UNIVERSIDADE D
COIMBRA

Maria João Leal Morais Soares

Relatórios de Estágio e Monografia intitulada "Cyclodextrins for the encapsulation of volatile compounds – pharmaceutical and cosmetic applications" referentes à unidade curricular "Estágio" sob a orientação da Dra. Ana Patrícia Rei, da Dra. Joana Martins de Carvalho 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.

Outubro de 2020

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Coimbra, 28 de outubro de 2020.

Maria João Leal Morais Soares

(Maria João Leal Morais Soares)

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Durante a escrita deste documento, pude refletir com todas as ferramentas que tive ao meu alcance e pude decidir com a única responsabilidade de estar a construir algo que me iria pertencer para a vida. No entanto, a conclusão deste documento levou-me a reconhecer que não é só a mim que ele pertence, mas também a todos aqueles que direta e indiretamente influenciaram a sua escrita.

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Estas páginas também são vossas!

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PARTE I

Relatórios de Estágio em Indústria Farmacêutica e em Farmácia Comunitária

No setor de *Portfolio Management* da Bluepharma Indústria, S.A

Sob orientação da Dra. Ana Patrícia Rei

Farmácia Rodrigues da Silva

Sob orientação da Dra. Joana Martins de Carvalho

Abreviaturas

FFUC	Faculdade de Farmácia da Faculdade de Coimbra
MICF	Mestrado Integrado em Ciências Farmacêuticas
SWOT	*Strengths, Weaknesses, Opportunities, and Threats*

Resumo

O plano curricular do Mestrado Integrado em Ciências Farmacêuticas (MICF) é provido de um amplo conteúdo programático que integra todo o ciclo do medicamento, o que oferece aos alunos uma vasta perspetiva daquilo que a atividade farmacêutica compreende.

No último semestre do curso, os conhecimentos teóricos são colocados em prática através de um estágio curricular em Farmácia Comunitária. A Faculdade de Farmácia da Universidade de Coimbra (FFUC) permite ainda que os alunos finalistas realizem um segundo estágio facultativo em Farmácia Hospitalar ou em Indústria Farmacêutica.

Neste âmbito, tive a oportunidade de realizar o estágio curricular na Bluepharma, uma indústria farmacêutica portuguesa e na Farmácia Rodrigues da Silva, em Coimbra. Estes dois estágios estão descritos no presente relatório, através de uma análise SWOT.

Palavras-chave: Farmácia comunitária; Farmácia Rodrigues da Silva; indústria farmacêutica; Bluepharma; investigação e inovação; gestão de portefólio; desenvolvimento do negócio

Abstract

The curricular program of the Integrated Master's in Pharmaceutical Sciences contains a programmatic content that integrates the entire cycle of the drug, offering to students a wide perspective of the pharmaceutical activity.

In the last semester of the course, the theoretical knowledge is put into practice through a curricular internship in Community Pharmacy. The Faculty of Pharmacy of the University of Coimbra also allows the final year undergraduates to choose a second and optional internship at Hospital Pharmacy or Pharmaceutical Industry.

Therefore, I had the opportunity to do the curricular internship at Bluepharma, Portuguese pharmaceutical industry, and at Rodrigues da Silva Pharmacy, in Coimbra. These two internships are described in this report, by undertaking a SWOT analysis.

Keywords: Community pharmacy; Rodrigues da Silva Pharmacy; Pharmaceutical Industry; Bluepharma; research and innovation; portfolio management; business development

1 Análise SWOT – Breve contextualização

A análise SWOT teve origem nos anos 60, numa época em que começara a surgir uma corrente de pensamento estratégica aliada à gestão de negócios e de projetos. Esta análise foi desenhada de forma a identificar as vantagens e as desvantagens intrínsecas de uma organização e a compreender a influência positiva e negativa dos fatores externos sobre essa mesma organização (GÜREL, 2017). A estrutura simples, clara e prática da análise SWOT tornou-a numa ferramenta bastante popular e muito utilizada ainda nos dias de hoje para realizar avaliações.

Assim sendo, os presentes relatórios de estágios são apresentados sob a forma de uma análise SWOT, em que aquilo que se pretende avaliar são "as atividades e os conhecimentos adquiridos durante o estágio".

A sigla SWOT refere-se a quatro fatores, dois internos, ***S****trengths* (Pontos Fortes) e ***W****eaknesses* (Pontos Fracos), e dois externos, ***O****pportunities* (Oportunidades) e ***T****hreats* (Ameaças) (GÜREL, 2017). Adaptando àquilo que se pretende avaliar, esta análise visa responder às seguintes questões:

- Pontos Fortes - Que características das atividades e dos conhecimentos adquiridos durante o estágio o colocaram em vantagem em relação aos outros estágios?
- Pontos Fracos - Que características das atividades e dos conhecimentos adquiridos durante o estágio o colocaram em desvantagem em relação aos outros estágios?
- Oportunidades - Que fatores externos tiveram impacto positivo nas atividades e nos conhecimentos adquiridos durante o estágio?
- Ameaças - Que fatores externos tiveram impacto negativo nas atividades e nos conhecimentos adquiridos durante o estágio?

2 Bluepharma – Indústria Farmacêutica, S.A

Desde cedo que os conhecimentos teóricos e laboratoriais adquiridos durante o curso me despertaram curiosidade em saber como era a realidade laboral de um farmacêutico que se encontrava no setor da indústria, o que me levou a escolher realizar um segundo estágio curricular na indústria farmacêutica.

A Bluepharma é uma indústria farmacêutica portuguesa sediada em Coimbra que surgiu em 2001. Aquando da sua abertura, apenas fabricava e comercializava formas farmacêuticas sólidas, mas rapidamente se alargou por outras áreas com foco na inovação e na internacionalização. Inaugurou o laboratório de Investigação e Desenvolvimento e iniciou a atividade do desenvolvimento de negócio, percorrendo, assim, toda a cadeia de valor do medicamento (BLUEPHARMA, 2018).

A história da Bluepharma e o seu foco em investir no desenvolvimento de novos produtos motivaram-me a candidatar-me para um estágio relacionada com a área de investigação e inovação nesta indústria. Assim, realizei uma entrevista e, após alguma análise das minhas preferências e do meu perfil, fui selecionada para a equipa de *Portfolio Management*. Iniciei o estágio no dia 6 de janeiro de 2020, tendo como tutora a responsável pelo *Portfolio Management,* Ana Patrícia Rei, que me acompanhou ativamente até ao fim do estágio a 30 de março de 2020.

2.1 Portfolio Management

No início da nova década, a Bluepharma passou por uma restruturação do seu organograma e foram criados alguns departamentos e setores de forma a responder mais eficazmente às necessidades da empresa, dos clientes e dos colaboradores. Foi o caso do setor de Gestão de Portefólio (*Portfolio Management*), que foi criado já este ano, ficando integrado no departamento de Desenvolvimento do Negócio (*Business Development)*.

O principal objetivo deste setor é centralizar numa única equipa gestão do atual e do futuro portefólio de produtos da Bluepharma.

Desta forma, as atividades desenvolvidas no *Portfolio Management* focaram-se, essencialmente, na análise de novas ideias de medicamentos genéricos convencionais, de medicamentos genéricos complexos e de *value added medicines*, geralmente referidos como VAMs, na procura de novos parceiros para o desenvolvimento farmacêutico e na pesquisa e organização da informação para a comunicação interna dos produtos do portefólio da Bluepharma, com foco nas necessidades da equipa do *Business Development.*

Figura I Análise SWOT do Estágio em Indústria Farmacêutica

2.2 Pontos Fortes

2.2.1 Processo de seleção

O processo de seleção da Bluepharma consiste na avaliação do *Curriculum Vitae* e da realização de uma entrevista presencial, o que tem a vantagem de providenciar aos alunos candidatos uma experiência muito similar àquela que é utilizada para aceder ao mercado de trabalho. Para além disso, este método permite que os alunos demonstrem as suas diferentes valências, que, geralmente, não são mensuráveis através da média curricular, possibilitando que estes sejam canalizados para as suas áreas de preferência e/ou para as quais tenham um perfil mais adequado.

2.2.2 Acolhimento e integração

No primeiro dia, fomos recebidos juntamente com os nossos colegas estagiários numa sessão de boas-vindas, onde nos deram breves orientações de como iria ser o nosso estágio.

De seguida, fomos levados a conhecer uma parte da empresa, onde fomos apresentados a todos os colaboradores dos diferentes departamentos. Nesse momento, fomos apresentados àqueles que iriam ser os nossos tutores. A existência desta sessão e deste acompanhamento no primeiro dia do estágio representaram uma enorme ajuda para nos integrarmos na empresa e nos sentirmos acolhidos pelos seus colaboradores. Além disso, ao longo do estágio, existiu sempre um espírito de confiança, de compreensão e de entreajuda para comigo e ainda uma preocupação com o meu bem-estar, o que me fez sentir efetivamente parte da equipa e não apenas uma estagiária.

2.2.3 Formações inicial e contínua

Desde o primeiro dia que foram marcadas várias formações tais como "Ambiente, Saúde e Segurança no Trabalho", "Farmacovigilância", "Assuntos Regulamentares", "Melhoria Contínua", que permitiram assimilar alguns conhecimentos já abordados durante o MICF mas com uma aplicação prática mais concreta, para além do nos alinhar com o espírito da empresa e com as suas diferentes atividades. Com o objetivo de obter aprendizagens mais específicas para desempenhar as atividades no *Portfolio Management*, tive ainda acesso a outro tipo de formações como "*Relevant sources of information for the pharmaceutical industry*", "Sistema de Gestão Integrado: Investigação, Desenvolvimento e Inovação" e ainda duas formações mais práticas que me permitiram aprender a trabalhar com o *Cortellis* e o IMS, duas bases de dados essenciais para o desempenho das atividades.

2.2.4 Participação nas sessões de Kaizen e reuniões

Todos os dias, durante 15 minutos, participava numa reunião baseada no método *Kaizen*, que tem como objetivo coordenar equipas e resolver problemas diários. Pude também assistir às reuniões de início de ano, em que se definia a estratégia do *Business Development* de forma a realizar um trabalho eficaz e com bons resultados. A oportunidade de participar nestes momentos foi extremamente útil para interligar conceitos e inteirar-me das atividades desenvolvidas e dos seus objetivos, dando-me uma visão de como se organiza e se trabalha em equipa.

2.2.5 Nível de Inglês

A globalização, mais do que uma perspetiva futura, é, atualmente, uma realidade. Além do mais, 90% do trabalho desenvolvido pela Bluepharma é exportado e nesse sentido, a utilização de uma língua simples e transversal, como o inglês, é indispensável. Assim, todo o trabalho desenvolvido foi elaborado em inglês. Este detalhe foi um dos grandes pontos fortes

do estágio, uma vez que fez da língua inglesa uma ferramenta de uso diário, ajudando-me a melhorar a minha capacidade de leitura, compreensão e de escrita.

2.2.6 Participação na criação de um setor

O setor de *Portfolio Management* foi criado no início do ano, o que me permitiu acompanhar este seu processo de criação. Assisti à sua apresentação ao *Business Development*, o que me trouxe uma visão mais clara do objetivo das atividades desenvolvidas no *Portfolio Management,* assisti a *Webinars* específicos sobre o conceito empresarial do *Portfolio Management* e ajudei na realização dos materiais de apoio para comunicação interna dos produtos da Bluepharma, participando e assistindo às pequenas modificações e melhoramentos que podiam ser feitos a essas apresentações. O meu envolvimento na génese deste departamento fez com que encarasse o estágio com mais motivação e como uma sensação de maior utilidade.

2.3 Pontos Fracos

2.3.1 Visão limitada da indústria farmacêutica

O estágio na Bluepharma permite conhecer muito bem uma área específica da empresa, aquela onde realizamos o estágio, mas não se tem uma visão abrangente de todos os departamentos da Bluepharma nem como funcionam entre si. Desta forma, é difícil ter uma clara noção das funções e das atividades desenvolvidas pelos colaboradores de outros departamentos, o que dificulta a aprendizagem do funcionamento geral de uma indústria farmacêutica.

2.3.2 Volatilidade de informação

Os conhecimentos que integram a dinâmica de uma indústria farmacêutica são muito vastos, muitas vezes vêm em forma de siglas e estão sempre em constante mudança. A velocidade com que se abordam temas e que se muda o paradigma é enorme e é-nos transmitido tudo rapidamente, o que torna moroso a aprendizagem e a compreensão dos conceitos de forma interligada e ordenada.

2.4 Oportunidades

2.4.1 Competências informáticas

Um dos fatores externos que tiveram impacto positivo no estágio foram os conhecimentos informáticos e a facilidade em aprender a lidar com novos programas. O

trabalho desenvolvido neste departamento é exclusivamente através de um computador, sendo necessário realizar pesquisas, compilar informações e analisar dados. Para tal, ter competências informáticas é essencial, tanto para realizar os trabalhos que nos são solicitados, como para aprender novas ferramentas de trabalho.

