UNIVERSIDADE D
COIMBRA

Ana Isabel Caldeira Freitas dos Santos

Relatórios de Estágio e Monografia intitulada "Dendrimers as Pharmaceutical Excipients" referentes à Unidade Curricular "Estágio", sob a orientação, da Dra. Joana Anastácio Ramos, da Dra. Ana Leite e Silva e pela Professora Doutora Ana Rita Ramalho Figueiras apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro de 2019

Faculdade de Farmácia da Universidade de Coimbra

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Coimbra, 2 de setembro de 2019.

Ana Isabel Caldeira Freitas dos Santos

(Ana Isabel Caldeira Freitas dos Santos)

Agradecimentos

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Índice

Resumo

O estágio curricular é um momento crucial de aplicação de todos os conhecimentos adquiridos, sendo também um período de grande aprendizagem. Com base nesta evidência, decidi repartir o meu estágio curricular por duas áreas diferentes (Indústria Farmacêutica e Farmácia Comunitária), de modo a contactar com várias realidades e ganhar uma nova visão sobre o mercado farmacêutico.

O estágio nos Laboratórios Basi possibilitou a perceção do papel do farmacêutico no funcionamento de uma indústria farmacêutica e no ciclo do medicamento, garantindo a sua eficácia, segurança e qualidade. Relativamente ao estágio na Farmácia Coimbra, este permitiu obter experiência na organização e gestão de uma farmácia, bem como experiência profissional na dispensa de medicamentos e no contacto com o público.

Os relatórios de estágio estão sob a forma de uma análise SWOT, tendo sido elaborada uma lista de pontos positivos e negativos, de âmbito quer interno, quer externo, relativo ao estágio.

A monografia tem como objetivo discutir as propriedades que tornam os dendrímeros em excipientes farmacêuticos e suas possíveis aplicações nos campos farmacêutico e biomédico. Embora os dendrímeros não tenham uma monografia descrita numa farmacopeia oficial e, deste modo, não poderem ser considerados excipientes farmacêuticos, estas nanoestruturas têm recebido grande atenção por parte dos investigadores. Devido às suas propriedades únicas, tais como dimensões em nanoescala, alto grau de ramificação, polivalência, alta solubilidade em água, presença de cavidades internas e alta biocompatibilidade, os dendrímeros são candidatos ideais como excipientes farmacêuticos. Estas características permitem aumentar, eficazmente, a solubilidade de fármacos com baixa solubilidade em água. O facto de as propriedades de um dendrímero poderem ser controláveis durante a sua síntese, torna estas nanoestruturas agentes promissores para aplicações de libertação controlada de fármacos, em várias formulações farmacêuticas. Além disso, os dendrímeros podem ser usados para reduzir a toxicidade do fármaco e para o aumento da eficácia do mesmo.

Palavras-chave: Laboratórios Basi, Farmácia Coimbra, Análise SWOT, Dendrímeros, Excipiente Farmacêutico, Nanotransportador, Propriedades Físico-Químicas.

Abstract

The curricular internship is a crucial moment of application of all the acquired knowledge, being also a period of great learning. Based on this evidence I decided to divide my internship into two different areas (Pharmaceutical Industry and Community Pharmacy), in order to contact with various realities and gain a new perspective on the pharmaceutical market.

The internship at Laboratórios Basi, enabled a more real perception of the pharmacist's role on the pharmaceutical industry and the on the product's lifecycle, ensuring its effectiveness, safety, and quality. Regarding the internship at Farmácia Coimbra, this allowed a gaining of experience in the organization and management of a pharmacy, as well as professional experience in dispensing medicines and on contact with the public.

The internship reports are written in the form of a SWOT analysis. Therefore, they provide a list of positive and negative aspects on the internship internal and external realm.

The monograph aims to discuss the properties that turn dendrimers into pharmaceutical excipients and their potential applications in the pharmaceutical and biomedical fields. Although dendrimers do not have a pharmacopoeia monograph and, officially, cannot be recognized as pharmaceutical excipients, these nanostructures have received enormous attention from researchers. Due to their unique properties, like the nanoscale uniform size, a high degree of branching, polyvalency, aqueous solubility, internal cavities and biocompatibility, dendrimers are ideal as excipients, enhancing the solubility of poorly water-soluble drugs. The fact that the dendrimer's properties are controllable during their synthesis renders them as promising agents for drug delivery applications in several pharmaceutical formulations. Additionally, dendrimers can be used for reducing the drug toxicity and for the enhancement of the drug efficacy.

Keywords: Laboratórios Basi, Farmácia Coimbra, SWOT Analysis, Dendrimer, PAMAM Dendrimer, Pharmaceutical Excipient, Nanocarriers, Physicochemical Properties.

Abreviaturas

API	Do inglês, *Active Pharmaceutical Ingredient*
BBB	Do inglês, *Blood-Brain Barrier*
BCS	Do inglês, *Biopharmaceutics Classification System*
cGMP	Do inglês, *Current Good Manufacturing Practices*
CNS	Do inglês, *Central Nervous System*
DCFPC	Delegação Centro da Fundação Portuguesa de Cardiologia
DCI	Denominação Comum Internacional
Dox	Do inglês, *Doxorubicin*
ELISA	Do inglês, *Enzyme Linked Immunosorbent Assay*
EMA	Do inglês, *European Medicines Agency*
FDA	Do inglês *Food and Drug Administration*
FI	Folheto Informativo
G	Do inglês, *Generation*
G5-^{3}H	Do inglês, *G5 PAMAM dendrimer coupled to the radioactive tritium marker*
G5-^{3}H-FA	Do inglês, *G5 PAMAM dendrimer coupled to folic acid and the radioactive tritium marker*
ICH	Do inglês, *International Conference on Harmonisation*
JD	Do inglês, *Janus Dendrimer*
miR-15	Do inglês, *MicroRNA-150*
MNSRM	Medicamentos Não Sujeitos a Receita Médica
MSRM	Medicamentos Sujeitos a Receita Médica
NM	Do inglês, *Nanomaterial*
PAMAM	Do inglês, *Poly(amidoamine)*
PEG	Do inglês, *Polyethylene glycol*
PLL	Do inglês, *Poly-L-lysine*
PPI	Do inglês, *Poly(propyleneimine)*
PrP	Do inglês, *Prion Protein*
RCM	Resumo das Características do Medicamento
ROS	Do inglês, *Reactive Oxygen Species*
SAMS	Serviço de Assistência Médico Social
SWOT	Do inglês, *Strengths*, *Weaknesses, Opportunities* e *Threats*

1. Relatório de Estágio

em Indústria Farmacêutica

basi®
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your health, our world

1.1. Introdução

De acordo com o artigo 77.º e 78.º do Decreto-Lei 288/2001 [1], o Ato Farmacêutico pode ser executado em diversas áreas para além do campo clássico da Farmácia de Oficina. Deste modo, o farmacêutico, sendo um profissional de saúde multidisciplinar, especialista do medicamento e agente de saúde pública, desempenha um papel chave na contribuição para o bem-estar da sociedade. Desta forma, optei pela realização de uma parte do estágio numa indústria farmacêutica, de modo a contactar com uma outra realidade da profissão farmacêutica, além da área de farmácia comunitária.

A indústria farmacêutica inclui inúmeras atividades relacionados com o medicamento que podem ser realizadas por farmacêuticos. Destas, destaca-se a farmacovigilância, os ensaios clínicos, a investigação e o desenvolvimento galénico, os assuntos regulamentares, a garantia de qualidade, a produção do medicamento, bem como o *marketing* farmacêutico.

Deste modo, os Laboratórios Basi foi a empresa escolhida para a realização do meu estágio, por forma a contactar com as diversas atividades inerentes à indústria farmacêutica. Foi no campo de controlo e garantia da qualidade que tive a oportunidade de estagiar e completar a minha formação enquanto farmacêutica.

O meu estágio em indústria farmacêutica teve início a 7 de janeiro e terminou a 2 de abril de 2019, sob a orientação da Dr.ª Joana Ramos.

O presente relatório encontra-se sob a forma de uma análise SWOT (do inglês, *Strengths*, *Weaknesses, Opportunities* e *Threats*). Esta ferramenta permite descrever, de forma crítica, toda a minha experiência como estagiária nos Laboratórios Basi, analisando os pontos fortes (*Strengths*), os pontos fracos (*Weaknesses*), as oportunidades (*Opportunities*) e as ameaças (*Threats*) relativamente à frequência do estágio curricular e à integração da aprendizagem teórica na prática profissional.

1.2. Laboratórios Basi

Laboratórios Basi é uma empresa portuguesa que iniciou a sua atividade em 1956, tendo como principal objetivo o desenvolvimento, fabrico e comercialização de medicamentos humanos e outros produtos farmacêuticos. Atualmente, encontra-se sedeado na Zona Industrial de Mortágua, sendo constituída, por cinco departamentos: i) o Departamento de Produção, ii) o Departamento de Controlo da Qualidade, iii) o Departamento de Garantia da Qualidade iv) o Departamento de Assuntos Regulamentares, e v) o Departamento de Desenvolvimento Galénico. Toda a empresa foi estruturada de forma a evitar a contaminação cruzada, tendo por base o fluxo lógico das diferentes atividades que decorrem no seu interior [2].

Relativamente ao departamento de produção, é nesta área que se fabricam as várias formas farmacêuticas. Nos Laboratórios Basi produz-se, essencialmente, formulações farmacêuticas líquidas (destacando-se a produção de xaropes, soluções, emulsões e suspensões) e formulações farmacêuticas semissólidas (tais como geles, cremes, pomadas e supositórios). Além destas formulações, os Laboratórios Basi dispõem de linhas para o acondicionamento secundário de outras formas farmacêuticas.

No que toca ao Departamento de Controlo de Qualidade, este é constituído por um laboratório de controlo físico-químico, onde se realizam as análises por HPLC (do inglês, *High performance liquid chromatography)*, por Cromatografia Gasosa, por espetroscopia de UV-VIS e infravermelho, por potenciometria e ainda os ensaios de desagregação e dissolução. Para além deste laboratório, o Departamento de Controlo de Qualidade possui também um laboratório de microbiologia, dotado de equipamentos e recursos necessários para realizar ensaios de esterilidade, ensaios de endotoxinas, contagem de microrganismos aeróbios viáveis totais, contagem de fungos e leveduras, pesquisa de microrganismos específicos e testes de eficácia de conservantes.

O Departamento de Garantia da Qualidade dedica-se a assegurar que as operações de produção e controlo da qualidade se encontram especificadas por escrito através de procedimentos operacionais, devidamente aprovados. Este departamento é também responsável pelo desenvolvimento de programas de validação de processos, validação de limpeza, calibração e qualificação dos equipamentos e instrumentos, em conjunto com os outros departamentos.

No Departamento de Desenvolvimento Galénico, realizam-se estudos no desenvolvimento de novas formulações e produtos farmacêuticos, bem como a realização de ensaios que tenham a finalidade de melhorar os diversos processos de fabrico do produto.

Por fim, é no Departamento de Assuntos Regulamentares que se tenta resolver quaisquer questões relacionadas com a aprovação regulamentar do medicamento, desde a sua fase de Investigação e Desenvolvimento, garantindo o desenvolvimento de forma célere, cobrindo todo o ciclo de vida do medicamento.

Exteriormente aos Laboratórios Basi, encontra-se uma cantina, uma sala de convívio e um gabinete médico.

No ano de 2016, o Laboratório Basi, deu início à implementação do projeto industrial para produção de soluções parentéricas de grande e pequeno volume.

Por fim, os Laboratórios Basi, sendo reconhecida como uma empresa de referência no seu setor de atividade, com uma história de mais de 50 anos, erigida sobre uma enorme diversidade de acontecimentos, experiências e aprendizagens, preocupa-se em consolidar uma imagem que reflita os seus principais valores.

1.3. Análise SWOT

1.3.1. Forças

Formação académica

O Mestrado Integrado em Ciências Farmacêuticas da Faculdade de Farmácia da Universidade de Coimbra proporcionou-me conhecimentos de vários temas integrados em diversas unidades curriculares, tais como Farmácia Galénica, Métodos Instrumentais de Análise, Tecnologia Farmacêutica, Assuntos Regulamentares e Gestão e Garantia da Qualidade. A dimensão teórica e prática das disciplinas, ao longo dos quatro anos e meio de curso, preparou-me para ter os conhecimentos fundamentais sobre a área industrial farmacêutica. Desta forma, estava familiarizada com muitos dos termos e técnicas com que me deparava no decurso do estágio.

Desenvolvimento das minhas competências

A realização do estágio nos Laboratórios Basi permitiu colocar as minhas competências à prova, devido ao rigor e à exigência presentes em todo o trabalho realizado diariamente no Departamento de Controlo de Qualidade. Deste modo, durante o estágio senti um ganho progressivo de autonomia (1.3.2. Fraquezas) no meu trabalho e aprendi a gerir o tempo de forma a dar uma resposta mais adequada ao volume de trabalho que me era exigido.

Capacidade de trabalho autónomo

Ao logo do estágio fui confrontada com várias situações em que demonstrei capacidade de trabalho autónomo. Em primeiro lugar, foi-me pedido para realizar a validação do teste de identificação de várias matérias primas, tais como aroma de laranja, aroma dos frutos do bosque, triglicéridos e Lanette® 16, utilizando a espetroscopia Raman portátil. Para a realização da validação, recorri não só à bibliografia disponível no Departamento de Controlo de Qualidade, tais como as farmacopeias Europeia e Britânica, bem como ao manual de instruções da espetroscopia Raman. Sempre que necessário recorri, também, aos colaboradores do laboratório físico-químico, que sempre se demonstraram disponíveis em me ajudar e a esclarecer as minhas dúvidas (1.3.3. Oportunidades). Atualmente, o método de identificação dos triglicéridos por via espetroscopia Raman, que desenvolvi e validei durante o meu estágio, encontra-se aprovado pela empresa e em uso.

Em segundo lugar, foi-me pedido para procurar informação sobre a validação do método de identificação de matérias primas pela espetroscopia de Infravermelho. Deste modo, desenvolvi um protocolo de validação de métodos de identificação de matérias primas por espetroscopia de infravermelho, tendo por base os requisitos descritos nas *guidelines* de validação analítica da Agência Europeia do Medicamento (EMA) e do Conselho Internacional para Harmonização (ICH). Este protocolo foi posteriormente apresentado e aceite. Contudo, devido a uma avaria do equipamento, o protocolo de validação de identificação de matérias primas não chegou a ser posto em prática durante a realização do estágio.

Conhecimentos da língua inglesa

Nos dias de hoje, dominar a língua inglesa é fundamental e indispensável, quer na vida pessoal, quer na vida profissional.

Durante o meu estágio na indústria, os meus conhecimentos da língua inglesa foram constantemente colocados à prova, pois traduzi um grande número de protocolos analíticos em português para inglês. Estes, por sua vez, eram corrigidos e verificados por um colega. Devido à minha facilidade de tradução, o meu trabalho tornou-se mais rápido e produtivo para a empresa.

Além da tradução de documentos, tive a oportunidade de estar presente numa reunião via *online*, com uma cliente estrangeira, em que a toda a comunicação foi feita em inglês.

1.3.2. Fraquezas

Insegurança Inicial

Tendo sido este estágio o meu primeiro contacto com uma indústria farmacêutica, no início surgiu-me uma certa timidez e insegurança normal na realização das tarefas que me foram propostas, o que limitou um pouco a minha autonomia. Contudo, com o decorrer do estágio, com a aquisição de conhecimentos, bem como com a observação do modo de proceder no laboratório, fui ganhando mais autonomia e confiança na realização das tarefas propostas, maximizando a minha aprendizagem (1.3.1. Forças).

Conhecimentos da língua francesa

No decorrer do meu estágio na indústria farmacêutica, deparei-me com documentos em francês. Foi-me pedido, várias vezes, para traduzir documentos em francês para inglês. Devido ao meu conhecimento apenas rudimentar da língua francesa, tive algumas dificuldades

na tradução e a entender certos termos científicos que estavam descritos no documento. Contudo, devido à ajuda e disponibilidade de um colega, a tradução era posteriormente analisada e corrigida.

Desta forma, a tradução de documentos de francês para inglês, permitiu melhorar o meu vocabulário, o que considero bastante benéfico.

1.3.3. Oportunidades

Entidade acolhedora

No primeiro dia de estágio fui recebida por uma pessoa dos recursos humanos, tendo tido uma receção igual à de um novo colaborador. Nessa receção, recebi um manual de acolhimento e um código de acesso para o almoço na cantina da empresa. Logo de seguida, fui encaminhada para uma sala onde decorreram duas apresentações sobre a empresa e suas normas internas, sobre gestão de resíduos e saúde ambiental, bem como informações acerca da higiene e segurança no trabalho. Após as apresentações, efetuei uma avaliação de escolha múltipla relacionada com os temas apresentados.

Seguidamente, fui visitar as diferentes secções do Laboratório de Controlo de Qualidade, onde explicaram, sucintamente, a função de cada sala do Laboratório.

Depois disto, encaminharam-me à minha orientadora de estágio, ficando a restante parte do dia a ler procedimentos úteis e fundamentais para a realização do estágio.

Deste modo, considero que a receção foi bem efetuada, na medida em que me ajudou a conhecer a empresa, bem como as suas políticas, normas e objetivos, facilitando, deste modo, a minha integração na equipa.

Equipa

No início do estágio, foi-me apresentada a equipa de cada secção do Laboratório de Controlo de Qualidade, constituída por farmacêuticos, engenheiros químicos, biólogos, gestores, técnicos e auxiliares. O facto de ser uma equipa multidisciplinar, favorece a resolução de múltiplas questões que possam eventualmente surgir.

