Ana Carolina de Oliveira Ferreira

Relatórios de Estágio e Monografia intitulada "Halloysite Clay Nanotubes for Life Sciences Applications - From drug/protein encapsulation to bioscaffold" referentes à Unidade Curricular "Estágio", sob orientação, respetivamente, da Dra. Cláudia Gama, da Dra. Isabel Folhas e do Professor Doutor Francisco Veiga e apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro 2017

· U C ·

Universidade de Coimbra

Ana Carolina de Oliveira Ferreira

Relatórios de Estágio e Monografia intitulada "Halloysite Clay Nanotubes for Life Sciences Applications - From drug/protein encapsulation to bioscaffold" referentes à Unidade Curricular "Estágio", sob orientação, respetivamente, da Dra. Cláudia Gama, da Dra. Isabel Folhas e do Professor Doutor Francisco Veiga e apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro 2017

· U C ·

UNIVERSIDADE DE COIMBRA

Eu, Ana Carolina de Oliveira Ferreira, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o nº 2012130691, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatórios de Estágio e Monografia intitulada "Halloysite Clay Nanotubes for Life Science Applications – From drug/protein encapsulation to bioscaffold" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade de Estágio Curricular.

Mais declaro que este Documento é um trabalho original e que toda e qualquer afirmação ou expressão, por mim utilizada, está referenciada na Bibliografia, segundo os critérios bibliográficos legalmente estabelecidos, salvaguardando sempre os Direitos de Autor, à exceção das minhas opiniões pessoais.

Coimbra, 13 de setembro de 2017.

Ana Carolina de Oliveira Ferreira

Agradecimentos

À Dra. Cláudia Gama, Diretora do Controlo de Qualidade da Bluepharma® e minha orientadora, o meu muito obrigado pela forma como me acolheu e me integrou na restante equipa, assim como por toda a disponibilidade demonstrada ao longo do meu estágio.

À restante equipa do Controlo de Qualidade, o meu agradecimento por todas as dúvidas esclarecidas, sempre com prontidão e paciência. Sei que foram muitas as perguntas e a todas elas me deram resposta.

À Dra. Isabel Folhas, Diretora Técnica da Farmácia Isabel Folhas e minha orientadora, o meu especial agradecimento pela vontade incansável com que me ajudou a ser melhor e pelos valores que conduziu ao longo do meu percurso. A sua energia e saber ficarão sempre comigo.

Ao Dr. António Pereira, Farmacêutico Substituto da Farmácia Isabel Folhas, um obrigado por me ter demonstrado da melhor forma como se processa a gestão de uma farmácia e quais as tarefas que o farmacêutico deve desempenhar. Terei sempre em conta a atenção aos pormenores, tal como me transmitiu.

À restante equipa da Farmácia Isabel Folhas, agradeço pela forma como me integraram e por tudo o que me foram ensinando ao nível de aconselhamento e do papel do farmacêutico na sociedade.

Ao Professor Doutor Francisco Veiga e meu orientador, um enorme agradecimento, quer pela disponibilidade, quer pela calma que sempre demonstrou ao longo da redação desta monografia.

À Professora Ana Cláudia Santos, é com gratidão que lhe mostro o fruto final deste trabalho. A sua disponibilidade e ajuda foram fundamentais, o meu sincero obrigado.

À Faculdade de Farmácia e a Coimbra, que me acolheram estes cinco anos.

À minha mãe e aos meus irmãos, pelos ensinamentos, pelos conselhos e pela paciência. A gratidão para com vocês nunca será suficiente.

Aos meus amigos, obrigada por tudo, festejos não faltarão.

ÍNDICE

PARTE I – RELATÓRIOS DE ESTÁGIOS

1. Introdução 10

2. Análise SWOT 11

3. Análise SWOT – Indústria Farmacêutica 11

3.1 Pontos Fortes 11

3.2 Pontos Fracos 15

3.3 Oportunidades 15

3.4 Ameaças 16

4. Análise SWOT – Farmácia Comunitária 17

4.1 Pontos Fortes 17

4.2 Pontos fracos 20

4.3 Oportunidades 22

4.4 Ameaças 24

5. Considerações Finais 25

Referências Bibliográficas 26

Anexos 28

1. Introduction 35

2. Selective drug loading, nanotubes' functionalization and drug release 37

2.1. Inside tube's lumen drug loading 38

a) Small drug molecules 39
b) Enzymes 44

2.2. Outside tube's lumen and dual drug loadings 45

a) Small drug molecules 45
b) DNA 47
c) Enzymes 48

3. Applications in medicine - administration routes 49

3.1. Topical 50

a) Creams 50
b) Sprays 50
c) Bandages 51
d) Pour powder 51

3.2. Oral 52

a) Tablets 53
b) Nanoparticles-in-microgel oral systems (NiMOS) 54

4. Additional applications in medicine 55

4.1. Implants 55

4.2. Tissue scaffolds 57

4.3. Would healing 61

5. Stability 62

6. Toxicity 64

7. Summary and future perspectives 67

Referências Bibliográficas 69

Resumo – Relatórios de Estágio

O Estágio Curricular é um elo de ligação entre a vida académica e a vida laboral, a primeira experiência com a qual o futuro farmacêutico se depara e onde deve ser capaz de interligar a sua formação teórica com as práticas laborais de diferentes áreas do medicamento. Como tal, optei por realizar estágio em Farmácia Comunitária e em Indústria Farmacêutica, entre os meses de janeiro a agosto de 2017.

O documento que se segue tem como objetivo analisar cada um dos estágios realizados de um modo retrospetivo e sob a forma de análise SWOT, salientando as forças, fraquezas, ameaças e oportunidades de cada percurso, procurando sempre aliar à formação de base que o Mestrado Integrado em Ciências Farmacêuticas me proporcionou.

Palavras-chave

Estágio Curricular, Farmácia Comunitária, Indústria Farmacêutica, Relatório de Estágio, Análise SWOT.

Abstract – Internship Reports

The Curricular Internship is a connecting link between the academic life and the work life, the first experience which the future pharmacist is faced with and where he must be able to interrelate his theoretical knowledge with the practices of different pharmaceutical fields. As such, I chose to practice Community Pharmacy and Pharmaceutical Industry, between the months of January and August of 2017.

The following report aims to analyze each internship from a retrospective way and through a SWOT Analysis, highlighting the strengths, weaknesses, threats and opportunities of each path, always regarding the knowledge foundation that the course provided me with.

Keywords

Curricular Internship; Community Pharmacy; Pharmaceutical Industry; Internship Report; SWOT Analysis.

Resumo – Nanotubos de Argila Haloisite para Aplicações em Ciências da Saúde – Desde a encapsulação de fármacos/proteínas até *bioscaffold*

Nas últimas décadas, os nanotubos de argila haloisite têm ganho bastante interesse entre a comunidade científica. Estes nanotubos são nanomateriais de argila com baixo impacto ambiental, que se têm demonstrado promissores em várias aplicações biomédicas. Adicionalmente, estes nanotubos são largamente disponíveis a baixo custo, sendo economicamente viáveis. Os nanotubos de argila haloisite são constituídos por camadas internas de carga positiva de alumínio e por camadas externas de carga negativa de sílica, que se curvam espontaneamente formando estruturas porosas tubulares ocas. Estas estruturas formam coloides estáveis em solventes aquosos devido ao seu potencial-zeta negativo.

Os nanotubos de argila haloisite permitem o controlo e o prolongamento da libertação de fármacos durante diversas horas, dias ou semanas. Em diversos estudos realizados, os nanotubos de argila haloisite foram funcionalizados com o objetivo de melhorar as suas propriedades, possibilitar a vectorização a um alvo terapêutico específico e ampliar o período de libertação de fármacos, aprimorando a sua *performance* como sistemas de entrega de fármacos. Os nanotubos de argila haloisite foram também reconhecidos como excelentes excipientes de compressão para comprimidos e são bons candidatos para a indústria de cosmética e *sprays* antibacterianos. Paralelamente, os nanotubos de argila haloisite demonstraram ser úteis na melhoria das propriedades mecânicas dos implantes e no desenvolvimento de *scaffolds* funcionais.

Perante o aumento acentuado do interesse na sua investigação, a toxicologia e biocompatibilidade dos nanotubos de argila haloisite foi avaliada aprofundadamente, e, no geral, os resultados desta pesquisa apoiaram a utilização segura destes nanomateriais relacionada com as suas aplicações biomédicas. Nesta monografia o potencial dos nanotubos de argila haloisite para aplicações em ciências da saúde foi discutido e demonstrado, destacando a sua utilização desde a entrega de fármacos, via administração tópica ou oral, até *scaffolds* e medicina regenerativa. Simultaneamente, a sua toxicidade, biocompatibilidade e estabilidade foram avaliadas, perspetivando, por fim, as futuras direções que a posterior investigação destes nanomateriais irá tomar nas aplicações farmacêutica e biomédica.

Palavras-chave

Nanotubos de argila haloisite; Sistemas de entrega de fármacos; Encapsulação de fármacos; Libertação de fármacos; Aplicações em ciências da saúde; *Scaffolds*; Implantes; Comprimidos; Biocompatibilidade; Toxicidade.

Abstract – Halloysite Clay Nanotubes for Life Sciences Applications – From drug/protein encapsulation to bioscaffold

Over the last few decades, halloysite clay nanotubes have gained great interest among the scientific community. These nanotubes are environmental friendly clay nanomaterials, which have shown to be promising for various biomedical applications. In addition, those nanotubes are widely available at low price, being economically viable. Halloysite clay nanotubes are composed by positively charged inner layers of alumina and negatively charged outer layers of silica, which naturally curve and form hollow tubular porous structures. These structures form stable colloids in water-based solvents due to their negative zeta-potential.

Halloysite clay nanotubes enable the attainment of controllable and prolonged drug release profiles for several hours, days or weeks. In several performed studies, halloysite clay nanotubes were functionalized in order to improve their properties, allow specific targeting and further extend drug release, therefore improving their performance as drug delivery systems. Halloysite clay nanotubes were also recognized as excellent compression excipients for tablets and constitute strong candidates for cosmetic industry and antibacterial sprays. Besides, halloysite clay nanotubes demonstrated to be useful in improving mechanical properties of implants and in developing functional tissue scaffolds.

Facing their increased research interest, the toxicology and biocompatibility of halloysite clay nanotubes were intensely assessed, and, overall, the results from those investigations supported the safe use of halloysite clay nanotubes related to their potential biomedical applications. This thesis discussed and demonstrated the potential of halloysite clay nanotubes for life sciences applications, highlighting their use from drug delivery, via oral or topical administration, to tissue scaffolds and regenerative medicine. Alongside, their toxicity, biocompatibility and stability were assessed, prospecting, ultimately, the upcoming directions for further research in the pharmaceutical and biomedical application of these nanomaterials.

Keywords

Halloysite Clay Nanotubes; Drug delivery systems; Drug loading; Drug Release; Life Sciences Applications; Scaffolds; Implants; Tablets; Biocompatibility; Toxicity.

Parte I

Relatórios de Estágios

Abreviaturas

CQ – Departamento do Controlo de Qualidade

DCI – Denominação Comum Internacional

FFUC – Faculdade de Farmácia da Universidade de Coimbra

FTIR – *Fourier Transform Infrared Spectroscopy*

HPLC – *High Performance Liquid Chromatography*

IMC – Índice de Massa Corporal

IV – Infravermelho

MICF – Mestrado Integrado em Ciências Farmacêuticas

MNSRM – Medicamentos Não Sujeitos a Receita Médica

MSRM – Medicamentos Sujeitos a Receita Médica

PON – Procedimento Operativo Normalizado

PSD – *Particle Size Distribution*

SWOT – *Strengths, Weaknesses, Opportunities, Threats*

UPLC – *Ultra Performance Liquid Chromatography*

USP-NF – *United States Pharmacopeia and The National Formulary*

UV – Ultravioleta

1. Introdução

Ao longo de nove semestres enquanto estudante do Mestrado Integrado em Ciências Farmacêuticas (MICF) na Faculdade de Farmácia da Universidade de Coimbra (FFUC), fui adquirindo as competências científicas através da formação curricular, assim como as ferramentas necessárias para poder exercer um bom papel enquanto futura profissional de saúde.

No decorrer do décimo semestre e com vista à conclusão do MICF, a FFUC proporciona aos seus alunos a unidade de Estágio Curricular, permitindo a aplicação destas competências em práticas laborais de ambiente supervisionado nas diferentes áreas do medicamento.

Como tal, optei por realizar estágio em Farmácia Comunitária e em Indústria Farmacêutica, os quais tiveram lugar na Farmácia Isabel Folhas e na Bluepharma®, respetivamente.

O meu percurso enquanto estagiária teve início no Departamento do Controlo de Qualidade (CQ) da Bluepharma®, no dia 9 de janeiro de 2017. Em primeira instância deram-me a conhecer todas as instalações e tive a oportunidade de me familiarizar com o circuito de informação e processos, desde a chegada das matérias-primas à saída dos medicamentos e tarefas posteriores. Após diversas formações e aprendizagem dos Procedimentos Operativos Normalizados (PON), fui inserida na equipa de análise físico-química da matéria-prima, na qual permaneci até ao término do meu estágio.

Em abril de 2017 tomei lugar na Farmácia Isabel Folhas, na qual, à semelhança do meu primeiro estágio, iniciei a atividade enquanto estagiária pelo conhecimento das instalações e do corpo da farmácia, assim como os diversos procedimentos que são realizados no âmbito desta atividade. O plano de estágio consistiu na passagem inicial pelas tarefas de *backoffice*, a nível de receção de encomendas, gestão de reservas e gestão de devoluções, progredindo para a disposição de lineares, preparação de manipulados, organização de receituário, análise de parâmetros antropométricos e bioquímicos, atendimento ao balcão e aconselhamento farmacêutico.

As tarefas por mim desempenhadas em cada um dos estágios foram monitorizadas pelos membros das respetivas equipas, os quais me auxiliaram e guiaram ao longo de toda a formação.

2. Análise SWOT

A análise SWOT é uma ferramenta que permite avaliar certo objeto e o seu meio envolvente, sendo um método que se divide numa componente interna, Forças e Fraquezas, e numa componente externa, Ameaças e Oportunidades. Neste caso, os Pontos Fortes e Fracos são relacionados com o objeto em análise, enquanto as Ameaças e Oportunidades são inerentes ao ambiente envolvente, mas influenciam o objeto de modo negativo e positivo, respetivamente[1].

A adaptação deste tipo de método para um âmbito pessoal, nomeadamente o percurso de estágio, permite-nos realizar uma análise sucinta, focando os aspetos mais importantes a nível do que executámos, o que correu pior, o que poderemos tomar como oportunidade num futuro próximo e do que nos devemos proteger.

Assim, em formato de Análise SWOT, segue-se a minha opinião pessoal acerca do meu trajeto enquanto estagiária em Indústria Farmacêutica, na qual interligo as componentes práticas que realizei com os conhecimentos teóricos que adquiri ao longo do meu percurso académico.

Esta retrospetiva é seguida de uma análise acerca do meu estágio em Farmácia Comunitária, a qual se rege pelos mesmos moldes e estrutura que a anterior.

3. Análise SWOT – Indústria Farmacêutica

3.1 Pontos Fortes

a) Identificação de matérias-primas

O circuito de operações de uma indústria farmacêutica inicia com a chegada de matérias-primas e respetiva amostragem. As amostras são então encaminhadas para o CQ, o qual procede à sua análise.

Em primeira instância dever-se-á identificar corretamente a matéria-prima, garantindo que corresponde aos seus requisitos. Cada composto possui as suas próprias caraterísticas a nível de aspeto, cor, espectro infravermelho (IV) e ultravioleta (UV), conteúdo em água e intervalo e ponto de fusão. Em alguns casos, quando aplicável, procedi também à análise da presença de certos grupos funcionais através de técnicas laboratoriais devidamente descritas nos procedimentos a seguir.

Na minha opinião, o farmacêutico como analista demonstra-se vantajoso devido ao seu conhecimento da substância a nível molecular, uma vez prevendo o comportamento de certa matéria-prima conduz para uma análise adequada e de confiança.

Este tipo de análise permitiu-me adquirir competências no manuseamento do FTIR, Espetrofotómetro UV/Visível e *Karl-Fischer*, equipamentos amplamente utilizados no meio analítico. Noutra perspetiva, este ponto também foi importante para instruir espírito crítico na análise comparativa entre os comportamentos da amostra e do padrão, nomeadamente na análise de espectros.

b) Certificação de parâmetros limite de matérias-primas

Aquando da análise das matérias-primas, torna-se fundamental assegurar o respeito pelos limites de certas substâncias e impurezas, de modo a garantir a segurança do utilizador final do medicamento.

Como tal, ao longo do meu estágio, tomei a responsabilidade de verificar que as concentrações de metais pesados, peróxidos, ferro, sulfatos e cinzas sulfatadas se encontravam dentro dos requisitos.

Estas análises podem ser realizadas através de técnicas laboratoriais com a adição de reagentes específicos, como é o caso dos metais pesados na qual o resultado é interpretado através da comparação de coloração final da amostra e do padrão, podendo ser utilizado um branco/controlo e um monitor. O tipo de análise de metais pesados é realizado consoante a matéria-prima que se pretende examinar.

Noutro tipo de impurezas pode proceder-se à quantificação direta através de métodos espectrofotométricos, como é o caso do índice de peróxidos ou através de métodos gravimétricos aquando do controlo da proporção de cinzas sulfatadas.

Este ponto permitiu-me adquirir destreza tanto nas diversas técnicas a realizar como na preparação de reagentes e soluções.

c) Análise quantitativa do conteúdo em água através do método *Karl-Fisher* e Perda por Secagem

A análise quantitativa do conteúdo em água de uma matéria-prima torna-se importante no cálculo da quantidade a utilizar em certos processos de fabrico que envolvam elevadas temperaturas ou nos quais a porosidade da matéria-prima possa ser um fator limitante.

Assim sendo, em cada análise quantifiquei o conteúdo em água através de um dos seguintes métodos: pelo equipamento de *Karl-Fischer* ou pela análise de Perda por Secagem[2].

No método *Karl-Fischer* é necessário escolher o titulante e o meio que melhor se adequam a cada matéria-prima, tendo sempre em conta o fator do titulante. Sempre que necessário realizei a factorização do mesmo, que se processa com a titulação de água ultra purificada. A análise é feita em duplicado, sendo que o conteúdo corresponde à média dos valores. Sempre que os valores diferem mais de 0,5% do limite máximo, é realizada uma terceira titulação[3].

O método de Perda por Secagem tem em conta o tempo e a quantidade de amostra que melhor se adequa a cada matéria-prima, sendo que nalguns casos foi necessário realizar Perda por Secagem a Peso Constante[4], seguindo os procedimentos descritos e cuja análise termina quando a diferença de massa de duas pesagens consecutivas seja menor que 0,5 mg/g. O conteúdo é calculado por método gravimétrico através da diferença de massa da amostra inicial e da amostra após secagem.

Na minha opinião, este ponto é importante uma vez que me permitiu praticar técnicas com as quais apenas entrei em contacto a nível observacional no meu percurso académico.

d) Utilização da Farmacopeia Europeia, United States Pharmacopeia and The National Formulary (USP-NF) e PON

Ao longo do estágio entrei em contacto com diversos tipos de procedimentos tais como a Farmacopeia Europeia (Anexo I), a USP-NF (Anexo II) e PON. A existência destes documentos é fundamental não só para definir os requisitos de cada matéria-prima como para harmonizar métodos de análise, permitindo assim resultados confiáveis e fidedignos.

Tendo em conta o objetivo acima referido, é na minha opinião importante seguir em direção à harmonização da USP-NF e da Farmacopeia Europeia, ressalvando que são muitos os ensaios que se encontram nessas condições. Os procedimentos internos vão de encontro quer a estes documentos de suporte quer aos requisitos dos clientes.

Este ponto proporcionou a aquisição de capacidades para a interpretação e manuseamento deste tipo de documentação, tornando-me uma profissional capaz de trabalhar com qualquer um destes modelos.

e) Utilização do Diário de laboratório e *Logbook*

A utilização do *Logbook* de um determinado aparelho permite a cada analista tomar conhecimento acerca das condições a que este se encontra aquando do tempo da análise a decorrer.

Do mesmo modo, a utilização do Diário de Laboratório não só promove a interpretação correta dos resultados, assim como permite a harmonização da organização de dados e facilita a rastreabilidade e a comunicação entre os diversos departamentos da indústria.