2.4.2 Conhecimentos do MICF

Os vários conceitos aprendidos no MICF são uma ferramenta essencial para lidar com toda a informação nova e atual com que nos deparamos num departamento onde é necessário conhecer novos medicamentos, interpretar novos mecanismos de ação em diferentes áreas terapêuticas, bem como ter conhecimentos de patentes e de assuntos regulamentares. Durante o estágio, senti que os conhecimentos adquiridos durante o MICF foram um fator muito importante para conseguir interpretar e relacionar conceitos que eram completamente novos e inovadores.

2.4.3 Pandemia de COVID-19

Sensivelmente a meio do período total do estágio, os primeiros casos positivos com Covid-19 surgiram em Portugal, o que fez com que a Bluepharma tomasse uma posição preventiva e adotasse a estratégia do teletrabalho para os colaboradores com a hipótese de o realizarem. Desta forma, toda a equipa de *Business Development* continuou as suas atividades em teletrabalho. Esta nova realidade fez com que eu tivesse a oportunidade de trabalhar em equipa, mas à distância. Como já tinha adquirido os conhecimentos mais basilares durante as semanas anteriores, o teletrabalho não transtornou o desempenho das atividades que me estavam delegadas, sendo por isso uma experiência positiva e que me permitiu ter mais algumas valências.

2.5 Ameaças

2.5.1 Excel

Quando entrei na Bluepharma, deparei-me com um grande desafio quando me foi proposto realizar uma tabela informativa pelo programa *Excel*. Não tinha conhecimentos suficientes para conseguir realizar essa tarefa, o que teve algum impacto negativo no desempenho das minhas funções mais iniciais. Ao longo do estágio, através da observação e da prática com o programa *Excel* acabei por evoluir bastante e ultrapassar esta lacuna. No entanto, percebi a importância que este programa tem na vida de qualquer profissional e que pode ser um fator de distinção entre farmacêuticos.

2.5.2 Estágio não creditado

A formação de um farmacêutico implica apenas a realização de um estágio em farmácia comunitária e/ou farmácia hospitalar (EUROPEU, 2013). No entanto, um farmacêutico atualmente é muito mais do que um farmacêutico comunitário ou um farmacêutico hospitalar e a sua forte presença na indústria farmacêutica é a prova disso. Desta forma, o estágio em indústria farmacêutica é uma enorme vantagem para a formação de um futuro farmacêutico e deveria fazer parte das opções creditadas de estágios. Embora, considere a realização deste estágio essencial para a minha formação e para a valorização do meu currículo, o facto de este não ser creditado constitui uma ameaça, uma vez que os alunos que pretendem realizar este tipo de estágios têm de realizar um número de horas totais superior e excessivo para o período de tempo que têm disponível.

3 Farmácia Rodrigues da Silva

A consagração da formação de um farmacêutico passa pela experiência prática numa farmácia comunitária. A Farmácia Rodrigues da Silva tem colaborado com a FFUC no sentido de ajudar neste processo de formação, recebendo, assim, estagiários, todos os anos. Este ano não foi exceção, sendo eu uma das estagiárias acolhidas por esta farmácia, desde o dia 25 de maio de 2020 até ao dia 11 de setembro de 2020, tendo como tutora de estágio a Dra. Joana Martins de Carvalho, cujo papel foi preponderante para a minha formação e crescimento pessoal.

Figura II Análise SWOT do Estágio em Farmácia Comunitária

3.1 Pontos Fortes

3.1.1 Gestão

Um dos pontos fortes deste estágio foi a transmissão de conhecimentos sobre a gestão de uma farmácia. Antes de o medicamento chegar à farmácia, há todo um processo de

monitorização de margens comerciais que envolve a seleção dos produtos a ter em *stock,* a escolha dos grossistas e a determinação das quantidades a encomendar. Essas decisões acabam por estar pré-estabelecidas e se não existir uma explicação desses processos, os estagiários não têm acesso a estes conhecimentos. Nesta farmácia, tive a oportunidade de adquirir essas noções através da elucidações feitas pelo farmacêutico proprietário e da diretora técnica, o que me permitiu perceber que área da gestão cada vez mais faz parte do trabalho de um farmacêutico comunitário e é essencial para manter uma farmácia dinâmica e funcional.

3.1.2 Organização

A Farmácia Rodrigues da Silva é dotada de uma grande organização aliada a um sentido de responsabilidade para com os utentes e para com as entidades reguladoras. Nesse sentido, fui aprendendo o fluido da organização dos procedimentos, como dos descontos praticados durante as campanhas, das reservas dos medicamentos aos utentes, das encomendas aos grossistas e das compras diretas aos laboratórios, das reclamações e das devoluções, do controlo dos psicotrópicos, entre outros. Todos estes documentos estavam devidamente guardados e armazenados, o que permitia facilmente consultar a documentação pretendida caso algum tipo de questão surgisse por parte de um utente, de um grossista ou uma entidade reguladora. Este tipo de organização contribui imenso para o sucesso deste estágio e transmitiu-me valores que podem ser aplicados em qualquer área de atuação farmacêutica.

3.1.3 Aconselhamento farmacêutico

Durante o MICF, as aprendizagens sobre os produtos farmacêuticos e cosméticos são feitas através da explicação das funções das substâncias ativas. Quando iniciamos o estágio na farmácia, a transposição dos conhecimentos é relativamente fácil para medicamentos sujeitos a receita médica, no entanto torna-se um grande desafio para os produtos de venda livre e cosméticos. O facto de os utentes recorrerem muito ao aconselhamento farmacêutico e confiarem no conhecimento dos colaboradores da farmácia permitiu-me ouvir o tipo de perguntas a fazer e as decisões a tomar na hora de ceder o produto. Além disso, os colaboradores da farmácia foram-me explicando os produtos de venda livre por secção de forma a me transmitirem o maior número de conhecimentos possíveis. Neste aspeto, senti que o estágio nesta farmácia me preencheu esta lacuna entre as substâncias e os produtos disponíveis no mercado.

3.1.4 Localização

A Farmácia Rodrigues da Silva localiza-se na baixa de Coimbra, uma das zonas mais turísticas da cidade. A nível pessoal, essa localização trazia algumas comodidades como o elevado número de transportes públicos e a facilidade de acesso a outros serviços. A nível profissional, teve um impacto muito positivo no estágio. Este fator faz com que a farmácia tenha dois tipos distintos de utentes, os utentes de passagem e os utentes fidelizados. Os utentes de passagem eram muitas vezes turistas que recorriam efetivamente ao aconselhamento farmacêutico, o que me obrigou a praticar oralmente inglês e a utilizar termos de saúde nesta língua. Os utentes fidelizados recorriam à farmácia várias vezes por mês, o que permitiu criar uma relação de empatia e de confiança com alguns utentes e fez com que a função de acompanhamento fosse desempenhada eficazmente.

3.1.5 Visitas de delegados

Durante o meu estágio pude assistir a algumas visitas de delegados comerciais de farmácia. Estes abordavam o farmacêutico proprietário ou a diretora técnica no sentido de perceberem como iam as vendas dos produtos, apresentavam as novidades do mercado, as campanhas e propunham encomendas. Além disso, motivavam os colaboradores, relembrando-os dos mecanismos de ação das substâncias ativas dos produtos, demonstrando as evidências científicas da sua eficácia e dizendo os seus principais argumentos de vendas. Ao ouvir estas abordagens foi possível deter um conhecimento mais detalhado de cada produto, bem como o marketing por detrás de cada um.

3.2 Pontos Fracos

3.2.1 Serviços e consultas

A Farmácia Rodrigues da Silva dispõe de um espaço reduzido o que limita a existência de algumas consultas e serviços, como a elaboração de manipulados, as consultas de nutrição ou a preparação individualizada de medicação, não havendo, portanto, oportunidade de perceber a forma de atuação do farmacêutico nestas atividades.

3.3 Oportunidades

3.3.1 Novo Módulo de Atendimento Sifarma®

Durante grande parte do meu estágio executei atendimentos e receções de encomendas através do programa informático Sifarma 2000®. Este é um programa que está

presente na maior parte das farmácias portuguesas, sendo de extrema importância saber manuseá-lo com destreza e rapidez, no entanto as suas conceções antigas dificultam esse processo. Sensivelmente duas semanas antes de terminar o estágio foi implementado na farmácia o Novo Módulo Atendimento Sifarma. O facto de este novo módulo ser muito parecido com o tipo de programas que estamos habituados a manusear atualmente e por ter tido, também, oportunidade de ter uma formação sobre o Novo Módulo Atendimento Sifarma, durante um evento proporcionado pelo Núcleo de Estudantes de Farmácia, fez com que a minha adaptação a este programa fosse rápida, o que teve um impacto positivo na utilização deste novo programa para o desempenho das atividades diárias na farmácia.

3.3.2 Interesse e curiosidade

Durante a realização do estágio em farmácia comunitária, há algumas tarefas fixas que é necessário realizar diariamente como a receção de encomendas e o aprovisionamento dos medicamentos. O atendimento aos utentes está dependente do fluxo de cada dia. Desta forma, existiam momentos de menos afluência. Nesses momentos, a motivação para aprender mais e o interesse em praticar diversas atividades são características chave para progredir no estágio. Assim, explorei os manuais de aconselhamento farmacêutico e os panfletos sobre diversos produtos que existiam na estante da farmácia, questionei os colaboradores sobre certas linhas de produtos, como os produtos cosméticos, os colírios oculares e auriculares e realizei algumas tarefas autonomamente. Esta atitude foi um fator externo que contribui positivamente para a minha experiência.

3.4 Ameaças

3.4.1 Capacidade de comunicação

A comunicação é um ponto-chave para o desempenho da profissão de farmacêutico comunitário e esse fator, embora seja passível de ser melhorado, está muito relacionado com a personalidade de cada um. O facto de me considerar uma pessoa mais introvertida não permitia muitas vezes ter a confiança para expor aos utentes alguns conhecimentos, assim como desenvolver conversas que pudessem ser úteis para o aconselhamento farmacêutico. Ao longo do estágio, consegui ultrapassar algumas dessas barreiras, no entanto, considero este pormenor uma ameaça uma vez que grande parte dos conhecimentos advém do contacto com o utente e das suas reações àquilo que lhes estamos a transmitir.

3.4.2 Pandemia de COVID-19

O início do estágio deu-se após o período de confinamento obrigatório devido a surgimento do coronavírus, o que fez com que não tivesse acesso à realidade da farmácia antes deste "novo normal". A Farmácia Rodrigues da Silva, por se localizar no centro da Baixa de Coimbra, foi extremamente afetada devido, essencialmente, à redução de turistas e dos estudantes. Dessa forma, o número de atendimentos era muito mais reduzido do que aquilo que costumava ser. Além disso, a pandemia obrigou ao uso de máscara e ao uso de acrílicos, afetando a proximidade entre os utentes e os farmacêuticos.

3.5 Caso Prático

3.5.1 Cessação Tabágica

Numa manhã bastante movimentada surgiu um utente a solicitar-me ajuda para parar de consumir tabaco. Explicou-me que já tinha realizado alguns tratamentos e que tinha conseguido ter sucesso durante um ano com a aplicação de uns adesivos transdérmicos à base de nicotina, cujo nome era Nicorette®. Apressei-me a procurar o produto pelo *stock* da farmácia e, posteriormente, pelo sistema informático Sifarma® e deparei-me com um grande leque de opções nas quais não tinha conhecimentos suficientes para me sentir à vontade para ceder o produto.

Tendo em conta a posição de grande responsabilidade que me estava a ser atribuída e na impossibilidade de pedir ajuda aos meus colegas pelo grande número de utentes que se encontravam na farmácia, expliquei ao utente que iria analisar bem as opções disponíveis e que se iriam adequar melhor à situação dele para, assim, poder encomendar o produto com certeza. Ele compreendeu e demonstrou-se disponível para voltar no dia seguinte para receber um aconselhamento mais instruído (SANTOS *et al.*, 2009). Fiquei com a informação de que ele consumia 20 cigarros por dia e no tempo disponível dediquei-me a pesquisar na base de dados do Sifarma, nas guias de tratamento europeias e nos *websites* dos produtos.

Quando o utente voltou à farmácia, apresentei-lhe as opções do Champix®, um medicamento sujeito a receita médica e que a partir de 2017 passou a comparticipado pelo estado (SNS, 2017), ficando bastante em conta para o utente, e do e Nicorette®. O Champix® é constituído por vareniclina, que se liga com mais afinidade que a nicotina aos recetores nicotínicos e provoca uma sensação desagradável quando o utente consome tabaco (EMA, 2016) e o Nicorette® é um adesivo transdérmico de libertação prolongada de nicotina para substituir completamente o consumo de tabaco (JOHNSON, 2019). O utente explicou que já tinha experimentado Champix® com acompanhamento médico, mas que lhe provocou um

efeito secundário na audição e por isso, abandonou o tratamento. Assim, demonstrou interesse nos adesivos e procedi à encomenda do Nicorette® na dose mais adequada para o seu consumo tabágico.

Até a encomenda chegar, comuniquei ao farmacêutico proprietário que iriamos ter um utente a seguir o tratamento com Nicorette® e este explicou-me alguns detalhes sobre este tratamento e que eram importantes de transmitir ao utente. Quando este voltou para adquirir o produto, expliquei-lhe que o tratamento poderia não ser linear ao que estava recomendado pela marca, podendo ser necessário aumentar a dose de nicotina ou prolongar o tempo de tratamento e que isso não podia ser visto como um motivo de desistência ou de fracasso. Além disso, questionei-lhe se as pessoas à sua volta já estavam informadas desta sua decisão e expliquei-lhe de como eram importantes neste processo.

