Nanosystèmes de délivrance pour l'administration orale de principes actifs : Microparticules polymères et microémulsions contenant des molécules anti-inflammatoires et anti infectieux

Acarilia Eduardo da Silva

UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE
Departamento de Farmácia
Laboratório de Sistemas Dispersos (LASID)

ÉCOLE DOCTORALE ED425
Du Fondamental à l'Appliqué

UNIVERSITE PARIS SUD
Faculté de Pharmacie
Centre d'Études Pharmaceutiques
UMR CNRS 8612

Numéro de série: 1220

THÈSE DE DOCTORAT SUR TRAVAUX

soutenue le 05/04/2013

par

Acarilia EDUARDO DA SILVA

Nanotechnological delivery systems for the oral administration of active molecules : Polymeric microparticles and microemulsions applied to anti-inflammatory and anti-infectious drugs

Directeurs de thèse :

Gillian BARRATT Directeur de recherche CNRS (UMR 8612, Châtenay-Malabry)

Eryvaldo SÓCRATES EGITO Professeur (UFRN)

Composition du jury :

Président du jury : Patrick SAULNIER Professeur (Université d'Angers)

Rapporteurs : Socorro ESPUELAS Professeur (Université de Navarre, Espagne)

Philippe LEGRAND Professeur (Université de Montpellier I)

"The only reason for time is so that everything doesn't happen at once."
Albert Einstein

ABSTRACT

This thesis was devoted to the development of innovative oral delivery systems for two different molecules. In the first part, microparticles (MPs) based on xylan and Eudragit® S-100 were produced and used to encapsulate 5-aminosalicylic acid for colon delivery. Xylan was extracted from corn cobs and characterized in terms of its physicochemical, rheological and toxicological properties. The polymeric MPs were prepared by interfacial cross-linking polymerization and spray-drying and characterized for their morphology, mean size and distribution, thermal stability, crystallinity, entrapment efficiency and *in vitro* drug release. MPs with suitable physical characteristics and satisfactory yields were prepared by both methods, although the spray-dried systems showed higher thermal stability. In general, spray-dried MPs would be preferable systems due to their thermal stability and absence of toxic agents used in their preparation. However, drug loading and release need to be optimized. In the second part of this thesis, oil-in-water microemulsions (O/W MEs) based on medium-chain triglycerides were formulated as drug carriers and solubility enhancers for amphotericin B (AmB). Phase diagrams were constructed using surfactant blends with hydrophilic-lipophilic balance values between 9.7 and 14.4. The drug-free and drug-loaded MEs presented spherical non-aggregated droplets around 80 and 120 nm, respectively, and a low polydispersity index. The incorporation of AmB was high and depended on the volume fraction of the disperse phase. These MEs did not reduce the viability of J774.A1 macrophage-like cells for concentrations up to 25 μg/mL of AmB. Therefore, O/W MEs based on propylene glycol esters of caprylic acid may be considered as suitable delivery systems for AmB.

Keywords: polymeric microparticles – lipid systems – colon delivery – oral administration – xylan – 5-aminosalicylic acid – amphotericin B.

RESUMO

Esta tese teve como objetivo o desenvolvimento de novos sistemas de liberação para duas moléculas distintas. Na primeira parte, micropartículas à base de xilana e Eudragit® S-100 foram produzidas para encapsular ácido 5-aminosalicílico visando à liberação cólon-específica. A xilana foi extraída de sabugos de milho e caracterizada quanto às suas propriedades físico-químicas, reológicas e toxicológicas. Em seguida, dois métodos de microencapsulação foram utilizados: reticulação interfacial polimérica e secagem por aspersão. Os sistemas produzidos foram caracterizados quanto à morfologia, tamanho médio e distribuição, estabilidade térmica, cristalinidade, taxa de encapsulação e liberação do fármaco *in vitro*. Foram obtidas micropartículas com adequadas características físicas e rendimentos satisfatórios através dos dois métodos, embora os sistemas aspergidos tenham apresentado maior estabilidade térmica e sejam considerados mais interessantes devido a sua maior estabilidade térmica e ausência de agentes tóxicos. No entanto, ajustes precisam ser feitos para melhorar a encapsulação e liberação do fármaco. Na segunda parte, microemulsões do tipo óleo em água (MEs O/A) com base em triglicerídeos de cadeia média (MCT) foram produzidas visando ao carreamento de anfotericina B (AmB) e aumento da sua solubilidade. Foram obtidas MEs O/A sem e com AmB com gotículas em torno de 80 e 120 nm, respectivamente, e índices de polidispersão de 0,25 e 0,31, respectivamente. A taxa de incorporação da AmB foi alta e dependente do volume da fase dispersa. A viabilidade celular não foi afetada até 25 μg/mL da AmB. Portanto, MEs O/A a partir de MCT podem ser promissores sistemas de liberação para AmB.

Palavras-chaves: micropartículas poliméricas – sistemas lipídicos – liberação colônica – administração oral – xilana – ácido 5-aminosalicílico – anfotericina B

RESUMÉ

Des systèmes efficaces de délivrance de médicaments ont été le sujet de la recherche pharmaceutique depuis plusieurs décennies. Le développement de systèmes qui transportent et libèrent une molécule active rapidement à une cible spécifique a été étudié de façon exhaustive, en tenant compte des facteurs complexes qui régissent l'efficacité d'un médicament. Ces facteurs comprennent les propriétés pharmaceutiques inhérentes de la molécule, telles que sa spécificité d'action, et les propriétés physico-chimiques telles que son poids moléculaire, sa composition chimique, sa solubilité globale dans les fluides corporels, sa pénétration dans les tissus et l'absorption par les cellules. En outre, le succès thérapeutique d'un médicament dépend également des processus biologiques qui se produisent après son administration, c'est-à-dire: l'absorption, la distribution, le métabolisme et l'élimination. Comme les médicaments ne sont que rarement des substances endogènes participant à l'homéostasie de l'organisme, la pharmacocinétique d'un médicament n'est pas nécessairement optimale par rapport à son action pharmacologique. Par conséquent, ce manque de sélectivité conduit parfois à des effets indésirables. Afin d'assurer une sécurité et une efficacité, les médicaments doivent être delivrés à leur site d'action sélectivement à une vitesse optimale qui permettrait le contrôle de leur profil de biodistribution. Afin d'y arriver, le système de distribution lui-même doit présenter une sélectivité et une spécificité vers les tissus ou cellules cibles.

L'utilisation de la nanotechnologie pour le développement de médicaments est d'une importance capitale en raison de la possibilité de surmonter un grand nombre de défis concernant non seulement la conception de nouveaux médicaments et des systèmes de délivrance, mais aussi d'améliorer les conditions d'utilisation des médicaments existants. Parmi les inconvénients rencontrés lors du développement pharmaceutique des ces systèmes, on retrouve une faible solubilité des médicaments, une biodisponibilité insuffisante, une instabilité *in vivo*, une absorption intestinale limitée, une efficacité thérapeutique modeste, des effets secondaires et des fluctuations plasmatiques de la concentration du médicament qui tendent à être soit au-dessous des concentrations minimales efficaces soit au-dessus des concentrations thérapeutiques de sécurité.

Les vecteurs de médicaments basés sur le concept de nanotechnologie ont un grand potentiel pour la reformulation de médicaments classiques dont l'utilisation est limitée par leurs profils biopharmaceutiques et du profil pharmacocinétiques. Ces nanovecteurs peuvent augmenter la solubilité, la biodisponibilité et, par conséquent réduire les effets secondaires et améliorer l'observance du traitement par le patient. En outre, les candidats

médicaments qui ont échoué pendant les phases d'essai peuvent devenir éligibles si délivrés sous forme d'un système nanométrique. Aussi, en fonction de leur composition, des propriétés physico-chimiques et du comportement biopharmaceutique, les nanovecteurs peuvent être conçus pour différentes voies, telles que les voies orale, parentérale, topique, nasale, etc..

Bien que chaque voie d'administration présente ses propres avantages, la voie orale est largement connue comme la plus pratique pour le traitement des maladies chroniques. Cependant, l'administration de 50% des médicaments par cette voie est limitée en raison de leur lipophilie qui limite leur solubilisation dans le milieu intestinal. Presque 40% des nouveaux médicaments candidats présentent une faible solubilité dans l'eau conduisant à une faible biodisponibilité par voie orale, une forte variabilité intra- et inter-sujets et un manque de proportionnalité de dose.

La délivrance colique est une des méthodes efficaces pour éviter l'absorption et/ou la dégradation du médicament au sein de l'environnement du tractus gastro-intestinal supérieur (TGS). L'objectif principal de la thérapie médicamenteuse des maladies inflammatoires de l'intestin (MII) est de réduire l'inflammation du côlon, mais cela nécessite la prise fréquente de médicaments anti-inflammatoires à des doses très élevées. Bien que ces médicaments soient très efficaces, ils sont absorbés très rapidement dans le TGS et habituellement ils ne parviennent pas à atteindre le côlon, entraînant des effets nocifs importants. Par conséquent, les microparticules à base du xylane semblent être des vecteurs de médicaments anti-inflammatoires prometteurs pour le traitement des MII, vu que ce polymère est exclusivement dégradé par des enzymes produit par les bactéries dans le côlon.

Récemment, les systèmes lipidiques de distribution de médicaments ont été largement considérés comme une approche appropriée pour l'amélioration de la biodisponibilité orale des médicaments peu solubles dans l'eau. Ces systèmes ont l'avantage de présenter le médicament sous forme de solution liquide stable, et, par conséquent, la molécule active demeure en solution tout au long du tractus gastro-intestinal.

En plus, l'absorption des médicaments lipophiles peut être améliorée par plusieurs autres mécanismes, tels que a) une meilleure dissolution et solubilisation grâce aux sécrétions biliaires et pancréatiques par la stimulation de la contraction vésiculaire par la présence de lipides dans la préparation; b) un temps de résidence gastrique prolongé grâce au retard de la vidange gastrique provoqué par les lipides présents dans le tractus gastro-

intestinal; c) l'amélioration de la perméabilité intestinale grâce à la modification de la barrière physique de l'intestin par certains lipides.

Cette thèse a été consacrée à la mise au point de deux systèmes d'administration destinés à la voie orale, pour deux molécules différentes.

Dans la première partie, des microparticules (MPs) à base de xylane et d'Eudragit® S-100 ont été produites pour encapsuler l'acide 5-aminosalicylique et permettre son absorption au niveau du colon. Le xylane a été extrait à partir de rafles de maïs et caractérisé selon ses propriétés physico-chimiques, rhéologiques et toxicologiques. Par la suite, les MPs ont été préparées soit par réticulation interfaciale, soit par séchage par atomisation et caracterisés quant à leur morphologie, leur taille moyenne et leur distribution, leur stabilité thermique, leur cristallinité, leur efficacité et leur profil de libération du médicament *in vitro*. Des MPs de caractéristiques physiques appropriées avec des rendements satisfaisants ont été préparées par ces deux méthodes, bien que les systèmes séchés par pulvérisation aient montré une plus grande stabilité thermique. En général, ces derniers systèmes étaient plus prometteurs en raison de leur stabilité thermique et de l'absence d'agents réticulants toxiques. Toutefois, la méthodologie doit être optimiser afin d'améliorer le chargement de principe actif ainsi que sa libération.

Dans la deuxième partie de cette thèse, des microémulsions huile-dans-l'eau (ME H/E) à base de triglycérides à chaîne moyenne ont été preparées afin de vectoriser et d'augmenter la solubilité de l'amphotéricine B (AmB). Des diagrammes de phases ont été construits en utilisant des mélanges de tensioactifs dont les valeurs de la balance hydrophile-lipophile variaient entre 9,7 et 14,4. Les MEs H/E sans et avec AmB étaient composées de gouttelettes sphériques non agrégées de diamètre moyen autour de 80 et 120 nm, respectivement, et avec une faible polydispersité. L'incorporation de l'AmB était élevée et dépendait de la fraction volumique de la phase dispersée. La viabilité des cellules J774.A1 n'était pas diminuée par l'exposition aux concentrations d'AmB encapsulée allant jusqu'à 25µg/mL. Par conséquent, les MEs H/E à base d'esters de propylèneglycol et d'acide caprylique peuvent être considéré comme des vecteurs adaptés pour l'AmB.

AGRADECIMENTOS

Como um fotógrafo que resolve partir em viagem para desbravar novas paisagens e capturar novas imagens, eu também me lancei em um novo projeto de tese e, além de estudar micropartículas poliméricas para liberação colônica de antiinflamatórios, estudei e desenvolvi microemulsões para o carreamento de anfotericina B. Para isso, deixei minha família, meu namorado, meus amigos e minha cidade para lidar não só com um novo tema de estudo mas também com um país e uma cultura diferentes.

Se eu fosse fotógrafa por profissão, diria que essa tese é a minha foto mais bonita. Ela teve como objeto principal vários elementos, dentre eles a busca por conhecimento, a motivação pelo desconhecido, a curiosidade das descobertas, a ansiedade pelos bons resultados, o desespero inicial frente a um obstáculo, seguido pelo conforto do aprendizado e a certeza do fortalecimento da minha segurança, autonomia e autoconfiança.

Além desses elementos abstratos, mas imensamente importantes e úteis na composição desta fotografia, outros objetos, estes concretos, firmes e genuínos, tiveram papel essencial na harmonia da foto: foram os amigos que encontrei nessa jornada e aqueles que permaneceram ao meu lado mesmo à distância.

Serei eternamente grata à minha querida amiga companheira de *bureau,* de *labô*, de *restô,* de 194, de saldos, de compras, Rachel Oliveira, a quem primeiro encontrei na Université Paris Sud XI e que sempre tive a alegria de reencontrar no dia seguinte no ambiente de trabalho e fora dele também. Ainda em Châtenay-Malabry sempre tive o sorriso e a cumplicidade das colegas de laboratório e de coração verde e amarelo Thaís Leite, Letícia Santiago e Flaviana Ribeiro, além dos meus colegas de equipe, Félix Sauvage, Hahn Pham, Cédrik Koffi, Serena Lombardi, Stéphanie Louguet, Emeline Servais, Juliette Vergnaud, Laurence Moine, Iuliana Popa, Stéphanie Denis, e doutorandos, Sabrina Valetti, Laura de Miguel, Chantal Sabbagh, Alice Gaudin, Bénédicte Pradines, Giovana Giacalone, Nadège Gabrowski, Valentina Agostoni. Felizmente, também tive grandes amigos colegas de residência e de corações estrangeiros, Ingrid Arriaga, Regina Lumbach, Johan Gustafsson, Niko Ro, Sophie Berner, Laura Gaya, Paola Rensi, Isaline Bard, Britta Labunh, Naoaki Kunishiro, Kara Hermez, Ariel Gutman, Solange Molu, Alexis Crawshaw que estavam sempre curiosos e atentos a ouvir sobre os últimos experimentos e frustrações e sucessos e que me fizeram me sentir em casa, mesmo estando na "Casa dos Estudantes Suecos" em Paris! Na Casa do Brasil, fui feliz em conhecer Josy Teixeira, Marcos Sosa, Andrea Betania, Reginaldo Carvalho, Vanda Ponte, Vitor Assis e Kaline, com quem pude compartilhar alguns passeios pela cidade e muitas risadas. No

Brasil, sempre tive o apoio moral, científico e filosófico (!) incondicional e imprescindível de Gyselle Holanda, a rápida e eficiente colaboração de Rosilene, Karol, Scheyla e Miguel e a palavra segura e amiga do meu eterno chefe, Elquio Eleamen. Também sou muito grata ao meu chefe mais antigo, Toshiyuki, que contribuiu enormemente com minha atual bagagem científica e dos meus fieis colegas de equipe, Monique, Larissa e Henrique. Na companhia de Amanda Brun e sua bela família, encontrei um abrigo quente e aconchegante em Paris.

Todos esses amigos participaram como cores vibrantes devidamente equilibradas e sem filtros que compuseram essa bela e edificante experiência que foi o doutorado em cotutela entre a Universidade Federal do Rio Grande do Norte e a Université Paris Sud XI. Na primeira, segui o exemplo do entusiasmo, do gosto pelo desafio e do espírito de vitória do meu orientador Prof. Sócrates Egito. Na segunda, encontrei a liberdade e a autonomia para exercer minha aptidão científica sem obstáculos através da orientação da Profª Gillian Barratt. Durante todo o meu doutorado, tive a honra e a felicidade de encontrar professores como Prof. Elias Fattal, Prof. Patrick Couvreur, Prof. Vincent Faivre, Profª. Claire Gueutin, Profª Monique Chéron, Prof. Nicolas Huang, Profª Vanessa Lievin Le Moal, Prof. Alejandro Ayala e sua aluna Gardênia de Sousa, que serviram como exemplos e me guiaram em determinados momentos com decisivas opiniões. Também preciso agradecer à Profª Socorro Espuelas, Prof. Philippe Legrand e ao Prof. Patrick Saulnier por aceitarem avaliar esta tese contribuindo com correções e observações pertinentes e enriquecedoras a este trabalho.

Agradeço à Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) que com sua visão especial e auxílio financeiro à Ciência concedeu a mim e tantos outros alunos a possibilidade de dedicação total aos estudos. Agradeço também à Pró-reitoria de Pós-graduação da UFRN, ao Programa de Pós-graduação em Ciências da Saúde, em especial à Profª Ivonete Araújo, e seus funcionários por me permitirem realizar meu estágio doutoral no exterior, sendo sempre muito prestativos quando precisei de autorização e determinados documentos.

Por fim, não poderia deixar de agradecer àqueles que foram e tem sido meu estável tripé: meus sábios pais, meus queridos irmãos e meu estimado amigo, Neto. Com eles a regra dos terços não funciona e sou gratidão, admiração e amor por inteiro a eles.

A todos,

Muito obrigada!

ACKNOWLEDGEMENTS

As a photographer who decides to brave traveling from new landscapes and capture new images, I also launched out a new thesis subject. Besides studying and developing polymeric microparticles for colonic release of inflammatory drugs in Brazil, I came to France to develop on microemulsions for the oral administration of amphotericin B, a very effective drug for the treatment of leishmaniasis, but very toxic and substantially insoluble in water. For that purpose, I left my family, my boyfriend, my friends and my city to deal not only with a new subject of study but also with a country and a different culture.

If I was a photographer by profession, I would say that this thesis is my most beautiful photo. It had several elements as main object, such as the search for knowledge, the motivation for the unknown, the curiosity of new findings, the anxiety for the good results, the initial despair in front of an obstacle, followed by the comfort and assurance of learning how to strengthen my autonomy and self-confidence.

In addition to those abstract elements, but immensely important and useful in the composition of this photograph, other objects, now firm, concrete and genuine played an important role in the harmony of this photo: the friends I met on this journey and those who stayed by my side even at a distance.

I will be forever grateful to my dear friend and workmate, Rachel Oliveira, whom I first met at the Université Paris Sud XI and I always had the joy of meeting again the next day in the workplace and outside it as well. Also in Châtenay-Malabry I could always find the smile and complicity of my green-and-yellow-hearted labmates Thais Leite, Leticia Santiago and Flaviana Ribeiro, besides my teammates, Felix Sauvage, Hahn Pham, Cédrik Koffi, Serena Lombardi, Stéphanie Louguet, Emeline Servais, Juliette Vergnaud, Laurence Moine, Iuliana Popa, Stéphanie Denis, and PhD students, Sabrina Valetti, Laura de Miguel, Chantal Sabbagh, Alice Guadin, Bénédicte Pradines, Giovana Giacalone, Nadège Gabrowski, Valentina Agostoni. Fortunately, I also had great foreign-hearted friends and colleagues of residence, Ingrid Arriaga, Regina Lumbach, Johan Gustafsson, Niko Ro, Sophie Berner, Laura Gaya, Paola Rensi, Isaline Bard, Britta Labunh, Naoaki Kunishiro, Kara Hermez, Ariel Gutman, Solange Molu, Alexis Crawshaw who were always curious and attentive to hear about the latest experiments and frustrations and successes and that made me feel at home, even though we lived at the warm and sweet "Swedish House" in Paris! In the Brazilian House, I was happy to meet Josy Teixeira, Marcos Sosa, Andrea Betania, Reginaldo Carvalho, Vanda Ponte, Vitor Assis and Kaline, with whom I shared

some city tours and lots of laughs. In Brazil, I always had the unconditional and indispensable moral, scientific and philosophical (!) support from Gyselle Holanda, the rapid and efficient collaboration from Rosilene, Karol, Scheyla and Miguel, and the safe word of my eternal boss and dear friend, Elquio Eleamen. I am also very grateful to my oldest boss, Toshiyuki, which contributed greatly to my current scientific background, as well as my loyal colleagues, Monique, Larissa and Henrique. At Amanda Brun's I definitely found a warm place to be in Paris with her and her beautiful family.

All these friends took part as vibrant colors properly balanced and with no filters, which composed this beautiful and uplifting experience that was the double doctorate between the Universidade Federal do Rio Grande do Norte and Université Paris Sud XI. In Brazil, I followed the example of the enthusiasm, the passion for challenge and the spirit of victory of my advisor Prof. Socrates Egito. In France, I found the freedom and autonomy to pursue my scientific aptitude unhindered through the guidance of Prof. Gillian Barratt. Throughout my PhD studies, I had the honour and happiness of finding professors such as Elias Fattal, Patrick Couvreur, Vincent Faivre, Claire Gueutin, Monique Chéron, Nicolas Huang, Vanessa Lievin Le Moal, Alejandro Ayala and his student Gardenia de Sousa, who served as examples and guided me in certain moments with decisive opinions. I also need to thank Prof. Socorro Espuelas, Prof. Philippe Legrand and Prof. Patrick Saulnier for accepting to evaluate this thesis contributing with corrections and relevant comments to enrich this work.

I thank the Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) which with its vision and special financial aid to science, provided me and many other students with the possibility of total dedication to their studies. I am also thankful for Pró-reitoria de Pós-graduação da UFRN, Programa de Pós-graduação em Ciências da Saúde and staff, in particular to Prof Ivonete Araújo for allowing me to perform my doctoral stage abroad, being always very helpful when I needed certain documents and authorization.

Finally, I would not forget to thank those who were and have been as a stable tripod in my life: my wise parents, Geraldo Eduardo e Dorinha, my dear sister and brothers, Acarízia, Acarílton and Acarírton, and my dear friend, Neto. With them the rule of thirds does not work and I am all gratitude, admiration and love for all of them.

Thank you all very much!

Acarilia

TABLE OF CONTENTS

TABLE OF FIGURES

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

CHAPTER VI

LIST OF TABLES

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER VI

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Targeted drug delivery

In the late Middle Age, when Paracelsus (1493-1541), a physician-alchemist, made his famous statement "All the substances are poisons; there is none that is not a poison. The right dose differentiates a poison from a remedy", he laid the groundwork for the later development of the modern toxicology by prompting the importance of the dose-response relationship (1). Many of his revolutionary views remain an integral part of toxicology, pharmacology and therapeutics, and this concept is considered as the basis for pharmaceutical therapy (2).

After Paracelsus's death in Salzburg, Austria, the field of chemistry advanced very slowly and two centuries later it could not yet be ranked as a science (3). However, a further century later, with the support of biologists, physiologists and pathologists, chemistry became a science again and important discoveries had a great impact on medicine. For instance, by using some Paracelsian concepts on the use of chemicals as therapeutical agents, Paul Erlich (1854-1915) made several contributions to the biochemistry and medicinal chemistry (3). He introduced the therapeutic index and the idea of the "magic bullet", which are directly connected to the importance of the dose, the incidence of toxic effects and the benefits of targeting drugs to their specific site of action. Therefore, the concept of drug targeting or site-specific drug delivery is attributed to Paul Ehrlich and proof of concept was given in 1909 when he and his colleagues synthesised the first man-made antibiotic, which after chemical modifications in its molecule had affinity for pathogens and act as "magic bullets" without affecting the host's cells (4).

While Paul Ehrlich fulfilled his goal of targeting a drug to its specific site of action by performing chemical modifications in the structure of the molecule, the selective delivery of molecules may also be achieved by the design and development of drug carriers with specific properties.

Effective drug delivery systems have been the focus of the pharmaceutical research field for several decades. The development of carriers that are able to transport and release an active molecule promptly to a specific target of interest has been exhaustively investigated, taking into account the complex factors that govern the efficacy of a drug. These factors include inherent drug properties, such as the specificity of action of the molecule itself, and physicochemical properties such as its molecular size, chemical composition, overall solubility in body fluids, penetration within tissues and uptake by cells. In addition, the

therapeutic success of a drug is also dependent on the biological processes occurring after its administration, that is: absorption, distribution, metabolism, and excretion (5). Since drugs are not usually endogenous substances taking part in maintaining homeostasis of the body, the pharmacokinetics of a drug is not necessarily optimal with respect to its pharmacological actions. As a consequence, the lack of selectivity in biodistribution sometimes leads to undesirable side effects (6). In order to ensure safety and efficacy, drugs are required to be delivered to their target site selectively at an optimal rate that allows the control of their biodistribution profile. Hence, the delivery system itself is supposed to present optimal selectivity and specificity towards the target tissues or cells. A number of techniques intended for targeted drug delivery have been proposed and developed so far.

Since the experiments performed by Ehrlich, the concept of drug targeting has greatly evolved and several approaches have been proposed. They range from relatively simple concepts to complex strategies involving the design of new materials with highly controlled properties (5). Such approaches are generally classified into four major categories, including passive, inverse, active and combined targeting.

Briefly, passive targeting relies on the accumulation of drugs in particular tissues of the body, for instance, in organs of the reticuloendothelial system, specially the liver and the spleen. Thus, drugs intended to treat pathological conditions in those organs are preferably accumulated in these sites passively. The enhanced permeability and retention (EPR) effect is another example of passive targeting associated with tumor and inflammatory vasculature. On the other hand, the inverse targeting is aimed at blocking the sites where drugs passively accumulate in order to provide their accumulation in other sites of interest. The active targeting comprises the design of drugs or drug carriers with specific features displaying affinity and, consequently, binding to a particular target. Finally, the combined targeting refers to the drug targeting enabled by the combination of more than one of the categories previously mentioned (5).

Nanotechnology in drug delivery systems

A great contribution to the field of drug targeting and drug delivery was possible with the breakthrough of nanotechnology. It comprises the design, characterization, synthesis and application of materials, structures, devices and systems by controlling shape and size at the nanometer scale. A large variety of subjects benefit from the advantages of nanotechnology, such as electronics, environment, metrology, energy, security, robotics, healthcare,

information technology, biomimetics, pharmaceuticals, manufacturing, agriculture, construction, transport, and food processing and storage (7).

In the pharmaceutical research, such discipline is of great interest due to the fact that an increase in the surface area and dominance of quantum effects provide changes in important properties of matter. Consequently, materials exhibit unique properties at nanoscale of 1 to 100 nanometer (nm). The quantum effects at nanoscale determine a material's magnetic, thermal, optical and electrical properties. Besides, it is generally expected that products at the nanoscale will be more cost effective due to the smaller quantity of materials required (7).

The use of nanotechnology in drug delivery is of utmost importance due to the potential to overcome a large number of challenges concerning not only the design of new drugs and delivery systems, but also the optimization of old drugs. Some of the drawbacks encountered by pharmaceutical development of most drug delivery systems are poor drug solubility, bioavailability, *in vivo* stability, intestinal absorption, sustained and targeted delivery to site of action, therapeutic effectiveness, side effects, and plasma fluctuations of drug concentration that tend to be either below the minimum effective concentrations or above the safe therapeutic concentrations (7).

Novel drug carriers based on the nanotechnology concept have a great potential for the reformulation of classical drugs whose use is restricted due to limitations in their biopharmaceutical and pharmacokinetic profile. Those nanocarriers can increase solubility, bioavailability and, as a consequence, reduce side effects and improve patient compliance. Additionally, drug candidates which have failed during trial phases may become eligible when delivered by a nanotechnological delivery system (8).

Additionally, depending on their composition, physicochemical properties and biopharmaceutical behavior, nanocarriers may be designed and applied to several routes of administration, such as oral, parenteral, topical, nasal, among others (9).

Although each route of administration has its own advantages, the oral route is widely known as the most convenient one for the treatment of chronic conditions. However, the delivery of 50% of drugs by this route is limited because of the high lipophilicity of the drugs themselves and nearly 40% of new drug candidates present low solubility in water leading to poor bioavailability and high intra- and inter-subject variability and lack of dose proportionality (10).

Polymeric delivery systems

Polymers are extremely versatile materials with applications in a large number of fields including packaging, engineering, coatings, textile, electronics and also pharmaceutical industry. Natural polymers generally exhibit low or no toxicity, low immunogenicity and, therefore, good biocompatibility, they have been preferably used in drug delivery systems (11).

Polymeric systems are able to carry and release drugs in various mechanisms depending on inherent properties of the polymer. Stimulus-responsive polymers have been used to produce not only externally regulated systems, which require an external stimuli to trigger the drug: magnetic, ultrasonic, thermal, or electric; but also self-regulated devices, from which the release rate is controlled by feedback information, without any external intervention, for instance, pH-sensitive polymers, enzyme-substrate reactions, pH-sensitive drug solubility, competitive binding and metal concentration-dependent hydrolysis (12).

Xylan is a polymer largely found in nature as the second most abundant polysaccharide present in plant cell wall of hardwoods and cereals (13). Because of its ability to remain intact in the physiological stomach environment and small intestine, xylan is believed to be a suitable raw material for the medical field, especially as a constituent of colon-specific drug carriers. They would protect the active molecule from early degradation to be released after enzymatic degradation of xylan by xylanases in the human colon (14-16).

Colonic delivery is one of the useful approaches to avoid absorption and/or degradation of the drug in the environment of upper gastrointestinal tract (GIT). In practice, the primary goal of drug therapy for inflammatory bowel diseases (IBD) is to reduce inflammation in the colon; however, this requires frequent intake of anti-inflammatory drugs at higher doses (17). Although these drugs are very effective in IBD, they are absorbed quite quickly in the upper gastrointestinal tract (GIT) and they usually fail to reach the colon, leading to significant adverse effects (18). Therefore, xylan-based microparticles seem to be promising drug carriers for antiinflammatory drugs for the treatment of IBD.

