UNIVERSIDADE D
COIMBRA

Sara Cabanas Coimbra

Relatório de Estágio e Monografia intitulada "Nanotechnology-based formulations towards the therapy and care of acne" referentes à Unidade Curricular "Estágio", sob a orientação da Dra. Carla Almeida Nobre Marques e do Professor Doutor António José Ribeiro, 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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Coimbra, 1 de setembro de 2020.

Sara Cabanas Coimbra

(Sara Cabanas Coimbra)

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"Aqueles que passam por nós não vão sós.
Deixam um pouco de si, levam um pouco de nós."

Antoine de Saint-Exupéry

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PARTE I

RELATÓRIO DE ESTÁGIO EM FARMÁCIA COMUNITÁRIA

Farmácia Tomás Ribeiro

Lista de Abreviaturas

AINE – Anti-inflamatório não esteróide.

ANF – Associação Nacional das Farmácias

DCI – Designação Comum Internacional

FFUC – Faculdade de Farmácia da Universidade de Coimbra

FTR – Farmácia Tomás Ribeiro

MICF – Mestrado Integrado em Ciências Farmacêuticas

MNSRM – Medicamentos Não Sujeitos a Receita Médica

MSRM – Medicamentos Sujeitos a Receita Médica

PUV – Produtos de Uso Veterinário

PVF – Preço de Venda à Farmácia

PVP – Preço de Venda ao Público

SWOT – Pontos Fortes (*Strenghts*), Pontos Fracos (*Weaknesses*), Oportunidades (*Opportunities*), Ameaças (*Threats*).

1. Introdução

O Estágio Curricular permite aos estudantes do Mestrado Integrado em Ciências Farmacêuticas (MICF) passar dos conhecimentos teóricos adquiridos para a aplicação prática dos mesmos. O estágio em farmácia comunitária possibilita não só a consolidação dos conhecimentos adquiridos ao longo do percurso académico, na Faculdade de Farmácia da Universidade de Coimbra (FFUC), como a apreensão de novos, num ambiente profissional controlado e adequado ao exercício da profissão farmacêutica.

A farmácia comunitária é uma instituição centenária que visa a prestação de serviços de interesse público, no sentido de promover a saúde e o bem-estar da população, aliado ao combate à doença. É dos locais mais comuns para o exercício da profissão tornando-se um espaço essencial para a interação com tarefas que competem ao farmacêutico. O farmacêutico tem como dever, no exercício das suas funções assegurar a qualidade, eficácia e segurança no tratamento que é providenciado ao utente (1).

O relatório de estágio que se segue foi realizado no âmbito da unidade curricular designada por Estágio Curricular, que decorreu entre o dia 6 de janeiro e o dia 3 de julho, na Farmácia Tomás Ribeiro (FTR) sob a orientação da Dra. Carla Marques.

2. Farmácia Tomás Ribeiro

A FTR situa-se em Tondela, distrito de Viseu, encontrando-se desde 2009 localizada na Rua José Bernardo da Silva. Esta farmácia era a antiga Farmácia Matos que mudou de proprietário em 2006.

O horário de atendimento é das 8:30 às 20 horas, nos dias úteis, e das 9 às 14 horas aos sábados, estando encerrada aos domingos e feriados. A FTR encontra-se em serviço permanente a cada seis semanas.

A equipa da FTR é constituída pela diretora-técnica, Dra. Carla Marques, pela farmacêutica substituta, Dra. Joana Matos e pelos técnicos de farmácia, Inês Silva, Luísa Coimbra, Rui Coimbra e Rui Gomes.

A maioria dos utentes são idosos, entre os quais, utentes que frequentavam a antiga Farmácia Matos, residentes da zona e alguns utentes pontuais devido a boa localização da farmácia, encontrando-se relativamente perto do Hospital de Tondela.

3. Análise SWOT

A análise SWOT (Tabela 1) foi utilizada para efetuar uma avaliação crítica ao estágio. Esta análise divide-se numa dimensão interna, onde são abordados os pontos fortes (*Strenghts*) e pontos fracos (*Weaknesses*) e externa, onde se englobam as oportunidades (*Opportunities*) e as ameaças (*Threats*).

Tabela 1 – Análise SWOT ao estágio realizado na Farmácia Tomás Ribeiro.

	Dimensão Interna		**Dimensão Externa**
Pontos Fortes	⇒ Autonomia, empatia, ética e responsabilidade. ⇒ Organização da farmácia. ⇒ Prestação de serviços. ⇒ Sistema SIFARMA 2000®.	**Oportunidades**	⇒ Contacto com diferentes tipos de prescrições. ⇒ A COVID-19. ⇒ Formação contínua. ⇒ Grande variedade de produtos de dermocosmética.
Pontos Fracos	⇒ Aconselhamento de determinados produtos. ⇒ Insegurança inicial. ⇒ Relação da DCI com as marcas comerciais.	**Ameaças**	⇒ Automedicação. ⇒ A imagem do estagiário. ⇒ Desconfiança aos genéricos. ⇒ Medicamentos esgotados.

3.1. Dimensão Interna

3.1.1. Pontos Fortes

3.1.1.1. Autonomia, Empatia, Ética e Responsabilidade

Apesar da autonomia que me foi sendo concedida ao longo do estágio, as tarefas foram realizadas com a consciência da repercussão que os atos poderiam ter. O exercício da profissão farmacêutica exige que haja um sentido de responsabilidade permanente, reconhecendo as possíveis consequências das decisões que se tomam. Para além disto, é também necessário que perante o atendimento de um utente haja a adoção de uma conduta ética e que se crie empatia no sentido de ouvir e reconhecer as dificuldades dos utentes.

3.1.1.2. Organização da Farmácia

Na sala de atendimento ao público da FTR encontram-se visíveis diversas secções entre as quais, puericultura, ortopedia, produtos de uso veterinário e cosmética. A exposição dos Medicamentos Não Sujeitos a Receita Médica (MNSRM) e suplementos alimentares encontra-se atrás dos balcões de atendimento. Nesta parte de *front-office*, para além da sala de atendimento ao público, existem ainda dois gabinetes de apoio ao utente sendo um deles usado para a prestação de serviços e o outro para consultas de nutrição e testes auditivos.

Na parte de *back-office* encontra-se o escritório da direção técnica, zona de receção de encomendas, armazém e laboratório. No armazém são armazenados os Medicamentos Sujeitos a Receita Médica (MSRM) tendo em conta as condições de conservação, entre as quais, a temperatura, humidade e integridade da embalagem, por forma a assegurar as Boas Práticas Farmacêuticas (2). Os MSRM são organizados nas gavetas tendo em conta a forma farmacêutica e por ordem alfabética, sendo seguida a regra de "*first in first out"* associada à regra de "*first expire first out"*. Para tal, eram tidas em conta as validades dos produtos que chegavam aquando da receção da encomenda e era, ainda, impressa a lista de gestão de prazos de validade de três em três meses sendo que os produtos com aproximação do término do prazo de validade eram colocados numa prateleira em destaque para que pudessem ser vendidos.

Numa fase mais inicial do estágio, foi o contacto com as tarefas realizadas no *back-office* e a arrumação de gôndolas e expositores do *front-office*, que permitiram o conhecimento de MNSRM e a associação dos princípios ativos aos respetivos medicamentos de marca, o que posteriormente foi facilitando o atendimento ao balcão.

3.1.1.3. Prestação de Serviços

Segundo o Decreto-Lei nº 307/2007, pelo que consta no Artigo 36.º, as farmácias podem efetuar a prestação de serviços no sentido de promover a saúde e o bem-estar dos utentes[1]. Na FTR existem diversos serviços que são prestados, entre os quais, pesagem de bebés, medição de parâmetros como o ácido úrico e a administração de injetáveis. No entanto, os mais prestados são a medição do peso e altura, com cálculo do respetivo IMC, medição de determinados parâmetros, tais como, a pressão arterial, a glicémia, o colesterol total e os triglicéridos. Os utentes deslocam-se com frequência à FTR para usufruírem destes serviços no sentido de efetuarem um registo com regularidade para posteriormente mostrar ao médico.

A prestação destes serviços individualizados permitiu a aplicação e a consolidação dos conhecimentos teórico-práticos adquiridos no MICF, estabelecendo uma proximidade com o utente.

3.1.1.4. Sistema SIFARMA 2000®

O *software* informático SIFARMA 2000® foi desenvolvido pela Glintt e pertence à Associação Nacional das Farmácias (ANF). É um *software* que auxilia grande parte das atividades que são processadas em farmácia comunitária, sendo uma ferramenta indispensável no quotidiano das farmácias. Deste modo, ao longo do estágio foi possível contactar com as diversas funcionalidades do SIFARMA 2000® e aplicá-las nas diversas tarefas efetuadas na FTR.

No *back-office* o sistema foi utilizado para a consulta do histórico de vendas realizadas, impressão das listas de verificação dos prazos de validade, verificação e correção de prazos de validade e de *stocks*. Na criação e receção de encomendas existia a preocupação de corrigir o Preço de Venda à Farmácia (PVF) e de confirmar o Preço de Venda ao Público (PVP) bem como os prazos de validade que constam nos medicamentos. Foi também através do sistema que se efetuaram as devoluções e respetivas regularizações, bem como, a preparação da recolha dos contentores da Sociedade Gestora de Resíduos de Embalagens e Medicamentos (VALORMED).

Relativamente ao atendimento ao público, o SIFARMA 2000® para além de permitir efetuar as vendas, tinha como vantagens a consulta da informação científica e posologias de alguns medicamentos. Serviu também para o registo dos serviços prestados e ainda consulta das fichas e históricos de venda do utente.

3.1.2. Pontos Fracos

3.1.2.1. Aconselhamento de Determinados Produtos

Apesar do plano curricular de MICF ser bastante extenso e proporcionar a apreensão de conhecimentos teóricos fundamentais para a aplicação no contexto de farmácia comunitária, este apresenta algumas lacunas que se refletiram no aconselhamento de determinados produtos.

A FTR apresenta uma gama variada de produtos de puericultura e de ortopedia havendo alguma afluência de utentes para a procura destes. Isto levou a que numa fase mais inicial, por falta de conhecimentos na área, sentisse dificuldades aquando do aconselhamento. No entanto, a equipa da FTR mostrou-se sempre disponível para me auxiliar.

Numa fase mais inicial, foram sentidas também algumas dificuldades no aconselhamento de colírios e pomadas para determinadas afeções descritas pelos utentes devido, por um lado, a uma abordagem superficial do aconselhamento destas formas farmacêuticas ao longo do MICF e por outro, à existência de uma grande variedade dos mesmos. Estas dificuldades foram sendo ultrapassadas com a experiência adquirida ao longo do estágio.

Por último, apesar de existir a unidade curricular de Preparações de Uso Veterinário (PUV) no MICF esta não incide sobre o aconselhamento dos PUV para determinadas situações, tais como, designação das vacinações de cães e coelhos e a posologia de diferentes formas farmacêuticas. Isto impediu, por vezes, a resposta imediata ao utente, no entanto, ao longo do decorrer do estágio com a experiência e com a participação em formações estas dificuldades foram sendo atenuadas.

3.1.2.2. Insegurança Inicial

As primeiras semanas de estágio são cruciais para haver uma correta adaptação à farmácia. É normal que o estagiário sinta uma insegurança inicial uma vez que é o primeiro contacto que tem com a realidade da atividade do farmacêutico em farmácia comunitária. Por outro lado, tem a consciência da responsabilidade do farmacêutico e de que qualquer erro nas suas decisões pode ter consequências para o utente.

Com o decorrer do estágio a confiança foi-se instalando, uma vez que diferentes situações foram ocorrendo ao longo dos atendimentos e com isto foi possível observar e perceber algumas técnicas de aconselhamento.

Posto isto, reforça-se a ideia de que o farmacêutico tem de adotar uma postura proativa no sentido de se manter atualizado, através de um estudo continuo dos novos princípios ativos que vão saindo para o mercado de modo a que o aconselhamento seja assertivo, com a qualidade, eficácia e segurança que é pretendida.

3.1.2.3. Relação DCI com as Marcas Comerciais

A principal dificuldade, inicialmente, foi a associação da Denominação Comum Internacional (DCI) à respetiva marca comercial.

Era frequente os utentes, perante a prescrição médica por DCI, questionarem se vinha prescrito um determinado medicamento, pronunciando o seu nome comercial. Devido ao reduzido contacto com os nomes comerciais ao longo do percurso académico, esta associação DCI com nome comercial não era imediata.

No entanto, o contacto com os medicamentos ao longo do estágio, tanto a nível do armazém, a rececionar e a arrumar as encomendas, como na dispensa no atendimento ao balcão permitiu consolidar esta associação.

3.2. Dimensão Externa

3.2.1. Oportunidades

3.2.1.1. Contacto com Diferentes Tipos de Prescrição

As prescrições podem ser efetuadas por meios eletrónicos, onde se incluem as modalidades de prescrição eletrónica desmaterializada e prescrição eletrónica materializada, ou manuais (3).