O produto Nicorette® apenas tem 14 adesivos transdérmicos, o que me corresponde a 14 dias de tratamento. Ao analisar o esquema de tratamento friamente não via razão lógica para o produto ter apenas estes adesivos mas depois de acompanhar o utente e de o voltar a receber passado duas semanas para ele voltar a encomendar o produto, percebi que o facto de o produto ter este número reduzido de adesivos, fazia com que o utente voltasse regularmente à farmácia e pudesse ser acompanhado e receber um incentivo à sua utilização.

Devido à duração do estágio, não me foi possível acompanhar o resto do tratamento deste utente, mas o facto de ter tido um papel tão importante e ativo na mudança de um hábito de consumo de uma pessoa fez-me sentir o verdadeiro impacto e a responsabilidade que um farmacêutico pode ter na vida de alguém.

4 Considerações Finais

A realização dos estágios curriculares foi, para mim, uma consolidação dos conhecimentos adquiridos durante o curso através da sua aplicação num contexto real, com a vantagem de nos estar associado um tutor, responsável por nos transmitir alguns saberes e por corrigir as nossas falhas.

A possibilidade de realizar parte do estágio curricular numa área diferente de farmácia comunitária dá a oportunidade de os alunos terem contacto com possíveis áreas de preferência com as quais, de outra forma, não seria possível de ter acesso. No meu caso, experienciei o dia-a-dia de um farmacêutico num escritório de uma indústria farmacêutica e contactei com o processo mais primordial da inovação e da investigação, algo que permaneceria numa ideia abstrata se não tivesse realizado este estágio curricular.

O estágio na farmácia comunitária é efetivamente uma etapa imprescindível no final do curso. Independentemente do caminho futuro que se pode seguir, a passagem pela farmácia é essencial para ter consciência da importância do farmacêutico na cedência do medicamento ao utente, que, no fundo, corresponde ao último passo sob controlo do farmacêutico dentro do ciclo do medicamento. Neste estágio, a minha maior aprendizagem veio do contacto com as pessoas e com as suas necessidades, o que me fez compreender que, embora o farmacêutico lide com o medicamento e com os seus diversos processos, a sua missão final é sempre melhorar a qualidade de vida das pessoas e ir ao encontro com as suas necessidades.

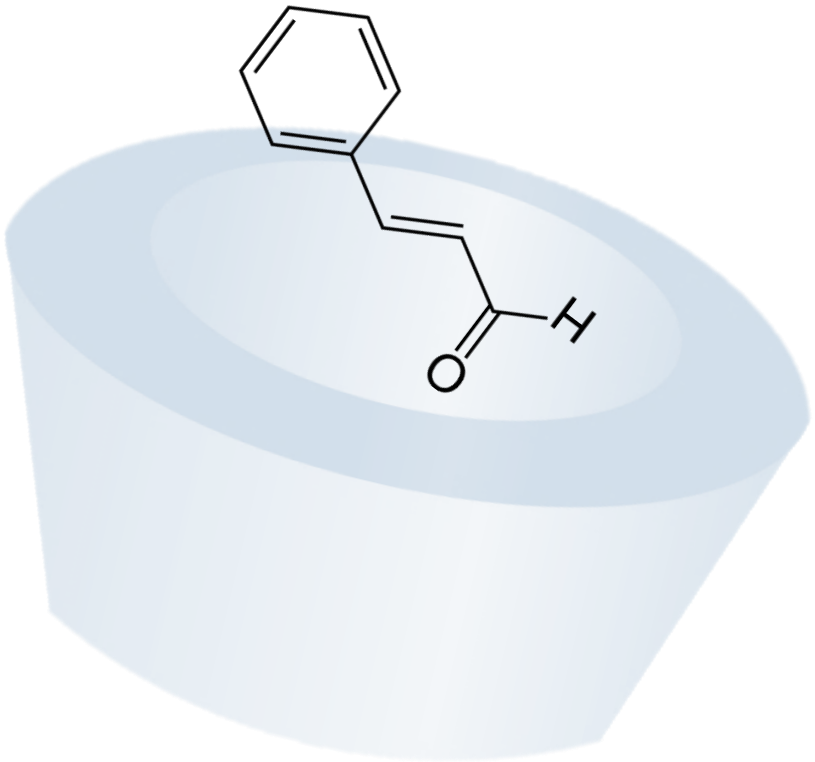
Desta forma, considero a passagem por estes estágios um momento chave para a completar a formação de um farmacêutico, dando-lhe tanto uma visão mais clara das suas competências e das suas preferências, como uma perspetiva mais rica daquilo que irá encontrar no mercado de trabalho.

PARTE II

Monografia

Cyclodextrins for the encapsulation of volatile compounds – pharmaceutical and cosmetic applications

Sob orientação do Professor Doutor António José Ribeiro



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Resumo

Os óleos essenciais têm vindo a assumir uma relevância crescente ao longo dos anos pelas suas diversas propriedades biológicas. Estas propriedades estão associadas à ação dos diferentes compostos voláteis presentes na composição dos óleos essenciais, razão pela qual é crucial o estudo dos compostos voláteis isolados, permitindo obter resultados mais concretos para a sua utilização terapêutica. Os compostos voláteis de origem natural, além do seu importante valor terapêutico, são considerados uma alternativa mais ecológica às substâncias químicas sintéticas. No entanto, os compostos voláteis são extremamente hidrofóbicos e sensíveis à degradação, o que diminui a sua biodisponibilidade e tem limitado o seu uso a nível industrial. As ciclodextrinas são utilizadas para encapsular moléculas e modificar as suas propriedades, como a baixa solubilidade e baixa estabilidade face à oxidação, calor e luz, o que representa uma solução viável, além de pouco dispendiosa, para encapsular compostos voláteis. Este artigo visa fornecer uma revisão do encapsulamento dos compostos voláteis em ciclodextrinas, destacando as técnicas de preparação mais exploradas, os resultados da eficácia dos compostos voláteis encapsulados, avaliando os fatores que influenciam estes valores e os estudos biológicos quer *in vitro* quer *in vivo* com foco no desenvolvimento de produtos farmacêuticos e cosméticos.

Palavras-chave: Compostos voláteis; óleos essenciais; ciclodextrinas; eficiência de encapsulação; constante de formação; atividades biológicas; técnicas de preparação; indústria farmacêutica; indústria cosmética.

Abstract

The use of essential oils (EO) has gained importance in the last years, due to their wide variety of biological properties, however, EOs are a complex mixture of volatile compounds (VC) which means that the study of VCs is often more precise and fruitful toward their use in therapeutics. These natural-origin molecules have an important therapeutic value, and they are considered a greener alternative to the synthetic compounds. Although, VCs are extremely hydrophobic and sensitive to degradation, limiting their bioavailability and their handling in the industry. The use of cyclodextrins (CD) has been used to surpass molecules' properties such as low solubility and low stability towards oxidation, heat, and light, which represents a viable and inexpensive solution for encapsulating volatile compounds. This article aims to provide a review of the VCs encapsulation in CDs, highlighting the preparation techniques most exploited, the efficacy results of the encapsulated VCs evaluating the factors that have influence these values, and the biological studies either *in vitro* and *in vivo* with a focus on the development of pharmaceutical and cosmetic products.

Keywords: Volatile compounds; essential oils; cyclodextrins; encapsulation efficiency; formation constant; biological activities; preparation techniques; pharmaceutical industry; cosmetic industry.

Abbreviations

CD	Cyclodextrin
CGTase	Cyclodextrin glycosyltransferase
CMC	Critical micellar concentration
CNS	Central nervous system
CRYSMEB	Low methylated-β-cyclodextrin
DM-β-CD	Dimethyl-β-cyclodextrin
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EE	Encapsulation Efficiency
EO	Essential oil
EPTAC-β-CD	Mono6-(2-hydroxy-3-(trimethylammonio)propyl)-β-cyclodextrin
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
GC-MS	Gas chromatography-mass spectrometry
GRAS	Generally recognized as safe
HP-ß-CD	Hydroxypropyl-β-cyclodextrin
HP-γ-CD	Hydroxypropyl-γ-cyclodextrin
IC	Inclusion complex
IL	Interleukin
iNOS	Inducible nitric oxide synthase
K_f	Formation constant
MAP	Mean arterial pressure
M-β-CD	Methyl-β-cyclodextrin
NA	Not available
PBS	Phosphate buffered saline
PC3	Prostate cancer cell line
RAMEB	Randomly methylated-β-cyclodextrin
ROS	Reactive oxygen species
SBE-β-CD	Sulfobutylated-β-cyclodextrin
Tmax	Maximum temperature
T-β-CD	Triazinyl-β-cyclodextrin
VC	Volatile compound

Introduction

The VCs are the chemical substances existing in the EOs (DHIFI *et al.*, 2016). They are produced by aromatic herbs and their wide range of biological properties makes them special useful and "greener" molecules to apply in the pharmaceutical and cosmetic industry (DE MATOS *et al.*, 2019; LEITE *et al.*, 2019). The study of the VCs rather than the complex mixture of the EOs provides more precise information about the pharmacology, the pharmacokinetics, and the toxicology of such compounds (AL-SHAR'I E OBAIDAT, 2018). VCs are extremely sensitive to degradation and rapidly loss by volatilization, which reduces their shelf life and limits their use in the industry. Besides their low solubility hampers their bioavailability and consequently their efficacy (TRINDADE *et al.*, 2019; YE *et al.*, 2019; YILDIZ *et al.*, 2019).

VCs can be protected and preserved through encapsulation in several delivery systems, such as lipidic micro- and nanoparticles and polymeric micro- and nanoparticles (DIMA E DIMA, 2015). However, these strategies have some limitations, lipidic nanoparticles lose high amounts of the encapsulated drug in topical administrations, their final formulation requires a high amount of water and they are unstable for long periods of storage (GHASEMIYEH E MOHAMMADI-SAMANI, 2018). Polymeric nanoparticles have issues to scale-up and lack toxicological assessment in the literature (KAHRAMAN *et al.*, 2017).

Among the several strategies to surpass these problems, stands out the encapsulation of VCs in CDs, a water-soluble nanoparticle starch-derived that have the capacity to incorporate hydrophobic substances in their interior cavity (HERRERA *et al.*, 2019). Among different types of CDs, the most suitable CD has been β-CD, since it has a size that allows entrapping most VCs. Besides, the CD can be functionalized, which greatly improves the solubility of the inclusion complex (IC) and the capacity to encapsulate the VCs (JANSOOK *et al.*, 2018; ZHANG *et al.*, 2017).

Currently, there are some drug products available in the market that contain CDs as an excipient. Nextrexone® is an injectable containing amiodarone as the active ingredient as well as CDs to improve the poor solubility of the drug, avoiding the use of the co-solvents polysorbate 80 and benzyl alcohol (CUSHING *et al.*, 2009). Nicorette®, a sublingual tablet, uses CDs with the purpose of taste masking the nicotine (SZEJTLI E SZENTE, 2005). Voltaren® Ophtha are eye drops with the diclofenac and the use of CDs improves the cornel tolerability and reduces the irritation to this drug (ABDELKADER *et al.*, 2018).

VCs present a greater challenge than other compounds as they are easily volatilized, which required appropriate conditions during the preparation of the ICs.

This review discusses the most recent encapsulated VCs with CDs and their preparation techniques with a focus on the efficacy results, based on encapsulation efficiency and ICs constants. In addition, we explore the possible pharmaceutical and cosmetic applications of these ICs, based on the evidence provided by *in vivo* and *in vitro* studies. Finally, toxicity and regulatory issues of CDs loaded with VCs are also addressed towards their commercialization.

1 Volatile compounds

1.1 Physicochemical properties

VCs are an interesting class of molecules, they cannot be seen but they can be felt through different receptors present in living beings, even humans. They have specific chemical properties that lead to their spread through vapor state at ambient conditions, like low molecular weight, below 300 Daltons, and high vapor pressure (DHIFI *et al.*, 2016; ROWAN, 2011). Therefore, VCs are mainly known for their odor and aroma.

