	IVRT	Membrane^{+} (12-14 KDa)	- NA; PBS (pH 7.4/37°C/800 rpm); spectrophotometry; 12h.	- Biphasic pattern with burst release followed by sustained release.	UPTRI *et al.*, 2013
	DTS	IVRT (Uncoated and chitosan-coated NLC designed as CSO-SA)	Nitrocellulose membrane (0.45 µm)	- NA; PBS containing 2% SDS (37°C/600 rpm); HPLC; 36h.	- Fast release for all NLC (NLC > CSO-SA NLC).	NOOR *et al.*, 2017
	MNX	IVRT (MNX-SLN, MNX-NLC and control)	Membrane (14 KDa)	- 19 mg; PBS (pH 7.4/37°C/300 rpm); HPLC; 24h.	- MNX release rate from NLC was faster than that from SLN.	WANG *et al.*, 2017
Squarticles	MNX	IVRT (Squarticles and control)	Celulose membrane (35 KDa)	- NA; NA; NA; 24h.	- Fast release in the first 8 h followed by plateau.	ALJUFFALI *et al.*, 2014
	MNX	IVRT (Squarticles and control)	Celulose membrane (35 KDa)	- NA; NA; NA; 24h.	- Biphasic release pattern; - MNX sustained and prolonged release.	ALJUFFALI *et al.*, 2015
Ethosomes	FNS	IVRT (Ethosomes)	Membrane (12-14 KDa)	- NA; 50 ml of PBS (pH7.4/37°C/50rpm); UV-Vis; 8h.	Biphasic release pattern: - burst phase; - sustained release for about 8 h.	WILSON *et al.*, 2018
Chitosan-decorated polymersomes	FNS	IVRT (Uncoated and chitosan-coated polymersomes)	Membrane (3.5 KDa)	- NA; 10 mL of PBS buffer (pH 7.4): ethanol solution (70/30, v/v); HPLC; 3h.	- Greatest control over the drug release profile with chitosan-coated polymersomes.	CAON *et al., 2014*
LCN	FNS	IVRT (FNS LCN and control) - Impact of GL, PG, PEG and OA on FNS release rate	Membrane (10 KDa)	- 500 µg; 10 ml of 20% (v/v) ethanol solution; HPLC; 24h.	- Slow FNS release with increasing MO concentration; - Higher release of FNS with glycerol, propylene glycol and polyethylene glycol; - FNS release was significantly reduced with 5% and 10% OA.	MADHEDWARAN *et al.*, 2013
	FNS	IVRT (FNS-loaded LCN prepared with varying concentration of surfactants) - Evaluation of surfactants addition on the FNS release	Membrane (10 KDa)	- NA;10 ml of 20%v/v ethanol in PBS (pH 7.4/32°C/stirring); HPLC; 24h.	- Higher surfactant concentration showed FNS faster release.	MADHESWARAN *et al.*, 2014