Apesar das prescrições eletrónicas desmaterializadas serem as mais comuns, ainda aparecem com alguma frequência prescrições eletrónicas materializadas e prescrições manuais.

Ao longo do estágio foi sendo possível o contacto e a familiarização com os diferentes tipos de prescrição, tendo notado que, com o decorrer do estágio, que era mais fácil o processamento e interpretação das mesmas.

3.2.1.2. A COVID-19

Embora tenha sido obrigatório o isolamento perante o estado de Emergência declarado devido à pandemia que se instalou pelo coronavírus SARS-CoV-2, a afluência às farmácias aumentou. As farmácias comunitárias, como referido na introdução, são locais onde se prestam serviços indispensáveis às necessidades de saúde e que demonstraram ser uma mais valia durante este período.

Apesar dos riscos associados à pandemia, lidar com esta situação foi uma oportunidade. Como profissionais de saúde, os farmacêuticos estiveram na linha da frente ao combate à COVID-19, tendo tido uma rápida capacidade de resposta, adotando medidas preventivas com vista à segurança dos seus utentes. Se as farmácias já eram muitas vezes o local preferido pelos utentes para o esclarecimento de dúvidas, perante esta situação foi ainda mais notório. Embora a informação que se obtinha à cerca da pandemia estivesse em constante atualização, os utentes procuravam junto da farmácia e era um dever do farmacêutico informar consoante as novas evidências científicas. Foi criada uma linha de apoio ao farmacêutico que visa responder a dúvidas e questões que iam surgindo durante a pandemia (4).

Na FTR as medidas para minimizar o risco de contaminação adotadas foram, para além do uso obrigatório de máscara, a aplicação de uma barreira física transparente ao balcão, a

colocação de linhas de segurança no chão e a disponibilização de uma solução antisséptica à base de álcool que se encontrava à entrada da farmácia, por forma a diminuir o risco de contaminação durante o aconselhamento (5). Todos os colaboradores da FTR tinham à sua disposição material de proteção como máscaras, viseiras, luvas descartáveis e álcool gel para efetuar a devida higienização das mãos. Foi também efetuado um reforço de *stocks* por forma a garantir a continuidade de tratamento dos utentes. E ainda no sentido de dar resposta aos utentes com medicação das farmácias hospitalares, durante a pandemia, a entrega destes medicamentos passou a ser feita nas farmácias comunitárias, inclusive na FTR.

3.2.1.3. Formação Contínua

Devido à existência de uma grande variedade de PUV e de produtos de Ortopedia na FTR, como referido anteriormente, foi possível assistir à exposição de apresentações acerca dos produtos comercializados na farmácia, por forma a que os aconselhamentos para cada situação exposta se tornassem mais elucidativos.

Foi possível assistir a outras formações fora da farmácia, tais como, o congresso organizado pela APCC "Sol e Pele", o *Workshop* de "Cosméticos para Antienvelhecimento. O Papel do Farmacêutico" organizado pela FFUC e ainda, uma formação dos suplementos alimentares da gama Bioactivo® da Pharmanord. Durante o período de isolamento, foi também possível efetuar formações em plataformas *online*.

As participações em formações permitiram não só o esclarecimento de dúvidas nas áreas abordadas como a aquisição de novos conhecimentos que permitem esclarecer o utente no aconselhamento.

3.2.1.4. Grande Variedade de Produtos de Dermocosmética

Apesar de haver algum desconforto inicial no aconselhamento destes produtos devido à grande variedade e quantidade de produtos de Dermocosmética que a FTR apresenta, foi com leitura da informação que consta nos produtos, com a ajuda e dicas dos colegas que os aconselhamentos se foram tornando mais fáceis.

Ao longo do estágio, foram diversas as situações em que o utente solicitava determinados produtos para a pele, tais como, um creme adequado ao tipo de pele, um corretor de olheiras ou um creme que ajudasse a disfarçar as manchas e imperfeições da pele. Foram poucas as situações em que existiam produtos de Dermocosmética prescritos com em diagnóstico prévio. Portanto, o aconselhamento adequado e assertivo do farmacêutico é muito

importante por forma a corresponder às necessidades do utente, tendo permitido complementar os conhecimentos adquiridos ao longo do MICF.

3.2.2. Ameaças

3.2.2.1. Automedicação

É importante que o farmacêutico tenha uma boa capacidade argumentativa, baseada em evidências científicas fortes, uma vez que cada vez mais os utentes dirigem-se à farmácia informados e muitas vezes decididos do que querem comprar. Muitas vezes, quando confrontados referem que foi recomendação de outrem ou que tinha lido na *Internet*.

Perante os MNSRM, o farmacêutico tem de ter a capacidade de avaliar a situação descrita pelo doente, relembrar ao utente os riscos associados à automedicação e consciencializando para a terapêutica farmacológica correta.

Não menos preocupante, são os utentes que solicitam MSRM referindo que é medicação não comparticipada pelo Estado ou que é medicação crónica. Perante esta situação é importante relembrar que os MSRM não devem ser dispensados sem prescrição médica e quanto muito verificando o histórico de vendas do utente no SIFARMA 2000® e comprovando que efetivamente é uma medicação habitual, a dispensa deste pode ser feita em suspensa na condição de que o utente trará a prescrição à posteriori.

É importante que se adapte o discurso em função da situação, mostrando sempre interesse em escutar e tentar ajudar, criando empatia com o utente, tendo presente a conduta ética e o sigilo profissional.

3.2.2.2. A Imagem do Estagiário

Apesar de a maioria das vezes o atendimento aos utentes ser gratificante, na medida em que foi criada uma empatia e o utente sentiu as suas necessidades correspondidas saindo satisfeito com o atendimento.

Existiam, porém, alguns utentes desconfiados por verem um elemento novo na equipa, sendo que muitas vezes acabavam por adquirir uma postura diferente, questionando tudo o que era dito. Muitas vezes, aguardavam para serem atendidos por outros colegas, ou referiam que tinham preferência para ser atendidos pelos colegas com quem se sentiam mais familiarizados.

Este tipo de comportamento por parte dos utentes, foi mudando por estímulo dos elementos da equipa, no entanto, constitui um entrave à aprendizagem do estagiário.

3.2.2.3. Desconfiança aos Genéricos

Perante a dispensa de medicamentos no atendimento ao balcão, uma das perguntas que é quase transversal a todos os atendimentos com prescrição médica por DCI é se o utente tem preferência pelo medicamento genérico ou de marca.

Esta questão gerava muita controvérsia e desconfiança por parte de alguns utentes, perguntando de imediato qual era a diferença ou afirmando que querem o de marca por considerarem ser melhor. Entre os que consideravam que o medicamento inovador, ou seja, o de marca, era melhor, argumentavam que tinha sido por conselho do médico ou porque já tomaram um genérico e sentiram algum efeito secundário.

Ainda assim, perante estas situações era importante esclarecer que o medicamento genérico contém a mesma substância ativa, forma farmacêutica e dose do medicamento de marca que lhe deu origem, tendo passado por testes de bioequivalência e servindo para mesma indicação terapêutica (6).

3.2.2.4. Medicamentos Esgotados

A existência de medicamentos esgotados pode comprometer a continuidade no tratamento do utente. Perante estas situações, em que a medicação é crónica e não podemos ceder a alternativa, é necessário encaminhar o utente para o médico para alterar o medicamento e dar continuidade ao tratamento da doença.

Esta situação dificulta muitas vezes o atendimento ao balcão, uma vez que os utentes não compreendem que a inexistência do medicamento na farmácia se pode dever aos distribuidores ou aos laboratórios.

4. Casos Práticos

4.1. Caso 1

Um utente do sexo masculino, com cerca de 40 anos, dirige-se à farmácia com queixas de que tem sintomas como rinorreia, dores de garganta e algumas dores no corpo. Solicita algo, não especificando, para aliviar os sintomas.

Perante este quadro é possível que esteja perante uma síndrome gripal. Foi necessário complementar o atendimento com algumas questões, tais como, se tinha dificuldades em deglutir ou se era diabético. Foi-lhe aconselhado a toma de Griponal® que contém 500 mg de paracetamol e 4 mg de maleato de clorofenamina, 3 a 4 vezes ao dia, podendo fazer 2 comprimidos efervescentes de 12 em 12 horas (7). O paracetamol é um analgésico e antipirético que ajuda no alívio de sintomas como dores corporais e febre, o maleato de clorofenamina é um anti-histamínico que conduz à secura das mucosas levando à diminuição do corrimento nasal, ou seja, a rinorreia (7). Para a dor de garganta, associada a dificuldade em deglutir e ausência da doença diabetes, foi aconselhado o Strepfen®, até 4 pastilhas por dia (sendo que o máximo é de 5). O flurbiprofeno presente nas pastilhas é um anti-inflamatório não esteróide (AINE) que possui potentes propriedades analgésicas, antipiréticas e anti-inflamatórias, sendo ideal para o alívio da dor de garganta (8).

Foram também referidas algumas medidas não farmacológicas, tais como, a ingestão regular de água e outros líquidos, repouso e a lavagem nasal com soro fisiológico ou uma solução salina.

4.2. Caso 2

Uma utente do sexo feminino, com cerca de 60 anos, confessa, durante o atendimento, que os sintomas de pernas inchadas e pesadas a deixam muito desconfortável. Refere que no quotidiano passa muitas horas em pé chegando ao final do dia cansada e com muitas dores nas pernas. Acrescenta ainda que costuma comprar Daflon®, mas que por questões económicas não consegue assegurar a toma diária. Pede então que se recomende algo mais acessível.

É importante começar por explicar que o cansaço que sente se deve às extensas horas que passa em pé, o que dificulta o retorno venoso do sangue. Foi realçado que efetivamente o Daflon® é muito bom ao nível das veias, uma vez que diminui a distensibilidade e a estase venosa ajudando no alívio dos sintomas causados pelo défice no sistema vascular de retorno (9). Contudo, foi aconselhado o uso do creme Cedraflon® que pode ser aplicado 2 a 3 vezes por dia. Este creme tem um efeito revitalizante para as pernas uma vez que a sua formulação contém cidra da Córsega e mentol que proporcionam um efeito refrescante e de alívio da

sensação de pernas pesadas e cansadas, logo após a sua aplicação (10). A sua aplicação deve ser feita com uma massagem no sentido de baixo para cima, com movimentos circulares. E ainda, se pretendesse um efeito mais refrescante poderia colocar o creme no frigorífico.

Como medidas não farmacológicas, foi aconselhado reduzir o número de horas que passa em pé durante o dia e à noite, como medida de alívio, elevar as pernas.

4.3. Caso 3

Jovem do sexo masculino, com cerca de 23 anos entra na farmácia com um inchaço notório na zona do tornozelo. Refere que tinha torcido o pé no trabalho e que não aguentava as dores ao caminhar. Perguntou o que teria para ajudar a aliviar as dores.

Foi-lhe aconselhado o uso de uma meia elástica para ajudar a estabilizar o pé e a aplicação de Voltaren Emulgelex® no local do inchaço, 2 vezes por dia, uma vez que o diclofenac é um AINE com propriedades analgésicas e anti-inflamatórias, levando a uma redução da dor e inchaço no local (11). Poderia também associar o anti-inflamatório oral, por exemplo Brufen® de 400 mg de 8 em 8 horas, uma vez que o ibuprofeno sendo um AINE ajuda não só no alívio da dor forte que sente, como na redução da inflamação e inchaço (12).

Como medida não farmacológica, foi aconselhado a aplicação de gelo, durante 20-30 minutos de 4 em 4 horas, para ajudar a reduzir a inflamação. Foi também advertido de que se nos próximos 4 dias não houvesse melhorias era aconselhado a ida ao médico.

5. Considerações Finais

A fase de estágio em farmácia comunitária foi o culminar de 5 anos de estudo de MICF. Na FFUC foram transmitidos conhecimentos que se tornaram essenciais para a atuação em farmácia comunitária. No entanto, apenas com a aplicação em meio profissional é que se conseguem aplicar e consolidar tais conhecimentos, adaptados ao ambiente. Cada atendimento ao balcão é diferente e deve ser personalizado para cada utente, o que torna a experiência desafiante e muito enriquecedora.

O estágio em farmácia comunitária permitiu-me valorizar a profissão farmacêutica, sendo notório o impacto da prestação de serviços essenciais à saúde, o esclarecimento de dúvidas não só sobre problemas de saúde, a terapêutica, mas também sobre a pandemia e promover a adesão e continuidade ao tratamento.

Para terminar, agradeço a toda a equipa da FTR por me ter acolhido na sua equipa durante este período de estágio, por me terem ajudado a ultrapassar as minhas inseguranças e se mostrarem disponíveis para esclarecerem as minhas dúvidas.