Durante o meu estágio, a equipa sempre mostrou simpatia, espírito de cooperação, motivação, paciência e preocupação. De facto, sempre que tinha alguma dúvida sobre a localização de um determinado reagente, matéria-prima ou acerca de um determinado procedimento técnico (1.3.2. Fraquezas), a equipa demonstrava disponibilidade para me ajudar a resolver as questões que surgissem durante a execução das minhas tarefas.

Cantina

A indústria farmacêutica na qual realizei o meu estágio, tem um refeitório acessível a todos os colaboradores. Todos os dias era possível escolher a ementa para o almoço do dia seguinte, de entre três opções: carne, peixe e dieta. Durante todo o meu estágio, tive acesso às refeições de forma gratuita, o que se revelou benefício a nível nutricional, contribuindo, desta forma, para um melhor desempenho nas minhas tarefas. Tendo em conta que a ementa incluía sopa, pão, prato principal, salada à discrição, bebida e sobremesa, solucionou a preocupação de ter de preparar o almoço para todos os dias de estágio.

Farmacoteca

Várias vezes, durante o meu estágio, tive acesso à Farmacoteca, cuja gestão está sob controlo do Laboratório de Controlo de Qualidade. Trata-se de um espaço de acesso restrito e que se destina ao aprovisionamento de amostras representativas de todos os lotes de medicamentos produzidos, até um ano após o término da validade do produto. Nesta secção, encontra-se também todas as matérias-primas e matérias de embalagem utilizadas no fabrico dos medicamentos.

Na Farmacoteca são mantidos dois tipos de amostras: as amostras de referência, que se destinam a eventuais reanálises necessárias durante o tempo de vida do lote do medicamento, e as amostras de retenção, que são exemplares de produto acabado mantidos para efeitos de consulta que, eventualmente, venha a ser necessária durante o tempo de vida do lote do medicamento.

O acesso a esta divisão permitiu-me conhecer em termos práticos a finalidade de uma Farmacoteca e compreender a sua organização e importância na rastreabilidade do medicamento.

Consciencialização ambiental e gestão de resíduos

Desde o início do estágio, e ao longo deste, um dos valores que me foi transmitido foi a valorização do ambiente. Para os Laboratórios Basi, a proteção do ambiente é uma das principais preocupações. Como tal, logo no meu primeiro dia de estágio, obtive, juntamente com outros novos colaboradores, uma apresentação sobre a importância e responsabilidade ambiental, prevenindo, deste modo, a poluição do meio ambiente.

Em cada sala da empresa, era-nos exigido a separação de resíduos, tais como o plástico, papel, vidro, resíduos halogenados e os não halogenados e os resíduos indiferenciados. Para tal, havia contentores diferenciados pela cor dos sacos, devidamente identificados.

Todo este esforço de separação de resíduos contribuiu para que me elucidasse do meu dever para com o meio ambiente, enquanto farmacêutica. Isto deveu-se ao facto de a empresa recorrer, frequentemente, ao uso de sustâncias tóxicas para o ambiente e para os seres vivos. Sendo o farmacêutico um agente de saúde pública, tem a obrigação e o dever de cuidar do meio ambiente.

Única estagiária

O facto de ser a única estagiária no período dos meses de janeiro a abril, no Departamento de Controlo de Qualidade, foi algo muito benéfico. Desta forma, o foco e o apoio dos colaboradores do Laboratório de Controlo de Qualidade estava unicamente destinado a mim. Como tal, a aprendizagem tornou-se mais célere, personalizada e adequada às minhas necessidades específicas.

1.3.4. Ameaças

Ausência de auditoria

Uma auditoria feita à indústria farmacêutica, quer seja interna ou externa, é sempre benéfica, pois permite encontrar e indicar possíveis pontos a melhorar, bem como identificar atividades que não estejam a ser desenvolvidas conforme estabelecido.

Infelizmente, durante o meu estágio no Laboratório de Controlo de Qualidade, não pude assistir a nenhuma auditoria, apesar de ter sido realizada uma auditoria nesse período. Teria sido vantajoso se o estágio me concedesse essa oportunidade, pois iria contribuir para uma maior perceção das exigências que são estipuladas pelos avaliadores e tudo aquilo que é alvo de uma auditoria numa indústria farmacêutica.

Poucas linhas de produção

Os Laboratórios Basi são uma indústria relativamente pequena, mas em constante crescimento. Atualmente, apresenta três linhas de produção, nomeadamente, uma linha de produtos líquidos (soluções, emulsões, suspensões e xaropes), uma linha de produtos semissólidos (cremes, pomadas, geles e supositórios) e uma linha de injetáveis de pequeno volume (ampolas) e de grande volume (frascos). Deste modo, em todo o meu estágio, apenas contactei com protocolos analíticos referentes a estas linhas de produção. Desta forma, não tive a oportunidade de aprofundar os meus conhecimentos noutras linhas de produção, particularmente, nas formas farmacêuticas sólidas.

Ausência de um plano de estágio

Desde o início do estágio, apercebi-me da ausência de um plano de atividades. Isto tornou-se um fator limitante pois, tendo em conta o tempo reduzido de estágio, teria sido importante planear as atividades de forma a permitir a rotatividade entre todos os segmentos do Controlo de Qualidade. Este plano teria permitido adquirir melhor as noções do tipo de trabalho nessas áreas.

Devido ao tempo limitado e à falta do planeamento do estágio, não tive oportunidade de trabalhar em três áreas do Laboratório de Controlo de Qualidade: na unidade de produção, no laboratório da microbiologia e na unidade de desenvolvimento galénico.

Todavia, o facto de ter permanecido mais tempo na área de controlo e garantia da qualidade, bem como no laboratório físico-químico, permitiu-me adquirir uma maior autonomia e conhecimento nessas áreas (1.3.1 Forças).

Curta passagem noutros sectores da indústria

A visita realizada no primeiro dia de estágio (1.3.3. Oportunidades) permitiu ter uma noção básica do que é feito nos outros setores da indústria farmacêutica. Contudo, nessa visita apenas se aflorou o trabalho que é realizado em cada secção do Laboratório de Controlo de Qualidade.

Durante o meu estágio no Departamento de Controlo de Qualidade, estive, maioritariamente, a trabalhar na parte documental relativa à gestão e garantia de qualidade. Deste modo, para atingir um maior conhecimento do que realmente é a indústria farmacêutica, teria sido importante, a passagem por outros setores da empresa, nos quais o farmacêutico desempenha um papel ativo, nomeadamente na unidade de produção, no laboratório da microbiologia, na unidade da cromatografia e na unidade de desenvolvimento galénico.

Todavia, compreendo que nem todos os setores estejam preparados para receber estagiários e que nuns possam existir tarefas com maior urgência e, por isso, com uma maior necessidade da ajuda de estagiários. Deste modo, para contornar este problema, sempre que tinha oportunidade, perguntava aos colaboradores responsáveis de cada secção, como funcionavam os diversos departamentos (1.3.1 Forças).

1.4. Conclusão

O estágio na indústria farmacêutica, mais concretamente, nos Laboratórios Basi, ofereceu-me uma nova experiência curricular. Este estágio de três meses permitiu conhecer melhor o papel do farmacêutico na indústria farmacêutica, como um especialista do medicamento.

Concluí que o farmacêutico representa um elemento fundamental na interligação entre os vários setores de empresa, tendo cargos de grande responsabilidade para o sucesso na obtenção de um produto final, garantindo a sua eficácia, segurança e qualidade. Adquiri novos conhecimentos não só da área farmacêutica, mas também sobre a segurança e respeito pelo ambiente. Todas as atividades que constituíram o meu estágio contribuíram para o aumento do meu sentido de responsabilidade e qualidade de trabalho.

Apesar de não ter sido possível o contacto com outros setores, a passagem pelo setor de controlo de qualidade foi bastante proveitosa e benéfica, pois permitiu-me conhecer a dinâmica de uma indústria farmacêutica. Esta oportunidade constituiu uma mais valia a nível profissional e pessoal, permitindo desenvolver a minha formação numa área de grande importância.

A oportunidade que a Faculdade de Farmácia da Universidade de Coimbra oferece de realização de estágios curriculares além da farmácia comunitária, mais concretamente em indústria farmacêutica, é, sem dúvida, uma oportunidade vantajosa. Esta oportunidade oferecida a cada estudante permite a obtenção de maior conhecimento e experiência contribuindo, deste modo, para nos tornarmos melhores profissionais de saúde.

2. Relatório de Estágio

em Farmácia de Oficina

farmáciacoimbra

2.1. Introdução

De acordo com o artigo 9º do Código Deontológico da Ordem dos Farmacêuticos [3], o farmacêutico é um agente profissional de saúde, tendo como principal objetivo, executar todas as tarefas relacionadas com o medicamento. Para além destas tarefas, cabe também ao farmacêutico realizar todas as ações suscetíveis de contribuir para a salvaguarda da saúde pública e educação dirigidas à comunidade no âmbito de promoção da mesma.

Por forma a exercer a sua atividade profissional, o farmacêutico é portador de uma sólida formação superior, teórica e prática, durante, pelo menos, quatro anos a tempo inteiro. Para ter a oportunidade de completar a formação recebida ao longo do curso, o candidato ao título de farmacêutico deve, conforme indicado no nº 2 do Artigo 44º da Diretiva 2013/55/EU [4], frequentar um estágio equivalente a seis meses de duração, em Farmácia de Oficina ou Farmácia Hospitalar.

Para um farmacêutico, a experiência profissional em local de trabalho, representa uma componente fundamental de aprendizagem e evolução científica, contribuído assim, para o aumento de experiência e sabedoria na área do medicamento, que serão colocados posteriormente em prova no mundo profissional. Para tais efeitos, selecionei o estágio curricular em farmácia comunitária na Farmácia Coimbra, entre 22 de abril e 18 de agosto de 2019, sob orientação da Dr.ª Ana Leite e Silva. A farmácia foi escolhida pela conveniência da sua localização, pela variedade alargada de produtos farmacêuticos bem como pela heterogeneidade populacional que a frequenta. Com esta escolha, pretendi ser confrontada com numerosos e diferentes casos de atendimento, adquirindo maior conhecimento e experiência sobre medicamentos e aconselhamento farmacêutico.

O presente relatório encontra-se sob a forma de uma análise SWOT. Esta análise possui como principal finalidade avaliar duas dimensões: a interna, que é avaliada pelos pontos fortes (*Strengths*) e pelos pontos fracos (*Weaknesses*); e a externa, que é descrita pelas oportunidades (*Opportunities*) e ameaças (*Threats*). Esta análise pretende avaliar a minha pessoa enquanto estagiária, tendo em conta aos ensinamentos adquiridos durante o percurso académico (dimensão interna), bem como estudar o que externamente influenciou o meu estágio, isto é, as oportunidades que a farmácia ofereceu e as ameaças.

2.2. Farmácia Coimbra

A Farmácia Coimbra encontra-se na Av. Dr. Mendes Silva, mais concretamente, no Centro Comercial Coimbra Shopping. A farmácia enquadra-se numa grande zona habitacional e escolar, encontrando-se também próxima do Centro de Saúde Norton de Matos e da Unidade de Saúde Familiar Briosa. Desta forma, a Farmácia Coimbra enquadra-se numa localização privilegiada devido à numerosa e variada afluência de utentes.

Uma outra particularidade vantajosa que a Farmácia Coimbra apresenta é o seu horário de funcionamento alargado: de segunda a quinta-feira é das 9h às 23h; sexta, sábado e nas vésperas de feriado das 9h às 24h e, relativamente aos feriados e domingos, das 9h às 22h. Adicionalmente ao horário alargado, a Farmácia Coimbra realiza periodicamente serviço permanente (24h), o que permite dar resposta a diversas situações de emergência dos utentes não só durante o período diurno, como também no período noturno.

A Farmácia Coimbra contempla uma equipa jovem, versátil e dinâmica, constituída por dez profissionais de saúde, cabendo a direção técnica à Dr.ª Ana Leite e Silva.

Relativamente à infraestrutura, a Farmácia Coimbra é constituída por dois pisos. No piso térreo encontra-se: i) cinco balcões de atendimento, ii) um gabinete de consulta, iii) um escritório de direção técnica, iv) um laboratório de manipulação de medicamentos, devidamente equipado, v) uma zona de receção e gestão de encomendas, vi) instalações sanitárias de uso exclusivo à equipa de trabalho e vii) uma porta de emergência com postigo para os dias de serviço permanente. Os medicamentos não sujeitos a receita médica (MNSRM) são visíveis ao utente, todavia encontram-se fora do seu alcance. No piso superior, encontra-se uma área destinada à receção e gestão de encomendas. Esta área é auxiliada por um robô. Fora da farmácia há um armazém onde ficam armazenados produtos de cosmética e MNSRM que vão sendo repostos, bem como os produtos associados a devoluções ou quebras.

Além da habitual dispensa de medicamentos, a Farmácia Coimbra possui uma ampla variedade de produtos de cosmética, puericultura e veterinária. A farmácia oferece, ainda, aos utentes, serviços de medição de parâmetros fisiológicos e bioquímicos, mais concretamente a tensão arterial, peso, glicémia e o colesterol total, bem como consultas de nutrição e dietética (com marcação prévia). É importante realçar ainda que a Farmácia Coimbra participa na recolha de medicamentos fora de uso em colaboração com a VALORMED.

2.3. Análise SWOT

2.3.1. Forças

Formação académica

A formação académica adquirida durante os quatro anos e meio, em diversas unidades curriculares desde a patologia, ao medicamento, passando pela organização e gestão farmacêutica, contribuiu para o aumento de conhecimentos que reconheço como essenciais em atividades como a organização da farmácia, para um melhor atendimento e aconselhamento farmacêutico. Este estágio forneceu bases teóricas e práticas para que os conhecimentos em falta (1.3.2. Fraquezas) fossem compreendidos e assimilados, completando assim o corpo de conhecimentos essenciais à realização da profissão farmacêutica.

Formação em medição de parâmetros bioquímicos e suporte básico de vida

Frequentei uma ação de formação, organizada pela Delegação Centro da Fundação Portuguesa de Cardiologia (DCFPC), sobre deteção e controle dos fatores de risco nas doenças cardiovasculares, bem como um curso de formação profissional de suporte básico de vida num adulto, planificada pelo Instituto Nacional de Emergência Médica (INEM).

Devido à formação obtida da DCFPC, pude executar, desde os primeiros dias de estágio, a medição dos parâmetros bioquímicos e fisiológicos, tais como colesterol, glicémia e pressão arterial, permitindo, ainda, uma maior autonomia no aconselhamento farmacêutico ao utente, desde os primeiros dias de estágio.

Relativamente ao curso de formação em suporte básico de vida, este permitiu que, em circunstâncias de urgência que surgissem na farmácia ou fora da mesma, quer por motivos de lesões, quer por outras condições de saúde, pudesse manter os sinais vitais, tentando evitar o agravamento do quadro no qual a pessoa se encontra.

Conhecimentos da língua inglesa

Algo que considero fundamental no atendimento ao público no geral, é a capacidade de compreender e saber expressar noutra língua para além da língua materna. Durante o estágio, a Farmácia Coimbra foi regularmente visitada por turistas cuja língua materna não é o português. Desta forma, na maioria dos casos, as competências na língua inglesa permitiram a compreensão do que foi solicitado e a realização de um aconselhamento farmacêutico com esses utentes.

Conhecimentos prévios sobre Sifarma 2000®

Durante o percurso académico, surgiu a oportunidade de assistir a uma formação introdutória sobre a aplicação do *Sifarma 2000®*. Para além desta formação, obtive aulas teóricas e práticas sobre o mesmo na unidade curricular Organização e Gestão Farmacêutica.

Sendo o sistema informático *Sifarma 2000®* o que se encontra instalado na farmácia, o conhecimento adquirido sobre o mesmo, apesar de pouco desenvolvido, permitiu o primeiro contacto com a aplicação do sistema informático. Isto provou ser uma mais-valia aquando da sua utilização na farmácia. Saliento ainda que, o conhecimento prévio da disponibilização de informação científica que o *Sifarma 2000®* disponibiliza, permitiu proceder ao esclarecimento de dúvidas pontuais relativas a medicamentos e outros produtos de saúde durante o atendimento.

Sentido de iniciativa

Durante o estágio, em diversos momentos, demonstrei sentido de iniciativa. Em momentos de menor atividade, procurava encontrar tarefas, tais como gestão e receção de encomendas, a reposição dos medicamentos sujeitos a receita médica (MSRM) no robô, a reposição de *stocks* dos MNSRM nas prateleiras e gavetas e a arrumação e reposição de produtos de dermocosmética e puericultura nas gôndolas e lineares.

Quando solicitado, oferecia-me para a medição de parâmetros bioquímicos e fisiológicos aos utentes, para o transporte de produtos para o armazém, para ajudar na correção de *stocks* de produtos, bem como para a verificação e recolha de medicamentos cujo prazo de validade terminava no mês seguinte.

Este sentido de iniciativa demonstrado foi positivo para o meu desenvolvimento durante o estágio, uma vez que possibilitou conhecer os locais onde se encontravam os diversos produtos e, por conseguinte, tornar o atendimento mais célere e eficiente.