Considero este um ponto forte uma vez que, para além de adquirir capacidades de organização, permitiu-me compreender a importância da comunicação numa área cujos departamentos se encontram interligados como peças de um mesmo motor – a máquina apenas funciona se todas as conexões estiverem corretamente ligadas e se a passagem de informação não detiver falhas.

f) Conteúdo Programático do MICF

A aquisição de conhecimentos na área do controlo de qualidade para realizar as diversas tarefas, foi facilitada graças à junção das competências teóricas e laboratoriais adquiridas ao longo do meu percurso académico.

Na minha opinião o curso de MICF proporciona uma boa formação ao nível analítico, nomeadamente nas unidades curriculares de Métodos Instrumentais de Análise I e II, que me deram o contacto inicial com diversos equipamentos, cujo manuseamento foi essencial para realizar as diversas análises. Destaco os aparelhos espetrofotométricos, potenciómetro e célula condutimétrica.

Ao longo do meu percurso enquanto estagiária no CQ foram também importantes os contactos anteriores com as unidades curriculares de Hidrologia, no âmbito da análise de água purificada, Química Analítica, a nível de análise de conteúdo, e Tecnologia Farmacêutica, relativamente à desagregação de cápsulas.

3.2 Pontos Fracos

a) Curto período de estágio

Tal como referi anteriormente, o meu estágio teve como início a familiarização à indústria farmacêutica e aos seus procedimentos e normas, após os quais fui inserida no CQ e iniciei o manuseamento das diversas técnicas analíticas.

A restante frequência do meu estágio teve lugar nesse mesmo sector, não tendo tido oportunidade de aprender todas as técnicas que me competiam na análise de matéria-prima. Penso que a causa desta desvantagem poderá ser o curto período de estágio, não permitindo um plano de estágio totalmente adequado que me permitisse adquirir aptidão e conhecimentos para realizar qualquer tipo de análise.

b) Contacto com apenas um sector do CQ

O CQ é um departamento que abrange diversas áreas entre as quais: amostragem, controlo microbiológico e físico-químico de matéria-prima, de produto semiacabado e de embalagem e documentação.

Este departamento engloba uma grande diversidade de funções. No entanto, no decorrer do meu estágio apenas contactei verdadeiramente com o controlo físico-químico de matéria-prima, excluindo as análises realizadas através de *High Performance Liquid Chromatography* (HPLC) e *Particle Size Distribution* (PSD).

Considero este um ponto fraco pois, embora me sinta preparada e capaz como futura profissional para realizar os diversos tipos de análises com os quais contactei, tal não corresponde com outros tipos de técnicas e funções realizadas por um analista.

3.3 Oportunidades

a) Manuseamento de equipamentos recentes

A nível analítico é importante conhecer e adquirir à vontade no manuseamento dos diferentes tipos de equipamentos largamente utilizados no âmbito da indústria farmacêutica.

Embora a FFUC seja uma faculdade bem equipada e me tenha permitido um primeiro contacto com os diversos tipos de aparelhos, o meu período de estágio proporcionou uma maior proximidade aos mesmos e aos seus sistemas associados, dando-me oportunidade para compreender o funcionamento da gama mais recente dos equipamentos utilizados em indústria.

b) Gestão equilibrada do tempo em cada sector do CQ

Como foi proferido anteriormente, o CQ é um departamento que abrange diversas funções distintas, todas elas importantes para um futuro profissional de saúde.

Assim sendo, na minha opinião, será vantajoso adequar o Plano de Estágio para uma passagem por cada sector do CQ e respetiva aquisição de conhecimentos a nível de técnicas de análise, aliando a gestão equilibrada do tempo ao aumento do período de estágio em indústria.

c) Acesso a formações

Ao longo do meu estágio tive a oportunidade de adquirir conhecimentos em determinadas áreas da indústria farmacêutica através de diversas formações que me foram proporcionadas.

Através destas, não só obtive consciência acerca da interligação e dependência dos diversos departamentos de uma indústria farmacêutica, como também adquiri algumas noções acerca de Assuntos Regulamentares, Segurança e Ambiente e Gestão da Qualidade.

3.4 Ameaças

a) Outros profissionais apresentam conhecimentos mais alargados a nível analítico

Embora o curso de MICF contemple todas as noções necessárias para as análises que realizei enquanto estagiária no CQ, na minha opinião será necessário aprofundar conhecimentos práticos a nível do sistema de HPLC, UPLC e PSD, tipos de equipamentos para os quais outros profissionais se encontram com melhor preparação.

Deste modo, para que a nossa classe profissional se demonstre a mais vantajosa ao nível de indústria farmacêutica, é indubitável a necessidade de um farmacêutico multidisciplinar que alie o seu conhecimento do medicamento às características que os seus componentes devem possuir e às novas tecnologias de análise, associando o espírito crítico dos conhecimentos teóricos à destreza dos conhecimentos práticos.

b) Estágio não creditado

O estágio curricular[5,6] pressupõe a realização de estágio em Farmácia Comunitária e em Farmácia Hospitalar. Embora a FFUC nos dê a oportunidade de realizar outro tipo de

estágio, tal como a minha opção em realizar estágio no ramo de Indústria Farmacêutica, este não é creditado como os restantes.

Na minha opinião este fator poderá refletir-se numa ameaça, uma vez que os estudantes que tomarem esta opção apenas possuirão creditação para estágio em Farmácia Comunitária.

Ainda assim, acredito não só que as competências que adquiri através deste estágio serão fundamentais enquanto futura profissional de saúde, bem como a indústria farmacêutica é uma das áreas do medicamento e como tal, num futuro próximo, deverá ser integrada no Plano de Estágio Curricular.

4. Análise SWOT – Farmácia Comunitária

4.1 Pontos Fortes

a) Plano de Estágio

O farmacêutico exerce uma vasta variedade de funções no âmbito da Farmácia Comunitária. Como tal, torna-se bastante importante estabelecer um plano de estágio bem delineado que dê oportunidade ao estagiário de passar por todas as etapas com uma boa gestão de tempo.

A minha experiência enquanto estagiária teve início no *backoffice,* na receção de encomendas. Para além de aprender a dinâmica e os passos desta função, esta atividade permitiu uma familiarização inicial com os nomes comerciais dos medicamentos, diferentes dosagens e laboratórios. Prossegui para a gestão de reservas, devolução dos medicamentos não conformes e regularização das devoluções. Todas estas funções têm como objetivo uma boa gestão de *stock* que permita satisfazer as necessidades dos utentes.

Fui gradualmente passando à organização de receituário, organização de lineares com a regra "*first in, first out*" e ao atendimento observacional, enquanto mantinha as minhas funções de *backoffice*. Com o aumento do conhecimento dos diversos produtos e suas aplicações passei para a preparação de manipulados, determinação de parâmetros bioquímicos e antropométricos e atendimento ao utente, atividades pelas quais fui sendo guiada até que pudesse adquirir autonomia.

Na minha opinião este ponto forte foi determinante no sucesso do meu estágio, assegurando que pudesse passar por todas as áreas da farmácia comunitária e assimilar todas as funções de um modo organizado, atempado e eficaz.

b) Programa de recolha de medicamentos VALORMED

O farmacêutico não deve ser apenas um profissional especialista do medicamento, mas também um agente de saúde pública, sendo o seu foco principal o bem-estar do utente e da sociedade em geral.

Deste mote nasceu a VALORMED, um sistema de gestão de resíduos medicamentosos que assegura o tratamento seguro dos mesmos, dando benefícios ao ambiente e prevenindo possíveis problemas de saúde pública[7].

A meu ver, a oportunidade de consciencializar os utentes para a entrega da sua medicação fora do prazo e obter tão boa resposta por parte dos mesmos tornou-se um bom elemento do meu estágio, possibilitando que a minha ação fosse além do estado físico da farmácia.

c) Preparação de Medicamentos Manipulados

A preparação de medicamentos manipulados está prevista quando não é possível encontrar nenhum medicamento com igual forma farmacêutica e dosagem, nos casos de aplicação cutânea, preparações de uso pediátrico e medicação destinada a indivíduos cuja farmacocinética se encontre alterada[8].

Esta é executada sob a responsabilidade do farmacêutico e segundo a descrição de receita médica, no caso de Fórmula Magistral, ou segundo os procedimentos do Formulário Galénico Português, no caso de Preparado Oficinal. Neste último caso, se não existir o protocolo pretendido, dever-se-á entrar em contacto com o Laboratório de Estudos Farmacêuticos para a cedência do mesmo[8].

O farmacêutico não só é responsável pela preparação destas formulações, como pela sua garantia de qualidade, assegurando que todo o processo de cedência seja realizado consoante as Boas Práticas de Farmácia. A garantia de qualidade é comprovada após produção do manipulado, segundo os ensaios descritos na Farmacopeia Portuguesa.

Aquando do meu estágio, tive a oportunidade de preparar diversos manipulados, nomeadamente de aplicação tópica para o tratamento de afeções cutâneas, tais como Vaselina Salicilada a 2 e 6%, Vaselina com Enxofre a 6% e ATL creme gordo com Dermovate pomada. Todos os processos realizados foram monitorizados pelo farmacêutico responsável, sendo posteriormente verificados pela Diretora Técnica da farmácia.

Este ponto permitiu-me contactar com um método de cedência distinto do habitual e recapitular os conhecimentos adquiridos através da unidade curricular Farmácia Galénica.

d) Determinação de parâmetros antropométricos e bioquímicos

Um dos papéis do farmacêutico é garantir a adesão à terapêutica, de modo a atestar o bem-estar do doente. Com este intuito, torna-se fundamental assegurar que os utentes cumpram a sua terapêutica corretamente ao nível da farmácia comunitária, uma vez que esta representa um dos principais pontos de comunicação com o doente. Para além de todo o aconselhamento e esclarecimento de dúvidas que o farmacêutico deve realizar, a determinação de parâmetros antropométricos e bioquímicos acaba por ser um elemento chave para assegurar a adesão à terapêutica.

Por exemplo, há maior probabilidade de um utente cumprir a sua terapêutica com estatinas se obtiver evidências diretas de que a medicação o está a ajudar a manter os níveis de colesterol total dentro do limite desejado.

Por outro lado, a realização destes testes também permite acompanhar a evolução de um utente com hipertensão ou hipercolesterolemia, anotando esses valores e permitindo que o doente possa transmitir a informação ao seu médico, evitando que alguma situação de descompensação se agrave caso seja necessário alterar a dose ou o intervalo de toma.

No decorrer do meu estágio, tive oportunidade de realizar tanto testes antropométricos para o cálculo do Índice de Massa Corporal, como também determinação dos valores de tensão arterial e ainda testes bioquímicos para a medição da glicémia, Colesterol Total e Colesterol HDL. Estes últimos realizam-se segundo os procedimentos descritos e através de métodos espetrofotométricos com reações enzimáticas.

e) Formação constante e aquisição de conhecimentos ao nível de aconselhamento farmacêutico

Na minha opinião, o estágio curricular deve possibilitar a prática laboral em ambiente monitorizado e instruir a aquisição de novos conhecimentos a aliar à nossa formação base proveniente do MICF.

Como tal, devemos ser confrontados com os mais variados problemas e adquirir ferramentas que nos permitam agilizar o processo de aconselhamento quer de Medicamentos Não Sujeitos a Receita Médica, como Suplementos Alimentares, Dermofarmácia e Cosmética (Anexo III), Preparações de Uso Veterinário e Dispositivos Médicos, de modo a satisfazer as necessidades de cada utente em cada caso (Anexo IV).

Nestes grupos de produtos e em situações menores, o farmacêutico consegue verdadeiramente marcar a diferença na terapêutica que um utente pretende seguir,

aconselhando-o acerca dos diferentes meios que este possui para a melhor resolução do problema, podendo este aconselhamento não passar pela cedência de nenhum produto.

Com o intuito de adquirir a melhor preparação possível para poder dar resposta a estas questões, ao longo do meu estágio foram-me colocadas diversas situações fictícias para as quais eu deveria ponderar as perguntas a colocar ao utente de modo a obter o máximo de informação relevante, associar sintomas com possíveis situações patológicas e aconselhar os produtos que melhor se adequavam na resolução da dita situação.

Este ponto foi deveras fundamental para que pudesse treinar o meu raciocínio e garantir um bom aconselhamento ao utente na chegada de uma situação real.

4.2 Pontos fracos

a) Conteúdo Programático do MICF

O conteúdo programático do MICF abrange diversas áreas e providencia bastantes conhecimentos base necessários ao nosso exercício profissional. No entanto, este não pode ser estático e deve evoluir conforme as necessidades e desafios da profissão farmacêutica, assegurando que os estudantes adquirem todas as competências essenciais.

Na minha opinião, este conteúdo apresenta algumas lacunas que dificultaram o meu percurso como estagiária, a nível de aconselhamento de Medicamentos de Uso Veterinário, Suplementos Alimentares, produtos ortopédicos e Dispositivos Médicos.

A unidade curricular "Preparações de Uso Veterinário" tem uma boa abordagem relativamente a antiparasitários, não se focando, no entanto, em patologias comuns, o que suscitou dificuldades no aconselhamento deste tipo de medicamentos em diversas situações.

No que concerne aos Suplementos Alimentares, é evidente o aumento da sua utilização ao longo dos últimos tempos, não só para satisfazer uma necessidade nutricional, como também a nível de *consumer health care*, isto é, para melhorar a qualidade de vida do utente em diversas vertentes. Assim sendo, foram variadas as situações em que foi necessário prestar um aconselhamento de qualidade acerca destes produtos, que seria facilitado caso o ensino destes se tornasse mais aprofundado a nível dos benefícios consoante determinada situação, interações, contraindicações e diferenças entre produtos através das suas composições.

A dificuldade no aconselhamento de produtos ortopédicos e Dispositivos Médicos prendeu-se com o fato destes conceitos não se encontrarem integrados no Plano Curricular do MICF. Este último tipo de produto é abordado numa Unidade Curricular Opcional, não

permitindo que todos os estudantes tenham acesso a esses conhecimentos e encontrem dificuldades no decorrer do estágio.

b) Dificuldades provenientes do desconhecimento do nome comercial dos medicamentos

A formação que obtemos através do MICF providencia-nos conhecimentos acerca dos princípios ativos e respetivos mecanismos de ação, e consequentemente na sua utilização para resolver uma determinada patologia. No entanto, a falta de familiarização com os nomes comerciais dos mesmos afeta em alguma parte no atendimento ao utente.

A nível de MSRM esta barreira é ultrapassada graças à prescrição por DCI, o que permite agilizar todo o processo de cedência do medicamento, esclarecendo todas as dúvidas que o doente possa suscitar.

No entanto, no âmbito dos MNSRM são raras as situações em que estes não sejam referenciados pelo seu nome comercial. Esta questão foi determinante nalguns processos de cedência e implicou um estudo mais aprofundado de todos os produtos existentes na farmácia de modo a ultrapassar esta dificuldade e evitar constrangimentos no atendimento ao utente, bem como a possível descredibilização do meu conhecimento enquanto estagiária. Em conclusão, considero que será vantajoso aplicar estes produtos e respetivas denominações comerciais em prática clínica simulada ao longo das unidades curriculares do Curso.

c) Imagem do estagiário perante a população

O estágio curricular é o marco entre o fim da aprendizagem dos conhecimentos teóricos base e o início da aprendizagem da prática laboral. Uma vez realizado como a última unidade curricular com vista à conclusão do Curso, é previsto que o estagiário detenha toda a formação necessária para pôr em prática no aconselhamento ao utente.

De modo a treinar o raciocínio e poder agilizar o processo da prática clínica, o estagiário deve ser confrontado com todo o tipo de situações, iniciando com o apoio de um farmacêutico até que seja capaz de atingir independência e adquirir a preparação necessária como futuro profissional de saúde.

No entanto, essa preparação é muitas vezes posta em causa devido à desconfiança dos utentes em que um estagiário consiga obter respostas a certas situações e que este lhes consiga fornecer um aconselhamento tão completo quanto um farmacêutico.

Ao longo do meu estágio, deparei-me diversas vezes com esta situação, tendo de pedir a um colega que me desse uma segunda opinião relativamente ao caso que me apresentavam, o que causou constrangimentos não só no tempo de atendimento como na própria qualidade do mesmo.

Por estas razões, na minha opinião é essencial desmascarar a imagem de que um estagiário não se encontra preparado para a prática das suas funções em farmácia comunitária, assim como enaltecer a ideia de que o incentivo da sua prática em cedência de medicamentos e aconselhamento é determinante para que este se torne um profissional de saúde de sucesso.

4.3 Oportunidades

a) Acesso a Formações

O farmacêutico deve ser um profissional atualizado relativamente a todas as inovações, produtos e terapêuticas que possam melhorar a qualidade de vida dos utentes. Como tal, a formação do farmacêutico deve ser constante de modo a aumentar a sua qualidade enquanto agente de saúde pública.

Ao longo do meu estágio tive a oportunidade de aprofundar conhecimentos acerca de diversas condições fisiológicas através de formações adicionais, das quais destaco a formação "A vida da Mulher na Pós-menopausa", na qual me esclareceram as alterações que esta sofre e as implicações que geram na sua qualidade de vida como também o aconselhamento acerca dos meios para atenuar estes sintomas. Nesta temática estão implícitos MSRM, dos quais o farmacêutico pode esclarecer as dúvidas à utente da terapêutica de substituição prescrita. No entanto, existem certas alternativas não hormonais e não sujeitas a receita médica para alguns dos sintomas, das quais o farmacêutico deve tomar conhecimento de modo a garantir um aconselhamento de qualidade.

As formações também me permitiram adquirir conhecimentos no âmbito da dermocosmética, em especial das novidades existentes no mercado a nível de gamas antienvelhecimento e medidas de proteção solar, consoante o tipo de pele e as suas necessidades.

A meu ver, este ponto foi bastante vantajoso para combater algumas lacunas do conteúdo programático de MICF, permitindo aumentar a minha instrução como futura profissional de saúde para poder prestar um serviço de qualidade aos utentes.

b) Aconselhamento farmacêutico

Cada vez mais assistimos a uma pirâmide demográfica envelhecida na nossa sociedade, o que se traduz numa grande percentagem da população na 3ªidade em Portugal, uma subpopulação à qual o farmacêutico deve prestar especial atenção e procurar os meios necessários para garantir a sua qualidade de vida.

Grande parte destes indivíduos encontram-se em regime de polimedicação, sendo fundamental a ação de um especialista do medicamento no seguimento da sua terapêutica. Assim, será possível garantir a relação risco-benefício positiva, esclarecendo todas as possíveis dúvidas quanto à medicação e sua correta utilização e promovendo a adesão à terapêutica, partilhando os seus pareceres com o médico.

A aposta neste papel garante a eficácia e a segurança da terapêutica, melhorando a qualidade de vida de cada doente e reduzindo custos hospitalares de internamentos associados a problemas de medicação.

Por estes motivos, considero que o futuro da profissão farmacêutica passará pelo foco de ação no doente e pelo acompanhamento farmacoterapêutico.

c) Farmacêutico como agente de saúde pública

Como já referi anteriormente, o foco de ação do farmacêutico deve ser o utente e a população em geral, e este deve garantir a sua importância na sociedade não só pelos seus conhecimentos especializados no medicamento, como também pela sua intervenção enquanto agente de saúde pública.

Na minha opinião, com a crescente facilidade de acesso à informação através de diversas fontes, aumenta a probabilidade de contradições e da passagem de afirmações erradas à população. Face a este problema, o farmacêutico deve garantir a informação que é disponibilizada aos utentes é fidedigna, providenciando formações alusivas a diversas temáticas como por exemplo proteção solar ou medidas não farmacológicas para situações não complicadas.

Noutra perspetiva, o farmacêutico também pode ajudar a população através da realização de rastreios, mantendo os utentes conscientes do seu estado de saúde e prevenindo o surgimento de complicações futuras.

4.4 Ameaças

a) Fácil acesso a informação errada

Na conjetura atual da *consumer health*, assistimos a uma população cada vez mais preocupada com o seu bem-estar e a sua saúde. Este facto é bastante benéfico, mas pode tornar-se uma ameaça à saúde pública devido à disseminação de informação pouco fidedigna que, consequentemente, gera falsas crenças e descredibiliza os profissionais de saúde cujo aconselhamento seja contraditório à opinião formada pelo utente.