	FNS + DTS	IVRT (Uncoated LCN and chitosan-coated LCN)	Membrane (10 KDa)	- NA; 20% vol/vol ethanol in PBS (pH 7.4/32°C/ stirring); HPLC; 24h.	- FNS and DTS controlled release from LCN and CHI-LCN (FNS > DTS).	MAHESWARAN *et al.*, 2017
NTF gel formulation	FNS	IVRT (FNS-NTF gel and control)	NA	-NA; NA; NA; 24h.	- FNS sustained release pattern.	AHMED *et al.*, 2018
Transfersomes	MNX	IVRT (Transfersomes containing different amounts of excipients)	Dialysis membrane (NA)	- NA; 33 ml of distilled water (37°C/ 50 rpm); UV-Vis; 24h.	- MNX release kinetics was first order.	RAMEZANI *et al.*, 2018
Cyclodextrins	MNX	IVRT (Methyl-β-CD-MNX dispersed in gels matrices were compared with MNX gel formulations)	Cellulose membrane (35 KDa)	- NA; 12.3 mL mixture of water:ethanol 60:40 v/v (37°C/ stirring); HPLC; NA.	- Methyl-β-CD containing gel increased MNX release.	LOPEDOTA *et al.*, 2015
	MNX	IVRT (HP-β-CD/MNX hydrogel formulations and control)	Cellulose membrane (0.1-0.5 KDa)	- 35 mg; water/ethanol 60:40 v/v (37°C); HPLC; 4h.	- Hydrogel formulation released MNX more slowly.	LOPEDOTA *et al.*, 2018
	MNX	IVRT Drug dissolution profile (HP-β-CD/MNX, CD/MNX hydrogel)	Rotating paddle (apparatus 2 Varian VK 7010)	- 30 mg; water 50 mL (25°C); HPLC; 6h.	- HP-β-CD enhanced the dissolute rate of MNX.	TRICARICO et al., 2018
Polymeric nanoparticles	FNS	IVRT	Aqueous system under stirring	- 5 mg; 10 mL of PBS (pH 7.4/25°C/130 rpm); UV-Vis; 3h.	- Prolonged release of FNS.	ROQUE *et al.*, 2017
	DTS	IVRT (Uncoated and Chitosan-coated nanoparticles)	Aqueous system under stirring	- NA; 24 mL of 60% water: 0.5% tween® 80: 40% ethylene glycol (32°C/600 rpm); 96h.	- Slow DTS diffusion; Chitosan coated nanoparticles exhibited more controlled release of DTS.	USHIROBIRA *et al.*, 2020
	MNX	IVRT (MNX chitosan nanoparticles and control)	Cellulose acetate membrane	- NA; NA; NA; 6h.	- MNX sustained release.	MATOS *et al.*, 2015
	MNX	IVRT (MNX-encapsulated PLGA nanoparticles and control)	Membrane (14 KDa)	- NA; 95 mL PBS (pH 7.4/32°C/30 rpm); HPLC; 8h.	- MNX cumulative and controlled release from nanoparticles.	TAKEUCHI *et al.*, 2018
	MNX	IVRT (HA-PLGA/MNX nanoparticles; PLGA/MNX nanoparticles and control)	Membrane (10 KDa)	- NA; 2 mL of PBS (pH 7.4/37°C/60 rpm); UV-Vis; 7-days.	- MNX controlled release with HA-PLGA nanoparticles.	JEONG *et al.*, 2019

Abbreviations: CD: Cyclodextrin; CHI-LCN: Chitosan-coated liquid crystalline nanoparticle;; CSO-SA: Chitosan oligomer stearic acid; DTS: Dutasteride; FNS: Finasteride; GL: Glycerol; HA-PLGA: Hyaluronate–poly (lactide-co-glycolide); HPLC: High-performance liquid chromatography; IVRT: *In vitro* release test; LCN: Liquid crystalline nanoparticle; MNX: Minoxidil; MO: Monoolein; NA: Not available; NLC: Nanostructured lipid carrier; NTF: Nano-transferosomal; OA: Oleic acid; PBS: Phosphate-buffered saline; PEG: Poly (ethylene glycol); PG: Propylene glycol; PLGA: Poly (lactide-co-glycolide); SDS: Sodium dodecyl sulfate; SLN: Solid lipid nanoparticle; UV-Vis: Ultraviolet-visible spectrophotometry.

[+]Authors did not provide more data

Annex 3. *In vitro* permeation and penetration studies of nanoparticles applied to topical administration of MNX, FNS and DTS in AGA treatment.