The first section of this thesis comprises the studies performed at Laboratório de Sistemas Dispersos (LASID), located at Universidade Federal do Rio Grande do Norte in Natal, RN, Brazil. In chapter one, both the theoretical framework of the research on the development of polymeric microparticles for colon delivery and experimental results are presented in the chapter titled “Xylan, a promising hemicellulose for pharmaceutical use” of the book “Products and Applications of Biopolymers” published by InTech. In chapter two,

the article “Influence of the lipophilic external phase composition on the preparation and characterization of xylan microcapsules: A Technical Note”, published in the journal AAPS PharmSciTech, describes the optimization of the production of xylan microcapsules by interfacial cross-linking polymerization regarding different oil phases. Chapter three concerns the article entitled “Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization” published in the journal “Bioresource Technology” and refers to the extraction and physicochemical and rheological characterization of xylan. Finally, in the fourth chapter entitled “Producing hemicellulose-based microparticles using chemical and physico-mechanical approaches as carriers for 5-aminosalicylic acid” accepted for publication in “Journal of Microencapsulation” the production of xylan-based microparticles by two techniques of microencapsulation is discussed.

Lipid-based formulations

Lipid-based formulations are regarded as a diverse group of formulations resulting from the blending of up to five classes of excipients; ranging from pure triglyceride oils, through mixed glycerides, lipophilic surfactants, hydrophilic surfactants and water-soluble cosolvents (19).

In recent years, lipid-based drug delivery systems have been widely considered as a suitable approach for improving the oral bioavailability of poorly water-soluble drugs (20). Such systems have the advantage of presenting the drug as a stable liquid solution, and, consequently, the active molecule is believed to remain in solution throughout its period in the gastrointestinal tract (21). Additionally, the absorption of lipophilic drugs would be enhanced due to several other mechanisms, such as a) improved dissolution and solubilization due to the biliary and pancreatic secretions by the stimulation of the gallbladder contractions because of the presence of the lipids in the formulation; b) longer gastric residence time because of a delay in gastric emptying owning to the lipids in the gastrointestinal tract; c) improved intestinal permeability after the change of the physical barrier of the gut by a variety of lipids, among others (22).

Although oral lipid-based formulations have been on the market for over 2 decades and currently comprise 2-4% of the commercially available drug products surveyed in 3 markets worldwide (23-25), there are a large number of oral lipid-based formulations under development and investigation. The options for such formulations include a) liquid lipid-formulations as a single emulsion or in the form of a self-micro-emulsifying drug delivery

system, self-nanoemulsified drug delivery system, microemulsions (MEs), nanoemulsions; b) solid lipid-based formulations as a multiparticulate system (powder, granules or pellets filled into sachets or capsules); and c) coloidal drug carriers, such as liposomes and nanoparticles (26).

MEs have emerged as novel vehicles for drug delivery allowing sustained or controlled release by several administration routes, such as oral, transdermal, topical, nasal, intravenous, ocular, parenteral and others. They have also been considered as a practical delivery platform for improving target specificity, therapeutic activity, and reducing toxicity of drugs (27).

MEs are thermodynamically stable nanoscale dispersions of water and an apolar phase, stabilized by a surfactant, usually in conjunction with a co-surfactant and/or short chain alcohol, resulting in mainly oil-in-water (O/W) and water-in-oil (W/O) formulations. In addition, MEs are characterized by certain properties such as spontaneous formation, clear appearance, high surface area, very low interfacial tension, small domain size (5–100 nm), and high solubilization capacity (28). Due to the inherent properties of MEs previously mentioned, they are considered to be a promising delivery system for amphotericin B (AmB).

AmB is an antifungal macrolide antibiotic whose efficacy in the treatment of visceral leishmaniasis has been attributed to its capacity of binding to the main sterol existing in *Leishmania* species, ergosterol. The AmB molecules interact with the cell bilayer and form transmembrane pores that modify the permeability of cations, glucose and water, resulting in cell lysis (29, 30). Nonetheless, because AmB also shows affinity with cholesterol present in membranes of mammalian cells, it exhibits high toxicity. This fact is explained by the insertion of aggregate forms of AmB into cell membranes which leads to the formation of pores through which important ions such as magnesium and potassium are leaked from the cell, causing renal tubular acidosis and nephrotoxicity (31, 32).

In addition to its high toxicity, low solubility in water, poor membrane permeability and instability at the low pH found in gastric fluid also seriously limit the oral bioavailability of AmB (33). Parenteral formulations have been developed in order to overcome the low solubility of AmB. However, they are very expensive and require, hospitalization during parenteral infusion, which also leads to a number of acute side effects (34). Therefore, oral lipid formulations of AmB have been considered as an attractive approach to improve its use by increasing its solubility and bioavailability and, consequently, to reduce its toxicity and enhance patient compliance.

The second section of this thesis addresses the research accomplished at Institut Galien Paris Sud, located at Université Paris Sud XI, in Châtenay Malabry, France. In chapter five, a literature review entitled "Challenges and recent advances on the delivery of poorly soluble drugs: An update on the development of carriers for amphotericin B", submitted to "Expert Opinion on Drug Delivery" provides an overview of publications from the latest ten years, with relevant findings in the pharmaceutical technology field, especially on the development of delivery systems for AmB. The chapter six is devoted to the paper titled "Development and characterization of O/W microemulsions for the delivery of amphotericin B for the treatment of leishmaniasis", submitted to "International Journal of Pharmaceutics" and describes the experimental work carried out for the development of AmB-loaded microemulsions and their characterization involving physicochemical and *in vitro* studies.

References

1. Gallo MA. History and scope of toxicology. In: Shanahan J, Naglieri C, editors. Casarett & Doull's Essentials of Toxicology. 2 ed: The McGraw Hill Companies; 2010. p. 1-4.
2. Hodgson E. Introduction to toxicology. In: Hodgson E, editor. A textbook of modern toxicology. 3 ed. Hoboken, NJ: John Wiley & Sons; 2004. p. 3-12.
3. Hajdu SI. Two pioneering chemists, three hundred years apart. Annals of Clinical and Laboratory Science. 2005;35(1):105-7.
4. Bosch F, Rosich L. The contributions of Paul Ehrlich to Pharmacology: A tribute on the occasion of the centenary of his Nobel prize. Pharmacology. 2008;82(3):171-9.
5. Muro S. Challenges in design and characterization of ligand-targeted drug delivery systems. Journal of Controlled Release. 2012;164(2):125-37.
6. Yamashita F, Hashida M. Pharmacokinetic considerations for targeted drug delivery. Advanced Drug Delivery Reviews. 2012 (In Press).
7. Ochekpe NA, Olorunfemi PO, Ngwuluka NC. Nanotechnology and drug delivery Part 1: Background and applications. Tropical Journal of Pharmaceutical Research. 2009;8(3):265-74.
8. Hughes GA. Nanostructure-mediated drug delivery. Nanomedicine: Nanotechnology, Biology and Medicine. 2005;1(1):22-30.
9. Heurtault B, Saulnier P, Pech B, Proust J-E, Benoit J-P. Physico-chemical stability of colloidal lipid particles. Biomaterials. 2003;24(23):4283-300.
10. Neslihan Gursoy R, Benita S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomedicine & Pharmacotherapy. 2004;58(3):173-82.
11. Rodrigues A, Emeje M. Recent applications of starch derivatives in nanodrug delivery. Carbohydrate Polymers. 2012;87(2):987-94.
12. Kost J, Langer R. Responsive polymeric delivery systems. Advanced Drug Delivery Reviews. 2012;64, Supplement(0):327-41.
13. Petzold-Welcke K, Schwikal K, Daus S, Heinze T. Xylan derivatives and their application potential Mini-review of own results. Carbohydrate Polymers. 2012;In Press, Corrected Proof.
14. Oliveira EE, Silva AE, Nagashima Jr T, Gomes MCS, Aguiar LM, Marcelino HR, et al. Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization. Bioresource Technology. 2010;101(14):5402-6.
15. Rubinstein A. Approaches and opportunities in colon-specific drug delivery. Critical reviews in therapeutic drug carrier systems. 1995;12(2-3):101-49.
16. Silva AKA, da Silva EL, Oliveira EE, Nagashima JT, Soares LAL, Medeiros AC, et al. Synthesis and characterization of xylan-coated magnetite microparticles. International Journal of Pharmaceutics. 2007;334(1-2):42-7.
17. Collnot E-M, Ali H, Lehr C-M. Nano- and microparticulate drug carriers for targeting of the inflamed intestinal mucosa. Journal of Controlled Release. 2012;161(2):235-46.
18. Mura C, Nácher A, Merino V, Merino-Sanjuán M, Manconi M, Loy G, et al. Design, characterization and in vitro evaluation of 5-aminosalicylic acid loaded N-succinyl-chitosan microparticles for colon specific delivery. Colloids and Surfaces B: Biointerfaces. 2012;94(0):199-205.
19. Colin W P. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and self-microemulsifying drug delivery systems. European Journal of Pharmaceutical Sciences. 2000;11, Supplement 2(0):S93-S8.

20. Han S-f, Yao T-t, Zhang X-x, Gan L, Zhu C, Yu H-z, et al. Lipid-based formulations to enhance oral bioavailability of the poorly water-soluble drug anethol trithione: Effects of lipid composition and formulation. International Journal of Pharmaceutics. 2009;379(1):18-24.
21. Pouton CW. Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. European Journal of Pharmaceutical Sciences. 2006;29(3-4):278-87.
22. Dahan A, Hoffman A. Rationalizing the selection of oral lipid based drug delivery systems by an in vitro dynamic lipolysis model for improved oral bioavailability of poorly water soluble drugs. Journal of Controlled Release. 2008;129(1):1-10.
23. Strickley R. Solubilizing excipients in oral and injectable formulations. Pharmaceutical Research. 2004;21(2):201-30.
24. Hauss DJ. Oral lipid-based formulations. Advanced Drug Delivery Reviews. 2007;59(7):667-76.
25. Strickley RG. Currently marketed oral lipid-based dosage forms: Drug products and excipients. Oral Lipid-Based Formulations2007. p. 1-32.
26. Chakraborty S, Shukla D, Mishra B, Singh S. Lipid - An emerging platform for oral delivery of drugs with poor bioavailability. European Journal of Pharmaceutics and Biopharmaceutics. 2009;73(1):1-15.
27. Fanun M. Microemulsions as delivery systems. Current Opinion in Colloid & Interface Science. 2012;17(5):306-13.
28. Rousseau D, Rafanan RR, Yada R. Microemulsions as nanoscale delivery systems. In: Murray M-Y, editor. Comprehensive Biotechnology. 2 ed. Burlington: Academic Press; 2011. p. 675-82.
29. Hartsel S, Bolard J. Amphotericin B: New life for an old drug. Trends in Pharmacological Sciences. 1996;17(12):445-9.
30. Rosenthal E, Delaunay P, Jeandel PY, Haas H, Pomares-Estran C, Marty P. Le traitement de la leishmaniose viscérale en Europe en 2009. Place de l'amphotéricine B liposomale. Médecine et Maladies Infectieuses. 2009;39(10):741-4.
31. Hillery AM. Supramolecular lipidic drug delivery systems: From laboratory to clinic A review of the recently introduced commercial liposomal and lipid-based formulations of amphotericin B. Advanced Drug Delivery Reviews. 1997;24(2-3):345-63.
32. Walker RJ, Endre ZH. Cellular mechanisms of drug nephrotoxicity. Seldin and Giebisch's The Kidney (Fourth Edition). San Diego: Academic Press; 2008. p. 2507-35.
33. Wasan EK, Bartlett K, Gershkovich P, Sivak O, Banno B, Wong Z, et al. Development and characterization of oral lipid-based Amphotericin B formulations with enhanced drug solubility, stability and antifungal activity in rats infected with Aspergillus fumigatus or Candida albicans. International Journal of Pharmaceutics. 2009;372(1-2):76-84.
34. Ibrahim F, Gershkovich P, Sivak O, Wasan EK, Wasan KM. Assessment of novel oral lipid-based formulations of amphotericin B using an in vitro lipolysis model. European Journal of Pharmaceutical Sciences. 2012;46(5):323-8.

SECTION I

POLYMERIC MICROPARTICLES

CHAPTER I

Book chapter: "Xylan, a promising hemicellulose for pharmaceutical use

Le premier chapitre de cette thèse est consacré au chapitre de livre intitulé «Xylan, a promising hemicellulose for pharmaceutical use ». Il a été publié le 7 Mars 2012 par InTech comme le chapitre quatre du livre intitulé «Produits et applications des biopolymères». Ce livre comporte dix chapitres et présente deux aspects des biopolymères, les produits potentiels et certaines applications de ces matériaux. Il s'agit d'un livre en libre accès qui peut être trouvé à http://www.intechopen.com/books/products-and-applications-of-biopolymers. Ce chapitre a été le deuxième plus téléchargé de ce livre (avec 659 téléchargements et 786 vues) dans les 11 mois suivant sa publication.

Le chapitre comprend cinq parties et contient l'ensemble des résultats et observations qui ont été réalisés sur le xylane et les vecteurs de médicaments basés sur ce polymère au cours de ma recherche scientifique au LASID-UFRN, au Brésil, sous la supervision du Professeur Sócrates Egito.

La première partie est une introduction générale qui résume l'importance des polymères et des microparticules polymère comme vecteurs de médicaments et qui expose l'objectif de ce chapitre.

La deuxième partie est intitulée « Xylan » et est divisée en deux sous-sections. La première sous-section - Sources, extraction et structure - se concentre sur les sources naturelles, les procédés d'extraction et de stratégies pour la modification structurelle de ce biopolymère prometteur. La deuxième sous-section est intitulée «Characterization of corn cob xylan» et concerne les caractéristiques physico-chimiques, les propriétés rhéologiques et le comportement thermique du xylane.

La troisième partie – « Xylan microparticles » – traite de l'utilisation du xylane comme matière première pour la production de microparticules comme système de délivrance colique de certains médicaments, tels que le diclofénac de sodium, l'acide 5-aminosalicilique et l'acide usnique. La délivrance colique de médicament est un mécanisme important pour éviter des effets secondaires et traiter efficacement des maladies inflammatoires de l'intestin. Il existe plusieurs techniques capables de produire des microcapsules et des microsphères polymère. Par exemple, les microparticules de xylane sont généralement produites par coacervation, réticulation interfaciale et séchage par atomisation. Ces différentes méthodes sont capables de former de nombreuses structures différentes telles que des microparticules sphériques ou oblongues avec des surfaces lisses ou rugueuses. Les différentes propriétés influent sur la libération du médicament.

Les biopolymères ont été étudiés et utilisés en nanotechnologie du fait de leur biocompatibilité et leur biodégradabilité. Afin de s'assurer de la biocompatibilité de

matériaux biomédicaux vis-à-vis de l'environnement cellulaire, des études de cytotoxicité sont essentielles lors du développement de vecteurs de médicaments. Notre équipe de recherche a mis au point une méthodologie d'extraction pour obtenir du xylane de rafles de maïs. Bien que les biopolymères soient considérés comme non-toxique et biocompatible, les résidus du processus d'extraction peuvent entraîner des problèmes de toxicité. Pendant le développement de produits pharmaceutiques, l'effet toxique de biomatériaux sur les cellules est considéré comme l'un des problèmes les plus importants à évaluer. Par exemple, la mort cellulaire, la prolifération cellulaire, la morphologie cellulaire et l'adhérence cellulaire sont des paramètres qui devraient être évalués in vitro. La perte de la viabilité pourrait indiquer un biomatériau toxique in vivo. La cytotoxicité du xylane et des microparticules a été évaluée par contact avec des cellules humaines et les résultats ont montré un effet limité sur la viabilité et la prolifération de ces cellules. Ces résultats sont exposés et discutés dans la quatrième partie de ce chapitre – « Biocompatibility of xylan and its products ».

La cinquième et dernière partie de ce chapitre contient des observations finales et des perspectives d'avenir concernant l'utilisation du xylane dans le développement de produits biomédicaux.

4

Xylan, a Promising Hemicellulose for Pharmaceutical Use

Acarília Eduardo da Silva[1], Henrique Rodrigues Marcelino[1], Monique Christine Salgado Gomes[1], Elquio Eleamen Oliveira[2], Toshiyuki Nagashima Jr[3] and Eryvaldo Sócrates Tabosa Egito[1]

[1]Universidade Federal do Rio Grande do Norte
[2]Universidade Estadual da Paraíba
[3]Universidade Federal de Campina Grande
Brazil

1. Introduction

Polymers are versatile materials with wide use in several industry fields, such as engineering, textile, automobile, packaging and biomedical. In the pharmaceutical industry, both natural and synthetic polymers have been largely used with different applications for the development and production of cosmetics and traditional dosage forms and novel drug delivery systems. For instance, a number of polymers are used as fillers, lubricants, disintegrants, binders, glidants, solubilizers, and stabilizers in tablets, capsules, creams, suspensions or solutions. Additionally, biodegradable and bioadhesive polymers may play an important role in the development of novel drug delivery systems, especially for controlled drug release.

Polymeric microparticles have been studied and developed for several years. Their contribution in the pharmacy field is of utmost importance in order to improve the efficiency of oral delivery of drugs. As drug carriers, polymer-based microparticles may avoid the early degradation of active molecules in undesirable sites of the gastrointestinal tract, mask unpleasant taste of drugs, reduce doses and side effects and improve bioavailability. Also, they allow the production of site-specific drug targeting, which consists of a suitable approach for the delivery of active molecules into desired tissues or cells in order to increase their efficiency.
Lately, the concern with environment and sustainability has been rising progressively and renewable sources of materials have been increasingly explored.

2. Xylan

For thousands of years, nature has provided humankind with a large variety of materials for the most diversified applications for its survival, such as food, energy, medicinal products, protection and defense tools, and others. The pharmaceutical industry has benefitted from such diversity of biomaterials and has exploited the use of natural products as sources of both drugs and excipients. One example of a promising biomaterial for pharmaceutical use is xylan, a hemicellulose largely found in nature, being considered the second most abundant polysaccharide after cellulose.

Xylan has drawn considerable interest due to its potential for packaging films and coating food, as well as for its use in biomedical products (Li et al., 2011). Because it is referred to as a corn fiber gum with a sticky behavior, xylan has been used as an adhesive, thickener, and additive to plastics. It increases their stretch and breaking resistance as well as their susceptibility to biodegradation (Ünlu et al., 2009). Xylan has also been studied because of its significant mitogenic and comitogenic properties, which enable it to be compared to the commercial immunomodulator Zymosan (Ebringerova et al., 1995). Another interesting application for xylan may be found in the food industry as an emulsifier and protein foam stabilizer during heating (Ebringerova et al., 1995). Previous papers have investigated the suitable use of xylan in papermaking (Ebringerova et al., 1994) and textile printing (Hromadkova et al., 1999). In the drug delivery field, xylan extracted from birch wood has been used for the production of nanoparticles after structural modification by the addition of different ester moieties, namely those with furoate and pyroglutamate functions (Heinze et al., 2007). On the other hand, the esterification of xylan from beech wood via activation of the carboxylic acid with N,N″-carbonyldiimidazole has been carried out in order to produce prodrugs for ibuprofen release (Daus & Heinze, 2010).

Egito and colleagues have been working for over a decade on the extraction of xylan from corn cobs and its use for the development of microparticles as drug carriers for colon-specific delivery of anti-inflammatory and toxic drugs, such as sodium diclofenac (SD), 5-aminosalycilic acid (5-ASA), and usnic acid (UA). Xylan-coated microparticles have also been developed by Egito and co-workers in order to deliver magnetite particles (Silva et al., 2007). Different microencapsulation techniques have been used for the production of xylan-based microparticles. Coacervation, interfacial cross-linking polymerization, and spray-drying have been shown to be the most successful methodologies for that purpose (Garcia et al., 2001; Nagashima et al., 2008).

Xylan degradation occurs by the action of hydrolytic enzymes named xylanases and β-xylosidases. Those enzymes are produced by a number of organisms, such as bacteria, algae, fungi, protozoa, gastropods, and arthropods (Kulkarni et al., 1999). The degradation of xylan in ruminants has been well reported, while some human intestinal bacteria have been investigated for their ability to produce xylan-polymer degrading enzymes. Among those intestinal species able to degrade complex carbohydrates, lactobacilli, bacteroides, and non-pathogenic clostridia have demonstrated that ability (Grootaert et al., 2007). Because of the presence of those bacteria in the human colon whether by induction of prebiotics or not, it is believed that xylan is a promising polymer for the composition of biodegradable drug carriers for colonic delivery. They would be able to undergo the upper gastrointestinal tract mostly intact, being degraded by xylanases when reaching the colon.

Additionally, corn cobs correspond to an abundant and low-cost renewable material in several countries worldwide and their recycling plays a very important role in the reduction of waste products. Consequently, such approach would lead to a relevant increase in the sustainability of agriculture around the world.

2.1 Sources, extraction, and structure

Hemicelluloses are the second most abundant polysaccharides in nature after cellulose. They occur in close association with cellulose and lignin and contribute to the rigidity of plant cell walls in lignified tissues. Hemicelluloses constitute about 20–30% of the total mass of annual and perennial plants and have a heterogeneous composition of various sugar units, depending on the type of plant and extraction process, being classified as xylans (β-1,4-linked D-xylose units), mannans (β-1,4-linked D-mannose units), arabinans (α-1,5-linked L-arabinose units), and galactans (β-1,3-linked D-galactose units) (Figure 1) (Belgacem & Gandini, 2008).

Xylans are the main hemicelluloses in hardwood and they also predominate in annual plants and cereals making up to 30% of the cell wall material and one of the major constituents (25–35%) of lignocellulosic materials. The most potential sources of xylans include many agricultural crops such as straw, sorghum, sugar cane, corn stalks and cobs, and hulls and husks from starch production, as well as forest and pulping waste products from hardwoods and softwoods (Ebringerova & Heinze, 2000; Kayserilioglu et al., 2003).

The structural diversity and complexity of xylans are shown to depend on the botanic source. Various suitable extraction procedures for the isolation of xylans from different plant sources are described and compared in the literature. It is suggested that certain structural types of xylans, such as glucuronoxylan, arabinoglucuronoxylan, and arabinoxylan, can be prepared from certain plant sources with similar chemical and physical properties. Its general structure has a linear backbone consisting of 1,4-linked D-xylopyranose residues, a reducing sugar with five carbon atoms. These may be substituted with branches containing acetyl, arabinosyl, and ıronosyl residues, depending on the botanic source and method of extraction (Den Haan n Zyl, 2003; Habibi & Vignon, 2005).

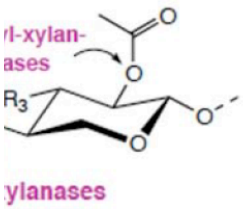


Fig. 1. Chemical structure of xylan (Shallom & Shoham, 2003).

A frequently used classification is based on the degree of substitution and types of side groups for characterization (Ebringerová, 2005; Sedlmeyer, 2011):

a. Homoxylans are linear polysaccharides common in some seaweeds.
b. Glucuronoxylans can be partly acetylated and have units substituted with α-(1→2)-4-O-methyl-D-glucopyranosyl uronic acid (MeGlcUA). They are found in hardwood, depending on the treatment.
c. (Arabino)glucuronoxylans have a substitution with α-(1→3)-L-arabinofuranosyl (ArbF) next to MeGlcUA. They are typical for softwoods.
d. Arabinoxylans with a substitution of the β-(1→4)-D-xylopyranose backbone at position 2 or 3 with ArbF can be esterified partly with phenolic acids. This type is frequently found in the starchy endosperm and the outer layers of cereal grains.
e. (Glucurono)arabinoxylans can be disubstituted with ArbF units, acetylated, and esterified with ferulic acid. This form is typical of lignified tissues of grasses and cereals.
f. Heteroxylans are heavily substituted with various mono- or oligosaccharides and are present in cereal bran, seed, and gum exudates.

Investigation of the xylan structure by various researchers is necessary. The use of xylan as a raw material is directly related to its structure. There is an interest in the application of the xylan polymer in the paper, pharmaceutical, cosmetic, biofuel and food industries. Several medical applications are cited in the literature. The films based on xylan show low oxygen permeability and thus have a potential application in the food packaging and pharmaceutical areas. Numerous studies use the xylan polymer as a specific substrate for xylanases. Besides that, xylan can be hydrolyzed into xylose and subsequently be converted into ethanol (Ebringerova & Heinze, 2000; Ebringerova & Hromadkova, 1999; Ebringerova et al., 1998; Garcia et al., 2000; Kayserilioglu et al., 2003; Oliveira et al., 2010; Sedlmeyer, 2011; Yang et al., 2005).

Previous studies on the corn cob xylan revealed the existence of at least two structurally different components. One is a low-branched arabinoglucuronoxylan, which is mostly water-insoluble (wis-X), and the second is a highly branched, water-soluble heteroxylan (ws-X), which possesses significant mitogenic and comitogenic activities (Ebringerova et al., 1995). The ws-X could be useful also as a food additive because of its emulsifying activity and ability to stabilize protein foam during heating. The wis-X has the ability to remain intact in the physiological stomach environment and small intestine. This property, together with the presence of xylanases (a group of enzymes which degrade the xylan) in the human colon, makes this polymer a suitable raw material for the medical field, especially as a constituent of colon-specific drug carriers (Oliveira et al., 2010; Rubinstein, 1995; Silva et al., 2007).

The most common method to extract xylan is the alkaline extraction. Several pretreatment methods can be used in association in order to break the covalent bonds that exist between xylan and other carbohydrates during the extraction (Wang & Zhang, 2006). A number of articles studied the use of ultrasound on the xylan extraction. Hromadkova and coworkers reported that 36.1% of xylan was extracted from corn cobs with 5% NaOH solution at 60˚C for 10 min of ultrasonication in comparison with 31.5% of xylan in the classical extraction. Both extractive methods yielded xylan with immunogenic properties (Hromadkova et al., 1999).

Wang and Zhang also investigated the effects on the xylan extracted from corn cobs enhanced by ultrasound at various lab-scale conditions. Results showed that the optimization conditions of xylan extraction should be carried out using (i) 1.8 M NaOH, (ii) corn cobs to NaOH solution ratio of 1:25 (w/w), (iii) sonication at 200 W ultrasound power for 30 min at 5 min intervals, and (iv) 60 °C (Wang & Zhang, 2006).

The process of the alkaline extraction of xylan from corn cobs was studied by Egito and colleagues (Unpublished data). The methodology applied in this work consisted of milling The corn cobs and separating the powder into different sizes. After that, the dried corn cobs were dispersed in water under stirring for 24h. The sample was treated with 1.3% (v/v) sodium hypochlorite solution in order to remove impurities. Then, an alkaline extraction was carried out by using NaOH solution. The bulk was neutralized with acetic acid, and xylan was extracted by settling down after methanol addition. Afterwards, several washing steps were performed by using methanol and isopropanol. Finally, the sample was filtered and dried at 50°C.

The efficiency of extraction was observed to be inversely proportional to the corn cob particle size. This was expected because the size reduction corresponds to an increase in total particle surface area. An increase in the time of the alkaline extraction and in the NaOH concentration also improves the efficiency of xylan extraction. This happened because when the NaOH concentration was lower, the xylan present in corn cobs could not be fully dissolved in the solution. Thus, it resulted in lower efficiency of xylan extraction. However, when the NaOH concentration was higher than 2 M, the yields decreased with continuously increasing of the NaOH concentration. This is probably due to the alkaline degradation of xylan chains, proceeding at the higher NaOH concentration, which indicated that the ideal NaOH concentration in the extraction was between 1.5 and 1.8 M (Unpublished data).

2.2 Characterization of corn cob xylan

Comprehensive physicochemical characterization of any raw material is a crucial and multi-phased requirement for the selection and validation of that matter as a constituent of a product or part of the product development process (Morris et al., 1998). Such demand is especially important in the pharmaceutical industry because of the presence of several compounds assembled in a formulation, such as active substances and excipients, which highlights the importance of compatibility among them. Besides, variations in raw materials due to different sources, periods of extraction and various environmental factors may lead to failures in production and/or in the dosage form performance (Morris et al., 1998). Additionally, economic issues are also related to the need for investigating the physicochemical characteristics of raw materials since those features may determine the most adequate and low-cost material for specific procedures and dosage forms.

After the extractive process described by Oliveira and colleagues, corn cob xylan appears to be an off-white fine powder with limited flowability. The xylan powder consists of a mixture of aggregated and non-aggregated particles with irregular morphology, a spherical shape, and a rough surface, as could be observed through the scanning electron microscopy (SEM) (Figure 2) (Oliveira et al., 2010).

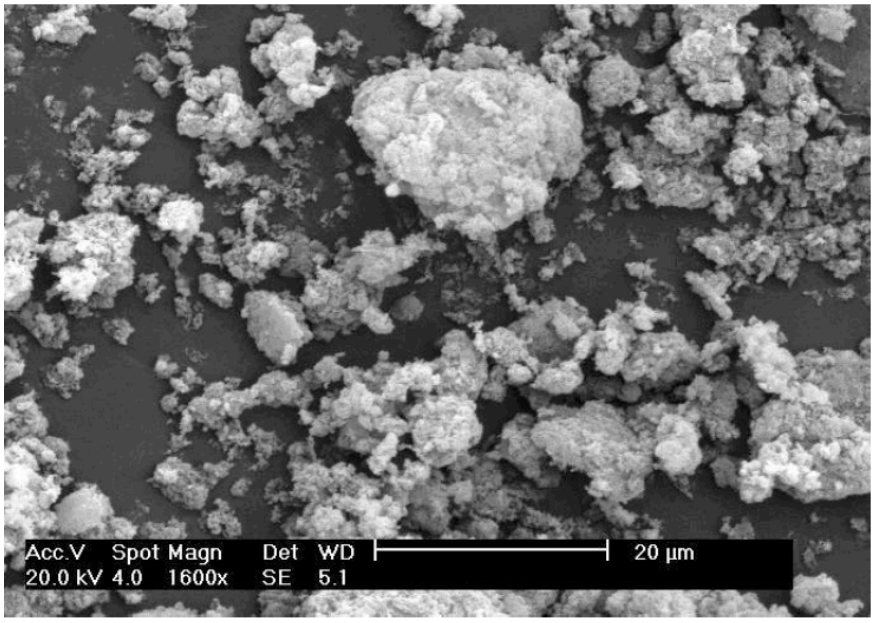


Fig. 2. SEM image of xylan powder after extraction from corn cobs (Oliveira et al., 2010).

y laser diffraction. It was observed act of xylan was smaller than 65.39 nile the mean particle size of xylan igure 3).