Interesse na aprendizagem

À medida que realizava a gestão ou receção de encomendas, quando efetuava um atendimento ou quando me deparava com certos fármacos ou designações comerciais (1.3.3. Oportunidades), com os quais não estava muito familiarizada, procurava informar-me com algum dos farmacêuticos, que sempre se mostraram disponíveis (1.3.3. Oportunidades). Por vezes, quando estes se encontravam ocupados com as suas tarefas, consultava a informação pretendida em diversas fontes, tais como folhetos informativos (FI), resumo das características do medicamento (RCM) ou a livros e cartazes sobre produtos farmacêuticos, que se encontravam organizados num armário ou fixados na parede da farmácia. Desta forma, as lacunas de conhecimento detetadas (1.3.2. Fraquezas) foram diminuídas.

2.3.2. Fraquezas

Lacunas de conhecimento

Apesar da aprendizagem adquirida ao longo do curso formar uma sólida base técnico-científica necessária à realização do estágio, havia algumas situações em que estava reticente, pois sentia algumas lacunas de conhecimento. Essa hesitação situava-se, principalmente, no que dizia respeito ao aconselhamento em produtos de dermocosmética, produtos de utilização veterinária e no reconhecimento de princípios ativos quando me eram solicitados pelos nomes comerciais (1.3.3. Oportunidades).

No entanto, as dificuldades referidas foram colmatadas devido à pesquisa autónoma (1.3.1. Forças), ao apoio das funcionalidades do *Sifarma 2000*, pelas formações em cosmética e pela prontidão e disponibilidade da minha orientadora e colaboradores da farmácia em me acompanhar e auxiliar (1.3.3. Oportunidades).

Timidez inicial

Durante a fase inicial do estágio e, por vezes, em novas tarefas que me eram propostas, sentia algum constrangimento em solicitar indicações e auxílio.

Nos primeiros atendimentos realizados ao balcão, ficava ligeiramente nervosa, tendo isto prejudicando-os, uma vez que os atendimentos ficavam mais demorados, e os utentes mais impacientes. Muitas vezes, o nervosismo juntamente com a não familiarização com os descontos, protocolos e/ou comparticipações especiais, tais como o Serviço de Assistência Médico Social (SAMS), conduziam a erros, sendo necessário anular e refazer as vendas.

No entanto, a forma como procurava superar a timidez e o nervosismo juntamente com a disponibilidade e simpatia dos colaboradores da farmácia (1.3.3. Oportunidades) permitiram a fácil superação desta fraqueza, ajudando a manter a calma e a resolver as questões que iam surgindo.

Inexperiência

Tendo sido este o primeiro contacto com o mundo real do trabalho em farmácia de oficina, é expectável que surjam erros associados a inexperiência. Um exemplo destes erros está associado com a contagem da caixa no fim do dia. Ocorreram várias situações em que o valor da caixa e o que se encontrava registado no programa informático (*Sifarma 2000*) não coincidiam. No entanto, todas as situações foram resolvidas e a farmácia não ficou prejudicada. Isto deveu-se ao sentido de iniciativa de aprendizagem (1.3.1. Forças) e, principalmente, à equipa de colaboradores da farmácia (1.3.3. Oportunidades), que sempre me orientou e permitiu que, de forma rápida, adquirisse a experiência necessária.

Falta de experiência nas receitas manuais

Quando iniciei a fase de atendimento ao público, uma das áreas em que sentia mais dificuldade era a receita manual. Atualmente, o receituário segue o modelo eletrónico, diminuído, deste modo, a probabilidade de erros na cedência do produto farmacêutico ao utente. No entanto, por vezes, surgiam receitas manuais, sendo necessário proceder à dispensa de MSRM pelo método tradicional. Devido à pouca experiência em manusear uma receita manual, alguns dos conhecimentos adquiridos sobre a mesma foram transmitidos quer pela minha orientadora, quer pelos colaboradores da farmácia (1.3.3 Oportunidades), permitindo perceber como se realiza a dispensa de medicamentos pelo método tradicional. Desta forma, a dispensa de MSRM a partir de uma receita manual, requer um nível de atenção máximo de modo a não cometermos ou, pelo menos, minimizar os erros.

2.3.3. Oportunidades

Grande variedade de produtos e serviços

A Farmácia Coimbra oferece aos utentes uma elevada variedade de produtos e serviços, tais como medicamentos, cosmética, veterinária, puericultura, suplementos alimentares e até maquilhagem. A farmácia realiza, frequentemente, a avaliação de risco cardiovascular e disponibiliza consultas de nutrição, testes de diagnóstico de pele e rastreios capilares, com marcação prévia. Durante o estágio, pude constatar que esta variedade de produtos aumenta a satisfação dos utentes e a sua fidelização à farmácia. Por outro lado, o estágio em farmácia de oficina, permitiu-me também, entrar em contacto com várias vertentes da profissão de um farmacêutico e aprofundar os meus conhecimentos em diversas áreas.

Equipa

A equipa de colaboradores da Farmácia Coimbra é composta por dez profissionais de saúde, altamente competentes e com grande consciência da sua responsabilidade como profissionais de saúde.

Desta forma, desde o primeiro dia até ao último, a equipa teve um papel fundamental no meu desenvolvimento enquanto farmacêutica, pois esta revelou-se compreensiva e, sobretudo, disposta a esclarecer quaisquer dúvidas e a ajudar em tarefas nas quais me defrontasse com dificuldades. Em suma, os colaboradores da Farmácia Coimbra contribuíram de forma significativa para ultrapassar certas lacunas de conhecimento tanto a nível prático e teórico, bem como a colmatar a inexperiência e o nervosismo inicial (em 1.3.2. Fraquezas).

Localização da farmácia e heterogeneidade de utentes

A Farmácia Coimbra encontra-se num local favorecido pois encontra-se num *shopping* e numa grande área habitacional e escolar, com elevada heterogeneidade de utentes. Desta forma, o estágio garantiu-me o contacto com diversas realidades económicas e socioculturais, o que contribuiu para me relacionar com várias classes de utentes e saber ir ao encontro das necessidades de cada pessoa. Em suma, após a realização do estágio, sinto-me em vantagem por ter tido a oportunidade de contactar com as diversas realidades que se apresentam na Farmácia Coimbra no dia-a-dia.

Um outro aspeto relevante a ter em consideração é o horário de funcionamento alargado e o grande número de utentes que frequentam a farmácia, sendo os sábados, os domingos e os feriados os dias mais propensos a maior movimento. Deste modo, era confrontada muitas vezes, com numerosos e variados atendimentos, o que contribuiu para o aumento do conhecimento e experiência no atendimento e aconselhamento farmacêutico.

Conta própria no Sifarma 2000® e caixa com plafond individual

Durante o estágio, foi-me concedida a oportunidade de ter uma conta própria no *Sifarma 2000®* e uma caixa própria com uma determinada quantia. No final de cada dia, tinha de verificar se, na caixa, o valor total obtido das vendas realizadas era o mesmo que o valor registado no *Sifarma 2000®*, permitindo desta forma, um melhor controlo e de mais fácil verificação. Em suma, esta oportunidade possibilitou um crescimento na autonomia e responsabilidade, uma vez que tinha de garantir que, no final do dia, o valor total tinha de estar correto.

Acesso a formações

Para completar a formação recebida na farmácia, pude participar em várias sessões de formação, conduzidas por entidades terceiras. Várias formações decorreram no próprio espaço da farmácia, enquanto que outras foram realizadas no Hotel Tivoli, na companhia da restante equipa técnica. Nestas visitas, os oradores forneciam informação sobre as gamas e os produtos das indústrias que representavam, nomeadamente a Oral-B®, a Norgine® Portugal Farmacêutica Unipessoal, o Laboratoires Théa Sa®, a Gedeon Richter®, a Aboca®, a Nestlé®, entre outras marcas e produtos.

Deste modo, o acesso às formações, colocou-me a par dos produtos existentes no mercado e permitiu que adquirisse o conhecimento necessário para que pudesse prestar ao utente um aconselhamento informado e cuidado, que se adequasse, da melhor forma, às suas necessidades.

Aprendizagem dos nomes comerciais

A formação académica a nível dos fármacos é feita, quase unicamente, recorrendo à Denominação Comum Internacional (DCI). Porém, ao realizar o estágio numa farmácia de oficina, tive a oportunidade de ter um período de adaptação dos nomes comerciais mais comuns. Foi frequente, durante os primeiros meses, desconhecer os nomes dos produtos que o utente pretendia. O estágio levou-me a ser confrontada, em vários atendimentos, com pedidos de medicamentos pelas suas respetivas designações comerciais. Desta forma, a realização do estágio permitiu-me uma progressiva adaptação e aprendizagem dos nomes comerciais, tornando a atendimento mais rápido e respondendo às necessidades dos utentes de uma forma mais eficaz.

Número de estagiários

Durante o período de estágio, de abril a agosto, eu fui, na maior parte das vezes, a única estagiária a frequentar estágio na Farmácia Coimbra. Embora haja mais um estagiário para além de mim, este era trabalhador-estudante, aparecendo no estágio só a partir das 18h, pelo que a minha aprendizagem se tornou mais célere, personalizada e adequada às minhas necessidades específicas. Por outro lado, nos fins de semana e feriados, havia alturas em que eu e o meu colega estagiário alternávamos nos horários. Desta forma, apenas um estagiário ficava a trabalhar na farmácia. Assim, o foco do apoio dos colaboradores da farmácia ao estágio estava destinado a um único estagiário.

2.3.4. Ameaças

Ausência de manipulados

Durante o meu estágio, não tive a possibilidade de observar nem de praticar a preparação de medicamentos manipulados. Normalmente, quando um determinado utente apresenta uma receita que remete à preparação de um manipulado, comunicava-se à farmácia Porto para que esta fizesse a preparação do respetivo medicamento. Quando preparado, a Farmácia Porto envia à Farmácia Coimbra, e, posteriormente, comunica-se ao utente a chegada do mesmo à farmácia.

Por outro lado, a diminuição da prescrição de formulações manipuladas em favor das preparações industrializadas, contribui como um dos principais fatores para o decrescimento desta prática a nível da farmácia de oficina.

Número reduzido de produtos ortopédicos

Apesar de a Farmácia Coimbra abranger uma grande variedade de medicamentos, de cosmética e de produtos veterinários, no que toca ao número de produtos ortopédicos, detém uma gama limitada. Deste modo, devido à falta de produtos de ortopedia, muitas vezes, durante o atendimento de utentes com necessidades de produtos ortopédicos, estes eram redirecionados para lojas ortopédicas ou para outras farmácias que tivessem materiais ortopédicos em maior número. Desta forma, como a farmácia vende poucos produtos ortopédicos, o aconselhamento farmacêutico na área foi insuficiente para que pudesse ocorrer uma verdadeira aquisição ou consolidação de conhecimentos.

Proximidade com uma parafarmácia

Como foi referido anteriormente, a Farmácia Coimbra situa-se no Coimbra Shopping, e, à frente das instalações da farmácia, encontra-se um hipermercado que inclui uma parafarmácia. Estas áreas comerciais não só constituem uma ameaça económica à farmácia, como também contribui para o aumento de risco para a saúde pública, pois a formação científica dos profissionais destes estabelecimentos é insuficiente, o que leva a um fraco aconselhamento e, por vezes, erróneo. Muitas vezes, era confrontada com utentes que tinham ficado com dúvidas ou ideias erradas, transmitidas nesse estabelecimento, sobre a posologia e modo de administração de um determinado MNSRM. Esses utentes ficavam, muitas das vezes, desconfiados quando lhes era apresentado um aconselhamento diferente.

Grande afluência de utentes

Devido à sua localização privilegiada (facilidade de acesso e estacionamento adequado), a Farmácia Coimbra conta com uma grande afluência de utentes. No entanto, este fator pode ser visto como uma ameaça ao estágio, uma vez que o atendimento não podia ser muito prolongado, para evitar que os utentes, que já estiveram à espera na fila, ficassem inquietos por aguardar durante muito tempo. Contudo, uma afluência abundante, permitiu a coexistência de momentos de atendimento aos utentes com períodos mais curtos, utilizados para discussão e consolidação dos conhecimentos adquiridos, resultando numa constante aprendizagem a um ritmo rápido, mas adequado.

2.4. Conclusão

Ao longo destes anos de curso fui confrontada com diversas pessoas que consideram que a farmácia de oficina está num patamar inferior a outras atividades profissionais no ramo das ciências farmacêuticas, tais como a investigação ou a indústria. Outras vezes, a farmácia é frequentemente definida como "vender medicamentos ao balcão". Porém, este estágio, na Farmácia Coimbra, constituiu uma prova evidente de que estas ideias relativas à farmácia de oficina, estão erradas, e de que esta profissão é tão importante como as restantes alternativas.

O tempo passado na Farmácia Coimbra teve como objetivo fornecer, além de noções práticas de gestão e organização de uma farmácia, numerosos e variados casos de atendimento, permitindo o aprofundamento das minhas bases científicas. Estas noções são fundamentais para o verdadeiro exercício da profissão de farmacêutico comunitário. Sendo assim, o estágio curricular deve ser interpretado como uma ferramenta valiosa na nossa formação preparando-nos, da melhor forma, para o mercado de trabalho.

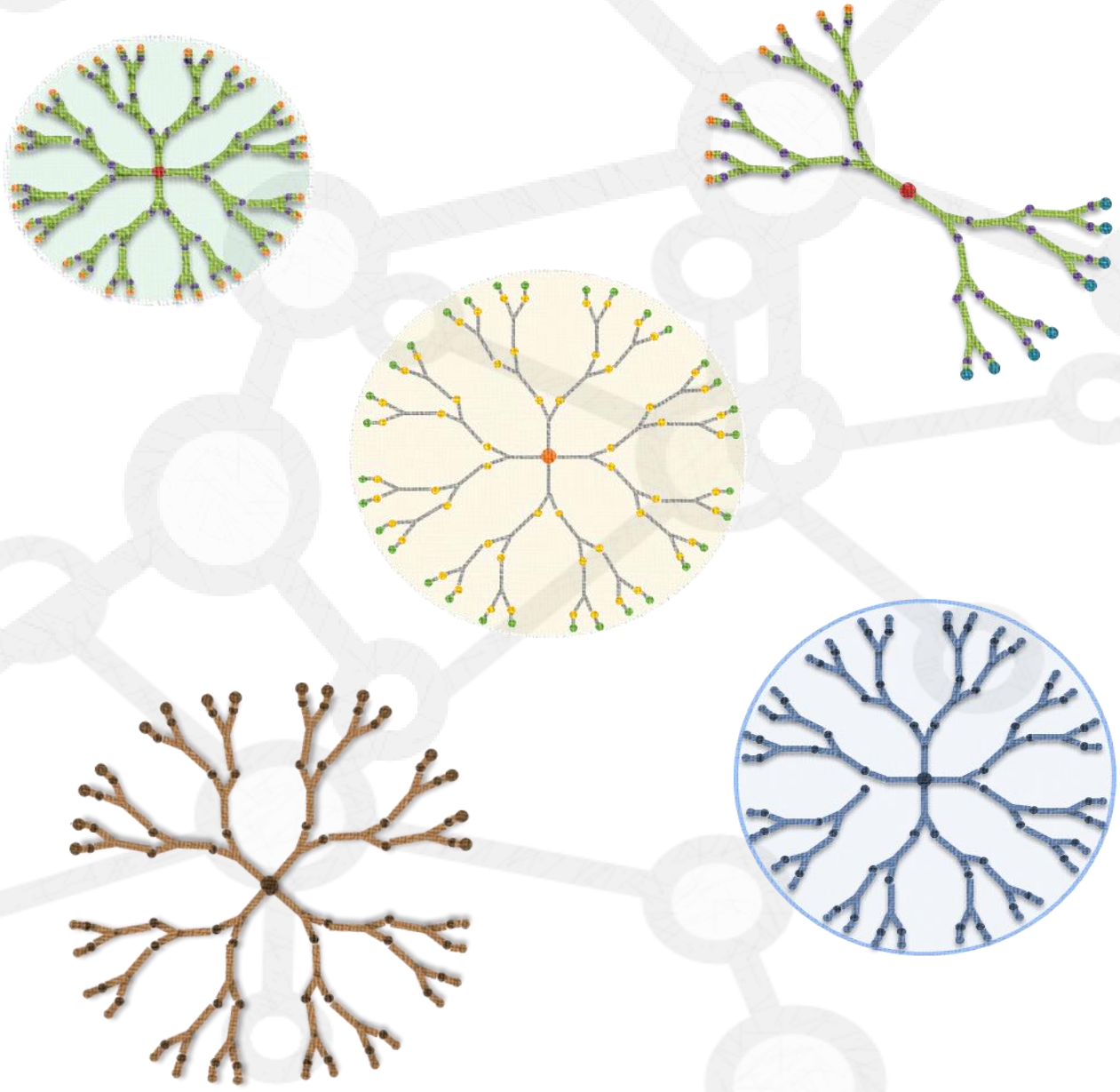
Devido ao profissionalismo, à organização e disponibilidade da equipa técnica da Farmácia Coimbra, o meu estágio tornou-se mais proveitoso e minimizou muitas das minhas dúvidas e inseguranças iniciais.

Terminado o estágio em farmácia de oficina, concluo que estes meses de aprendizagem foram enriquecedores e gratificantes, o que me permitiu perceber, verdadeiramente, que os farmacêuticos são mais que um simples "vendedor de medicamentos". Permitiu, ainda, compreender o real papel de um farmacêutico, como um especialista do medicamento e agente da saúde pública, que preza pelo bem-estar geral da sociedade.

Em conclusão, realço que a realização do estágio na Farmácia Coimbra revelou ser uma mais-valia na minha aprendizagem e uma oportunidade de crescimento a nível pessoal.