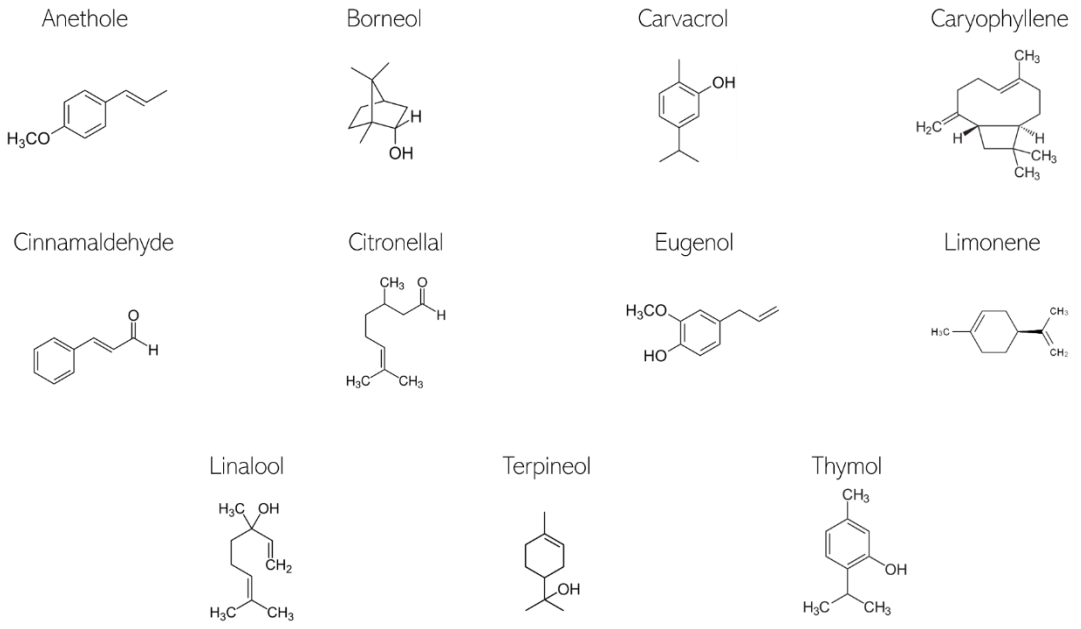

Figure 1 The structure of the most encapsulated volatile compounds in cyclodextrins.

In nature, they play a vital role, since these substances are used by plants as mechanisms of survival and reproduction (DHIFI *et al.*, 2016; ROWAN, 2011). These VCs are obtained generally from aromatic plants, resulting in EOs (JOHN DE OLIVEIRA MELO *et al.*, 2020; SIVA *et al.*, 2019). The EOs are a complex mixture of VCs, such as alcohols, aldehydes, amides,

amines, esters, ethers, heterocycles, ketones, oxides, phenols, and mainly the terpenes (Figure 1) (DHIFI *et al.*, 2016).

1.2 Therapeutic properties

VCs have an antihyperalgesic effect (JOHN DE OLIVEIRA MELO *et al.*, 2020), antiproliferative activity in numerous types of neoplastic cells (TRINDADE *et al.*, 2019), potential in decreasing the inflammatory response, cardiovascular effect and gastroprotective properties (DOS SANTOS NEGREIROS *et al.*, 2019). Moreover, VCs have antibacterial and antifungal activity (BOUCHEMELA *et al.*, 2019; YILDIZ *et al.*, 2019) and these properties are believed to be the result of the VCs interactions with the microbial cells' membrane promoting those structures' loss of function and permeability (AYTAC *et al.*, 2016; YUAN *et al.*, 2019). VCs also have activity in the central nervous system (CNS) since they are lipophilic compounds thus able to cross the blood-brain barrier without difficulty. Thereby, they interact with the signaling pathways associated with depression, insomnia, anxiety, and epilepsy (DINIZ *et al.*, 2019).

These widespread properties have contributed to a major interest in VCs to treat and control diseases. Furthermore, they may represent a more sustainable alternative to existing drugs (DE MATOS *et al.*, 2019; LEITE *et al.*, 2019).

1.3 Major volatile compounds drawbacks

The physicochemical properties of VCs significantly limit its pharmaceutical and cosmetic applications. They are extremely volatile, which leads to high mass loss during manipulation (MENEZES *et al.*, 2016), and they have low oral bioavailability due to their hydrophobic characteristics (KFOURY *et al.*, 2016a). Furthermore, VCs have a high photo, heat, and oxygen sensibility (TRINDADE *et al.*, 2019; YE *et al.*, 2019; YILDIZ *et al.*, 2019), which hinders long term shelf life (CELEBIOGLU *et al.*, 2018a). In topical products specifically, the instability of the VCs is an obstacle, since it is required a high degradation resistance when a VC-containing product is spread on the skin (PIRES *et al.*, 2018).

2 Cyclodextrins

2.1 Physicochemical properties

CDs are truncated hollow cone-like nanostructures produced from the degradation of starch, through the enzyme cyclodextrin glycosyltransferase (CGTase) (DA ROCHA NETO

et al., 2018; RECIO *et al.*, 2018). They are cyclic oligosaccharides with D-glucose monomers linked by α-1,4 bonds (TRINDADE *et al.*, 2019). As can be seen in Figure 2, CDs have outer secondary hydroxyl groups at the larger border of the cone (carbon 2 and carbon 3) and primary hydroxyl groups at the narrow border (carbon 6), which gives it a hydrophilic character. In the interior surface, the presence of apolar hydrogens in carbon 3 and carbon 5, ethereal oxygens and, skeletal carbons results in a hydrophobic cavity (DA ROCHA NETO *et al.*, 2018; RECIO *et al.*, 2018). This central cavity allows encapsulating a wide range of hydrophobic molecules among which VCs (HERRERA *et al.*, 2019). The lipophilic part of the hydrophobic molecules, generally referred to as guest molecules, establishes noncovalent interactions (electrostatic, van der Waals, hydrophobic and H-bond interactions) with the interior of the CDs thus leading to an IC, which is water-soluble, due to the hydrophilic exterior (ALVIRA, 2018; AYTAC *et al.*, 2016; JANSOOK *et al.*, 2018).

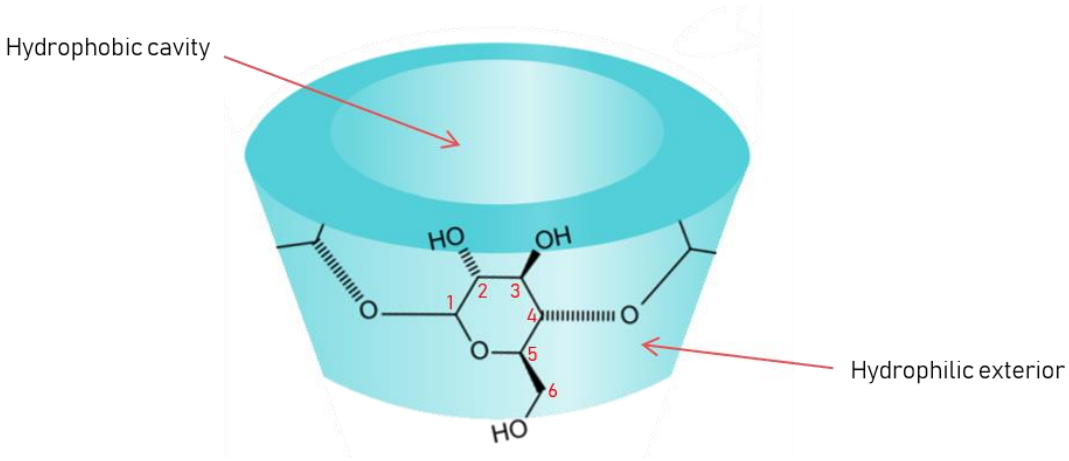

Figure 2 Schematic 3D illustration of a cyclodextrin.

2.2 Types and most common cyclodextrins

There are several types of CDs. Unmodified natural CDs can have a different number of D-glucose: α-CDs have the lowest number of monomers, six, followed by β-CDs with seven monomers whilst γ-CDs have the highest number of D-glucose monomers, eight (Figure 3). β-CDs are the most used (around 70% of annual overall production) since they have an appropriate cavity size to accommodate a high number of guests, as well as cost related to their purification, is low when compared to other natural CDs (AL-NASIRI *et al.*, 2018).

Due to their larger cavity, γ-CDs are used often to trap bigger molecules. On the opposite, the use of α-CDs is somewhat limited to small molecules additionally to long alkyl

chains (JANSOOK *et al.*, 2018; ZHANG *et al.*, 2017). There are natural CDs with more than eight D-glucose monomers, but they lack functionality. Furthermore, there are CDs functionalized with reagents with the main purpose of improving the solubility of molecules. Thus, among CDs derivatives we can find hydroxypropyl-β-cyclodextrins (HP-β-CD), hydroxypropyl-γ-cyclodextrins (HP-γ-CD), randomly methylated-β-cyclodextrins (RAMEB), sulfobutylated-β-cyclodextrins (SBE-β-CD), and overall they represent on average 10% of annual CD production (JANSOOK *et al.*, 2018).

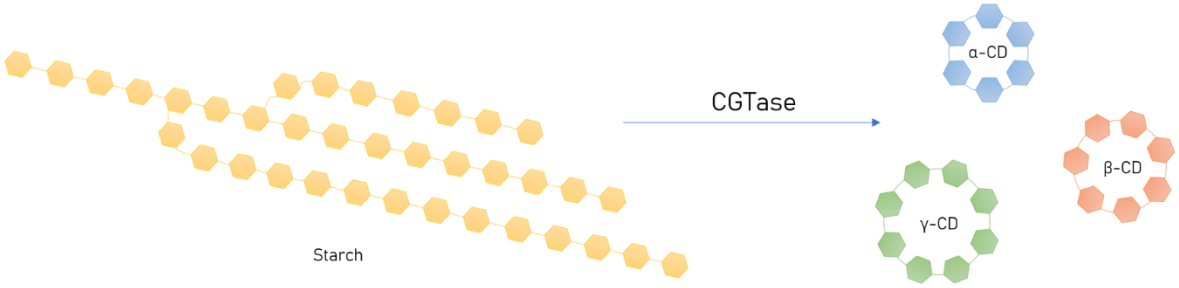

Figure 3 Schematic illustration of the reaction between starch and cyclodextrin glycosyltransferase (CGTase), that naturally can result in α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), or γ-cyclodextrin (γ-CD).

2.3 Specificity

Due to their unique structure and their physicochemical properties, CDs are able to encapsulate hydrophobic molecules but they can also be used to improve molecules' solubility and bioavailability, to preserve molecules stability against oxidation, and to provide molecules a controlled release profile (CELEBIOGLU *et al.*, 2018a; RECIO *et al.*, 2018; YE *et al.*, 2019). Moreover, CDs are biocompatible, having low toxicity as they provide no activation of the immune response. They can be used both in solution and in solid-state and they are available at a low price (RECIO *et al.*, 2018; ZHANG *et al.*, 2017).

CDs are included in the group of pharmaceutical excipients, and they represent one of the complexing agents most commonly used by the pharmaceutical industry because they are inexpensive, biocompatible, and also capable of improving the biological, chemical, and physical properties of bioactive molecules (GUIMARAES *et al.*, 2015).

CDs are considered as "generally recognized as safe" (GRAS), which makes them excellent encapsulating agents for non-polar drugs (OLIVEIRA *et al.*, 2017). As pharmaceutical raw-material, it is approved by the Food and Drug Administration (FDA), described in pharmacopeias of several countries and it can be found in at least 35 pharmaceutical products,

as an anticancer agent and anti-inflammatory drugs, predominantly as β-CD (GUIMARAES *et al.*, 2015).

3 Encapsulation of volatile compounds in cyclodextrins

The use of CDs to encapsulate VCs has been proposed (CAMARGO *et al.*, 2018; SIVA *et al.*, 2019; TRINDADE *et al.*, 2019) to circumvent most of the aforementioned obstacles and thus to improve VCs' bioavailability, shelf-life, and release control (DE MATOS *et al.*, 2019; LI *et al.*, 2020; YE *et al.*, 2019).

There are various preparation techniques to form ICs with CD and VCs and their role in the yield and the efficacy of encapsulation is of relevance regarding towards e the quality of the IC and the efficacy of the VC. The selection of the preparation and characterization techniques for VCs complexation has been pursued for several years (CEBORSKA *et al.*, 2013; KFOURY *et al.*, 2014) and some of the techniques are used as references to prepare the ICs and to assess the improvement of the physicochemical and biological properties of VCs (SILVA *et al.*, 2016; SOUZA *et al.*, 2018; TIEFENSEE RIBEIRO *et al.*, 2019; TRINDADE *et al.*, 2019; XIAO *et al.*, 2020).

3.1 Preparation techniques

There is not a conventional method to prepare ICs with CDs (PIRES *et al.*, 2018). The most common and recently used preparation techniques to form ICs involve the use of co-solvents. These methods achieve better efficacy results, however, they are time-consuming as the evaporation of the solvent in a later step must be performed (AL-SHAR'I E OBAIDAT, 2018).

The first steps involve the solubilization of the components. In the case of the VCs, the co-solvent most used is ethanol. The CDs are usually dissolved in water. BOUCHEMELA *et al.*, (2019) reported that the water provides some flexibility to the CD, leading to their deformation, which facilitates the process complexation and improves the formation constant. (ARAUJO-FILHO *et al.*, 2017; BOUCHEMELA *et al.*, 2019; GUIMARAES *et al.*, 2015). However, alternative solvents have been studied among which hydroalcoholic solutions and phosphate buffered saline (PBS) solutions. AL-NASIRI *et al.* (2018) verified that the addition of ethanol to water was effective to dissolve CDs but less effective to form ICs with VCs, due to the less polar environment and consequently the hydrophobic interactions binding the VC to the central cavity of CD are weaker (AL-NASIRI *et al.*, 2018). KFOURY *et al.* (2014) studied the differences between encapsulation efficiency (EE) of VC/CD ICs in water and PBS and they

conclude that the ionic strength of PBS does not modify the interaction between CDs and the VCs (KFOURY *et al.*, 2014). The concentration of the CDs solution also has importance in the efficacy results of the IC. The increase of the CDs solution concentration is directly proportional to the EE of the IC, however, very high concentrations of CDs promote the self-association of the CDs, which compromises the effectiveness of the encapsulation between CDs and VCs (YILDIZ *et al.*, 2018).