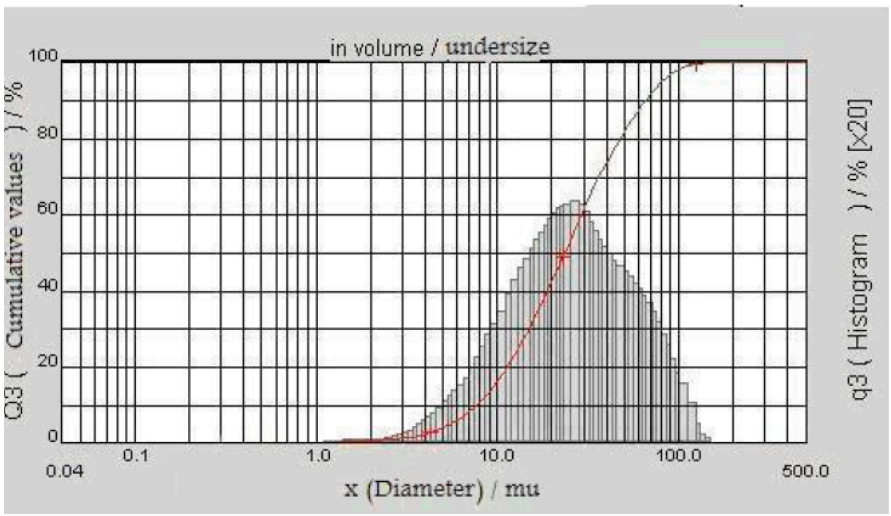


Fig. 3. Particle size distribution of xylan powder after extraction from corn cobs (Oliveira et al., 2010).

As a consequence of the irregular and rough structure of the xylan particles, entanglements between particles are promoted and this fact may explain the poor flow properties of this polymer (Kumar et al., 2002; Nunthanid et al., 2004). Additionally, rheological parameters of xylan powder have also been studied, such as bulk and tapped densities, Hausner ratio, Carr's index, and angle of repose values, and they are summarized in Table 1.

romising Hemicellulose for Pharmaceutical Use

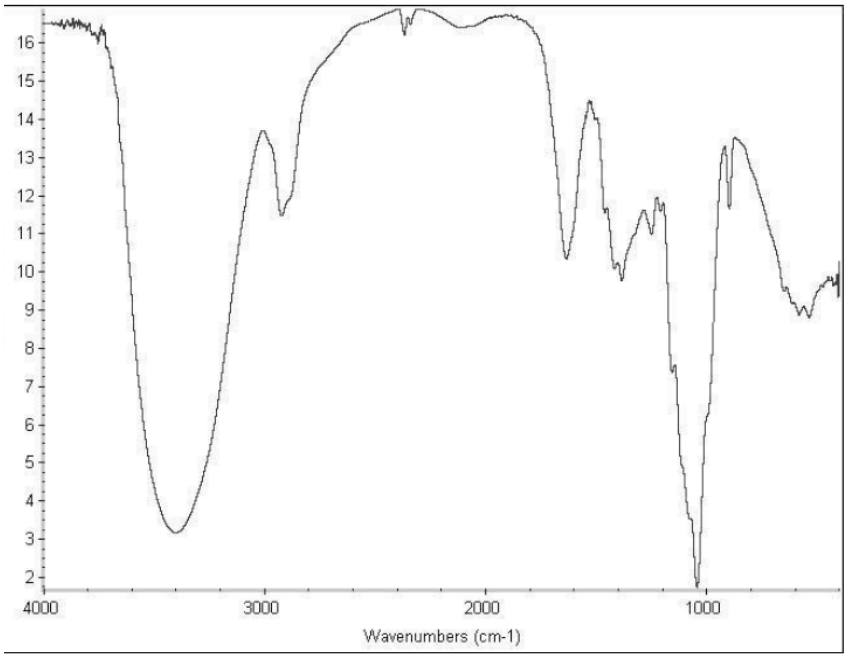
andard deviation)
± 0.0029) g/ml
± 0.0059) g/ml
(± 0.0035) %
58 (± 0.01)
(± 0.1) mL[a]
0 (± 3.2318)°

ble 1. Rheological properties of xylan powder extracted from corn cobs

The bulk density of a powder is calculated by dividing its mass by the volume occupied by the powder (Abdullah & Geldart, 1999). Tapped bulk density, or simply tapped density, is the maximum packing density of a powder achieved under the influence of well-defined, externally applied forces (Oliveira et al., 2010). Because the volume includes the spaces Between particles as well as the envelope volumes of the particles themselves, the bulk and tapped density of a powder are highly dependent on how the particles are packed. This fact is related to the morphology of its particles and such parameters are able to predict the powder flow properties and its compressibility.

Hausner ratio and the compressibility index measure the interparticle friction and the potential powder arch or bridge strength and stability, respectively (Carr, 1965; Hausner, 1967). They have been widely used to estimate the flow properties of powders. A Hausner ratio value of less than 1.20 is indicative of good flowability of the material, whereas a value of 1.5 or higher suggests a poor flow (Daggupati et al., 2011). The compressibility index is also called the Carr index. According to Carr, a value between 5 and 10, 12 and 16, 18 and 21, and 23 and 28 indicates excellent, good, fair, and poor flow properties of the material, respectively. The Hausner ratio and Carr's index values obtained for xylan are listed in Table 1 and suggest that xylan presents extremely poor flow properties. Although the Hausner ratio and the Carr index correspond to indirect measurements of flowability of materials during preliminary studies, the values obtained for xylan suggest the characterization of this biopolymer as a cohesive powder.

Another parameter of the flow behavior of a powder is the angle of repose, which evaluates the flowability of powders through an orifice onto a flat surface. It is considered a direct measurement. Angles of repose below 30° indicate good flowability, 30°-45° some cohesiveness, 45°-55° true cohesiveness, and > 55° sluggish or very high cohesiveness and very limited flowability (Geldart et al., 2006). The angle of repose for xylan is 40.70°, which confirms its cohesive nature predicted by the aforementioned indirect measurements. This is due to the irregular shape of the xylan particles. Besides, the fine particles of xylan, having high surface-to-mass ratios, are more cohesive than coarser particles; hence, they are more influenced by gravitational force. In addition, it is generally believed that the flowability of powders decreases as the shapes of particles become more irregular (Oliveira et al., 2010).



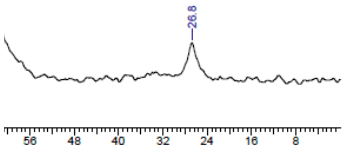
by Fourier-transform infrared (FT-IR) m^{-1} and 1160 cm^{-1} are revealed. They can glycosidic groups and to CC and COC

Fig. 4. FT-IR spectrum of xylan powder extracted from corn cobs.

Moreover, an absorption band near 1375 cm^{-1} is detected and it is assigned to the CH bending vibration present in cellulose and hemicellulose chemical structures (Sun et al., 1998). The prominent band at 1044 cm^{-1} is also associated with hemicelluloses and is attributed to the C–OH bending. Finally, a sharp band at 897 cm^{-1}, which is typical of b-glycosidic linkages between the sugar units in hemicelluloses, was detected in the anomeric region (Sun et al., 2005).

A solid-state ^{13}C nuclear magnetic resonance (NMR) experiment was carried out in 4 mm double bearing rotor made from ZrO_2 on a Bruker DSX 200 MHz spectrometer with resonance frequency at 75.468 MHz. The pulse length was 3.5 μs and the contact time of 1H–13C CP was 2–5 ms. The NMR spectrum of the dry sample showed broad unresolved peaks that correspond to a typical mixture of 4-O-methyl-D-glucuronic acid, L-arabinose and D-xylose, and proteins (Oliveira et al., 2010) (Figure 5).

Concerning the analysis of crystallinity of xylan, the X-ray diffraction detects a few and small peaks, which indicate that xylan presents a low crystallinity (Figure 6). On the other hand, thermal analysis of xylan by thermogravimetry demonstrates a first event of 8.9% weight loss detected in the range of 62 and 107ºC due to dehydration. The second and most relevant event of 49.8% weight loss appears in the range of 250 and 300ºC due to the polymer decomposition (Figure 7). The differential scanning calorimetry curve reveals an endothermic peak at 293.04ºC, which is attributed to the melting point of the polymer (Figure 7).



id-state ^{13}C nuclear magnetic resonance spectrum of corn cob xylan.

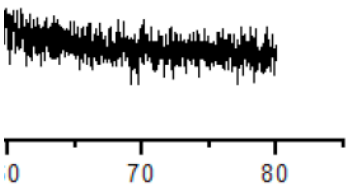


Fig. 6. X-ray diffraction pattern for corn cob xylan (Unpublished data).

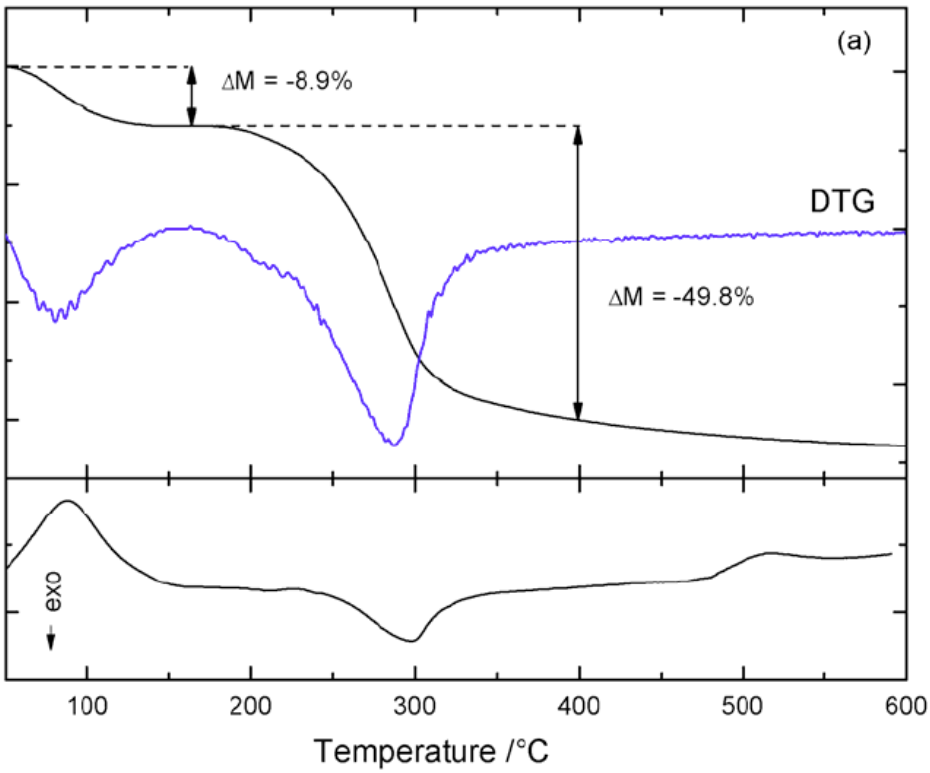
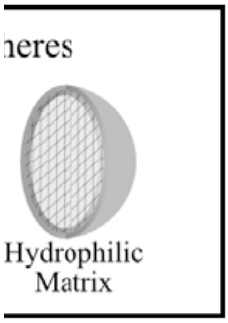


Fig. 7. Thermogravimetry and differential scanning calorimetry curves for corn cob xylan (Unpublished data).

3. Xylan microparticles

As previously described, xylan has been considered as a suitable raw material to produce colonic drug delivery systems due to the ability of enzymes produced by the colonic microflora to degrade the β-glycosidic bonds between the sugar units of the polymer backbone (Kacurakova et al., 2000; Oliveira et al., 2010; Saha, 2000). Regarding the colonic environment, it presents a neutral pH range of the colon and a local blood circulation that prevents the rapid distribution of the drug into the body before circulating into the intestinal blood vessels. As a result, the colonic absorption of drugs is an alternative approach to deliver molecules that are degraded in the stomach medium and are toxic in small quantities in the body (Luo et al., 2011).

A large variety of drug delivery systems are described in the literature, such as liposomes (Torchilin, 2006), micro and nanoparticles (Kumar, 2000), polymeric micelles (Torchilin, 2006), nanocrystals (Muller et al., 2011), among others. Microparticles are usually classified as microcapsules or microspheres (Figure 8). Microspheres are matrix spherical microparticles where the drug may be located on the surface or dissolved into the matrix. Microcapsules are characterized as spherical particles more than 1 μm containing a core substance (aqueous or lipid), normally lipid, and are used to deliver poor soluble molecules in hydrophilic medium (Couvreur et al., 2002; Kumar, 2000; Ribeiro et al., 1999).



ermore, microcapsules may have one or more cores while the microspheres may show ıogenous or heterogeneous aspect with the drug distributed equally or aggregated ıe particle.

Fig. 8. Structural differences between microcapsules and microspheres.

In the past, microparticles were considered as mere carriers, usually micronized dry material without sophisticated attributes (Vehring, 2008). However, nowadays they have found a number of applications in the pharmaceutical field. For instance, microparticles have been used in order to achieve controlled release of drugs, deliver two or more agents in the same system, improve the bioavailability and the biodistribution of molecules, target drugs to specific cells or issues, or mask the unpleasant taste of some active molecules (Simó et al., 2003; Tran et al., 2011; Vehring, 2008). Xylan microparticles have been successfully produced by the following methods: coacervation (Garcia et al., 2001), interfacial cross-linking (Nagashima et al., 2008) and spray-drying (Unpublished data), all of which are described in the following subsection.

3.1 Methods of production

3.1.1 Coacervation

The coacervation technique is defined as a partial desolvation of a homogeneous polymer solution into a polymer-rich phase (coacervate) and the poor polymer phase (coacervation medium). It was the first process to be scaled-up to an industrial process (Jyothi et al., 2010). However, for the optimization of this method, some changes in the methodology were made and the technique was classified into two types: simple and complex. In simple coacervation the desolvation agent is added to form the coacervate, while the complex coacervation process is guided by the presence of two polymers with different charges, and divided into three steps: (i) formation of three immiscible phases, (ii) deposition of the coating, and (iii) strengthening of the coating (Gouin, 2004; Jyothi et al., 2010; Qv et al., 2011).

After the first step, which includes the formation of three immiscible phases (liquid manufacturing vehicle, core material, and coating material), the core material is dispersed in a solution of the coating polymer. The coating material phase, which corresponds to an immiscible polymer in liquid state, is formed by (i) changing the temperature of the polymer solution, (ii) adding a salt, (iii) adding a non-solvent, (iv) adding an incompatible polymer to the polymer solution, and (v) inducing polymer-polymer interaction. The second step includes deposition of the liquid polymer upon the core material. Finally, the prepared microcapsules are stabilized by cross-linking, desolvation, or thermal treatment (Jyothi et al., 2010; Stuart, 2008).

Xylan-based micro- and nanoparticles have been produced by simple coacervation (Garcia et al., 2001). In the study, sodium hydroxide and chloride acid or acetic acid were used as solvent and non-solvent, respectively. Also, xylan and surfactant concentrations and the molar ratio between sodium hydroxide and chloride acid were observed as parameters for the formation of micro- and nanoparticles by the simple coacervation technique (Garcia et al., 2001). Different xylan concentrations allowed the formation of micro- and nanoparticles. More precisely, microparticles were found for higher concentrations of xylan while nanoparticles were produced for lower concentrations of the polymer solution. When the molar ratio between sodium hydroxide and chloride acid was greater than 1:1, the particles settled more rapidly at pH=7.0. Regarding the surfactant variations, an optimal concentration was found; however, at higher ones a supernatant layer was observed after 30 days (Garcia et al., 2001).

3.1.2 Interfacial cross-linking polymerization

The production of microparticles by this technique involves basically two experimental steps: (i) emulsification and (ii) cross-linking reaction (Figure 9). In fact, the emulsification is the major step of the process to determine the particle size distribution and the aggregation arrangement of the microparticles. Therefore, the chemical reactivity of the cross-linking agent is also important to determine the required time to complete the entire process (Chang, 1964; Jiang et al., 2006; Levy & Andry, 1990; Li et al., 2009).

In the first step of the interfacial cross-linking polymerization, the polymer is dissolved into the solvent, which is the internal phase of the emulsion, and another phase with a non-solvent to the polymer is produced; then the aqueous phase is poured to the organic phase ntaining the cross-linking agent is added roparticles (Couvreur et al., 2002; Rao &

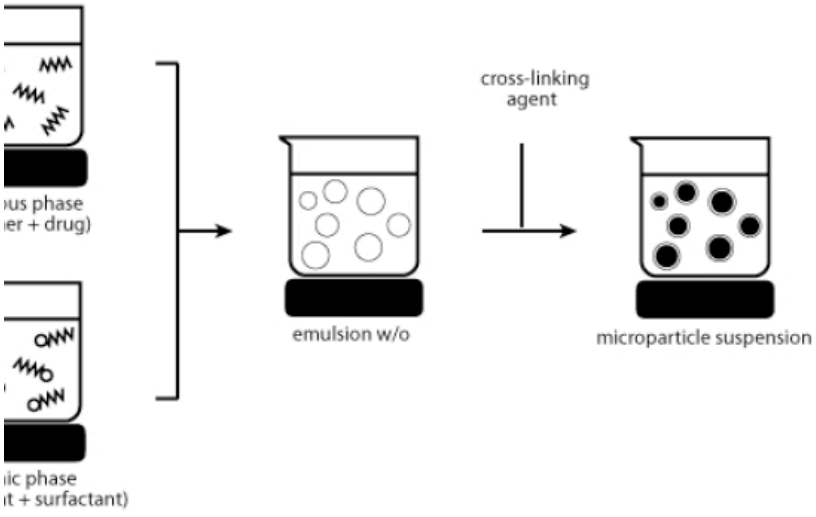
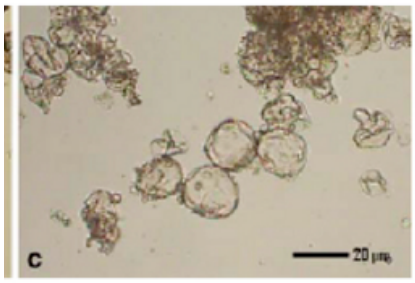


Fig. 9. Scheme for interfacial cross-linking polymerization.

The influence of the lipophilic external phase on the production of xylan-based microparticles by interfacial cross-linking polymerization has been investigated (Nagashima et al., 2008). Three different external phases were investigated: a 1:4 (v/v) xane mixture, soybean oil, and a medium chain triglyceride, with 24, and 52 cP, respectively. It was observed that the use of these different in different macroscopic and microscopic aspects of the system (Figure



a) 1:4 (v/v) Chloroform: cyclohexane mixture;
b) Soybean oil;
c) Medium chain triglycerides.

Fig. 10. Optical microscopy images of xylan microcapsules produced by interfacial cross-linking polymerization with different lipophilic external phases (Nagashima et al., 2008).

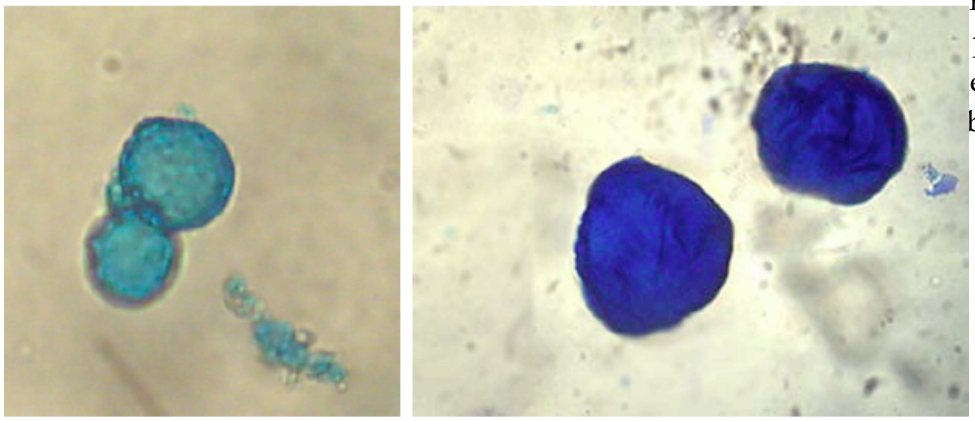
Because emulsions are susceptible to many destabilizing phenomena occurring since the formation of these systems, such as Ostwald ripping (Anton et al., 2008) and coalescence (Li et al., 2009), the formation of the microcapsules may be influenced by those phenomena, which can form aggregates and agglomerates, respectively. Also, the higher viscosity of the lipid phase may support the shaping of microcapsules with a bigger size than the oil phases with a lower viscosity (Nagashima et al., 2008).

The cross-linking agent is present in the interfacial area, where the polymer should be adsorbed due to the poor solubility of the polymer at the external medium. It is known that the chemical reactivity of the cross-linking agent is a limiting parameter to determine the duration and the yield of the process (Li et al., 2009). Terephthaloyl chloride is a cross-linking agent used to produce microcapsules based on polysaccharides, and it was extensively studied by Levy to produce starch derivate microcapsules for pharmaceutical uses. According to Levy, the pH medium, the concentration of the polymer, the stirring speed, and the concentration of terephthaloyl chloride are significant parameters for the formation of the microparticles and their structure (Andry et al., 1996; Andry & Lévy, 1997; Edwards-Lévy et al., 1994; Levy & Andry, 1990).

Cross-linked xylan-based microparticles are produced by the emulsification of an alcaline solution of xylan with a lipophilic phase formed by a mixture of chloroform and cyclohexane by using 5% (w/v) sorbitan triesterate as the surfactant. Subsequently, the cross-linking reaction is carried out for 30 minutes with 5%(w/v) terephthaloyl chloride in order to yield a hard and rigid polymeric shell (Nagashima et al., 2008). The interfacial cross-linking polymerization has been demonstrated to be a suitable method for the production of xylan microcapsules with high drug encapsulation efficiency. SD- loaded cross-linked xylan microcapsules have been produced with three different amounts of the drug (3.1, 6.2, and 60mg).

At the end of the process, yellowish suspensions of spherical polymeric microcapsules were produced. The mean particle size was found to be approximately 12.5 μm (Figure 11). Regarding the encapsulation efficiency, high and inversely concentration-dependent rates were achieved. While the SD concentration of 3.1 mg induced a load ability of 99 ± 2%, 6.2 mg of SD promoted 75.8 ± 1 %, and 60mg of SD yielded a 30.4 ± 6 % load efficiency. Accordingly, the results demonstrated the feasibility of producing xylan microcapsules with and without SD, presenting the same aspect and homogeneity, but concentration-dependent encapsulation rates (Unpublished data).

Regarding the stability of those formulations after storage, studies have been performed in order to evaluate the SD release. As a result of storage for 30 days, it was found that approximately 30 ± 5% of SD had been released to the external medium. This fact may be evidence that some adjustments in the methodology need to be made. One approach that release to the external medium product instead of an aqueous ement to the interfacial cross-bsection.



(a) (b)

a) SD-loaded cross-linked xylan microcapsules containing 60 mg of SD
b) SD-loaded cross-linked xylan microcapsules containing 3.1 mg of SD

Fig. 11. Optical microscopy of SD-loaded cross-linked xylan microcapsules at 40x magnification.

Cross-linked xylan microcapsules have also been successfully developed in order to protect superparamagnetic particles from gastric dissolution (Silva et al., 2007). First, magnetic particles were synthesized by coprecipitation using solutions of ferric chloride and ferrous sulphate as a source of iron. Subsequently, xylan was dissolved in 0.6 M NaOH solution and the magnetic suspension was added to the xylan solution after neutralization and sonication. Finally, the emulsification was carried out in chloroform:cyclohexane containing 5% (w/v) sorbitan tristerate followed by the cross-linking reaction with terephthaloyl chloride. As a result, polymeric microparticles with a mean diameter of 25.26 ± 0.42 μm and roughly spherical in shape were produced. They were suggested to involve more than one magnetic particle entity due to their five-fold larger size. Additionally, dissolution studies revealed that only 2.3% of the magnetite content was dissolved in 0.1 M HCl solution at 37 ± 0.1 ºC after 120 min. This fact corroborates the feasibility of xylan as a material for colon delivery.

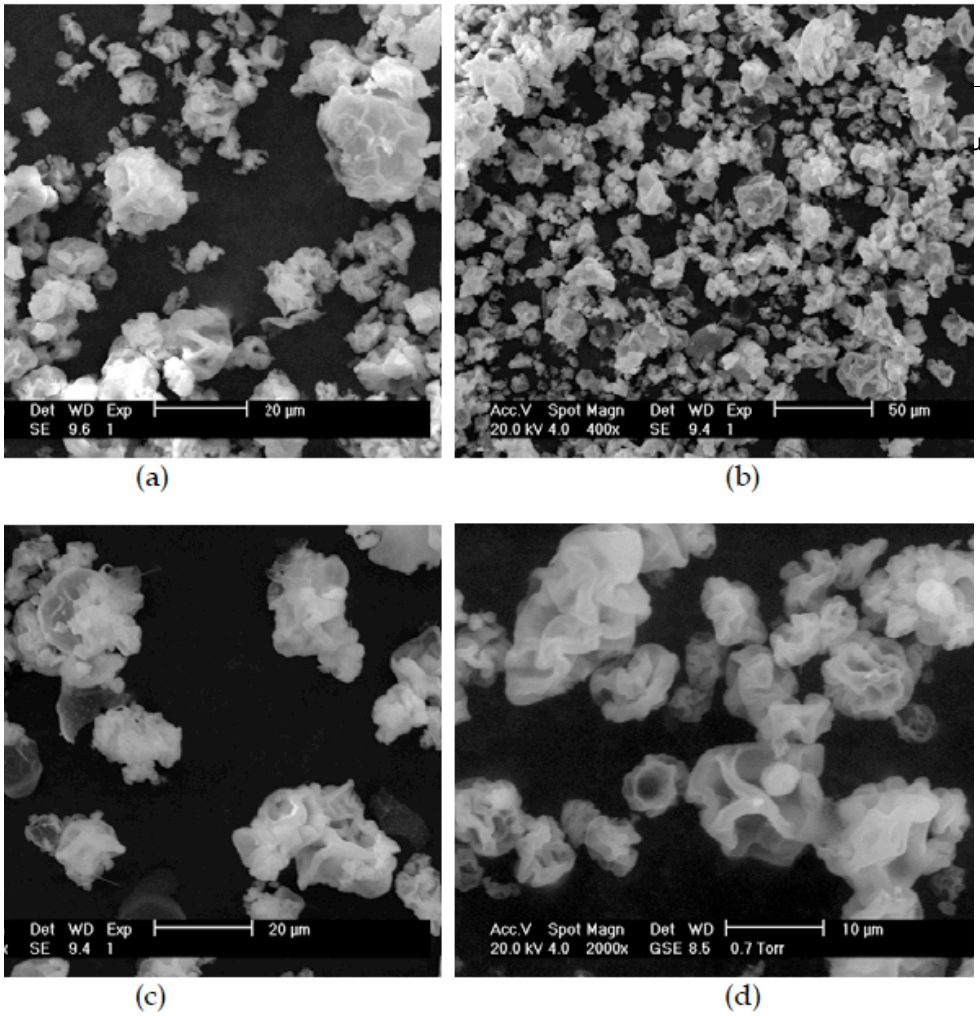
3.1.3 Spray-drying

The spray-drying technique is a one-step continuous operation characterized by the atomization of suspensions or solutions into fine droplets followed by a drying process that leads to the formation of solid particles (Tewa-Tagne et al., 2007). When compared to other approaches for producing and drying systems, this technique exhibits the advantages of low price, rapid process, and the possibility of modulating the physicochemical properties of particles, such as particle size, polydispersity, bulk and tapped densities, and cohesion (Raffin et al., 2006; Tewa-Tagne et al., 2006; Vehring, 2008). Briefly, the main steps of the process are (1) atomization of the feed into a spray, (2) spray-air contact, (3) drying of the spray, and (4) separation of the dried product from the drying gas (Tewa-Tagne et al., 2007; Tewa-Tagne et al., 2006). Because of the dry state of the final product obtained by the spray-drying technique, this method is highly appropriate to improve the stability of microparticulate systems due to the reduction of microbiological contamination, polymer hydrolysis, and physicochemical instability because of the elimination of the water content.

The production of xylan-based microparticles by spray drying has provided useful results. Although some limitation may be observed due to the sticky nature of xylan, which may lead to scarce amounts of final dry product, the use of other materials is very helpful. With that purpose, derivatives of methacrylic acid and methyl-methacrylate, also known as Eudragit®, have been used to prepare suitable xylan-based microparticles. In addition, Eudragit® S-100 (ES100) plays an additional role in the pharmacokinetic properties of the polymeric microparticles. ES100 is a synthetic gastroresistant polymer that has been largely used in the pharmaceutical industry due to its safety and degradation behavior. It is a pH-sensitive copolymer and, because of that, it is able to prevent drug release until the formulation passes through the stomach and reaches some distance down the small intestine (Friend, 2005).

Thus, spray-dried xylan/ES100 microparticles were produced at different polymer weight ratios dissolved in alkaline and neutral solutions, separately. More precisely, xylan and ES100 were dissolved in 1:1 and 1:3 weight ratios in 0.6 N NaOH and phosphate buffer (pH 7.4). Then, the suspensions were spray-dried at the feed rate of 1.2 mL/min (inlet temperature of 120ºC) using a Büchi Model 191 laboratory spray-dryer with a 0.7 mm nozzle, separately. Cross-linked xylan microcapsules were also coated by ES100 after spray- drying at the same conditions.

It was observed that this technique was able to produce microparticles with a mean diameter of approximately 10.17 ± 3.02 μm in a reasonable to satisfactory yield depending on the formulation. This value was observed to be higher for the polymer weight ratio of 1:3 (87.00 ± 4.25 %), which indicates that ES100 improves the final result of the spray-drying process. According to the SEM analysis, the polymeric microparticles were shown to be quite similar in shape. Regardless of the formulation, they appeared to be mostly concave and asymmetric (Figure 12).



a) 1:1 (w/w) xylan/ES100 microparticles (solvent: NaOH) at 938x magnification.
b) 1:3 (w/w) xylan/ES100 microparticles ((solvent: NaOH) at 400x magnification.
c) 1:3 (w/w) xylan/ES100 microparticles (solvent: phosphate buffer) at 1000x magnification.
d) 1:3 (w/w) xylan/ES100 microparticles (solvent: phosphate buffer) at 2000x magnification.