Durante o meu estágio esta problemática gerou dificuldades na prestação de serviços a alguns utentes que não se mostraram recetivos aos esclarecimentos que tentei transmitir. No entanto, devo ressalvar que foram muitos os utentes que demonstraram gratidão pelo cuidado e atenção prestados.

b) Elevado número de produtos para o mesmo efeito

Como profissional de saúde especialista na área do medicamento, o farmacêutico deve possuir os conhecimentos necessários acerca de cada produto para poder exercer um aconselhamento de qualidade visando dar resposta às necessidades de cada utente.

A elevada quantidade de produtos existente na farmácia comunitária para resolver o mesmo problema de saúde, essencialmente a nível de MNSRM, representa um obstáculo para esta prestação de serviços. A constante formação que o farmacêutico desenvolve, não só pelo estudo individual como pela formação providenciada por delegados de informação médica, pode revelar-se insuficiente face à quantidade de novos produtos que vão surgindo no mercado.

Estas questões, aliadas à publicidade de certos produtos junto do consumidor, podem surtir dúvidas na escolha do produto mais indicado devido às elevadas semelhanças das composições de cada um, colocando o farmacêutico numa situação propícia à sua descredibilização por parte do utente e à desvalorização da opinião dos profissionais de saúde.

5. Considerações Finais

O meu percurso enquanto estagiária curricular foi o culminar destes 5 anos de formação, permitindo-me consolidar os conhecimentos adquiridos ao longo de todo o plano do MICF.

Ambos os estágios, quer em indústria farmacêutica, quer em farmácia comunitária, permitiram-me desenvolver novas competências sob a forma de práticas laborais de ambiente controlado.

O estágio na Bluepharma® possibilitou-me ganhar conhecimentos da enorme extensão de funções que esta área engloba e como o circuito de processos deve funcionar ao nível de uma indústria farmacêutica. Para além desta questão, este estágio mostrou-me o farmacêutico como trabalhador no âmbito laboratorial e de que modo a sua formação se pode mostrar vantajosa.

Por outro lado, o estágio na Farmácia Isabel Folhas, demonstrou-me o farmacêutico ligado ao utente, capaz de dar resposta a cuidados de saúde primários e velando pelo bem-estar da população em geral.

As diferentes funções que exerci em cada um permitiram-me percecionar que o farmacêutico é um profissional com diversas valências que deve investir constantemente na sua formação de modo a enaltecer a sua valorização profissional e a prestar o melhor serviço possível no meio em que se insere.

No fim desta etapa, encaro tudo o que aprendi como ferramentas a utilizar ao longo da minha vida profissional, salvaguardando a importância destes estágios na minha formação para me tornar numa farmacêutica de sucesso.

Referências Bibliográficas

1- Economic Times – Definition of 'SWOT Analysis' [acedido a 9 de agosto de 2017]. Disponível na Internet: http://economictimes.indiatimes.com/definition/swot-analysis

2- Karl Fischer vs. Loss on Drying – Which method is the best? [acedido a 10 de agosto de 2017]. Disponível na Internet: http://www.cscscientific.com/csc-cientific-blog/karl-fischer-vs-loss-on-drying-which-method-is-the-best

3- FFCR – Water Determination – Karl Fischer Method [acedido a 10 de agosto de 2017]. Disponível na Internet: http://www.ffcr.or.jp/zaidan/ffcrhome.nsf/7bd44c20b0dc562649256502001b65e9/146fd852cd5e269049256f32001a133e/$file/b43.pdf

4- USP – Loss On Drying [acedido a 10 de agosto de 2017]. Disponível na Internet: https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c731.pdf

5- DGES – Directiva 2005/36/CE, de 07 de setembro do Parlamento Europeu [acedido a 11 de agosto de 2017]. Disponível na Internet: https://www.dges.gov.pt/sites/default/files/directiva36_2005.pdf

6- FCT – Decreto-Lei n.º 74/2006, de 24 de março – Ministério da Ciência, Tecnologia e Ensino Superior [acedido a 11 de agosto de 2017]. Disponível na Internet: https://www.fct.pt/apoios/bolsas/DL_74_2006.pdf

7- VALORMED – Quem somos [acedido a 11 de agosto de 2017]. Disponível na Internet: http://www.valormed.pt/pt/conteudos/conteudo/id/5

8- INFARMED – Decreto-Lei n.º 95/2004, de 22 de abril – Regula a prescrição e a preparação de medicamentos manipulados [acedido a 12 de agosto de 2017]. Disponível na Internet: http://www.infarmed.pt/documents/15786/1070327/067-A-DL_95_2004.pdf

9- ORDEM DOS FARMACÊUTICOS – Boas Práticas Farmacêuticas para a farmácia comunitária [acedido a 12 de agosto de 2017]. Disponível na Internet: http://www.ordemfarmaceuticos.pt/xFiles/scContentDeployer_pt/docs/Doc3082.pdf

10- ISDIN – Nutraisdin Reparador perioral [acedido a 12 de agosto de 2017]. Disponível na Internet: http://www.isdin.com/pt-PT/produto/nutraisdin/reparador-perioral

11- URIAGE – Uriage Pruriced Gel [acedido a 12 de agosto de 2017]. Disponível na Internet: http://www.uriage.com/PT/pt/produtos/pruriced-gel

12- INFARMED – Resumo das Características do Medicamento [acedido a 12 de agosto]. Disponível na Internet: https://www.lundbeck.com/upload/pt/files/pdf/RCMs/Cipralex%20FCT_09%202013.pdf

Anexos

Anexo I – Estrutura de uma monografia da Farmacopeia Europeia

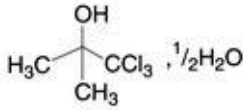
maximum at about 276 nm (2.4.7). Calculate the content of $C_{15}H_{15}Cl_2N_2NaO_8$ taking 220 as the specific absorbance at 276 nm. 1 mg of $C_{15}H_{15}Cl_2N_2NaO_8$ is equivalent to 0.7257 mg of $C_{11}H_{12}Cl_2N_2O_5$.

Storage. Store protected from light and moisture.

Labelling. The label states the quantity of Chloramphenicol Sodium Succinate in the sealed container in terms of the equivalent amount of chloramphenicol.

Chlorbutol

Chlorobutanol



$C_4H_7Cl_3O, ½H_2O$ Mol.Wt. 186.5

Chlorbutol is 1,1,1-trichloro-2-methylpropan-2-ol hemihydrate.

Chlorbutol contains not less than 98.0 per cent and not more than 101.0 per cent of $C_4H_7Cl_3O$, calculated on the anhydrous basis.

Category. Pharmaceutical aid (antimicrobial preservative), analgesic; local anaesthetic.

Description. Colourless crystals or a white, crystalline powder; odour, characteristic and somewhat camphoraceous; sublimes readily.

Identification

A. To 5 ml of a freshly prepared 0.5 per cent w/v solution add 1 ml of *1 M sodium hydroxide* and then, slowly, 2 ml of *iodine solution*; a yellow precipitate of iodoform is produced.

B. Heat about 20 mg with 2 ml of *10 M sodium hydroxide* and 1 ml of *pyridine* on a water-bath and shake; the separated pyridine layer becomes red.

C. Warm gently about 20 mg with 5 ml of *ammoniacal silver nitrate solution*; a black precipitate is produced.

Tests

Appearance of solution. A 50.0 per cent w/v solution in *ethanol (95 per cent)* is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS5 (2.4.1).

Acidity. Dissolve 2.0 g in 20 ml of *ethanol (95 per cent)*, add 0.1 ml of *bromothymol blue solution* and titrate with *0.1 M sodium hydroxide*; not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Chlorides (2.3.12). 0.5 g dissolved in 10 ml of *ethanol (95 per cent)* complies with the limit test for chlorides (500 ppm). Use 5 ml of *ethanol (95 per cent)* in place of 5 ml of *water* to prepare the standard.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

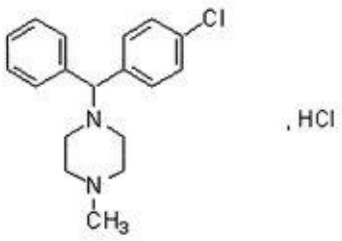
Water (2.3.43). 4.5 per cent to 6.0 per cent, determined on 0.3 g.

Assay. Weigh accurately about 0.2 g and dissolve in 5 ml of *ethanol (95 per cent)*. Add 5 ml of *sodium hydroxide solution* and boil under a reflux condenser for 15 minutes. Cool, dilute with 20 ml of *water*, add 5 ml of *nitric acid*, 1 ml of *nitrobenzene* and 50.0 ml of *0.1 M silver nitrate* and shake vigorously for 1 minute. Add 4 ml of *ferric ammonium sulphate solution* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate*.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005917 g of $C_4H_7Cl_3O$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Chlorcyclizine Hydrochloride



$C_{18}H_{21}ClN_2,HCl$ Mol. Wt. 337.3

Chlorcyclizine Hydrochloride is 1-(4-chlorobenzhydryl)-4-methylpiperazine hydrochloride.

Chlorcyclizine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of the stated amount of $C_{18}H_{21}ClN_2,HCl$, calculated on the dried basis.

Category. Antihistaminic.

Dose. 50 mg thrice daily.

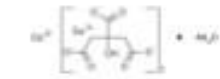
Description. A white crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

Anexo II – Estrutura de uma monografia da USP-NF

Calcium Citrate



$C_{12}H_{10}Ca_3O_{14} \cdot 4H_2O$ 570.49

1,2,3-Propanetricarboxylic acid, 2-hydroxy-, calcium salt (2:3), tetrahydrate;

Calcium citrate (3:2), tetrahydrate [5785-44-4].

DEFINITION

Calcium Citrate contains four molecules of water of hydration. When dried at 150° to constant weight, it contains NLT 97.5% and NMT 100.5% of $Ca_3(C_6H_5O_7)_2$.

IDENTIFICATION

- **A.**

  Analysis: Dissolve 0.5 g in a mixture of 10 mL of water and 2.5 mL of 2 N nitric acid. Add 1 mL of mercuric sulfate TS, heat to boiling, and add 1 mL of potassium permanganate TS.

  Acceptance criteria: A white precipitate is formed.

- **B.**

  Sample: 0.5 g of Calcium Citrate

  Analysis: Ignite completely the *Sample* at as low a temperature as possible, cool, and dissolve the residue in dilute glacial acetic acid (1:10). Filter, and add 10 mL of ammonium oxalate TS to the filtrate.

  Acceptance criteria: A voluminous white precipitate that is soluble in hydrochloric acid is formed.

ASSAY

- **Procedure**

  Sample solution: Dissolve 350 mg of Calcium Citrate, previously dried at 150° to constant weight, in 12 mL of 0.5 M hydrochloric acid, and dilute with water to about 100 mL.

  Analysis: While stirring the *Sample solution*, add 30 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 8.307 mg of calcium citrate ($Ca_3(C_6H_5O_7)_2$).

  Acceptance criteria: 97.5%–100.5% on the dried basis

IMPURITIES

- **Arsenic,** *Method I* ⟨211⟩

  Test preparation: Dissolve 1 g of Calcium Citrate in 5 mL of 3 N hydrochloric acid, and dilute with water to 35 mL.

  Acceptance criteria: NMT 3 ppm

- **Heavy Metals,** *Method I* ⟨231⟩

  Test preparation: Dissolve 1 g of Calcium Citrate in a mixture of hydrochloric acid and water (2:20). Add 1.5 mL of ammonium hydroxide, and dilute with water to 25 mL.

  Acceptance criteria: NMT 20 ppm

- **Lead** ⟨251⟩

  Test preparation: Dissolve 0.5 g of Calcium Citrate in 20 mL of 3 N hydrochloric acid. Evaporate this solution on a steam bath to 10 mL, dilute with water to 20 mL, and cool. Use 5 mL of *Diluted Standard Lead Solution* (5 µg of Pb) for the test.

  Acceptance criteria: NMT 10 ppm

- **Limit of Fluoride**

  [Note—Prepare and store all solutions in plastic containers.]

  Standard stock solution: 1000 µg/mL of fluoride ion from USP Sodium Fluoride RS in water

  Standard solution: 5 µg/mL of fluoride ion from *Standard stock solution*. [Note—Prepare on the day of use.]

  Linearity solution A: Transfer 1.0 mL of the *Standard solution* to a 250-mL plastic beaker. Add 50 mL of water, 5 mL of 1 N hydrochloric acid, 10 mL of 1.0 M sodium citrate, and 10 mL of 0.2 M edetate disodium. If necessary, adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 5.5. Transfer to a 100-mL volumetric flask, and dilute with water to volume. This solution contains 0.05 µg/mL of fluoride.

  Linearity solution B: Transfer 5.0 mL of the *Standard solution* to a 250-mL plastic beaker, and proceed as directed for *Linearity solution A* beginning with "Add 50 mL of water,". This solution contains 0.25 µg/mL of fluoride.

  Linearity solution C: Transfer 10.0 mL of the *Standard solution* to a 250-mL plastic beaker, and proceed as directed for *Linearity solution A* beginning with "Add 50 mL of water,". This solution contains 0.50 µg/mL of fluoride.

  Sample solution: Transfer 1.0 g of Calcium Citrate to a 100-mL beaker. Add 10 mL of water and, while stirring, 10 mL of 1 N hydrochloric acid. When dissolved, boil rapidly for 1 min, transfer the solution to a 250-mL plastic beaker, and cool in ice water. Add 15 mL of 1.0 M sodium citrate and 10 mL of 0.2 M edetate disodium, and adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 5.5. Transfer this solution to a 100-mL volumetric flask, and dilute with water to volume.

  Electrode system: Use a fluoride-specific, ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see *pH* ⟨791⟩).

  Analysis

  Samples: *Linearity solution A*, *Linearity solution B*, *Linearity solution C*, and *Sample solution*

  Transfer 50 mL of each *Linearity solution A*, *Linearity solution B*, and *Linearity solution C* to separate 250-mL plastic beakers, and measure the potential of each solution with the *Electrode system*. Between each reading wash the electrodes with water, and absorb any residual water by blotting the electrodes dry. Plot the logarithms of the fluoride concentrations (0.05, 0.25, and 0.50 µg/mL, respectively) versus potential to obtain a Standard response line.

  Transfer 50 mL of the *Sample solution* to a 250-mL plastic beaker, and measure the potential with the *Electrode system*. From the measured potential and the Standard response line determine the concentration, *C*, in µg/mL, of fluoride ion in the *Sample solution*. Calculate the percentage of fluoride in the specimen taken by multiplying *C* by 0.01.

  Acceptance criteria: NMT 0.003%

- **Limit of Acid-Insoluble Substances**

  Sample solution: Dissolve 5 g of Calcium Citrate by heating with a mixture of hydrochloric acid and water (10:50) for 30 min.

  Analysis: Filter, wash, and dry at 105° for 2 h the residue so obtained.

  Acceptance criteria: The weight of the residue is NMT 10 mg (0.2%).

SPECIFIC TESTS

- **Loss on Drying** ⟨731⟩: Dry a sample at 150° for 4 h. It loses from 10.0% to 13.3% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards** ⟨11⟩

  USP Sodium Fluoride RS

Anexo III – Caso Prático I – Dermofarmácia

Um casal dirigiu-se à farmácia com o seu filho de 18 meses, devido a umas manchas avermelhadas que lhe começaram a surgir na pele.

A mãe começou por explicar a sua desconfiança em se poder tratar de uma reação alérgica uma vez que tinham introduzido recentemente mirtilos na alimentação do filho. O pai acrescentou que o filho, ao comer, passou os mirtilos na zona perioral, que agora apresentava as determinadas manchas.

Comecei por perguntar se os pais tinham reparado em algum outro sintoma a nível de prurido ou alterações do sistema respiratório, ao qual me confirmaram a primeira questão e negaram a segunda.

Ao examinar a pele da criança reparei que para além da zona perioral, as manchas exibiam-se na zona da nuca e na zona lombar.

Esclareci que, tratando-se de uma reação alérgica, seria expectável que esta se demonstrasse numa das seguintes situações: ou apenas na zona perioral devido ao contacto direto com a pele; ou abrangendo todo o corpo, revelando uma reação sistémica.

Ponderando outra possibilidade, questionei os pais se a criança ainda utilizava chupeta, à qual me responderam que apenas lhe dava uso para dormir. Perguntei também se o prurido era agravado pela manhã e se após o banho o seu filho se acalmava, questões ambas respondidas afirmativamente pelos pais.

Devido às zonas da pele afetadas e às respostas às questões, expliquei ao casal que a sensibilização da pele poderia estar a ser causada pela sudorese, estando agora agravada devido ao calor. Tal explicava a zona lombar devido ao contacto com a fralda, a zona perioral pelo contacto com a chupeta e a zona da nuca pela almofada.

Face a isto, aconselhei os pais em darem banho à criança em água tépida e vestirem roupas frescas, aconselhando dois produtos para aliviar os sintomas do filho. Em primeiro lugar o Nutraisdin® Reparador perioral, para aplicar na zona agredida pela chupeta 3 vezes ao dia na pele limpa e seca, sendo uma delas necessariamente antes de deitar[10]. Este produto é especialmente formulado para a pele do bebé e acalma a irritação da pele e do prurido.

Para alivar o prurido nas outras zonas aconselhei a aplicação do Uriage Pruriced Gel® após o banho da criança, uma vez que para além da sua composição com calamina, uma substância calmante para a comichão e irritação, hidrata a pele e dá a sensação de frescura devido à sua textura leve[11].

Não obstante, sensibilizei os pais para a ida ao médico pediatra caso os sintomas da criança persistissem.

Considero que este caso prático demonstra a necessidade do farmacêutico em prestar atenção aos pormenores e a prezar pela recolha de toda a informação necessária para poder dar resposta às necessidades do utente e garantir o seu bem-estar.

Anexo IV – Caso Prático II – Adesão à Terapêutica

Uma Senhora de 40 anos dirigiu-se à farmácia na sequência da terapêutica iniciada duas semanas antes. Aquando do atendimento, a utente começou por referir que por indicação do médico tinha iniciado a terapêutica com Cipralex®[12]. Ao conversar com a utente, percebi que a mesma não achava que a medicação estivesse a surtir efeito; e pondo a hipótese de suspender o tratamento, pretendia pedir a opinião do farmacêutico face a esta situação. A utente mostrava-se ainda bastante ansiosa devido ao diagnóstico que lhe foi feito.

Calmamente esclareci a utente de que a baixa de humor resulta da diminuição da atividade certas substâncias do cérebro, podendo ocorrer a qualquer pessoa e que a medicação receitada visava a estabilização da atividade destas mesmas substâncias, o que a longo prazo a iria fazer sentir-se bem, melhorando assim a sua qualidade de vida.

Ao percecionar a recetividade da utente ao meu aconselhamento e a demonstrar compreensão face ao referido, expliquei ainda que o efeito antidepressivo pretendido demora um pouco a ser percetível, pelo que seria fundamental a adesão à terapêutica de modo correto para sentir melhorias.

Tomei oportunidade para alertar a utente que, quando chegasse o término do tratamento, a supressão da medicação deveria ser realizada de modo gradual para a mesma não notar nenhuma alteração abrupta.

Ao obter maior esclarecimento acerca da sua situação, a utente mostrou-se mais descansada e agradeceu por toda a atenção e cuidado prestados. Terminei o atendimento pedindo à utente que dispusesse qualquer dúvida adicional que pudesse ter.

Considero este caso prático importante de referir pois um dos deveres do farmacêutico é promover a adesão à terapêutica, garantindo a efetividade e segurança do tratamento de modo a melhorar a qualidade de vida do utente. Não obstante, este caso prático representa que um atendimento ao doente não tem necessariamente de culminar na cedência de um produto, sendo o farmacêutico um agente de saúde pública com dever cívico de prestar os seus serviços à comunidade onde se insere.