3. Monografia

Dendrimers as Pharmaceutical Excipients



3.1. Introduction

Nanotechnology is an emergent area that studies materials with a nanometer-scale [5]. During the recent decades, nanotechnology received great interest of researchers in the field of biomedical engineering, pharmaceutical technology and medicine. Nanomaterials (NM) are structures with less than 100 nm in diameter that have unique physical, chemical and/or biological properties associated with its nanostructure [6]. Nanomedicine is one of the sub-topics of nanotechnology, having as its main purpose the treatment and prevention of diseases through nanoformulation [7, 8]. One of the major goals of nanotechnology and nanomedicine is to develop a good pharmaceutical formulation, i.e., to produce a safe and effective drug formulation, with quality, while enhancing the bioavailability of the active pharmaceutical ingredients (API).

Some APIs have inherent bioavailability due its good solubility and permeation through biological membranes. However, many of them belong to the class II of the Biopharmaceutics Classification System (BCS) (i.e., low solubility, high permeability) or to the class IV (low solubility, low permeability), which is translated into low bioavailability [9]. Almost 40% of the APIs developed by the pharmaceutical industry are rejected due to bioavailability problems [10]. One way to overcome bioavailability drawbacks of the API is by choosing appropriate excipients for the pharmaceutical formulation, improving the dissolution profile of the drug.

The European Medicines Agency (EMA) "Guideline on excipients in the dossier for application for marketing authorisation of a medicinal product" [11] and the United States Current Good Manufacturing Practices (cGMP) [12] define excipient or inactive Ingredient as the constituents of the pharmaceutical form other than the active ingredient (i.e., "any component that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of human or other animals"). The European Commission guideline on "Excipients in the labelling and package leaflet of medicinal products for human use" [13] defines excipients as "any constituents of a medicinal product, other than the active substance and the packaging material". This guideline recognises that, although excipients are usually inert and with little or no pharmacological effect, some may have some action in certain circumstances.

Due to the unique properties that NMs offer, they can be used to reduce some of the limitations found in traditional pharmaceutical formulations. In other words, these NMs may be the key to solve the bioavailability problems of the API referred above, being used as excipients.

Among the various NMs, dendrimers have been highlighted in recent years as promising nanostructures and the applicability as pharmaceutical excipients has been explored, due to their distinctive physicochemical and structural properties [14]. Dendrimers are highly branched polymers with surfaces that are easily modifiable [15]. In the pharmaceutical technology, these polymers can be considered as excipients in the development of several pharmaceutical forms. Due to its properties such as nanoscale size, high degree of branching, polyvalency, biocompatibility, high water solubility, absence of immunogenicity, precise molecular weight and available internal cavities, dendrimers are excellent vehicles for safely and effectively transporting drugs [15]. Additionally , the possibility of combining different polymeric excipients, such as polyethylene glycol (PEG), enables the production of controlled drug delivery systems and the development of novel pharmaceutical formulations [16].

The unique properties of dendrimers, which differentiate them from other NMs, render them as widely applicable in diagnostic and biomedical engineering, including drug and gene delivery systems. The use of dendrimers for drug targeting and delivery has proven to have an important role in improving drug safety and reducing drug related toxicity [17].

Another interesting application of dendrimers is in inflammatory diseases because these nanostructures present anti-inflammatory activity by themselves, being useful in the treatment of atherosclerosis, rheumatoid arthritis and other associated diseases [17, 18]. Additionally, dendrimers may also have antimicrobial and antiviral activities [17, 19].

Although the properties of dendrimers render them suitable as pharmaceutical excipients, they present less advantageous properties which may hinder their use, namely, the cytotoxicity, the limitation of incorporation of the drug into the dendrimer cavities and the inability to control the rate of drug release. Furthermore, this type of polymer also presents high manufacturing costs and the need for specialised workforce [17, 20]. However, some of these problems are nowadays less significant due to the evolution of nanotechnology and increasing knowledge about dendrimers. These developments allowed to increase the industrial manufacturing efficiency, and lowering production costs [20].

In order to reduce the cytotoxicity and to increase the space on dendrimer cavity, numerous modifications have been proposed to the structure of the dendrimers. Several studies have shown that cytotoxicity of dendrimers could be significantly reduced by surface modifications with inert particles such as PEG [21] and fatty acids [17]. These alterations make dendrimers more suitable for use as excipients. Additionally, PEGylation of dendrimers increases their blood circulation time [21]. The lack of control of the rate of drug release from the dendrimer can be avoided by covalent conjugation of the drug to the dendrimer surface. Drug release is then dependent on the cleavage of the dendrimer-drug linkage [17].

Poly(amidoamine) (PAMAM) dendrimers are the best studied and most used nanostructures. The structure and properties of PAMAMs render them suitable for the fixation and encapsulation of drugs and can be used to improve the solubilization, residence time, bioavailability and permeation through the skin. Consequently, PAMAM dendrimers play an important role in various pharmaceutical applications, more particularly, in new ocular, pulmonary, transdermal and oral drug delivery systems [22, 23]. Table 1 summarizes the effects of the use of PAMAM dendrimers in several pharmaceutical applications.

Table 1: Overview of the effects of the use of PAMAM dendrimers in different pharmaceutical applications.

API	Application	Observed effects	Reference
Pilocarpine	Ocular Drug Delivery	Improved residence time of Pilocarpine in the eye.	[24]
Enoxaparin	Pulmonary Drug Delivery	Increased bioavailability of enoxaparin by 40 %.	[25]
Ketoprofen	Transdermal Drug Delivery	Improved drug permeation through the skin.	[26]
Diflunisal	Transdermal Drug Delivery	Improved drug permeation through the skin.	[26]

In 1991, a major breakthrough was made in the area of NMs with the discovery of a new class of dendrimers, known as Janus Dendrimers (JDs) or diblock dendrimers. These amphiphilic dendrimers attracted much attention of researchers because of their asymmetric structure, which grants them unusual properties, when compared to the conventional symmetrical forms. JDs are composed of two hemispheres (hydrophobic and hydrophilic) with different numbers of terminal groups and sizes. Diblock dendrimers play a significant role in medical and drug delivery applications because of their capacity to increase drug solubility, the ability to effectively carry drugs and for their potential as transdermal penetration enhancers [27]. Ouyang *et al.* [28] and Pan *et al.* [29] studied the performance of the JDs for bone-targeted drug delivery of Naproxen, a class II drug according with BCS. They concluded that this amphiphilic dendrimer has the ability to enhance the water solubility, to carry the drug and to target it to the bone. Therefore, this amphiphilic JD revealed to be a promising approach to delivery of poor water-soluble drugs in specific targets. Another study was conducted by Liu *et al.* [30] demonstrated that JDs act as a potential siRNA delivery systems. They confirmed that these amphiphilic dendrimers have the ability to be a robust and versatile siRNA delivery system to various cell lines, including human primary and stem cells.

Although dendrimers do not have a pharmacopoeia monograph and cannot yet be recognized as a pharmaceutical excipient [20], significant progress has been made towards establishing dendrimers as pharmaceutical excipients.

3.2. Dendrimers

3.2.1. Definition and Structure

In 1985, the synthesis of "nanocascade spheres" and "starburst dendritic macromolecules" was reported, introducing a new class of macromolecules, known today as dendrimers [31]. The word dendrimer has its routes from the Greek word *Dendron*, meaning "tree" or "branch", and the word *meros*, meaning "part" [23]. Dendrimers or dendritic polymers are nanoparticles based drug delivery systems, with a size between 1 nm to 100 nm [10, 32]. These nanodelivery platforms can increase drug solubility and, subsequently, improve drug bioavailability. Additionally, they can reduce the therapeutic dosage by increasing the drug time of exposure, minimizing adverse drug side effects [33].

Dendrimers are three-dimensional, hyper-branched and monodisperse structures containing a central core surrounded by peripheral groups. These characteristics are fundamental for their physicochemical and biological properties. Normally, dendrimers possess three distinguishing architectural components, as represented in Figure A1: (i) core; (ii) branches (an interior layer composed of repeating units attached to the core) and (iii) terminal groups attached to the branches [31]. The dendritic polymer arrangement creates internal cavities, in which the drug can be deposited, increasing its solubility and stability. The referred characteristics turn these macromolecules into good candidates for pharmaceutical excipients [23, 34].

The core of dendrimers consists of an atom or group of atoms with branches of carbon that are added through a sequence of chemical reactions, producing a spherical dendritic structure. However, some of the dendrimers, such as PAMAMs, do not have proper spherical structure [35].

Dendritic polymers are synthesized stepwise around the core. During the synthesis, each successive reaction step leads to an additional generation of branching and the number of repeated steps is defined as dendrimer generation (denoted as G), as represented in Figure A2. Normally, dendrimers with $G < 4$ and $G \geq 4$ are termed low- and high-generation dendrimers, respectively [36]. The core of the dendrimer is sometimes denoted generation zero or G0 [37].

The terminal groups (also called as functional groups or surface groups) are responsible for the interaction of the dendrimers with the external groups or molecules. Consequently, the physicochemical properties of dendrimers depend not only on the branching units, but

also on the surface functional groups [38]. As the control of the physicochemical properties of these structures can be achieved during synthesis by controlling the core groups, the extent of branching, and the nature and/or number of terminal groups on the surface, dendrimers became popular in the NMs research area [39]. This leads to the possibility of acquiring, at the end of the chemical synthesis, a desired chemical structure with an appropriate role: as API or as pharmaceutical excipient (Figure B1).

When dendrimers act as APIs, the structure determines if they possess anti-inflammatory, antiviral or antimicrobial activity. Park *et al.* [40] studied the anti-inflammatory effect and reported that dendrimers with glucosamine conjugates inhibited the synthesis of pro-inflammatory chemokines and cytokines of cells associated with the inflammatory process (such as monocytes, macrophages and lymphocytes). Their results demonstrated that such conjugates also blocked the proliferation and migration of endothelial cells mediated by a growth factor produced by fibroblasts.

Frequently, antiviral and antimicrobial treatment is hampered by drug resistance and the appearance of adverse effects, increasing the need of new therapeutic strategies. Dendrimers demonstrate good antiviral and antimicrobial activity due to the strong interactions that they make with virus and the bacterial membranes, respectively, preventing infection of the host. Consequently, they became an important tool in the treatment of viral infections, especially in Human Immunodeficiency Virus (HIV) and Influenza Virus infections [17]. An example of a dendrimer with antiviral action is the SPL7013 (VivaGel®) [41, 42, 43]. This nanostructure is a poly(l-lysine) dendrimer which presents an anionic naphthalene disulphonate surface. They can block the entry of the virus by binding to the viral envelope protein gp120, preventing the formation of the CD4-gp120 complex [44]. They also exhibit potent activity against bacterial vaginosis when administered as a topical gel [45]. VivaGel® was used as the first dendrimer-based commercial medical product and many clinical trials with dendrimers are now being conducted [38]. Nowadays, VivaGel® is also used in condoms, already available on the market in Australia and Japan [46] with 99.9% effectiveness against HIV [45].

As excipients, dendrimers may be used to improve the physicochemical properties of a pharmaceutical formulation. In fact, the concept of excipients evolved: at first, excipients were used simply as substances added to complete a volume in the formulation; currently, these substances also serve as vehicle and to incorporate the API. Excipients are classified as solubilisers, permeation enhancers, dyes, emulsifiers, diluents, flavours, preservatives, wetting agents, solvents, and sustained release matrices [13]. In order to be good excipients,

dendrimers should not alter the safety, efficacy and stability of the formulation, while guaranteeing that the dose is administered and delivered with precision and accuracy [47].

Besides PAMAM dendrimer and JD, there are other types of dendrimer candidates that can to be excipients, as listed in Table 2. These dendrimers share some common characteristics, namely, biocompatibility, high aqueous and non-polar solubility, being monodisperse and the non-existence of a linear relationship between viscosity and molecular weight [23].

Table 2: Summary of different types of dendrimer with potential for being excipients, their chemical structure and their use in pharmaceutical formulations.

Dendrimer Name	Chemical structure	Mechanism	Reference
Poly(propyleneimine) (PPI) dendrimers	Terminal groups with primary amines and the interior of PPI contains tertiary tris-propylene amines.	Increased drug solubility through electrostatic interactions.	[48]
Frechet-type dendrimers	Hyper-branched architecture of polybenzyl ether. Contains -COOH groups as terminal groups.	Helps to enhance solubility in aqueous media and other polar solvents.	[38]
Peptide dendrimers	Peptidyl branching core and/or covalently attached as surface functional units.	Acts as surfactant and carrier for drug and gene delivery.	[38, 49]
Glycodendrimers	Contains saccharide residues as terminal groups and a core with sugar units.	Site-specific drug delivery to the lectin-rich organs.	[38, 50]
Hybrid dendrimers	A blend of linear and dendritic polymers.	Acts as surfactant and drug delivery system.	[38]
Polyester dendrimers	Polyester-based dendrimers.	Drug targeting, improved biodistribution, and modulation of drug release.	[38, 51]
Poly-L-lysine (PLL) dendrimers	Core and branching units are based on the amino acid lysine.	Gene carriers and increased drug solubility.	[15]

3.2.2. Synthesis

There are two basic types of polymers that consist entirely of branched repeat units, namely, dendrimers and classical polymers. The classical polymers exhibit an irregular architecture with incompletely reacted branch points throughout the structure. Dendrimers, on their turn, are highly ordered, regularly branched globular macromolecules. Another difference in these two polymers lies on the way to synthesize them: all classical polymers are obtained by polymerization reactions, whereas dendrimers are not [52, 53].

Dendrimer's synthesis is related with the molecular and the polymer chemistry. They associate to the molecular chemistry world by their step-by-step controlled synthesis, and they are related to the polymer world due to their use of repetitive structure, i.e. monomers. These globular structures are synthesized in cascade by sequence of reactive steps to grow from the first generation (G1) to the second generation (G1 + 1), and so on [35].

The first synthesized dendrimers were PAMAMs, introduced in 1980. However, various other dendrimers including PPI and PPL, glycodendrimers, polyester dendrimers and amphiphilic dendrimers, were synthesized in the later years [54].

3.2.2.1. Classical Synthesis Pathways

Dendrimers are usually synthesized through methods that allow the control of the structure at every stage of construction. The dendritic structures are mostly synthesized by two main different methods: divergent or convergent [55].

3.2.2.1.1. Divergent Growth Method

The divergent growth method was the first one proposed and is currently the most widely used [53]. This method arises from the seminal work of Tomalia and Newkome, as well as the branched model work of Vögtle [52]. In the divergent process, the construction of the dendrimer is starting from the core up to the periphery. This method requires two essential steps: (i) coupling of monomer and (ii) activation of the monomer end-group, to promote the reaction with a new monomer [37]. The divergent growth method consists on the repetition of the two aforementioned steps, until the obtention of the desired dendrimer generation, as represented in Figure C1.

The divergent processing starts by activation or modification of the core and coupling of the first monomer, creating the first generation of the dendrimer. The next step is the deprotection or activation of this first generation (G1) to react with other branched monomers in order to couple the second generation (G2), and so on. When a new layer of branching units is created, a new generation is obtained, i.e. the number of the generation corresponds to the number of branched layers from the core [53]. On the divergent method it is important that every step of the reaction is fully completed before the addition of a new generation so as to avoid deficiently formed branches [15]. The surface of the dendrimer may be easily functionalized and modified at each step obtaining the desired pharmaceutical excipient at the end of the synthesis.

Usually, the divergent approach leads to the synthesis of highly symmetric dendrimer molecules. However, recently, researchers have taken up the possibility to create heterogeneously functionalised dendrimers by the divergent growth method, leading to dendrimers with several types of functional groups bound to the surface [37].

3.2.2.1.2. Convergent Growth Method

An alternative method used for the synthesis of dendrimers is the convergent process, proposed by Fréchet and Hawker in 1989 – 1990 [15]. In contrast to the divergent process, the convergent method synthetises dendrimers starting from what will eventually become the exterior of the structure, i.e. the surface, and not from the core (Figure C2).

The convergent growth method also includes the repetition of the coupling and activation steps in order to obtain the desired dendritic structure. Primarily, the surface groups, generally two, are coupled to a monomer to give the dendritic segment (dendron generation zero). The second step consists of the activation of this fragment so that it can react with other monomers, thus creating the first generation dendron, that is, a dendritic wedge. This synthetic procedure can be repeated to give larger generation dendrons and use them to be coupled in the final step to a multifunctional core, producing the final dendrimer. The final part of the convergent synthesis ends up at the core, where two or more dendrons are joined together, creating the dendrimer. As the coupling reaction occurs at the focal point of the growing dendron, the preparation of large dendrimers (usually above the sixth generation) is difficulted by steric inhibition, resulting in decreased yields [52, 53].

In convergent synthesis, greater structural control is achieved than with the divergent approach due to its relatively lower number of coupling reactions at each growth step, allowing the synthesis dendritic products of unmatched purity. In addition, this strategy enables

synthesis of asymmetric dendrimers, where different segments are coupled together to create dendrimers with heterogeneous morphologies, e.g. JDs. Due to the referred advantages, this synthesis process opens up to intriguing fields of incorporating several active sites in one dendrimer to create a multifunctional excipient [37].

3.2.2.2. Accelerated Approaches

There is a large number of reports about dendrimer synthesis. However, only a few have reached the market. This is justified by the large number of reaction steps that not only increase the chemical waste of valuable starting materials but also increases the probability of introducing structural defects in the dendritic form. Therefore, the dendrimers production becomes slow and costly. The most important difficulty when a high generation dendrimer is synthesized is to ensure the full substitution of all reactive groups in order to avoid defects within the structure. To overcome those problems, accelerated approaches were developed with the intention to minimize the number of reaction steps, the reaction time, the starting materials and the production associated costs [55].