Fig. 12. SEM images of 5-ASA-loaded spray-dried xylan and ES100 microparticles in different polymer weight ratios (Unpublished data).

4. Biocompatibility of xylan and its products

Among other natural products, biopolymers have been largely studied, due to their numerous applications in which their contact to cells and tissues via their surface is of utmost importance. For instance, micro- and nanocapsules, film coatings, excipients for traditional dosage forms, and novel drug delivery systems have taken much advantage by using biopolymers, especially due to their biocompatibility and biodegradability properties (Drotleff et al., 2004; Villanova et al., 2010). Biopolymers are subject to degradation in vivo by hydrolysis or enzymatic attack. The use of these polymers may represent a lower cost compared to other conventional biodegradable polymers (Villanova et al., 2010).

During the development of pharmaceutical products, the toxic effect of biomaterials on cells is considered one of the most important issues to be evaluated. For instance, cell death, cell proliferation, cell morphology, and cell adhesion are features directly with

correlated with the toxicity in vitro. Therefore, loss of viability could be a consequence of a toxic biomaterial (Marques, 2005). Although biopolymers are considered non-toxic and biocompatible, residues from their extraction methodology may cause toxicity issues.

In order to assess the effect of the corn cob xylan on the cell viability and proliferation rate, xylan solutions at concentrations of 0.1, 0.25, 0.50, 0.75, and 1 mg/ml were placed in contact with human cervical adenocarcinoma cells (HeLa cells) for 24 and 72 h. Finally, the cell viability was determined by the MTT assay. It was observed that regardless of the xylan concentration, the samples tested did not affect the viability of HeLa cells after incubation for 24 h (Figure 13) (Unpublished data).

Besides, the statistical analysis of the results obtained confirmed that the xylan samples did not present a significant effect on the cell viability and cell proliferation rate when in direct contact with HeLa cells at the concentrations used in this study and compared to the control.

Similarly, after a longer time of incubation, no significant changes in the cell proliferation rate was detected, as can be seen in the data for 72 h (Figure 13). In fact, this was expected due to the biocompatible nature of xylan. As a natural polyssacharide, this biomaterial is considered to be highly stable, non-toxic and hydrophilic (Liu et 8). Accordingly, the alkaline extraction of xylan from corn has proved to be a safe ch for obtaining the polymer with no relevant toxicity (Unpublished data).

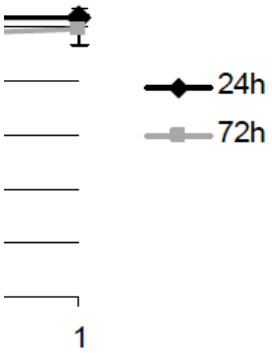


Fig. 13. Viability of HeLa cells after incubation for 24 and 72h with solutions of xylan at different concentrations.

Xylan-based microparticles were also evaluated regarding their in vitro toxicity. In fact, cross-liked (CLM) and spray-dried microparticles (SDM) based on xylan and ES100 were produced in order to carry UA and avoid its side effects, namely hepatotoxicity and nephrotoxicity. Additionally, CLM and SDM dispersions at concentrations of 50, 125, 250, and 500 μg/ml were placed in contact with human embryonic lung fibroblasts (MRC-5 cells) for 24 h and the MTT

assay was carried out to assess the cell viability. According to the MTT assay results, the cells treated with CLM presented an initial decrease in the cell viability of 56% at the lowest tested concentration (50 µg/mL) while the cell viability rate reached only 12.6% at the highest concentration (500 µg/mL) (Figure 14).

Nevertheless, SDM showed a maximum decrease in the cell survival rate of approximately 12% and 27% at the lowest and highest concentrations of microparticles, respectively (Figure 14). The massive cytotoxicity induced by CLM may be explained by the presence of remaining molecules of terephthaloyl chloride, which plays the role of cross-linking agent during the formation of CLM and is well known as a toxic substance.

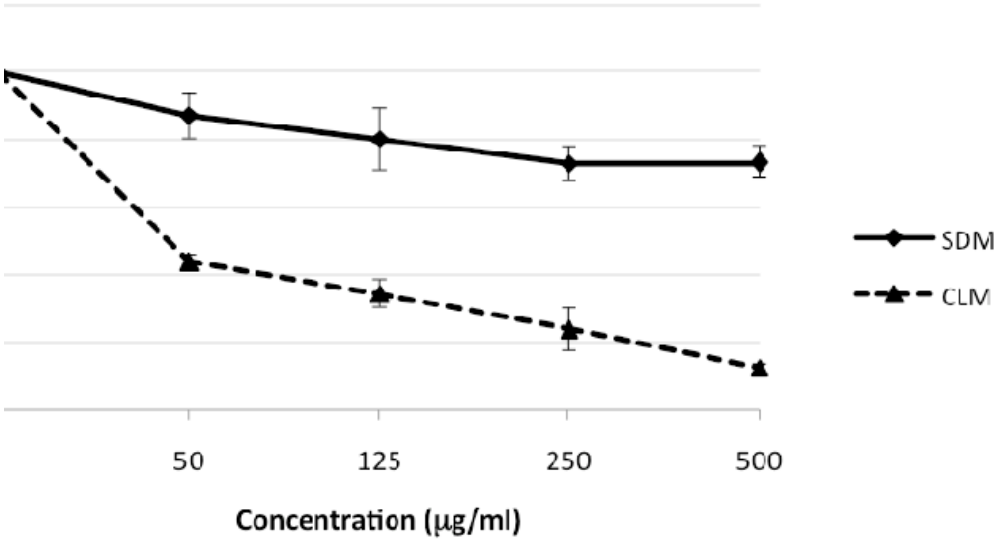


Fig. 14. Viability of MRC-5 cells after incubation for 24h with spray-dried (SDM) and cross-linked xylan microparticles (CLM) containing UA.

5. Conclusions

The need of modern science to achieve a sustainable future development has been shown in many circumstances in society. Finding strategies less harmful to the environment has been a quest for research in several areas, such as pharmaceuticals, biotechnology, and food industries. With that purpose, the increase in research and development of more applications of xylan and its derivatives has shown the versatility of this biopolymer, thus helping the search for sustainable alternatives.

Xylan may be extremely useful in the pharmaceutical field, especially for the production of colon-specific drug carriers, such as micro- and nanoparticles, and film coatings. In addition, because of its abundant sources in nature, its use would bring many benefits, including reducing costs to industry, optimizing the use of natural resources, and reducing environmental damage due to its biodegradability and biocompatibility.

Large amounts of agricultural waste products, such as corn cobs, are continuously provided in several developing countries. Xylan is considered to be a green polymer that may play an essential role in the renewability of waste products due to its biodegradable and biocompatible nature. Furthermore, as shown in this chapter, xylan presents particular properties that allow a wide range of applications.

6. Acknowledgements

The authors are grateful to Professor Dr. Lucymara Fassarela Agnez Lima and Acarízia Eduardo da Silva for the contribution with the cytotoxicity assay.

7. References

Abdullah, E. C. & Geldart, D. (1999). The use of bulk density measurements as flowability indicators. Powder Technology, Vol. 102, 2, (March 1999), pp. (151-165), ISSN 0032-5910

Andry, M. C., Edwards-Lévy, F. & Lévy, M. C. (1996). Free amino group content of serum albumin microcapsules. III. A study at low pH values. International Journal of Pharmaceutics, Vol. 128, 1-2, (February 1996), pp. (197-202), ISSN 0378-5173

Andry, M. C. & Lévy, M. C. (1997). In vitro degradation of serum albumin microcapsules: Effect of process variables. International Journal of Pharmaceutics, Vol. 152, 2, (June 1997), pp. (145-151), ISSN 0378-5173

Anton, N., Benoit, J. P. & Saulnier, P. (2008). Design and production of nanoparticles formulated from nano-emulsion templates - A review. Journal of Controlled Release, Vol. 128, 3, (June 2008), pp. (185-199), ISSN 0168-3659

Belgacem, M. N. & Gandini, A. (Ed(s).). (2008). Monomers, polymers and composites from renewable resources, Elsevier, ISBN 978-0-08-045316-3, Oxford

Carr, R. L. (1965). Classifying flow properties of solids. Chemical Engineering, Vol. 72, 3, 1965), pp. (69-72)

Chang, T. M. S. (1964). Semipermeable microcapsules. Science, Vol. 146, 364, (October 1964), pp. (524-&), ISSN 0036-8075

Couvreur, P., Barratt, G., Fattal, E., Legrand, P. & Vauthier, C. (2002). Nanocapsule technology: A review. Critical Reviews in Therapeutic Drug Carrier Systems, Vol. 19, 2, (March 2002), pp. (99-134), ISSN 0743-4863

Daggupati, V. N., Naterer, G. F., Gabriel, K. S., Gravelsins, R. J. & Wang, Z. L. (2011). Effects of atomization conditions and flow rates on spray drying for cupric chloride particle formation. International Journal of Hydrogen Energy, Vol. In Press, Corrected Proof, pp. 0360-3199, ISSN 0360-3199

Daus, S. & Heinze, T. (2010). Xylan-based nanoparticles: Prodrugs for ibuprofen release. Macromolecular Bioscience, Vol. 10, 2, (November 2010), pp. (211-220), ISSN 1616-5195

Den Haan, R. & Van Zyl, W. H. (2003). Enhanced xylan degradation and utilisation by Pichia stipitis overproducing fungal xylanolytic enzymes. Enzyme and Microbial Technology, Vol. 33, 5, (October 2003), pp. (620-628), ISSN 0141-0229

Drotleff, S., Lungwitz, U., Breunig, M., Dennis, A., Blunk, T., Tessmar, J. & Gopferich, A. (2004). Biomimetic polymers in pharmaceutical and biomedical sciences. European Journal of Pharmaceutics and Biopharmaceutics, Vol. 58, 2, (September 2004), pp. (385-407), ISSN 0939-6411

Ebringerová, A. (2005). Structural diversity and application potential of hemicelluloses. Macromolecular Symposia, Vol. 232, 1, (February 2005), pp. (1-12), ISSN 1521-3900

Ebringerova, A. & Heinze, T. (2000). Xylan and xylan derivatives - Biopolymers with valuable properties, 1 - Naturally occurring xylans structures, procedures and properties. . Macromolecular Rapid Communications, Vol. 21, 9, (June 2000), pp. (542-556), ISSN 1022-1336

Ebringerova, A. & Hromadkova, Z. (1999). Xylans of industrial and biomedical importance, In: Biotechnology and Genetic Engineering Reviews, pp. (325-346), Intercept Ltd Scientific, Technical & Medical Publishers, ISBN 0264-8725, Andover

Ebringerova, A., Hromadkova, Z., Alfodi, J. & Hribalova, V. (1998). The immunologically active xylan from ultrasound-treated corn cobs: extractability, structure and properties. Carbohydrate Polymers, Vol. 37, 3, (November 1998), pp. (231-239), ISSN 0144-8617

Ebringerova, A., Hromadkova, Z. & Hribalova, V. (1995). Structure and mitogenic activities of corn cob heteroxylans. International Journal of Biological Macromolecules, Vol. 17, 6, (December 1995), pp. (327-331), ISSN 0141-8130

Ebringerova, A., Hromadkova, Z., Kacurakova, M. & Antal, M. (1994). Quaternized xylans: Synthesis and structural characterization. Carbohydrate Polymers, Vol. 24, 4, (May 1994), pp. (301-308), ISSN 0144-8617

Edwards-Lévy, F., Andry, M. C. & Levy, M. C. (1994). Determination of free amino group content of serum-albumin microcapsules. II. Effect of variations in reaction-time and terephthaloyl chloride concentration. International Journal of Pharmaceutics, Vol. 103, 3, (March 1994), pp. (253-257), ISSN 0378-5173

Friend, D. R. (2005). New oral delivery systems for treatment of inflammatory bowel disease. Advanced Drug Delivery Reviews, Vol. 57, 2, (January 2005), pp. (247-265), ISSN 0169-409X

Garcia, R. B., Ganter, J. & Carvalho, R. R. (2000). Solution properties of D-xylans from corn cobs. European Polymer Journal, Vol. 36, 4, (April 2000), pp. (783-787), ISSN 0014-3057

Garcia, R. B., Nagashima Jr, T., Praxedes, A. K. C., Raffin, F. N., Moura, T. F. A. L. & Egito, E. S. T. (2001). Preparation of micro and nanoparticles from corn cobs xylan. Polymer Bulletin, Vol. 46, 5, (May 2001), pp. (371-379), ISSN 1436-2449

Geldart, D., Abdullah, E. C., Hassanpour, A., Nwoke, L. C. & Wouters, I. (2006). Characterization of powder flowability using measurement of angle of repose. China Particuology, Vol. 4, 3-4, (July 2006), pp. (104-107), ISSN 1672-2515

Gouin, S. (2004). Microencapsulation: Industrial appraisal of existing technologies and trends. Trends in Food Science & Technology, Vol. 15, 7-8, (July-August 2004), pp. (330-347), ISSN 0924-2244

Grootaert, C., Delcour, J. A., Courtin, C. M., Broekaert, W. F., Verstraete, W. & Van de Wiele, T. (2007). Microbial metabolism and prebiotic potency of arabinoxylan oligosaccharides in the human intestine. Trends in Food Science & Technology, Vol. 18, 2, (February 2007), pp. (64-71), ISSN 0924-2244

Habibi, Y. & Vignon, M. R. (2005). Isolation and characterization of xylans from seed pericarp of Argania spinosa fruit. Carbohydrate Research, Vol. 340, 7, (May 2005), pp. (1431-1436), ISSN 0008-6215

Hausner, H. H. (1967). Friction conditions in a mass of metal powders. International Journal of Powder Metallurgy, Vol. 3, (February 1967), pp. (7-13), ISSN 0888-7462

Heinze, T., Petzold, K. & Hornig, S. (2007). Novel nanoparticles based on xylan. Cellulose Chemistry and Technology, Vol. 41, 1, January 2007), pp. (13-18), ISSN 0576-9787

Hromadkova, Z., Kovacikova, J. & Ebringerova, A. (1999). Study of the classical and ultrasound-assisted extraction of the corn cob xylan. Industrial Crops and Products, Vol. 9, 2, (Januar 1999), pp. (101-109), ISSN 0926-6690

Jiang, B. B., Hu, L., Gao, C. Y. & Shen, J. C. (2006). Cross-linked polysaccharide nanocapsules: Preparation and drug release properties. Acta Biomaterialia, Vol. 2, 1, (January 2006), pp. (9-18), ISSN 1742-7061

Jyothi, N. V. N., Prasanna, P. M., Sakarkar, S. N., Prabha, K. S., Ramaiah, P. S. & Srawan, G. Y. (2010). Microencapsulation techniques, factors influencing encapsulation efficiency. Journal of Microencapsulation, Vol. 27, 3, (June 2010), pp. (187-197), ISSN 0265-2048

Kacurakova, M., Capek, P., Sasinkova, V., Wellner, N. & Ebringerova, A. (2000). FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses. Carbohydrate Polymers, Vol. 43, 2, (October 2000), pp. (195-203), ISSN 0144-8617

Kayserilioglu, B. S., Bakir, U., Yilmaz, L. & Akkas, N. (2003). Use of xylan, an agricultural by-product, in wheat gluten based biodegradable films: mechanical, solubility and water vapor transfer rate properties. Bioresource Technology, Vol. 87, 3, (May 2003), pp. (239-246), ISSN 0960-8524

Kulkarni, N., Shendye, A. & Rao, M. (1999). Molecular and biotechnological aspects of xylanases. FEMS Microbiology Reviews, Vol. 23, 4, (July 1999), pp. (411-456), ISSN 0168-6445

Kumar, M. (2000). Nano and microparticles as controlled drug delivery devices. Journal of Pharmacy and Pharmaceutical Sciences, Vol. 3, 2, (May-August 2000), pp. (234-258), ISSN 1482-1826

Kumar, V., de la Luz Reus-Medina, M. & Yang, D. (2002). Preparation, characterization, and tabletting properties of a new cellulose-based pharmaceutical aid. International Journal of Pharmaceutics, Vol. 235, 1-2, (March 2002), pp. (129-140), ISSN 0378-5173

Levy, M. C. & Andry, M. C. (1990). Microcapsules prepared through interfacial cross-linking of starch derivatives. International Journal of Pharmaceutics, Vol. 62, 1, (July 1990), pp. (27-35), ISSN 0378-5173

Li, B.-z., Wang, L.-j., Li, D., Chiu, Y. L., Zhang, Z.-j., Shi, J., Chen, X. D. & Mao, Z.-h. (2009). Physical properties and loading capacity of starch-based microparticles crosslinked with trisodium trimetaphosphate. Journal of Food Engineering, Vol. 92, 3, (June 2009), pp. (255-260), ISSN 0260-8774

Li, X., Shi, X., Wang, M. & Du, Y. (2011). Xylan chitosan conjugate - A potential food preservative. Food Chemistry, Vol. 126, 2, (May 2011), pp. (520-525), ISSN 0308-8146

Liu, Z., Jiao, Y., Wang, Y., Zhou, C. & Zhang, Z. (2008). Polysaccharides-based nanoparticles as drug delivery systems. Advanced Drug Delivery Reviews, Vol. 60, 15, (December 2008), pp. (1650-1662), ISSN 0169-409X

Luo, J. Y., Zhong, Y., Cao, J. C. & Cui, H. F. (2011). Efficacy of oral colon-specific delivery capsule of low-molecular-weight heparin on ulcerative colitis. Biomedicine & Pharmacotherapy, Vol. 65, 2, (March 2011), pp. (111-117), ISSN 0753-3322

Marques, A. P. C., H. R.; Coutinho, O. P.; Reis, R. L. (2005). Effect of starch-based biomaterials on the in vitro proliferation and viability of osteoblast-like cells. Journal of Materials Science : Materials in Medicine, Vol. 16, 1, (September 2005), pp. (833-842), ISSN 0957-4530

Morris, K. R., Nail, S. L., Peck, G. E., Byrn, S. R., Griesser, U. J., Stowell, J. G., Hwang, S.-J. & Park, K. (1998). Advances in pharmaceutical materials and processing. Pharmaceutical Science & Technology Today, Vol. 1, 6, (September 1998), pp. (235-245), ISSN 1461-5347

Muller, R. H., Gohla, S. & Keck, C. M. (2011). State of the art of nanocrystals - Special features, production, nanotoxicology aspects and intracellular delivery. European Journal of Pharmaceutics and Biopharmaceutics, Vol. 78, 1, (May 2011), pp. (1-9), ISSN 0939-6411

Nagashima, T., Oliveira, E. E., Silva, A. E., Marcelino, H. R., Gomes, M. C. S., Aguiar, L. M., Araujo, I. B., Soares, L. A. L., Oliveira, A. G. & Egito, E. S. T. (2008). Influence of the lipophilic external phase composition on the preparation and characterization of xylan microcapsules - A technical note. AAPS PharmSciTech, Vol. 9, 3, (September 2008), pp. (814-817), ISSN 1530-9932

Nunthanid,J.,Laungtana-Anan,M.,Sriamornsak,P.,Limmatvapirat, Puttipipatkhachorn, S., Lim, L. Y. & Khor, E. (2004). Characterization of chitosan acetate as a binder for sustained release tablets. Journal of Controlled Release, Vol. 99,
1, (September 2004), pp. (15-26), ISSN 0168-3659

Oliveira, E. E., Silva, A. E., Nagashima Jr, T., Gomes, M. C. S., Aguiar, L. M., Marcelino, H. R., Araujo, I. B., Bayer, M. P., Ricardo, N. M. P. S., Oliveira, A. G. & Egito, E. S. T. (2010). Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization. Bioresource Technology, Vol. 101, 14, (July 2010), pp. (5402-5406), ISSN 0960-8524

Qv, X. Y., Zeng, Z. P. & Jiang, J. G. (2011). Preparation of lutein microencapsulation by complex coacervation method and its physicochemical properties and stability. Food Hydrocolloids, Vol. 25, 6, (August 2011), pp. (1596-1603), ISSN 0268-005X

Raffin, R. P., Jornada, D. S., Ré, M. I., Pohlmann, A. R. & Guterres, S. S. (2006). Sodium pantoprazole-loaded enteric microparticles prepared by spray drying: Effect of the scale of production and process validation. International Journal of Pharmaceutics, Vol. 324, 1, (October 2006), pp. (10-18), ISSN 0378-5173

Rao, J. P. & Geckeler, K. E. (2011). Polymer nanoparticles: Preparation techniques and size-control parameters. Progress in Polymer Science, Vol. 36, 7, (July 2011), pp. (887-913), ISSN 0079-6700

Ribeiro, A. J., Neufeld, R. J., Arnaud, P. & Chaumeil, J. C. (1999). Microencapsulation of lipophilic drugs in chitosan-coated alginate microspheres. International Journal of Pharmaceutics, Vol. 187, 1, (September 1999), pp. (115-123), ISSN 0378-5173

Rubinstein, A. (1995). Approaches and opportunities in colon-specific drug-delivery. Critical Reviews in Therapeutic Drug Carrier Systems, Vol. 12, 2-3, 1995), pp. (101-149), ISSN 0743-4863

Saha, B. C. (2000). Alpha-L-arabinofuranosidases: Biochemistry, molecular biology and application in biotechnology. Biotechnology Advances, Vol. 18, 5, (August 2000), pp. (403-423), ISSN 0734-9750

Sedlmeyer, F. B. (2011). Xylan as by-product of biorefineries: Characteristics and potential use for food applications. Food Hydrocolloids, Vol. In Press, Corrected Proof, pp. ISSN 0268-005X

Shallom, D. & Shoham, Y. (2003). Microbial hemicellulases. Current Opinion in Microbiology, Vol. 6, 3, (June 2003), pp. (219-228), ISSN 1369-5274

Silva, A. K. A., Silva, E. L., Oliveira, E. E., Nagashima, J. T., Soares, L. A. L., Medeiros, A. C., Araujo, J. H., Araujo, I. B., Carriço, A. S. & Egito, E. S. T. (2007). Synthesis and characterization of xylan-coated magnetite microparticles. International Journal of Pharmaceutics, Vol. 334, 1-2, (April 2007), pp. (42-47), ISSN 0378-5173

Simó, C., Cifuentes, A. & Gallardo, A. (2003). Drug delivery systems: Polymers and drugs monitored by capillary electromigration methods. Journal of Chromatography B, Vol. 797, 1-2, (November 2003), pp. (37-49), ISSN 1570-0232

Stuart, M. A. C. (2008). Supramolecular perspectives in colloid science. Colloid and Polymer Science, Vol. 286, 8-9, (August 2008), pp. (855-864), ISSN 0303-402X

Sun, R., M. Fang, J., Goodwin, A., M. Lawther, J. & J. Bolton, A. (1998). Fractionation and characterization of polysaccharides from abaca fibre. Carbohydrate Polymers, Vol. 37, 4, (December 1998), pp. (351-359), ISSN 0144-8617

Sun, X. F., Xu, F., Sun, R. C., Geng, Z. C., Fowler, P. & Baird, M. S. (2005). Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw. Carbohydrate Polymers, Vol. 60, 1, (April 2005), pp. (15-26), ISSN 0144-8617

Tewa-Tagne, P., Briançon, S. & Fessi, H. (2007). Preparation of redispersible dry nanocapsules by means of spray-drying: Development and characterisation. European Journal of Pharmaceutical Sciences, Vol. 30, 2, (April 2007), pp. (124-135), ISSN 0928-0987

Tewa-Tagne, P., Briançon, S. & Fessi, H. (2006). Spray-dried microparticles containing polymeric nanocapsules: Formulation aspects, liquid phase interactions and particles characteristics. International Journal of Pharmaceutics, Vol. 325, 1-2, (November 2006), pp. (63-74), ISSN 0378-5173

Torchilin, V. P. (2006). Multifunctional nanocarriers. Advanced Drug Delivery Reviews, Vol. 58, 14, (December 2006), pp. (1532-1555), ISSN 0169-409X

Tran, V. T., Benoît, J. P. & Venier-Julienne, M. C. (2011). Why and how to prepare biodegradable, monodispersed, polymeric microparticles in the field of pharmacy? International Journal of Pharmaceutics, Vol. 407, 1-2, (December 2011), pp. (1-11), ISSN 0378-5173

Ünlu, C. H., Günister, E. & Atici, O. (2009). Synthesis and characterization of NaMt biocomposites with corn cob xylan in aqueous media. Carbohydrate Polymers, Vol. 76, 4, (May 2009), pp. (585-592), ISSN 0144-8617

Vehring, R. (2008). Pharmaceutical particle engineering via spray-drying. Pharmaceutical Research, Vol. 25, 5, (May 2008), pp. (999-1022), ISSN 0724-8741

Villanova, J. C. O., Orefice, R. L. & Cunha, A. S. (2010). Pharmaceutical applications of polymers. Polimeros - Ciência e Tecnologia, Vol. 20, 1, (January-March 2010), pp. (51-64), ISSN 0104-1428

Wang, Y. & Zhang, J. (2006). A novel hybrid process, enhanced by ultrasonication, for xylan extraction from corncobs and hydrolysis of xylan to xylose by xylanase. Journal of Food Engineering, Vol. 77, 1, (November 2006), pp. (140-145), ISSN 0260-8774

Yang, R., Xu, S., Wang, Z. & Yang, W. (2005). Aqueous extraction of corn cob xylan and production of xylooligosaccharides. LWT - Food Science and Technology, Vol. 38, 6, (September 2005), pp. (677-682), ISSN 0023-6438

CHAPTER II

Article 1: “Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization”

Le deuxième chapitre de cette thèse est consacré à l'article intitulé « Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization ». Il a été publié dans le journal « Bioresource Technology ».

Le xylane, composant naturel de l'hemicellulose, est l'un des biopolymères les plus abondants dans le monde végétal. C'est aussi le principal polysaccharide non cellulosique de la paroi cellulaire des angiospermes, des graminées et des céréales, où il existe sous diverses compositions. En conséquence de ces multiples sources botaniques, les xylanes présentent une diversité considérable avec des structures et complexités différentes.

Plusieurs procédés d'extraction pour l'isolement des xylanes à partir de diverses sources végétales sont disponibles. Selon la source, les propriétés et la structure du polymère obtenu peuvent différer. En particulier, certains types de xylanes : le glucuronoxylane, l'arabinoglucuronoxylane et l'arabinoxylane, pourraient être obtenus à partir de sources végétales avec des compositions chimiques et des propriétés physico-chimiques reproductibles.

Toutes les matières premières entrant dans la formulation des produits pharmaceutiques doivent subir une caractérisation approfondie. Compte tenu de l'utilité potentielle du xylane comme excipient pour des systèmes de délivrance colique de médicaments et de l'absence d'études antérieures sur le xylane extrait de rafles de maïs, l'objectif de cet article était de fournir une caractérisation complète du xylane. De nombreuses techniques de caractérisation ont été mises en œuvre : spectroscopie infrarouge et résonance magnétique nucléaire, études de la morphologie, granulométrie et rhéologie, mesures telles que l'angle de repos, densités aérée et tapée, indice de compressibilité, indice d'Hausner et compactibilité.

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization

Elquio Eleamen Oliveira [a,b], Acarília Eduardo Silva [a], Toshiyuki Nagashima Júnior [c], Monique Christine Salgado Gomes [a], Larissa Muratori Aguiar [a], Henrique Rodrigues Marcelino [a], Ivonete Batista Araújo [a], Marc P. Bayer [d], Nágila M.P.S. Ricardo [e], Anselmo Gomes Oliveira [f], Eryvaldo Sócrates Tabosa Egito [a,*]

[a] Programa de Pós-Graduação em Ciências da Saúde (PPGCSa), Universidade Federal do Rio Grande do Norte, Natal – R, Brazil
[b] Centro de Ciências Biológicas e Sociais Aplicadas (CCBSA), Universidade Estadual da Paraíba, João Pessoa – PB, Brazil
[c] Unidade Acadêmica de Saúde, UAS-CES, Universidade Federal de Campina Grande, Cuité – PB, Brazil
[d] Universität Tübingen, Institut für Organische Chemie, D-72076, Germany
[e] Universidade Federal do Ceará, Departamento de Química Orgânica e Inorgânica, Laboratório de Polímeros, Fortaleza – CE, Brazil
[f] Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas, UNESP, Rodovia Araraquara – Jaú km 01, 14801-902 Araraquara, SP, Brazil

ARTICLE INFO

Article history:
Received 10 November 2008
Received in revised form 26 January 2010
Accepted 27 January 2010
Available online 19 February 2010

Keywords:
Xylan
Biopolymer
Fourier transform infrared
Flow properties
Bioresource

ABSTRACT

Although many authors have reported several beneficial effects ascribed to xylan, such as inhibitory action on mutagenicity activity, antiphlogistic effects, and mitogenic and comitogenic activities, few papers have investigated a systematic study on the technological properties of this polymer. The aim of the present work was to evaluate xylan as a promise raw material for the pharmaceutical industry. The water-insoluble xylan samples were extracted from corn cobs following several steps. The obtained powered sample was analyzed by infrared and RMN spectroscopy, and characterized regarding their particle size, bulk and tap densities, compressibility index, compactability, Hausner ratio, and angle of repose. According to the results, infrared and RMN spectroscopy were shown to be able to evaluate the xylan structural conformation and composition, respectively. In addition, rheological data demonstrated that xylan powder obtained from corn cobs may be characterized as a material with low density and very cohesive flow properties.

1. Introduction

Xylan is the second most abundant biopolymer in the plant kingdom (Ebringerova and Hromadkova, 1999; Garcia et al., 2000). It is not only the most common hemicellulose but also the major non-cellulosic cell wall polysaccharide of angiosperms, grasses, and cereals, where it occurs in many different compositions and structures. Its main chain is constituted of D-xylopyranose units in the backbone linked through 1 → 4 glycosidic bonds. The majority of D-xylans have other sugars in side chains, such as 4-O-methyl-D-glucuronic acid, O-acetyl-L-arabinose, L-arabinose, and D-glucuronic acid. Concerning, specifically, the xylan from corn cobs, it has been demonstrated that such polymer presents a chemical composition of 4-O-methyl-D-glucuronic acid, L-arabinose and D-xylose in the proportion of 2:7:19, respectively (Ebringerova and Hromadkova, 1999; Garcia et al., 2000; Karucákova et al., 1994; Silva et al., 1998; Whistler and Smart, 1953).