		***In vitro* study**				
Nanoparticle	**Drug**	**Study objective**	**Model**	**Experimental conditions (Drug dose;medium release;drug assay;time study**	**Output**	**Ref.**
NLC	MNX	Drug skin penetration	Franz apparatus (pig ear cells)	- NA; PBS (pH 7.4/37°C/stirring); UV-Vis; 24h.	- Small quantity of MNX penetrated into the skin.	GOMES *et al.* 2014
	FNS	Drug skin penetration	Franz apparatus (pig ear cells)	- NA; PBS (pH 7.4/37°C/stirring); HPLC; 24h.	- Short quantity of FNS penetrate into the skin.	
	DTS	IVPT (DTS-NLC, uncoated and coated chitosan (CSO-SA)/(CSO) and control)	Franz apparatus (pig ear cells)	- NA; PBS containing 2% SDS and 0.02% sodium azide (pH 7.4/37°C/ stirring); HPLC; 48h.	- Amount of DTS in the skin was higher with DTS-NLC either uncoated and coated NLC.	NOOR *et al.*, 2017
	MNX	Drug skin permeation (MNX-SLN, MNX-NLC and control)	Franz apparatus (rat abdominal skin)	- 19 mg; PBS (pH 7.4/32°C/300 rpm); HPLC ;72h.	- Compared to the MNX-SLN, the MNX-NLC showed the highest MNX retention in the skin.	WANG *et al.*,2017
Squarticles	MNX	IVPT (NLC, NE and control)	Franz apparatus (mice skin)	- NA; 30% ethanol in buffer (pH 7.4/37°C/600 rpm); HPLC; 24h.	- Increased the skin uptake of MNX; - Increased the skin MNX deposition; - Improved the MNX accumulation in the skin; - Increased the MNX follicular uptake and accumulation.	ALJUFFALI *et al.*, 2014
	MNX	IVPT (Squarticles and control)	Franz apparatus (porcine and mouse skin)	- NA; 30% ethanol in buffer (pH 7.4/37°C/600 rpm); HPLC; 24h.	- Enhanced MNX skin deposition and accumulation.	ALJUFFALI *et al.*, 2015
Niosomes	MNX	Drug skin deposition and permeation (MNX niosomal gel and control)	Modified Keshary–Chien-apparatus (porcine and mice skin)	- 500 mg; 10 mL of PBS (pH 7.4/37°C/stirring); HPLC; 24h.	- Increased deposition and skin permeation of MNX as cholesterol concentration increased in the formulation.	MALI *et al.*, 2013
Ethosomes	FNS	Drug skin penetration (Liposomes; ethosomes and control)	Franz apparatus (human cadaver skin)	- NA; 6.8 ml of isotonic saline; HPLC; 24h.	- Highest FNS skin permeation with ethosomes; - Ethosomes increased transdermal fluxes.	RAO *et al.*, 2008; RAO *et al.* 2015
		Drug skin accumulation (Liposomes; ethosomes and control)		- Small pieces of epidermis and dermis skin; - NA; 2/4 mL of methanol; HPLC; 24H.	- Highest FNS skin accumulation with ethosomes.	
	FNS	Drug permeation (Ethosomes and control) - Evaluation of permeation enhancers PG, OA, thymol and isopropyl myristate	Franz apparatus (rat dorsal skin)	- NA; 15 ml of PBS (pH7.4/37°C); UV-Vis spectroscopy; 8h.	- Higher skin permeation for FNS-loaded ethosomes containing permeation enhancers (OA showed the highest transdermal flux).	WILSON *et al.*, 2018