3.2.2.2.1. Double Exponential Growth Technique

A fundamental breakthrough in the synthesis of the dendrimer is the double exponential growth technique, introduced by Moore in 1995. This method is a mixture of both the divergent and the convergent method. The double exponential technique allows formation of two types of monomers that are prepared by convergent and divergent growth method from a single starting material, as represented in Figure C3 [56, 57].

Theoretically, the double exponential strategy requires a XY_2 monomer with protected X and Y functional groups. Then, this monomer is activated selectively, i.e. the focal point or the periphery, to give two differently activated monomers which are coupled together to obtain a G2 protected dendron. Repeating the selective activation and coupling process allows the formation of G4, then G8 dendron, and so on. On the final step, the focal points of the dendrons are activated and coupled to a multifunctional core obtaining a true dendrimer. The double exponential growth technique is similar to the convergent method, being very versatile because of the possibility of supramolecular preparation, classical or asymmetrical dendrimers [55].

3.2.2.2.1. Double-Stage Convergent Method or Hypercore Approach

The hypercore approach is derived from the classical convergent technique. This double-stage method consists of three simple steps, as represented in Figure C4: (i) low generation dendrons, with protected terminal groups, are coupled to a multifunctional core through their focal point; (ii) terminal groups of the obtained dendrimer (hypercore) are activated or deprotected and (iii) dendrons (different or the same) react with the hypercore, leading to the desired dendrimer [55, 58].

The hypercore method enables the formation of dendrimers with chemically differentiated external and internal branches, due to the use of two different types of monomers in the synthesis of the dendrons and the hypercore. When compared to the convergent technique, the double-stage method uses a hypercore, which reduces the steric hindrance and helps obtaining higher generations and monodisperse dendrimers [58].

3.2.2.2.3. Hypermonomer Method or the Branched Monomer Approach

In the branched monomer approach, the monomers, called hypermonomers, have a higher number of functional groups than conventional monomers. Using theses XY_4 hypermonomers (sometimes XY_8), dendrimers with a high number of functional groups are obtained in a few steps. A great advantage of this method is the ability to obtain high generation dendrimers in a smaller number of steps (Figure C5) [59].

3.2.2.3. Advantages and Limitations of Synthetic Methods

Nowadays, it is important that the excipients conform to the regulations and expectations of the pharmaceutical market. Ideally, they are produced on a large scale, at a low cost and with high quality standards, so that these compounds can guarantee the performance of the drug and optimize of the therapeutic effect.

The choice of the synthesis method of the dendrimer depends on the intended structure, the industrial goals and large scale production feasibility, which will impact on the way the branching, is introduced. In this way, for the obtained dendrimer to have quality and exert its function as an excipient, the advantages and limitations of each synthesis method should be considered. Table D1 summarizes some important aspects that should be considered for each type of synthesis used on the production of dendrimers as pharmaceutical excipients.

3.2.3. Physicochemical Properties

Dendrimers are a relatively new class of compounds when compared to the traditional linear polymers and they are characterized by their unique molecular architecture and dimensions. In comparison with other types of delivery systems, the advantages of using dendrimers include: (i) three-dimensional and globular architecture, (ii) controllable structure and size, (iii) lower molecular volume when compared with linear polymers of similar molecular weight, and (iv) provides a perfect opportunity for a wide variety of applications, including drug encapsulation [60, 61]. Additionally, these nanosystems have important physicochemical properties which render them good candidates for pharmaceutical excipients. To better understand the potential of dendrimers as pharmaceutical excipients, a discussion of the physicochemical properties is necessary.

3.2.3.1. Nanoscale Size

Due to the fact that dendrimers have dimensions on a nanometric scale and because they have other protein-like properties, the dendritic polymers can be recognized as artificial proteins with biomimetric properties [62]. The size and shape of the dendrimers are close to the proteins found in the human body. Dendrimer´s size can be controlled through molecular engineering to closely resemble enzymes, antibodies, and globular proteins. PAMAM dendrimer generations 3, 4 and 5, with ammonia core, may present the same dimensions of insulin (3 nm), cytochrome C (4 nm) and hemoglobima (5.5 nm), respectively [63].

Another important feature is that the dendrimers can cross biobarries like the Blood-Brain Barrier and membranes of tumour cells. The nanometric scale and uniformity of size enhance their ability to cross cell membranes, while reducing the risk of undesired clearance from the body through the liver or spleen [64].

Therefore, due to this similarity and to their high permeability in biological membranes, these polimeric nanocarriers are excellent excipients because they are capable of transporting the drug effectively through the organism.

3.2.3.2. Higher Solublization Potential

Generally, dendrimers, in comparison to the analogous classical polymers, are more soluble and, for this reason, this nanosystem has been studied as solubilizer of drugs with low solubility [65]. Ionic interactions, hydrogen bonding and hydrophobic interactions are the mechanisms by which dendrimers exert their solubility enhancement. Therefore, dendrimers are capable of improving the solubility, biodistribution and efficacy of a number of therapeutic agents as well as being used as diagnostic and imaging molecules in animal models bearing brain tumours [66].

The presence of many terminal groups is responsible for the high solubility and reactivity of the dendrimers. For higher generation dendrimers, solubility property depends mainly on the properties of their surface groups. Dendrimers with a surface with hydrophilic groups are soluble in polar solvents whereas dendrimers with hydrophobic groups are soluble in non-polar solvents. However, in addition to the functional groups, there are other intrinsic properties that can also influence the solubility of the dendrimer: (i) the nature of the repetition units, (ii) the generation number, and (iii) the core. It is noteworthy that the type of medium in which the dendrimer is found will also influence the solubility of this nanocarrier. Therefore, during exposure of the carrier system to a drug, the drug may or may not encapsulate into the dendrimer depending on the medium properties. If the drug molecules are poorly soluble in water, and the dendrimer provides a more hydrophobic environment, the drug will tend to encapsulate [20, 67].

3.2.3.3. Multivalent Surface

Multivalence is a property that dendrimers possess and is an important characteristic that leads them to be considered a good excipient. Multivalency or polyvalency refers to the number of reactive zones that a dendrimer presents on its surface (terminal groups) in order to interact with biological receptor sites, such as proteins, polymers, cells and virus. The surface modification may allow the design of dendrimers mimicking biological exo-receptors, substrates, cofactors or inhibitors. Free surface groups can form complex or conjugates with drug or ligands by using cross linking agents. In general, these interactions are reversible and occur in both inhibition and activation of biological processes. In contrast to weak monovalent receptor-ligand binding, multivalent interaction may amplify the signal transduction. Such augmentation is due to the enhanced affinity and cooperativity between the receptor and the ligand [66, 68].

As the dendrimer generation increases, the number of functional groups present on the surface also increases, favouring the interaction of these with the biological targets. Svenson *et al.* [69] confirmed that drugs transported and delivered by dendrimers show a greater therapeutic response when compared to conventional drugs, due to the polyvalence that the dendrimers demonstrate (Figure E1).

3.2.3.4. Monodispersity

One of the advantageous properties in using dendrimers as pharmaceutical excipients is precisely the obtention of monodisperse nanoparticles with low levels of impurities. Dendrimers are highly branched molecules with a well-defined structure, exhibiting a high degree of monodispersibility, contrasting with the traditional polymers. This is possibly due to their controlled synthesis and purification processes, which these dendritic polymers are subjected to during their synthesis [70].

The great advantage of monodispersibility is that it is possible to predict their pharmacokinetic behaviour in a biological organism. The pharmacokinetic properties are one of the most important aspects that need to be considered for the successful pharmaceutical application of a dendrimer. For example, the pharmaceutical industry uses the pharmacokinetic information to select the best drug that suits a specific pathology, the vehicle and the route of administration, aiming to achieve the maximum therapeutic response and the lowest possible toxicity [71].

3.2.3.5. Low Viscosity

One of the most important properties of dendritic macromolecules is the low viscosity. Dendrimers in solution have a significantly lower viscosity than linear polymers. Although viscosity increases with the number of monomers, in the dendritic macromolecules, from a certain generation (usually from generation 4), the viscosity decreases. Thus, higher generation dendrimers have more funcional groups, with lower viscosity than the low generation dendrimers. This behavior differs from linear polymers because on these structures, the intrinsic viscosity increases continuously with the molecular mass. The low viscosity is an advantageous property because the preparation of the dendrimer-drug complex becomes easier and the immediate release of drugs is facilitated [65, 72].

3.2.3.6. High Loading Capacity

The structure of the dendrimers can be used to load and store a wide range of inorganic or organic molecules by: (i) absorption on the surface by electrostatic interactions, (ii) conjugation with the surface groups through covalent bonding, or (iii) encapsulation of the drug into the cavities of the dendrimer, as shown in Figure E2 [73].

Although the number of molecules incorporated into a dendrimer depends on the architecture of this nanocarrier, the loading capacity increases with the number of functional groups on the dendrimer surface. The number of terminal groups available for drug interactions doubles with each increasing generation of dendrimer.

The presence of a large numbers of ionisable groups on the surface of the dendrimers, such as the amine and carboxyl groups, provides an opportunity for electrostatic attachment of numerous ionisable drugs. An example of this is the electrostatic interaction between PAMAM dendrimers and the nonsteroidal anti-inflammatory drug Ibuprofen. It has been proved that approximately 40 Ibuprofen molecules interact with a G4 PAMAM dendrimer at pH 10.5, leading to a considerable enhancement of drug solubility [74].

The covalent attachment of the molecules to the surface groups of dendrimers, through hydrolysable or biodegradable linkages, offers the opportunity for greater control over the drug release. Such an event may be justified by the fact that this type of interaction is stronger and more difficult to break when compared to other attachment. Yang and Lopina [75] have conjugated Penicillin V with both G2.5 and G3 PAMAM dendrimers through amide and ester bonds, respectively. They demonstrated that the amide linkage provided more stability than liposome-based drug-delivery systems, whereas the ester linkage of the drug to the dendrimer demonstrated to increase the drug circulation time in the body via hydrolysis.

The technique of drug encapsulation within a dendrimer may be a purely physical entrapment or it can involve interactions with specific structures within the nanocarrier [76]. For example, the existence of atoms of oxygen and nitrogen in the internal structure of the dendrimers allows interactions by hydrogen bonds with the drug. Generally, the empty internal cavities oh the dendrimer are hydrophobic, allowing interactions with poorly soluble drugs [77]. Encapsulation is a general technique for low molecular weight molecules and to bioactive molecules which, if carried on the surface of the dendrimer, induce undesired immunogenicity [78]. The Table 3 summarizes examples of incorporation of the drug into the dendrimer as well as the observed effects.

Table 3: Examples of incorporation of the drug into the dendrimer as well as the observed effects.

Dendrimer	Drug loaded	Formulation Type	Results	Reference
PEG-PAMAM-G4	Silybin	Encapsulation	Increased solubility.	[79]
PAMAM-Biotin	SB-T-1214	Conjugation	High potency and targeted drug delivery.	[80]
PAMAM-G4-DHA	Paclitaxel	Conjugation	Increased pharmacological activity in Upper Gastrointestinal Cancer.	[81]
PAMAM	Berberine	Conjugation and Encapsulation	Improved pharmacokinetic profile.	[82]
PAMAM	Gallic acid	Conjugation	Improved bioavailability.	[83]
Silica-PAMAM	Black carrot anthocyanin	Encapsulation	Sustained release; Less toxicity and enhanced activity.	[84]
PAMAM-G4	Resveratrol	Encapsulation	Improved solubility.	[85]

3.2.3.7. Conformational Behaviour

Dendrimers, like biological macromolecules, respond to the surrounding chemical environment showing altered conformational behaviour. Consequently, as its function is intimately related to its structure, it is important to be aware of the type of effect the surrounding environment exerts on the dendrimer.

3.2.3.7.1. Dendrimers and the Effect of pH

Dendrimers have been reported to act as solubilizing agents to host both hydrophilic and hydrophobic drugs. However, the mechanism of solubilization largely depends on the protonated/deprotonated state of the dendrimer. Amino-terminated PPI and PAMAM dendrimers have basic terminal groups as well as a basic interior. For these types of dendrimers, with interiors containing tertiary amines, the low pH (pH < 4) region generally leads to extended conformations, based on highly ordered structure. At this pH, the interior is getting increasingly "hollow" as the generation number increases, as a result of repulsion between the positively charged amines both at the dendrimer surface and the tertiary amines in the interior. In addition, at neutral pH, back-folding occurs which may be a consequence of hydrogen bonding between the positively charged surface amines and the uncharged tertiary amines in the interior of the dendrimer. At higher pH (pH ≥ 10) the dendrimer contract as the charge of the molecule becomes neutral, acquiring a more globular structure based on a compact network. At this point, the repulsive forces between the dendrimer arms and between the terminal groups reaches a minimum [86].

3.2.3.7.2. Dendrimers and the Effect of Salts

The high ionic strength, i.e., high concentration of salts, has a strong effect on charged dendrimers, such as PPI, and favours a contracted conformation of dendrimers, with a high degree of back-folding, somewhat similar to what is observed upon increasing pH. At low salt concentrations, the repulsive forces between the charged dendrimer segments results in an extended conformation in order to minimise charge repulsion on the structure [87].

3.2.3.7.3. Dendrimers and the Effect of the Solvent

The solvation power of any solvent to solvate the dendrimer is a very important criterion when investigating the conformation state of a dendrimer. Generally, dendrimers of all generations exhibit a larger extent of back-folding with decreasing solvent quality, i.e., decreasing solvation. Nevertheless, the low generation dendrimers show the highest tendency towards back-folding, as a result of poor solvation, when compared to the higher generation dendrimers. Chai *et al.* [88] studied the solvent effect on PPI dendrimers. They concluded that a nonpolar solvent (benzene), poorly solvates the dendrimers favouring intramolecular interactions between the dendrimer segments and the back-folding. However, a weak acid solvent (chloroform) can act as a hydrogen donor for the interior amines in a basic dendrimer such as PPI, leading to an extended conformation of the dendrimer. This is due to the hydrogen bonding between the solvent and the dendrimer amines. Studies on polar dendrimers, such as amino-terminated PPI and PAMAM dendrimers, show the tendency that nonpolar solvents ("poor") induce higher molecular densities in the core region as a result of back-folding, whereas polar solvents ("good") solvate the dendrimer arms and induce a higher molecular density on the surface of the dendrimer.

3.3. Biodistribution and Toxicity

Nanosystems, especially dendrimers, have been used to overcome certain limitations of most conventional drugs such as (i) low water solubility, (ii) narrow therapeutic index, (iii) low concentration at the target, (iv) high affinity to plasma proteins, (v) rapid elimination of the drug, and (vi) low specificity on the biodistribution. For the dendrimer to be considered a good excipient, it needs to be able to overcome the biological barriers of the organism. The size, chemical composition, surface structure and the shape of the dendrimer influence both its biodistribution and its toxicity. In addition, these properties allow us to understand how they are metabolised and what is the long term impact of the use of dendrimers at a cellular level [89].

3.3.1. Biodistribution of Dendrimers

Some *in vivo* studies have been performed to evaluate the biodistribution of dendrimers administered by the parenteral route. Kukowska-Latallo *et al.* [90] analysed, *in vivo*, the biodistribution and elimination of G5 PAMAM dendrimer coupled to folic acid and the radioactive tritium marker (G5-^{3}H-FA). In this study, two different dendrimers were synthesized and labelled with a radioactive compound: a dendrimer A, which was coupled to folic acid (G5-^{3}H-FA), and a dendrimer B (control), which was not coupled to folic acid (G5-^{3}H). They concluded that, on the first four days after administration, the clearance of G5-^{3}H-FA dendrimers was lower than G5-^{3}H dendrimers because the dendrimers A were found in tissues expressing the folic acid receptor. The kidney, being the main organ responsible for the elimination of these dendrimers, also expresses high amounts of the folic acid receptor. Thus, the kidney levels of the G5-^{3}H dendrimers rapidly decreased, whereas the dendrimers A slightly increased on the first 24 hours, due to the presence of the folic acid receptors on the renal tubules.

The biodistribution was evaluated on other studies, whose conclusions indicated that lower generation dendrimers (G3-G4) are cleared exclusively by the kidneys without further metabolization, G5 both by direct excretion by the kidney and excretion after liver conjugation, and higher generation dendrimers (G6-G9) are excreted only after hepatic metabolization. Therefore, it is possible to control the mode of excretion of a dendrimer by changing the number of generations of the nanoparticle, providing the dendrimer with an advantageous property as a pharmaceutical excipient [91].

Another study was performed with I^{125}- labelled PAMAM dendrimers, *in vivo*, to study the biodistribution of this nanocarrier. Dendrimers with charged, either anionic or cationic surface groups, and hydrophobic dendrimers are rapidly cleared from the circulation, particularly by the liver [92]. However, Malik *et al*. [93] have further demonstrated that the anionic dendrimers remain in blood circulation for a longer period of time than the cationic dendrimers. On their turn, dendrimers with a hydrophilic surface (e.g., hydroxy-terminated or PEGylated dendrimers), and dendrimers with a higher number of generations, remain in circulation for longer periods.

Therefore, biodistribution is an important factor to be considered, and the dendritic conjugates should remain in the bloodstream for a sufficient period of time to achieve therapeutic efficacy and allow accumulation at the target, such as tumour cells. On the other hand, nanoparticles must be easily removed from the human body, in order to avoid unacceptable long-term accumulation [94].