Corn cobs contain a considerable amount of xylan-type hemicelluloses, which were recognized as a satisfactory source of xylose by early studies (Ai et al., 2005; Collins et al., 2005). The corn cob xylan can be characterized by two different structural types.One is a low-branched arabinoglucuronoxylan, which is mostly water-insoluble (wis-X), and the second is a highly branched water soluble heteroxylan (ws-X) (Hromadkova et al., 1999).

In previous papers, the xylan isolated from corn cobs has been shown to be applicable as an additive in papermaking and textile printing, as well as in the pharmaceutical industry (Hromadkova et al., 1999). In addition, the fermentative process of xylan and other hemicelluloses has been studied as a method for production of biofuels (Pauly and Keegstra, 2010; York and O'Neill, 2008). Several beneficial effects associated to xylans have been reported by many authors. For instance, inhibitory action on mutagenicity activity and heating seems to increase the detoxification ability of dietary fibers, antiphlogistic effects, and both mitogenic and comitogenic activities (Ebringerova and Hromadkova, 1997;

* Corresponding author. Address: UFRN – CCS – Departamento de Farmácia, Rua Praia de Areia Branca, 8948, CEP: 59094-450, Brazil. Tel.: +55 84 94 31 88 16; fax: +55 84 32 15 43 55.
E-mail address: socrates@ufrnet.br (E.S.T. Egito).

0960-8524/$ - see front matter
doi:10.1016/j.biortech.2010.01.137

Ebringerova et al., 1998, 1995, 2002; Kayserilioglu et al., 2003). An important characteristic of wis-X is its ability to remain intact in the physiological stomach environment and small intestine. This property, together with the presence of specific enzymes for colon biodegradability, makes this copolymer a suitable raw material for the medical field, especially as a colon-specific drug carrier (Ebringerova and Heinze, 2000; Rubinstein, 1995; Silva et al., 2007; Sinha and Kumria, 2001).

Because of the distal location of colon at the gastrointestinal tract (GIT), a colon-specific drug delivery system should prevent drug release in the stomach and small intestine, and provide an abrupt onset of drug release upon entry into the colon. For that purpose, a triggering element in the system that can respond to physiological changes in the colon is needed. Overall, the physiological changes along the GIT can be generally characterized as continuum, with decreases in enzymatic activity, motility, and fluid content and an increase in pH. These gradual changes in physiological parameters are not suitable for triggering elements to perform a sudden and dramatic change in the performance of a delivery system in order to obtain colon-specific delivery. However, the presence of specific bacterial populations in the colon is the exception that has been extensively explored as triggering components for initiating colon-specific drug release. This strategy is highly promising because non-starch polysaccharides, like xylan, remain undigested in the stomach and the small intestine and can only be degraded by the vast anaerobic colon microflora, like Bifidobacterium. Because of these characteristics, xylan may be considered as a promising polymer for drug delivery systems (Rubinstein, 1995; Sinha et al., 2004; Yang et al., 2002a,b).

Nevertheless, only a few papers have investigated the properties of this polymer and the influence of such characteristics on the application of xylan in the pharmaceutical field. Such data may be applied either by the pharmaceutical industry in the development of drug delivery systems based on xylan or by scientific research groups on colon-specific carriers. The aim of the present work was to extract the wis-X xylan from corn cobs, a renewable raw material which is widely cultivated around the world. In addition, an analytical method to identify the extracted xylan was proposed by using the infrared spectroscopy, which is a usual and low-cost technique, and RMN spectroscopy. The physical properties of this polymer were also evaluated.

2. Methods

2.1. Materials

Ethanol, polysorbate 20, polysorbate 80, and sodium hydroxi were purchased from Vetec Chemical (Brazil); acetic ac methanol, and isopropanol were purchased from Sigma Chemi Co. (USA). Xylan samples, from corn cobs, were obtained after a single extraction process in our laboratory.

2.2. Xylan extraction

The polymer was extracted from corn cobs following the technique described by Garcia et al. (2000) with some modifications. After grinding, the dried corn cobs were dispersed in water under stirring for 24 h. Then, the sample was treated with 1.3% (v/v) sodium hypochlorite in order to remove impurities. Afterwards, an alkaline extraction was carried out by using 4% (v/v) sodium hydroxide solution. The extract was neutralized with acetic acid, and xylan was separated by settling down after methanol addition. Subsequently, several washing steps were performed by using methanol and isopropanol. Finally, the sample was filtered and dried at 50 °C. The characterization process was made from the same single bulk of polymer.

2.3. Fourier transform infrared FT-IR spectroscopy and NMR spectroscopy

The powered samples were analyzed by infrared spectroscopy measured in KBr translucent pellets using a Thermo Nicolet Nexus 470 FT-IR spectrophotometer. 13C-Solid-State NMR experimente was carried out in 4 mm double bearing rotor made from ZrO2 on Bruker DSX 200 MHz spectrometer with resonance frequency at 75.468 MHz. The pulse length was 3.5 μs and the contact time of 1H–13C CP was 2–5 ms.

2.4. Morphology and particle size analysis

Morphology analysis of xylan dry powder was conducted by microscopy on a scanning electron microscope (XL 30 ESEM, Philips, The Netherlands). The frequency of the size distribution and mean particle diameter of xylan were analyzed using a laser light scattering particle size analyzer (Cilas, 920L – France). The mean diameter was calculated by ''The Particle Expert'' software built on the Cilas equipment, and consisted of the ''De Brouckere'' mean diameter. The technique is based on the principle of Fraunhofer diffraction to determine the particle size. Xylan powder samples were pretreated using a liquid dispersing agent (sodium hexametaphosphate) to avoid the flocculation process and, then, dispersed in distilled water (Silva et al., 2007).

2.5. Flow properties

2.5.1. Powder densities

Samples of 2 g of xylan, from the same bulk, were placed into a 25 mL glass graduated cylinder and their volumes were measured. Then, the graduated cylinder was fixed to a mountain plate autotap apparatus (Varian Inc., USA) and run for 1250 taps. The volume (V) and number of taps were recorded after 10, 500 and 1250 taps. The bulk density (ρbul) and the initial

$$\rho bul = \frac{m}{V_0} \quad (1)$$

alculated as

$$\rho tap = \frac{m}{V_{1250}} \quad (2)$$

me powder (V1250), as illustrated in Eqs. (1) and (2) below (Foster and Leatherman, 1995).

$$\frac{\rho tap - \rho bul}{\rho tap} \times 100 \quad (3)$$

$$\frac{\rho tap}{\rho bulk} \quad (4)$$

The compressibility index and Hausner ratio were calculated using Eqs. (3) and (4), respectively.

2.5.3. Angle of repose

The samples of xylan, from the same bulk, were sifted through a glass funnel with 8 mm in diameter. A constant distance of 7 cm was maintained between the funnel and the base for all analyses. The powder was allowed to flow through the funnel onto the base, forming a cone-shaped powder heap. A graduated ruler was used to measure the height of the powder cone and the diameter of the circle. The angle of repose was measured using the height

and the radius of the cone to calculate its tangent (Foster and Leatherman, 1995).

The physicochemical analyses describe above were made in triplicate from the same bulk of produced xylan. The main and standard deviation calculation were made with the software Statistica 6.0 (Statsoft, USA).

3. Results and discussion

3.1. Extraction process

The process of solubilization of the xylan polymer with sodium hydroxide solution followed by the polymer precipitation by methanol provided yield around 11 ± 1.4% (n = 3), which is a satisfactory value when compared with those of classical procedures previously reported (Ebringerova et al., 1998, 2002; Hromadkova et al., 1999). The obtained sample appeared to be a yellowish fine powder. Such properties were similar for three bulks of polymer made in sequence (results not shown).

3.2. Fourier transform infrared spectroscopy and NMR spectroscopy

The xylan infrared spectrum revealed a broad absorption band at 3405 cm^{-1} that can be attributed to the OH stretching associated to polar groups linked through intra- and intermolecular hydrogen bonding (Sun et al., 2005b). Normally, this band occurs as a result of the association between the polymers and its intensity is influenced by the polymer concentration present in the analyzed sample. Furthermore, not only the absorption band at 3405 cm^{-1} but also the band at 1160 cm^{-1} is characteristic of glycosidic groups, the latter being assigned to CC and COC stretching vibrations in hemicelluloses (Sun et al., 1998; Xu et al., 2004). Additionally, a band at 2920 cm^{-1} was detected and is indicative of CH stretching vibrations due to CH2 and CH3 groups. A sharp band at 1635 cm^{-1} was also detected and is related to HOH stretching, which occurs mainly in the amorphous state and crystalline spectra measured in KBr, and belongs to the absorbed water (Kacurakova et al., 1998). According to previous studies, the absorption band observed at 1637 cm^{-1} has been attributed to hydration water present in xylan-type polysaccharides (Kacurakova et al., 1998). A band due to CH2 stretching vibrations was observed near 1460 cm^{-1}. Moreover, an absorption band near 1375 cm^{-1} was detected and it is due to the CH bending vibration present in cellulose and

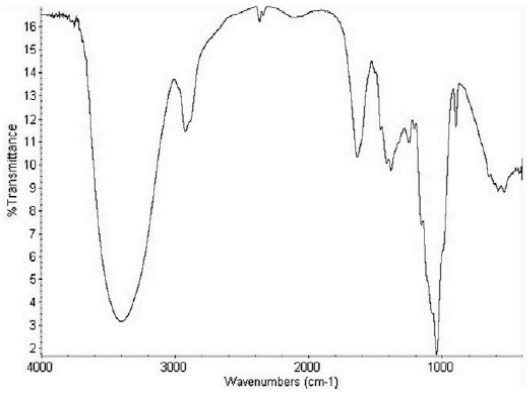


Fig. 1. FT-IR spectrum of xylan powder extracted from corn cobs.

the sugar units in hemicelluloses, was detected in the anomeric region (Sun et al., 2005a) (Fig. 1). The infrared spectroscopy was shown to be able to identify the main organic functions and chemical bonds present in the main and side chains of corn cob xylan. Therefore, such analytical method may be considered a useful and suitable tool for the characterization of xylan.

The RMN spectrum of the dry sample showed broad unresolved peaks that corresponding to be a typical mixture of 4-O-methyl-D-glucuronic acid, L-arabinose and D-xylose and proteins.

3.3. Morphology and particle size analysis

The scanning electron photomicrograph of the xylan extracted from corn cobs is shown in Fig. 2. As can be seen, the xylan powder consists of a mixture of aggregated and non-aggregated particles with irregular morphology, a spherical shape, and a rough surface.

Laser diffraction was employed to analyze the size distribution of xylan (Fig. 3). The mean particle size of the xylan was found to be 30.53 ± 1.5 lm. It was also determined that about 90%, 50%, and 10% of the sample was smaller than 65.39 ± 1.76, 23.34 ± 1.2, and 7.68 ± 0.54 lm, respectively. Fig. 2 shows that the particle size of the xylan was uniformly distributed around the average value with a unimodal distribution.

The span index was used to analyze the polydispersity in the particle size distribution. It is defined as (D90–D10)/D50, where D10, D50, and D90 are the respective particle sizes at 10%, 50%, and 90% cumulative percentage undersize. The span index of xylan particles was 2.47, indicating a low polydispersity for this material.

Properties such as particle size and morphology are important factors in designing composite materials for medical purposes, such as biomedical implants, ceramics, and pharmaceutical tablets. For solid dosage forms, it is well known (Narayan and Hancock, 2005) that the particle properties of excipients and drug compounds affect their brittle–ductile transition characteristics, packing behavior, and tableting performance. The characteristics seen in the morphology and size of xylan particles are attributed to manufacturing conditions employed. However, it is expected that the morphology of the particles will not only affect the bulk density and moisture levels, but it can also influence the rehydration characteristics and volatile losses (Foster and Leatherman, 1995).

3.

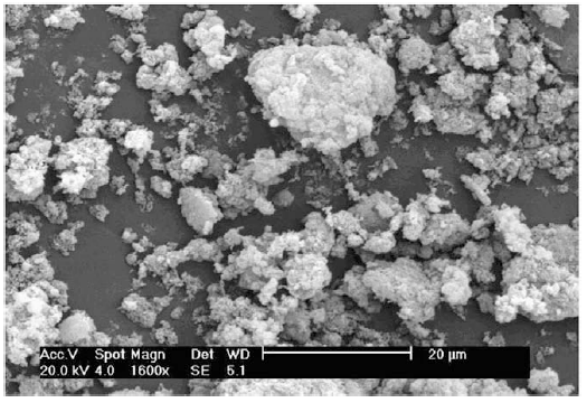


Fig. 2. SEM photomicrograph of xylan powder after extraction from corn cobs.

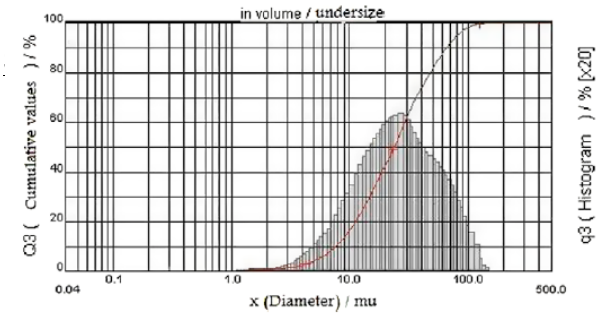


Fig. 3. Size distribution of xylan powder extracted from corn cobs.

The minimum packed volume thus achieved depends on a number of factors including particle size distribution, true density, particle shape, and cohesiveness due to surface forces including moisture. Therefore, the tap density of a material can be used to predict both its flow properties and its compressibility.

On the other hand, the Hausner ratio (Hausner, 1967) and the compressibility index (Carr, 1995), which are measures of interparticle friction and the potential powder arch or bridge strength and stability, respectively, have been widely used to estimate the flow properties of powders. According to Wells, a Hausner ratio value of less than 1.20 is indicative of good flowability of the material, whereas a value of 1.5 or higher suggests a poor flow displayed by the material (Wells, 1988). The compressibility index is also called the Carr index. According to Carr (1995), a value between 5 and 10, 12 and 16, 18 and 21, and 23 and 28 indicates excellent, good, fair, and poor flow properties of the material, respectively. The Hausner ratio and Carr's index values obtained for xylan are listed in Table 1 and suggest that xylan presents extremely poor flow properties. The higher Hausner ratio and the Carr index values of xylan were expected because of its irregular and rough structure, which facilitates entanglements between particles, and consequently, displays poor flow properties (Kumar et al., 2002; Nunthanid et al., 2004).

Although the Hausner ratio and compressibility index have been extensively used in preliminary evaluation of the flow behavior of particulate systems, they are presented as indirect measurements. Therefore, other methods have been frequently applied, as can be seen in the literature. Such methods tend to reach a higher fidelity among the results and the powder technological characteristics since they are known as rheological dynamic measurements. The angle of repose, which evaluates the flowability of powders through an orifice onto a flat surface, is an example of direct measurement (Foster and Leatherman, 1995).

When a free-flowing powder or granulate material is passed through an orifice onto a flat surface, a cone-shaped pile of the

Table 1
Rheological properties of xylan powder extracted from corn cobs.

Property	Value (±standard deviation)
Bulk density	0.1336 (±0.0029) g/ml
Tap density	0.2256 (±0.0059) g/ml
Compressibility index	40.77 (±0.0035)%
Hausner ratio	1.68 (±0.01)
Compactability	32.6 (±0.1) mL[a]
Angle of repose	40.70 (±3.2318)°

[a] Extrapolating the values to 100 mL.

since angles of repose higher than 30° are typical of fair flowing to very cohesive powders (Foster and Leatherman, 1995).

According to the mean angle of repose determined for the xylan particles (40.70°), it may be concluded that the powder has a cohesive flow behavior. This is due to the small and irregular shape of the sample obtained in this study. The fine particles of xylan, having high surface-to-mass ratios, are more cohesive than coarser particles; hence, they are more influenced by gravitational force. In addition, it is generally believed that the flowability of powders decreases as the shapes of particles become more irregular.

4. Conclusions

The main purpose of this work was to obtain and characterize xylan powder from corn cobs. Shape and morphology, size distribution, densities, compressibility index, Hausner ratio, and angle of repose of the powder were investigated, resulting in useful information for medical and pharmaceutical applications of this polymer. Fourier transform infrared and RMN spectroscopy were found to be an eligible technique to identify the xylan polymer.

The xylan powder was characterized as a material with low density and poor and non-free flow. Those properties may provide relevant information and guidelines for processes which require the knowledge of the flow properties of a xylan powder, particularly for that obtained from corn cobs.

In fact, although xylan powder presented unfavorable properties for its application in solid dosage form, this polymer has been shown as a promising material for the development of colon-specific delivery systems. Kayserilioglu et al. (2003) showed that xylan can be used as an additive to produce biodegradable films. Silva et al. (2007) and Nagashima et al. (2008) developed xylan-based a microparticulate system with applications for image analyses and for treatment of Crohn's disease, respectively.

In spite of all these results, the production of xylan from corn cobs could be an important issue for the environment because a ''green'' polymer from a renewable source was, actually, a great challenger for the pharmaceutical industry.

Acknowledgements

The authors are grateful to BNB, CNPq, and Capes for the financial support, CTPETRO-INFRA I and FINEP/LIEM for the SEM analyses; Professor Dr. Luiz Alberto Lira Soares for the contribution of the mechanical characterization, and for the Department of Chemistry of UFRN for FTIR analyses. The authors are also grateful to Glenn Hawes, M.Ed., The University of Georgia, for editing this manuscript.

References

Ai, Z.L., Jiang, Z.Q., Li, L.T., Deng, W., Kusakabe, I., Li, H.S., 2005. Immobilization of Streptomyces olivaceoviridis E-86 xylanase on Eudragit S-100 for xylooligosaccharide production. Process Biochemistry 40, 2707–2714.

Carr, L.L., 1995. Classifying flow properties of solids. Chemical Engineering, 2091–2111.

Collins, T., Gerday, C., Feller, G., 2005. Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiology Reviews 29, 3–23.

Ebringerova, A., Heinze, T., 2000. Xylan and xylan derivatives – biopolymers with valuable properties, 1 – naturally occurring xylans structures, procedures and properties. Macromolecular Rapid Communications 21, 542–556.

Ebringerova, A., Hromadkova, Z., 1997. The effect of ultrasound on the structure and properties of the water-soluble corn hull heteroxylan. Ultrasonics Sonochemistry 4, 305–309.

Ebringerova, A., Hromadkova, Z., 1999. Xylans of industrial and biomedical importance. Biotechnology and Genetic Engineering Reviews, vol. 16. Intercept Ltd. Scientific, Technical and Medical Publishers, Andover, pp. 325–346.

Ebringerova, A., Hromadkova, Z., Hribalova, V., 1995. Structure and mitogenic activities of corn cob heteroxylans. International Journal of Biological Macromolecules 17, 327–331.

Ebringerova, A., Hromadkova, Z., Alfodi, J., Hribalova, V., 1998. The immunologically active xylan from ultrasound-treated corn cobs: extractability, structure and properties. Carbohydrate Polymers 37, 231–239.

Ebringerova, A., Kardosova, A., Hromadkova, Z., Malovikova, A., Hribalova, V., 2002. Immunomodulatory activity of acidic xylans in relation to their structural and molecular properties. International Journal of Biological Macromolecules 30, 1–6.

Foster, T.P., Leatherman, M.W., 1995. Powder characteristics of proteins spray-dried from different spray-dryers. Drug Development and Industrial Pharmacy 21, 1705–1723.

Garcia, R.B., Ganter, J., Carvalho, R.R., 2000. Solution properties of D-xylans from corn cobs. European Polymer Journal 36, 783–787.

Hausner, H.H., 1967. Friction conditions in a mass of metal powders. International Journal of Powder Metallurgy 7, 13.

Hromadkova, Z., Kovacikova, J., Ebringerova, A., 1999. Study of the classical and ultrasound-assisted extraction of the corn cob xylan. Industrial Crops and Products 9, 101–109.

Kacurakova, M., Belton, P.S., Wilson, R.H., Hirsch, J., Ebringerova, A., 1998. Hydration properties of xylan-type structures: an FTIR study of xylooligosaccharides. Journal of the Science of Food and Agriculture 77, 38–44.

Karucákova, M., Ebringerova, A., Hirsch, J., Hromadkova, Z., 1994. Infrared study of arabinoxylans. Journal of the Science of Food and Agriculture 66, 423–427.

Kayserilioglu, B.S., Bakir, U., Yilmaz, L., Akkas, N., 2003. Use of xylan, an agricultural by-product, in wheat gluten based biodegradable films: mechanical, solubility and water vapor transfer rate properties. Bioresource Technology 87, 239–246.

Kumar, V., de la Luz Reus-Medina, M., Yang, D., 2002. Preparation, characterization, and tabletting properties of a new cellulose-based pharmaceutical aid. International Journal of Pharmaceutics 235, 129–140.

Nagashima Jr., T., Oliveira, E.E., da Silva, A.E., Marcelino, H.R., Gomes, M.C.S., Aguiar, L.M., Araujo, I.B., Soares, L.A.L., Oliveira, A.G., Egito, E.S.T., 2008. Influence of the lipophilic external phase composition on the preparation and characterization of xylan microcapsules - A technical note. American Association of Pharmaceutical Scientists (AAPS) PharmSciTech. 9, 814–817.

Narayan, P., Hancock, B.C., 2005. The influence of particle size on the surface roughness of pharmaceutical excipient compacts. Materials Science and Engineering: A 407, 226–233.

Nunthanid, J., Laungtana-Anan, A., Sriamornsak, P., Limmatvapirat, S., Puttipipatkhachorn, S., Lim, L.Y., Khor, E., 2004. Characterization of chitosan acetate as a binder for sustained release tablets. Journal of Controlled Release. 99, 15–26.

Pauly, M., Keegstra, K., 2010. Plant cell wall polymers as precursors for biofuels. Current Opinion in Plant Biology 13, 1–8.

Rubinstein, A., 1995. Approaches and opportunities in colon-specific drug delivery. Critical Reviews in Therapeutic Drug Carrier Systems 12, 101–149.

Silva, S.S., Carvalho, R.R., Fonseca, J.L.C., Garcia, R.B., 1998. Extração e caracterização de xilanas de sabugo de milho. Polímeros: Ciência e Tecnologia 2, 1–9.

Silva, A.K.A., da Silva, E.L., Oliveira, E.E., Nagashima, J.T., Soares, L.A.L., Medeiros, A.C., Araujo, J.H., Araujo, I.B., Carrico, A.S., Egito, E.S.T., 2007. Synthesis and characterization of xylan-coated magnetite microparticles. International Journal of Pharmaceutics 334, 42–47.

Sinha, V.R., Kumria, R., 2001. Polysaccharides in colon-specific drug delivery. International Journal of Pharmaceutics 224, 19–38.

Sinha, V.R., Mittal, B.R., Bhutani, K.K., Kumria, R., 2004. Colonic drug delivery of 5-fluorouracil: an in vitro evaluation. International Journal of Pharmaceutics 269, 101–108.

Sun, R.C., Fang, J.M., Goodwin, A., Lawther, J.M., Bolton, A.J., 1998. Fractionation and characterization of polysaccharides from abaca fibre. Carbohydrate Polymers. 37, 351–359.

Sun, X.F., Xu, F., Sun, R.C., Geng, Z.C., Fowler, P., Baird, M.S., 2005a. Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw. Carbohydrate Polymers 60, 15–26.

Sun, X.F., Xu, F., Zhao, H., Sun, R.C., Fowler, P., Baird, M.S., 2005b. Physicochemical characterisation of residual hemicelluloses isolated with cyanamide-activated hydrogen peroxide from organosolv pre-treated wheat straw. Bioresource Technology 96, 1342–1349.

Wells, J.I., 1988. In: Horwood, E. (Ed.), Pharmaceutical Preformulation: The Physicochemical Properties of Drug Substances. Halsted Press, Chichester, New York.

Whistler, R.L., Smart, C.L., 1953. Polysaccharide Chemistry. Academic Press Inc., New York.

Xu, F., Sun, R.C., Sun, X.F., Geng, Z., Xiao, B., Sun, J.X., 2004. Analysis and characterization of acetylated sugarcane bagasse hemicelluloses. International Journal of Polymer Analysis and Characterization 9, 229–244.

Yang, L., Chu, J.S., Fix, J.A., 2002a. Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. International Journal of Pharmaceutics 235, 1–15.

Yang, L.B., Chu, J.S., Fix, J.A., 2002b. Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. International Journal of Pharmaceutics 235, 1–15.

York, W.S., O'Neill, M.A., 2008. Biochemical control of xylan biosynthesis – which end is up? Current Opinion in Plant Biology 11, 258–265.

CHAPTER III

Article 2: “Influence of the lipophilic external phase composition on the preparation and characterization of xylan microcapsules: A Technical Note”

Le troisième chapitre de cette thèse est constitué de l'article intitulé «Influence of the lipophilic external phase composition on the preparation and characterization of xylan microcapsules - A technical note». Il a été publié dans le journal « AAPS PharmSciTech ».

Cet article décrit la production de microcapsules à base de xylane par réticulation du polymère à l'interface. Ce procédé comprend d'abord une étape d'émulsification de type eau dans huile suivie d'une réaction de réticulation entre les chaînes de polysaccharide. Une solution alcaline contenant du xylane et de l'hydroxyde de sodium est préparée et émulsionnée dans une phase lipophile contenant 5% (p/v) de sorbitane de triestérate. La réaction de réticulation est déclenchée par l'ajout d'une solution à 5% (p/v) de chlorure de téréphtaloyle et terminée par dilution avec du cyclohexane. Puis, les microcapsules sont séparées par centrifugation et lavées plusieurs fois : d'abord avec une solution alcoolique de 2% (v/v) de polysorbate (HLB = 15,85), puis avec de l'éthanol, et enfin avec de l'eau.

Bien qu'il s'agisse d'un des procédés les plus couramment utilisés pour la production de microcapsules, il mérite encore d'être optimisé. Notamment, la phase externe utilisée dans l'étape d'émulsification est l'un des paramètres critiques du procédé de microencapsulation. En effet, la nature de cette phase peut influencer la morphologie des microcapsules, leur état d'agrégation, et surtout la libération du composé actif associé.

Compte tenu de l'importance de la phase externe pour les propriétés des microcapsules obtenues, trois différentes phases lipophiles ont été testées : un mélange 1:4 (v/v) chloroforme : cyclohexane, des triglycérides à chaîne moyenne (Miglyol® 810N) et de l'huile de soja. Par ailleurs, l'influence de la composition de cette phase sur la production, l'aspect macroscopique, la morphologie, la granulométrie, le pH et la stabilité des microcapsules à base de xylane produites par réticulation de polymères à l'interface a été évaluée.

AAPS PharmSciTech, Vol. 9, No. 3, September 2008 (# 2008)
DOI: 10.1208/s12249-008-9115-z

Brief/Technical Note

Influence of the Lipophilic External Phase Composition on the Preparation and Characterization of Xylan Microcapsules—A Technical Note

Toshiyuki Nagashima Jr[1], Elquio Eleamen Oliveira[1,2], Acarília Eduardo da Silva[3], Henrique Rodrigues Marcelino[4], Monique Christine Salgado Gomes[4], Larissa Muratori Aguiar[4], Ivonete Batista de Araújo[4], Luiz Alberto Lira Soares[3], Anselmo Gomes de Oliveira[5], and E. Sócrates Tabosa do Egito[1,3,4,6]

Received 31 January 2008; accepted 23 April 2008; published online 8 July 2008

KEY WORDS: characterization; lipophilic phase; microcapsules; xylan.

INTRODUCTION

Scientific studies on new drug delivery systems have significantly increased in the past few years and this growth is expected to continue in the near future (1). Such systems are of great interest because of their ability to improve drug performance in terms of efficacy, safety, and patient compliance (1). In many cases, conventional drug delivery provides an increase of drug concentration at potentially toxic levels (2). Additionally, the need for delivering drugs with fewer side effects has prompted the development of new drug delivery systems (1).

Xylan is the second most abundant polymer found in hardwoods and annual plants (3), particularly in agricultural residues such as grain hulls, corn cobs, and corn stalks (4). Depending on the botanical source, the backbone chemical structure may vary. However, the majority of xylans present side chains of different sugars such as 4-O-methyl-D-glucoronic acid, O-acetyl-L-arabinose, L-arabinose, and D-glucoronic acid bond by a glycosidic linkage to the backbone (3). Because of its complex structure, the complete degradation of xylan requires the activity of several enzymes, which are specifically produced by human colonic microflora (5). Therefore, xylan microcapsules may represent a novel and promising colon- specific drug delivery system.

[1] Programa de Pós-graduação em Ciências da Saúde, Universidade Federal do Rio Grande do Norte (UFRN), Centro de Ciências da Saúde (CCS), Laboratório de Sistemas Dispersos (LaSid), Natal, RN, Brazil.

[2] Centro de Ciências Biológicas e Sociais Aplicadas (CCBSA), Universidade Estadual da Paraíba, João Pessoa, PB, Brazil.

[3] Programa de Pós-graduação em Ciências Farmacêuticas, UFRN, CCS, LaSID, Natal, RN, Brazil.

[4] Departamento de Farmácia, UFRN, CCS, LaSid, Natal, RN, Brazil.

[5] Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas, UNESP, Rodovia Araraquara, Jaú km 01, 14801-902 Araraquara, SP, Brazil.

[6] To whom correspondence should be addressed. (e-mail: socrates@ufrnet.br)

1530-9932/08/0300-0814/0 # 2008 American Association of Pharmaceutical Scientists

Microcapsules based on natural polymers may be produced by means of a variety of methods. Emulsion solvent extraction, emulsion solvent evaporation and interfacial cross-linking polymerization are the most commonly employed processes for the production of microcapsules (4). One of the critical parameters in the microencapsulation process is the external phase used in the emulsification step (6). In fact, the external phase can influence the microcapsules morphology, their aggregation state, and mainly the release of the microencapsulated active compound (7). Because the production of xylan-based microcapsules is a subject barely studied by scientists worldwide, the aim of this work was to evaluate the influence of the lipophilic external phase composition on the production and mean particle size of xylan microcapsules produced by interfacial cross-linking polymerization.