Parte II

Halloysite clay nanotubes for life sciences applications from drug/protein encapsulation to bioscaffold

Abreviaturas

ALT – *Alanine transaminase*

APTES – *3-aminopropyltriethoxysilane*

AST – *Aspartate transaminase*

ASODNs – *Antisense Oligodeoxynucleotides*

bNiMOS – *Halloysite Nanotubes-based modified Nanoparticles-in-Microgel Oral Systems*

BSA – *Bovine Serum Albumin*

BSA-MIPs/HNTs – *Halloysite Nanotubes Molecular Imprinted Polymer for Bovine Serum Albumin*

C. elegans – *Caenorhabditis elegans*

CLSM – *Confocal Laser Scanning Microscopy*

DA – *Dopamine*

DeTAB – *Decyltrimethylammonium bromide*

FAM – *Flourescein*

f-HNTs – *Functionalized Halloysite Clay Nanotubes*

GO – *Graphene Oxide*

HA – *Hydroxyapatite*

HNTs – *Halloysite Nanotubes*

HPMCAS – *Hydroxypropylmethylcelulose acetate succinate*

LbL – *Layer-by-Layer*

LDPE – *Low Density Polyethylene*

MIPs – *Molecular imprinted polymers*

NaL – *Sodium Dodecanoate*

NiMOS – *Nanoparticles-in-Microgel Oral Systems*

NSAIDs – *Non-steroid Anti-inflammatory Drugs*

P. caudatum – *Paramecinum caudatum*

PCL – *ε-caprolactone*

PDA – *Polydopamine*

PEG – *Polyethylene glycol*

PLA – *Poly lactic Acid*

PMMA – *Poly(methyl methacrylate)*

PMVEMA – *Poly(methyl vinyl ether-co-maleic acid)*

PNIPAAM – *Poly(N-isopropylacrylamide)*

1. Introduction

In the past few decades, pharmaceutical research has gained a great deal of interest in the application of nanotechnology for the development of new drug delivery systems endowed with enhanced safety and efficiency in diagnostics and therapeutics[1]. Advances in this field provided the obtainment of drug formulations with unique physical properties, capable of promoting the durability on blood circulation, targeting specific sites, stimuli-responsive and cellular internalization, revealing a great potential in several biomedical applications[2, 3]. In this context, the potential environmental hazard and toxicity associated with the use of synthetic materials constitute a critical issue, endorsing the use of green nanotechnology by exploring friendly materials with natural origins. Clay nanomaterials are recognized as green nanotechnology materials candidates with immense potential, which position has been recovered against synthetic materials, leading to an attracting widespread interest within the scientific community[4]. In addition to this gain, clay nanomaterials are abundantly available at low prices[1, 5]. When used in drug delivery, clay nanomaterials have been shown the ability to protect drugs against degradation by chemicals and enzymes, while modifying the drug release rate, thus becoming promising functional drug carriers[4].

Halloysite is a tubular clay nanomaterial which offers great advantage towards additional widely used materials, such as kaolin and montmorillonite, becoming the subject of numerous studies in the past few years[6]. It is a naturally occurring raw material found in soils worldwide, that is formed by adjacent sheets of alumina and silica together with an additional monolayer of water between the adjacent layers[7]. These aluminosilicate layers curve into the format of tubes owing to their hydroxyl groups, forming halloysite nanotubes (HNTs)[8]. In contrast to kaolin, bentonite or montmorillonite, HNTs are easily dispersed in single particles on water or polar polymers[6, 8, 9].

A key aspect of these aluminosilicate nanoparticles is their porous structure, which resembles a two-layered hollow cylinder, with a positively charged inner lumen composed of alumina and negatively charged silica outer surface. The two layers are divided with hydroxyl groups groups that may be modified to improve HNTs characteristics (Figure 1)[6, 10].

Figure 1 3D representation of rolled halloysite nanotubes, which are composed by two layers: alumina in the inner layer (green) and silica in the outer surface (red). Source: Lvov *et al*[6]

Generally, HNTs are used in their completely rolled form (Figure 2), presenting a 15 nm inner diameter and an external diameter between 40 and 60 nm, which dimensions are more advantageous for sustained drug release[6, 8]. However, HNTs with an inner diameter up to 100 nm can be used as well. The interlayer space measures 0.7 nm, and a wide range from 500 nm to 5 μm is reported for the length[8], although, in terms of safety and biocompatibility, researchers assume 1 μm as the ideal length[6].

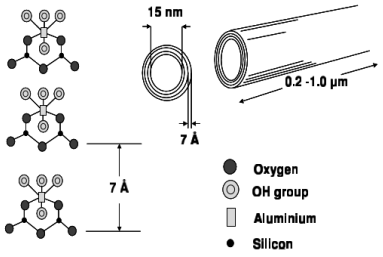


Figure 2 Schematic of the chemical and physical structure of completely rolled halloysite nanotubes. Source: Veerabadran *et al.*[8]

Moreover, the behavior of HNTs in bioliquids and their colloidal properties are similar to silica nanoparticles with 100 nm, forming a steady dispersion in water due to their negative electrical zeta-potential, which is ca. -30 mV[6, 11, 12]. The zeta-potential of HNTs is lower than that reported for pure silica particles of -50 mV, due to the superposition of the negative silica outer surface layer with the positive alumina lumen layer[6].

This way, the inner diameter of HNTs allows these structures to load not only small drug molecules but also heavier molecules, including proteins and DNA[6, 8, 12]. The silica outer surface may also be functionalized allowing for dual loading of positive and negative molecules[12]. The HNTs lumen can retain and deliver molecules in a manageable way, initially by desorption from the surface and ends of the tubule and far along by pore diffusion[8, 13]. Therefore, HNTs can increase drug effectiveness without changing its concentration, being able to perform a sustained drug release up to 250 hours[12, 14]. Thanks to their great drug loading capacity and biocompatibility features, HNTs may be used to perform intracellular drug-delivery, e.g., by being applied as transmembrane carriers through enzyme-activated drug delivery mechanisms, representing very attractive strategies regarding conventional diffusion-controlled drug delivery carriers[6].

Several studies have shown good perspectives regarding the use of HNTs formulated into tablets as the final dosage form for oral administration, and also for the topical administration by the development of HNTs-based topical dosage forms[15, 16]. Moreover, such nanomaterial is not only promising in drug delivery but also represents a good candidate for

several biomedical applications, such as tissue scaffolds, antimicrobial platforms, self-healing composites, strengthening of microvascular networks and bone implants[6, 8, 17].

The increasing use of HNTs urged the need to assess their toxicity towards living organisms[6]. Overall, HNTs were considered non-hazardous in a widespread concentration range, at least up to 0.2 mg/mL[4]. The biocompatibility of this nanomaterial was supported through toxicity assessment from different human cell lines, like epithelial adenocarcinoma cells or dermal fibroblasts, to yeast, bacteria, algae and microworms, among others[4, 12, 18]. Besides these studies, halloysite is considered the safest clay nanomaterial, based on a previous analysis comparing its toxicity to silica, graphene, montmorillonite, kaolin and bentonite. Therefore, HNTs may be employed without harm and are extremely promising for biomedical applications[19].

This essay aims, thereby, to demonstrate and discuss the potential of HNTs for life sciences applications, highlighting their use from drug delivery – via oral or topical administration – to tissue scaffolds and regenerative medicine, while assessing their toxicity and biocompatibility, and prospect the upcoming directions for further research.

2. Selective drug loading, nanotubes' functionalization and drug release

The positively charged alumina-based inner layer (lumen) together with the negatively charged silica-based outer layer (surface) of HNTs offer a particular and interesting structure to these carriers which enables for selective loading[6, 20, 21]. In addition, HNTs detain high capillary forces, which provides the capability to easily adsorb various materials within a broad range of pH[12].

Thus, HNTs have gained great interest from pharmaceutical research over their potential as drug delivery systems. Even though most reports had positive outcomes, unfortunately some results, precisely the loading capacity and release behavior, weren't as good as expected[8]. These outcomes were due to the weak interaction forces between the drug and the HNTs, such as Van der Waals forces and hydrogen bonding[22]. This problem has been undertaken by enhancing the affinity between the drug and the carrier, through the conjugation of polymeric materials compatible with the molecule of interest in the inner or in the outer surface of the HNTs, by, e.g., the surface modification with 3-aminopropyltriethoxysilane (APTES) to perform a successful bondage to antisense oligodeoxynucleotides (ASODNs)[13, 23]. Yah *et al.* were able to perform a selective modification of HNTs lumen using a dopamine derivative, whose catechol group was able to

form a covalent bond with alumina but did not has affinity towards de composition of the outer surface[24]. Furthermore, a study conducted by Massaro *et al.* has shown that the functionalization of either surface did not compromise the physical characteristics of these nanostructures[9]. These functionalities allow to control drug loading and release rate by various methods, such as nanocoatings formed by Layer-by-Layer (LbL) self-assembly, transformation of multilayer films and selective polymer binding to HNTs[3, 9, 24, 25]. In essence, this technique is a simple water-based process which enables control over thickness, permeability and modification of the surface properties of HNTs. The formation of nanocoatings using LbL self-assembly differs from additional coating methods due to its simplicity, suitability to most substrates, capacity of surface labelling via targeting molecules application and reduced need of coating materials to form a successful coating[3].

The loading of various molecules into HNTs is generally reported regarding two approaches: 1) selective loading inside tubular lumen and 2) linkage to the outer surface[26]. The first method broadly consists in applying vacuum to load the drug inside the lumen of HNTs, enabling better loading capacity than the second[26]. The second method relies on the covalent bond between the drug of interest and the wide outer surface area, which allows the attachment of larger drug molecules than the first approach[12]. Nevertheless, the two strategies may be merged enabling a primary release of the drugs from the outer surface followed by an extended release from the inner lumen of HNTs[25]. Aside from this issue, some researchers have reported that small drug molecules may also be loaded into interlayer spaces, e.g., urea dislocates the hydroxyl groups, increasing the interlayer space up to 1.1 nm[6, 23]. However, after drying, it was noticed that the distance between the layers had gone back to 0.7 nm, demonstrating that the molecules of urea were removed[6]. In the next subsections we have described in detail the drug loading in HNTs regarding both referred methods.

2.1. Inside tube's lumen drug loading

Selective loading within the inner alumina-based layers of HNTs is performed due to the positive charge of the formers or to the affinity between de drug and alumina[6]. This drug loading method enables a slower and longer drug release and overcomes technological obstacles associated with low soluble drugs[27].

The use of halloysite as a drug carrier was first carried out by Price *et al.* by combining a dry powder of HNTs with saturated drug water/alcohol-based solutions in order to entrap hydrophilic drugs (e.g. oxytetracycline HCl). In the case of low soluble drugs

with low melting point, HNTs were previously heated at 100 °C in ethylene glycol to remove the water in their composition and were vacuum-dried, followed by the blend between the resulting powder with the melted drug (e.g. khellin)[13]. The resulting mixtures were then subjected to a vacuum pumping technique with the aim of exchanging the entrapped air with the drug solution. After drug loading, to remove the excess of hydrophilic drug connected to the outer surface HNTs were rinsed in water, whereas to remove hydrophobic drugs those were subjected to a rinse in ethanol[13].

As aforementioned, completely rolled HNTs present an inner diameter of ca. 15 nm. In this case, the lumen volume represents ca. 10 wt% of the HNTs[6]. However, for the purpose of enhancing the drug loading capacity, the lumen volume can be increased up to 30 wt%, becoming close to the polymeric microcapsules loading capacity. This can be achieved by etching through the contact of HNTs with acid, e.g., applying 0.1 M H_2SO_4 at 60 °C during 8h, enlarging the inner diameter precisely from 14 nm to 25 nm without changing the external diameter[6, 28]. The interesting conserved morphology demonstrates that the etching occurs solely in the inner lumen, as pretended. Nevertheless, at a higher level of alumina etching, precautions should be taken as HNTs have shown to lost their natural structure[28].

This way, successful HNTs-based drug delivery systems have already been achieved with several drugs of interest, including small drug molecules, therapeutic nucleic acids and proteins[14, 23]. The procedures, implications and outcomes of these studies are going to be discussed hereinafter.

a) Small drug molecules

Halloysite-based nanoformulations provide numerous advantages for the delivery of free small drug molecules. Researchers have studied the behavior of HNTs-based models involving different therapeutic classes, as non-steroid anti-inflammatory drugs (NSAIDs), antimicrobial and chemotherapy agents.

HNTs can considerably enhance the efficiency of active agents with low solubility in water. As an illustration, khellin was loaded in the inner lumen at very high concentrations and its release profile was characterized by an initial burst in the first 4 h and a further slow release rate over 192 h[13]. The entrapment of this drug in HNTs allowed its presence in physiological media during a significant longer time-period, increasing the released quantity and consequently improving its therapeutic profile[13].

In another case, Vergaro *et al.* investigated the advantages of employing HNTs as a drug carrier for resveratrol, a low soluble drug with recognized antioxidant and antineoplasic

properties[29]. The loading procedure was based on vacuum cycling of HNTs powder in a resveratrol-saturated alcoholic solution. The product obtained was then rinsed, to eliminate loosely attached molecules to the outer layer, and it was ultimately dried[29]. The calculated loading efficiency was 99.7%, and the resveratrol release lasted ca. 48 h, with an initial fast release phase during 15 min and a subsequent slower release phase.

Facing these data, for the purpose of better controlling resveratrol release rate and decrease the detected initial burst release, the authors proceeded to the functionalization of HNTs with biocompatible polyelectrolytes through the LbL self-assembly technique, as their outer surface presents an ideal platform for functionalization with polyelectrolytes[29]. This technique was thus promptly applied to HNTs, producing a coating multilayer which was shown to be effectively able of decreasing even further the resveratrol release rate[3, 29]. LbL technique was also applied more recently in curcumin-loaded HNTs, allowing to decrease the released drug amount in 24 h from 40% for the case of pristine HNTs to less than 20% in LbL-coated HNTs[30]. This supports the hypothesis that LbL procedure helps to better modulate drug release, precisely by varying the number of coating layers upon HNTs surface[30].

NSAIDs, such as ibuprofen and aspirin, are widely used low soluble drugs in the clinical practice[22]. In the first attempt of loading these drugs in HNTs, the results were unsatisfactory due to the weak hydrogen bonds-based interaction between the drugs and HNTs[22, 31]. To circumvent these issues, HNTs inner surface was functionalized with APTES and the APTES-modified HNTs were applied as drug carriers for aspirin and ibuprofen[22, 31]. HNTs modification with APTES improved the loading capacity of both drugs by enhancing the affinity between the drugs and HNTs, specifically by means of electrostatic attraction between the aminopropyl groups of APTES and the carboxyl groups of the NSAIDs (Figure 3)[31].

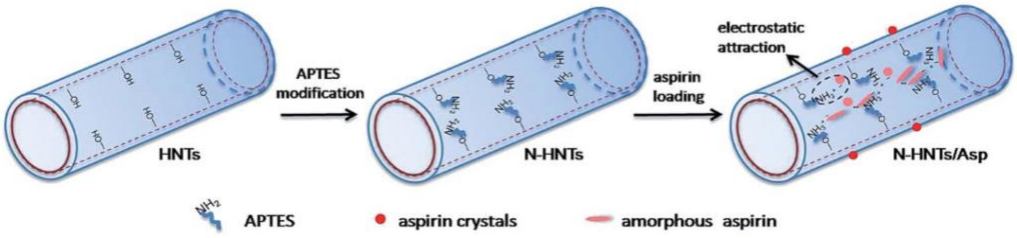


Figure 3 Halloysite nanotubes modification and subsequent aspirin drug loading. Source: Lun *et al. (2014)* [31]

This way, APTES-modified HNTs increased the loading capacity of aspirin from 3.84 to 11.98 wt% and considerably improved the aspirin dissolution rate[31]. The loading capacity of ibuprofen was improved as well. In terms of drug release, the initial burst of ibuprofen was reduced due to the low drug concentration on HNTs outer surface, diminishing its adverse effects; and the release rate was much slower comparing to the profile relating to free ibuprofen[22]. Overall, these outcomes emphasized the great potential for APTES-modified HNTs as drug delivery systems[22, 31].

Veerabadran *et al.* studied the influence of the solvent in the loading capability of HNTs by altering the alcohol/water ratio and the pH value of the solvent, determining the optimal conditions to achieve the best loading capacity. For the case of dexamethasone, the optimized conditions consisted in as solvent composed of 50% ethanol and a pH of 1.4, corresponding to 12 wt% loading capacity, a very close value to the maximum theoretical loading capacity. In a second stage of the study, the release profiles of dexamethasone, furosemide and nifedipine loaded in HNTs. The resultant release profiles of the free drugs and the drugs-loaded into HNT are shown in Figure 4[8].

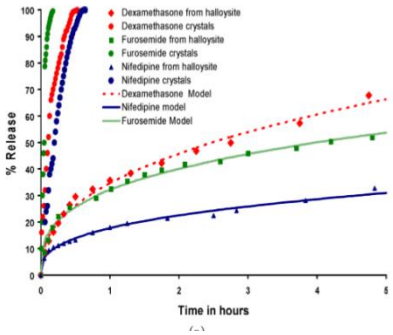


Figure 4 Release Profiles from furosemide, nifedipine and dexamethasone from halloysite nanotubes and microcrystals. Source: Veerabadran *et al. (2007)* [8]

Comparing each drug-concerning curves pair, it may be concluded that the release rate of nifedipine was 25 times longer in the corresponding HNT model, while regarding furosemide and dexamethasone HNT models such difference was up to 75 times. All three drug release profiles were characterized by an initial drug burst of ca. 10 min followed up with a 6-10 h prolonged drug release. On the basis of these results, the same research group applied further the LbL self-assembly technique in order to decrease the release rate of dexamethasone (Figure 5). Multilayer shells of cationic chitosan and anionic gelatin-B were assembled at the surface of dexamethasone-loaded HNTs, whose process was properly monitored by zeta-potential measurements and transmission electron microscopy (TEM) assessments. The calculated loading capacity of dexamethasone was ca. 7 wt% and a great

decrease of the release rate was encountered, prolonging the release from 7 h in unmodified dexamethasone-loaded HNTs to 30 h after the LbL polymeric assembly[32].

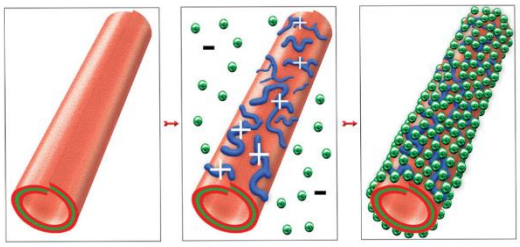


Figure 5 Layer by Layer self-assembly application upon halloysite nanotubes surface. Source: *Lvov et al. (2016)* [6]

In respect to the encapsulation of toxic drugs, the use of HNTs allows the administration of lower drug doses capable of performing the same therapeutic effect, the localized drug release preventing the hazard over healthy tissue, and, therefore, diminishing side effects while improving the biocompatibility[10]. In this context, to determine the effects of multifunctional HNTs on targeted and controlled release of doxorubicin, Hu *et al.* performed *in vitro* studies, in which the loading capacity and release profile were assessed[10]. HNTs were initially modified with thiol groups and then grafted with (β-CD-$(SH)_7$) through disulfide bonds. This assembly is broken by the glutathione, which concentration is increased in tumor cells, thus enabling a target release. Moreover, given that the folate receptor is over-expressed in tumor cells, folic acid was used as the targeted molecule in the functionalized HNTs (f-HNTs). Polyethylene glycol was also conjugated with HNTs to enhance their solubility and biocompability[10]. The loading percentage of doxorubicin was 14.2 wt% and only 40% of doxorubicin was released within 79 h. On the opposite, in a study performed with free doxorubicin, up to 55% of the drug was released in the first 10 h. A comparison of the two previous results revealed that HNTs demonstrated a very slow release rate and an outstanding stimuli-responsive release profile, as desirable[10].

A study performed by Patel *et al.* established that HNTs were capable of drug loading and prolonged drug release using a wide range of antimicrobial agents[12]. Even though the outcomes were different regarding each drug, their general release profile from HNTs was similar, showing an initial fast release and subsequent slow release rate[12]. Concerning the analyzed antiseptics, 4.76% of povidone iodine was released within 6.5 h; 84.95% of chlorhexidine was released after 4 h; and in 5 h, 96.52% of brilliant green and 92.68% of iodine were released. Regarding antibiotics, HNTs loaded with doxycycline showed a release of 98.73% in 4 h, and 94.83% of the amoxicillin-potassium calvulanate association was released within 5 h[12]. The release profile of vancomycin from HNTs and APTES f-HNTs was assessed by Kurczewska *et al.*[7]. The modification of the HNTs surface allowed a significant

improvement of the release profile of the drug, specifically from released 90% of released drug from unmodified HNTs to only 15% released from f-HNTs in the same 30 minutes-period. In fact, authors verified that after 24 h only 70% of vancomycin had been released from f-HNTs, emphasizing the modified drug release role attributed to this functionalization[7].