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PARTE II

MONOGRAFIA

NANOTECHNOLOGY-BASED FORMULATIONS
TOWARDS THE THERAPY AND CARE OF ACNE

Abbreviations

ADA – Adapalene

AgNP – Silver Nanoparticle

AuNP – Gold Nanoparticle

AuNR – Gold Nanorod

AZA – Azelaic Acid

BENP – Benzoyl Peroxide

CD – Cyclodextrin

CLY – Clindamycin

CPA – Cyproterone Acetate

CRYP – Cryptotanshinone

CUB – Cubosome

DAP – Dapsone

DC – Decreased

EC – European Commission

EE – Entrapment Efficiency

ERY – Erythromycin

ETO – Ethosome

EU – European Union

FULL – Fullerene

HaCaT – Human Immortalized Keratinocyte

ITR – Isotretinoin

LAH – Lauric Acid

LBNCA – Lipid Based Nanocapsule

LIP – Liposome

MIC – Minimum Inhibitory Concentration

NA – Non-Applicable

NC – Inconclusive

NCA – Nanocapsule

NCRY – Nanocrystal

ND – Not Detected

NE – Nano-emulsion

NF – Nanofiber

NP – Nanoparticle

NIO – Niosome

NLC – Nanostructured Lipid Carrier

NSP – Nanosphere

PDI – Polydispersity Index

POLM – Polymeric Micelle

PS – Particle Size

PSU – Pilosebaceous Unit

QbD – Quality by Design

SC – Stratum Corneum

SiNP – Silica Nanoparticle

SLN – Solid Lipid Nanoparticle

TEY – Tetracycline

TiO_2NP – Titanium Oxide Nanoparticle

TRA – Transfersome

TRE – Tretinoin

ZnONP – Zinc Oxide Nanoparticle

ZP – Zeta Potential

Abstract

Acne is one of the most common skin conditions that affects teenagers and can persist in adulthood, leading to physical and social impacts that compromise their life quality. There are effective conventional treatments for acne, although low tolerability and the appearance of side effects such as skin irritation have been related. Nanotechnology-based formulations have been developed as new strategies for acne treatment in order to overcome the difficulties of conventional treatments. Critical analysis of compiled nanosized anti-acne strategies strongly support a controlled active ingredient release, better skin permeation and lower skin irritation. As they are relatively recent, the lack of evidence about their safety, toxicity and regulatory support is also addressed.

Keywords: Acne, Nanoparticle, Nanosystem, Nanotechnology, Regulatory, Toxicology.

Resumo

A acne é uma das condições cutâneas que mais afeta os adolescentes e que pode persistir na idade adulta, podendo levar a impactos físicos e sociais que comprometem a sua qualidade de vida. Apesar de existirem tratamentos convencionais que são eficazes para o tratamento da acne, estes estão associados à baixa tolerabilidade e ao aparecimento de efeitos secundários reportados dos quais é exemplo a irritação da pele. Como novas estratégias para o tratamento da acne, têm vindo a ser desenvolvidas formulações baseadas em nanotecnologia, por forma a ultrapassar as dificuldades relacionadas com a terapia convencional. A análise crítica feita à compilação das nano-estratégias utilizadas para o tratamento da acne suportam fortemente a existência de uma libertação controlada do princípio ativo, uma melhor permeação na pele e menor irritação da pele. Como são relativamente recentes, existe falta de evidência relativamente à sua segurança, toxicidade e suporte regulamentar, sendo também abordados.

Palavras-Chave: Acne, Nanoparticula, Nanosistema, Nanotecnologia, Regulamentação, Toxicologia.

1. Introduction

Acne vulgaris, widely known as acne, is characterized by several lesions that affect the pilosebaceous unit (PSU). These lesions are related not only to increased sebum production, follicular epithelial desquamation, immunological host reactions and bacterial colonization in the hair follicles, but also to a dietary regimen with high amounts of sugar, proteins or fat and personal behaviors such as smoking (1), (2), (3). The PSU, represented in Figure 1, can be affected by inflammatory lesions such as papules, pustules, nodules and cysts, or as well by non-inflammatory lesions as comedones occurring, frequently, on the face, neck and back areas, as shown in Figure 2 (4), (5).

The basement membrane zone between the epidermis and dermis is the same of PSU and provides support for structures and appendages including hair follicles, erector pili muscles, nerve endings, sebaceous and sweat glands. This basement membrane is lined with basal stem cells that change into sebaceous cells or keratinocytes responsible for sebum production and hair growth. The proliferation of these cells is regulated by hormones, specifically androgens (2). There are pathogenic processes that affect the PSU and lead to the development of acne associated lesions. An abnormal over-keratinization can lead to the appearance of comedones. It can occur when there is a hormonal dysregulation in which sebum production and hair growth became increased. The pressure created within this area makes the oxygen diffusion difficult, creating an anoxic environment, that when allied to the overproduction of sebum providing a nutrient source, it becomes favorable to the colonization and proliferation of commensal gram-positive bacteria, such as *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*) and *Cutibacterium acnes* (*C. acnes*), previously known with the taxonomic classification *Propionbacterium acnes* (*P. acnes*) (6). In response to the damage within the PSU, there are immunological host reactions that are activated and cause inflammation (2), (7), (8).

Acne classification, graphically summarized in Figure 2, is commonly based on the characterization of the injury caused by this chronic inflammatory disease (9), (10). Comedonal acne appears regularly on the facial area and most of the time it has a non-inflammatory nature that include closed comedones (whiteheads) or open comedones (blackheads) (11). Papular acne is localized predominantly on the facial and back areas, involving around 10 to 25 papules with a soft scarring appearance (12). Pustular acne is more frequently localized on the face, neck and chest areas, involving more than 25 pustules that are described as small bumps filled with pus or fluid (9). Severe pustulocystic acne is described by the existence of nodules and

cysts localized on the groin, buttocks and axillary areas, and these cysts usually affect profounder skin tissue (9), (12).

Acne affects around 85% of the teenager population, reaching its acme during adulthood and despite of being considered a common cosmetic disorder among teenagers, it can affect life quality by influencing anxiety, depression and social withdrawal, leading to its indispensable treatment (13). Effective conventional acne treatments are available for topical or systemic use. The choice of treatment is not only made in consideration of acne severity, categorized as mild, moderate or severe, but also on the sort of acne lesion, severity of inflammation and body area involved (11).

Topical treatment is used as first choice in mild to moderate situations of acne. As topical treatments there are retinoids, benzoyl peroxide (BENP), azelaic acid (AZA), topical antibiotics and combined therapy. Topical retinoids such as adapalene (ADA), tretinoin (TRE) and isotretinoin (ITR), are used as first line therapy for primary acne lesions, by reversing the formation of microcomedones, decreasing the proliferation of keratinocytes and having an anti-inflammatory effect (14), (15), (16). Nevertheless, they are associated to some side effects, such as erythema, eczematous irritation, skin peeling, dryness and sunlight sensitivity (17), (18), (19). For inflammatory lesions, it is recommended the use of BENP since it has a bactericidal effect when applied on the inflamed lesions by releasing oxygen radicals. However, it can cause irritant dermatitis with dryness, erythema and peeling as side effects (11), (14). AZA has anti-inflammatory effect and reduces the formation of comedones, by regulating the differentiation of keratinocytes, so it can be used on inflamed areas. Still, it can cause some photosensitivity, skin irritation and redness (9), (11). Clindamycin (CLY), erythromycin (ERY) and tetracycline (TEY) are topical antibiotics used to treat acne. They are applied for mild to moderate acne, having a great anti-inflammatory impact and causing a big reduction in the number of *C. acnes*, though one of the biggest concerns is the increasing resistance of *C. acnes* to antibiotic therapies, besides this, they can cause skin irritation (14). To conclude this part of topical treatments for acne, it is also possible to use them as a combined therapy, towards a synergistic effect. It is common to associate TRE to CLY or ERY to produce a synergistic effect and it is shown that TRE promotes skin penetration of other topical agents used (19). Other combinations are topical antibiotics with BENP or retinoids, which are also frequent, because in this way an anti-inflammatory effect is also provided (11).

Systemic treatment is used in all severities of acne. As systemic therapies there are systemic antibiotics, hormonal therapies or retinoids. Systemic antibiotic treatment has a great anti-inflammatory impact and decreases the number of *C. acnes*. To prevent the increase of resistant *C. acnes*, antibiotics must be prescribed on the right dosage. The use of hormonal

treatments, such as, antiandrogen therapy or cyproterone acetate (CPA), can be convenient when there is resistance to other therapies. Oral contraceptives are widely used since they have shown an anti-acne effect by reducing the circulating androgens and they have revealed a direct consequence on reducing comedogenesis (11). Oral retinoid, TRE, is one of the most effective treatment for severe acne. TRE has a significant influence on sebum production reduction, some anti-inflammatory impact and declining of *C. acnes*. However, it is related to some serious side effects such as inflammatory bowel disease, depression and mucosa drying (14).

Nano-sized technological strategies have been investigated as alternatives to regular acne treatments. Their efficacy as controlled active ingredient delivery systems is better than most regarding the limitations of conventional administration systems such as low biodistribution, poor effectivity and toxicity (2). In addition, nanosystems are advantageous due to preserving the properties of entrapped active ingredients, having a great entrapment efficiency, promoting their penetration and delivering the active ingredients to the targeted areas without tissue damage. They also allow the accommodation of molecules with a large variety of solubility, accepting both lipophilic and hydrophilic active ingredients (12). So, according to nanosystems physico-chemical properties such as particle size (PS), entrapment efficiency (EE), zeta potential (ZP) and polydispersity index (PDI) they can be capable of permeate the skin releasing the active ingredients (3). Novel nanotechnology-based formulations against acne provide the delivery of the active ingredients to specific skin localizations, contributing to less side effects (17). Figure 3 is a schematic compilation of nanotechnology-based formulations for acne treatment that will be addressed bellow.

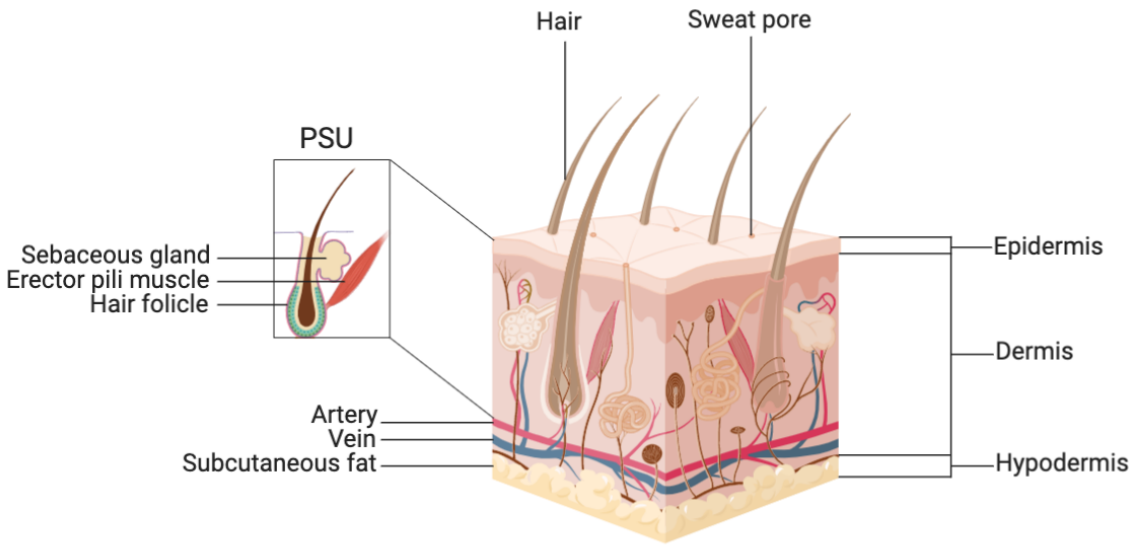

Figure 1 – Schematic illustration and localization of the pilosebaceous unit (PSU) in the skin.

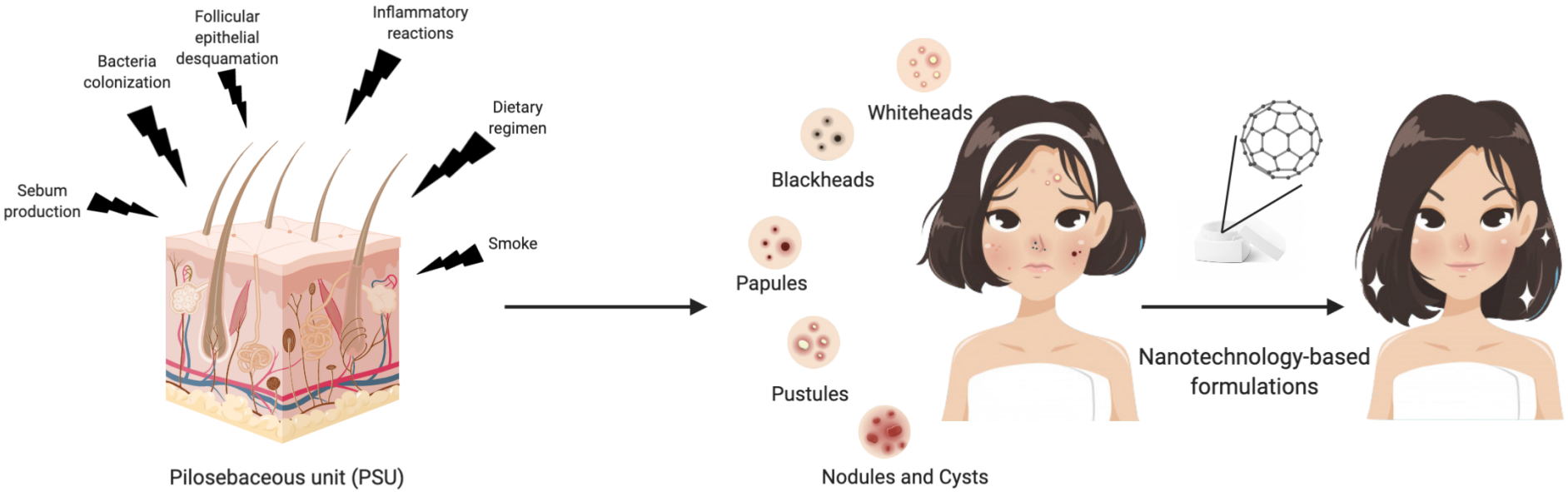

Figure 2 – Schematic illustration representing the factors that can lead to acne (increased sebum production, bacteria colonization, follicular epithelial desquamation, inflammatory reactions, dietary regimen and smoke), acne lesions (white heads, blackheads, papules, pustules, nodules and cysts) and the application of nanotechnology-based formulations for its treatment.