MATERIALS AND METHODS

Materials

Terephthaloyl chloride and sorbitan triesterate were purchased from Sigma chemical, USA. Isopropanol, chloroform, cyclohexane, ethanol, Polysorbate® 20, and Polysorbate® 80 were obtained from Vetec chemical, Brazil. Medium Chain Triglycerides (MCT), Miglyol 810N, was obtained from Sasol, Germany, and Soybean oil was purchased from Lipoid, Germany. All the chemicals were used as received from manufacturers. Xylan samples were obtained after extraction from corn cobs in our laboratory (8).

Xylan Extraction

The polymer was isolated from corn cobs (8). Briefly, after grinding, the dried corn cobs were dispersed in water under stirring for 24 h. The sample was then treated with 1.3% (v/v) sodium hypochlorite solution in order to remove possible impurities.

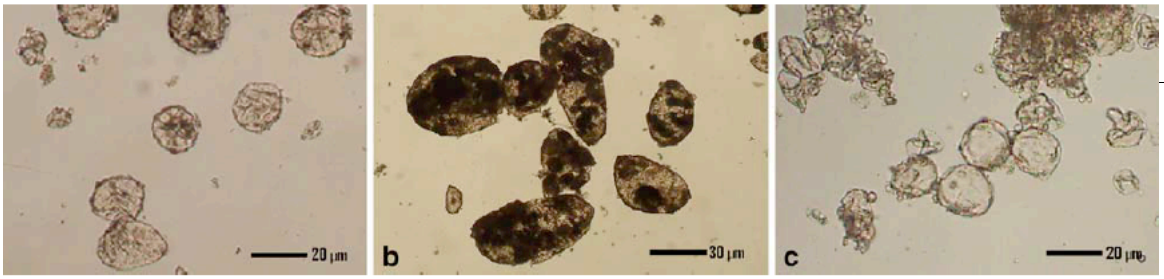


Fig. 1. Optical microscopy of CCM (**a**), SBM (**b**) and MCTM (**c**) at 40×, 40× and 10× magnification, respectively

Then, an alkaline extraction was carried out by using 4% (v/v) sodium hydroxide solution. The bulk was neutralized with acetic acid, and xylan was extracted by settling down after methanol addition. Afterwards, several washing steps were performed by using methanol and isopropanol. Finally, the sample was filtered and dried at 50 °C.

Preparation of Microcapsules

Xylan microcapsules were produced by means of interfacial cross-linking polymerization (8). This method comprises a w/o emulsification step followed by a polymer cross-linking reaction (6). They were produced employing three different lipophilic phases in each experimental setup: 1:4 (v/v) chloroform/cyclohexane, MCT and soybean oil. First, 6 ml of an alkaline solution containing xylan and sodium hydroxide was prepared and, then, emulsified in 30 ml of the lipophilic phase also containing 5% (w/v) sorbitan triesterate. After 10 min, under stirring, the interfacial cross-linking reaction was triggered by adding 40 ml of a 5% (w/v) terephthaloyl chloride. Stirring was maintained for 30 min at room temperature. The reaction was ended by dilution with 30 ml of cyclohexane. Afterwards, the microcapsules were separated by centrifugation and washed several times: first with a 2% (v/v) polysorbate (HLB=15.85) solution in ethanol, then with ethanol, and finally with water.

Characterization of the Microcapsules

Microscopic analysis

Homogeneity and morphological examination of the microcapsules were performed using an optical microscope (Leica, Germany). The samples were observed at 10X and 40X magnification.

pH evaluation

The pH value of the samples was measured at 25°C by a pHmeter (model pH303I, WTW Inc., Germany). The measurement was made by the direct insertion of the electrode probe into the aqueous suspensions containing the microcapsules.

Particle size analysis

The microcapsules were subjected to particle analysis under optical microscopy (Leica microscopic). The samples were placed on glass slides and size measurements of 1,500 microcapsules of each formulation sample were performed according to Ferret's diameter principle using an optical microscope calibrated with a stage micrometer scale (9). The mean particle size was estimated by statistical analysis assuming a normal distribution (graphic method; 10), non-normal distribution (RRSB-net; 11) and considering diameter values in terms of an equivalent sphere (geometric method). For each sample analysis, 500 particles were counted in triplicate.

Table I. Characterization of the Microparticulate Systems

	CCM			MCTM			SBM		
	M1	M2 (R^2)	M3	M1	M2 (R^2)	M3	M1	M2 (R^2)	M3
n diameter	21.2±8.0	21.7±1.8	23.7±8.2	61.3±2.3	71.7±2.9	74.7±4.1	9.6±1.4	13.3±2.1	16.3±1.5
m)		(0.992)			(0.964)			(0.952)	
osis		0.62			3.19			19.52	
litude		46.8			198.0			89.0	
oscopic aspect		Good			Good			Poor	
		4.5			2.8			4.1	

th different lipophilic rature (25±2 °C) for 3 d mean particle size of

RESULTS AND DISCUSSION

Regarding the macroscopic aspect, all the systems presented distinct visual features. The samples obtained by using chloroform/cyclohexane (CCM) and MCT (MCTM) as lipophilic phases were shown to be white suspensions whereas soybean oil (SBM) as a lipophilic phase yielded a system presenting a white flocculated aspect. Furthermore, while CCM and MCTM were relatively homogeneous, SBM showed a higher agglomeration tendency (Fig. 1). Both CCM and SBM systems presented pH values of nearly 4.0 (4.5 and 4.1, respectively), while MCTM system showed a pH value of 2.8 (Table I).

Microscopy analysis showed that both CCM and SBM (Fig. 1) microcapsules were quite spherical in shape while MCTM microcapsules were observed to be larger and oblong in shape (Fig. 1). Also, according to macroscopic aspects, CCM and MCTM showed to remain very stable after storage. However, the SBM presented a high agglomeration with the visual aspect of a cream.

On the other hand, the microcapsules morphology was found to vary depending on the oil phase used for each experimental setup. The MCTM presented to be large and oblong in shape when compared with the other formulations probably due to the phenomenon of coalescence in the emulsion. This phenomenon leads to the formation of large oil droplets which increases the size of the xylan microcapsules (12). Comas et al. (13) studied this phenomenon and the influence of pH on the diameter value of different systems. When pH value underwent a decrease, an increase in the diameter was observed as a consequence of a diminution in their surface activity or due to the fact that the interfacial film formed was less resistant to avoid the coalescence during the homogenization and the cross-linking reaction.

Concerning particle size analysis, the graphic method (M1), RRSB grid (M2), and the geometric method (M3) indicated the following values as mean particle sizes of CCM, MCTM, and SBM, respectively: (1) 21.2±8.0, 21.7±1.8, 23.7± 8.2 μm; (2) 61.3±2.3, 71.7±2.9, 74.7±4.1 μm; and (3) 9.6±1.4, 13.3±2.1, 16.3±1.5 μm. In addition, kurtosis and amplitude were determined by the software Statistica 6.0 (Statsoft Inc., USA; Table I).

As presented above, SBM showed the smallest diameter regardless of the particle size analysis method applied (Table I). For samples of the same formulation, however, the results differed according to the calculation method, disregarding the morphology characteristics presented by the samples (Fig. 1). This profile was imputed to the asymmetrical particle size distribution (non-normal), which was corroborated by the kurtosis parameter determined for CCM, MCTM, and SBM (Table I). In order to describe this type of distribution, the RRSB-net (Rosin–Rammler–Sperling–Bennet) is widely applied (13). Thus, the diameter values obtained by this methodology for CCM, MCTM and SBM, respectively, 21.7± 1.8-, 71.7±2.9-, and 13.3±2.1 μm, were considered more accurate (11,14). The CCM distribution curve amplitude (46.8) was narrower if compared with those of SBM (89) and MCTM (198). These results agreed with the CCM, MCTM, and SBM kurtosis values of 0.62, 3.19, and 19.5, respectively, and indicated that the particle size distributions show a higher probability of extreme values.

After the same period of storage (3 months), SBM systems showed deterioration evidence, probably as a consequence of oxidative reactions of lipids present in the soybean oil. Furthermore, the system containing soybean oil appeared to have low dispersity properties and high tendency to agglomeration. This fact can be imputed to the washing process, which was inefficient due to the high viscosity presented by this oil. Therefore, the residual amount of soybean oil was responsible for the physicochemical changes of the product. On the other hand, CCM and MCTM remained very stable after storage for 3 months, suggesting that the polymeric cross-linking reaction may be carried out with a suitable efficiency in such lipophilic phases. The hypothesis for such approach is based on the characteristic of the diffusibility of these lipophilic phases, which can allow a suitable diffusion of the cross-linking agent on the interfacial surface of the emulsion. Additionally, the viscosity of these oils can contribute to the characteristics obtained in the microcapsules. Soybean oil, MCT, and chloroform/cyclohexane have the viscosity values of 52, 24, and <1 cP, respectively (15,16). The physicochemical properties of the microparticles followed the gradient of viscosity of the oils. The higher the viscosity value, the worse the product characteristics.

SUMMARY AND CONCLUSIONS

This work demonstrates the influence of three different lipophilic phases on the production of xylan microcapsules for medical purposes by means of interfacial cross-linking polymerization with different lipophilic phases. Stability data have indicated the feasibility of the method to produce xylan microcapsules with a suitable stability using MCT or chloroform/cyclohexane as lipophilic phases. However, MCT may be more advantageous than chloroform/cyclohexane due to its well-known use with reduced toxicity in the pharmaceutical industry (17).

ACKNOWLEDGMENTS

This work was supported by CNPq, BNB, and CAPES. The authors are grateful to Glenn Hawes, from the University of Georgia, American Language Program, for editing this manuscript.

REFERENCES

1. R. K. Verma, and S. Garg. Current status of drug delivery technologies and future directions. Pharm. Technol. Online. 2001, pp. 251–14.
2. S. Freiberg, and X. Zhu. Polymer microspheres for controlled drug release. Int. J. Pharm. 282(1–2):1–18 (2004).
3. R. B. Garcia, J. Ganter, and R. R. Carvalho. Solution properties of D-xylans from corn cobs. Eur. Polym. J. 36(4):783–787 (2000).
4. B. Conti, P. Giunchedi, and U. Conte. Cellulose microparticles in drug delivery. STP Pharma. Sci. 7(5):331–342 (1997).
5. E. Schacht, A. Gevaert, E. R. Kenawy, K. Molly, W. Verstraete, P. Adriaensens, R. Carleer, and J. Gelan. Polymers for colon specific drug delivery. J. Control Release. 39(2–3):327–338 (1996).
6. M. C. Levy, and M. C. Andry. Microcapsules prepared through interfacial cross-linking of starch derivatives. Int. J. Pharm. 62 (1):27–35 (1990).

7. A. Billon, L. Chabaud, A. Gouyette, J. M. Bouler, and C. Merle. Vancomycin biodegradable poly(lactide-co-glycolide) microparticles for bone implantation. Influence of the formulation parameters on the size, morphology, drug loading and in vitro release. J. Microencapsul. 22(8):841–852 (2005).
8. R. B. Garcia, T. Nagashima, A. K. C. Praxedes, F. N. Raffin, T. Moura, and E. S. T. do Egito. Preparation of micro and nanoparticles from corn cobs xylan. Polym. Bull. 46(5):371–379 (2001).
9. S. Al-Thyabat, N. J. Miles, and T. S. Koh. Estimation of the size distribution of particles moving on a conveyor belt. Miner. Eng. 20(1):72–83 (2007).
10. DIN66141 (1997) Darstellung von Korn-(teilchen-)grössenverteilungen–Grundlagen, 3 Edition. Berlin, Beuth.
11. DIN66145 (1997) Darstellung von Korn-(teilchen-)grössenverteilungen–RRSB-Netz, 3 Edition. Berlin, Beuth.
12. C. Washington. Stability of lipid emulsions for drug delivery. Adv. Drug Deliv. Rev. 20(2–3):131–145 (1996).
13. D. I. Comas, J. R. Wagner, and M. C. Tomas. Creaming stability of oil in water (O/W) emulsions: Influence of pH on soybean protein–lecithin interaction. Food Hydrocoll. 20(7):990–996 (2006).
14. K. C. B. De Souza, P. R. Petrovick, V. L. Bassani, and G. G. Ortega. The adjuvants Aerosil 200 and Gelita-Sol-P influence on the technological characteristics of spray-dried powders from Passiflora edulis var. flavicarpa. Drug Dev. Ind. Pharm. 26
(3):331–336 (2000).
15. R. Dey, A. K. Singh, and J. D. Pandey. A temperature-dependent viscometric study of binary liquid mixtures. J. Mol. Liq. 137:88–91 (2008).
16. F. Cournarie, M. P. Savelli, W. Rosilio, F. Bretez, C. Vauthier, J. L. Grossiord, and M. Seiller. Insulin-loaded W/O/W multiple emulsions: Comparison of the performances of systems prepared with medium-chain-triglycerides and fish oil. Eur. J. Pharm. Biopharm. 58(3):477–482 (2004).
17. J. P. F. Macedo, L. L. Fernandes, F. R. Formiga, M. F. Reis, T. Nagashima, L. A. L. Soares, and E. S. T. Egito. Micro-emultocrit technique: A valuable tool for determination of critical HLB value of emulsions. AAPS Pharm. Sci. Technol. 7(1):E1–E7 (2006).

CHAPTER IV

Article 3: “Producing hemicellulose-based microparticles using chemical and physico-mechanical approaches as carriers for 5-aminosalicylic acid”

Le quatrième chapitre de cette thèse est dédié à l'article intitulé «Producing hemicellulose-based microparticles using chemical and physico-mechanical approaches as carriers for 5-aminosalicylic acid». Il a été accepté pour publication dans le « Journal of Microencapsulation » le 5 décembre 2012.

Plusieurs méthodes ont été décrites pour la production de microparticules pour l'encapsulation de médicaments, comme la coacervation, l'émulsification et l'extraction de solvant, l'émulsification et l'évaporation de solvant, la réticulation de polymère à l'interface et le séchage par atomisation. Cette dernière est reconnue comme la méthode la plus simple et est maintenant largement utilisée pour produire des microparticules.

Brièvement, le procédé de séchage par atomisation consiste en la pulvérisation d'un liquide d'alimentation sous forme d'un jet en contact avec de l'air chaud suivi par une phase de séchage initiée par le transfert de chaleur. Puis, les particules séchées sont recueillies. Ainsi, cette méthode est donc considérée comme se réalisant en une seule étape, qui est néanmoins relativement complexe car le résultat final dépend d'un ensemble de paramètres.

D'autre part, la réticulation de polymère à l'interface est basée sur une réaction chimique qui se produit entre les groupes fonctionnels des polymères à l'interface autour des gouttelettes et nécessite l'utilisation de solvants organiques ainsi que des agents de réticulation. Il en résulte des microcapsules contenant un noyau aqueux entouré d'une enveloppe polymère.

L'objectif de cette étude était de produire des microparticules à base de xylane et d'Eudragit® S100 contenant de l'acide 5-aminosalicylique (5-ASA). Leur préparation a été réalisée par deux méthodes, la réticulation de polymère à l'interface et le séchage par atomisation, afin d'évaluer l'influence de la méthode de préparation sur la stabilité thermique des microparticules, leur morphologie, leur taille moyenne, le rendement, le taux de chargement et la libération du principe actif. Le 5-ASA a été choisi comme molécule modèle à cause de son utilisation comme traitement de première intention pour les maladies inflammatoires de l'intestin. Le xylane n'est dégradé que par des enzymes bactériennes au niveau du colon. Ainsi, les microparticules à base de xylane sont aptes à délivrer le 5-ASA spécifiquement au niveau de son site d'action.

Producing xylan/Eudragit® 100-based microparticles by chemical and physicomechanical approaches as carriers for 5-aminosalicylic acid

AE Silva[1], EE Oliveira[2], MCS Gomes[3], HR Marcelino[1], T Nagashima Jr[4], AP Ayala[5], AG Oliveira[6], EST Egito[1,3*]

[1] UFRN – Programa de Pós-graduação em Ciências da Saúde (PPgCCSa) – Natal/RN, Brazil

[2] UEPB - Centro de Ciências Biológicas e Sociais Aplicadas – Laboratório de Síntese e Vetorização de Moléculas - João Pessoa/PB, Brazil

[3] UFRN – Departamento de Farmácia (DFAR) – Natal/RN, Brazil

[4] UFCG – Centro de Educação e Saúde - Campus Avançado de Cuité, Cuité/PB, Brazil

[5] UFC – Departamento de Física, Fortaleza/CE, Brazil

[6] UNESP – Departamento de Fármacos e Medicamentos, Araraquara/SP, Brazil

* Corresponding author: Dr. Eryvaldo Sócrates Tabosa do Egito

UFRN – CCS – Departamento de Farmácia

Rua Praia de Areia Branca, 8948.

ZIP code: 59094-450

Phone: +55 84 9431 8816

Fax: + 55 84 3342 9808

E-mail address: socratesegito@gmail.com or socrates@ufrnet.br

Keywords: spray-drying, cross-linking polymerization, polymer interaction, thermal analysis, infrared spectroscopy.

Abstract

Xylan is a biopolymer found in a variety of cell wall plants. Eudragit® S-100 (ES100), a pH-dependent polymer, is used as a coating material in gastroresistant delivery systems. In this study, microparticles based on xylan and ES100 were produced by interfacial cross-linking polymerization and a spray-drying technique in order to investigate their feasibility and the stability of the systems. Size and morphology of the microparticles were characterized by optical and SEM while IR, thermal analysis (TG/DTA), and X-ray diffraction (XRD) evaluated the drug-polymer interactions and the thermal behaviour of the systems. IR confirmed the absence of chemical interaction between the polymers. TG/DTA analysis showed a higher stability for spray-dried microparticles and XRD data proved the amorphous feature of both carriers. The results reveal that xylan/ES100 microparticles can be produced by chemical or physicomechanical ways, the latter being the best option due to the lack of toxic cross-linking agents and easy scale-up.

1. Introduction

Polymeric drug carriers have been largely designed to release drugs, cells and proteins for the treatment of several conditions, such as neurodegenerative disorders and infections as well as inflammatory bowel diseases (IBD) (1-4). As the first-line therapy for patients with IBD, 5-aminosalicylic acid (5-ASA) has been recommended since the 1960s by the rectal administration of gels, foams and enemas with satisfactory efficacy due to its successful action in the induction and maintenance of clinical remission in patients with ulcerative colitis (5). However, those dosage forms offer some issues regarding inconvenience and difficulty of administration, problems with retention and leakage and, consequently, low patient compliance (5). In addition to aminosalycilates, corticosteroids are also very effective for the treatment of mild to severe IBD. Nevertheless, systemic side effects after oral and intravenous administration limit their use. Therefore, targeted delivery systems have been suggested as a new way of treatment for IBD (6).

Biopolymers have been extensively studied and used for drug development in the pharmaceutical field due to their biodegradability and biocompatibility properties. As a hemicellulose found in a variety of cell wall plants, xylan is considered one of the most abundant biopolymer in hard wood and grass (7). Besides the great advantage of a renewable material, this natural polymer has also been related to several profitable properties in the pharmaceutical field such as anti-phlogistic effects, immune function, inhibitory action on the growth rate of tumours, mutagenicity activity and use in the preparation of pH-responsive hydrogels for the controlled release of oral drugs (8). Because it is degraded by enzymes exclusively presented in the colon, xylan seems to be an eligible polymer for colon-specific drug carriers (9). On the other hand, Eudragit® S-100 (ES100) is a synthetic polymer based on methacrylic acid and methyl methacrylate in the ratio of 1:2 of the free carboxyl groups to the ester groups. Among several applications in the pharmaceutical field such as its use as a coating material and in drug delivery systems, ES100 has been utilized in the development of colonic drug carriers due to its pH-dependent dissolution properties (10). Therefore, the association of two polymers with different responsive activities, such as biodegradability or pH-sensitive degradation, corresponds to a very interesting approach to develop novel drug delivery systems (11).

Depending on the formulation and the application of microparticulate drug carriers, several methods have been used for their production, such as coacervation, emulsion solvent extraction, emulsion solvent evaporation, interfacial cross-linking polymerization and spray-

drying (9). This latter one has become the easiest and widely used method to produce microparticles, which is a general term to address polymeric particles in the range of μm in size (12). Briefly, the spray-drying method consists of the atomization of a liquid feed into a spray under hot air contact followed by the drying stage initiated by heat transfer. After the drying process, the dried particles are collected (13). Accordingly, it is considered a one-step, but complex method whose output control depends on a combination of many parameters (14). On the other hand, the interfacial cross-linking polymerization is based on a chemical reaction occurring in the interface around droplets between functional groups of polymers and requires the use of organic solvents and cross-linking agents (15-17). As a result, this method produces microparticles regarded by this paper as microcapsules due to their aqueous inner enclosed by a polymeric shell.

The interactions among drugs and excipients in pharmaceutical dosage forms and their thermal stability are key points to be investigated during pre-formulation studies. For that purpose, the aim of this work was to produce 5-ASA-loaded microparticles based on xylan and ES100 and prepared by a chemical and a physicomechanical approach (cross-linking polymerization and spray-drying, respectively) in order to evaluate the influence of the spray-drying process on the thermal stability of the microparticles. 5-ASA was chosen as a model molecule because of its traditional use as the first-line therapy in IBD (18, 19).

2. Materials and methods

2.1. Materials

Terephthaloyl chloride, sorbitan triesterate and 5-aminosalicylic acid (5-ASA) were purchased from Sigma Aldrich, USA. Chloroform, cyclohexane, ethanol, sodium hydroxide (NaOH), Polysorbate® 20 and Polysorbate® 80 were obtained from Vetec chemical, Brazil. Eudragit® S-100 was purchased from Degussa Röhm Pharma Polymers, Germany. All the chemicals were used as received from manufacturers. Xylan samples were obtained after extraction from corn cobs as reported in the literature (9, 20).

2.2. Methods

2.2.1. Preparation of microparticles

2.2.1.1. Interfacial cross-linking polymerization method

Xylan microcapsules (F1) were produced by means of interfacial cross-linking polymerization (21). This method comprises a w/o emulsification step followed by a polymer

cross-linking reaction (22). First, 6 mL of an alkaline solution containing xylan, 5-ASA and NaOH was prepared and, then, emulsified in 30 mL of 1:4 (v/v) chloroform:cyclohexane containing 5% (w/v) sorbitan triesterate. After 10 min, under stirring, the interfacial cross-linking reaction was triggered by adding 40 mL of a 5% (w/v) terephthaloyl chloride chloroform:cyclohexane 1:4 (v/v) solution. Stirring was maintained for 30 minutes at room temperature. The reaction was ended by dilution with 30 mL of cyclohexane. Afterwards, the microcapsules were separated by centrifugation at 2300 g and withdraw of the supernatant after three washing steps: first with a 2% (v/v) Polysorbate® 20 and 80 mixture (1:1) (HLB=18.85) in ethanol, then with ethanol, and finally with water.

2.2.1.2. Spray-drying technique

Spray-drying was used for preparing three formulations: one, in which cross-linked xylan microcapsules were coated with ES100 at the polymer weight ratio of 1:3, producing formulation F2; and two others in which xylan and ES100 at two weight ratios (1:1 and 1:3), named F3 and F4, respectively, were spray-dried generating microparticles in a one single-step process. F3 and F4 have a 5-ASA loading weight of 15 mg.

In order to prepare ES100-coated xylan microcapsules (F2), ES100 was solubilised in 0.6N NaOH. Subsequently, F1 was dispersed in this alkaline solution and spray-dried at the feed rate of 1.2 mL/min (inlet temperature: 120ºC) using a laboratory spray-dryer (Büchi, Model B-191, Geneva, Switzerland) with a 0.7 mm nozzle.

On the other hand, F3 and F4 were produced by spray-drying dispersions containing 5-ASA and the polymers xylan and ES100 at the weight ratios of 1:1 and 1:3, respectively, in 0.6 N NaOH solution. Table I summarizes the composition of all prepared formulations.

Table I: Composition of the studied formulations

Formulation*	Xylan (mg)	ES100 (mg)	Method
F1	124	-	Interfacial cross-linking polymerization
F2	74.4	223.2	Interfacial cross-linking polymerization followed by spray-drying
F3	150	150	Spray-drying
F4	150	450	Spray-drying

* For all formulations the solvent was a 0.6N NaOH solution containing 15 mg of 5-ASA.

2.2.2. Determination of entrapment efficiency and drug loading

Entrapment efficiency is the percentage of drug encapsulated in the microcapsules compared to the initial (nominal) quantity of the drug loaded in the formulation. 20 mg of microcapsules were weighed and crushed in a glass mortar-pestle. Following, 3 mL of phosphate buffer were added, one by one mL, to the grounded microcapsule powder and the crushing process were continued for 5 minutes each time to get the maximum extraction of 5-ASA in the solvent. The sample so obtained was centrifuged (Excelsa ^{TM} II centrifuge, Model 206 BL, FANEM^{TM} , São Paulo, Brazil), at 2,300 g for 3 min to obtain a clear solution and assayed for the drug content spectrophotometrically at 328 nm. Entrapment efficiency was determined by using the formula below:

$$\text{Entrapment efficiency} = \frac{\text{Real drug content}}{\text{Nominal drug loading}} \text{ X } 100$$

For F1, which was not a dried powder, 3 mL of the sample were withdrawn for this study.

2.2.3. Characterization of the microparticles

2.2.3.1. Microscopic evaluation

The shape and the surface of the microparticles were analysed by optical microscopy (Zeiss, Model Axioscope 50, Oberkochen, Germany) and scanning electronic microscopy (SEM) (Philips, Model XL30, Eidhoven, Netherlands), respectively.

2.2.3.2. Determination of particle size

The microparticles were subjected to particle analysis under optical microscopy (Leica, Model 020507.010, Olympus, Center Valley, PA, United States). The samples were placed on glass slides and size measurements of 1500 microparticles of each sample formulation were performed according to Feret's diameter principle using an optical microscope calibrated with a stage micrometer scale (23).

2.2.3.3. Fourier transform infrared (FT-IR) spectroscopic analysis

FT-IR spectroscopic analysis of the polymers, their physical mixture, and microparticulate formulations (F2 – F4) were carried out at room temperature, in the range of 400-4000 cm^{-1} using KBr pellets in a FT-IR spectrometer, 470 FT-IR (Thermo Nicolet Nexus, Model 470 FT-IR, Waltham, MA, USA).

2.2.3.4. Thermal analysis

Thermogravimetric analysis (TGA) and differential thermoanalysis (DTA) were carried out for xylan, ES100 and 5-ASA, separately. Additionally, the thermal behaviour of the microparticulate systems was also evaluated. TGA and DTA curves were obtained from approximately 20 mg of samples with a thermobalance (NETZCH, Model STA 409 PC/PG, Selb, Germany), using platinum pans under dynamic nitrogen atmosphere (50 mL min^{-1}) at a heating rate of 10°C min^{-1} and temperature range from 25 to 600°C.

2.2.3.5. X-ray diffraction (XRD)

XRD experiments were performed for all the microparticulate systems on a diffractometer (Shimadzu, Model XRD-6000, Kyoto, Japan) with Cu-Kα radiation (30 kV × 30 mA). The XRD pattern was recorded for 2θ of 2º/min ranging from 10° to 80°.

2.2.4. In vitro drug release studies

For the *in vitro* drug release profile of the formulations F2, F3 and F4, 40 mg of the microparticles were weighed and placed in the beaker containing 30 mL of phosphate buffer, pH 7.4. The release study was started by stirring (magnetic) the system at 75 r.p.m and continued for a period of 24 hours. 3 mL of sample was withdrawn after every 0.5, 1, 2, 3, 4, 5, 24 hours and analysed spectrophotometrically at 328 nm. After withdrawing all the samples, they were previously centrifuged at 2,300g for 3 min to settle down any microparticles and only the limpid supernatant was spectrophotometrically evaluated. The cumulative drug release was calculated and expressed as the percentage of release. For F1, which was not a dried sample, the release profile was performed with the 50 mL of the formulation and the time of study was set up between 15 and 2400 minutes.

3. Results and discussion

3.1. Preparation of microparticles

3.1.1. Interfacial cross-linking polymerization

According to the particle size analysis, the mean diameter of xylan microcapsules produced by means of interfacial cross-linking polymerization (F1) was found to be 21.2 ± 8.0 μm. Optical microscopy analysis also confirmed the capsular structure of the microparticles, which were shown to be quite spherical in shape (Fig. 1). The entrapment efficiency for this formulation was 24.98 ± 0.12%. The loss of 75% of the 5-ASA on this process can be inferred for the several washing process, which is mandatory to avoid residual terephthaloyl chloride.

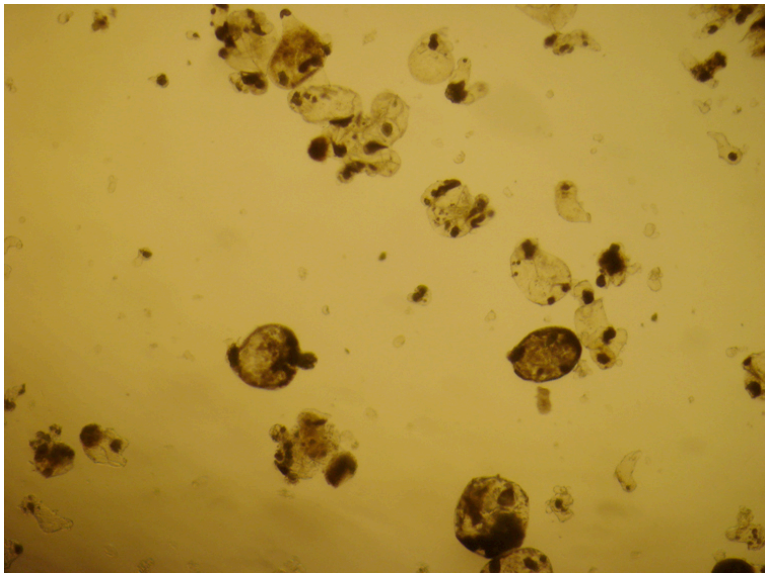


Fig. 1. Optical microscopy image of xylan microcapsules produced by interfacial cross-linking polymerization at 100χ.