3.3.2. Toxicity of Dendrimers

Although there are numerous advantages in the use of these nanosystems as a pharmaceutical excipient, it is important to evaluate the toxicity associated with dendrimers. Due to the size of the dendrimers (1–100 nm), they interact with some cellular elements, such as the cell membrane, nucleus and proteins, as these cellular constituents are on the same dimension span. Furthermore, the dendrimers may also complex some metal ions, such as iron and zinc, affecting the biological action of haemoglobin and the renal function, respectively. However, the main determining factor for dendrimer induced toxicity is its surface charge. The toxicity of a polymer *in vivo* is influenced by pharmacokinetics and biodistribution. In this way, biodistribution tests become indispensable to analyse which tissues or organs have a greater storage capacity of the drug and which, therefore, are potential targets of toxicity [89; 92]. In the next sections, some important toxic effects will be mentioned in more detail.

3.3.2.1. Membrane Interaction

Some investigators have demonstrated that cytotoxicity is highly related to the terminal groups present on the surface of the dendrimer. Lee and Larson [96] demonstrated that these cationic nanostructures interact with the negative charges of the phosphate groups of the lipid bilayer through electrostatic interactions, leading to the formation of small pores (nanopores),

contributing to lower stability and increased cellular permeability. In addition, they concluded that cell membrane rupture is more pronounced at higher concentration, molecular weight and generation of the dendrimer, because these features are intimately related to the ability of the nanosystem to form nanopores. Thus, the results indicate that the spheroidal form of the dendrimers seems to be more efficient in drug transport than linear polymers, due to the increased permeability of the membrane. However, this increase of permeability can also lead to harmful effects and, ultimately, cellular lysis. It should be noted that other researchers also realized that the induction of permeability by dendrimers was not permanent and leaked cytoplasmic proteins returned to normal levels upon removal of dendrimers [95].

3.3.2.2. Haemolytic Toxicity

The dendrimers which exhibit terminal cationic groups on their surface interact with the red blood cells, leading to haemolysis. Bhadra *et al.* [97] performed an *in vivo* study of toxicity and concluded that the main limitation of cationic amine terminated PPI dendrimers was the haemolytic effect. They concluded that dendrimers with free amine groups on their surface caused 35.7 % and 49.2 % of haemolysis for PPI G4 and G5 dendrimers, respectively. On the other hand, when the PPI dendrimers are peripherally coated with galactose, there is a significant reduction in the haemolysis to 10 % and 7.1 %, respectively, in the dendrimers G4 and G5. In contrast, studies with anionic dendrimers show absence of haemolytic activity. Some authors suggested that the higher the generation of the cationic dendrimer, the greater the observed haemolysis, attributing this direct proportionality to the fact that these dendrimers have higher cationic charge [92].

3.3.2.3. Cytokine Release

Dendrimers can modulate the release of the reactive oxygen species (ROS) and, therefore, increase cytokine production, which may be a useful as a therapeutic tool or lead to significant toxic effects. Naha *et al.* [98] studied the inflammatory mediators, namely, the macrophage inflammatory protein-2 (MIP-2), tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6). These inflammatory mediators were measured by the enzyme linked immunosorbent assay (ELISA) after exposure of macrophage cells to PAMAM dendrimers (G4, G5 and G6). The investigators demonstrated that the toxic response of the PAMAM dendrimers correlated well with the number of surface primary amino groups, i.e., with the

increase of the dendrimer generation. The response consists of an increased intracellular ROS production and cytokine-induced cytotoxicity which, in high concentrations, can lead to cell death.

It has been shown that both the anti-inflammatory properties and the toxic effect of dendrimers are intimately related to their terminal groups, charge and number of generations. Pramod *et al.* [99] demonstrated that PAMAM dendrimer terminated with amine (-NH_2), hydroxyl (-OH) or carboxylic (-COOH) groups conjugated to glucosamine inhibit the release of cytokines. As a consequence, these dendrimers have potential as tools for various therapies, such as in rheumatoid arthritis. Although there is not enough information to establish the structure-activity relationships of dendrimers, these nanosystems show great potential in the future as possible anti-inflammatory therapeutic agents.

3.3.2.4. Immunogenicity

Several studies aimed to test if dendrimers exhibit an exaggerated immune response when administrated in the body. However, the literature suggests that dendrimers, depending on the size and end groups of the dendrimer´s surface, may provide a weak immunogenic response or even no response at all, not inducing the production of specific antibodies against the dendrimers. Roberts *et al.* [100] examined the immunogenicity of PAMAM dendrimers but observed no signs of immunogenicity within the dose range of 0.1 - 0.0001 μM. Agashe *et al.* [101] investigated *in vivo* the immunogenicity of G5 PPI dendrimers using ELISA for monitoring the antibody production. They reported that dendrimers were unable to provoke any detectable humoral immune response under the experimental conditions. This means that these nanosystems are not recognized by the host immune system as "foreign" particles, rendering them suitable for the drug transportation across the body.

3.3.3. Solutions for Toxicity Issues

After the recognition that some dendrimers could induce cytotoxicity and haemolytic effect, the investigators developed several methods to overcome the adverse effects associated with the use of dendrimers [95]. Figure F1 provides a general overview of the various strategies that can be used to minimize the toxicity associated with dendrimers, which can be organised on biocompatible or biodegradable dendrimers and surface engineered dendrimers.

3.3.3.1. Biocompatible or Biodegradable Dendrimers

One of the possible ways to decrease the toxicity associated with the dendrimers is based on the development of biodegradable central core, branches and surface groups. However, researchers believe that the construction of dendrimers with biodegradable surface functionality is probably the most effective strategy to solve the toxicity issues of the dendrimer. These dendrimers are intended to be degraded into non-toxic compounds and subsequently eliminated from the circulation. Consequently, the use of these dendrimers allows the performance of their functions as pharmaceutical excipients without toxic or immunological effects [102]. Some examples of biocompatible dendrimers include: i) the peptide dendrimer, ii) the polyester dendrimer, iii) the triazine dendrimer, iv) the phosphate dendrimer, v) the polyether dendrimer, vi) the melamine dendrimer, and vii) the polyether imine dendrimer [95]. These dendrimers have been studied and the investigators have been able to demonstrate that these nanocarriers can be used as drug delivery vehicles in a safe and effective manner. These biocompatible dendrimers are mentioned in more detail in Table 4.

Table 4: Examples of biocompatible dendrimers as well as the observed effects.

Biocompatible Dendrimer	Chemical structure	Results	Reference
Peptide dendrimer	PLL-Lactose G4 dendrimer.	Reduces haemolysis.	[103]
Polyester dendrimer	Polyester dendrimer with ethylene oxide as the branching unit.	Absence of toxicity in cells and decreased drug toxicity.	[104]
Polyether dendrimer	Carboxylate and malonate as terminal groups.	Absence of haemolysis in the erythrocytes one hour after its administration.	[93]
Polyether imine dendrimer	Carboxylic acid as terminal group.	Absence of toxicity in cells.	[105]
Phosphate dendrimer	5G thiophosphate dendrimer.	Dendrimer is neither hemotoxic nor cytotoxic.	[106]
Melamine dendrimer	Melamine as the branching unit.	Significant reduction in hepatotoxicity.	[107]
Triazine dendrimer	Triazine dendrimer with hydrazone linkages.	No toxic effect and degradable into small molecules.	[108]

3.3.3.2. Surface Engineered Dendrimers

Surface engineering appears to be one of the best strategies for decreased dendrimer's toxicity. This strategy is based on the modification of the surface groups in order to protect the cationic groups such as the amine groups. By using neutral or anionic molecules, the

electrostatic interactions of these molecules with the cell membrane are prevented, thus avoiding the cytotoxicity of the cationic groups. In addition to the decreased toxicity, this technique also enables: i) to improve drug encapsulation efficiency, ii) to improve biodistribution and pharmacokinetic properties, iii) to increase solubility, iv) targeting to specific site, v) better transfection efficiency, vi) to sustain and control drug release, vii) improvement of the stability profile, and viii) to improve potential of the anti-viral and anti-bacterial activity [95]. Table 5 presents several possible strategies to modifying the dendrimer surface groups.

Table 5: Examples of strategies to modifying the dendrimer surface groups.

Technique	Conjugated Molecule	Results	Reference
PEGylation	Polyethylene glycol (PEG)	Improved drug loading and decreased haemolytic toxicity of the PAMAM dendrimer.	[109]
Carbohydrate conjugated dendrimer	Maltose	Decreased haemolytic activity inherent to the PPI dendrimers.	[110]
Acetylation	Acetyl groups	Decreased PAMAM dendrimers toxicity and maximizing their transepithelial permeability.	[111]
Half generation	Carboxylic groups	Decreased cytotoxicity associated with PAMAM dendrimer.	[112]
Peptide conjugated dendrimer	Arginine-glycine-aspartate peptide	The conjugation of tripeptides minimized the cytotoxicity of the cationic PAMAM dendrimer.	[113]
Drug conjugated dendrimer	Flurbiprofen	The drug-dendrimer complex showed lesser haemolytic toxicity than PAMAM dendrimer.	[114]
Antibody conjugated dendrimer	Human epidermal growth factor receptor-2 monoclonal antibody (Anti-HER2 mAb)	Rapid and efficient cellular internalization of the dendrimer-antibody conjugate with low systemic toxicity.	[115]
Tuftsin conjugated dendrimer	Threonyl-lysyl-prolyl-arginine peptide (Tuftsin)	Tuftsin-PPI complex possessed lower cytotoxicity than the PPI dendrimer.	[116]
Folic Acid conjugated dendrimer	Folic Acid and Polyethylene glycol (PEG)	Folic acid-PEG-PAMAM has lower haemolytic toxicity compared to the PEG-PAMAM and the PAMAM dendrimer.	[117]

3.4. Application of Dendrimers as Drug Delivery Systems

Over the past years, growing attention has been drawn to the development of controlled and sustained drug delivery systems. Dendrimers, due to their unique properties, like the globular shape, well-defined three-dimensional structure, high functionality, presence of cavities, and small size, are suitable nanocarriers for drug delivery applications. Consequently, these nanocarriers have stimulated wide interest in the field of nanotechnology, as the diverse biomedical applications, represented in Figure G1, testify.

The PAMAM dendrimer is the most well-studied and well-characterized class of dendrimers and was the first dendrimer that was synthesized and commercialized. For these reasons, this dendrimer has several medicinal and practical applications [118]. In the next sections, some applications of PAMAM dendrimer as drug delivery systems will be mentioned.

3.4.1. Dendrimers in Ocular Drug Delivery

The topical application of APIs to the eye is the most prescribed route of administration for the treatment of various ocular disorders. However, the intraocular bioavailability of topically applied drugs is extremely poor. This is due to the drainage of the excess fluid via the nasolacrimal duct and the elimination of the solution by the tears. By using specialized delivery systems such as dendrimers, the referred difficulties of ocular drug delivery are diminished. Ideal ocular drug delivery systems should be sterile, non-irritating, isotonic, biocompatible, biodegradable and should not run out from the eye. Trivedi *et al.* [119] improved the bioavailability of the pilocarpine in the eye recurring to dendrimers, and the results demonstrated that the resident time of the drug increased by using PAMAM dendrimers with hydroxyl or carboxylic groups.

3.4.2. Dendrimers in Oral Drug Delivery

Generally, the oral route is considered the favourite via for drug administration. Dendrimers are suitable candidates in oral drug delivery because these nanosystems improve drug solubility and absorption. Oral drug delivery studies using the human colon adenocarcinoma, Caco-2 cell line, have shown that low generation of PAMAM dendrimers cross cell membranes. Additionally, P-glycoprotein efflux transporter does not appear to affect dendrimers. Therefore the drug dendrimer complex is able to bypass the efflux transporter [74]. In another study, Kolhe *et al.* [120] synthesized a fourth-generation PAMAM (PAMAM-G4-OH) dendrimer covalently linked to ibuprofen. The results suggested that the dendrimer–

ibuprofen complex improved the drug efficacy by enhancing cellular delivery and that the complex produced a more rapid pharmacological response when compared to pure Ibuprofen. Furthermore, these dendrimer-drug conjugates can potentially be modified by attaching ligands and antibodies for targeted drug delivery.

3.4.3. Dendrimers in Intravenous Drug Delivery

The intravenous route is the most direct method for delivering drugs into the body. However, the poor water solubility of many drugs (especially anti-cancer drugs) limits the application of the intravenous administration route. Additionally, intravenous administration of these drugs may result in several side effects, such as haemolysis and cytotoxicity. Numerous studies have been made to develop new formulations that are suitable for the intravenous route. Dendrimer-drug formulation had proved to be a useful nanocarrier of drugs with low solubility in different routes of administration because they can provide drugs with greater water-solubility, bioavailability, and biocompatibility. Consequently, there is a growing interest concerning the application of dendrimers as targeting carriers in cancer therapy [121]. Malik and collaborators [122] conjugate PAMAM dendrimer with cisplatin, a potent anti-cancer drug with some toxicity and poor water solubility. This conjugate showed, *in vivo,* increased solubility, decreased systemic toxicity and enhanced permeation as well as being able to selectively accumulate cisplatin in solid tumours.

3.4.4. Dendrimers in Pulmonary Drug Delivery

Lungs represent an attractive alternative route and site of drug administration due to their large surface area, thin alveolar region, extensive vasculature as well as avoidance of the first-pass metabolism. This advantage leads to increased systemic bioavailability of the drug and more effective therapeutic action. Due to their unique structure, many types of dendrimers have been designed, developed and studied for pulmonary delivery of various therapeutics. These nanosystems have demonstrated to have good potential as inhalable drug delivery alternatives for the treatment of pulmonary disorders [123]. Bai *et al.* [124] studied PAMAM dendrimer as a carrier for pulmonary delivery of enoxaparin, a low molecular-weight heparin, to treat vascular thromboembolism. They concluded that the positively charged PAMAM dendrimers are a suitable nanocarrier for pulmonary delivery of enoxaparin, without damage to the lungs. Additionally, these investigators showed that heparin encapsulated in pegylated dendrimers has a longer circulating half-time and increased pulmonary absorption.

3.4.5. Dendrimers in the Central Nervous System Drug Delivery

The brain is a challenging organ for drug delivery because of the blood-brain barrier (BBB) that is the best gatekeeper, protecting the central nervous system (CNS) of the exogenous substances. Consequently, the drug delivery to the brain is challenging because many drugs have inadequate solubility, lipophilicity, limited bioavailability and the BBB can block 98 % of drugs. Due to the ineffectiveness of conventional drug therapies, finding ways to deliver therapeutic drugs to the CNS safely and effectively is indispensable. Nanomedicine has shown great potential for the treatment of many CNS diseases with nanocarrier delivery systems such as dendrimers. These nanocarriers have demonstrated promising properties in CNS drug delivery with low toxicity and low immunogenicity as well as increased solubility, stability, and permeability of drugs. Furthermore, dendrimers have more efficient paracellular and transcellular transport across the BBB, which makes them ideal carriers for targeting water-insoluble drugs to the brain [125]. Katare *et al.* [126] investigated the efficiency of water-insoluble antipsychotic drug haloperidol via the intranasal route using PAMAM dendrimer. They demonstrated that aqueous solubility of haloperidol was increased with the dendrimer-based formulation and showed a significantly higher distribution of haloperidol in the brain and plasma compared to a control formulation of the drug. This study demonstrated the potential of dendrimers in improving the delivery of water-insoluble drugs to the brain.

3.4.6. Dendrimers in Transdermal Drug Delivery

Dendrimers were designed to be highly water-soluble and biocompatible. It was demonstrated that dendrimers are able to improve drug properties, such as plasma circulation time and permeation through the skin, thus delivering drugs efficiently on transdermal formulations. PAMAM dendrimer complex with nonsteroidal anti-inflammatory drug, such as ketoprofen or indomethacin, could improve the drug permeation through the skin as penetration enhancers. Chauhan and co-workers [127] investigated the enhanced bioavailability of PAMAM dendrimer by using indomethacin as the model drug in transdermal drug application. They demonstrated that PAMAM dendrimer showed to be effective as a drug delivery system because this nanosystem increased the flux of indomethacin across the skin *in vitro* as well as *in vivo*.

3.4.7. Dendrimers in Nasal Drug Delivery

Intranasal delivery is distinguished from the various strategies currently available for drug targeting. It is non-invasive and reduces the exposure of non-target sites to the API, thus increasing the efficiency and safety of the drug. Drug molecules can be targeted to the brain via the nasal cavity through the trigeminal nerve pathway and the olfactory nerve pathway. Dendrimers have been reported to enhance the aqueous solubility of drugs by forming a complex with them. This complex would provide a high concentration of drug at the nasal area. PAMAM dendrimers, for example, have caught the attention of researchers regarding nose-to-brain targeting [125]. Perez *et al.* [128] studied intranasal delivery with dendrimers by coupling radioactive small interfering ribonucleic acid (siRNA) to PAMAM dendrimers to form dendriplexes (siRNA−dendrimer complexes) and formulated these particles into mucoadhesive gels. Several concentrations of the different gels were tested, and no toxicity was observed. Moreover, dendriplexes showed increased radioactivity in the brain. Thus, this study demonstrated the potential of PAMAM dendrimers in improving the delivery of drugs to the brain via intranasal administration.