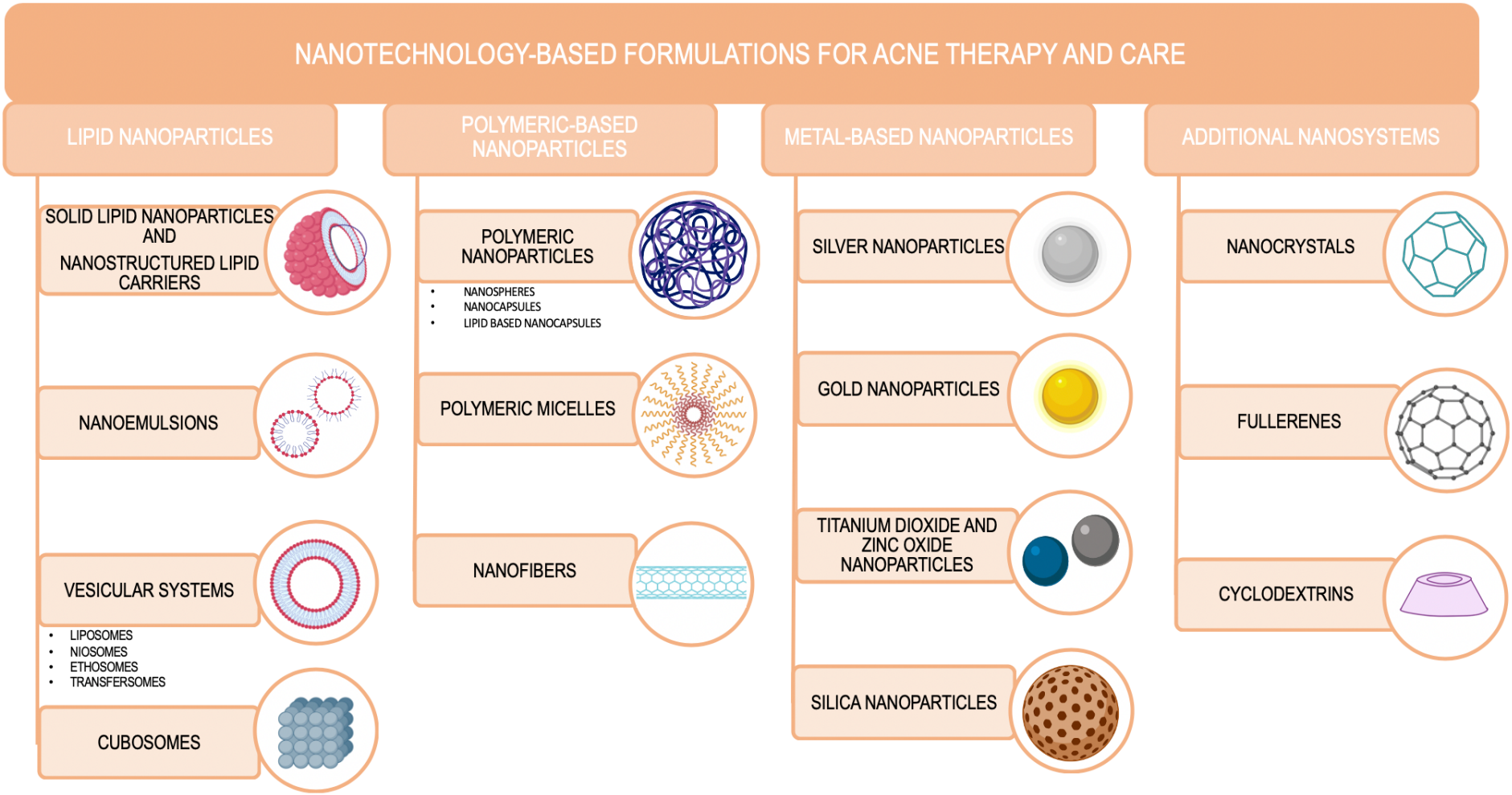

Figure 3 – Schematic compilation of nanotechnology-based formulation for acne treatment.

2. Novel Acne Technological Strategies: Nanosystems

Table 1 is a data compilation that englobes the physico-chemical properties as EE, PS, ZP and polydispersity index PDI of the nanotechnology-based formulations. Other studies such as active ingredient release, skin permeation, skin irritation and anti-acne activity, where the minimum inhibitory concentration (MIC) is used to measure the anti-bacterial activity, of the nanotechnology-based formulations are presented on the Table 2 and will be discussed ahead.

2.1. Lipid Nanoparticles

Lipid nanoparticles can be sorted according to their internal structure, as solid lipid nanoparticles (SLNs), as nanostructured lipid carriers (NLCs), nano-emulsions (NEs), vesicular systems and cubosomes (CUBs) (20). They have positive aspects as sustained active ingredients release during a larger period of time, prevention from active ingredients degradation, good tolerability and physico-chemical stability (21).

2.1.1. Solid Lipid Nanoparticles (SLNs)

SLNs were introduced in 1991, as an alternative to traditional colloidal carriers like emulsions, polymeric nanoparticles and vesicular nanosystems (22). They are sub-micron colloidal carriers with dimensions reaching from 50 to 1000 nm, composed by a biocompatible lipid core, containing lipids between 0.1-30% (w/w), that are solid at body and room temperatures and at the outer core by an amphiphilic surfactant, comprising surfactants between 0.5-5% (w/w) that have a stabilizing role (23), (24), (25). Lipids have an important function by defining the colloidal properties since they are the main constitutive material present in SLNs, contributing for their small size, controlled active ingredients releasing, good skin permeation and high active ingredients loading (21). The most common solid lipids used are free fatty acids, glycerol esters and waxes (21). SLNs have brought a new input toward skin diseases such as acne, due to its capacity to encapsulate not only polar but also non-polar therapeutic molecules (17).

Ridolfi *et al*., (2012) managed to improve SLNs properties as a carrier by the addition of chitosan to TRE loaded SLNs which has turned them statistically larger and has given them a higher polydisperse distribution. Also, they have demonstrated that low TRE concentrations in TRE loaded SLNs with chitosan inhibits acne causing bacteria, such as *C. acnes* and *S. aureus*, with a MIC of 40 μg/ml and 300 μg/ml respectively (26). In 2013, neem oil SLNs were developed by double emulsification (w/o/w), and the one using 100 mg of lecithin and 10 mg

of cholesterol as lipid associated to a 4% of Tween® 80 as surfactant showed less active ingredient release over time which is a great result for acne treatment during a prolonged period of time (22). In 2014 has been developed BENP loaded SLNs gel using Percinol® ATO 5 as solid lipid and chloroform as solvent to enhance the BENP dissolved in the gel, which when submitted to an *ex vivo* permeation study showed a low permeation rate which explains the increased skin deposition of active ingredient and the reduced skin irritation that is important for acne treatment. Also, BENP loaded SLNs gel demonstrated a robust antibacterial activity against acne causing bacteria such as *C. acnes* and *S. aureus*, having a MIC of 2.15 μg/ml and 2.51 μg/ml respectively due to the particle physico-chemical characteristics that provide them an increased surface area and a more controlled active ingredient release (16). *In vitro* permeation study of ADA loaded SLNs gel, performed in 2014, demonstrating a low systemic uptake of ADA by targeting the active ingredient to a specific location (9). Also, ADA loaded SLNs showed a good penetration into the stratum corneum (SC), a sustained release, a better skin targeting due to the small PS and the presence of soya lecithin which are important attributes for nanotechnology-based systems applied on acne treatment (9). Anti-acne potential of ITR loaded SLNs and of 0.1% ADA loaded SLNs gel were tested on acne-induced mice, showing not only a significant reduction in number of acne lesions but also less skin toxicity when compared to commercial formulation (27), (28). Additionally, *ex vivo* permeation study of dapsone (DAP) loaded SLNs demonstrated a higher permeation rate when compared to the commercial formulation, which is associated to their small size that increases their surface area (29).

2.1.2. Nanostructured Lipid Carriers (NLCs)

NLCs are very similar to SLNs, the difference between them is that NLCs have a liquid lipid content entrenched within the solid lipid matrix, allowing greater active ingredient loading and better stability compared to SLNs. This can be explained due to the fact that NLCs don´t allow recrystallization of solid lipids, and consequently the size during the storage time remains almost unaffected (20), (30), (21). The carriers are mostly composed by non-toxic and biodegradable blends of solid and liquid lipids and nanoparticles with a dimension reaching from 50 nm to 1000 nm, upon mixing solid and liquid lipids in a weight ratio ranging between 70:30 and 90:10 (23), (21). The active ingredient release mainly depends on NLCs composition, which has a lipid matrix that is less organized than SLNs and are composed by medium-chain triglycerides, triglycerides of caprylic and capric acid and/or oleic acid used as the liquid state lipid (10), (24). When compared to other nanocarriers they show many advantages not only

related to the scale up potential but also because NLCs have a low degradation rate of the entrapped active ingredients (23).

ITR loaded NLCs have shown a decreasing effect on the MIC of ITR due to a better interaction with the *C. acnes* cell wall provided by the increased contact time and sustained active ingredient release (4). The *in vitro* skin permeation flux of TRE loaded NLCs is achieved due to the use of isopropyl myristate (31). TRE and TEY loaded NLCs showed that TRE is mainly located in the inner core and since it has amphiphilic properties TRE provides NLCs a smaller size due to an emulsification effect. Also, TEY is an hydrophilic molecule and has the possibility to reside in the lipid/water interface (32). The EE of TRE and TEY loaded NLCs was high having an advantageous effect on reducing skin irritation. It was observed a *C. acnes* and *S. aureus* growth inhibition, with a MIC of 1.64 μg/ml for both, due to the antioxidant effect of TRE and the antibacterial activity of TEY (32). Cationic charged DAP loaded NLCs have been confirmed to be safer and to have a better topical active ingredient delivery because they have demonstrated a controlled pattern *in vitro* skin permeation (33). NLCs showed to be capable of limiting the systemic absorption of CPA preventing systemic adverse effects. Also CPA loaded NLCs with an average diameter of 300 nm are promising for treating androgenic skin diseases such as acne because *in vitro* follicular targeting showed that they are more capable of targeting hair follicles and sebaceous glands (34). The small size of clindamycin loaded NLCs allied to the lipid monolayer allows skin occlusion that benefits the prolonged active ingredient release (35). *In vitro* rat skin permeation studies of ADA loaded NLCs supported the importance of the lipid matrix used and that NLCs formulations reduce systemic uptake of ADA because the amount of ADA in the receptor chamber was lower when compared to the conventional gel (9). *In vivo* anti-acne tests of ADA loaded NLCs were performed on rats with testosterone induced acne showing that after 4 weeks of treatment papules and swelled sebaceous glands almost disappeared (9). Lacatusu *et al*., (2020) studied the synergic effect of plant extract and vegetable oils based NLCs, that can be applied for acne treatment. The lipid phase contained a 10% concentration of solid fats, vegetable oils as rosehip, black cumin and carrot extract or marigold extract. *In vitro* anti-inflammatory effect of ADA loaded NLCs containing carrot extract or marigold extract demonstrated that both had an anti-inflammatory effect that is related to the bioactive composition of black cumin oil, this effect was more pronounced on ADA loaded NLCs prepared with marigold extract. *In vitro* antibacterial effect was determined against *C. acnes* and *S. epidermidis* of ADA loaded NLCs containing carrot extract or marigold extract showing that both are very effective against *C. acnes* and *S. epidermidis* with a inhibition diameter of 17.50 mm and 20.00 mm respectively and that only ADA loaded NLCs containing marigold extract had a moderate activity against *S.*

epidermidis. Also, *in vivo* anti-acne potential was tested on humans by applying ADA loaded NLCs containing marigold extract during 28 consecutive days and demonstrated to have a significant decrease on sebum production rate and a drastic remission on non-inflammatory and inflammatory acne lesions (36).