		Drug permeation (Ethosomes and control) - Evaluation of permeation enhancers PG, OA, thymol and isopropyl myristate	Franz apparatus (human cadaver frontal scalp skin)		- Higher skin permeation for FNS-loaded ethosomes containing permeation enhancers (OA showed the highest transdermal flux).	
Chitosan-decorated polymersomes	FNS	Drug skin permeation and retention (Undecorated, decorated polymersomes and control)	Franz apparatus (pig ear skin)	- NA; 10 mL of PBS buffer (pH 7.4/37°C/800 rpm); (pH 7.4): ethanol solution (70/30, v/v); HPLC; 24h.	- Decorated polymersomes increased FNS retention in the skin; - Low permeation flux values.	CAON *et al., 2014*
LCN	FNS	Drug skin permeation and retention (FNS-loaded LCN dispersion and control) - Evaluation of additives GL, PG, PEG and OA	Franz apparatus (mice skin)	- 500 µg; 10 ml of 20% (v/v) ethanol solution at 37°C/stirring; HPLC; 24h.	- Permeation and retention increased with GL, PG, and PEG and decreased upon addition of OA.	MADHESWARAN *et al.*, 2013
	FNS	Drug skin permeation and retention (FNS-loaded LCN and control) - Effect of surfactant concentration	Franz apparatus (porcine abdominal skin)	- 500 µg; 10 ml of 20%v/v ethanol in the PBS (pH 7.4/32°C/stirring); HPLC; 24h.	- Higher surfactant concentrations increased skin permeation; - Lowest surfactant concentrations increased skin retention.	MADHESWARAN *et al.*, 2014
	FNS + DTS	Drug skin permeation and retention (Uncoated and chitosan NLC and control)	Franz apparatus (porcine skin)	-500 µg of FNS and 250 µg of DTS; 20% vol/vol ethanol in PBS (pH 7.4/32°C/400 rpm); HPLC; 24h.	- Higher cumulative FNS/DTS permeation with CHI-LCN; - Higher retention and distribution into skin layers (LCN > CHI-LCN).	MADHESWARAN *et al.*, 2017
NTF gel formulation	FNS	IVPT (FNS-NTF and control)	Vertical diffusion cells (rat abdominal skin)	- NA; PBS (pH 5.8/32°C /400 rpm); HPLC; 3h.	- FNS-NTF gel formulations enhanced the amount of FNS permeated skin.	AHEMD *et al.*, 2018
Cyclodextrins	MNX	Drug skin penetration and permeation (Hydrogel methyl-β-CD/MNX and control)	Franz apparatus (pig skin)	- NA;12.3 ml of 0.05 M PBS (pH 7.4/37 °C/ stirring); HPLC; 24h.	- Hydrogel methyl-β-CD increased retention and accumulation of MNX.	LOPEDOTA *et al.*, 2015
	MNX	Drug permeation and accumulation ((HP-β-CD/MNX hydrogel formulations and control)	Franz apparatus (pig skin)	- NA; 12.3 mL of 0.05 M PBS (pH 7.4/37 °C/stirring); HPLC; 24h.	- Hydrogel formulation increased MNX accumulation and permeation in the skin.	LOPEDOTA *et al.*, 2018
Polymeric nanoparticles	FNS	IVPT (FNS nanoparticle in the different vehicles)	Franz apparatus (PDMS membrane[a])	- NA; PBS (pH 7.4/32°C/130 rpm); UV-Vis; 24h.	- Low levels of FNS penetration.	ROQUE *et al.*, 2017
	DTS	IVPT (Uncoated and chitosan-coated nanoparticles)	Franz-type apparatus (porcine ear skin)	- NA; 75 mL of 0.5% SDS aqueous solution; NA; 12h.	- Enhanced permeation skin layers; - Increased hair follicles targeting; - DTS accumulation; - Uncoated nanoparticles favored DTS penetration into the hair follicles.	USHIROBIRA *et al.*, 2020

	MNX	IVPT (MNX chitosan nanoparticles and control)	Franz apparatus (porcine ear skin)	- NA; 15 mL of 0.01 M PBS (pH 7.4); HPLC;12h.	- Higher MNX accumulation into the follicles; - Increased MNX penetration into hair follicles; - MNX release targeted to the hair follicles.	MATOS *et al.*, 2015
	MNX	IVPT (PLGA and HA-PLGA nanoparticles)	Franz apparatus	- 300 μg; NA; NA; 24h.	- Higher skin permeation and efficiency with HA-PLGA/MNX.	JEONG *et al.*, 2019