3.1.2. Coating of cross-linked microcapsules by spray-drying

ES100-coated xylan microcapsules by spray-drying (F2) were successfully obtained and they were shown to be regular in shape. They appeared to be concave and shrivelled in a manner that is typically related to the particles derived from macromolecules, such as starch, after the spray-drying process (Fig. 2) (24).

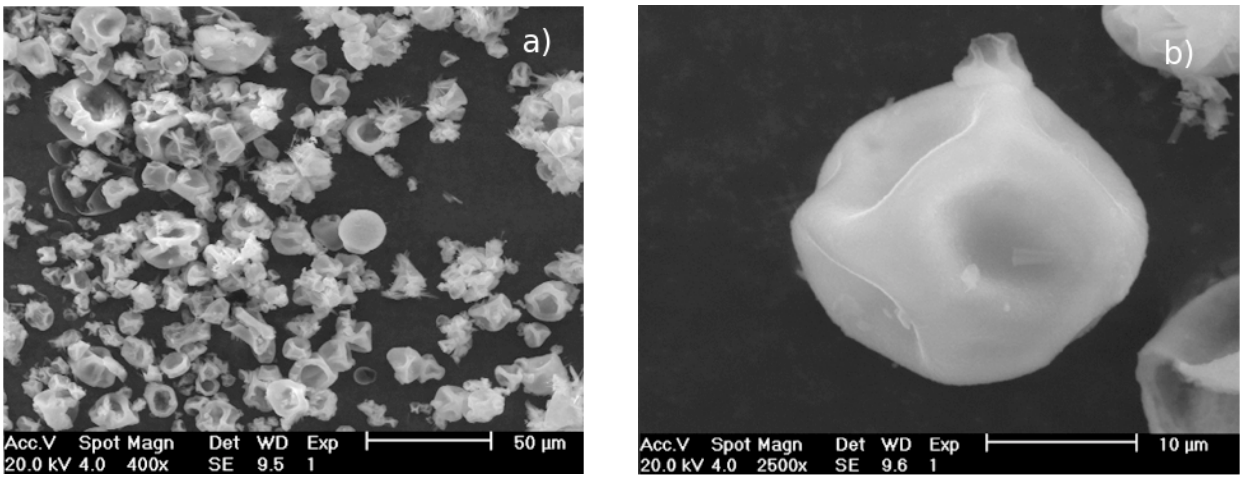


Fig. 2. SEM images of cross-linked xylan microcapsules containing 5-ASA and coated with ES100 by means of spray-drying in the weight polymer ratio of 1:3 in 0.6N NaOH (F2) at 400χ (a), and 2500χ (b), respectively.

The mean particle size of F2 was found to be 10.17 ± 3.02 µm, which is half value of F1, showing that the spray-drying process reduces the particle size of xylan microparticles probably due to the dehydration followed by the coating process with the ES100 polymer. At the end of the spray-drying process, the yield provided for F2 was 50.56 ± 0.15%. The entrapment efficiency of this formulation was 23.61 ± 0.15%, which is quite similar to the F1 formulation. Therefore, as expected, the spray drying process although generating a loss of the totality of microparticles (represented by the 50% of yield) did not degrade the 5-ASA.

3.1.3. One-step spray-drying technique

Concerning the xylan/ES100 microparticles produced directly by the spray-drying technique, all the proposed formulations were satisfactorily produced. They appeared to be an off-white to yellowish fine powder with good apparent flowability. According to the SEM analysis, the spray-dried microparticles were shown to be quite similar in shape, mostly shrivelled and asymmetric (Fig. 3). Such characteristic profile is quite similar to the ones found by Moretti et al. for microspheres of ketoprofen (25) and Kim et al. for the thermo-sensitive microparticles of PNIPAM-grafted ethylcellulose (26), both produced by the spray-drying technique. According to the polymer ratio used for each formulation, the following yields were provided for F3 and F4: 87.00 ± 4.25% and 74.03 ± 8.81%, respectively. The entrapment efficiency for these formulations was 47.30 ± 0.68% and 91.06 ± 0.42% for F3 and F4, respectively. Such variation on the content of 5-ASA on these formulations can be

attributed to the final density of both systems. In fact, the formulation F4 possess two times more mass than F3 due to the high content on Eudragit ES100 on the latter one (Table 1). Therefore, F4 is much denser and more 5-ASA can be entrapped on the polymer network.

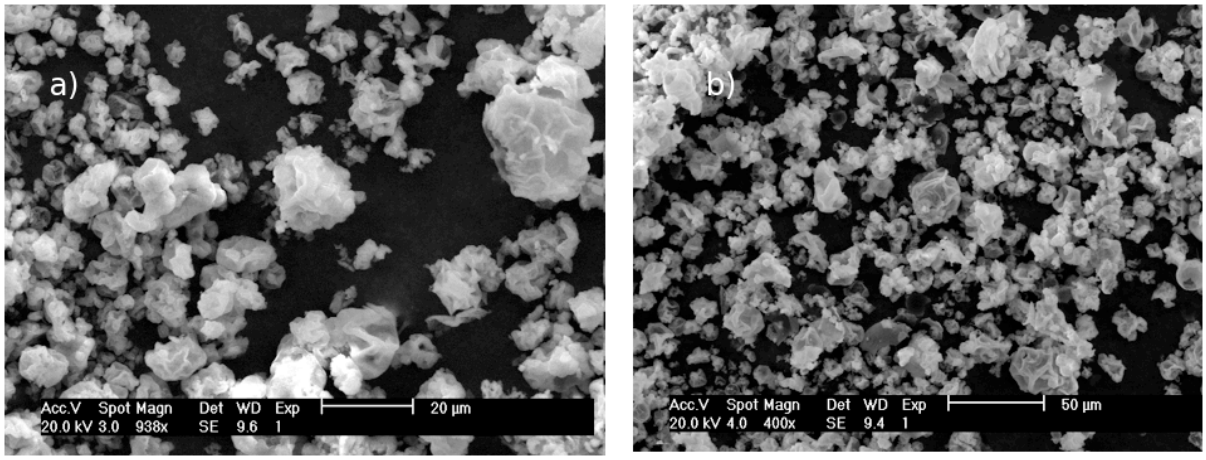


Fig. 3. SEM images of spray-dried xylan and ES100 microparticles in the weight polymer ratio of (a) 1:1 and (b) 1:3 in 0.6N NaOH (F3 and F4, respectively) at 938χ and 400χ, respectively.

The feasibility of spray-drying as a technique for successful production of microparticulate delivery systems has also been evaluated in several studies. It has been used not only as an effective method for scale-up, but also for improvement of photo-stability of drugs, such as pantoprazole (27, 28) or ketoprofen (25). However, such microencapsulation technique has not been reported in the literature as a method the produce microparticles based on xylan.

3.2. Polymer interaction

3.2.1. FT-IR spectroscopy

FT-IR analyses were performed in order to investigate the interaction between the polymers. Therefore, the analyses were carried out for xylan, ES100, their physical mixture, and the microparticles produced by both interfacial cross-linking polymerization and spray-drying.

As expected, the spectra of the raw materials were similar to those found in the literature (9, 29). Thus, the FT-IR spectrum of xylan (Oliveira et al., 2010) showed a broad absorption band at 3405 cm^{-1}, attributed to the OH stretching associated with polar groups

linked through intra- and intermolecular hydrogen bonding (30). This band in addition to the one found at 1160 cm^{-1} is characteristic of glycosidic groups, the latter being assigned to CC and COC stretching vibrations in hemicelluloses (31, 32). Additionally, a band at 2920 cm^{-1}, indicative of CH stretching vibrations due to CH_2 and CH_3 groups, and a band due to CH_2 stretching vibrations, near 1460 cm^{-1}, were also observed. A sharp band around 1635 cm^{-1} can be ascribed to the HOH stretching due to hydration water present in xylan-type polysaccharides (33). Moreover, other bands at 1375 cm^{-1}, 1044 cm^{-1} and a sharp one at 897 cm^{-1} were detected and were typically composed of cellulose and hemicellulose chemical structures (30, 32). Concerning ES100, the FT-IR spectrum showed a typical absorption band at 1730 cm^{-1} that is attributed to C=O vibration from esterified carboxylic groups, besides the bands related to ester at 1150, 1190 and 1275 cm^{-1}. Stretching vibrations attributed to methyl groups were observed between 2900 and 3000 cm^{-1}. Additionally, the presence of bands at 1385, 1450 and 1485cm^{-1} corroborates those findings while a broad band near 3500 cm^{-1} may be attributed to the presence of hydroxil groups and water (9, 29).

The spectrum of the physical mixture (Fig. 4a) is dominated by the bands of ES100 (2920, 1730, 1450, 1275, 1190 and 1150 cm^{-1}). However, the xylan content can be fingerprinted by the wide OH-stretching band at 3405 cm^{-1}, the shoulder of the carbonyl stretching around 1630 cm^{-1} (HOH stretching), and the low wavenumber structure around 550 cm^{-1}.

The FT-IR spectra of powdered samples of cross-linked xylan microcapsules (F1) showed a prominent absorption band at 1276 cm^{-1} that can be attributed to the formation of terephthalic esters (34) due to the contribution of terephthaloyl chloride as the cross-linking agent. This band was observed to be less intense at the FT-IR spectra of cross-linked xylan microcapsules coated with ES100 by spray-drying (F2) (Fig. 4b).

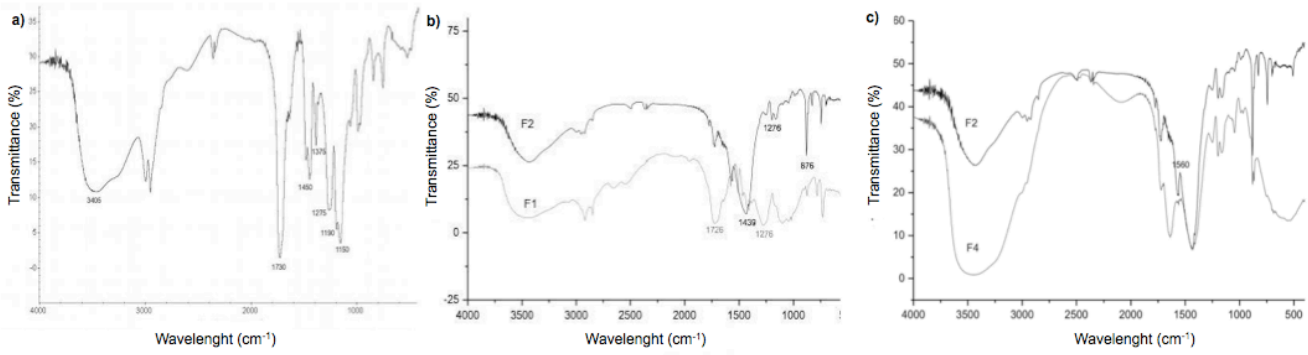


Fig. 4. FT-IR spectrum of a) the physical mixture of xylan and ES100 in the weight ratio of 1:1, b) cross-linked xylan microcapsules (F1) and c) ES100-coated cross-linked xylan microparticles (F2).

Moreover, the absorption band at 1276 cm^{-1} did not appear at the spectra of xylan/ES100 directly spray-dried microparticles (F4) probably because of the absence of terephthaloyl chloride. Also, absorption bands at 1717 and 1276 cm^{-1}, which would reflect the formation of ester bonds from hydroxyl groups of xylan (35), could be detected at neither F2 nor F4 (Fig. 4c). This fact may be evidence that xylan and ES100 do not present chemical interaction during the spray-drying process. In addition, when ES100 is ionized, the carboxylate band shifts from 1728 to 1560 cm^{-1}, corresponding to the anti-symmetrical vibration of COO^- (36). As this band did not show any shift at F2 or F4 spectra, it is suggested that no new chemical bond was formed after spray-drying and the results would confirm that the polymers are physically aggregated to the microparticles (Fig. 4c).

3.2.2. Thermal analysis

TGA curves for xylan, ES100 and 5-ASA revealed relevant events of weight loss for each compound in the range of 250-350°C, 360-430°C and 280-300°C, respectively (Fig. 5a). In order to evaluate the influence of the spray-drying process on the polymers and the drug during the microencapsulation process, TGA and DTA data of these compounds were correlated with their physical mixture (Fig. 5a,b).

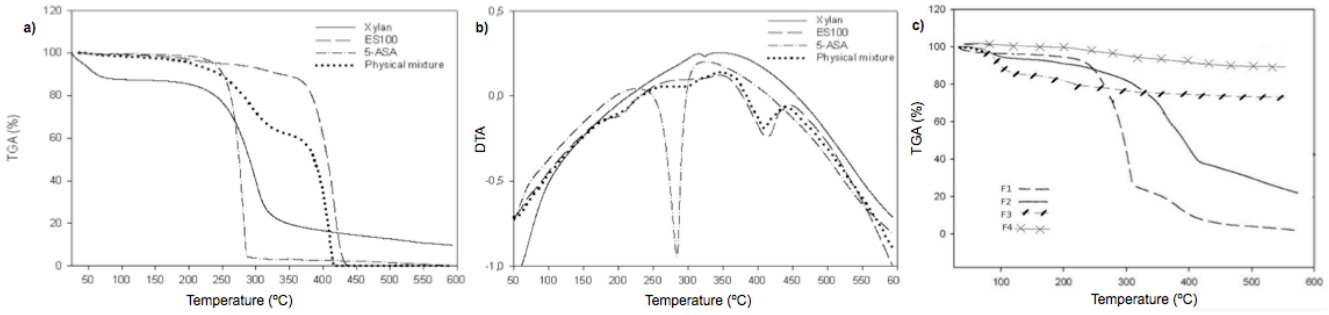


Fig. 5. a) TGA and b) DTA curves for xylan, ES100, 5-ASA and the physical mixture of the polymers and the drug in the weight ratio of 1:1:1, and c) TGA curves for F1, F2, F3 and F4.

From the TGA/DTA data for xylan, a first event of 6.62% weight loss was detected in the range of 62 and 107ºC due to dehydration, and the second and most relevant event of 45% weight loss in the range of 250 and 300ºC due to the polymer decomposition. TGA/DTA data for ES100 revealed a first event between 60 and 104ºC with weight loss of 2.73% due to dehydration and the second endothermic event around 360 and 430ºC with weight loss of

approximately 90% corresponding to the polymer decomposition. Regarding the thermal behaviour of 5-ASA, one single and well-defined endothermic event was observed between 270 and 280ºC with weight loss of 85% due to decomposition of the drug.

Data obtained from the thermogravimetric experiments were used to determine the reaction order by Arrhenius equation (Eq. 1):

$$k = Ae^{(-E/RT)} \qquad \text{(Eq. 1)},$$

where E is the activation energy, A is the pre-exponential factor, R is the universal gas constant, T is the absolute temperature and k is the rate coefficient. Thus, the decomposition events of xylan, ES100 and 5-ASA were found to present zero-order kinetics and the Arrhenius constant for those materials were 7.428×10^{-2}, 1.31×10^{-2}, and 4.426×10^{-2}, respectively. As expected, xylan presented lower stability when compared to ES100 and 5-ASA most likely due to the fact that it is a natural organic material with variable composition depending of its source and its extraction procedure.

The TGA/DTA curves for the physical mixture of both polymers and the drug at a proportional weight ratio revealed behaviour similar to that demonstrated by TGA/DTA curves for xylan, ES100 and 5-ASA, separately. Endothermic events attributed to weight losses were detected at approximately the same temperature ranges for each compound. An interesting fact is that the decomposition of xylan and 5-ASA occurred at very similar temperature ranges and because of that it may not be possible to clearly identify the decomposition events individually.

Concerning the thermal behaviour of the microparticulate systems, ES100-coated cross-linked xylan microparticles (F2) and directly spray-dried ES100/xylan microparticles (F3 and F4) were evaluated and thermal data was correlated with cross-linked xylan microcapsules (F1) (Fig. 5c). Table II summarizes the thermal analysis data.

Table II. Thermal analysis data for xylan, ES100, 5-ASA and the microparticulate systems

Formulation	First event	Second event
F1	T = 200 – 300ºC Δm = 80%	T = 300 – 400ºC Δm = 20%
F2	T = 85 – 110°C Δm = 20%	T = 290 – 330°C Δm = 10%
F3	T = 97.9 – 130.4ºC Δm = 17.06%	T = 229.5 – 356.2ºC Δm = 4.16%

F4	T = 78 – 110ºC Δm = 5.04%	T = 220 – 600ºC Δm = 7.32%
Xylan	T = 62.7 – 107.4ºC Δm = 6.62%	T = 249.2 – 288.6ºC Δm = 45.37%
ES100	T = 62.5 – 104.2ºC Δm = 2.73%	T =362.4 – 427-0ºC Δm = 87.53%
5-ASA	T = 274.2 – 283.4ºC Δm = 82.27%	-

* T = Temperature range; # Δm = Weight loss

By analysing the TGA curve for F1, it was possible to observe two events. The first one corresponded to a weight loss of approximately 80% at the range of 200°C to 300°C while the second event occurred from 300°C to 400°C and corresponded to the final 20% of the sample weight. Such events may be attributed to both the decomposition of 5-ASA and non-cross-linked xylan likely present in the formulation and the decomposition of cross-linked xylan, respectively. The fact that the decomposition of 5-ASA occurred at the same temperature may be explained by the absence of chemical interaction with the polymer during interfacial cross-linking polymerization (37). When ES100 was added to the formulation in order to coat the cross-linked xylan microcapsules or produce spray-dried xylan microparticles, a higher thermal stability was clearly evidenced by the weight loss of only 60% for F2 and at more than 400°C. This fact may be explained by the high stability of ES100, corroborated by its low Arrhenius constant when compared to xylan and 5-ASA.

F3 and F4 showed a weight loss of approximately 20 and 10% at nearly 200 and 300ºC, respectively, probably due to dehydration of xylan, and their masses remained unchanged until 600ºC. A possible reason for the absence of weight loss at the 5-ASA temperature of decomposition is its complete entrapment in the polymer matrix as reported elsewhere (38). Additionally, it is possible that a significant reduction in drug crystallinity happened after encapsulation as reported by other studies and corroborated by the absence of the crystallinity profile of the drug in the XRD curves (38-40). According to the Arrhenius equation, the decomposition of F3 presents first-order kinetics. Thus, concentration of its formulation has a considerable influence on the decomposition of the system. On the other hand, concerning F4, this phenomenon did not occur because it presents zero-order kinetics decomposition.

When interfacial cross-linking polymerization (F2) was compared to the spray-drying technique (F4) regarding the thermal stability, TGA curves demonstrated a relatively slight increase in the stability of the spray-dried system (F4). Although some studies have demonstrated the importance of adding a cross-linking agent after the spray-drying process in order to stabilize the drug release from the microparticles (39, 41), depending of the polymer and the drug encapsulated, the strategy of spray-drying previously cross-linked microparticles has proven to be a suitable method to increase the stability of drug release by polymeric carriers (38, 42, 43). No other studies focusing on the concurrent comparison between the thermal stability of cross-linked and spray-dried polymeric particles were found in the literature. Nevertheless, Silva-Junior et al. performed a comprehensive thermal analysis of spray-dried particles containing ciprofloxacin hydrochloride and triamcinolone (44, 45), which gave rise to this work.

3.3. X-ray diffraction (XRD)

Physical properties of drugs and pharmaceutical excipients in the solid state are of great interest in that they can affect not only the product development and formulation, but also their biological effect. Crystallinity of a substance may affect its stability in the solid state, its flowability properties and its dissolution rate, which affects the substance bioavailability. Total or partial loss of crystallinity results in a significant increase in solubility and dissolution rate of drugs (46).

The spray-drying technique generally produces amorphous compounds. This fact is usually attributed to the speed of the drying process, which hinders the organization of a crystalline structure. It is well known that amorphous solid molecules are organized randomly and, thus, low energy is required to separate them and, consequently, their dissolution is faster (47, 48). Based on this fact, the development of formulation containing a drug in its amorphous form is normally advantageous regarding dissolution and bioavailability concerns.

By analysing the XRD curves for both cross-linked (F1 and F2) and spray-dried (F3 and F4) microparticulate systems, one broad peak related to the presence of ES100 at approximately 30º could be detected for F2, F3 and F4 (Fig. 6). Besides, the intensity of this peak increased according to the increment in the amount of ES100 in the formulation (Fig. 6). Typical peaks of 5-ASA could also be detected for all the samples, although they indicated a

less intense drug crystallinity probably due to their partial solubilization in the amorphous polymer, xylan, as similarly occurs during the hot-melt extrusion (49).

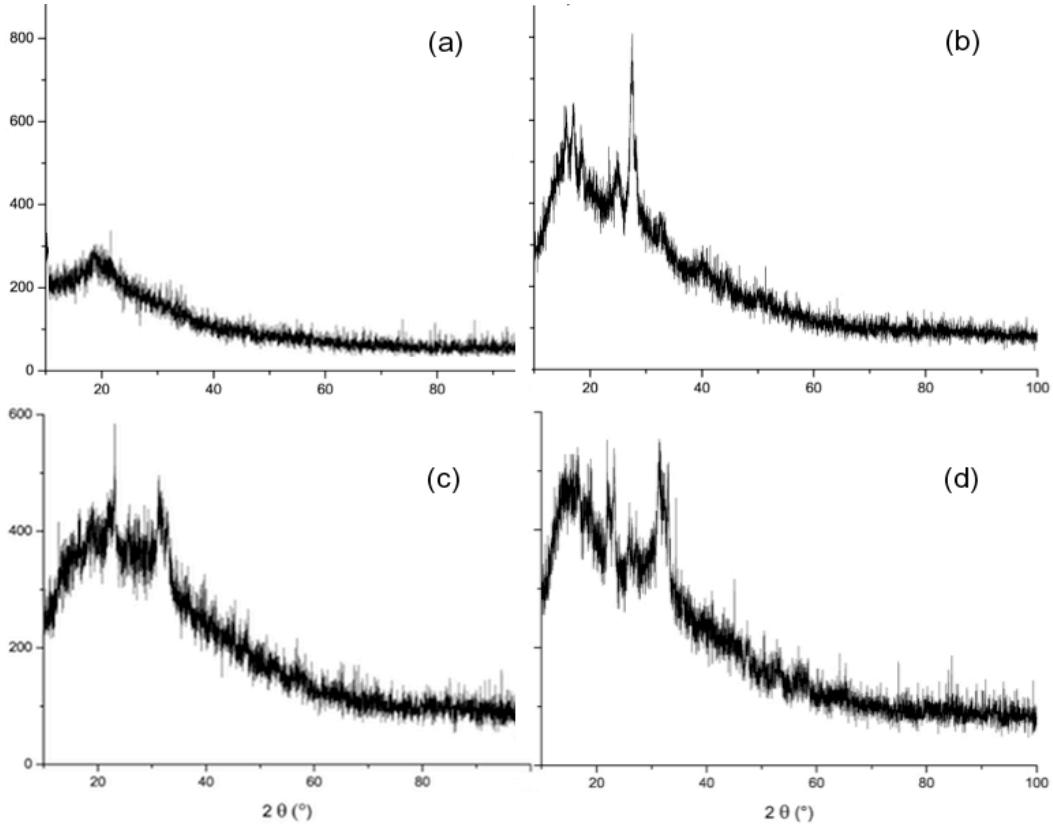


Fig. 6. XRD curves for xylan microparticles. (a) Microparticles produced by single cross-linking reaction (F1), (b) Microparticles produced by cross-linking reaction followed by coating with ES100 polymer by spray-drying (F2), (c) Microparticles produced with xylan and ES100 (1:1) by the spray-drying technique (F3) and (d) Microparticles produced with xylan and ES100 (1:3) by the spray-drying technique (F4).

Additionally, in the XRD curves for xylan/ES100 microparticles produced directly by the spray-drying technique, it was possible to observe the profile of a predominantly amorphous compound with a slight crystallinity due to the presence of a large halo at 2θ from 10º to 40º, which is typical of materials subjected to the spray-drying process (Fig. 6c, d).

3.4. In vitro drug release studies

The release profile of the formulation F1 obeys the Higuchi model with the almost totality of release at around 40 hours (2,400 minutes) (Figure 7). It is interesting to note that although presenting low entrapment efficiency, F1 releases 5-ASA into the media and this

phenomenon happens at pH 7.4. Our team has previously demonstrated that the xylan polymerisation process by itself is not able to produce microparticles with no pores on their structure, probably due to the intrinsic molecular weight variation of this polymer produced during its extraction process (Nagashima et al., 2008). In fact, this is the reason why on this work it was attempted to produce xylan microparticles combined with Eudragit S100. The objective was to avoid the pore formation on the microparticles structure by using another polymer with pH sensitive property.

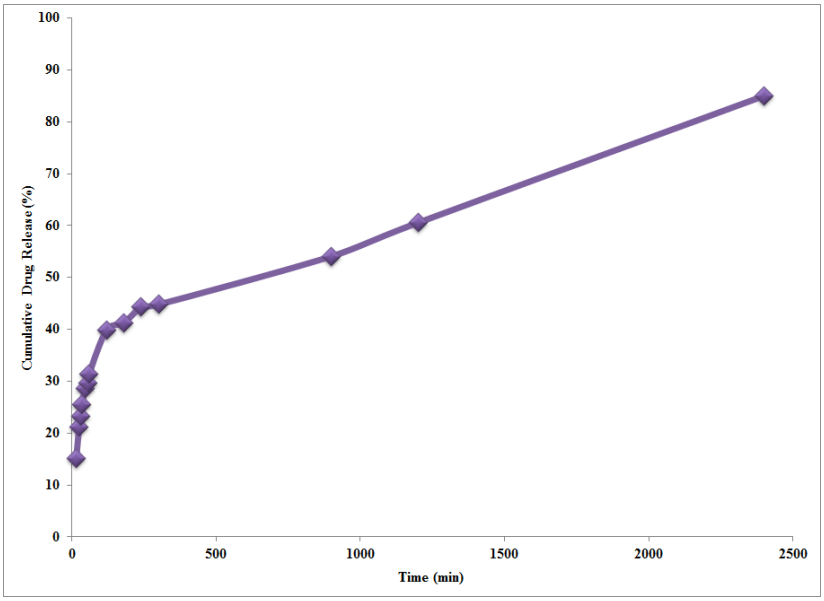


Fig. 7. Cumulative drug release profile of microparticles prepared by interfacial cross-linking polymerization (Formulation F1).

On the other hand, the release profile of F2, F3 and F4 formulations was quite dissatisfying (Figure 8). All the formulations released the 5-ASA as soon as the powder was dispersed into the phosphate buffer media. The reason for such release was that the NaOH used to dissolve the polymers and the drug was still present on the spray-dried formulation and the buffer was not strong enough to neutralize it. In fact, the pH measured on the release media was around 9.0, which completely dissolves the microparticles into the media.

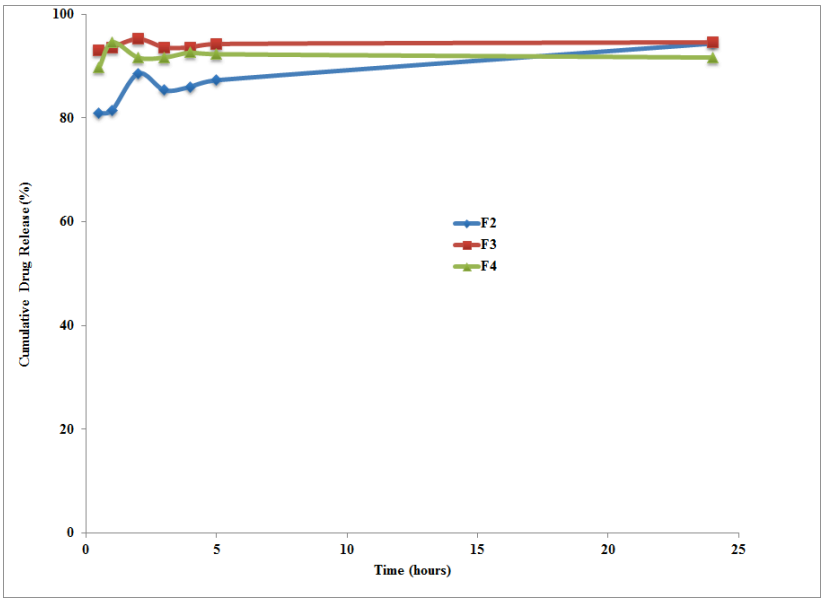


Fig. 8. Cumulative drug release profile of microparticles prepared by interfacial cross-linking polymerization and spray-drying (Formulation F2) or spray-drying alone (Formulations F3 and F4).

Besides the unfortunate data revealed by the release studies, the totality of physicochemical data presented here, demonstrated that the xylan based microparticles containing ES100 can be a very promising system to treat IBD in the colon. The NaOH presented on the formulation prepared by the spray drying process can be easily removed by the use of a diluent with an acid pH to neutralize it.

4. Conclusions

Both cross-linking interfacial polymerization and the spray-drying technique successfully produced well-defined microparticles based on xylan and ES100 containing 5-ASA and presenting suitable physical characteristics and satisfactory yields. However, the spray-drying technique produced more stable microparticles regarding the thermal behaviour when compared to the cross-linked ones. In addition, it was also possible to coat cross-linked xylan microcapsules by means of the spray-drying technique.

Thermogravimetric analysis provided data to the prediction of the higher stability of spray-dried microparticles when compared to the cross-linked ones. FT-IR spectroscopy demonstrated the lack of relevant interactions among the compounds of the formulation. Finally, XRD was able to evidence the influence of the microencapsulation methods on the crystallinity of the systems.

The totality of the results presented here reveals that although the biopolymer xylan can be used to produce microparticles by chemical or physicomechanical ways, the latter could be the better option because it avoids the use of cross-linking agents, frequently responsible for important side effects in pharmaceutical products. In addition, spray-drying is a technique easily transposable for an industrial scale.

5. Acknowledgements

This work was supported by CNPq and CAPES. The authors are grateful to Prof. Dr. Josue Mendes, from the Universidade Federal do Ceará, and Prof. Dr. Arnóbio Antonio Silva-Júnior, from the Universidade Federal do Rio Grande do Norte, for the thermogravimetric analysis; and CTPETRO-INFRA I and FINEP/LIEM for the SEM images and XRD analysis. The authors also thank Glenn Hawes, from the University of Georgia – American Language Program, for editing this manuscript.