2.1.3. Nano-emulsions (NEs)

NEs are composed by an oil phase, an aqueous phase and an active ingredient. The hydrophilic active ingredient is retained in the aqueous phase while oily active ingredients are kept in the oil phase. There are available both water in oil and oil in water formulations, but most of them are oil in water (23). NE have the advantage of an increased effect on solubility of the water-soluble active ingredients, by dispersing them on the oily phase (37).

Isopropyl myristate composed oil phase of DAP loaded NEs provided an increased *in vitro* skin permeation, because DAP has a partition coefficient of 1.32 that favours its dissolution in this oily phase. The oily regions of NEs have the ability to deliver the active ingredient to lipid regions on the skin (38). Also, ITR loaded NEs demonstrated a better active ingredient release when compared to the *in vitro* ITR release from the oily phase per se isolated, in this case coconut oil (39). Tween® 80 and Span® 80 were used as surfactants in ADA loaded tea tree oil NEs and showed the capacity to formulate the smallest droplets. ADA loaded tea tree oil NEs MIC was assessed *in vitro* against *C. acnes* and showed to be lower when compared to tea tree oil NEs, although ADA doesn´t have any antibacterial effect this can be explained by the interaction between NEs and the bacterial cells (40). ADA loaded tea tree oil NEs *in vivo* skin irritation tests were performed on rabbit skin and showed the absence of skin irritation nor erythema (40). *In vivo* clinical study were performed using TRE loaded NEs in 10 patients with mild to moderate acne lesions, during 6 weeks showing a significative reduction of inflammatory and non-inflammatory lesions (11).

2.1.4. Vesicular Nanosystems

2.1.4.1. Liposomes (LIPs)

LIPs are colloidal vesicular structures discovered in 1970 (41). They are composed by multiple layers that contain phospholipids and cholesterol. Cholesterol is frequently added to increase the stability of the bilayers (25). The phospholipids, such as phosphatidylethanolamine, phosphatidylserine and phosphatidylcholine form a layer or multiple layers, which are separated by aqueous phases and encompass an aqueous center. Due to these characteristics,

liposomes are successfully used to deliver both lipophilic and/or hydrophilic active ingredients (23), (21).

LIPs are characterized based on their size and number of layers. Multilamellar vesicles have a size that is superior than 0.5 μm, while small unilamellar vesicles have a size that ranges from 20 nm to 100 nm and large unilamellar vesicles have a size superior than 100 nm (25). They offer many advantages such as capability to be produced in a big scale, great biocompatibility, low toxicity and ability to incorporate both lipophilic and amphiphilic substances. But there are some disadvantages too, such as, their physical and chemical instability (23), (41).

The presence of cholesterol induces the bilayer to be more compact turning TRE loaded LIPs smaller and it also increases incorporation efficiency of TRE because of the cementing effects that cholesterol has on membrane packing and the increased hydrophobicity (42). The high ZP value of TRE loaded LIPs prevents LIPs from aggregate because of strong electrostatic repulsion interaction, being indicative of the LIPs stability (42). Also TRE loaded LIPs showed a remarkable anti-acne effect *in vivo* by showing a reduction of papules and comedones on the face, after 4 weeks treatment with TRE loaded LIPs (42). TEY is a positively charged molecule that when entrapped in LIPs with lecithin in the inner and outer surface of the LIP can lead to the positive ZP presented (43). *Ex vitro* skin penetration studies supported a higher skin penetration of TEY loaded LIPs when compared to TEY in aqueous solution, because TEY characteristics such as molecular weight and the partition coefficient are not compatible with skin penetration (43). Also, *ex vivo* TEY deposition studies suggested that TEY loaded LIPs can cause less systemic effects being there was less TEY passing to the receiver compartment of the Franz diffusion cell, supporting that LIPs are a good choice for anti-acne treatment when using antibiotics such as TEY (43). *In vitro* anti-bacterial studies were performed using TEY loaded LIPs and showed that LIPs can increase the penetration of antimicrobial agents such as TEY and reduce the MIC (43). Lauric acid (LAH) loaded LIPs can be stable at acid pH, avoiding the flocculation of the active ingredient and being an interesting characteristic for applying them on the hair follicle for acne treatment (44).

2.1.4.2. Niosomes (NIOs)

NIOs are vesicular structures that have been developed in the recent years (21). They can be either single or multilayer that comprehend non-ionic surfactants, cholesterol and charge inducing agents. Cholesterol is mostly used to promote the rigidity of the bilayer, non-ionic surfactants such as polyoxyethylene alkyl ethers and esters promote stability and prevent

chemical degradation, and charge inducing agents among which dicetyl phosphate and stearylamine, contribute to electrostatic repulsion and to increase the stability (41), (23). They are an encouraging active ingredients delivery option for acne skin disorders treatment because NIOs augment the topical effect time of active ingredients on the skin, reducing its systemic absorption. They also have some discommodities related to their physical instability, leading to sedimentation, aggregation and fusion (45).

In vitro active ingredient release of ERY loaded NIOs were performed on human cadaver skin and shows a sustained ERY release when compared to the conventional gel because of the NIOs "reservoir" effect caused by its constitution being ERY:cholesterol:surfactant, that can be useful for acne treatment (46).

2.1.4.3. Ethosomes (ETOs)

ETOs are phospholipidic vesicles composed of phosphatidylcholine, cholesterol, ethanol and water, described by the first time by Touitou *et al*., (2000) (10). The abundant amount of ethanol, 20-50%, an organic solvent, improves skin permeation, both ethanol and cholesterol contribute to their elastic properties (23), (21). ETOs are advantageous by having the faculty of carrying both lipophilic and/or hydrophilic active ingredients and by providing an efficient active ingredient delivery in both occlusive and non-occlusive conditions (21).

EE is a parameter that evaluates the delivery potential of cryptotanshinone (CRYP) from ETOs and in this case the greater retention of the active ingredient is attributed to the ethanol retention capacity, present in the ETOs core (47). *In vitro* skin permeation showed that CRYP loaded ETOs have a better skin permeation when compared to conventional gels, but the precise mechanism for the better skin permeation isn't yet clear, although ethanol is considered to have the major role in permeation because it interacts with lipid molecules in the polar head group region (47). Also *in vivo* anti-acne effect of CRYP loaded ETOs was tested in rabbits with oleic acid induced acne and showed that they all recovered from acne lesions (47).

2.1.4.4. Transfersomes (TRAs)

TRAs were introduced in the first years of 1990 by Ceve and Blaume in response to the necessity of creating a vesicular system with an increased permeation capacity (24). They are ultra-deformable vesicles composed by an aqueous core enclosed by a complex phospholipidic bilayer and an edge activator (10). Edge activators such as sodium deoxycholate, sodium cholate, Span® 60, Span® 65 and Span® 80, confer the bilayer´s the ability to deform, because

they have the capability to change the interfacial tension (21). Due to their composition, they offer many advantages such as the ability to encapsulate a variety of active ingredients, being more deformable and the capacity to pass through small sized pores (23). Besides this, hydrophobic active ingredient loading is still a hard task since they compromise the elasticity of TRAs (21).

Ex vitro skin penetration demonstrated that TEY loaded TRAs penetrate deeply through the skin layers, making TRAs good dermal carriers for acne treatment (43). *In vitro* anti-bacterial activity showed a reduction on MIC against *S. epidermidis* when compared to TEY aqueous solution, because TRAs favours TEY penetration into bacteria (43).

2.1.5. Cubosomes (CUBs)

CUBs comprise rounded bicontinuous lipid bilayers that are arranged in a three-dimensional structure, ranging between 10-500 nm in diameter. They are self-assembled liquid crystalline particles composed by surfactants and lipids with the appropriate relation of water. CUBs are thermodynamically stable and they can carry not only hydrophilic but also hydrophobic active ingredients. CUBs are nanostructures that constitute a novel alternative for acne treatment by minimizing side effects and preserving active ingredient efficacy. They have also the capacity of reducing the toxicity of encapsulated active ingredient and dose related side effects (48).

In 2018, studies concluded that TRE loaded CUBs have a sustained active ingredient release which is favourable for acne treatment, allied to TRE lipophilic properties which provide a better depository effect and retention properties (49). Also, in 2018 were obtained ERY loaded CUBs with an EE at about 95.29% and an average PS of 264.50 nm. *In vitro* active ingredient release was performed through a cellophane membrane and showed a cumulative percentage of 88.64%, after 24 hours and 91.55%, after 36 hours. They concluded that ERY loaded CUBs have a sustained release, which can easily penetrate into the skin and increase the active ingredient retention time, which is positive for acne treatment (48).

2.2. Polymeric-based Nanoparticles

Polymeric-based nanoparticles such as polymeric nanoparticles, polymeric micelles and nanofibers, have been investigated for acne treatment and earned a lot of attention because they demonstrate higher physical stability and relatively smaller size when compared to lipid nanocarriers (10).

2.2.1. Polymeric Nanoparticles

Polymeric nanoparticles englobe nanospheres (NSPs), nanocapsules (NCAs) and lipid based nanocapsules (LBNCAs), with sizes that range between 20 and 1500 nm. NSPs are matrix particles in which are adsorbed lipophilic active ingredients at the surface. NCAs are vesicular systems that have a case layered with solid material, in which hydrophilic active ingredients are encased in the aqueous core. LBNCAs are nanocapsules that also have a case layered with solid material, but within which lipophilic active ingredients are encased in an oily core (10).

Desaminotyrosyl-tyrosine octyl ester suberate copolymer was used to form micellar-like ADA loaded tyrosine-derived NSPs, in aqueous media, with spherical shape due to its amphiphilic nature (50). *In vitro* skin irritation of ADA loaded tyrosine derived NSPs were tested using human immortalized keratinocyte (HaCaT) cells showing that these cells had a higher metabolic activity, resulting in a decreased irritation potential. *In vitro* skin irritation of ADA loaded tyrosine derived NSPs were also performed on a three dimensional epidermal skin model which demonstrated to have no irritant potential due to the less secretion of pro-inflammatory cytokines when ADA loaded tyrosine-derived NSPs were applied during 24 hours (50).

2.2.2. Polymeric Micelles (POLMs)

POLMs are self-assembled systems with core-shell structures forming amphiphilic copolymers at a specific concentration, known as critical micelle concentration, in order to decrease surface-free energy in an aqueous solution. They have gained particular consideration due to their high solubility of lipophilic active ingredients and changeable physico-chemical properties (10).

BENP loaded POLMs in which acetonitrile was used as organic phase were selected for *in vitro* skin permeation study because they have shown to be more stable. *In vitro* skin permeation tests of BENP loaded POLMs were performed with porcine skin and demonstrated to be efficient when compared to the commercial gel, because POLMs enhances the skin penetration of lipophilic active ingredients such as BENP, that will be optimal for acne treatment (51).

2.2.3. Nanofibers (NFs)

NFs have gained attention in recent years due to their mechanical properties, controllable pore size and flexibility that can be advantageous for acne treatment. They have normally a diameter that ranges from 50 to 100 nm (52).

In vitro active ingredient release was performed using the total immersion method showing that TRE and ERY loaded NFs achieved an active ingredient release of 76.00% after 100 hours of its application (53). TRE have shown to enhance antibiotics, as ERY activity *in vivo* by inhibition of trans-glutaminase activity, which reduces cellular adhesion and follicular plugging and leads to drainage of excess sebum and bacteria. Also TRE may help to create a more aerobic condition which hinders the growth of anaerobic bacteria like *C. acnes* (53).

2.3. Metal-based Nanoparticles

Metal-based nanoparticles such as gold, silver, titanium oxide or zinc oxide, can bring some benefits to anti-acne treatment among which the improvement of biological performance, a good active ingredient loading and delivery capacity, skin lesions targeting and systemic invasion prevention (54).

2.3.1. Silver Nanoparticles (AgNPs)

AgNPs are characterized by having a great electrical conductivity, advantageous optical assets, good biological and thermal properties due to their remarkable physico-chemical qualities. They are largely used as antibacterial agents due to its morphology (55).