Abbreviations: CD: Cyclodextrin; CHI-LCN: Chitosan-coated liquid crystalline nanoparticle; CSO: Chitosan oligomer; CSO-SA: Chitosan oligomer stearic acid; DPC: Dermal papilla cell; DTS: Dutasteride; FNS: Finasteride; GL: Glycerol; HA-PLGA: Hyaluronate–poly (lactide-co-glycolide); HP-β-CD: hydroxypropyl-β-cyclodextrin; HPLC: High-performance liquid chromatography; IVPT: *In vitro* skin permeation test; LCN: Liquid crystalline nanoparticle; MNX: Minoxidil; NA: Not available; NE: Nanoemulsion; NLC: Nanostructured lipid carrier; NTF: Nano-transferosomal; OA: Oleic acid; PBS: Phosphate-buffered saline; PDMS: Polydimethylsiloxane; PEG: Polyethylene glycol; PG: Propylene glycol; PLGA: Poly (lactide-co-glycolide); SDS: Sodium dodecyl sulfate; SLN: Solid lipid nanoparticle; UV-Vis: Ultraviolet-visible spectrophotometry.

[a] A simplified model of human epidermis

Annex 4. *In vitro* and *in vivo* studies concerning the toxicity, skin distribution and hair effects of nanoparticles applied to topical admistration of MNX, FNS and DTS in AGA treatment.

***In vitro* or *In vivo* study**						
Nanoparticle	**Drug**	**Study objective**	**Animal model**	**Experimental conditions**	**Output**	**Ref.**
NLC	MNX	Visual inspection for edema and erythema (NLC-containing gel and saturated drug solution)	Albino mice	- hair removal 3 days before the experiment; - 7-day period (once a day).	- NA.	UPTRI *et al*,2013
	DTS	*In vitro* MTT cytotoxicity (Encapsulated and free DTS nanoparticles)	Human hair follicle DPCs	- 5-day period.	- DTS NLC enhanced the maximum non-toxic concentration approximately 20-fold.	NOOR *et al*., 2017
	MNX	Skin irritation test (Visual inspection for erythema) (MNX- NLC and conventional formulation)	Male rats	- shaved surfaces with circular areas; - 3-day period (twice a day).	- Erythemal scores of conventional formulations were higher than those of MNX-NLC.	WANG *et al*,2017
Squarticles	MNX	Skin irritation test (Squarticles (NE and NLC) and control)	Female nude mice (ICR-Foxn1nu)	- applied onto dorsal region of animal; - 7-day period (repeated application); -the treated site was measured by TEWL, skin erythema and skin pH.	- No erythema was detected for the squarticles.	ALJUFFALI *et al*,2014
		Skin delivery (Squarticles compared with control)		- nile red dye as tracer; - 6h study; - glass cylinder (diffusion area of 0.785 cm^2); - skin sample was analyzed by fluorescence microscopy and CLSM.	- Squarticles were mainly distribution to the skin surface, hair shaft and in the hair follicles.	
		VEGF Amount of DPCs	Human hair DPCs	- 48h incubation.	- Upregulated VEGF expression DPCs.	
		Viability of DPCs *In vitro* MTT cytotoxicity (NE, NLC and control)		- 24h incubation.	- NE and NLC were nontoxic.	
	MNX	Skin permeation and distribution (Squarticles; PDGF-squarticles and control)	Female nude mice	- nile red as tracer; - 4h study - glass cylinder (diffusion area of 0.785 cm^2).	- Squarticles increased MNX accumulation in hair shafts; - Squarticles and PDGF-squarticles increase MNX follicular deposition; - Squarticles increased MNX follicular uptake; - Maximum MNX uptake with PDGF-squarticles.	ALJUFFALI *et al*,2015
		Viability and VEGF amount of DPCs	Human hair DPCs	- 72h study.	- Increased viability; - Enhanced VEGF expression; - Higher cell proliferation.	
		Squarticles internalization into DPCs		- 24h study.	- Enhanced internalization by DPCs.	