HNTs may be used as drug delivery systems for volatile substances, such as essential oils, since the associated drug loading procedure does not require the use of high-temperatures[33]. Carvacrol, an essential oil with antimicrobial properties, was firstly loaded into HNTs by Shemesh *et al.* in order to improve its thermal stability (Figure 6), being then the loaded HNTs high-temperature compounded with low-density polyethylene (LDPE) by melting means without the risk of carvacrol volatilization[33]. The resulting LDPE/(HNTs/carvacrol) nanocomposite was studied together with the following composites: LDPE/HNTs, LDPE/carvacrol and LDPE/HNTs/carvacrol (model without the primary loading of carvacrol into HNTs)[33]. LDPE/(HNTs/carvacrol) showed ca. 3.1 wt% of carvacrol, and, therefore, evidenced a greater loading efficiency than LDPE/carvacrol and LDPE/HNTs/carvacrol nanocomposites, whose carvacrol content consisted of 1.8 wt% and 2.1 wt%, respectively. This outcome demonstrated not only the importance of using HNTs as carvacrol drug delivery systems, but also the importance of the pre-compounding production of HNTs/carvacrol[33]. From these results, Shemesh *et al.* confirmed that HNTs play an important role in the protection of volatile substances during high-temperature procedures[33].

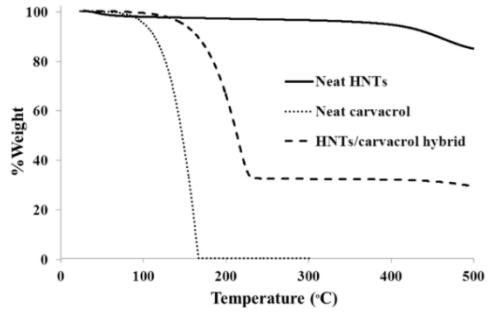


Figure 6 Evaporation of neat carvacrol completed at 165 °C and
halloysite nanotubes /carvacrol hybrids evaporation at 220 °C. Source: Shemesh *et al.* [33]

More recently, Yendluri *et al.*[14] performed a study involving HNTs as drug carriers for paclitaxel in a pH range from 1.2 to 6.8 to simulate gastric and intestinal conditions, respectively. Paclitaxel-loaded HNTs showed ca. 7.5 wt% of drug content, which was released at a steady rate within 24 h, independently of the pH value. Moreover, as paclitaxel was shown to be very susceptible to degradation in acidic pH, the authors performed the coating of HNTs with poly(methacrylic acid-co-methyl methacrylate (PMMM), a pH-sensitive

polymer insoluble in acidic pH. In this case, the release of paclitaxel started after 6 h and at basic pH, due to the polymer dissolution. The drug release went up to 24 h, by a sustained rate from 9 h to 24 h. These results established the opportunity to expand the application of HNTs to oral administration forms[14].

b) Enzymes

Enzymes are promising in therapeutic fields through the catalysis of chemical reactions. Thanks to their exquisite efficiency and selectivity, enzymes bear a great potential for intra-cellular delivery. The use of a carrier for enzymes delivery enables their protection against proteases and the easy recovery from the products to reusability[34]. When using HNTs as enzymes delivery systems researchers controlled the pH range, by using it above the enzymes isoelectric point. In this case, enzymes presented a negative charge and were mainly loaded into the positively charged inner lumen of HNTs[6].

To determine the effects of HNTs on the release profile of pepsin, laccase, glucose oxidase and lipase, Tully *et al.* compared the outcomes of these enzymes in their free and HNTs-encapsulated forms[35]. Enzyme-loaded HNTs showed more stability to pH and temperature changes than pristine enzymes, demonstrating the protection that HNTs offer to their loaded drugs[35]. With respect to the loading capacity of the enzymes into HNTs, the values corresponded to ca. 4 wt%. The release profile from HNTs begun with a burst phase of 4 h resulting in the release of approximately 70% of the encapsulated enzyme, followed by a slow release from 20 to 30 h[35].

Bovine serum albumin (BSA) was loaded into HNTs resulting in an enzyme content of 10 wt%. Within 30 min, 70% of BSA was released from the HNTs, with an initial burst release. The drug released continued further at a slower rate, releasing the BSA up to 95% in 24 h[36].

In terms of public health, it is of interest to refer the use of lysozyme-loaded HNTs in a polymeric film matrix in order to inhibit bacterial grow in food formulations. This application was developed to promote the inhibition of the bacterial growth for longer periods without needing high enzyme concentrations[37]. Bugatti *et al.* incorporated HNTs loaded with lysozyme into a matrix of poly(lactic) acid (PLA)[37]. The film of HNTs evidenced longer release profile, which was characterized by three stages. In the first phase, lysozyme free molecules localized at the film surface were burst released. This stage was followed by a slower diffusion of free lysozyme from the bulk of the film. Next, the third stage consisted on an even lower release rate, represented by the diffusion of lysozyme from the HNTs[37].

Further on, the same research group assessed the release profile of lysozyme using a matrix of ε-caprolactone (PCL), whose outcomes were found to be similar to the previous. The authors verified that a burst release phase occurred in the first 24 h, followed by a second release phase that took place between 24 to 60 h, and finally by the third release phase in which only 50% of the lysozyme was released after 720 h[38].

2.2. Outside tube's lumen and dual drug loadings

Selective loading onto the outer surface of HNTs is formed thanks to the affinity between de drug and silica or the negative charge this layer presents[6]. Furthermore, the external layer is easily functionalized, which enables the improvement of HNTs physical characteristics and the affinity towards adsorbed drugs. Surface modification helps targeting delivery through cellular recognition as well[9]. Moreover, as a result of HNTs unique chemical structure it is possible to perform dual loading of drugs to synergistically increase the pretended action, improve target selectivity and exceed drug resistance, e.g., loading of positive lipase into the lumen and posterior adsorption of negative lysozyme onto the silica layer (Figure 7)[1, 34]. The co-loaded HNTs demonstrated high stability, possible reusability and great biocatalytic activity[34].

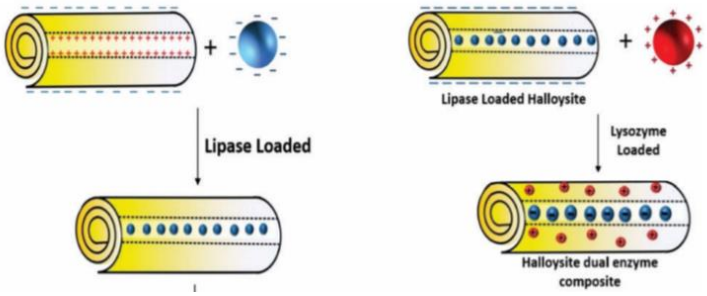


Figure 7 Dual enzyme loading of lipase and lysozyme into halloysite nanotubes. Source: Sun *et al.* [34]

a) Small drug molecules

In 2017, Fizir *et al.* developed a novel formulation aiming a controlled release of cationic drugs from HNTs[1]. Polymer grafted-magnetic HNTs loaded with norfloxacin were synthetized by three steps. First, HNTs powder was stirred with a solution of Fe^{2+}/Fe^{3+} salt followed by the addition of ammonia, resulting in the precipitation of magnetic HNTs. Researchers then proceeded to the norfloxacin drug loading onto the magnetic HNTs. To conclude the synthesis, the polymerization process was performed by grafting polymers in

the outer surface via hydrogen bounds[1]. This formulation showed a great sustained release of norfloxacin. Within 10 h only 38% of norfloxacin was released from the HNTs, which increased up to nearly 50% in 24 h. After 60 h, 22% of norfloxacin was still found to be adsorbed to HNTs[1].

A modification of HNTs silica surface with carbohydrate-cyclodextrin was studied as a potential drug delivery system for curcumin[9], and, in another study, amphiphilic-cyclodextrin units were used to modify HNTs with the purpose of dual loading of two different drugs with different characteristics[39]. This procedure was possible because of the cyclodextrin cavity created onto the HNTs, which is able to interact and encapsulate drug molecules. The amphiphilic-cyclodextrin f-HNTs were used for dual loading of silibinin and quercetin, two anticancer drugs. It was shown that quercetin interacted preferably with the cyclodextrin cavity, in which it was 2.2% incorporated, while 6.1% of silibinin was loaded into the HNTs lumen[39].

To assess the influence of HNTs surface modification, Massaro *et al.* studied the properties of cardanol-loaded HNTs functionalized with triazolium salts[27]. f-HNTs demonstrated two times more loading efficiency of cardanol (10 wt%), compared to pristine HNTs (ca. 5 wt%). The released quantity of cardanol from f-HNTs was found to be highly dependent of the pH value. Even though the release profile pattern from f-HNTs was quite similar in diverse pH environments, the quantity of released cardanol was significantly different depending on this parameter. Overall, the release profiles were characterized by an initial burst in the first 200 min, remaining at a slower rate up to 6 h[27].

In 2014, Riela *et al.* used HNTs modified with triazolium salts to load curcumin[20]. Although curcumin tends to be loaded into the lumen, in this study this drug was absorbed on the outer layer due to the positive charge created in the exterior surface by the triazolium salts[20]. A slow drug release rate was preceded by an initial fast release within 100 min. Moreover, the drug release rate was shown to be superior in acidic pH because of established electrostatic repulsions between curcumin and f-HNTs, both positively charged in this environment[20]. Studying the functionalization of HNTs with poly(N-isopropylacrylamide), a temperature-responsive material, for the delivery of curcumin, Cavallaro *et al.* also obtained a drug release profile sensitive to pH changes[40]. Within 50 min in acidic pH, none of the 3.6 wt% content of curcumin loaded into HNTs was released. On the opposite, a higher drug release was observed at a higher pH. Given the previous outcomes, the latter authors have speculated that this drug delivery system may be complementary to f-HNTs with triazolium salts, which release profile evidence a contrary behavior[40].

Through HNTs functionalization, researchers synthetized a dual-stimuli-responsive prodrug of curcumin-loaded HNTs[21]. These formulations are able to facilitate the target delivery, improving the therapeutic action, while diminishing adverse effects[21]. To form the prodrug, HNTs outer surface was first connected to cysteamine by a disulfide bond, which is known to be broken in the presence of GSH, acting by a reduction-responsive mode. Subsequently curcumin was attached to the amino groups from cysteamine, creating a pH-responsive imine bond[21]. The drug release behavior of the resulting prodrug was then evaluated. In the presence of GSH, 25% of curcumin was released from the f-HNTs in 100 min. By contrast, only 5% was released in the absence of the reductive agent. Studying the stimuli response into pH range, it was demonstrated that the release of curcumin was substantially faster in acidic environment over neutral environment. These results supported the efficiency of stimuli-responsive prodrugs using HNTs as drug delivery systems[21].

b) DNA

In spite of the widely research with nucleic acid based therapeutics, such as ASODNs, their practical application has faced challenges due to the struggle related with delivering an undamaged nucleic acid to the *in vivo* action site[23]. Various obstacles must be overcome before the therapeutic reaches its target, e.g., limited intracellular uptake and degradation by nucleases present in the blood stream, saliva, among others[23]. In order to solve those problems, several drug delivery systems have been applied to deliver nucleic acid therapeutics, but each system evidenced in parallel their own limitations, e.g., cell toxicity of cationic carriers. Owing to such, a vigorous and nontoxic delivery system, such as HNTs, is greatly sought after in order to defend the nucleic acid from degradation and enhance their intracellular uptake[23].

Shi *et al.* developed a HNT-based drug delivery system comprising ASODNs as a therapeutic agent against survivin activity, which is an inhibitor of apoptosis gene selectively overexpressed in tumor cells[23]. HNTs outer surface was first functionalized with APTES, while ASODNs were connected to fluorescein (FAM) to provide fluorescent labeling. Afterwards, ASODNs were linked to the outer surface of the functionalized HNTs (f-HNTs) by electrostatic interactions[23]. Shi *et al.* proceeded to the measurement of the surface zeta-potential of the samples. HNTs initial zeta-potential of -14.3 mV increased up to +44.8 mV, demonstrating that APTES coated completely the negative surface of HNTs[23]. The high charged surface was due to the amine groups of APTES, which offer suitable sites for further conjugation of biomacromolecules. f-HNTs loaded with ASODNs-FAM presented a zeta

potential value of +35.5 mV, indicating the successful linkage onto the drug carriers[23]. This hypothesis was afterwards confirmed through fluorescence spectra analysis, given that both samples, free ASODNs-FAM and loaded ASODNs-FAM, showed a similar fluorescence at the characteristic emission peak of FAM conjugated with ASODNs[23].

The association between nucleic acids and HNTs may also be useful to enhance the physical properties of this drug delivery system[41]. Regarding these premises, a study was performed by Lee *et al.* to assess the employment of DNA-wrapped HNTs as drug delivery systems for doxorubicin[42]. The outer surface modification of HNTs with DNA allowed a significantly superior water-dispersion of doxorubicin-loaded f-HNTs[42]. The activity results of free doxorubicin and f-HNTs loaded with doxorubicin were compared within a 3-day treatment. Free doxorubicin showed a higher cytotoxicity activity relatively to doxorubicin-loaded f-HNTs. Nevertheless, the cytotoxicity of loaded doxorubicin demonstrated to increase throughout the treatment. These outcomes supported de hypothesis of slow and prolonged drug release from f-HNTs[42]. Furthermore, comparing the drug release profiles between the release of doxorubicin from f-HNTs and free doxorubicin, f-HNTs were capable of reducing the initial burst and extend the drug release period for more than two weeks[42].

c) Enzymes

Zhu *et al.* used HNTs as core agents of molecular imprinted polymers (MIPs) of BSA to promote targeting to its binding sites and, consequently, increase their adsorption capacity[43]. MIPs are promising for recognition and separation of enzymes, although their original crosslinked structure complicates the connection with binding sites. In this work, dopamine was self-polymerized to form polydopamine (PDA), a biocompatible polymer with multifunctional groups, ideal for the recognition and adsorption of enzymes. To synthetize this nanocomposite (Figure 8), HNTs were functionalized with APTES to acquire amino groups. f-HNTs were then bonded to BSA, followed by the addition of dopamine to the solution. After adding ammonium persulfate to the mixture, dopamine started to polymerize, coating the BSA-loaded f-HNTs. The coating PDA layer interacted with BSA through its functional groups. Subsequently, by removing BSA from the formed nanocomposite, the imprinted cavities created by the PDA layer corresponded to BSA in terms of physical characteristics, such as orientation of functional groups, shape and size. To assess the recognition selectivity of the resulting HNTs molecular imprinted polymer for BSA (BSA-MIPs/HNTs), lysozyme, ovalbumin and trypsin were employed in the analysis as competitive

proteins. BSA-MIPs/HNTs showed more affinity and adsorption capability towards BSA, which reached easily the binding sites. Furthermore, BSA-MIPs/HNTs were reusable and simply reproduced, demonstrating as very promising for proteomics applications[43].

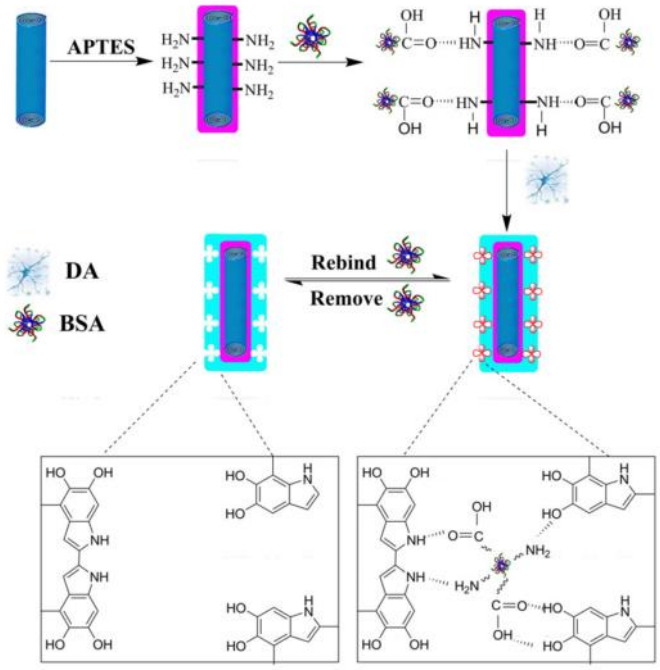


Figure 8 Synthesis of Bovine serum albumin-Molecular imprinted polymers/Halloysite nanotubes. Source: Zhu *et al.* [43]

3. Applications in medicine - administration routes

Several administration routes are available to introduce a drug in the body towards its therapeutic target. Depending on the pretended therapeutic action and on the physical and chemical properties of the drug, a particular administration route may be more appropriate in comparison to others. For example, internal organ illnesses are treated mainly by oral administration or intravenous injection, whereas skin conditions are usually treated topically. So far, HNTs have been developed mainly for oral administration. The great availability and low cost of HNTs render them fit to be used in tablet and capsule forms[26]. HNTs are not biodegradable in blood, which offers a limitation for their use by intravenous injection, given the risk of thrombosis[6, 44]. However, in a different kind of application, HNTs showed to be promising for topical applications, not only in creams but also as sprays due to their high stability as aqueous colloids[6, 26]. Hereinafter the considered administration routes for drug delivery employing HNTs are going to be discussed.

3.1. Topical

In topical administration, the drug is applied externally and locally, diffusing through the skin to the site of action. This may be achieved via a cream, a spray or a patch for wound dressing[6, 7, 16, 45].

a) Creams

Research in cosmetics aims to create products able to provide a good looking and healthy skin[45]. To achieve better and better results, researchers began introducing new delivery systems, such as HNTs. These carriers performed a controlled release of the active agent, conferring a slow absorption onto the skin[45].

In the US Patent 20070202061 A1, it was claimed a method for cosmetic skin care involving HNTs for the sustained release of loaded emollient agents and nutrients, e.g. vitamins[45]. HNTs also enabled a combination of several active agents in only a single product, fact that is markedly advantageous for cosmetic applications as these types of products may contain over 40 ingredients[45]. In this work, glycerin was loaded into HNTs, which provided a controlled release and skin absorption from 4 h up to 18 h, whose ideal intervening period consisted from 8 h to 15 h[45]. Besides enhancing the moisturizing action of the product, the drug loading of glycerin into HNTs also improved the product sensorial characteristics[45].

Suh *et al.* evaluated the use of HNTs as glycerol drug delivery systems, enlightening their retention capacity and release profile[16]. Glycerol was loaded mainly into the lumen but it was also incorporated via adsorption onto the outer layer, resulting in a loading efficiency of ca. 20 wt%[16]. The release profile did not display an initial fast release, demonstrating to be steady slow rate for over 20 h. As a matter of fact, this release period is extremely well-suited for cosmetic applications. Although the use of LbL technique, to further extend the release was shown to be insignificant, authors believed that coating HNTs with higher molecular weight polymers or capping small nanoparticles may decrease the release rate of glycerol from HNTs[16].

b) Sprays

Thanks to their high zeta-potential magnitudes, HNTs give origin to highly stable colloids and, therefore, they may be used to form antibacterial sprays by drug loading antiseptics[26]. Brilliant green-loaded HNTs spray may enable prolonged antimicrobial action and avoid infections[26]. In addition, Wei *et al.* performed a study in which they demonstrated

the extended efficiency of antiseptic drugs owing to the use of HNTs as drug carrier[46]. To assess the influence of HNTs in the release profile, the research group compared HNTs release profile with the one regarding free brilliant green. The total release time of free brilliant green only reached 15 min. On the contrary, only 70% of brilliant green was released from HNTs within 3 h, and the release period was prolonged up to 5 h[46].

c) Bandages

HNTs may be loaded with antibacterial agents and subsequently embedded in a gel matrix to form modern wound dressing. These products provide appropriate conditions for wound healing, protecting the affected zone from pathogens. Additionally, these dressings also improve the user's comfort[7]. In this context, aiming the creation of a bandage for wound healing with high capability to perform a slow and steady release, a research group introduced HNTs into matrices of alginate and gelatin/alginate[7]. Kurczewska *et al.* modified the outer surface of HNTs with APTS, whose aminopropyl groups granted a stronger bond to drugs with carboxyl groups, such as the used vancomycin, due to electrostatic interactions. The loaded f-HNTs were afterwards included into alginate and gelatin/alginate. It was shown that after 24 h only 44% and 37% of vancomycin was released, for alginate and gelatin/alginate matrices, respectively. From this outcome, researchers concluded that the addition of gelatin onto de matrix aided to further slow the drug release rate. Moreover, an additional analysis demonstrated that the dose of vancomycin had no significant influence in the release profile. However, it was confirmed that delivery systems with lower content of HNTs showed lower stability, supporting the idea of the fundamental role of these structures on drug sustained release[7].

d) Pour powder

The application of HNTs and chitosan oligosaccharides to form pour powders was performed by Sandri *et al.*[17]. The pour powder was created with the purpose of improving wound healing. To synthetize the pour powder, a simple procedure was applied: HNTs powder was mixed with an aqueous solution of chitosan oligosaccharide, resulting in spontaneous electrostatic interactions between the two materials thanks to chitosan oligosaccharide positive charge and HNTs negatively charged outer surface[17].