In vitro antimicrobial activity of AgNPs biosynthesized from *Lawsonia inermis* (*L. inermis*) extract was evaluated against *C. acnes* showing to be the most sensitive when compared to the other bacteria that were also studied and having a MIC of 8 μg/ml demonstrating that *L. inermis* mediated synthesis of AgNPs have a maximum activity against *C. acnes* (56). Also, AgNPs biosynthesized from *Pinus densiflora* demonstrated a moderated *in vitro* antibacterial activity against *C. acnes* and *S. epidermidis* showing a MIC of 10 μg/ml and 40 μg/ml respectively (57). *In vitro* anti-acne activity of AgNPs biosynthesized from *Coriandrum sativum* leaf extract was tested against *C. acnes* demonstrating a MIC of 3.1 μg/ml, despite the mechanism of antibacterial activity of AgNP not being clear yet there is literature that establishes the adsorption of silver cation (Ag^+) to the cell wall as an explanation (18). *In vitro* antibacterial activity of AgNPs biosynthesized from *Citrus maxima* (*C. maxima*) fruit, leaf and peel extracts, showing a MIC for *C. acnes* of 85 μg/ml with the fruit extract, 155 μg/ml with the leaf extract and 150 μg/ml with the peel extract. So, AgNPs biosynthesized from *C. maxima* fruit extract

showed the highest antibacterial activity against *C. acnes* (58). *In vitro* antibacterial activity of AgNPs biosynthesized from *Salvia miltiorrhiza* was performed against *S. aureus*, *S. epidermidis* and *C. acnes* showing that the AgNPs biosynthesized with blue LED light had a maximum inhibition zone of 9 mm against *C. acnes* when compared to the ones byosintetized with red, green and white LED lights (59). *In vitro* antibacterial activity of AgNPs biosynthesized from *Bacillus nakamurai* against *C. acnes* showed a MIC of 10 μg/ml (60).

2.3.2. Gold Nanoparticles (AuNPs) and Gold Nanorods (AuNRs)

AuNPs are extensively investigated due to the different advantages they offer for skin and follicular active ingredient delivery, diagnostic and therapeutic use (54). AuNRs are gold nanoparticles with non-spherical shape that are acquiring particular attention due to their antibacterial potentials. Despite these attractive attributes, AuNRs have high propensity to aggregate within the presence of high salts and biomolecules (61).

In vitro antibacterial activity was performed with cystamine AuNR showed to have a MIC of 210 μg/ml against *C. acnes* and of 800 μg/ml against *S. aureus* (61).

2.3.3. Titanium Dioxide Nanoparticles (TiO_2NPs) and Zinc Oxide Nanoparticles (ZnONPs)

Metal oxide nanoparticles, particularly ZnONPs and TiO_2NPs, have received a lot of attention and are being studied for anti-acne products as they possess distinctive properties like being less toxic when compared to AgNPs, larger surface area per mass unit that gives them a strong catalytic activity, diverse morphology, antibacterial activity and long-term thermodynamic stability (62-66).

2.3.4. Silica Nanoparticles (SiNPs)

SiNPs are also known as silicon dioxide (SiO_2) nanoparticles and have been investigated for active ingredient delivery systems because they have physico-chemical parameters that can be modified for specific applications such as acne treatment (54). Silica-gold nanoshells with a spherical form were studied for acne treatment. They contain a 120 nm silica core with a gold shell which give them a total PS of 150 nm. *Ex vivo* delivery of these nanoparticles was performed using an arrangement comprising a Franz cell and porcine ear skin showing that the nanoshells size is good for skin penetration. Human clinical studies were also conducted *in vivo* showing safety of ultrasound and laser exposure and acne improvement (67).

2.4. Additional Nanosystems

2.4.1. Nanocrystals (NCRYs)

NCRYs have been receiving significant attention in acne employment justified by its great aqueous active ingredient saturation solubility and good dissolution rate because of its extensive surface area. Other interesting qualities are not only the raised concentration gradient achieved between the epidermis and the formulation with nanocrystals, but also the great adhesion they have to the skin. Commonly NCRYs are produced as nanosuspensions in water where polymers and/or ionic or non-ionic surfactants are used as stabilizing agents and hydrogels are commonly used as useful vehicles. NCRY suspensions can be turned into solid NCRYs using methods such as spray-drying or freeze-drying (68).

In vitro active ingredient release of AZA loaded NCRY showed to be ten times higher when compared to the conventional formulation 20% AZA cream for the same period of time leading to an improved bioavailability of AZA (68). *Ex vivo* follicular penetration study was performed on pig ear skin and demonstrated possible to target BENP to the hair follicle, which is useful in acne treatment (69).

2.4.2. Fullerenes (FULLs)

FULLs are spherical carbon molecules discovered in 1985 (70). They have a favourable configuration that allows entrapping the active ingredient followed by their release on the targeted places in the epidermis (15). Polyhydroxylated FULLs tested in hamster sebocytes have demonstrated inhibitory effects *in vitro* against *C. acnes* and reduced sebum production as well. *In vivo* studies performed on 11 patients with facial acne lesions, using 0.1% lipo-FULL gel, twice a day for 8 weeks have shown a relevant reduction on acne lesions (71), (70). In conclusion, FULLs have demonstrated capacity to reduce *C. acnes* lipase activity and suppress sebum production in the inflammatory state, turning them into an interesting nanosystem to control acne (15).

2.4.3. Cyclodextrins (CDs)

CDs are cyclic oligosaccharides that are comprised of 6, 7 or 8 glucopyranose units, that attributes them a hydrophobic core and a hydrophilic outer surface, which provides them an aqueous solubility (72). CDs allow active ingredient complexation in order to increase its solubility controlling its release, minimizing its degradation and contributing for its stability (73). ITR-hydroxypropyl-beta-CD and TRE-dimethyl-beta-CD can be applied for acne treatment (72), (73). A skin permeation study of ITR-hydroxypropyl-beta-CD was performed

in vitro using Franz diffusion cell method and albino abdomen rat skin as animal model, showing a transdermal flux of 13.9 μg/cm^2/h. Also, skin irritation studies were conducted using male albino rabbits as animal model and showed an absence of erythema and less skin irritation when compared to free ITR applications it was concluded that ITR-hydroxypropyl β loaded CDs can be used for topical delivery and has an easy preparation method (73).

Table I – Physico-chemical properties of nanotechnology-based formulations for acne treatment.

Active Ingredient	NBF	Preparation Method	EE (%)	PS (nm)	ZP (mV)	PDI	Ref.
ADA	SLN	Solvent injection	89.90	148.30	-12.00	0.17	(9)
ADA	SLN	Hot melt homogenization	>85.00	102.00	-12.40	0.18	(28)
BENP	SLN	Solvent evaporation	26.65 to 64.61	246.00 to 783.00	-5.21 to -7.66	0.17 to 0.28	(16)
DAP	SLN	Microemulsion followed by sonication	87.40 to 95.60	168.50	-16.70	0.34	(29)
ITR	SLN	Microemulsification	58.92 to 98.99	57.70 to 300.00	-22.40	0.12 to 0.50	(27)
Neem oil	SLN	Double emulsification	82.10	221.60	NA	0.95	(22)
TRE	SLN	Hot high pressure homogenization	99.60	284.80	55.90	0.38	(26)
ADA	NLC	Hot high pressure homogenization	87.29	268.30	-16.35	0.22	(13)
AZA	NLC	Melt-emulsification and high pressure homogenization	93.48	139.10	-58.40	0.11	(36)
CLY	NLC	Melt-emulsification followed by sonication	80.00	136.00	-41.90	0.26	(35)
CPA	NLC	Solven diffusion evaporation	99.03	100.00	-35.00	NA	(34)
DAP	NLC	Emulsification followed by sonication	76.50 to 91.10	106.20 to 151.30	NA	NA	(33)
ITR	NLC	Microemulsification	78.60	80.00	-15.00	NA	(4)
TRE	NLC	Thin-film hydration	92.13	79.50	-23.50	NA	(31)
TRE and TEY	NLC	Hot homogenization followed by sonication	76.19	213.40	-8.00	NA	(32)

Table 1 – Physico-chemical properties of nanotechnology-based formulations for acne treatment. (Continuation)

Active Ingredient	NBF	Preparation Method	EE (%)	PS (nm)	ZP (mV)	PDI	Ref.
ADA	NE	Spontaneous emulsification	NA	105.00	0.07	0.20	(40)
DAP	NE	Homogenization followed by sonication	NA	11.90	NA	< 1.00	(38)
ITR	NE	Nanoemulsification	NA	21.00	NA	0.35	(39)
TRE	NE	Emulsification and high pressure homogenization	NA	116.20	-47.10	0.11	(11)
ITR	LIP	Thin-film hydration	90.10	100.00 to 200.00	NA	NA	(73)
LAH	LIP	Thin-film hydration and extrusion	NA	100.00	NA	< 0.2	(44)
TRE	LIP	Thin-film hydration	73.40	318.00 to 485.50	-41.20	0.43- 0.67	(42)
TEY	LIP	Thin film hydration	44.90	78.00	17.20	< 0.3	(43)
ERY	NIO	Thin film hydration	56.60 to 86.40	3.40 to 6.90	NA	NA	(46)
CRYP	ETO	Homogenization followed by water-bath sonication	9.10 to 40.30	69.10 to 82.90	NA	< 0.30	(47)
TEY	TRA	Thin film hydration	54.60	0.28	7.53	< 0.3	(43)
TRE	CUB	Emulsification	95.29	264.50	NA	NA	(48)
ADA	NSP	Self-assembly	27.90 to 69.30	64.70 to 81.60	NA	0.14 to 0.19	(50)
BENP	POLM	Thin film hydration	81.94	25.30	-2.00 to -13.00	0.30	(51)
TRE and ERY	NF	Electrospinning	89.00 to 99.00	64.67 to 80.70	NA	NA	(53)
NA	AgNP	Biosynthesized from *B. nakamurai*	NA	379.20	NA	NA	(60)
NA	AgNP	Biosynthesized from *L. inermis* leaf extract	NA	6.00 to 72.00	-32.80	NA	(56)

Table 1 – Physico-chemical properties of nanotechnology-based formulations for acne treatment. (Continuation)

Active Ingredient	NBF	Preparation Method	EE (%)	PS (nm)	ZP (mV)	PDI	Ref.
NA	AgNP	Biosynthesized from *C. sativum* leaf extract	NA	37.00	NA	NA	(18)
NA	AgNP	Biosynthesized from *C. maxima* fruit extract	NA	121.00	-17.80	NA	(58)
NA	AgNP	Biosynthesized from *C. maxima* leaf extract	NA	396.00	-15.40	NA	(58)
NA	AgNP	Biosynthesized from *C. maxima* peel extract	NA	243.10	-14.40	NA	(58)
NA	AgNP	Biosynthesized from *S. miltiorrhiza*	NA	10.00 to 80.00	NA	NA	(59)
NA	AgNP	Biosynthesized from *P. densiflora* cone extract	NA	40.00 to 70.00	NA	NA	(57)
NA	AuNR	Seed-mediated surfactant-assisted wet-chemical	NA	49.50	+26.00	NA	(61)
AZA	NCRY	Wet media milling technique	NA	59.20	NA	0.14	(68)
BENP	NCRY	Film hydration	NA	200.00	NA	< 0.2	(74)

ADA - Adapalene; AgNP - Silver Nanoparticle; AuNR - Gold Nanorod; AZA - Azelaic Acid; BENP - Benzoyl Peroxide; CLY - Clindamycin; CPA - Cyproterone Acetate; CRYP - Cryptotanshinone; CUB - Cubosome; DAP - Dapsone; EE - Entrapment Efficiency; ERY - Erythromycin; ETO - Ethosome; ITR - Isotretinoin; LAH - Lauric Acid; LIP - Liposome; N_2 - Nitrogen; NA - Non-Applicable; NBF - Nanotechnology-based Formulation; NCRY - Nanocrystal; NE - Nano-emulsion; NF - Nanofiber; NIO - Niosome; NLC - Nanostructured Lipid Carrier; PDI - Polydispersity Index; POLM - Polymeric Micelle; PS - Particle Size; SLN - Solid Lipid Nanoparticle; TEY - Tetracycline; TRE - Tretinoin; TRA - Transfersome; ZP - Zeta Potential.

B. nakamurai - Bacillus nakamurai; *C. maxima - Citrus maxima*; *C. sativum - Coriandrum sativum*; L. *inermis - Lawsonia inermis*; *P. densiflora - Pinus densiflora*; *S. miltiorrhiza - Salvia miltiorrhiza.*

Table 2 – Biological effects of nanotechnology-based formulations for acne treatment. Most of the formulations were characterized for active ingredient *in vitro* release and skin uptake and permeation properties but main focus was on their skin irritation and antibacterial activity.