3.4.8. Dendrimers in Gene Delivery

The ability to transfer genetic material efficiently, into the cytoplasm and the nucleus of eukaryotic cells may allow the treatment of a variety of genetic disorders. Dendrimers are one of the most useful gene delivery systems and play a significant role in the development of vectors for gene delivery due to their ability to transfect genes without inducing toxicity. Additionally, the high charge density in the surface of the nanocarrier allows optimal condensation and formation of nanostructures with deoxyribonucleic acid (DNA). Among the several commercially available dendrimers, PAMAM dendrimers have received the most attention as potential gene delivery agents due to their cationic nature which enables DNA binding at physiological pH by electrostatic interactions. Literature suggests that functionalized dendrimers are much less toxic than the native dendrimers [125]. Luo *et al.* [129] revealed the low cytotoxicity of PEG-modified PAMAM dendrimers and their efficiency on the DNA delivery to the cells. They demonstrated that the PEG-modified PAMAM dendrimer is an extremely efficient, highly biocompatible, low-cost DNA delivery system and it can be readily used in basic research laboratories as well as in future clinical applications.

3.4.9. Dendrimers in Vaccines

Most low molecular weight substances are not immunogenic and, consequently, when it is desired to rise induce antibody production against small molecules, they must be conjugated to a macromolecule. Nowadays, a possible alternative strategy to solve this problem is to use dendrimers as nanocarriers of these small antigens. Dendrimers have optimal characteristics as efficient immunostimulant compounds (adjuvants) that can increase the efficiency of vaccines. Several studies have been performed to verify if the PAMAM dendrimer is an ideal carrier of small antigens. The results demonstrated that this nanosystem does not induce adverse host responses, including immune and/or inflammatory reactions after administration. This demonstrates that PAMAM dendrimers can be used successfully in conjugates with antigens [125, 130].

3.4.10. Patents

Although dendritic polymers have a history of nearly three decades, the amount of papers and patents is increasing every year, which is indicative of the continuous progress on their applications in academic researches, industrial processes as well as in the biomedical field [131]. In Table 6, some recent examples of patents for dendrimers as drug delivery systems will be mentioned.

Table 6: Examples of patents of dendrimers as drug delivery systems.

Pharmaceutical Application	Dendrimer	Drug loaded	Summary	Publication Date	Patent	Reference
Gene delivery	PAMAM	MicroRNA-150 (miR-150)	A PAMAM dendrimer was designed for sustained delivery of miR-150 to FLT3-overexpressing acute myeloid leukaemia cells. Preclinical animal model studies have demonstrated good therapeutic efficacy.	2019	US20190175754	[132]
CNS drug delivery	PAMAM	Prion protein (PrP)	PrP was conjugated to PAMAM dendrimers for Alzheimer's therapy. This complex will inhibit β-amyloid plaque formation (they act as potent neurotoxins *in vitro* and *in vivo* in Alzheimer's disease).	2019	US20190092837	[133]
Tumour drug delivery	PAMAM	Disulfiram	Disulfiram and photosensitizer indocyanine green were entrapped into PAMAM-G0 dendrimer for anti-tumour therapy. This prepared a nano-drug delivery system that can simultaneously play roles of chemotherapy and photodynamic therapy.	2018	CN108888764	[134]
Tumour targeting and controlled drug release	PAMAM	Doxorubicin (DOX)	Tumour targeting and controlled drug release of the DOX-PEG-PAMAM dendritic complex is controlled by the pH.	2017	CN107596385	[135]
Tumour targeting	PAMAM	Erlotinib	The Erlotinib-PAMAM dendrimer will target tumour cells with a high expression of CD44 and can specifically deliver more drugs to the tumour site.	2017	CN107281164	[136]
Targeted drug delivery	PLL	Polynucleotides	The Rabies virus glycoprotein was conjugated to PLL dendrimer to provide effective and safe delivery of polynucleotides to target cells.	2012	KR1020120067168	[137]
Gene delivery	PLL	Plasmid DNA	A PLL system containing a vector with intracellular nuclear protein binding and reducible polymers is provided to stabilize plasmid DNA in an extracellular region and to promote its absorption to the target cell.	2012	KR1020120007208	[138]
Cancer targeting	Peptide-dendrimer	Docetaxel	The peptide was conjugated with the dendrimer for targeting, imaging, and treatment of prostate cancer.	2018	EP3402484	[139]
Vaccine	Positively charged dendrimer	Antigen	Branched polymeric dendrimers (e.g., PAMAM and other dendrimers) were used as vehicles for the targeted delivery of antigen to specific cells, giving rise to a new nanoparticle-based method for genetic or protein vaccination.	2018	US20180099032	[140]
Drug delivery system	Asymmetric dendrimer	Paclitaxel	Paclitaxel-loading asymmetric dendrimer nanometer drug carrier system has the anti-tumour treatment index and biosecurity enhanced compared with those of free Paclitaxel during the *in vivo* treatment.	2017	CN106512021	[141]
Transdermal drug delivery and permeation enhancer	Second generation oleodendrons	Diclofenac	Oleic acid-based dendron is used as a potential chemical penetration enhancer in transdermal drugs.	2013	IN1749/MUM/2010	[142]

3.5. Conclusion and Future Perspectives

Dendrimers, as excipients, are predicted to have a foremost role in both the pharmaceutical industries and for medicine. Due to their structural properties (like nanoscale uniform size, the high degree of branching, polyvalency, water solubility and availability of internal cavities), and the fact that they can be almost precisely controllable during their synthesis, dendrimers may be used as excipients to improve the drug formulation properties. Additionally, there is a possibility of extending the patent lifetime.

The drug may be attached to the dendrimer by covalent bonds, electrostatic interactions or encapsulation. The choice of drug-dendrimer interaction is dependent on the needs and properties of the drug and the type of pathology. Dendrimers are excellent drug carriers and can be carefully engineered for the delivery of biomolecules to target cells, allowing the use of smaller drug doses, with smaller side effects.

Another advantage of the dendrimer is that this nanosystem has been shown in various studies, to be a compatible, safe and effective nanocarrier in various routes of administration. Consequently, it allows us to increase the range of drugs that have therapeutic value but are rejected by the pharmaceutical industry because of their low water-solubility.

Many studies already did show evidence that dendrimers are promising excipients in several therapeutic formulations. However, it should be noted that the studies carried out so far have been performed on in vitro experimental models or animal models. Thus, it is not yet possible to extrapolate these results to humans. Although this new technology is promising, further in vivo studies are required to understand with more accuracy the biocompatibility and dendrimer-associated toxicity. Only then clinical studies in humans are possible to evaluate the medium/long-term toxicological impact.

Although dendrimers are relatively new structures (approximately 30 years) and the fact that they cannot be recognized as a pharmaceutical excipient yet, they present a promising future in the pharmaceutical and biomedical field.

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5. Annexes

Annex A - Structure of the dendrimer

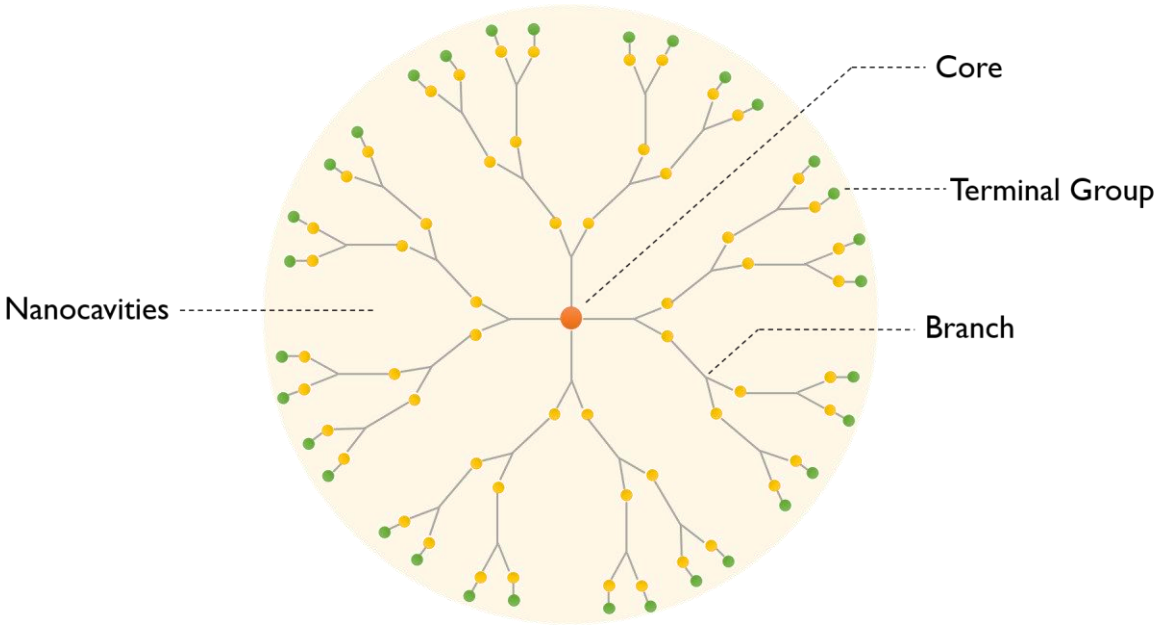

Figure A1: Basic structure of a dendrimer.

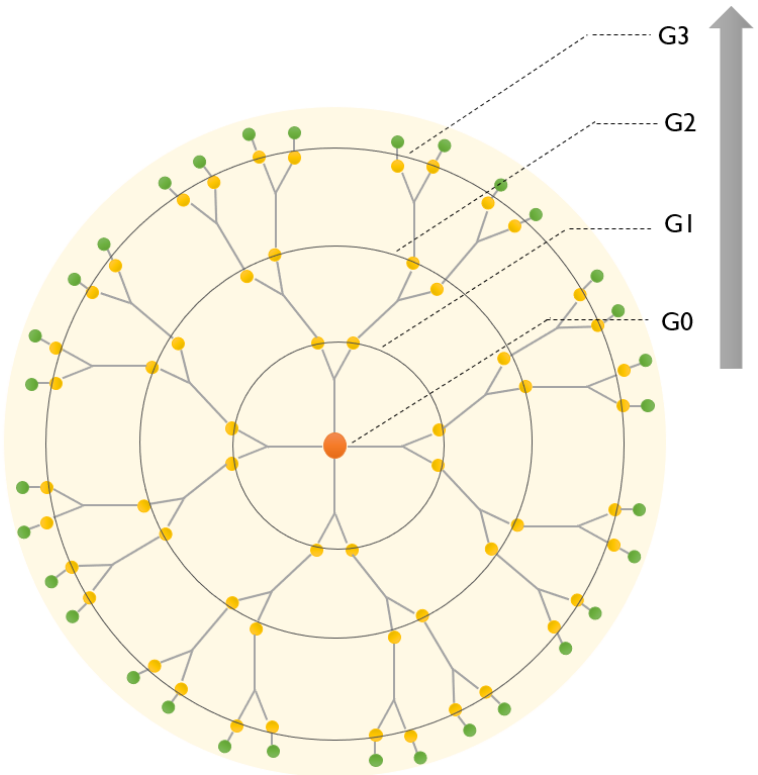

Figure A2: Schematic representation of the increasing generations of the dendrimer: from first (G1) to third generation (G3).

Annex B - Dendrimer's classification

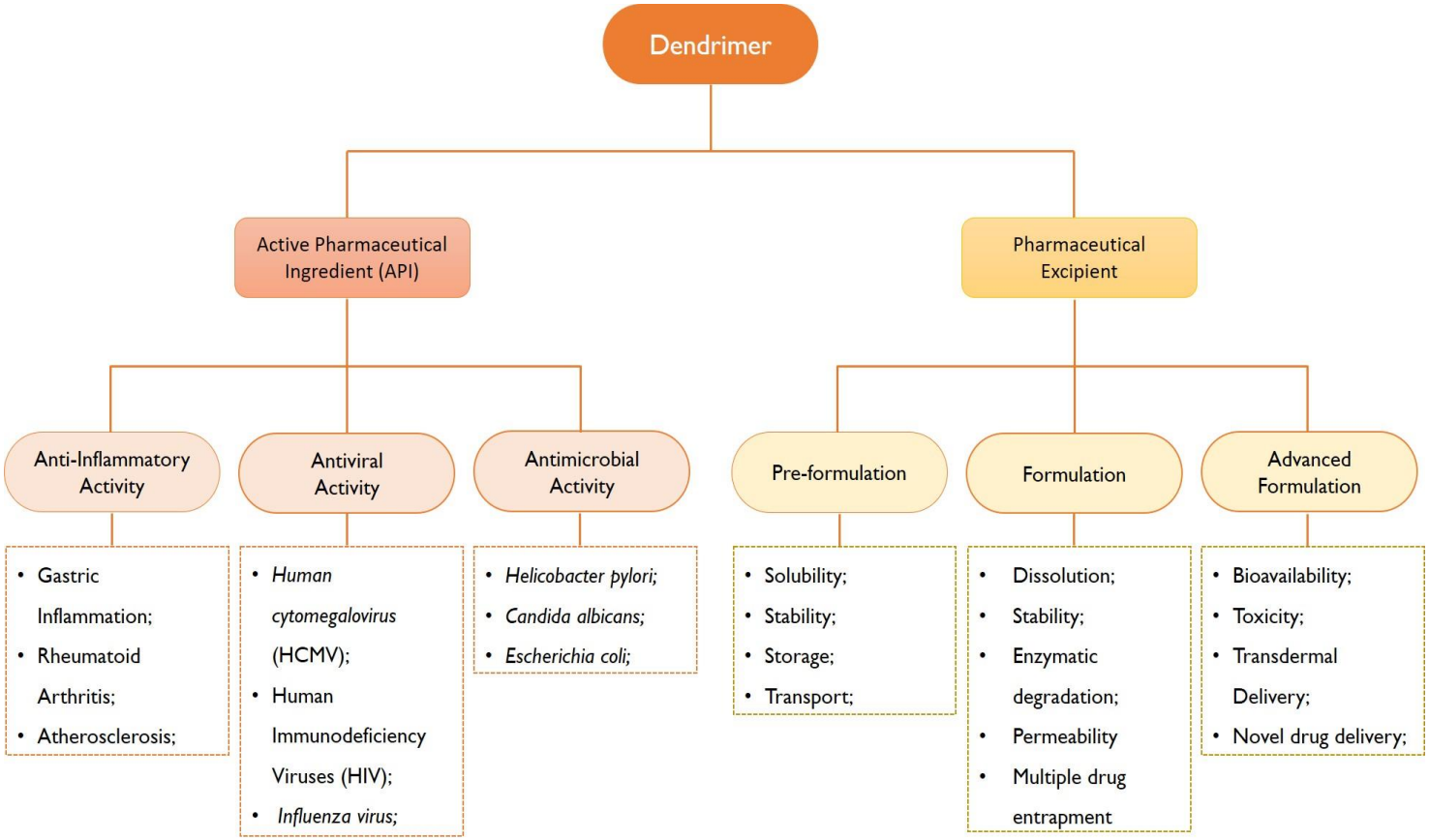

Figure B1: Dendrimer's classification according to their role in the formulation. The constitution of the dendrimer may confer different activities, namely, anti-inflammatory activity, antiviral activity and antimicrobial activity. As pharmaceutical excipient, dendrimers may enhance distinct properties of the formulation according to the phase of drug product development.

Annex C - Synthesis of dendrimers

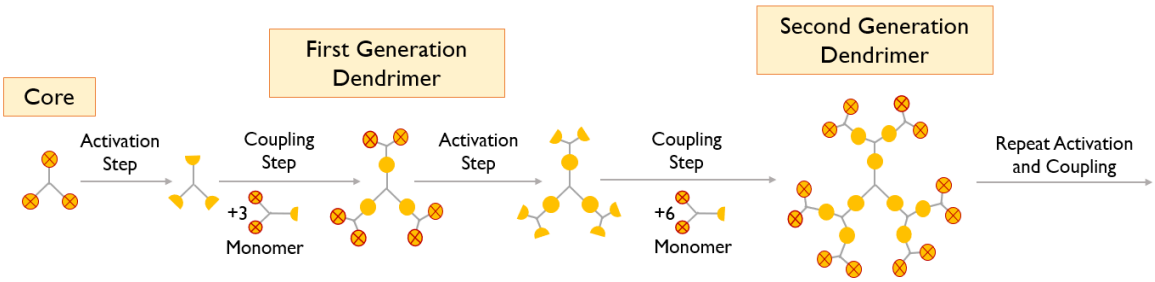

Figure C1: Synthesis of dendrimers by the divergent growth method.

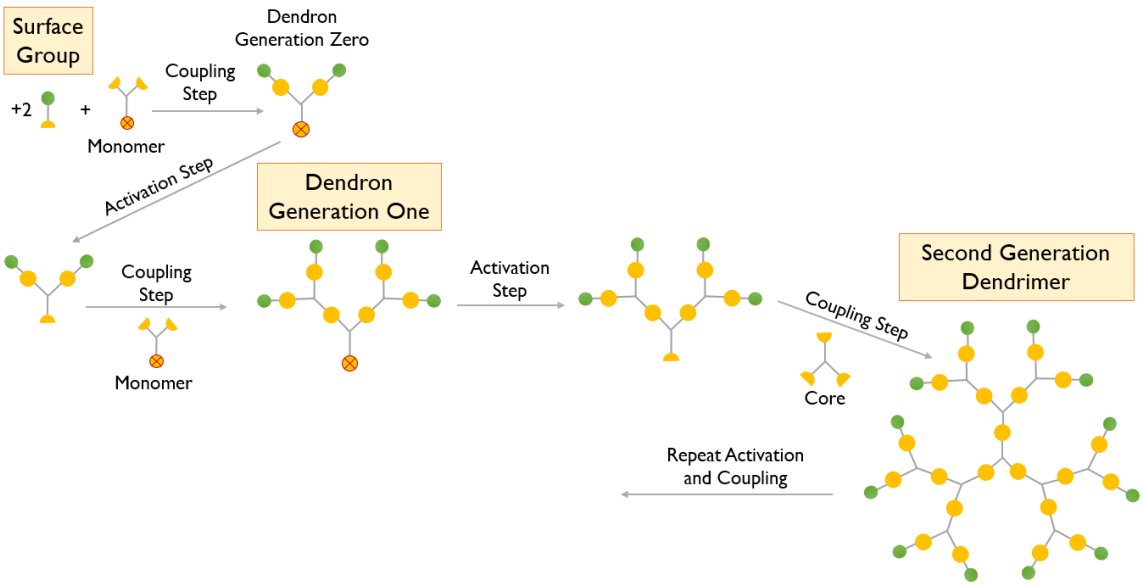

Figure C2: Synthesis of dendrimers by the convergent growth method.