3.2. Oral

The use of HNTs for oral drug administration provides several advantages in comparison to traditional delivery systems: HNTs protect their cargo upon severe conditions, strengthen drugs solubility, improve its absorption and diminish its metabolism, thus improving the oral bioavailability of the loaded drug. Furthermore, HNTs are suited to satisfy the high demands of excipients in oral formulations, since they are widely available at low cost[15]. Therefore, HNTs may be used as compression excipients in tablets and in capsules by blending with polymers[44]. Even though HNTs are promising for oral administration, these novel formulations evidenced some setbacks in terms of precision of drug release in appropriate absorption sites and interaction with intestinal epithelium to enhance drugs absorption[15]. To overcome these issues, Kerdsakundee *et al.* developed multifunctional nanocomposites by modification of HNTs outer surface with APTES and poly(methyl vinyl ether-co-maleic acid) (PMVEMA), followed by further encapsulation of f-HNTs into an hydroxypropyl methylcelulose acetate succinate (HPMCAS) matrix[15]. APTES confered amino groups on HNTs outer surface, supporting the conjugation with the carboxyl groups of PMVEMA. PMVEMA is a mucoadhesive polymer, which is recognized to enable stronger interactions with the intestinal epithelium. HPMCAS, in turn, is a polymer stimuli-responsive to pH range and insoluble to strong acidic pH[15]. To assess the influence of this functionalization on the drug release profile, curcumin was employed as the loaded drug. The analysis took place in a pH 1.2 solution for 2 h, followed by 6 h in a pH 6.8 solution. Within 2 h, free curcumin, curcumin-loaded HNTs and curcumin-loaded f-HNTs showed an almost complete release. On the contrary, no release of curcumin was shown from curcumin-loaded f-HNTs in HPMCAS matrix in the first 2 h. With the pH rise to 6.8, curcumin initiated its release. These outcomes supported, thus, the hypothesis of gastro protection conferred by the HPMCAS matrix[15]. In addition, drug permeability strongly influences the respective oral absorption, thus becoming important to assess the effect of the novel formulation regarding this characteristic. HNTs were shown to increase curcumin permeability, which was further improved using f-HNTs thanks to the presence of PMVEMA. By its mucoadhesive action, PMVEMA enables the closer interaction between the f-HNTs and the epithelium cells[15]. This novel formulation revealed this way to be promising for oral administration[15].

The outcomes explained below demonstrate the potential of HNTs for efficient oral administration associated to various exposed drug formulations. These are supported by studies performed in piglets, in which HNTs were capable of removing zearalenone, a

mycotoxin present in animal feed[47]. Thanks to their adsorption efficiency, HNTs may be used to reduce the toxic effects on the reproductive system caused by zearalenone. Zhang *et al.* assessed the HNTs adsorption *in vivo* capacity of zearalenone[47]. Since zearalenone presents a low solubility in water, HNTs outer surface was modified with the surfactant stearyldimethylbenzylammonium to support the adsorption. By introducing 1% f-HNTs to zearalenone-contaminated grain feed, it was shown the protection of physical conditions of female sows and growth performance of their offspring[47]. Further along, the researchers extended their investigation on this issue[48]. Even though f-HNTs did not completely reestablish the healthy physiologic characteristics, they alleviated the zearalenone effects on muscle fibers and on development of piglets[48].

a) Tablets

Beyond controlling drug-release, HNTs also detain outstanding compression properties and good powder flow, being extremely promising as an excipient of tablet formulations[44]. Yendluri *et al.* performed a study in which they compared the release profile of nifedipine from HNTs with extended release commercially available tablets[44]. All the tablets were disintegrated past 30 min under *sink* conditions. Within 2 h, less than 10% of nifedipine was released, after 4 h the released quantity increased between 10 and 20%, after 12 h, 70% of nifedipine was released, and ca. 9% of the drug was released after 24 h[44]. Overall, the release profile was similar to commercial available targets for extended release. Furthermore, the released in the first 2 h was much lower for HNTs tablets than pristine HNTs, corresponding to 10% and 80%, respectively[44]. During the performed study, it was also shown that loaded nifedipine demonstrated higher photostability, which is a strong indicator that HNTs not only allow for controlled release but also are able to reinforce drugs stability[44]. In addition, the same research group studied the drug release characteristics of paclitaxel from HNTs tablets[14]. Release studies were assessed using tablets with paclitaxel-loaded HNTs (group 1) and tablets with paclitaxel pre-mixed with HNTs (group 2). By analyzing Figure 9, it can be observed that the release rate of group 2 was substantially faster. After 72 h, 80% of paclitaxel was released from group 2 and only 25% was released from group 1 in the corresponding period. Regarding group 1, the total release period was over 250 h, in which only 40% of paclitaxel was released within 120 h[14]. By modifying HNTs outer layer with pH-responsive polymers, a control of the release starting point was achieved in basic pH[14]. Relatively to paclitaxel activity, the functionalization of HNTs with

dextrin enabled specific target drug delivery to tumor cells, in which dextrin was decomposed via enzymatic degradation[14].

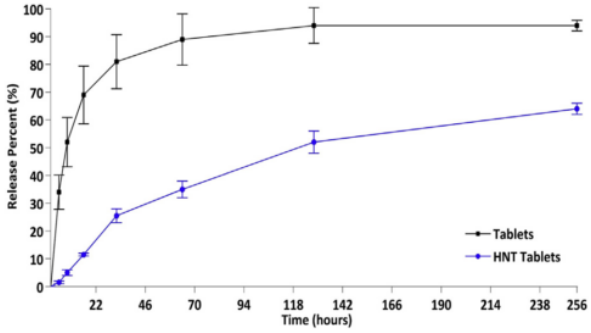


Figure 9 Release profiles from tablets with paclitaxel-loaded halloysite nanotubes (blue) and tablets with paclitaxel pre-mixed with halloysite nanotubes (black). Source: Yendluri *et al.* [14]

b) Nanoparticles-in-microgel oral systems (NiMOS)

Nanoparticles-in-microgel oral systems (NiMOS) are strong candidates for oral administration due to their structure, which allows for drug multi-compartment. Moreover, their simple manufacture process enables their scale-up production[36]. In 2016, Kruif *et al.* designed a novel formulation of NiMOS by incorporating HNTs in a microgel matrix for the oral administration of proteins[36]. NiMOS were synthetized by a simple microencapsulation method called priling. A polymeric solution of loaded-HNTs was pumped into the prilling device, in which those suffered vibration and passed through an electrode ring until reaching a hardening bath[36]. The performed study aimed to assess protein stability after prilling, the protection from enzymatic action and the drug release profile[36]. It should be noticed that a group of HNTs suffered etching prior to the NiMOS synthesis, forming HNTs-based modified NiMOS (bNiMOs)[36]. Researchers pretended a local action of the protein in the intestinal mucosa, in order to avoid the absorption phase and the associated limiting factors[36]. Concerning the release profiles, the initial release from NiMOS was very distinct from the HNTs release. Compared to pristine HNTs, NiMOS showed a faster initial release, which rate decreased after ca. 90 min, resulting in 89% of released BSA in a 24 h period. bNiMOS showed an unusual slow initial release in the first 15 min, contrary to NiMOS which evidenced an initial burst release. This case may be explained by the result of a higher concentration of HNTs near the core than to the surface[36]. Overall, the outcomes were found to be very favorable, since NiMOS showed to be efficient in the protection of their cargo from enzyme degradation and also by promoting a controlled drug release, while their manufacture did not show harm regarding the proteins viability. In authors opinion, NiMOS

may be further employed as fixed-dose combination dosage forms or as a two-drug compartment delivery system[36].

4. Additional applications in medicine

HNTs are auspicious materials with immense potential for several medicine applications. Henceforth, studies regarding the use of HNTs in implants, tissue scaffold and wound healing are explored.

4.1. Implants

On the basis of the aforementioned, it is clear that HNTs are robust materials, assuming themselves as a potential candidate for distinct applications, particularly to improve bone cement structure and to extend the durability of dental adhesive interfaces, while performing their ability for steady and sustained drug release[49, 50]. Furthermore, the development of innovative restorative materials requires the enhancement of biological and physical properties, which can be together improved by using biocompatible materials[51].

Combinations of prophylactic antibiotics with poly(methyl methacrylate) (PMMA) cement are largely employed in orthopedic surgery[49]. However, the addition of antibiotics may lead to loss of the cement strength[52]. In the US Patent 9192912 B1, it was claimed an implantable enlarged ceramic composite involving the dispersion of radiated HNTs into a PMMA cement. The HNTs subjected to radiation were disaggregated in methyl methacrylate, and posteriorly mixed with PMMA through a vacuum technique. The inventors claimed that the created composite included a combination of drugs, which could be promoters of bone growth, antibiotics or chemotherapy agents, among others[52].

HNTs shown to be able to support a good polymerization rate of methacrylate due to the interaction between PMMA carbonyl groups and the electron deficient alumina present in HNTs inner layer[51]. This way, with the same purpose of enhancing bone cement characteristics, Wei *et al.* created nanocomposites of HNTs/PMMA and analyzed the properties of the resulting products[49]. 5% of gentamicin-loaded HNTs were incorporated and evenly distributed into the PMMA cement. The latter was stored in water for 50 days to mimic the interstitial fluid and the *in vivo* environment in order to access its influence on the mechanical characteristics of the resulting nanocomposite[49]. HNTs/PMMA nanocomposites showed greater tensile strength than pure cement and good flexural strength. Moreover, those nanocomposites increased almost five times the adhesion force between bone cement

and bovine femoral bone[49]. The increased adhesion force must have been due to enhanced hydrophilic forces provided by HNTs and also to the anchoring process of HNTs into the pores of the bone surface[49]. This outcome was also noticed in resin-dentin bond in a study conducted by Bottino *et al.*[53]. The loading efficiency of gentamicin into HNTs lumen was ca. 10 wt%, and a prolonged gentamicin release from nanocomposites was found. In fact, within 250 h, only 60% of gentamicin was released from bone cement. By adding free gentamicin, a double antibiotic release was achieved, together with the induction of an initial burst release without jeopardizing tensile and flexural strength. It is worth noting that the addition of free gentamicin did not affect the adhesion[49]. Besides these outcomes, the produced nanocomposite demonstrated to be efficient in the inhibition of bacterial growth (Figure 10), which supports the use of HNTs into bone cement to help solving the infection problem and maintain the optimal mechanical properties[49]. In a similar test performed by Abdallah, HNTs/PMMA nanocomposites showed an increase in hardness. Notwithstanding, HNTs/PMMA did not show a significative influence in the flexural strength[54].

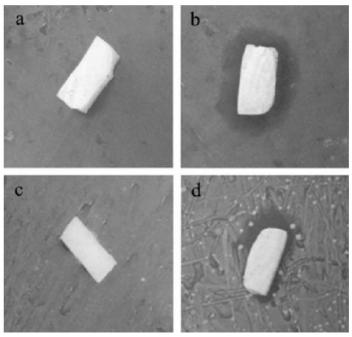


Figure 10 One week of bacterial culture growth of *E. coli* (a, b) and *S. aureus* (c, d) around poly(methyl methacrylate) (a, c) and gentamicin-loaded halloysite nanotubes/poly(methyl methacrylate) (b, d). Source: Wei *et al.* [49]

In another study, Jammalamadaka *et al.* studied the effect of barium sulfate coating of HNTs prior to their introduction in PMMA cement[55]. Barium sulfate, a radiopacifying agent, enhanced contrast properties and enabled the obtainment of potential imaging techniques[55]. From the performed analysis, it was possible to assess that the barium coating did not compromise mechanical properties. Although, more extended studies are effectively required to assess coating thickness uniformity, durability and surface adhesion[55].

HNTs have been developed as resin fillers to reinforce the bond strength between resin and dentin, simultaneously allowing antibiotic release to avert caries recurrence[51, 53]. The research team of Alkatheeri *et al.* demonstrated the enhancement of the adhesive force thanks to the use of HNTs[56]. Furthermore, triclosan-loaded HNTs incorporated into a dental methacrylate material demonstrated to be efficient in remineralization of cavities,

being considered good candidates as dental fillers, with more advantageous than the market available materials[51].

Feitosa *et al.* concluded that high concentrations of HNTs in adhesives may compromise the mechanical properties of this material. For example, the introduction of 20 wt% HNTs diminished significantly the adhesive flexural strength[50]. Nevertheless, the analysis performed involving small amounts of HNTs, up to 10 wt%, depicted better mechanical results than the original adhesives, improving its physical properties. In the opinion of the authors, HNTs must increase the biological activity through drug loading preceding the incorporation into the adhesive[50].

4.2. Tissue scaffolds

Tissue engineering aims to produce functional composites to substitute or rebuild damaged tissues, representing a vital filed in regenerative medicine[57]. The development of 3D scaffolds is crucial to provide tissue prototypes that detain biomimetic functional properties and, consequently, allow for an effective transplantation. Transplantation from donors is not able to satisfy the high demand for replacement of damaged tissues, either by the unavailability of donor organs or by the lack of immunological compatibility. In contrast to cell monolayers, 3D scaffolds allow the formation of complex cellular structures, as the placenta or trophoblast vesicles[58]. High porosity and pore size are determinant properties of tissue scaffolds for cell attachment and nutrients diffusion[59].

In this context, Naumenko *et al.* produced scaffolds with chitosan, agarose and gelatin as biopolymers, which were incorporated with HNTs to improve their mechanical strength, biocompability and adhesion force between the cells and scaffolds[58]. In order to make the comparison of results, researchers produced three different scaffolds by dissolving the biopolymers in an acidic solution with different concentrations of HNTs, including a scaffold without HNTs, a scaffold with 3 wt% of HNTs and a scaffold with 6 wt% of HNTs. The resulting products were then freeze-dried and did not need cross-linkers for their production. The mechanical characteristics analysis showed that scaffolds exhibited shape reconstitution after being submitted to deformation, and it was also verified the HNTs capacity to increase scaffolds robustness. Water uptake capability was also assessed in order to confirm the scaffolds capacity of exchanging nutrients and water. It was shown that HNTs improved the hydrophilicity of the scaffold, thus improving its biocompability. To test cell viability *in vitro*, cells were seeded on the scaffolds, resulting in their growth and dispersion. The cells also maintained their morphology intact. Scaffolds' biocompability was confirmed

for various cell lines, thus supporting their use for tissue engineering. Figure 11 demonstrates the results from an *in situ* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. It is possible to see that metabolically active cells performed MTT reduction into formazan (purple). As shown, all scaffolds enabled the viability of adherent cells (Figure 11)[58].

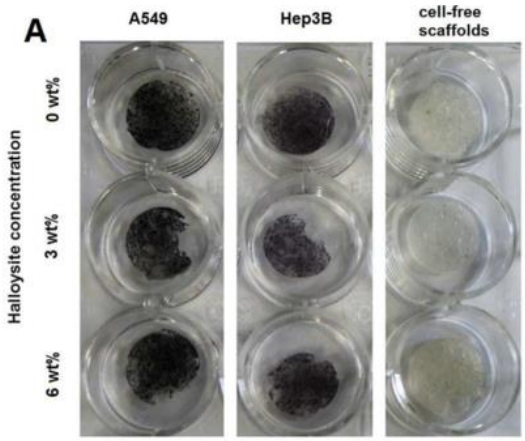


Figure 11 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay results of A549 and Hep3B seeded on scaffolds. Source: Naumenko *et al.* [58]

The biocompability was also evaluated in *in vivo* with rat models, in whom the scaffolds were implanted subcutaneously for 3 and 6 weeks. After each of those periods, the rat models were euthanized and the scaffold-surrounding formed tissue was prepared for the obtainment of histological sections (Figure 12). No abnormalities or inflammatory reactions were observed after the implantation of scaffolds, and it was noticed the presence of neoangiogenesis surrounding the scaffold. After 6 weeks, a complete degradation of the scaffold material was observed, as pretended. The authors stated that their outcomes suggested that HNTs evidence a great potential as fillers for tissue scaffold[58].

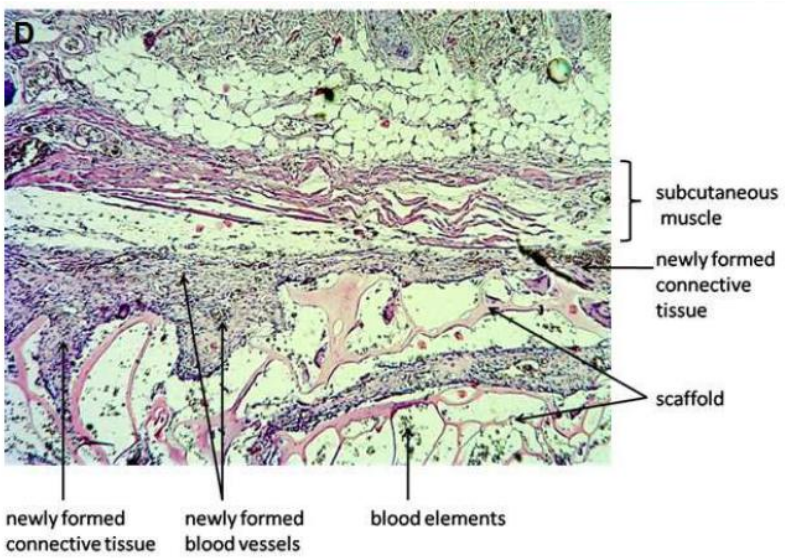


Figure 12 Histological section of the subcutaneous area 3 weeks after the implantation. Source: Naumenko *et al* [58]

Liu *et al.* studied as well the effects of HNTs in chitosan-based tissue scaffolds[59]. The incorporation of HNTs did not meaningfully alter the scaffolds structure, although more uniform pore walls were presented due to the increased mechanical strength provided by HNTs. Thanks to strong electrostatic interactions between HNTs and chitosan, the thermal stability of the scaffold was enhanced. However, chitosan scaffolds comprising HNTs demonstrated to decrease the water uptake capability and higher density[59]. In fact, the influence of HNTs in the water uptake behavior of scaffold is in line with other studies[60]. Despite this disadvantage, the cell attachment was facilitated in chitosan-HNTs scaffolds[59]. Overall, the properties of the scaffold were significantly improved, and the outcomes pinpointed HNTs as excellent materials for tissue engineering purposes.

An analysis performed by Afshar *et al.* involved the construction of chitosan/alginate scaffolds with embedded HNTs, evidencing similar results to the study abovementioned concerning mechanical properties, scaffold structure and water uptake[61]. In this particular analysis, the scaffold preparation involved an amination treatment, which provided greater fibroblast attachment trough electrostatic interactions. Scaffolds which were subjected to amination process also demonstrated superior cell viability and proliferation[61].

In a work conducted by Huang *et al.* a hydrogel scaffold containing HNTs and Sodium alginate (SA) solution was developed by cross-linking with $CaCl_2$ solution[60]. The presence of HNTs has allowed to increase the viscosity of the solution, thus enabling the preparation of uniform hydrogels without occurring precipitation[60]. In 2017, Bonifacio *et al.* developed a gellan gum-based hydrogel scaffold comprising HNTs as fillers and glycerol as a molecular spacer[62]. The biological behavior of this scaffold was tested regarding cell attachment and viability, metabolic activity and capability to encapsulate cells to future prospected applications[62]. Gellan gum-glycerol (GG-Gly) hydrogel with incorporated HNTs stimulated the fibroblasts metabolic activity and helped cells growth and proliferation. However, high concentrations of HNTs demonstrated a major decrease in the cell viability. HNTs-containing GG-Gly hydrogel also have shown to be capable to encapsulate cells and ensure their viability during the incubation period[62]. In general, these results suggested the developed scaffold as a feasible and strong strategy to carry out for soft tissue engineering[62].