Active Ingredient	NBF	Study Objective	Studies						Ref.
			In vitro release	Skin Permeation		Skin Irritation		Anti-Acne Activity	
			(%)	Testing Method	(%)	Testing Method	(%)		
ADA	SLN	Skin uptake and permeation	16.82	*Ex vivo*	76.00[a]	NA	NA	NA	(9)
ADA	SLN	Release and tolerability on skin	30.00	*In vitro*	NA	*In vitro*	DC	< Papules density	(28)
BENP	SLN	Antibacterial activity	28.21	*Ex vivo*	41.10[b]	*In vitro*	DC	2.15[c] and 2.51[e]	(16)
DAP	SLN	Skin uptake and permeation	61.04	*Ex vivo*	39.27[a]	NA	NA	NA	(29)
ITR	SLN	Skin permeation	NA	*In vitro*	8.85 to 27.29[a]	NA	NA	< Papules density	(27)
Neem oil	SLN	Release	52.54	NA	NA	NA	NA	NA	(22)
TRE	SLN	Antibacterial activity	NA	NA	NA	NA	NA	40.00[c] and 300.00[e]	(26)
ADA	NLC	Release	40.32	*In vitro*	NC	NA	NA	< Papules density	(13)
AZA	NLC	Release and anti-inflammatory activity	54.00	NA	NA	*In vitro, In vivo*	DC	Comedolytic and sebo-regulating	(36)
CLY	NLC	Skin uptake and permeation	72.23	*In vitro*	2.16[a]	NA	NA	NA	(35)
CPA	NLC	Release and skin permeation	50.00	*Ex vivo*	NA -[b]	NA	NA	NA	(34)
DAP	NLC	Skin permeation and safety	96.00	*Ex vivo*	5.30[a]	*In vitro*	ND	NA	(33)

Table 2 – Biological effects of nanotechnology-based formulations for acne treatment. Most of the formulations were characterized for active ingredient *in vitro* release and skin uptake and permeation properties but main focus was on their skin irritation and antibacterial activity. (Continuation)

Active Ingredient	NBF	Study Objective	Studies						Ref.
			In vitro release	Skin Permeation		Skin Irritation		Anti-Acne Activity	
			(%)	Testing Method	(%)	Testing Method	(%)		
ITR	NLC	Skin permeation and antibacterial activity	NA	*In vitro*	14.39[a]	NA	NA	62.50[c]	(4)
TRE	NLC	Modulate active ingredient irritation potential	NA	*In vitro*	15.48[a]	*In vitro*	ND	NA	(31)
TRE and TEY	NLC	Multiple active ingredient therapy	NA	*In vitro*	< 0.1[a]	NA	NA	1.64[c, e]	(32)
ADA	NE	Skin tolerability and antibacterial activity	17.21	*Ex vivo*	NA	*In vivo*	ND	700.00[c]	(40)
DAP	NE	Skin uptake and permeation	NC	*In vitro*	59.74[a]	NA	NA	NA	(38)
ITR	NE	Release	10.39	NA	NA	NA	NA	NA	(39)
TRE	NE	Stablility and effectiveness of anti-acne activity	NA	NA	NA	*In vivo*	ND	Comedolytic, < papule density	(11)
ITR	LIP	Skin permeation and irritation	NA	*In vitro*	13.90[a]	*In vitro*	ND	NA	(73)
LAH	LIP	Skin permeation	NA	*In vitro*	0.3[a]	NA	NA	NA	(44)
TRE	LIP	Skin tolerability	55.00	NA	NA	*In vivo*	DC	Comedolytic, < papule density	(42)
TEY	LIP	Skin permeation and antibacterial activity	21.60	*Ex vivo*	0.9[b]	NA	NA	89.20[d]	(43)

Table 2 – Biological effects of nanotechnology-based formulations for acne treatment. Most of the formulations were characterized for active ingredient *in vitro* release and skin uptake and permeation properties but main focus was on their skin irritation and antibacterial activity. (Continuation)

Active Ingredient	NBF	Study Objective	Studies						Ref.
			In vitro release	Skin Permeation		Skin Irritation		Anti-Acne Activity	
			(%)	Testing Method	(%)	Testing Method	(%)		
ERY	NIO	Skin permeation	NA	*In vitro*	1.23[a]	NA	NA	NA	(46)
CRYP	ETO	Skin permeation and tolerability	NA	*In vitro*	0.019[a]	*In vivo*	ND	< inflammation of PSU	(47)
TEY	TRA	Skin permeation and antibacterial activity	54.00	*Ex vivo*	0.6[b]	NA	NA	89.20[d]	(43)
TRE	CUB	Skin permeation and antibacterial activity	88.64	NA	NA	NA	NA	NA	(48)
ADA	NSP	Skin tolerability	NA	NA	NA	*In vitro*	DC	Comedolytic	(50)
BENP	POLM	Skin permeation	NA	*In vitro*	0.15[a]	NA	NA	NA	(51)
TRE and ERY	NF	Anti-acne effect of the formulation	76.00	NA	NA	NA	NA	Antibacterial activity	(53)
NA	AgNP	Antibacterial activity	NA	NA	NA	NA	NA	0,10[c, e]	(60)
NA	AgNP	Antibacterial activity	NA	NA	NA	NA	NA	8.00[c]	(56)
NA	AgNP	Antibacterial activity	NA	NA	NA	NA	NA	3.10[c]	(18)
NA	AgNP	Antibacterial activity	NA	NA	NA	NA	NA	85.00[c], 25.00[e] and 90.00[d]	(58)

Table 2 – Biological effects of nanotechnology-based formulations for acne treatment. Most of the formulations were characterized for active ingredient in vitro release and skin uptake and permeation properties but main focus was on their skin irritation and antibacterial activity. (Continuation)

Active Ingredient	NBF	Study Objective	Studies						Ref.
			In vitro release	Skin Permeation		Skin Irritation		Anti-Acne Activity	
			(%)	Testing Method	(%)	Testing Method	(%)		
NA	AgNP	Antibacterial activity	NA	NA	NA	NA	NA	155.00[c], 55.00[e] and 150.00[d]	(58)
NA	AgNP	Antibacterial activity	NA	NA	NA	NA	NA	150[c], 45[e]and 125[d]	(58)
NA	AgNP	Antibacterial activity	NA	NA	NA	NA	NA	200[c]	(59)
NA	AgNP	Antibacterial activity	NA	NA	NA	NA	NA	10[c]and 40[d]	(57)
NA	AuNR	Antibacterial activity	NA	NA	NA	NA	NA	210[c]	(61)
AZA	NCRY	Antibacterial activity	59.00	NA	NA	NA	NA	NA	(68)
BENP	NCRY	NA	NA	NA	NA	NA	NA	NA	(74)

ADA - Adapalene; AgNP - Silver Nanoparticle; AuNR - Gold Nanorod; AZA - Azelaic Acid; BENP - Benzoyl Peroxide; CLY - Clindamycin; CPA - Cyproterone Acetate; CRYP - Cryptotanshinone; CUB - Cubosome; DAP - Dapsone; DC - Decreased; EE - Entrapment Efficiency; ERY - Erythromycin; ETO - Ethosome; ITR - Isotretinoin; LAH - Lauric Acid; LIP - Liposome; MIC - Minimum Inhibitory Concentration; NA - Non-Applicable; NBF - Nanotechnology-based Formulation; NC - Inconclusive; NCRY - Nanocrystal; ND - Not Detected; NE - Nano-emulsion; NF - Nanofiber; NIO - Niosome; NLC - Nanostructured Lipid Carrier; PDI - Polydispersity Index; POLM - Polymeric Micelle; PS - Particle Size; SLN - Solid Lipid Nanoparticle; TEY - Tetracycline; TRE - Tretinoin; TRA - Transfersome.

a -1% represents 1μg/cm^2/h; b - Percentages related to the reference values used in the referenced article; c - MIC in μg/ml for *C. acnes*; d - MIC in μg/ml for *S. epidermidis*; e - MIC in μg/ml for S. aureus.

Table 3 – Characteristics of nanotechnology-based formulations as delivery systems for anti-acne ingredients with regard to encapsulated active ingredients' release profile, skin penetration/targeting, activity and toxicity. Nanotechnology-based formulations are generically referred as nanoparticles (NPs).

NP	Active Ingredient Release	Skin Permeation/Penetration and Follicular Targeting	Anti-Acne Activity	Toxicity
SLN	- Burst release of ADA followed by a slower and sustained release (9), (28); - BENP release exhibited a controlled release pattern up to 24 hours (16); - Neem oil release followed a zero order release kinetics (22).	- Zero order release kinetics for ADA (9); - Higher skin permeation of DAP (29); - ADA NPs demonstrated minimal penetration across epidermis (9).	- ADA NPs reduced severe acne lesions, after 3 weeks of treatment (28); - BENP NPs exhibited the strongest antibacterial activity against all the species associated with acne (22).	- ADA NPs decreased skin irritation (28); - After 24 hours BENP NPs showed no signs of erythema** (16).
NLC	- Burst effect release followed by a sustained release pattern of ADA (13); - CLY NPs showed a burst release followed by sustained release up to 24 hours (35); - CPA release from NPs was significantly slower* (34); - Higher release rate of DAP followed by a steady release phase (33).	- Higher skin targeting and permeation of ADA from NPs (13); - ADA NPs did not penetrate skin (13); - NPs enhanced permeation and targeting properties to the skin (35); - CLY skin permeation 1.6 fold higher** (35); - Higher level of DAP permeated**(33); - NPs with less than 200 nm improved skin penetration of DAP (33); - NPs increased skin permeation of TEY (32); - 100-300 nm NPs with CPA could target hair follicles and sebaceous glands but 600 nm NPs had most dermal-epidermal penetration (34).	- ADA NPs decreased the papule density by the fourth week of treatment (13); - CLY NPs penetrated to the dermal-epidermal layer required for the effective treatment of acne (35);	- No irritation or inflammation in the area of application (33).
NE	- Lower cumulative release of ADA NPs (40).	- In ADA loaded tea tree oil NPs ADA penetrated through epidermis and it was confined in the dermis (40); - The presence of oil and surfactants increased NPs the penetration through SC (40).	- Tea tree oil NPs improved antibacterial activity against *C. acnes* (40).	- No skin irritation and erythema (40).

Table 3 – Characteristics of nanotechnology-based formulations as delivery systems for anti-acne ingredients with regard to encapsulated active ingredients' release profile, skin penetration/targeting, activity and toxicity. Nanotechnology-based formulations are generically referred as nanoparticles (NPs). (Continuation)

NP	Active Ingredient Release	Skin Permeation/Penetration and Follicular Targeting	Anti-Acne Activity	Toxicity
LIP	- Percentage of TEY from NPs formulation was higher*** (43).	- Low relative amount of LAH reaching the receptor fluid in the time selected. Retention of LAH in the SC, hair follicle, dermis, and epidermis were considered to represent cutaneous permeation (44); - Low skin permeation of LAH (44); - Higher skin penetration of NPs TEY*** (43); - Deposition and skin targeting effect of ITR NPs (73).	- ITR loaded NPs are likely improve the penetration of antimicrobial agents into bacteria thus decreasing the MIC value (43).	- Decreased skin irritation of encapsulated ITR (73).
NIO	- NPs followed Higuchi´s diffusion controlled model (46).	- NPs improved ERY permeation across the skin (46).	NA	NA
ETO	NA	- The cumulative permeation and transdermal flux increased 2.5 times with NPs (47).	- CRYP NPs used to treat acne lesions lead to the recovery of the normal skin structure (47).	- NPs loaded CRYP showed no signs of erythema or edema (47).
TRA	- Lower TEY release from TRAs*** (43).	- TEY in the aqueous solution permeated lower*** (43); - Higher penetration of NPs loaded TEY*** (43); - Lower amount of TEY in receptor phase of Franz cell from NPs indicated lower systemic absorption potential (43).	- MIC value decreased (43).	NA
CUB	- The cumulative percentage of ERY NPs was 89 after 24 hours and 92 after 36 hours (48).	NA	- Higher antibacterial activity of ERY NPs (48).	- NPs reduced the toxicity of encapsulated ERY and the dose related side effects (48).
NSP	NA	NA	- ADA NPs reduced the size of lesions (50).	- NPs decreased skin irritation (50).

Table 3 – Characteristics of nanotechnology-based formulations as delivery systems for anti-acne ingredients with regard to encapsulated active ingredients' release profile, skin penetration/targeting, activity and toxicity. Nanotechnology-based formulations are generically referred as nanoparticles (NPs). (Continuation)

NP	Active Ingredient Release	Skin Permeation/Penetration and Follicular Targeting	Anti-Acne Activity	Toxicity
POLM	NA	- Higher deposition of BENP in the skin** (51); - Higher BENP delivery efficiency into PSU (51).	NA	- BENP NPs demostrated any cytotoxicity (51).
NF	- First 3-hour phase with 38% of total release followed by a gradual TRE release over the next 4 days (53).	NA	- TRE created a more aerobic condition which hinders the growth of anaerobic bacteria like *C. acnes* (53).	NA
AgNP	NA	- 0.002-0.02 ppm concentration of NPs penetrate (60).	-Efficacy even at the lowest NPs concentrations (60); -NPs inhibited the acne causing bacteria (59); - NPs showed moderate antibacterial activity against odor and skin infection causing bacteria (57).	- 0.002 to 0.02 ppm concentration of NPs didn't show cytotoxicity (60).
AuNP	NA	NA	- NPs revealed bacteriostatic and bactericidal activity against *S. aureus* and *C. acnes* (61).	NA
NCRY	- Lag phase of AZA followed by a mathematical relationship between the AZA released per unit area time (68).	NA	NA	NA

ADA - Adapalene; AgNP - Silver Nanoparticle; AuNP - Gold Nanoparticle; AZA - Azelaic Acid; BENP - Benzoyl Peroxide; CLY - Clyndamicine; CPA - Cyproterone Acetate; CRYP - Cryptotanshinone; CUB - Cubosome; DAP - Dapsone; ERY - Erythromycin; ETO - Ethosome; ITR - Isotretinoin; LAH - Lauric Acid; LIP - Liposome; NCRY - Nanocrystal; NE - Nano-emulsion; NF - Nanofiber; NLC - Nanostructured Lipid Carrier; NIO - Niosome; NSP - Nanosphere; POLM - Polymeric Micelle; PSU - Pilosebaceous Unit; SC - Stratum Corneum; SLN - Solid Lipid Nanoparticle; TEY - Tetracycline; TRA - Transfersome; TRE - Tretinoin.