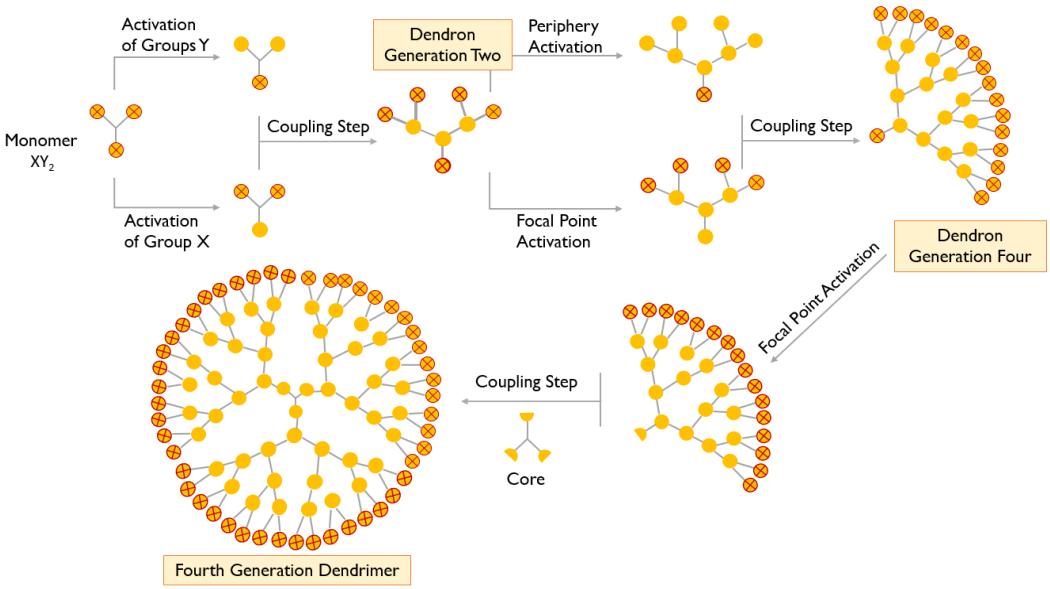

Figure C3: Synthesis of dendrimers by the double exponential growth technique.

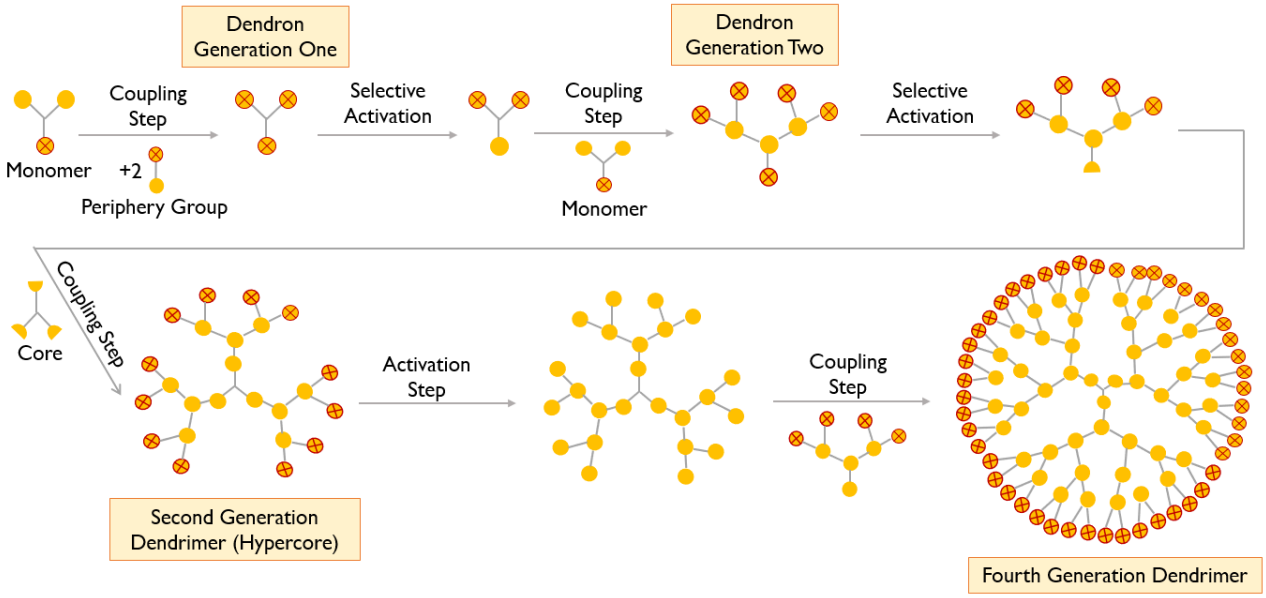

Figure C4: Synthesis of dendrimers by the double-stage convergent method or the hypercore approach.

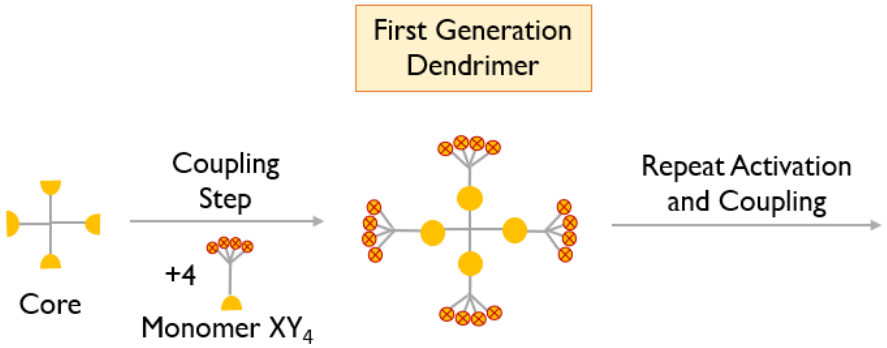

Figure C5: Synthesis of dendrimers by the hypermonomer method or the branched monomer approach.

Annex D - Advantages and disadvantages of the dendrimers preparation method

Table D1: Summary of the main advantages and disadvantages of the dendrimers preparation method.

Method of Preparation	Advantages	Disadvantages	Examples of dendrimers
Divergent Growth Method	Fast synthesis; Production of large quantities; Synthesis of highly symmetric dendrimers; The surface of the dendrimer can be easily modified with desired functional groups; Allows formation of high generation dendrimers.	Possibility of defects in the higher generation dendrimers product; Difficult in the separation of the desired product from reactants; Excess of reagents; Requires numerous steps to form a large structure; Requires a large quantity of starting material; Possible incomplete reaction of the terminal groups.	PAMAMs; PPI; Poly(arylalkyl ether);
Convergent Growth Method	Easy to purify the desired product; Occurrence of defect is minimised; Possibility of synthesis of asymmetric dendrimers; Involves only a small number of reactions per molecule; Provides greater structural control than the divergent approach.	Does not allow formation of high generation of dendrimers; Lower yield; Difficult to modify the terminal groups.	JDs; Poly(aryl ether); Poly(aryl alkyne); Poly(phenylene); Poly(alkyl ester); Poly(alkyl ether);
Double Exponential Growth Technique	Elaboration of large multifunctional dendrons or dendrimers; Preparation of symmetric, supramolecular, or asymmetrical dendrimers; High synthetic yields; Large number of dendrimers using same monomers for 2-3 times.	The process is time-consuming, as the method uses both convergent as well as divergent process.	Poly(phenylacetylene); Poly(amide); Poly(ether urethane); Poly(ester); JDs;
Double-Stage Convergent Method	Allows the formation of high generation dendrimers; Uses a hypercore that reduces the steric effect; Helps obtaining more monodisperse dendrimers; Enables the formation of dendrimers with chemically differentiated internal and external branches.	The synthesis of the hypercore, the dendrons, and the final dendrimers is slow.	Phenylacetylene; Poly(amide);
Hypermonomer Method	Dendrimers showing a high number of functional groups in fewer steps; Allows the formation of high generation dendrimers in few steps.	Synthesis requires several growth and activation steps; The acceleration is limited to generating dendrimers; Monomers synthesis is a time-consuming process.	Poly(aryl ether); Triazines;

Annex E - Physicochemical Properties of the dendrimers

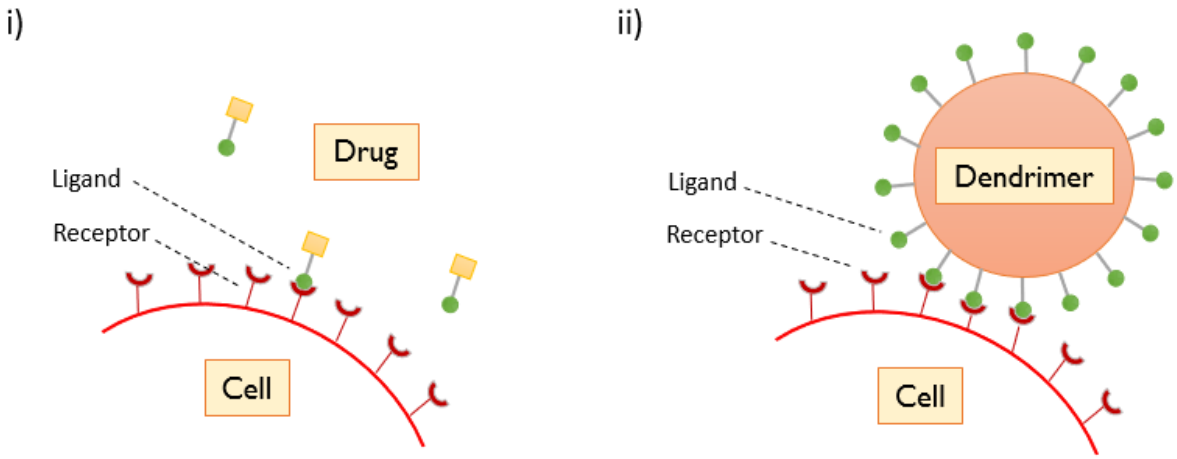

Figure E1: Comparison of the (i) classical interaction of the free drug with the cell receptor with the (ii) enhanced dendrimer interaction.

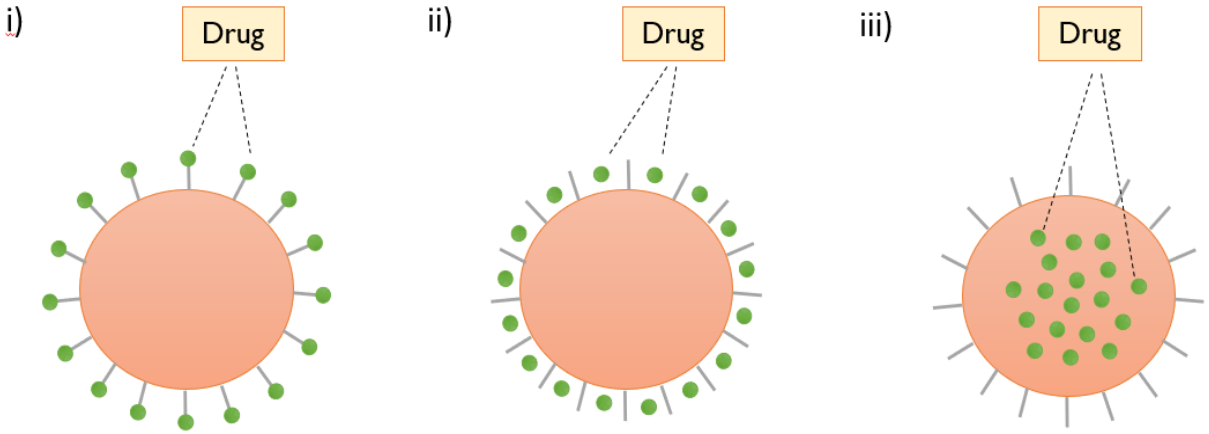

Figure E2: Schematic representation of the three ways of incorporation of the drug in the dendrimer: i) Covalent binding, ii) electrostatic interactions, and iii) encapsulation.

Annex F - Strategies to decrease the toxicity associated with dendrimers

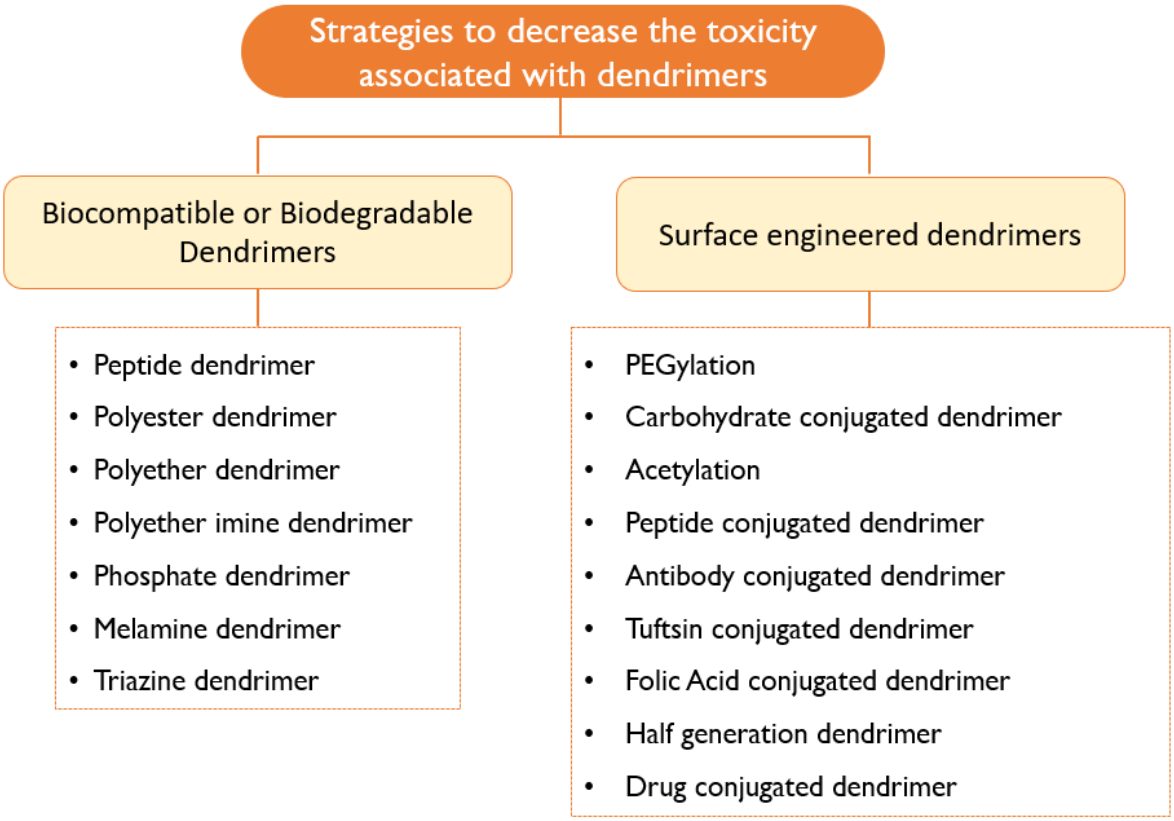

Figure F1: Various strategies to decrease the toxicity related with dendrimers.

Annex G - Application of dendrimers as drug delivery systems

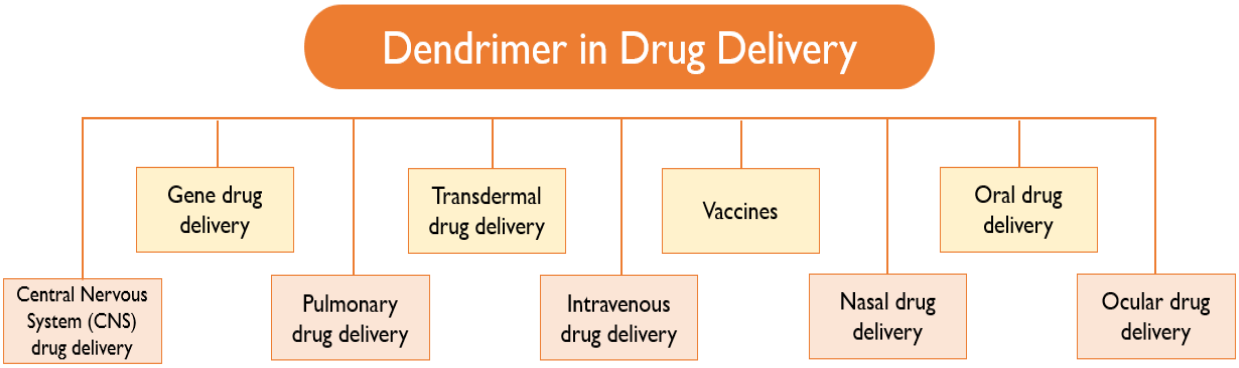

Figure G1: Several applications of dendrimers in drug delivery.