Usually, the CDs and the VCs solutions are prepared upon an equimolar ratio (1:1). Most of the VCs have a molecular weight between 120-160 g/mol, allowing one molecule of CD to entrap one molecule of a VC (DAS *et al.*, 2019; RAKMAI *et al.*, 2017). However, there are exceptions, as reported by CEBORSKA *et al.* (2015) that suggests a molar ratio of 2:2 between geraniol molecules and β-CD, where the two terpineol molecules are accommodated in the same two CDs, forming a unique IC (Figure 3) (CEBORSKA *et al.*, 2015).

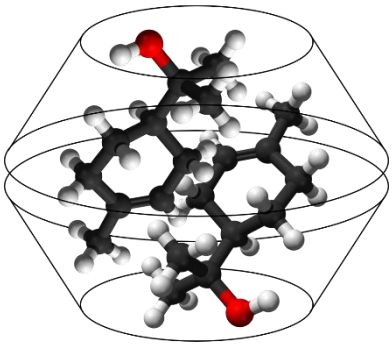

Figure 4 Terpineol/β-Cyclodextrin inclusion complex with a 2:2 molar ratio – side view.

Upon dissolution of the CDs and the VCs with their respective co-solvents, the resultant solutions are mixed together and prepared by different methods, such as freeze-drying, co-precipitation, ultrasound, slow diffusion, spray drying, rotary evaporation, that are described in detail the Table 1.

In addition, some preparation techniques do not use co-solvents, among which physical mixture, supercritical fluid technology, and sealed-heating method, or use a small amount of water to help the mixture of the CDs powder with the pure VC, among which slurry complexation, paste complexation, and kneading method, as displayed in Table 1. OLIVEIRA *et al.* (2016) demonstrated that when using the slurry complexation it was visible changes in the shape of the CD during the process of complexation, helping the encapsulation process, while during the physical mixture, the CDs maintain their rectangular crystal shape (OLIVEIRA *et al.*, 2016).

Table 1 Comparison of preparation techniques of CD-complexes.

Preparation techniques	VC and CD co-solvents	Steps	T_{max}	Time Duration	Considerations	References
Aqueous solution	Ethanol/Water	1) Stirring 2) Filtration	25°C	12h-24h	NA	(GHARIB *et al.*, 2018; YILDIZ *et al.*, 2018)
Freeze-drying	Ethanol/Water	1) Stirring 2) Filtration 3) Lyophilization	30°C	3 days	• Strong interaction between CDs and VCs • High quality and stability product • Optimal for heat-sensitive compounds • Possible to scale up	(KFOURY *et al.*, 2014; LI *et al.*, 2020; PUJIASTUTI *et al.*, 2017; SANTOS *et al.*, 2017; TRINDADE *et al.*, 2019; XIAO *et al.*, 2020)
Co-precipitation	Ethanol/Water	1) Stirring under heat 2) Storing at low temperature 3) Vacuum filtration 4) Vacuum desiccator	55°C	Long	• One of the simplest techniques • Widely used in laboratory • Problems to large-scale production [a]	(AL-NASIRI *et al.*, 2018; HERRERA *et al.*, 2019; SU *et al.*, 2012)
Ultrasound	Ethanol/Water	1) Stirring with an ultrasonicator 2) Filtration 3) Washing [b] 4) Lyophilization	60°C	1h	• Very simple and easy to control	(SU *et al.*, 2012; TRINDADE *et al.*, 2019)
Spray drying	Ethanol/Water	Atomization of the VC solution and the CDs solution	125°C	NA	• Low drug content • The high temperatures probably lead to the evaporation of the compound	(PIRES *et al.*, 2018)
Rotary Evaporation	Ethanol/Water	1) Stirring 2) Filtration 3) Rotary evaporation	80°C	NA	NA	(PIRES *et al.*, 2018)
Slow diffusion	Water	Diffusion of the VC vapor to the CDs solution	25°C	2 weeks	NA	(CEBORSKA *et al.*, 2015)
Slurry complexation	Small amount of water	1) Stirring 2) Desiccator 3) Manual trituration	25°C	1 week	• Strong interaction between CDs and VCs • Simple technique	(DOS PASSOS MENEZES *et al.*, 2017; GUIMARAES *et al.*, 2015; MENEZES *et al.*, 2016; SOUZA *et al.*, 2018)

Preparation techniques	VC and CD co-solvents	Steps	T_{max}	Time Duration	Considerations	References
Paste complexation	None	1) Manual agitation helped by water 2) Desiccator 3) Manual trituration	25°C	2 days	NA	(DOS PASSOS MENEZES *et al.*, 2017; MENEZES *et al.*, 2016)
Physical mixture	None	Manual agitation	25°C	15 min	• No homogeneous structure • Lack of interaction	(DOS PASSOS MENEZES *et al.*, 2017; GUIMARAES *et al.*, 2015; KFOURY *et al.*, 2014; MENEZES *et al.*, 2016; SANTOS *et al.*, 2016; TRINDADE *et al.*, 2019)
Kneading Method	None	1) Kneading helped by water 2) Vacuum oven 3) Grinding and sieving	70°C	13h	• ICs with high stability	(AL-SHAR'I E OBAIDAT, 2018)
Supercritical Fluid Technology	None	Submitting to supercritical conditions	50°C	6h	• Unsuccessful • Low interaction	(AL-SHAR'I E OBAIDAT, 2018; PIRES *et al.*, 2018)
Sealed-heating method	None	1) Sealing at acidic medium and heating 2) Lyophilization	28°C	Long	• Suitable technique • Simple technique	(NIEDDU *et al.*, 2014)

Cyclodextrin (CD); inclusion complex (IC); maximum temperature (T_{max}); not available (NA); volatile compound (VC).

[a] high water amounts required and large reaction tanks needed (AL-NASIRI *et al.*, 2018)

[b] to remove all the VC molecules connected to the surface of the CD (SU *et al.*, 2012; TRINDADE *et al.*, 2019)

3.2 Most encapsulated volatiles and efficacy results

In recent years, several VCs were encapsulated in CD through the preparation techniques above explained, and the efficacy results were expressed mostly as EE and formation constant (K_f) (Table 2). These parameters have been extensively used to characterize the quality of the IC. The values obtained are influenced by the physicochemical properties of the VC, and the type of interaction with the CD and the preparation technique (AL-SHAR'I E OBAIDAT, 2018; KFOURY *et al.*, 2016b; MENEZES *et al.*, 2016).

The EE is a quantitative parameter to calculate the quantity of the guest molecule present in the IC (MENEZES *et al.*, 2016; TRINDADE *et al.*, 2019) and it is used as a leading indicator in selecting the optimum formulation or preparation technique (LOU *et al.*, 2017). Usually, the mass of VC, required to calculate its EE, is determined by a gas chromatography method (HERRERA *et al.*, 2019; LOU *et al.*, 2017) and EE value is obtained using the following equation:

$$EE(\%) = \frac{m_{volatile\ compound\ exp}}{m_{volatile\ compound\ i}} \times 100$$

where $m_{volatile\ compound\ exp}$ is the mass of VC experimentally determined in the IC and $m_{volatile\ compound\ i}$ is the mass of the VC initially used to prepare the IC (GHARIB *et al.*, 2018; TRINDADE *et al.*, 2019). The differences between the EE values can be attributed to several factors such as the molecular weight of VC and the interaction level between the VCs and the CD cavity (HERRERA *et al.*, 2019) but the latter is directly associated with the cavity size of CDs also (CELEBIOGLU *et al.*, 2018a).

The K_f is another parameter relevant to evaluate the success of VC encapsulation in CDs. The K_f denotes the binding strength between the guest molecule and the CD (CELEBIOGLU *et al.*, 2018b). K_f values within 100–10,000 M^{-1} are considered ideal to practical applications (SANTOS *et al.*, 2017) once indicates that CDs are appropriate to allow drug-controlled release as well as adequate to improve the bioavailability of the poorly soluble drugs (KFOURY *et al.*, 2014; KFOURY *et al.*, 2016a). The formation constant (K_f), also referred to as stability constant, can be determined from the following equation:

$$K_f = k/S_0(1-k)$$

where S_0 is the concentration in $mol.L^{-1}$ of the VC solubilized in deionized water without CDs, in other words, the intrinsic solubility, and k is the slope of the linear function of the phase solubility diagram (CELEBIOGLU *et al.*, 2018a; PILETTI *et al.*, 2017).

The borneol/β-CD IC had a high value of K_f, indicating a very strong interaction between the VC and the β-CD (SU *et al.*, 2012), as well as the encapsulation of citronellal with the β-CD, which revealed better efficacy when compared to HP-β-CD (ABRIL-SANCHEZ *et al.*, 2019).

In the case of anethole, its encapsulation with natural CDs showed lower performance compared to the CDs derivatives. RAMEB showed an IC with higher EE and K_f results (KFOURY *et al.*, 2014).

The most suitable CD to encapsulate cinnamaldehyde was the DM-β-CD as EE value was higher when compared to the β-CD and HP-β-CD (SUN *et al.*, 2018).

The M-β-CD stood out, achieving the best results when compared with other derivative CDs while encapsulating eugenol, limonene, linalool, and thymol (AYTAC *et al.*, 2016; AYTAC *et al.*, 2017; CELEBIOGLU *et al.*, 2018a).

The size of the CD seems to play an important role. Carvacrol, cinnamaldehyde, eugenol, limonene, linalool, and thymol encapsulated in HP-γ-CDs demonstrated a lower K_f compared to the K_f of the respective VCs encapsulated in HP-β-CDs, indicating HP-γ-CD weaker interactions with the VCs due to CD larger size (YILDIZ *et al.*, 2019).

The preparation techniques also influence the EE and K_f results as it can be seen when the authors prepare the same ICs using different preparation techniques. The EE values of the ICs prepared by co-precipitation and freeze-drying methods stand out, while the physical mixture showed very low results of success.

OLIVEIRA *et al.* (2016) referred that stirring and amount of water are essential factors to promote the replacement of water molecules for guest molecules in the CDs, which can explain the better results obtained with the preparation techniques involving the use of co-solvents or a substantial amount of water (OLIVEIRA *et al.*, 2016).

In some cases, different authors prepare the same VC with the same CDs and the same preparation techniques and the EE and K_f values are quietly different, which difficulty the comparison with different authors and lead to assume the existence of others factors that might influence these parameters, like the analytical methods and the treatment of the experimental data (Kfoury- Molecules 2018). An example of these discrepancies is the encapsulation of thymol in β-CD in an aqueous solution, where the K_f results were calculated

Table 2 Recently encapsulated volatile compounds in cyclodextrins and their preparation techniques, with respective efficacy results, based on the encapsulation efficiency and formation constant.

VC	Type of CD	Preparation Techniques	EE (%)	K_f (M^{-1})	References
Anethole	HP-ß-CD	Aqueous solution	100,0	NA	(GHARIB *et al.*, 2017)
Anethole	HP-ß-CD	Aqueous solution	100,0	NA	(GHARIB *et al.*, 2018)
Anethole	CRYSMEB	Freeze-drying	27,0	877	(KFOURY *et al.*, 2014)
Anethole	HP-β-CD	Freeze-drying	25,0	981	(KFOURY *et al.*, 2014)
Anethole	RAMEB	Freeze-drying	34,0	1110	(KFOURY *et al.*, 2014)
Anethole	α-CD	Freeze-drying	25,0	710	(KFOURY *et al.*, 2014)
Anethole	β-CD	Freeze-drying	17,0	497	(KFOURY *et al.*, 2014)
Anethole	β-CD	Freeze-drying	12,0	NA	(KFOURY *et al.*, 2016b)
Anethole	β-CD	Co-precipitation coupled to freeze-drying [a]	20,0	NA	(KFOURY *et al.*, 2016b)
Borneol	RAMEB	NA	NA	1784	(DAS *et al.*, 2019)
Borneol	EPTAC-β-CD	Ultrasound method	94,3	NA	(FAN *et al.*, 2019)
Borneol	β-CD	Ultrasound method	96,5	38812	(SU *et al.*, 2012)
Carvacrol	β-CD	Co-precipitation	99,3	NA	(AL-NASIRI *et al.*, 2018)
Carvacrol	β-CD	Supercritical fluid technology	67,0	NA	(AL-SHAR'I E OBAIDAT, 2018)
Carvacrol	β-CD	Kneading method	81,0	NA	(AL-SHAR'I E OBAIDAT, 2018)
Carvacrol	β-CD	Physical mixture	3,0	NA	(MENEZES *et al.*, 2016)
Carvacrol	β-CD	Paste complexation	34,3	NA	(MENEZES *et al.*, 2016)
Carvacrol	β-CD	Slurry complexation	71,7	NA	(MENEZES *et al.*, 2016)
Carvacrol	β-CD	Physical mixture	1,2	NA	(TRINDADE *et al.*, 2019)
Carvacrol	β-CD	Ultrasound method	34,0	NA	(TRINDADE *et al.*, 2019)
Carvacrol	β-CD	Freeze-drying	81,2	NA	(TRINDADE *et al.*, 2019)
Carvacrol	HP-β-CD	Aqueous solution	NA	3321	(YILDIZ *et al.*, 2018)
Carvacrol	HP-γ-CD	Aqueous solution	NA	288	(YILDIZ *et al.*, 2018)
Caryophyllene	β-CD	Co-precipitation	62,0	NA	(LIU *et al.*, 2013)
Caryophyllene	HP-β-CD	Freeze-drying	60,0	NA	(LOU *et al.*, 2017)
Cinnamaldehyde	HP-β-CD	Aqueous solution	NA	140	(YILDIZ *et al.*, 2019)