* - Compared to free active ingredient; ** - Compared to the commercial formulation; *** - Compared to another NP.

3. Characteristics of nanotechnology-based formulations as delivery systems for anti-acne ingredients

There have been several efficient nanotechnology-based formulations that have been proved for treatment of acne. A systematic comparison of available results associated with release, skin permeation/penetration, toxicity and anti-acne activity of nanoparticles loaded with the afore mentioned active ingredients is shown in Table 3. However, it should be emphasized that details pertaining to used methodologies are not the scope of this review.

ADA loaded SLNs prepared by hot homogenization revealed an initial burst effect followed by a sustained and continuous active ingredient release (9), (28). The SLNs loaded with BENP have shown differences from the marketed formulation, showing a controlled realease up to 24 hours (16). Neem oil realease SLN has shown to follow a zero order kinetics (22). The *in vitro* skin permeation studies revealed minimal penetration across epidermis and low potential of systemic uptake of ADA. SLN of ITR optimized by quality by design (QbD) approach were able to transport ITR to several skin layers effectively and showed an active ingredient reservoir effect (27). These SLN revealed an anti-acne potential and favorable tolerability profile on mouse skin when compared with the marketed product (27). Preclinical findings must be extrapolated to human models so their clinical utility is corroborated. TRE was encapsulated in SLN, uncoated and coated with chitosan, and submitted to *in vitro* cytotoxicity and antibacterial activity assays (26). Chitosan improved SLN properties as a carrier for TRE since no cytotoxicity to keratinocytes was found as well as a high activity against bacteria associated with acne (26).

Several NLC have been proposed to deliver active ingredients for the treatment of acne. Accordingly, ADA loaded NLCs showed an initial burst release, tailed by a sustained release over 24-hour period (13). The gel containing CLY loaded NLCs presented a biphasic release, starting with an initial burst that was then followed by a sustained release up to 24 hours (35). Following the same biphasic pattern, the DAP loaded NLCs showed an initial higher release rate then followed by a steady release phase (33). On the other hand, it was verified in NLCs carrying CPA a significantly slower release when compared with free CPA (34). Upon their loading into NLC, an increased degree of penetration through the skin of CLY (35) and DAP (33) was observed because of the occlusive effect caused by the lipid monolayer and the hydrophilic domain that moisturizes the SC. The lipid matrix is very important for active ingredient deposition and as well as to influence the permeation rate (13). Besides, there was a significant increase in the skin permeation of CLY loaded NLCs when compared to the

market formulation (35). There have been results providing evidence that these nanoparticles can increase the skin permeation probably due to an occlusive effect provided by NLC. The enhancement effect on active ingredient skin permeation was more significant in the case of NLCs loaded with TEY (32). ADA (13) loaded NLC and CPA (34) loaded NLC have been shown to decrease systemic uptake, minimizing systemic adverse side effects ADA (13) and CPA (34) loaded NLCs demonstrated an enhanced skin and hair follicular targeting, respectively. During the period of this study NLCs showed no irritation or inflammation towards the skin (33).

ADA loaded NEs gel presented a smaller cumulative release in comparison with the marketed gel, penetrating through the epidermis and did not show any skin irritation or erythema, nor systemic side effects. As a matter of fact no measurable ADA was found in plasma samples (40).

The skin permeation of LAH loaded LIPs was low, although the active ingredient release percentage of TC from liposomal formulation was higher than that of tranferosomal formulations (44). ITR loaded LIP demonstrated to have a great skin targeting, no skin irritation or edema was detected, because of the encapsulation of the active ingredients in LIP (73). All NIOs formulations have stayed true to the Higuchi's diffusion controlled model, that being said, ERY permeation across the skin was increased after encapsulation into NIOs (46). CRYP loaded ETOs have demonstrated to have an effective skin delivery of the active ingredient, with a higher skin permeation in comparison to simple ETOs gel and unwanted effects such as erythema or inflammation in the PSU (47). TEY loaded TRAs have a higher skin permeation when compared to TEY loaded LIPs. The flexibility of the bilayer structure of TRAs may have led to a lower active ingredient release. TRAs allows the active ingredient to target the skin leading to less systemic side effects (43).

CUBs showed a sustained ERY release over a 24-hour period. ERY release from CUBs gel followed the Korsmeyer Peppas kinetic making skin penetration easier and providing ERY skin retention. ERY loaded CUBs were considered suitable for acne treatment and bacterial infections (48).

ADA loaded NSP demonstrated no irritation potential as no difference in the release of pro-inflammatory cytokines was verified when compared to controls. The concentration gradient between the formulation and the targeted area was the driving force for NSP to permeate the skin. The improved ADA efficacy was due to the penetration and accumulation of NSP, associated with a controlled active ingredient release in the target. Moreover, NSP did not penetrate into intact epidermis instead they accumulate in the PSU (50).

BENP loaded POLM showed an enhanced skin penetration of this low water solubility and high lyophilic active ingredient due to the structure of the nanosystem (51). POLM demonstrated an enhanced active ingredient delivery to the PSU when compared to the commercial gel (51).

TRE loaded NFs demonstrated a stabilizing effect on TRE and a biphasic pattern, being that the initial burst phase up to 3 hours, followed by a sustained release of the active ingredient over the next 4 days in the skin target (53).

AgNP nanosystems have shown bactericidal effects but the mechanism of action remains to be elucidated. The deactivation of enzymes in the cell membrane as well as the interactions with the cell's ions are believed to promote protein denaturation and the opening of porous structures, giving Ag its antibacterial effects (18), (57). All AgNP tested repressed the growth of *C. acnes*, *S. aureus* e *S. epidermidis* (59). Also AuNP demonstrated to be promising for acne treatment because of its bacteriostatic and bactericidal effect against *C. acnes* and *S. aureus* (61).

AZA loaded NCRY and BENP loaded NCRY did not penetrate deep into the skin, only the SC layer thus providing active ingredient release in the target area (68), (74).

The anti-acne potential of lipid nanoparticles, polymeric-based nanoparticles and additional nanosystems are likely to be greatly influenced by the active ingredient entrapped. As summarized in Table 2, ADA loaded SLNs (28), ITR loaded SLNs (27) and ADA loaded NLCs (13) demonstrated to have influence in reducing papule density in a 4 cm^2 of skin area. Also, AZA loaded NLCs (36) showed a comedolytic and sebo-regulating effect. The number of non-inflammatory lesions, open and closed comedones, and inflammatory lesions (papules) were reduced with TRE loaded NEs (11) and TRE loaded LIPs (42). CRYP loaded ETOs demonstrated to diminish keratoplasia and inflammation of the PSU (47). The thickness of viable epidermal layers improved and the less comedonal density was obtained with ADA loaded NSP (50). Only the metal-based nanoparticles presented antibacterial activity against the bacteria that cause acne by themselves. Most studies compare the efficacy of the nanostructures with the commercial formulations, but a comparison among nanosystems would be more relevant. Also, a clear and objective explanation of any targeting mechanisms and its benefits towards the anti-acne effect would help to better understand any new input brought by them.

4. Toxicity

It can´t be excluded the chance that nanotechnology-based formulations could be toxic to some extent, especially once they are small sized, which make them hard to track and also because of the skin's potential to process exogenous material. Although, there have been technological advancements, particularly when it comes to fluorescent microscopy where better fluorescent probes and dyes, allowing a more detailed insight on cellular uptake and the course of the nanoparticles in cellular environment have emerged. In the meantime, the long-term effects of nanotechnology-based formulations remains to be studied (24).

Besides these facts, there are some studies where nanotechnology-based formulations toxicity is tested. For example, SLNs containing TRE have demonstrated to be not cytotoxic to *in vitro* human immortalized keratinocyte (HaCaT) cell culture, even when using the highest concentration of 500 g/ml (26). After 24 hours of LIPs containing ITR application to Sprague Dawley rats it was observed a significant reduction in skin toxicity upon skin histopathology analysis (73). AgNPs showed to have a minimal toxicity on normal human erythrocytes. In order to study this, AgNPs were tested on human erythrocytes through a hemolysis assay and results demonstrated a hemolysis rate lower than 55%, using a maximum concentration of 1600 μg/ml. These results assure that AgNPs can be used directly, at low doses, or in combination with a vehicle so that the safety of human cells can be assured (58). Studies performed in a mouse embryonic cell line in order to evaluate potential skin toxicity related to polymeric micelles usage and results demonstrated that BENP loaded polymeric micelles can be safely used (51).

Some penetration studies showed that nanotechnology-based formulations accumulate in higher concentrations in the epidermis and lower concentrations in deeper skin layers (63). But there are some authors that disagree with this point of view, defending that nanotechnology-based formulations are able to penetrate not only through epidermis and dermis, but also into hypodermis and blood vessels (63). Studies results about nanotechnology-based formulations toxicity are of outmost importance not only limited to skin irritation but broadened to bloodstream and skin layers effects related to their long-term usage, and genotoxicity in order to prove the safety of these formulations.

5. Regulatory Framework

Nanotechnology is considered an important key in the development of technology and it will positively contribute for the European Union (EU) (75). EU will benefit not only from

nanotechnology-based formulation innovation and the economic growth associated but also with investments for their application (76). Nevertheless, there are some uncertain issues related to the environmental impact, health, safety and toxicity risks associated to the production and usage of these nanomaterials. Therefore, in order to assure consumers' safety by using nanotechnology-based formulations, as cosmetic or medical products used for acne therapy and care, it is necessary to have an effective regulation based in scientific studies that evaluate their potential impacts.

In EU, medical products for human use are regulated by EU directive 2001/83/EC (77). In relation to nanotechnology-based medication regulatory, the European Technology Platform settled up with the European Commission (EC) was created in 2005 in order to standardize the legislation and also to solve ethical, safety, toxicity and environmental questions related to nanomaterial applications. According as well to EC, nanotechnology-based formulations for acne therapy and care, as cosmetic products must obey the n°1223/2009 regulation (78). In 2011, the EC defined nanomaterial as "natural, incidental or manufactured material containing particles in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1-100 nm (79). In 2012, EC published instructions on the Guidance on the Safety Assessment of Nanomaterials in Cosmetics, in order to meet the expected demand for safety of nanomaterials. In 2017, EC published a study where 12 frameworks and 48 tools used in Europe were evaluated, to measure the possible health and environmental risks of the produced nanomaterials. Still, none of the frameworks met all the criteria and it is recommended that the investigators should integrate human health, environmental risks and track the whole life cycle of nanomaterial-based medications or cosmetics.

6. Concluding Remarks and Future Perspectives

Human skin structure is frequently a barrier for the employment of treatments, particularly SC from epidermis that makes the penetration harder of the active ingredients used in conventional treatments.

Topical treatment of acne is advantageous by mitigating systemic side effects, through a correct active ingredient amount so bacterial resistance is not increased. However, conventional administration systems present limitations such as low biodistribution, poor effectivity, active ingredient dose dumping, low targeting ability, skin irritability and the correspondent toxicity.

Recently, nanotechnology-based formulations were developed in order to overcome the difficulties of conventional treatments used for acne treatment.

These nanosized formulations have demonstrated a remarkable potential as new strategies for acne treatment. Through the various studies compiled in this review, it is noticeable that nanostructures can be advantageous when compared to conventional acne treatments because they have a regulated active ingredient release, better skin permeation and lower skin irritation. The highlights of these nanocarriers are the possibility to entrap lower concentrations of active ingredients promoting a targeted effect, which can be prolonged in time due to physico-chemical properties of the chosen nanostructure, allied to the pretended anti-acne effect and less skin irritation.

It is important to notice that most of the permeation studies were conducted *in vitro* and there are factors that influence skin permeation *in vivo* that cannot be excluded, as skin condition (intact or with acne lesions), nanotechnology-based formulations characteristics (with penetration enhancers or not), the nanotechnology-based formulations physico-chemical properties and application conditions (presence or not of mechanical enhancers such as massage). In order to clarify the permeation of these novel anti-acne strategies it is necessary to perform and record, for longer periods of time, studies that can reply *in vivo* conditions.

Also, novel nanotechnological alternatives for acne treatment can lead to the appearing of new side effects, not only related to their small size and facility to achieve deeper skin layers that may cause cell and tissue destruction, but also environmental nano-pollution (80). As previously referred, very little is known about the toxicity of nanotechnology-based formulations.

As future perspectives, more studies should be conducted to assure the consumers' health and environmental safety, so that the part of society directly involved with the healthcare can be instructed and consequently recommend the safe use of them, based in scientific evidence. Also, a consensus about nanotechnology regulatory framework should be achieved. To conclude, combined active ingredient anti-acne therapy using nanotechnology and other nanosystems can also be explored.

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