Torres *et al.* demonstrated the improvement of thermal stability and mechanical strength of PCL matrices through the addition of hydroxyapatite (HA) and HNTs to the scaffold structure[63]. As HA has shown osteoconductive and osteoinductive properties,

thus ensuring the compatibility of the scaffold with human bone. For their part, HNTs enabled the drug loading and enhanced the robustness of the scaffold[63]. In order to reduce the costs of tissue engineering, Jing *et al.* assessed the possibility of substituting HA for HNTs as filler for scaffolds[64]. Although the morphology of neat PCL scaffold did not alter significantly with the addition of HA or HNTs, in the case of higher loading levels of these fillers, the porosity of scaffolds decreased (Figure 13), which is not supportive for vascularization, nutrients transportation and bone ingrowth. Nevertheless, almost all samples showed porosity levels greater than 75%, which equaled the minimum value for 3D scaffolds requirements (Figure 13) [64]. Regarding thermal properties, HNTs revealed to be more helpful for thermal-stability. Even though both fillers improved the mechanical properties of scaffolds, HNTs were shown to be more effective. Concerning cell viability and proliferation, after 28 days, 5% of HA and 1% of HNTs scaffolds presented far better results (Figure 14). HA and HNTs also showed to be appropriate for cell growth, excluding high concentrations of HNTs. PCL scaffolds filled with HA or HNTs showed an increased cell differentiation, which was supported by the increased activity of alkaline phosphate and expression of osteocalcin. The authors concluded that silica ions from HA and HNTs are the key aspect for the stimulus of osteoblast and osteoblast-like cells to secret type I collagen and other markers. These results offer interesting insights for the use of HNTs as scaffold fillers, capable of substituting HA[64].

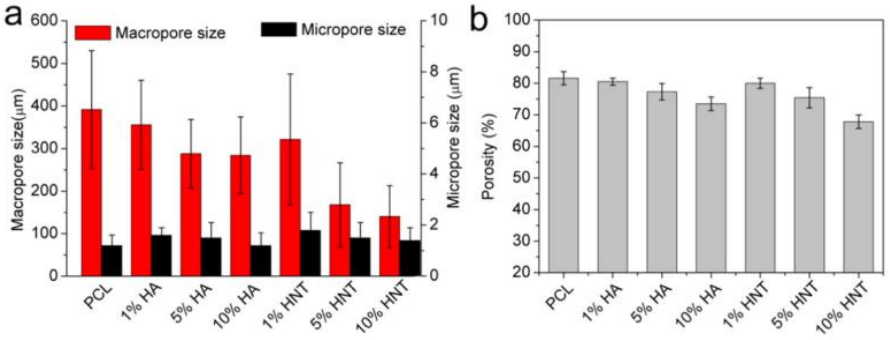


Figure 13 Scaffold pores size (a) and porosity (b) reliant on hydroxyapatite/halloysite nanotubes concentrations. Source: Jing *et al.* [64]

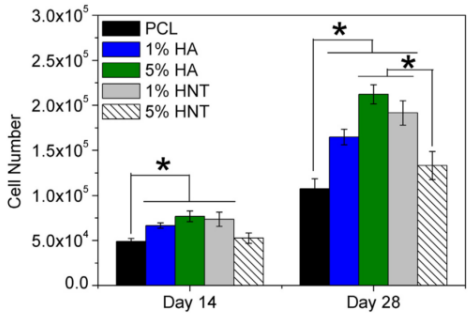


Figure 14 Proliferation of human mesenchymal stem cells on scaffolds. Source: Jing *et al.* [64]

Xue *et al.* embedded metronidazole-loaded HNTs in a polymeric solution for electrospinning to form HNTs/electrospun microfibers composites[65]. This study aimed to produce efficient scaffolds with sustained antibiotic release, good mechanical strength and biocompatibility[65]. Prior to this procedure, HNTs outer surface was functionalized with APTES to increase their dispersivity in the polymer solution. The release of metronidazole from free HNTs was completed after 24 h. However, the introduction of HNTs into the electrospun microfibers, with 20 wt% of metronidazole inserted directly in the microfibers and 5 wt% of metronidazole loaded into HNTs, allowed to further extend the drug release for 15 days [65]. As for the case of the antibacterial activity, the inhibition zone around the membrane evidenced a long durability in *in vitro* assays, indicating the possibility of using HNTs/electrospun microfibers in applications that require a long period functionality[65]. By incorporating ibuprofen loaded-HNTs into gelatin scaffolds, Ji *et al.* were able to extend the drug release of ibuprofen from 8 h to over 100 h[66].

4.3. Would healing

The incidence of chronic cutaneous wounds constitutes a main concern due to the prevalence of certain diseases, such as type 2 diabetes and the metabolic syndrome[17]. As has been explained previously, Sandri *et al.* developed a pour powder aiming wound healing[17]. To assess the efficacy of this formulation, the research group used rat model for an *in vivo* study and compared its results in the treatment of skin lesions with the saline solution, simple HNTs and simple chitosan oligosaccharides. After 7 days of treatment, the results of pour powder stood out from the others, showing re-epithelialization in the wound borders, hemostasis, macrophage infiltrate and neoangiogenesis[17]. After 18 days, all samples showed complete re-epithelialization, besides the fact that the pour powder performed a faster healing process[17].

In a study conducted by Wei *et al.*, brilliant green-loaded HNTs were coated with benzotriazole-copper complexation, resulting in a total release time of 200 h[46]. The release rate was controlled through the modification of benzotriazole-copper ratio, e.g., higher concentrations of benzotriazole and lower of copper form a coat with less porosity, thus diminishing the release rate. By performing a *S. aureus* viability assay, it was revealed that brilliant green-loaded HNTs provided bacteria suppression over 72 h, suggesting the application of this nanocomposite in bandages for wound healing[46].

Wound dressing of calcium alginate and vancomycin has particularly been gaining attention for surgical wounds. To warrant a sustained drug release, Kurczewska *et al.* combined HNTs to the wound dressing[7]. HNTs were first loaded with vancomycin and were thereafter introduced in an alginate or in an alginate-gelatin matrix. The biological activity of the resulting product was later assessed. Specifically, given the activity spectrum of vancomycin, the microbiological study involved solely gram-positive bacteria. Overall, alginate matrices demonstrated a higher inhibition activity. This difference was attributed to the drug release rate associated to each matrix, which has shown to decrease with the addition of gelatin. Although the quantity of vancomycin released within 24 h from alginate leaded to the desired effect, in the case of alginate-gelatin matrix the amount of vancomycin released in the same period was found to be insufficient to inhibit bacterial growth. Given these results, together with the characteristic high stability of this nanocomposites which promotes a long storage durability, vancomycin-loaded HNTs in an alginate matrix were considered very interesting platforms for wound dressing materials[7].

5. Stability

Colloidal stability is a critical aspect for pharmaceutical applications[67]. In their unmodified form, HNTs form stable dispersions in water-based solvents for ca. 3 h, thanks to their surface zeta-potential[6]. To optimize HNTs stability, researchers have functionalized HNTs with anionic species in order to neutralize the positive lumen and thus to further decrease the zeta-potential to -70 mV[6, 67]. This way, Cavallaro *et al.* combined HNTs with ionic surfactants to develop composites appropriate to carry low soluble drugs[67]. To explore the stability properties of negative and positive f-HNTs, two models were developed through layers modification: one model with sodium dodecanoate (NaL) and the other model with decyltrimethylammonium bromide (DeTAB). In fact, NaL is able to increase HNTs zeta-potential, while DeTAB inverts it to a positive charge[67]. As it is shown in Figure 15, NaL showed to be an efficient candidate to enhance HNTs dispersion and prolong their

colloidal stability. On the contrary, DeTAB destabilized the HNTs dispersion in water and promoted its sedimentation[67]. The increased stability of NaL f-HNTs may be explained through the augmentation of electrostatic repulsion between NaL and f-HNTs. In addition, to better understand the importance of electrostatic interactions in the stability of HNTs dispersions, the researchers measured the destabilization under a range of ionic strength conditions. NaL f-HNTs dispersion was found to be highly debilitated by the addition of KCl to the solvent. On the other hand, KCl barely affected DeTAB f-HNTs dispersion, given that the dispersion was already destabilized[67]. Another study performed by Cavallaro *et al.* showed that HNTs modified with poly(N-isopropylacrylamide) (PNIPAAM) formed dispersions stable for at least 1 day[40].

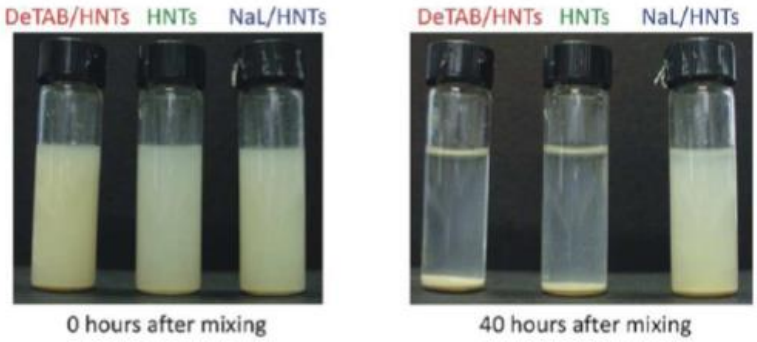


Figure 15 Pristine and f-halloysite nanotubes dispersions in water. Source: Cavallaro *et al.* [67]

Shamsi *et al.* studied the solubilization and dispersion of HNTs through a solid-state mechanochemical reaction, which conducts to the formation of highly reactive centers, enabling the synthesis of DNA f-HNTs[41]. To synthetize the f-HNTs, equal amounts of HNTs and DNA were mixed and milled for 1 h at 25 °C. The resulting powder was dissolved in water and centrifuged, whose supernatant was collected and stored for supplementary analysis. The deposited material was dried in a vacuum oven for 36 h. This procedure was repeated with shorter milling periods in order to compare the properties of the various prepared f-HNTs. This technique is simple and consists to a friendly green nanotechnology, which implies shorter periods of time and minor amounts of DNA to form the intended product. The synthetized f-HNTs within 40 min to 60 min demonstrated a water-stability up to 2 months. However, their original structure was found to remain not intact. In contrast, f-HNTs produced from 20 min to 30 min retained their structure and showed to keep their stability until one month and a half. Authors believed that the improvement in the water-solubility and dispersion of f-HNTs was due to the DNA phosphate groups or the interaction between the DNA backbones with the silica outer surface of the HNTs[41].

In 2004, Kelly *et al.* developed a nanocomposite comprising HNTs and poloxamer 407 for the treatment of periodontitis[68]. The stability of the novel nanocomposite was examined at ca. 25 °C and at 4 °C. Samples stored at 4 °C did not show positive and steady results. Due to the poloxamer 407 low viscosity at lower temperature, HNTs tended to aggregate, with no capacity to maintain a uniform dispersion. However, the formulation was shown to maintain its stability for over 9 months when stored at room temperature[68].

6. Toxicity

The verification of the safety of a pharmaceutical formulation is crucial before proceeding to clinical use. In this context, the study of HNTs has been subject to a large evaluation about their biocompatibility and cytotoxicity[26]. The first studies usually consist in *in vitro* assays, in order to evaluate the effects of HNTs in cell lines in a bid to predict their behavior in additional models[6]. Generally, these tests are performed via colorimetric or fluorescent methods to confirm if the plasma membrane is intact or to measure the cellular metabolic activity, thus estimating their viability. For example, HNTs were functionalized with APTES and fluorescent polyelectrolyte layers to study their interaction with HeLa cells (cervical adenocarcinoma cells) and MCF-7 cells (breast cancer cells)[69]. Using confocal laser scanning microscopy (CLSM) was possible to observe and analyze HNTs cellular uptake. Although it occurred HNTs accumulation in the intracellular environment, it did not effectively harm cells nor inhibited their proliferation, as it was showed by monitorization[69]. Furthermore, even when applied high concentrations, e.g., 75 μg/mL, HNTs did not show toxicity and up to 90% of the cells were viable[69]. In a study conducted by Ahmed *et al.,* HNTs evidenced a similar behavior on HCT116 cells (human colon cancer cells) and HepG2 cells (human liver cancer cells) cell lines[70]. In Vergaro *et al.* perspective, the secondary effects on tissue triggered by HNTs may be useful, e.g., the augmentation of fibrogenicity for bone cements[69]. Thereafter, the same research group studied the effects of resveratrol-loaded HNTs in MCF-7 cell culture, estimating their viability at 24, 48, 72 and 96 h using samples with different resveratrol concentrations[29]. Hazzard effects on cells increased in a dependent manner with the resveratrol concentration and longer exposure periods. After 24 h, the viability of all samples was quite high, ca. 80%, due to the small amount of released resveratrol. In the other hand, the cell viability diminished almost to 5% at 72 h and 96 h[29]. The authors attributed the observed toxic effects to resveratrol[29].

According to their inherent and observed meritorious visages of HNTs, an interest for their oral administration was awakened, becoming important to evaluate their

gastrointestinal effects. For that purpose, Lai *et al.* assessed the HNTs toxicity using an *in vitro* model to mimic large intestine epithelium with a monolayer of Caco-2/HT29-MTX co-culture[71]. Even at low doses, e.g., 1 μg/mL, HNTs showed proinflammatory effects. At high levels of exposure, e.g., 100 μg/mL, HNTs increased the up-regulation of proteins related to the processes of cell growth, proliferation and responses to cell stress, such as cell infection or injury[71]. On the whole, the obtained results indicated that HNTs were unlikely toxic at moderate doses. In another investigation, Ahmed *et al.* verified that only concentrations of HNTs over 1000 μg/mL triggered significant proliferation inhibition on human peripheral lymphocytes, which suggested them suitable for oral drug delivery[70]. To interpret this outcome, authors took notice of some important facts: overall, HNTs showed lower toxicity *in vivo* than in *in vitro* tests. Furthermore, authors considered the average percentage of diluent in conventional large tablet forms, ca. 50%, which represents 250 mg. Upon dilution in gastrointestinal fluids this concentration turns into ca. 125-165 μg/mL, which values do not harm human peripheral lymphocytes[70].

The applications HNTs in tissue scaffolds and bone cements crucially demands the assessment of the HNTs hemocompatibility. With this purpose, Liu *et al.* examined the interactions of HNTs with anti-coagulated rabbit blood[72]. HNTs hemolysis ratio was less than 5 %, thus matching the safety requirements. Moreover, HNTs promoted clotting activity, reducing plasma re-calcification time with the increase of HNTs dose. However, with low concentrations of HNTs it was not observed platelet aggregation. These outcomes suggested a good hemocompatibility using low doses of HNTs and provided important information for further clinical research[72].

HNTs *in vivo* toxicity was evaluated in various models. *Paramecinum caudatum (P. caudatum)*, a common fresh water protozoan, was used as model to assess the toxicity effects arising from HNTs, kaolin, montmorillonite, silica, bentonite and graphene oxide (GO)[19]. This microorganism is a viable model to evaluate both acute and long-term toxicity, whose simple behavior patterns enable a proper monitorization. *P. caudatum* cells preferentially chose the clay nanomaterials over GO, indicating that the cells are able to distinguish different compounds and avoid those which are more toxic. As a matter of fact, most performed studies involved *in vitro* models and concentrations up to 1 mg/mL, which are considered relatively low. However, this *in vivo* test applied a significantly higher concentration range up to 10 mg/mL, in order to understand which nanomaterials were safe to be used at an industrial level[19]. Both kaolin and HNTs did not show to be cytostatic at low concentrations. However, making an overall evaluation, HNTs were considered the

safest of the tested nanomaterials, demonstrating to be safe to *P. caudatum* cells with doses up to 10 mg/mL[19].

Fakhrullina *et al.* studied the *in vivo* toxicity of HNTs with *Caenorhabditis elegans* (*C. elegans)* as a model[18]. *C. elegans* is a microworm that naturally populate soils, whose behavior-related parameters facilitate the estimation of toxic effects, while, due to their transparence, possibly the visualization of HNTs distribution upon the application of increased dark-field microscopy. In high concentrations, HNTs irritated the intestinal cells of *C. elegans*, disturbing its ingestion and, consequently, its body length, even though the longevity of *C. elegans* was not affected. Thereby, the data indicated that HNTs triggered no severe toxic effects towards *C. elegans,* only causing irritation in the alimentary system. To overcome this drawback, authors proposed to coat the HNTs outer surface with pH-responsive polymers[18].

Toledano-Magaña *et al.* tested the effects of HNTs on macrophage cultures and on mice, performing *in vitro* and *in vivo* assays, respectively[73]. HNTs demonstrated to trigger only half of the toxic effects caused on macrophage cultures by other clay nanomaterials. Moreover, in comparison with multi-walled carbon nanotubes (MWNTs), which affected severely the viability in very low doses, HNTs showed to be far safer, only exhibiting minor effects when using thousand-times higher doses. At high doses, HNTs induced apoptosis on macrophages, which did not cause inflammation nor tissue damage. Besides the very interesting and promising results, further research is required to better understand the macrophages apoptotic mechanism. In addition, the diminished observed inflammation exerted on macrophages by HNTs was in accordance with the results from the *in vivo* test. The administration of HNTs in mice did not show increased body temperature or weight changes. Furthermore, renal and hepatobiliary functions did not suffered changes, whilst AST (aspartate transaminase), ALT (alanine transaminase), creatine and total bilirubin maintained their normal serum levels[73].

Overall, cytotoxic studies involving HNTs have pointed out for a low toxicity inherent to their use, providing a significant enlightenment on this issue, although further research is required to understand the toxic effect of HNTs on living organisms in higher detail[6]. This way, further *in vitro* and *in vivo* investigation is effectively warranted to assess the safety of the chronic use of HNTs[71].

7. Summary and future perspectives

In recent years, a great deal of effort has been put forth to the research of HNTs as a drug delivery system, especially regarding its functionalization and deployment. HNTs are tubular clay nanomaterials constituted by alumina and silica, which can be used for drug loading and sustained drug release[20]. The inherent lumen may be enlarged with an etching procedure, enabling HNTs to load not only small drug molecules, but also macromolecules[4, 28]. Furthermore, HNTs outer layer detains a great surface area and has shown to be appropriate to adsorb positively charged large molecules. Due to their unique and interesting structure, HNTs can be easily functionalized on both inner and outer layers to improve their drug delivery system properties, in order to enable for dual loading, specific targeting and/or control the drug release, and, ultimately, improve the implemented therapeutics[25, 26].

Given their inherent promising composition and architecture, HNTs have been successfully applied to develop drug delivery systems, specifically for small drugs molecules, enzymes and nucleic acids. Pristine HNTs are able to prolong drug release for several hours, and, upon functionalization, the release may be further extended to days, weeks and months[3, 6, 13]. Owing to such, extensive *in vitro* testing has proved the concept of potential advantage to use HNTs for the formulation of tablets, cosmetics, antibacterial sprays, bone cements, dental fillers and bioscaffolds[26]. Thanks to their zeta-potential, HNTs are highly stable as colloidal dispersions, which consists in a very promising feature in the submicron particles-based formulations field[45, 67]. Moreover, their excellent characteristics as compression excipients for tablet formulations makes them suitable for traditional dosage forms adapting to the classical pharmaceutical technology demands[14]. Regarding the more recent technologies, HNTs were demonstrated to improve mechanical properties of implants[51], and to be successfully incorporated in polymer matrices to comprise functional tissue scaffolds with sustained drug release and increased cell proliferation[57]. In addition to those pharmaceutical gains, this nanomaterial is vastly available at low cost, and is considered a friendly green nanotechnology and non-toxic in a wide concentration range, as proved with different cell cultures and animal models[4, 18]. Nevertheless, some issues remain yet to be solved and additional pharmaceutical applications require further research[4]. In fact, to the best of our knowledge, intravenous administration route is not allowed for HNTs administration since those are not biodegradable. In addition, the limited capability of drug loading and the absence of studies in human subjects also represent drawbacks for HNTs

use[26]. Facing these outcomes and challenges, the ultimate aim is to increase the use of HNTs in drug delivery systems for distinct administration routes, owing to their exhibited potential for a plethora of biomedical applications. Experts in this field believe that in a nearby future HNTs will be applied in cosmetic formulations, animal treatment, antimicrobial sprays and traditional oral formulations[26].