VC	Type of CD	Preparation Techniques	EE (%)	K_f (M^{-1})	References
Cinnamaldehyde	HP-γ-CD	Aqueous solution	NA	110	(YILDIZ *et al.*, 2019)
Cinnamaldehyde	β-CD	NA	85,0	NA	(DAVAATSEREN *et al.*, 2017)
Cinnamaldehyde	β-CD	Co-precipitation	91,0	NA	(HERRERA *et al.*, 2019)
Cinnamaldehyde	DM-β-CD	Ultrasound method	53,6	NA [b]	(SUN *et al.*, 2018)
Cinnamaldehyde	HP-β-CD	Ultrasound method	46,5	NA [b]	(SUN *et al.*, 2018)
Cinnamaldehyde	β-CD	Ultrasound method	29,4	NA [b]	(SUN *et al.*, 2018)
Citronellal	HP-β-CD	NA	NA	525	(ABRIL-SANCHEZ *et al.*, 2019)
Citronellal	β-CD	NA	NA	1472	(ABRIL-SANCHEZ *et al.*, 2019)
Eugenol	HP-β-CD	Aqueous solution	71,0	217	(CELEBIOGLU *et al.*, 2018a)
Eugenol	HP-γ-CD	Aqueous solution	68,0	63	(CELEBIOGLU *et al.*, 2018a)
Eugenol	M-β-CD	Aqueous solution	95,0	259	(CELEBIOGLU *et al.*, 2018a)
Eugenol	β-CD	Co-precipitation	66,0	NA	(HERRERA *et al.*, 2019)
Eugenol	β-CD	Freeze-drying	85,0	NA	(LI *et al.*, 2020)
Eugenol	β-CD	Freeze-drying	77,0	NA	(PHUNPEE *et al.*, 2016)
Limonene	HP-β-CD	Aqueous solution	42,0	NA	(AYTAC *et al.*, 2016)
Limonene	HP-γ-CD	Aqueous solution	38,0	NA	(AYTAC *et al.*, 2016)
Limonene	M-β-CD	Aqueous solution	78,0	NA	(AYTAC *et al.*, 2016)
Limonene	α-CD	Physical mixture	-0,66 [c]	NA	(DOS PASSOS MENEZES *et al.*, 2017)
Limonene	α-CD	Slurry complexation	8,0	NA	(DOS PASSOS MENEZES *et al.*, 2017)
Limonene	α-CD	Paste complexation	12,1	NA	(DOS PASSOS MENEZES *et al.*, 2017)
Limonene	β-CD	Paste complexation	-8,18 [c]	NA	(DOS PASSOS MENEZES *et al.*, 2017)
Limonene	β-CD	Physical mixture	-0,26 [c]	NA	(DOS PASSOS MENEZES *et al.*, 2017)
Limonene	β-CD	Slurry complexation	9,4	NA	(DOS PASSOS MENEZES *et al.*, 2017)
Linalool	β-CD	Co-precipitation	99,3	NA	(AL-NASIRI *et al.*, 2018)
Linalool	β-CD	Supercritical fluid technology	70,0	NA	(AL-SHAR'I E OBAIDAT, 2018)
Linalool	β-CD	Kneading method	84,0	NA	(AL-SHAR'I E OBAIDAT, 2018)
Linalool	HP-β-CD	Aqueous solution	84,0	NA	(AYTAC *et al.*, 2017)

VC	Type of CD	Preparation Techniques	EE (%)	K_f (M^{-1})	References
Linalool	HP-γ-CD	Aqueous solution	77,0	NA	(AYTAC *et al.*, 2017)
Linalool	M-β-CD	Aqueous solution	89,0	NA	(AYTAC *et al.*, 2017)
Linalool	β-CD	Co-precipitation	NA	290	(BONETTI *et al.*, 2015)
Linalool	RAMEB	NA	NA	688	(DAS *et al.*, 2019)
Terpineol	β-CD	Slow diffusion	NA	399	(CEBORSKA *et al.*, 2015)
Thymol	β-CD	Co-precipitation	99,8	NA	(AL-NASIRI *et al.*, 2018)
Thymol	β-CD	Aqueous solution	NA	2300	(BOSE *et al.*, 2019)
Thymol	HP-β-CD	Aqueous solution	82,0	430	(CELEBIOGLU *et al.*, 2018b)
Thymol	HP-γ-CD	Aqueous solution	88,0	163	(CELEBIOGLU *et al.*, 2018b)
Thymol	M-β-CD	Aqueous solution	91,0	477	(CELEBIOGLU *et al.*, 2018b)
Thymol	RAMEB	NA	NA	1206	(DAS *et al.*, 2019)
Thymol	β-CD	Aqueous solution	NA	28000	(LOZANO *et al.*, 2017)
Thymol	HP-β-CD	Spray-drying	59,1	NA	(PIRES *et al.*, 2018)
Thymol	HP-β-CD	Freeze-drying, rotary evaporation, supercritical fluid technology	90,0	NA	(PIRES *et al.*, 2018)
Thymol	α-CD	Aqueous solution	NA	23803	(PIRES *et al.*, 2018)
Thymol	β-CD	Aqueous solution	NA	73879	(PIRES *et al.*, 2018)
Thymol	γ-CD	Aqueous solution	NA	9341	(PIRES *et al.*, 2018)
Thymol	HP-β-CD	Aqueous solution	NA	125063	(PIRES *et al.*, 2018)

Dimethyl-β-cyclodextrin (DM-β-CD); encapsulation efficiency (EE); formation constant (K_f); hydroxypropyl-β-cyclodextrin(HP-β-CD); hydroxypropyl-γ-cyclodextrin(HP-γ-CD); inclusion complex (IC); low methylated-β-cyclodextrin (CRYSMEB); methyl-β-cyclodextrin (M-β-CD); mono6-(2-hydroxy-3-(trimethylammonio)propyl)-β-cyclodextrin (EPTAC-β-CD); not available (NA); randomly methylated-β-cyclodextrin (RAMEB); volatile compound (VC).

[a] The co-precipitation coupled to the freeze-drying method is executed as the co-precipitation method described in Table 2 but the freeze-drier is used instead of the vacuum filtration and vacuum desiccator (AL-NASIRI *et al.*, 2018; NIEDDU *et al.*, 2014);

[b] The author only ordered the ICs by the formation constant results (DM-β-CD > HP-β-CD > β-CD) (SUN *et al.*, 2018);

[c] The negative results are explained due to the linalool volatilization during the complexation process and due to the linalool retained on the CV surface (DOS PASSOS MENEZES *et al.*, 2017);

by different methods such as the Benesi-Hildebrand relation (BOSE *et al.*, 2019; LOZANO *et al.*, 2017) and Higuchi and Connors's method (PIRES *et al.*, 2018).

The characterization of the inclusion complex through the determination of the Kf value is fundamental while selecting the best CD for the encapsulated VC guest. However, for some VCs, no data related to K_f are available thus hampering an appropriate characterization of ICs. In some publications (NIEDDU *et al.*, 2014; OLIVEIRA *et al.*, 2016), the ICs characterization is only performed by qualitative methods, which is useful to indicate if an IC has been formed or not but it does not quantify the efficacy of that event, thus explaining the absence of some EE and K_f values in Table 2.

Thus, the lack of data regarding preparation methods and CDs or its uniformization limits in some cases a straightforward comparison of values in each experiment.

3.3 Pharmaceutical and cosmetic applications

Nowadays, rather than assessing ICs constants, *in vitro* and *in vivo* studies are of utmost relevance when selecting the best formulation and preparation parameters (Table 3) (LI *et al.*, 2020; TIEFENSEE RIBEIRO *et al.*, 2019).

KFOURY *et al.* (2016a) demonstrated the activity of the anethole and eugenol on the reduction of the pro-inflammatory cytokines, interleukin-6 (IL-6) and IL-8, on the pulmonary cells and carcinoma liver cells exposed to the airborne particulate matter, i.e. air pollution (KFOURY *et al.*, 2016a). The encapsulation of VCs increased their aqueous solubility and preserved their anti-inflammatory activity, however, no significant improvements in the pharmacological profile were observed (KFOURY *et al.*, 2016a). Nevertheless, KFOURY *et al.* (2016a) considered the HP-β-CD an ecological alternative to surfactants and solvents for the formulation and dissolution of these VCs (KFOURY *et al.*, 2016a).

Borneol was encapsulated in the SBE-β-CD and an increasing effect on the stability and solubility of this VC was observed (XIAO *et al.*, 2020). The administration of borneol/SBE-β-CD IC promoted the opening of the blood-brain barrier and, when co-administered with tetramethylpyrazine, enhanced the brain absorption of this substance, increasing the protection of middle cerebral artery occlusion (XIAO *et al.*, 2020).

Following borneol encapsulation with EPTAC-β-CD its sublimation was reduced and its aqueous solubility was increased (FAN *et al.*, 2019). Its use in a wound dressing provided analgesia, stimulated the muscle growth, and in combination with tobramycin improved its antibacterial effect against skin infections, promoting wound healing (FAN *et al.*, 2019).

Borneol, linalool, and thymol encapsulated in RAMEB enhanced VCs aqueous solubility and provided them protection from the external conditions. These ICs increased the biological properties of the VCs and the results suggested their use as efficient natural preservatives and antioxidants (DAS *et al.*, 2019).

The encapsulation of carvacrol in β-CDs enhanced the bioavailability and the solubility of carvacrol, and it was demonstrated a higher had anticancer activity on prostate cancer cells for carvacrol/β-CD IC compared to free carvacrol (TRINDADE *et al.*, 2019).

The carvacrol/β-CD IC demonstrated higher oral bioavailability without inducing systemic toxicity (TIEFENSEE RIBEIRO *et al.*, 2019). Moreover, IC revealed an efficient anti-inflammatory and antioxidant activity, thereby preventing the loss of dopaminergic neurons, a condition-specific to Parkinson's disease (TIEFENSEE RIBEIRO *et al.*, 2019).

The carvacrol/β-CD IC increased carvacrol's aqueous solubility, stability, and bioavailability. This IC inhibited the nociception and demonstrated an anti-inflammatory activity similar to the dexamethasone, representing a potential to develop new strategies to reduce muscular inflammatory pain (SOUZA *et al.*, 2018).

As a model two-phase study of orofacial pain management, the formalin test was executed in mice. The administration of free carvacrol did not produce an effect in the first phase, while in the second phase produced 28.7% of analgesic inhibition. The administration of carvacrol/β-CD IC led to the reduction of the nociception during the two phases, with 49.3% analgesic inhibition. These results were associated with the higher aqueous solubility and bioavailability provided by the encapsulation of carvacrol (SILVA *et al.*, 2016).

Due to the increased solubility effect on carvacrol, the IC decreased effectively the hyperalgesia at lower doses and for a longer period compared to free carvacrol, thus reducing carvacrol toxicity potential (GUIMARAES *et al.*, 2015).

The caryophyllene/β-CD IC promoted an improvement of the caryophyllene pharmacological profile on the reduction of the hyperalgesia (QUINTANS-JUNIOR *et al.*, 2016). IC reduced the pain by both activation of the cannabinoid receptors type 2 and by reduction of the levels of Fos expression in the spinal cord (QUINTANS-JUNIOR *et al.*, 2016).

The caryophyllene was also encapsulated with M-β-CD, improving the aqueous solubility of this VC (SANTOS *et al.*, 2017). The anti-inflammatory effect of the IC was evaluated by analyzing the edema in mice and the reduction of edema was bigger with the IC compared to the free caryophyllene (SANTOS *et al.*, 2017). A gastric damage was induced by ethanol in mice and the pre-treatment with the caryophyllene/M-β-CD IC prevented this damage more efficiently than free caryophyllene and omeprazole (SANTOS *et al.*, 2017).

Table 3 *In vitro* and *in vivo* characterization studies and results of volatile compounds/cyclodextrins inclusion complexes.