LCN	FNS + DTS	Drug skin retention (LCN; FNS and DTS control)) Drug skin distribution (rhodamine-6G-loaded LCN and CHI-LCN)	Hairless mice	- drugs analysis in 24-exposed skin; - rhodamine 6G as a tracer.	- Increased FNS retention (CHI-LCN > LCN); - DTS retention was higher than FNS; - FNS and DTS distribution in SC and epidermis layer of skin treated with LCN; - FNS and DTS homogenous distribution on the skin layers with CHI-LCN.	MADHESWARAN *et al*., 2017
Transfersomes	MNX	Hair growth: Hair length and weight (Transfersome containing MNX and caffeine; transfersome placebo; aqueous solution of minoxidil and caffeine; conventional MNX)	Wistar rats	- area of 4 cm^2 was shaved; - 30-day period.	- Transfersome containing MNX and caffeine: - Promoted uniform hair growth; - Higher efficacy of MNX delivery to follicles; - Enhanced MNX residence in the skin.	RAMEZANI *et al*, 2018
Cyclodextrins	MNX	Gene expression (HP-β-CD/MNX hydrogel and MNX solutions)	Wistar rats	- 4-week period.	- HP-β-CD/MNX hydrogel formulations increase mRNA levels of the AKT2 gene.	TRICARICO *et al*., 2018
Polymeric nanoparticles	FNS	Cytotoxicity test	*Saccharomyces cerevisiae model*	- 24h study.	- Safe for FNS delivery to the skin surface.	ROQUE *et al*., 2017
		Safety of the formulation excipients (Occluded patch test) (3 different formulations with and without empty nanoparticles, all cases without FNS)	10 healthy female volunteers	- 2 adhesives were applied in the lumbar area; - 24h study; - visual observations 2h after removal of the adhesives.	- No signs of erythema; - No signs of adverse reactions.	
	MNX	Drug transdermal delivery (MNX PLGA nanoparticles and MNX solution)	C3H/He mice	- 8h study.	- Improved MNX delivery amount to stratum corneum and hair follicles.	TAKEUCHI *et al*., 2018
	MNX	Cell viability test	Mouse fibroblast cells	- 24h study.	- No cytotoxicity.	JEONG *et al*., 2019
		Cellular uptake test	Hair follicle DPCs	- HA-PLGA/rhodamine B and PLGA/rhodamine B incubated with 10 μM concentration of rhodamine B 2h.	- HA-PLGA/MNX demonstrated that can regulated hair growth and follicle development.	
Metalic nanoparticles	MNX	Hair-growth effect and MNX levels in the hair, skin, and blood	C57BL/6 mice	- hair was cut to a length of c.a. 2 mm (dorsal area administration); - 4h formulation contact; - MNX quantification by HPLC.	- High MNX amounts in the hair; - Lower MNX levels in the skin; - No MNX was detected in plasma; - Promoted hair growth.	NAGAI *et al*., 2019
		Therapeutic efficiency for hair growth		- 10-day period (once a day) (dorsal area administration); - measurement of IGF-I and VEGF.	- Increase of mRNA, protein of IGF-1, and VEGF levels.	

Abbreviations: AKT2: ATP-sensitive potassium channel opener gene code; CD: Cyclodextrin; CHI-LCN: Chitosan-coated liquid crystalline nanoparticle; CLSM: Confocal laser scanning microscopy; CSO-SA: Chitosan oligomer stearic acid; DPC: Dermal papilla cell; DTS: Dutasteride; FNS: Finasteride; HA-PLGA: Hyaluronate–poly (lactide-co-glycolide); HP-β-CD: Hydroxypropyl-β-cyclodextrin; HPLC: High-performance liquid chromatography; IGF-1: Insulin-like growth factor-1; LCN: Liquid crystalline nanoparticle; MNX: Minoxidil; mRNA: Messenger ribonucleic acid; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NA: Not available; NE: Nanoemulsion; NLC: Nanostructured lipid carrier; PBS: Phosphate-buffered saline; PDGF: Platelet-derived growth factor; PLGA: Poly (lactide-co-glycolide); SLN: Solid lipid nanoparticle; TEWL: Transepidermal water loss; UV-Vis: Ultraviolet-visible spectrophotometry; VEGF: Vascular endothelial growth factor.