Referências Bibliográficas

1. Fizir, M., Dramou, P., Zhang, K., Sun ,C., Pham-Huy, C. and He, H. - *Polymer grafted-magnetic halloysite nanotube for controlled and sustained release of cationic drug.* Journal of Colloid and Interface Science, 2017. **505**: p. 476-488.

2. Torchilin, V.P. - *Multifunctional nanocarriers.* Advanced Drug Delivery Reviews 2006. **58**: p. 1532-1555.

3. De Villiers, M.M., Otto, D.P., Strydom, S.J. and Lvov, Y.M. - *Intoduction to nanocoatings produced by layer-by-layer (LBL) self-assembly.* Advanced Drug Delivery Reviews, 2011. **63**: p. 701-715.

4. Lazzara, G., Riela, S. and Fakhrullin, R.F. - *Clay-based drug delivery systems: What does the future hold?* NANO: Brief Reports and Reviews, 2007. **2**: p. 115-120.

5. Ruiz-Hitzky, E., Aranda, P., Darder, M. and Rytwo, G. - *Hybrid materials based on clays for environmental and biomedical applications.* Journal of Materials Chemistry, 2010: p. 9306-9321.

6. Lvov, Y., Wang, W., Zhang, L. and Fakhrullin, R. - *Halloysite Clay Nanotubes for Loading and Sustained Release of Functional Compounds.* Advanced Materials, 2016. **28**: p. 1227-1250.

7. Kurczewska, J., Pecyna, P., Ratajczak, M., Gajecka, M. and Schroeder, G. - *Halloysite nanotubes as carriers of Vancomycin in alginate-based wound dressing.* Saudi Pharmaceutical Journal, 2017.

8. Veerabadran, N.G., Price, R.R. and Lvov, Y.M. - *Clay Nanotubes for Encapsulation and Sustained Release of Drugs.* NANO: Brief Reports and Reviews 2007. **2**: p. 115-120.

9. Massaro, M., Riela, S., Lo Meo, P., Noto, R., Cavallaro, G., Milioto, S. and Lazzara, G. - *Functionalized halloysite multivalente glycocluster as a new drug delivery system.* Journal Materials Chemistry B 2014. **2**: p. 7732-7738.

10. Hu, Y., Chen, J., Li, X., Su, Y., Huang, S., Li, Y., Liu, H., Xu, J. and Zhong, S. - *Multifunctional Halloysite Nanotubes for Targeted Delivery and Controlled Release of Doxorubicin In-Vitro and In-Vivo Studies.* Nanotechonology, 2017: p. 1-40.

11. Dzamukova, M.R., Naumenko, E.A., Lvov, Y.M. and Fakhrullin, R.F. - *Enzyme-activated intracellular drug delivery with tubule clay nanoformulation.* Scientific Reports, 2015. **5**: p. 1-11.

12. Patel, S., Jammalamadaka, U., Sun, L., Tappa, K. and Mills, D.K. - *Sustained Release of Antibacterial Agents from Doped Halloysite Nanotubes.* Bioengineering 2016. **3**: p. 1-14.

13. Price, R.R., Gaber, B.P. and Lvov, Y. - *In-vitro release characteristics of tetracycline HCL, khellin and nicotinamide adenine dineculeotide from halloysite; a cylindrical mineral.* Journal of Microencapsulation 2001. **18**: p. 713-722.

14. Yendluri, R., Lvov, Y., Devilliers, M., Vinokurov, V., Naumenko, E., Tarasova, E. and Fakhurullin, R. - *Paclitaxel Encapsulated in Halloysite Clay Nanotubes for Intestinal and Intracellular Delivery.* Journal of Pharmaceutical Sciences 2017. **30**: p. 1-9.

15. Kerdsakundee, N., Li, W., Martins, J.P., Liu, Z., Zhang, F., Kemell, M., Correia, A., Ding, Y., Airavaara, M., Hirvonen, J., Wiwattanapatapee, R. and Santos, H.A. - *Multifunctional Nanotube–Mucoadhesive Poly(methyl vinyl ether-co-maleic acid)@Hydroxypropyl Methylcellulose Acetate Succinate Composite for Site-Specific Oral Drug Delivery.* Advanced Healthcare Materials 2017: p. 1-12.

16. Suh, Y.J., Kil, D.S., Chung, K.S., Abdullayev, E., Lvov, Y.M. and Mongayt, D. - *Natural Nanocontainer for the Controlled Delivery of Glycerol as a Moisturizing Agent.* Journal of Nanoscience and Nanotechnology 2011. **11**: p. 661-665.

17. Sandri, G., Aguzzi, C., Rossil, S., Bonferonil, MC., Bruni, G., Bosellil, C., Cornaglia, A.I., Riva, F., Viseras, C., Caramellal, C. and Ferraril, F. - *Halloysite and chitosan oligosaccharide nanocomposite for wound healing.* Acta Biomater 2017. **57**: p. 216-224.

18. Fakhrullina, G.I., Akhatova, F.S., Lvov, Y.M. and Fakhrullin, R.F. - *Toxicity of halloysite clay nanotubes in vivo: a Caenorhabditis elegans study.* Environmental Sciences: Nano 2015. **2**: p. 54-59.

19. Kryuchkova, M., Danilushkina, A., Lvov, Y. and Fakhrullin, R. - *Evaluation of toxicity of nanoclays and graphene oxide in vivo: a Paramecium caudatum study.* Environmental Sciences: Nano 2016. **3**: p. 442-452.

20. Riela, S., Massaro, M., Colleti, C.G., Bommarito, A., Giordano, C., Noto, R., Poma, P. and Lazzara, G. - *Development and characterization of co-loaded curcumin/triazole-halloysite systems and evaluation of their potential anticancer activity.* International Journal of Pharmaceutics 2014. **475**: p. 613-623.

21. Massaro, M., Amorati, R., Cavallaro, G., Guernelli, S., Lazzara, G., Milioto, S., Noto, R., Poma, P. and Riela, S. - *Direct chemical grafted curcumin on halloysite nanotubes as dual-responsive prodrug for pharmacological applications.* Colloids and Surfaces B: Biointerfaces, 2016. **140**: p. 505-513.

22. Tan, D., Yuan, P., Annabi-Bergaya, F., Liu, D., Wang, L., Liu, H. and He, H. - *Loading and in vitro release of ibuprofen in tubular halloysite.* Applied Clay Science 2014. **30**: p. 1-6.

23. Shi, Y., Tian, Z., Zhang, Y., Shen, H. and Jia, N. - *Functionalized halloysite nanotube-based for intracellular delivery of antisense oligonucleotides.* Nanoscale Research Letters, 2011. **6**: p. 1-7.

24. Yah, W.O., Xu, H., Soejima, H., Ma, W., Lvov, Y. and Takahara, A. - *Biomimetic Dopamine Derivative for Selective Polymer Modification of Halloysite Nanotube Lumen.* Journal of the American Chemical Society, 2012. **134**: p. 12134-12137.

25. Fan, L., Zhang, J. and Wang, A. - *In situ generation of sodium alginate/hydroxyapatite/halloysite nanotubes composite hydrogel beads as drug-controlled release matrices.* Journal Materials Chemistry B 2013. **45**: p. 6261-6270.

26. Lvov, Y.M., DeVilliers, M.M. and Fakhrullin, R.F. - *The application of halloysite tubule nanoclay in drug delivery.* Expert Opin. Drug. Deliv. , 2016. **13**: p. 977-986.

27. Massaro, M., Colletti, C.G., Noto, R., Riela, S., Poma, P., Guernelli, S., Parisi, F., Milioto, S. and Lazzara, G. - *Pharmaceutical properties of supramolecular assembly of co-loaded cardanol/triazole-halloysite systems.* International Journal of Pharmaceutics, 2015. **478**: p. 476-485.

28. Abdullayev, E., Joshi, A., Wei, W., Zhao, Y. and Lvov, Y. - *Enlargement of Halloysite Clay Nanotube Lumen by Selective Etching of Aluminum Oxide.* ACS Nano, 2012. **6**: p. 7216-7226.

29. Vergaro, V., Lvov, Y.M. and Leporatti, S. - *Halloysite Clay Nanotubes for Resveratrol Delivery to Cancer Cells.* Macromolecular Bioscience 2012. **12**: p. 1265-1271.

30. Dionisi, C., Hanafy, N., Nobile, C., De Giorgi, M.L., Rinaldi, R., Casciaro, S., Lvov, Y.M. and Leporatti, S. - *Halloysite Clay Nanotubes as Carriers for Curcumin: Characterization and Application.* Nanotechonology, 2016. **15**(5).

31. Lun, H., Ouyang, J., Yang, H. - *Natural halloysite nanotubes modified as an aspirin carrier.* The Royal Society of Chemistry Advances, 2014. **4**: p. 44197-44202.

32. Veerabadran, N.G., Mongayt, D., Torchilin, V., Price, R.R. and Lvov, Y.M. - *Organized Shells on Clay Nanotubes for Controlled Release of Macromolecules.* Macromol. Rapid Commun., 2009. **30**: p. 99-103.

33. Shemesh, R., Krepker, M., Natan, M., Danin-Poleg, Y., Banin, E., Kashi, Y., Nitzan, N., Vaxman, A. and Segal, E. - *Novel LDPE/Halloysite Nanotubes Composites with Sustained Carvacrol Release for Broad-Spectrum Antimicrobial Activity.* The Royal Society of Chemistry Advances, 2015. **5**: p. 87108-87117

34. Sun, J., Yendluri, R., Lui, K., Guo, Y., Lvov, Y. and Yan, X. - *Enzyme-immobilized clay nanotube-chitosan membranes with sustained biocatalytic activities.* Physical Chemistry Chemical Physics, 2017. **19**: p. 562-567.

35. Tully, J., Yendluri, R. and Lvov, Y. - *Halloysite Clay Nanotubes for Enzyme Immobilization.* Biomacromolecules, 2016. **17**: p. 615-621.

36. Kruif, J.K., Ledergerber, G., Garofalo, C., Fasler-Kan, E. and Kuentz, M. - *On prilled Nanotubes-in-Microgel Oral Systems for protein delivery.* European Journal of Pharmaceutics and Biopharmaceutics, 2016. **101**: p. 90-102.

37. Bugatti, V., Sorrentino, A. and Gorrasi, G. - *Encapsulation of Lysozyme into halloysite nanotubes and dispersion in PLA: structural and physical properties and controlled release analysis.* European Polymer Journal, 2017. **93**: p. 495-506.

38. Bugatti, V., Viscusi, G., Naddeo, C. and Gorrasi, G. - *Nanocomposites Based on PCL and Halloysite Nanotubes Filled with Lysozyme: Effect of Draw Ratio on the Physical Properties and Release Analysis.* Nanomaterials, 2017. **7**(213).

39. Massaro, M., Piana, S., Colletti, C.G., Noto, R., Riela, S., Baiamonte, C., Giordiano, C., Pizzolanti, G., Cavallaro, G., Milioto, S. and Lazzara, G. - *Multicavity halloysite-amphiphilic cyclodextrin hybrids for co-delivery of natural drugs into thyroid cancer cells.* Journal Materials Chemistry B, 2015. **3**: p. 4074-4081.

40. Cavallaro, G., Lazzara, G., Massaro, M., Milioto, S., Noto, R., Parisi, F. and Riela, S. - *Biocompatible Poly(N-isopropylacrylamide)-halloysite Nanotubes for Thermoresponsive Curcumin Release.* The Journal of Physical Chemistry, 2015. **119**(16): p. 8944-8951.

41. Shamsi, M.H. and Geckeler, K.E. - *The first biopolymer-wrapped non-carbon nanotubes.* Nanotechonology, 2008. **19**(075604): p. 1-5.

42. Lee, Y., Jun, G., Cho, S.J., Geckeler, K.E., Fuchs, H. - *Cellular interactions of doxorubicin-loaded DNA-modified halloysite nanotubes.* Nanoscale, 2013. **5**: p. 8577-8585.

43. Zhu, X., Li, H., Liu, H., Peng, W., Zhong, S. and Wang, Y. - *Halloysite-based dopamine-imprinted polymer for selective protein capture.* Journal of Separation Science, 2016. **39**(12): p. 2431-2437.

44. Yendluri, R., Otto, D.P., De Villiers, M.M. and Vinokurov, V. - *Application of halloysite clay nanotubes as a pharmaceutical excipient.* International Journal of Pharmaceutics, 2017. **521**: p. 267-273.

45. Riedlinger, M. and Corkery, R. - *Cosmetic skincare applications employing mineral-derived tubules for controlled release*, in *Google Patents*. 2007, Naturalnano, Inc.: United States.

46. Wei, W., Minullina, R., Abdullayev, E., Fakhrullin, R., Mills, D. and Lvov,Y. - *Enhanced Efficiency of Antiseptics with Sustained Release from Clay Nanotubes.* RSC Advances, 2014. **4**: p. 488-494.

47. Zhang, Y., Gao, R., Liu, M., Shi, B., Shan, A. and Cheng, B. - *Use of modified halloysite nanotubes in the feed reduces the toxic effects of zearalenone on sow reproduction and piglet development.* Theriogenology, 2015. **83**: p. 932-941.

48. Gao, R., Meng, Q., Li, J., Liu, M., Zhang, Y., Bi, C. and Shan, A. - *Modified halloysite nanotubes reduce the toxic effects of zearalenone in gestating sows on growth and muscle development of their offsprings.* Journal of Animal Science and Biotechnology, 2016. **7**(14).

49. Wei, W., Abdullayev, E., Hollister, A., Mills, D. and Lvov, Y.M. - *Clay Nanotube/Poly(methyl methacrylate) Bone Cement Composites with Sustained Antibiotic Release.* Macromolecular Materials and Engineering, 2012. **297**: p. 645-653.

50. Feitosa, S.A., Münchow, E.A., Al-Zain, A.O., Kamocki, K., Platt, J.A. and Bottino, M.C. - *Synthesis and characterization of novel halloysite-incorporated adhesive resins.* Journal of Dentistry, 2015. **43**: p. 1316-1322.

51. Degrazia, F.W., Leitune, V.C.B., Takimi, A.S., Collares, F.M. and Sauro, S. - *Physiochemical and bioactive properties of innovative resin-based materials containing functional halloysite-nanotubes fillers.* Dental Materials, 2016. **32**(9): p. 1133-1143.

52. Mills, D. and Lvov, Y.M. - *Ceramic nanotube composites with sustained drug release capability for implants, bone repair and regenaration*, in *Google Patents*. 2015, Lousiana Tech University Research Foundation; a Division of Louisiana Tech University Foundation: United States.

53. Bottino, M.C., Batarseh, G., Palasuk, J., Alkatheeri, M.S., Windsor, L.J. and Platt, J.A. - *Nanotube-modified dentin adhesive - Physiochemical and dentin bonding characterizations.* Dental Materials, 2013. **29**: p. 1158-1165.

54. Abdallah, R.M. - *Evaluation of polymethyl methacrylate resin mechanical properties with incorporated halloysite nanotubes.* The Journal of Advanced Prosthodontics, 2016. **8**: p. 167-171.

55. Jammalamadaka, U., Tappa, Karthik, T., Weisman, J.A., Nicholson, J.C. and Mills, D.K. - *Effect of barium-coated halloysite nanotube addition on the cytocompatibility, mechanical and contrast properties of poly(methyl methacrylate) cement.* Nanotechonology, Science and Applicantions, 2017. **10**: p. 105-114.

56. Alkatheeri, M.S., Palasuk, J., Eckert, G.J., Platt, J.A. and Bottino, M.C. - *Halloysite nanotube incorporation into adhesive systems - effect on bond strenght to human dentin.* Clinical Oral Investigations, 2015. **19**: p. 1905-1912.

57. Fakhrullin, R.F. and Lvov, Y.M. - *Halloysite clay nanotubes for tissue engineering.* Nanomedicine, 2016. **11**(17): p. 2243-2246.

58. Naumenko, E.A., Guryanov, I.D., Yendluri, R., Lvov, Y.M. and Fakhrullin, R.F. - *Clay nanotube - biopolymer composite scaffolds for tissue engineering.* Nanoscale, 2016. **8**(13): p. 7257-7271.

59. Liu, M., Wu, C., Jiao, Y., Xiong, S. and Zhou, C. - *Chitosan-halloysite nanotubes nanocomposite scaffolds for tissue engineering.* Journal Materials Chemistry B, 2013. **1**: p. 2078-2089.

60. Huang, B., Liu, M., Long, Z., Shen, Y. and Zhou, C. - *Effects of halloysite nanotubes on physical properties and cytocompability of alginate composite hydrogels.* Materials Science and Engineering C, 2017. **70**: p. 303-310.

61. Afshar, H.A. and Ghaee, A. - *Preparation of Aminated chitosan/alginate scaffold containing halloysite nanotubes with improved cell attachment.* Carbohydrate polymers, 2016. **151**: p. 1120-1131.

62. Bonifacio, M.A., Gentile, P., Ferreira, A.M., Cometa, S. and Giglio, E.D. - *Insight Into Halloysite Nanotubes-Loaded Gellan Gum Hydrogels For Soft Tissue Engineering Applications.* Carbohydrate polymers, 2017. **163**: p. 280-291.

63. Torres, E., Fombuena, V., Vallés-Lluch, A. and Ellingham, T. - *Improvement of mechanical and biological properties of Polycaprolactone loaded with Hydroxyapatite and Halloysite Nanotubes.* Materials Science and Engineering C, Materials for biological Applications, 2017. **75**: p. 418-424.

64. Jing, X., Mi, H. and Turng, L. - *Comparison between PCL/hydroxyapatite (HA) and PCL/halloysite nanotube (HNT) composite scaffolds prepared by co-extrusion and gas foaming.* Materials Science and Engineering C, 2017. **72**: p. 53-61.

65. Xue, J., Niu, Y., Gong, M., Shi, R., Chen, D., Zhang, L. and Lvov, Y. - *Electrospun Microfiber Membranes Embedded with Drug-Loaded Clay Nanotubes for Sustained Antimicrobial Protection.* ACS Nano, 2015. **9**(2): p. 1600-1612.

66. Ji, L., Qiao, W., Zhang, Y., Wu, H., Miao, S., Cheng, Z., Gong, Q., Liang, J. and Zhu, A. - *A gelatin composite scaffold strengthened by drug-loaded halloysite nanotubes.* Materials Science and Engineering C, 2017. **78**: p. 362-369.

67. Cavallaro, G., Lazzara, G. and Milioto, S. - *Exploiting the Colloidal Stability and Solubilization Ability of Clay Nanotubes/Ionic Surfactant Hybrid Nanomaterials.* The Journal of Physical Chemistry C, 2012. **116**(41): p. 21932-21938.

68. Kelly, H.M., Deasy, P.B., Ziaka, E. and Claffey, N. - *Formulation and preliminary in vivo dog studies of a novel drug delivery system for the treatment of periodontitis.* International Journal of Pharmaceutics, 2004. **274**: p. 167-183.

69. Vergaro, V., Abdullayev, E., Lvov, Y.M., Zeitoun, A., Cingolani, R., Rinaldi, R. and Leporatti, S. - *Cytocompability and Uptake of Halloysite Clay Nanotubes.* Biomacromolecules, 2010. **11**: p. 820-826.

70. Ahmed, F.R., Shoaib, M.H., Azhar, M., Um, S.H., Yousuf, R.I., Hashmi, S. and Dar, A. - *In-vitro assessment of cytotoxicity of halloysite nanotubes agaisnt HepG2, HCT116 and human peripheral blood lymphocytes.* Colloids and Surfaces B: Biointerfaces, 2015. **135**: p. 50-55.

71. Lai, X., Agarwal, M., Lvov, Y.M., Pachpande, C., Varahramyan, K. and Witzmann, F.A. - *Proteomic profiling of halloysite clay nanotube exposure in intestinal cell co-culture.* Journal of Applied Toxicology, 2013. **33**(11): p. 1316-1329.

72. Liu, H., Du, L., Zhao, YT. and Tian, WQ. - *In Vitro Hemocompability and Cytotoxicity Evaluation of Halloysite Nanotubes for Biomedical Application.* Journal of Nanomaterials, 2015: p. 1-9.

73. Toledano-Magaña, Y., Flores-Santos, L., Oca, G.M., González-Montiel, A., Jiménez-Durán, K., Ramos, J.C.G., Mora, C., Saavedra-Ávila, N., Laclette, J.P., Gudiño-Zayas, M. and Carrero, J.C. - *Toxicity of Functionalized and Non Functionalized Halloysite Clay Nanotubes.* Toxicology Research, 2016.