VC	CD	Biological study	Experimental Model	Results[1]	References
Anethole Eugenol	HP-β-CD	Pulmonary and hepatic cells inflammation reduction	*In vitro* (Pulmonary and hepatic cell lines)	Free and both encapsulated VCs decreased by about 90% the levels of pro-inflammatory cytokines after air pollution exposition	(KFOURY *et al.*, 2016a)
Borneol	SBE-β-CD	Middle cerebral artery occlusion prevention	*In vivo* (Rats)	IC co-administered with tetramethylpyrazine enhanced the protection of middle cerebral artery occlusion	(XIAO *et al.*, 2020)
Borneol	EPTAC-β-CD	Wound healing	*In vivo* (Mice Skin)	IC in combination with tobramycin showed the highest reduction in the wound area	(FAN *et al.*, 2019)
Borneol Linalool Thymol	RAMEB	Antioxidant activity; Antimicrobial activity	*In vitro* (DPPH assay); *S. pombe*, *E. coli,* and *S. aureus*	Higher antimicrobial and antioxidant activity using smaller amounts of the three VCs	(DAS *et al.*, 2019)
Carvacrol	β-CD	Inhibition of proliferation of prostate cancer cells	*In vitro* (cancer cell line)	IC effectively reduced cell viability, proliferation, and migration of the PC3	(TRINDADE *et al.*, 2019)
Carvacrol	β-CD	Protection against the dopaminergic denervation	*In vivo* (Rats)	IC prevented the loss of dopaminergic neurons	(TIEFENSEE RIBEIRO *et al.*, 2019)
Carvacrol	β-CD	Reduction of inflammation and nociception	*In vivo* (Rats)	IC protected rats from acute inflammatory and nociceptive alterations	(SOUZA *et al.*, 2018)
Carvacrol	β-CD	Orofacial antinociceptive	*In vivo* (Formalin test in mice)	IC reduced the nociceptive during the two phases of the formalin test	(SILVA *et al.*, 2016)
Carvacrol	β-CD	Cancer pain reduction	*In vivo* (Mice)	IC (50 mg/kg) decreased the hyperalgesia in mice with tumors for 24 h, while free carvacrol (100 mg/kg) promoted effects until 9 h.	(GUIMARAES *et al.*, 2015)
Caryophyllene	β-CD	Anti-hyperalgesic effect	*In vivo* (Mice)	IC inhibited the mechanical hyperalgesia in all tested doses	(QUINTANS-JUNIOR *et al.*, 2016)
Caryophyllene	M-β-CD	Anti-inflammatory effect; Gastric protection	*In vivo* (Mice)	IC reduced the edema in a higher percentage IC reduced efficiently the gastric lesion	(SANTOS *et al.*, 2017)
Caryophyllene	HP-β-CD	Cognitive deficits in vascular dementia	*In vivo* (Rats)	IC improved the memory retention and abilities of spatial learning in rats with induced vascular dementia IC treated groups recovered faster the cerebral blood flow IC reduced the number of abnormal neurons and nerve fibers	(LOU *et al.*, 2017)

VC	CD	Biological study	Experimental Model	Results[1]	References
Cinnamaldehyde	HP-β-CD	Antibacterial activity	*In vitro (E. coli* culture*)*	Nanofibers of IC showed antibacterial activity against *E. coli*.	(YILDIZ *et al.*, 2019)
Cinnamaldehyde	β-CD	Antimicrobial wound dressing	*In vitro (E. coli and S. aureus)*	The highest effective antibacterial activity of IC was well preserved for 60 h	(LIU *et al.*, 2017)
Cinnamaldehyde	β-CD	Antioxidant activity; Anti-inflammatory activity	*In vivo* (Murine)	IC reduced the antioxidant activity Both free cinnamaldehyde and cinnamaldehyde/β-CD reduced the inflammation	(DAVAATSEREN *et al.*, 2017)
Cinnamaldehyde	T-β-CD	Mosquito repellents	*In vitro* and i*n vivo*	The encapsulation in T-β-CD improved the cinnamaldehyde activity in protecting against mosquito	(DELONG *et al.*, 2016)
Citronellal	β-CD HP-β-CD	Antimicrobial activity	*In vitro (Escherichia coli*; *Bacillus subtilis)*	The antimicrobial activity of citronellal was impaired with the encapsulation on both CDs	(ABRIL-SANCHEZ *et al.*, 2019)
Citronellal	β-CD	Anti-hyperalgesic effect	*In vivo* (Chronic muscle pain model in mice)	IC significantly reduced the hyperalgesia, and its effect was long-lasting	(SANTOS *et al.*, 2016)
Eugenol	β-CD	Antibacterial activity Diabetic wound healing	*In vitro* and *in vivo* (Rats Skin)	A hydrogel with eugenol/β-CD inhibited the bacterial proliferation and improve all the parameters that promote wound healing	(LI *et al.*, 2020)
Eugenol	M-β-CD	Antioxidant activity	*In vitro* (DPPH assay)	IC demonstrated a higher and faster antioxidant activity	(CELEBIOGLU *et al.*, 2018a)
Eugenol	β-CD	Antibacterial effect	*In vitro (E. coli* and *S. aureus*)	IC preserved the antibacterial activity of eugenol	(PILETTI *et al.*, 2017)
Limonene	β-CD	Antibacterial and drug-modulatory effect	*In vitro (S. aureus* and *P. aeruginosa)*	Free limonene was efficient against *S. aureus* and *P. aeruginosa* and it promoted a synergistic effect when combined with gentamicin, while IC did not demonstrate any efficacy	(COSTA *et al.*, 2019)
Limonene	β-CD	Anti-hyperalgesic effect	*In vivo* (Chronic muscle pain model in mice)	The anti-hyperalgesic effect was superior and more prolonged with the IC	(ARAUJO-FILHO *et al.*, 2017)
Limonene	M-β-CD	Antibacterial activity	*In vitro (E. coli* and *S. aureus*)	The nanofibers with IC had a stronger antibacterial activity against *E. coli* and *S. aureus*	(AYTAC *et al.*, 2016)
Linalool	β-CD	Antihypertensive effect	*In vivo* (Spontaneously hypertensive rats)	MAP in 20th day with free Linalool: 160.90 ± 7.54 mmHg MAP in 15th day with Linalool/β-CD: 145.66 ± 4.13 mmHg	(CAMARGO *et al.*, 2018)

VC	CD	Biological study	Experimental Model	Results[1]	References
Linalool	HP-β-CD M-β-CD HP-γ-CD	Antibacterial activity	*In vitro (E. coli* and *S. aureus*)	Growth inhibition rate against *E. coli* and *S. aureus*, respectively: linalool/HP-β-CD: 84% and 70% linalool/M-β-CD: 93% and 79% linalool/HP-γ-CD: 95% and 88%	(AYTAC *et al.*, 2017)
Linalool	β-CD	Gastroprotective effect	*In vivo* (Ulcers induced by absolute ethanol in mice)	The dose of linalool in the IC was lower and with a significantly superior effect	(DA SILVA *et al.*, 2016)
Linalool	β-CD	Anti-hyperalgesic activity	*In vivo* (Chronic muscle pain model in mice)	IC showed antihyperalgesic activity for a longer period	(NASCIMENTO *et al.*, 2014)
Terpineol	β-CD	Anti-hyperalgesic activity	*In vivo* (Chronic muscle pain model in mice)	IC had a long-lasting analgesic effect	(OLIVEIRA *et al.*, 2016)
Thymol	HP-β-CD	Antifungal activity	*In vitro (Trichophyton mentagrophytes* and *Candida albicans)*	The antifungal activity against *Trichophyton mentagrophytes* of the IC increased about 22 times A better antifungal action against *Candida albicans* was achieved with the IC at lower concentrations	(PIRES *et al.*, 2018)
Thymol	HP-β-CD HP-γ-CD M-β-CD	Antioxidant activity	*In vitro* (DPPH assay)	The IC had an antioxidant activity over 95% while free thymol had only 25%	(CELEBIOGLU *et al.*, 2018b)
Thymol	α-CD β-CD	Antioxidant activity; Antimicrobial activity	*In vitro* (DPPH assay); *B. subtilis*	IC showed a higher antioxidant activity Thymol/α-CD did not improve the antibacterial activity of thymol and the results of thymol/β-CD IC were inconclusive	(BOSE *et al.*, 2019)
Thymol	β-CD	Genotoxic activity	*In vitro* (DNA)	IC significantly reduced the interactions between thymol and DNA	(LOZANO *et al.*, 2017)
Thymol	β-CD	Intestinal bacterial diseases	*In vivo* (Pigs)	IC prolonged the gastrointestinal transit time of thymol, promoting a topical effect on the intestine against the bacterial diseases	(NIEDDU *et al.*, 2014)

Cyclodextrin (CD); deoxyribonucleic acid (DNA); 2,2-diphenyl-1-picrylhydrazyl (DPPH); hydroxypropyl-β-cyclodextrin (HP-β-CD); hydroxypropyl-γ-cyclodextrin (HP-γ-CD); inclusion complex (IC); mean arterial pressure (MAP); methyl-β-cyclodextrin (M-β-CD); mono6-(2-hydroxy-3-(trimethylammonio)propyl)-β-cyclodextrin (EPTAC-β-CD); not available (NA); prostate cancer cell line (PC3); randomly methylated-β-cyclodextrin (RAMEB); reactive oxygen species (ROS); sulfobutylated-β-cyclodextrin (SBE-β-CD); triazinyl-β-cyclodextrin (T-β-CD); volatile compound (VC).

[1]Unless noticed biological effect refers its comparison with free VC

Besides the anti-inflammatory action, the antioxidant effect of caryophyllene promoted protective effects on the gastric mucosa (SANTOS *et al.*, 2017).

The HP-β-CD was used to encapsulate the caryophyllene towards an improvement of the solubility and bioavailability of the VC. Moreover, caryophyllene/HP-β-CD improved the cerebral blood supply, suppressed neuronal apoptosis, and decreased the deterioration of nerve fibers in vascular dementia model rats, resulting in an improvement of memory and spatial learning. The IC activated the cannabinoid receptor type 2, which is associated with neuroprotection against brain disorders (LOU *et al.*, 2017).

The cinnamaldehyde/β-CD IC improved the stability and the aqueous solubility of the cinnamaldehyde (YILDIZ *et al.*, 2019). This IC was incorporated in nanofibers and showed antimicrobial activity against *E. coli* (YILDIZ *et al.*, 2019). LIU *et al.* (2017) used the same delivery system to form a wound dressing, which showed antibacterial activity against *E. coli* and *S. aureus* for 60h . One of the hypotheses for this long antibacterial activity is the cell permeability enhancing effect promoted by the cinnamaldehyde and consequently the death of microorganisms. Besides, cinnamaldehyde/β-CD IC incorporated in nanofibers contrarily to cinnamaldehyde did not show cytotoxicity in human cells (LIU *et al.*, 2017).

Following the encapsulation of cinnamaldehyde in β-CDs, higher stability to oxidation and a controlled release profile at higher temperatures was observed for the IC when compared to the free cinnamaldehyde. The IC did not demonstrate immediate antioxidant activity, probably because the encapsulation hampered the reaction of the cinnamaldehyde with the free radicals, but over time the release of the VC has translated into antioxidant activity. The anti-inflammatory activity of the IC studied by measuring the levels of nitric oxide production showed a similar effect compared to the free cinnamaldehyde (DAVAATSEREN *et al.*, 2017).

Cinnamaldehyde is a mosquito repellent and this activity was improved following its incorporation in a hydrogel coupled to T-β-CD. The encapsulation in T-β-CD improved the duration of the maximum protection of the VCs against the mosquitos by 2h (DELONG *et al.*, 2016).

The citronellal was encapsulated with β-CD and HP-β-CD and the critical micellar concentration (CMC), the maximum concentration of a compound in a solution that does not form aggregates, was significantly reduced, indicating an increase of the citronellal solubility. The citronellal has an intense scent, but its rapid volatilization limits its applicability. The presence of the CDs increased its permanence from 2h to 24h, corroborated by the gas chromatography-mass spectrometry (GC-MS) and a sensorial analysis by humans (ABRIL-

SANCHEZ *et al.*, 2019). Another characteristic of the citronellal is the antimicrobial activity, however, this process was impaired with the addition of the CDs. One of the hypotheses to this occurrence was the digestion by amylases of the CDs, which means a carbon source to the microorganisms. When the authors added a substance to prevent the degradation of the CDs, the results demonstrated a significant improvement in the antimicrobial activity and the duration of the efficacy (ABRIL-SANCHEZ *et al.*, 2019).

SANTOS *et al.* (2016) encapsulated citronellal in β-CD and the anti-hyperalgesic effect of the citronellal was improved, with values that can be compared to the standard drug tramadol (SANTOS *et al.*, 2016). The mechanism subjacent is possibly due to the interaction between the citronellal and the glutamate receptors, leading to the activation of the descending inhibitory pathway. Usually, these type of drugs affects muscular strength, but that secondary effect was not confirmed with the citronellal/β-CD IC. Besides, the daily treatment did not present signs of addiction or tolerance (SANTOS *et al.*, 2016).

LI *et al.* (2020) created a wound dressing based on a hydrogel with eugenol/β-CD ICs, increasing the stability and the aqueous solubility and promoting a sustained release of the VC, remaining at higher concentration and for an extended time in the wound dressing (LI *et al.*, 2020). The results *in vitro* demonstrated an increase in the number of migrated cells, a higher tube formation, and inhibition of the inflammation mediator's activation, increasing the angiogenesis. *In vivo* results showed an accelerated wound contraction and an enhanced wound re-epithelialization rate at day 10. In addition, the bacterial proliferation was totally inhibited for 10 days (LI *et al.*, 2020).

The eugenol is a natural VC with a relevant antioxidant activity (CELEBIOGLU *et al.*, 2018a). To assess this property, eugenol was encapsulated in CDs and nanofibers by electrospinning and results revealed an improving effect on the aqueous solubility and the thermal stability of the eugenol. The M-β-CD showed better results providing a faster antioxidant reaction and a total antioxidant activity in the given period compared to free eugenol (CELEBIOGLU *et al.*, 2018a).

PILETTI *et al.* (2017) also demonstrated the increase of the thermal stability of eugenol by following their encapsulation in β-CD, which preserved the eugenol antibacterial activity at higher temperatures (PILETTI *et al.*, 2017).

The antibacterial studies whit free limonene revealed its efficacy against *S. aureus* and *P. aeruginosa*, as well as a synergistic effect when combined with gentamicin. When encapsulated in a β-CD, the limonene did not have an influence on the gentamicin antibacterial

effect, nor were there improvements in the activity of the limonene, probably because the β-CD disturb the interaction between the VC and the bacterial cells (COSTA *et al.*, 2019).

The limonene/β-CD demonstrated a higher aqueous solubility, stability, and a sustained release of the limonene, showing a higher antihyperalgesic effect at the same dose and a long-lasting effect compared with the free limonene. This IC reduced the expression of Fos protein, reducing the transmission of the pain impulses through the spinal cord. Besides, its antihyperalgesic effect is related to its interaction with GABAergic receptors. In addition, the Limonene/β-CD did not affect muscular strength (ARAUJO-FILHO *et al.*, 2017).

AYTAC *et al.* (2016) developed a nanofiber containing limonene/M-β-CD ICs, increasing the aqueous solubility, stability, and providing a sustained release to the limonene. The growth inhibition rate was 20% higher compared to the free limonene, demonstrating an improvement in the antibacterial activity against the *E. coli* and *S. aureus* (AYTAC *et al.*, 2016).

The antihypertensive effect of linalool was studied in spontaneously hypertensive rats and compared to the effect provided by the Linalool/β-CD IC. IC showed a more pronounced reducing effect on the arterial blood pressure and the beneficial effects were observed earlier when compared to the free linalool. Besides, the IC provided linalool higher stability and increased bioavailability (CAMARGO *et al.*, 2018).

Linalool was encapsulated in three different types of modified CDs, HP-γ-CD, HP-β-CD, and M-β-CD to produce antibacterial nanofibers. The results obtained demonstrated that nanofibers loaded with linalool/M-β-CD ICs had the highest thermal stability and K_f, promoting a controlled release effect. The nanofibers loaded with linalool/HP-γ-CD ICs demonstrated a higher aqueous solubility, achieving the best antibacterial results against *E. coli* and *S. aureus* (AYTAC *et al.*, 2017).

Linalool/β-CD demonstrated a higher aqueous solubility and stability. Its administration at a dose of 40 mg/kg reduced the gastric lesion area in 94.2%, showing a gastroprotective effect in a dose ten times lower than free linalool. The antioxidant activity of the linalool was enhanced due to its encapsulation, increasing the levels of sulfhydryl compounds, which protect the gastric mucosa from free radicals. Besides, the levels of neutrophil infiltration were reduced, as well as the lipid peroxidation, that causes oxidative stress and it is strongly associated with gastric ulcers (DA SILVA *et al.*, 2016).

Linalool and linalool/β-CD IC demonstrated an antihyperalgesic effect without affecting the muscular strength, probably due to an interaction with the descending inhibitory pain pathway once it was verified a significant reduction of the Fos protein in spinal cord neurons. The most important difference between the free linalool and the IC was the half-life. The

antihyperalgesic effect remained for 24h after the administration of the IC whereas the free linalool revealed a shorter half-time. This effect was due to the improvement of the water solubility provided by the encapsulation of the VC (NASCIMENTO *et al.*, 2014).

The encapsulation of terpineol with the β-CD promoted a higher aqueous solubility, a higher bioavailability, and a higher shelf-life. The IC had a long-lasting analgesic effect compared with the free terpineol. Its effects were associated with the modulation of the opioid and serotoninergic systems, which promotes the activation of the inhibitory descending pathway and reduces the transmission of pain. Although the terpineol mechanism of action involves the non-specific muscle relaxation effects and CNS depression, no changes in muscle strength and motor coordination were observed (OLIVEIRA *et al.*, 2016).

Thymol was encapsulated in different CDs and HP-β-CD was the most appropriate. The antifungal activity of the thymol was significantly improved following its encapsulation in HP-β-CD, probably due to the improvement of the solubility of thymol. In addition, the encapsulation increased 354% of the shelf-life compared to the free thymol, as well as higher stability at high temperatures (PIRES *et al.*, 2018).

CELEBIOGLU *et al.* (2018b) produced for the first-time nanofibers without polymers, by using the CDs and taking advantage of their capacity to form aggregates and self-assemble in highly concentrated solutions (CELEBIOGLU *et al.*, 2018b). The incorporation of the thymol in the CDs improved its aqueous solubility and thermal stability, resulting in an antioxidant activity close to 100%. Nanofibers loaded with the linalool/M-β-CD IC showed an antioxidant activity at lower doses concentrations, probably to the higher EE of thymol encapsulated by the M-β-CD (CELEBIOGLU *et al.*, 2018b).

Thymol encapsulation complexes with α-CD and β-CD did not significantly improve the antioxidant activity compared to the free thymol. About antibacterial activity, the results did not demonstrate much improvement in comparison to free thymol, leading to conclude that the CDs did not hinder the thymol (BOSE *et al.*, 2019).

LOZANO *et al.* (2017) demonstrated that thymol interacts with deoxyribonucleic acid (DNA), confirming its genotoxicity potential (LOZANO *et al.*, 2017). To reduce the interaction between thymol and DNA, this VC was encapsulated in a β-CD. After encapsulation thymol became partially unavailable decreasing its interaction establishment with DNA and consequently its genotoxicity (LOZANO *et al.*, 2017).

Thymol is also known for its antimicrobial properties, but its low aqueous solubility limits their efficacy. The thymol encapsulation with a β-CD was suggested by NIEDDU *et al.* (2014) to surpass these barriers (NIEDDU *et al.*, 2014). Besides, the polymer Eudragit® E PO

was added to the Thymol/β-CD IC to mask the strong unpleasant taste and smell of thymol. This additive did not affect the encapsulation process of thymol; thus, this formulation was the one chosen to proceed with the *in vitro* and *in vivo* studies. In pharmacokinetic studies, compared to the free thymol, the IC had a faster absorption rate and a long half-life (22h), allowing to reduce the number of administrations per day. Also, the results demonstrated that thymol is mainly absorbed by the stomach and it is poorly absorbed by the intestinal mucosa. Furthermore, the IC showed an increasing effect on the gastrointestinal transit time of thymol thus promoting the accumulation of the not absorbed thymol along the intestinal tract surface. This phenomenon may allow thymol to exert a local therapeutic effect on the intestinal mucosa against intestinal bacterial diseases (NIEDDU *et al.*, 2014).

4 Toxicity issues

One important concern is the safety of the use of VC/CD ICs in pharmaceutical and cosmetic products.

CDs are pharmacological inert, since do not present therapeutic effects by themselves (COSTA *et al.*, 2019; KFOURY *et al.*, 2016a; LI *et al.*, 2020). They are classified as GRAS by the FDA (OLIVEIRA *et al.*, 2017). They are biocompatible and no activation of the immune response following oral administration has been described (RECIO *et al.*, 2018; ZHANG *et al.*, 2017). However, in parenteral formulations, α-CD, β-CD, and RM-β-CD showed renal toxicity at relatively low doses (STELLA E HE, 2008), while HP-β-CD and SBE-β-CD are considered safe at relatively high doses (IRIE E UEKAMA, 1997).

Studies in human volunteers have shown CDs have a significant safety margin in the dermal application (IRIE E UEKAMA, 1997). HP-β-CD was considered as safe as materials currently being used in perfumes and cosmetics (IRIE E UEKAMA, 1997).

Although the VCs are classified as GRAS by the FDA a high therapeutic dose may be required to achieve adequate bioavailability thus rising toxicity issues. The encapsulation of the VCs with CDs increases their aqueous solubility thus increasing oral bioavailability. Accordingly, decreasing the dose of VC will reduce eventually toxicity phenomena (GUIMARAES *et al.*, 2015). There have been several publications regarding the toxicity of VC encapsulation by CD. Cinnamaldehyde demonstrated cytotoxicity while the cinnamaldehyde/β-CD IC incorporated in nanofibers did not show any cytotoxicity in human cells (LIU *et al.*, 2017). TIEFENSEE RIBEIRO *et al.* (2019) studied the toxicity of the carvacrol/β-CD IC and no alterations in blood cell profile or hepatotoxicity were observed (TIEFENSEE RIBEIRO *et al.*, 2019). LOU *et al.* (2017) highlighted the requirement of additional studies to

determine the safety and possible side effects of caryophyllene/HP-β-CD. In other cases, the purpose of the encapsulation was specifically to reduce the genotoxicity of the thymol by its encapsulation in β-CD (LOZANO *et al.*, 2017).

5 Regulatory issues

The presence of CDs can be found in at least 35 approved pharmaceutical products, (GUIMARAES *et al.*, 2015) and in cosmetic products (SHARMA E BALDI, 2016). CDs are considered as "generally recognized as safe" (GRAS), as food additives on the World Health Organization's website (JANSOOK *et al.*, 2018) and they include FDA Inactive Ingredients Database (FDA, 2020), as well as in European Medicines Agency (EMA) in the annex with the excipients known to have a recognized action or effect (EMA, 2019).

CDs are also described in Japanese Pharmaceutical Codex, in the United States Pharmacopeia/National Formulary, and the European Pharmacopoeia (JANSOOK *et al.*, 2018; OLIVEIRA *et al.*, 2017; SUVARNA *et al.*, 2017). Although VCs have a natural origin, they are pure isolated single compounds thus they can be approved as new drugs by the regulatory agencies such as EMA and FDA, demonstrating quality, security, and efficacy as any other chemical substance (CECHINEL-FILHO, 2012).

6 Conclusions

The number of studies targeting VCs of EOs has been increasing, including their inclusion in cyclodextrins towards a deeper and time-depth physicochemical and biological evaluation of ICs when compared to their origin essential oils.

With the increase in consumer concerns regarding environment protection, natural-origin VCs encapsulated in CDs are a potential alternative therapeutic to chemical substances. Some ICs, such as carvacrol/β-CD, caryophyllene/M-β-CD, and citronellal/β-CD ICs perform equivalent therapeutic effects to approved drugs, like dexamethasone, omeprazole, and tramadol, respectively. Also, certain ICs, when co-administered with other drugs, improve their pharmacological profile, reducing the drug amount required to have a therapeutic effect. For example, borneol/SBE-β-CD and borneol/EPTAC-β-CD ICs increased the tetramethylpyrazine absorption and improved the tobramycin effect, respectively.

The VC/CD ICs also represent a relevant and new therapeutic approach in the management of several diseases, particularly chronic pain conditions. The encapsulation of caryophyllene, citronellal, limonene, linalool, and terpineol demonstrated a significant reduction of the hyperalgesia, by the activation of the descending pain inhibition pathway, while

not affecting the muscular strength, a possible secondary effect of some antihyperalgesic drugs. Besides these ICs have the advantage to provide a long-lasting anti-hyperalgesic effect, reducing the diary doses and the toxicity of VCs.

The CDs significantly improve the aqueous solubility of the VCs and the results *in vivo* and *in vitro* demonstrated that this improvement had an impact on the increase of the bioavailability and the pharmacological effect of VCs. Among the VC-containing ICs effects, stands out the anticancer effect of the carvacrol, the antihypertensive effect of linalool, the neuroprotection against brain disorders of limonene, and the anti-inflammatory and anti-oxidant promoted by most of the VCs. The antibacterial effects of VCs were not always improved by their encapsulation with CDs once the bacteria with amylases are able to degrade CDs and use them as a carbon source to grow.

The physical stabilization provided by the CDs is of utmost relevance since numerous pharmaceutical and cosmetic products are produced using some level of heating and in topical preparations, the product is spread on the skin and must support environmental hard conditions. Besides, several authors applied VC/CD ICs in wound dressings to wound healing, by exploiting and improving the antibacterial and anti-inflammatory characteristics of the VCs, as borneol, eugenol, and cinnamaldehyde and the results were very satisficing due to the protection to external conditions and the extended-release.

The CD with more potential to encapsulate volatile compounds is the β-CD once this CD has a suitable cavity to compounds with the size of the volatile compounds. However, this CD has low solubility, which can be surpassed by its functionalization, thereby suggesting RAMEB, DM-β-CD, and M-β-CD as valid alternatives.

Generally, the preparation techniques that use co-solvents to dissolve the CDs and VCs have shown better efficacy results, with a highlight of the freeze-drying method. This technique also has the advantages of easy scale-up and it is optimal to use with thermolabile compounds, as the VCs.

The stability and pharmacological improvements demonstrated by the ICs, the high biocompatibility and biodegradability of the CDs as their simplicity and low cost of synthesis and the increasing number of studies realized with CDs and VCs reveal that CDs are one of the most adequate encapsulation materials for VCs.

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