6. References

1. Leonardi D, Salomón CJ, Lamas MC, Olivieri AC. Development of novel formulations for Chagas' disease: Optimization of benznidazole chitosan microparticles based on artificial neural networks. International Journal of Pharmaceutics. 2009;367(1-2):140-7.
2. Misra A, Hickey AJ, Rossi C, Borchard G, Terada H, Makino K, et al. Inhaled drug therapy for treatment of tuberculosis. Tuberculosis. 2011;91(1):71-81.
3. Tan ML, Choong PFM, Dass CR. Recent developments in liposomes, microparticles and nanoparticles for protein and peptide drug delivery. Peptides. 2010;31(1):184-93.
4. Wilson B, Samanta MK, Santhi K, Sampath Kumar KP, Ramasamy M, Suresh B. Significant delivery of tacrine into the brain using magnetic chitosan microparticles for treating Alzheimer's disease. Journal of Neuroscience Methods. 2009;177(2):427-33.
5. Sonu I, Lin MV, Blonski W, Lichtenstein GR. Clinical pharmacology of 5-ASA compounds in inflammatory bowel disease. Gastroenterology Clinics of North America. 2010;39(3):559-99.
6. Varshosaz J, Ahmadi F, Emami J, Tavakoli N, Minaiyan M, Mahzouni P, et al. Microencapsulation of budesonide with dextran by spray drying technique for colon-targeted delivery: an in vitro/ in vivo evaluation in induced colitis in rat. J Microencapsulation. 2011;28(1):62-73.
7. Kacuráková M, Capek P, Sasinková V, Wellner N, Ebringerová A. FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses. Carbohydrate Polymers. 2000;43(2):195-203.
8. Sun X-F, Wang H-h, Jing Z-x, Mohanathas R. Hemicellulose-based pH-sensitive and biodegradable hydrogel for controlled drug delivery. Carbohydrate Polymers. 2013;92(2):1357-66.
9. Oliveira EE, Silva AE, Nagashima Jr T, Gomes MCS, Aguiar LM, Marcelino HR, et al. Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization. Bioresource Technology. 2010;101(14):5402-6.
10. Yoo JW, Giri N, Lee CH. pH-sensitive Eudragit nanoparticles for mucosal drug delivery. International Journal of Pharmaceutics. 2011;403(1-2):262-7.

11. Tian H, Tang Z, Zhuang X, Chen X, Jing X. Biodegradable synthetic polymers: Preparation, functionalization and biomedical application. Progress in Polymer Science. 2012;37(2):237-80.
12. Kreuter J. Nanoparticles and microparticles for drug and vaccine delivery. Journal of anatomy. 1996;189 (Pt 3):503-5.
13. Tewa-Tagne P, Briançon S, Fessi H. Spray-dried microparticles containing polymeric nanocapsules: Formulation aspects, liquid phase interactions and particles characteristics. International Journal of Pharmaceutics. 2006;325(1-2):63-74.
14. Durrigl M, Kwokal A, Hafner A, Segvic Klaric M, Duminic A, Cetina-Cizmek B, et al. Spray dried microparticles for controlled delivery of mupirocin calcium: Process-tailored modulation of drug release. Journal of Microencapsulation. 2011;28(2):108-21.
15. Salaun F, Bedek G, Devaux E, Dupont D, Gengembre L. Microencapsulation of a cooling agent by interfacial polymerization: Influence of the parameters of encapsulation on poly(urethane-urea) microparticles characteristics. Journal of Membrane Science. 2011;370(1-2):23-33.
16. Yufen Z, Rochefort D. Comparison of emulsion and vibration nozzle methods for microencapsulation of laccase and glucose oxidase by interfacial reticulation of poly(ethyleneimine). Journal of Microencapsulation. 2010;27(8):703-13.
17. Li BZ, Wang LJ, Li D, Chiu YL, Zhang ZJ, Shi J, et al. Physical properties and loading capacity of starch-based microparticles crosslinked with trisodium trimetaphosphate. Journal of Food Engineering. 2009;92(3):255-60.
18. Ford AC, Khan KJ, Sandborn WJ, Hanauer SB, Moayyedi P. Efficacy of Topical 5-Aminosalicylates in Preventing Relapse of Quiescent Ulcerative Colitis: A Meta-analysis. Clinical Gastroenterology and Hepatology. 2012;10(5):513-9.
19. Markowitz J. Current treatment of inflammatory bowel disease in children. Digestive and Liver Disease. 2008;40(1):16-21.
20. Garcia RB, Nagashima Jr T, Praxedes AKC, Raffin FN, Moura TFAL, Egito EST. Preparation of micro and nanoparticles from corn cobs xylan. Polymer Bulletin. 2001;46(5):371-9.
21. Nagashima T, Oliveira EE, da Silva AE, Marcelino HR, Gomes MC, Aguiar LM, et al. Influence of the lipophilic external phase composition on the preparation and characterization of xylan microcapsules--a technical note. AAPS PharmSciTech. 2008;9(3):814-7.
22. Lévy MC, Andry MC. Microcapsules prepared through interfacial cross-linking of starch derivatives. International Journal of Pharmaceutics. 1990;62(1):27-35.
23. Al-Thyabat S, Miles NJ, Koh TS. Estimation of the size distribution of particles moving on a conveyor belt. Minerals Engineering. 2007;20(1):72-83.
24. Fu ZQ, Wang LJ, Li D, Adhikari B. Effects of partial gelatinization on structure and thermal properties of corn starch after spray drying. Carbohydrate Polymers. 2012;88(4):1319-25.
25. Moretti MDL, Gavini E, Juliano C, Pirisino G, Giunchedi P. Spray-dried microspheres containing ketoprofen formulated into capsules and tablets. Journal of Microencapsulation. 2001;18(1):111-21.
26. Kim BY, Kang HS, Kim JD. Thermo-sensitive microparticles of PNIPAM-grafted ethylcellulose by spray-drying method. Journal of Microencapsulation. 2002;19(5):661-9.
27. Raffin RP, Colomé LM, Schapoval EES, Pohlmann AR, Guterres SS. Increasing sodium pantoprazole photostability by microencapsulation: Effect of the polymer and the preparation technique. European Journal of Pharmaceutics and Biopharmaceutics. 2008;69(3):1014-8.

28. Raffin RP, Jornada DS, Ré MI, Pohlmann AR, Guterres SS. Sodium pantoprazole-loaded enteric microparticles prepared by spray drying: Effect of the scale of production and process validation. International Journal of Pharmaceutics. 2006;324(1):10-8.
29. Cilurzo F, Minghetti P, Selmin F, Casiraghi A, Montanari L. Polymethacrylate salts as new low-swellable mucoadhesive materials. Journal of Controlled Release. 2003;88(1):43-53.
30. Sun XF, Xu F, Sun RC, Geng ZC, Fowler P, Baird MS. Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw. Carbohydrate Polymers. 2005;60(1):15-26.
31. Xu F, Sun RC, Sun XF, Geng Z, Xiao B, Sun J. Analysis and characterization of acetylated sugarcane bagasse hemicelluloses. International Journal of Polymer Analysis and Characterization. 2004 2004/02/23;9(4):229-44.
32. Sun R, M. Fang J, Goodwin A, M. Lawther J, J. Bolton A. Fractionation and characterization of polysaccharides from abaca fibre. Carbohydrate Polymers. 1998;37(4):351-9.
33. Kačuráková M, Belton PS, Wilson RH, Hirsch J, Ebringerová A. Hydration properties of xylan-type structures: an FTIR study of xylooligosaccharides. Journal of the Science of Food and Agriculture. 1998;77(1):38-44.
34. Larionova NV, Kazanskaya NF, Larionova NI, Ponchel G, Duchene D. Preparation and characterization of microencapsulated proteinase inhibitor aprotinin. Biochemistry. 1999;64(8):857-62.
35. Devy J, Balasse E, Kaplan H, Madoulet C, Andry MC. Hydroxyethylstarch microcapsules: A preliminary study for tumor immunotherapy application. International Journal of Pharmaceutics. 2006;307(2):194-200.
36. Raffin RP, Colomé LM, Pohlmann AR, Guterres SS. Preparation, characterization, and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles. European Journal of Pharmaceutics and Biopharmaceutics. 2006;63(2):198-204.
37. Agnihotri SA, Aminabhavi TM. Controlled release of clozapine through chitosan microparticles prepared by a novel method. Journal of Controlled Release. 2004;96(2):245-59.
38. Simonoska Crcarevska M, Glavas Dodov M, Goracinova K. Chitosan coated Ca-alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa. European Journal of Pharmaceutics and Biopharmaceutics. 2008;68(3):565-78.
39. Mladenovska K, Cruaud O, Richomme P, Belamie E, Raicki RS, Venier-Julienne MC, et al. 5-ASA loaded chitosan-Ca-alginate microparticles: Preparation and physicochemical characterization. International Journal of Pharmaceutics. 2007;345(1-2):59-69.
40. Stulzer HK, Tagliari MP, Parize AL, Silva MAS, Laranjeira MCM. Evaluation of cross-linked chitosan microparticles containing acyclovir obtained by spray-drying. Mater Sci Eng C. 2009;29(2):387-92.
41. Desai KGH, Park HJ. Preparation of cross-linked chitosan microspheres by spray drying: Effect of cross-linking agent on the properties of spray dried microspheres. J Microencapsulation. 2005;22(4):377-95.
42. Desai KGH, Park HJ. Encapsulation of vitamin C in tripolyphosphate cross-linked chitosan microspheres by spray drying. J Microencapsulation. 2005;22(2):179-92.
43. Möbus K, Siepmann Jr, Bodmeier R. Zinc-alginate microparticles for controlled pulmonary delivery of proteins prepared by spray-drying. European Journal of Pharmaceutics and Biopharmaceutics. 2012;81(1):121-30.
44. Silva-Junior AA, de Matos JR, Formariz TP, Rossanezi G, Scarpa MV, Egito EST, et al. Thermal behavior and stability of biodegradable spray-dried microparticles containing triamcinolone. International Journal of Pharmaceutics. 2009;368(1-2):45-55.

45. Silva-Junior AA, Scarpa MV, Pestana KC, Mercuri LP, Matos JR, Oliveira AG. Thermal analysis of biodegradable microparticles containing ciprofloxacin hydrochloride obtained by spray drying technique. Thermochim Acta. 2008;467(1-2):91-8.
46. Shoukri RA, Ahmed IS, Shamma RN. In vitro and in vivo evaluation of nimesulide lyophilized orally disintegrating tablets. European Journal of Pharmaceutics and Biopharmaceutics. 2009;73(1):162-71.
47. Cheow WS, Hadinoto K. Self-assembled amorphous drug-polyelectrolyte nanoparticle complex with enhanced dissolution rate and saturation solubility. Journal of Colloid and Interface Science. 2012;367(1):518-26.
48. Van den Mooter G. The use of amorphous solid dispersions: A formulation strategy to overcome poor solubility and dissolution rate. Drug Discovery Today: Technologies. 2012;9(2):e79-e85.
49. Bruce LD, Shah NH, Waseem Malick A, Infeld MH, McGinity JW. Properties of hot-melt extruded tablet formulations for the colonic delivery of 5-aminosalicylic acid. European Journal of Pharmaceutics and Biopharmaceutics. 2005;59(1):85-97.

SECTION II
AmB-LOADED MICROEMULSIONS

CHAPTER V

Article 4: “Challenges and recent advances on the delivery of poorly soluble drugs: An update on the development of carriers for amphotericin B”

Le cinquième chapitre de cette thèse est constitué par un article de revue intitulé « Challenges and recent advances on the delivery of poorly soluble drugs: An update on the development of carriers for amphotericin B ».

L'amphotéricine B (AmB) est une molécule très efficace contre les infections fongiques systémiques. Elle est également utilisée en traitement secondaire de la leishmaniose, une maladie parasitaire qui affecte des centaines de millions de personnes dans plus de 60 pays. En dépit de son importance thérapeutique, les propriétés physico-chimiques de l'AmB posent des problèmes critiques à sa formulation et son utilisation thérapeutique. L'inconvénient le plus important réside dans sa solubilité très faible dans l'eau ; ce qui complique la conception de nouvelles formulations et conduit également à une très faible biodisponibilité par voie orale. L'utilisation de l'AmB est ainsi limitée à la perfusion intraveineuse (IV) ou à l'application locale. En outre, un certain nombre d'effets secondaires aigus sont induits par la perfusion, ce qui amène à raccourcir le traitement et nuit au succès thérapeutique.

Plusieurs formulations lipidiques ont été développées comme systèmes de délivrance potentiels pour AmB. Toutefois, les produits commercialisés sont extrêmement coûteux et toujours conçus pour l'administration IV.

Cet article tente à donner un aperçu des publications parues pendant les dix dernières années présentant des résultats pertinents dans le domaine de la technologie pharmaceutique et en particulier sur le développement de systèmes de délivrance d'AmB rapportant des résultats prometteurs. Dans ce but, une enquête systématique de la littérature a été réalisée sur la base de données compilées par l'ISI Web of Knowledge et National Center for Biotechnology Information et publiées entre 2002 et 2012 utilisant comme mots-clés « nanotechnologie », « amphotéricine B », « système colloïdal d'administration de médicaments », « microémulsion », « nanoémulsion », « émulsion », « micelles », « amphiphile », « liposomes », « nanoparticules », et « nanotubes de carbone ».

Challenges and recent advances on the delivery of poorly soluble drugs:

An update on the development of carriers for amphotericin B

Acarília Eduardo da Silva[1,2], Kattya Gyselle de Holanda e Silva[3], Gillian Barratt[2] and E. Sócrates T. Egito[1]

[1] Universidade Federal do Rio Grande do Norte, Natal-RN, Brazil.

[2] Université Paris Sud XI, Châtenay-Malabry, France

[3] Universidade Federal do Rio de Janeiro, Rio de Janeiro-RJ, Brazil

* Corresponding author: Dr. Eryvaldo Sócrates Tabosa do Egito

UFRN – CCS – Departamento de Farmácia

Rua Praia de Areia Branca, 8948.

Zip code: 59094-450

Phone: +55 84 94 31 88 16

Fax: + 55 84 32 15 43 55

E-mail address: socratesegito@gmail.com

Keywords: amphotericin B, amphiphiles, carbon nanotubes, emulsions, liposomes, lipid complexes, micelles, solid lipid nanoparticles

ABSTRACT

Amphotericin B (AmB) is a very effective molecule against systemic fungal infections. It has also been considered the secondary therapy for leishmaniasis, a parasitic disease affecting hundreds of million people in over 60 countries. Despite its therapeutic importance, the physicochemical properties of AmB lead to critical problems in its formulation and therapeutic use. The most important drawback to the design of novel formulations of AmB lies in its very poor solubility in water. This low solubility in aqueous media also leads to low bioavailability by the oral route. The use of AmB is therefore limited to either IV infusion or local application. In addition, a number of acute side effects are induced by the IV infusion, which is highly inconvenient and reduces patient compliance and therapeutic success. Lipid formulations have emerged as potential alternative delivery systems for AmB. However, the marketed products are extremely expensive and still designed for the IV administration. This review provides a literature survey on the most interesting findings over the last ten years in pharmaceutical research and development of AmB formulations according to physicochemical, preclinical and clinical perspectives.

1. INTRODUCTION

The development of effective, safe and low-cost drug and delivery systems has been a key goal of the pharmaceutical research and industry for decades. Recently, the use of combinatory chemistry and high-throughput screening have provided an increasing number of drug candidates with poor aqueous solubility [1]. Low solubility in water represents an important factor limiting the drug dissolution rate. Consequently, absorption and bioavailability after oral administration of these drugs is also unsatisfactory, thus reducing the therapeutic efficacy and safety of these poorly water-soluble molecules [2].

Amphotericin B (AmB) is a polyene antibiotic, first isolated from *Streptomyces nodosus* in 1945 [3]. The molecule consists of an elongated circular structure presenting hydrophilic polyhydroxyl and hydrophobic polyene domains. As a consequence of having both apolar and polar sides to its lactone ring combined with the presence of ionizable carboxyl and amine groups, AmB molecule presents both amphoteric and amphiphilic behavior (Figure 1). Hence, AmB is poorly soluble in aqueous solvents and in many organic solvents. At a pH below 2 or above 11, AmB is water-soluble, although it is unstable [4]. As a result of its very low solubility and membrane permeability, AmB is characterized as a type IV drug by the Biopharmaceutical Classification System [5, 6].

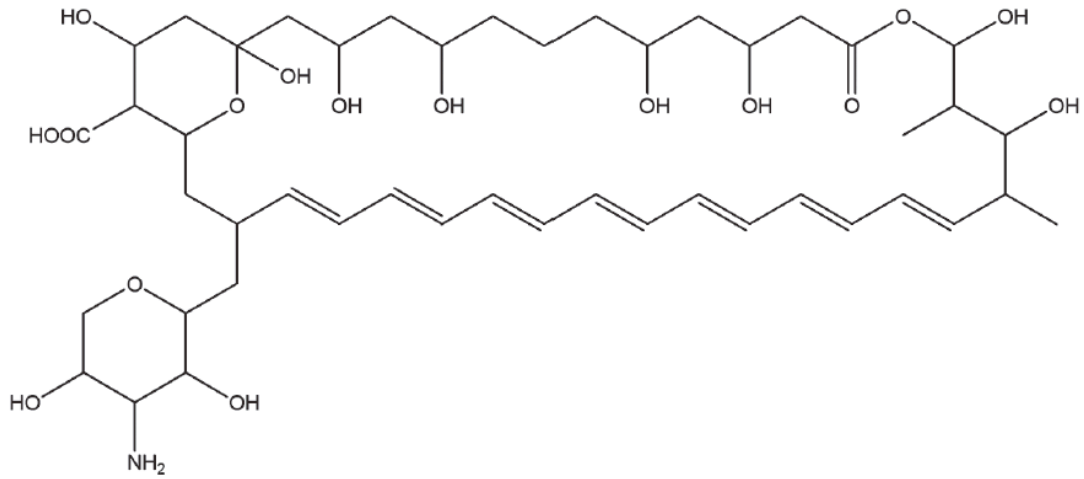


Figure 1. Chemical structure of amphotericin B [7].

The amphipathic nature of AmB causes it to self-associate and aggregate according to its concentration in water [8, 9]. Thus, only monomers are formed at concentrations of 5 x 10^{-}

^{8} M and below while from 5 x 10^{-5} M to higher concentrations, only aggregates are found. The two forms co-exist between 5 x 10^{-6} M and 5 x 10^{-7} M [3] (Figure 2). Interestingly, depending on its concentration, AmB in water may form a mixture of water-soluble monomers and oligomers with insoluble aggregates. The AmB aggregate forms are usually described as "water-soluble oligomers" and "water-insoluble aggregates" [9]. These different aggregate forms of AmB have been shown to be directly related to its toxicity. Water-soluble oligomers are defined as the most toxic form of the drug while larger water-insoluble aggregates are believed to be less toxic [9].

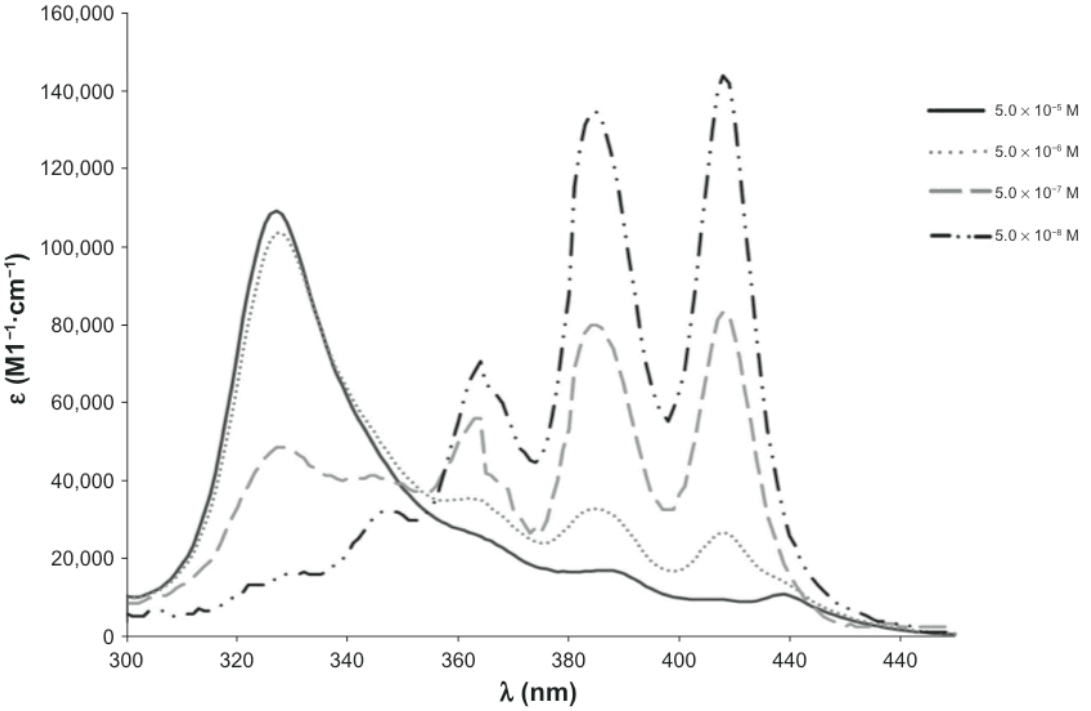


Figure 2. Absorption spectra of aqueous solutions of AmB-DOC at different concentrations at 25ºC. Peaks at 363, 385 and 408 nm are due to the monomeric form while the peak at 327 nm indicate the presence of aggregates of AmB [10].

AmB provokes both acute and chronic toxic side effects. The acute toxicity of AmB is infusion-related and caused by the production of proinflammatory cytokines by innate immune cells. As a microbial product, it is believed that AmB can stimulate mammalian immune cells via toll-like receptors [11]. Nausea, vomiting, rigors, fever, hypertension/hypotension, and hypoxia are observed as the main symptoms of acute toxicity of AmB deoxycholate, the "conventional" formulation [12]. Among these side effects, hyperkalemia is considered the most life-threatening consequence due to the potential for

developing fatal cardiac arrhythmias because of the leakage of potassium (K^+) from the intracellular compartment [12]. Among the chronic toxicities caused by therapy with AmB, distal renal tubular acidosis with hypomagnesaemia and hypokalemia is the most important side effect [13]. Shigemi and coworkers have evaluated retrospectively the frequency of anemia, thrombocytopenia, nephrotoxicity, hepatotoxicity and hypokalemia induced by the administration of liposomal formulations of AmB (L-AmB) [14]. The relationship between the daily dose of L-AmB and these side effects was also investigated. They observed that both anemia and thrombocytopenia occurred in a dose-dependent manner [14]. Nephrotoxicity was seen to be associated with a greater cumulative dose of AmB and concomitant administration of other nephrotoxic drugs, such as cyclosporine and streptomycin [14]. However, the study was not powerful enough to determine the influence of AmB on the hepatotoxicity and hypokalemia.

For over 50 years, AmB deoxycholate (AmB-DOC), the conventional colloidal dispersion marketed as Fungizone®, has been the treatment of choice for fungal infections, despite significant adverse effects, notably severe nephrotoxicity. As well as AmB-DOC, several other formulations incorporating amphiphiles have been developed in order to improve the drug solubility and bioavailability and decrease its toxicity. In the last 15 years, several new formulations have emerged. For example, in AmBisome® the drug is incorporated into small unilamellar liposomes to overcome its toxic effects. Colloidal drug delivery systems have incited great interest for decades due to their multiple advantages for the administration, stability and efficacy of active molecules. These drug carriers are able to provide improved biodistribution and bioavailability, reduced toxicity and better selectivity to both hydrophilic and hydrophobic molecules.

Different approaches have been adopted with a view towards decreasing the toxicity of AmB, such as its complexation with other agents such as surfactants, cyclodextrins, lipids, polymers and carbon nanotubes [15-25]. The aim of this article is to provide an overview of publications from the last ten years, which have reported relevant findings in the pharmaceutical technology field, especially on the development of delivery systems for AmB showing promising results. For this purpose, a systematic literature survey was performed on the database compiled by ISI Web of Knowledge and National Center for Biotechnology Information and published between 2002 and 2012 using “nanotechnology”, “amphotericin B”, “colloidal drug delivery system”, “microemulsion”, “nanoemulsion”, “emulsion” “micelles”, “amphiphile”, liposomes”, “nanoparticles”, and “carbon nanotubes” as search

terms. The quantitative results are shown in Figure 3.

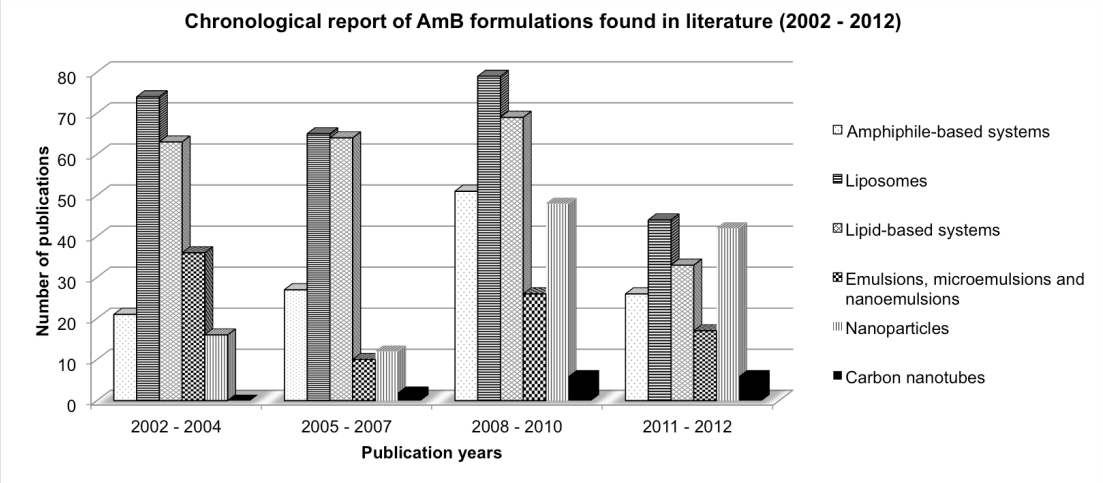


Figure 3. Timeline of publications on AmB formulations, such as amphiphile-based systems, liposomes, lipid-based systems, emulsions, microemulsions and nanoemulsions, nanoparticles and carbon nanotubes, indexed in Web of Science from 2002 until 2012.

2. AMPHIPHILE-BASED SYSTEMS

Amphiphiles are also known as surface active agents or surfactants due to their ability to reduce the surface tension between immiscible compounds by spreading over the hydrophobic substrates through the adsorption of the surfactant molecule at all the interfaces involved [26]. In general, surfactants present a hydrophilic group (or "head"), which can be ionic or highly polar, and a hydrophobic group (or "tail"), which is usually a long-chain aliphatic hydrocarbon group. Surfactants are classified as anionic, cationic, nonionic and amphoteric according to the nature of the hydrophilic "head" [27].

A dynamic and spontaneous self-association of amphiphilic molecules occurs when the critical micelle concentration (CMC) is reached and yields a variety of thermodynamically stabilized microstructures such as micelles, emulsions, microemulsions, nanosuspensions, solid lipid nanoparticles, cyclodextrins, niosomes and others [2, 28-31].

In fact, for technological and toxicological reasons, AmB solubilized with deoxycholate (AmB-DOC) has been available for the treatment of fungal infections since 1958 [32]. Fungizone® and Amfocan® are examples of formulations for intravenous (IV) administration [6]. Deoxycholate is a non-toxic fat emulsifier naturally occurring in the human body where it is released from the bile into the intestine forming mixed micelles with fatty acids [33, 34]. It is a biosurfactant with facial polarity due to hydrophilic groups on the

concave face of the molecule and most of the steroidal skeleton with its methyl groups situated on the convex face [35].

It has been reported that, when subjected to controlled heating, AmB-DOC forms a new type of aggregate, which is less susceptible to oxidative degradation and less selective for the cholesterol in the mammalian cell membrane, thus making it less toxic [36]. Based on this information, Egito and co-workers evaluated the relationship between controlled heat treatment, the absorption spectra, and the cytotoxicity of a Brazilian brand of AmB-DOC in aqueous micellar solutions before and after heat treatment (AmB-DOC-H). The heating process was aimed at rebuilding a pre-formed micelle system. The results showed similarity in the AmB-DOC-H and AmB-DOC spectra. A toxicity study comparing the lytic effects of AmB-DOC and AmB-DOC-H against red blood cells (RBCs) revealed that the two formulations behaved identically with respect to the leakage of K^+, and no significant hemoglobin (Hb) leakage occurred below 0.5 $mg.L^{-1}$ for both formulations. However, while the AmB-DOC revealed a sharp increase in their toxicity at concentrations above 0.5 $mg.L^{-1}$, reaching 100% lysis at 5 $mg.L^{-1}$, AmB-DOC-H showed no toxic effect on the whole range of the concentrations tested. Therefore, it was inferred that heating AmB-DOC was able to modify the aggregation state of the drug forming AmB super-aggregated species which may be considered a reservoir of monomeric AmB species that releases only a limited amount of monomeric AmB in the aqueous media [10].

Since most formulations containing AmB do not penetrate the central nervous system [37], the use of amphiphilic block copolymers in the development of micelles is a suitable strategy to deliver water-insoluble drugs to the brain. For example, 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] (DSPE-PEG_{2000})-based micelles loaded with AmB and surface-modified with a peptide were seen to be able to improve the solubilization of the drug, enhance its permeation across the blood-brain barrier in vivo and reduce its cytotoxicity [37]. Nanosuspensions of AmB with different surfactants were also prepared for brain delivery and they showed better physicochemical characteristics, such as particle size, stability storage and protein adsorption pattern, when using Tween® 80 and sodium cholate as surfactants instead of Tween® 20, Pluronic® F127 and Pluronic® F68 [38].

It is well known that hydrophobic blocks are required to obtain stable micelles. Due to the fact that fluorinated compounds are characterized by both lipophobicity and extreme hydrophobicity, also known as fluorophilicity, it was hypothesized that the incorporation of

fluorinated segments into polymeric micelles should enhance their thermodynamic stability by increasing the hydrophobicity of the micelle core, while kinetic stability would be enhanced by the lipophobic fluorinated segment by reduced binding to blood proteins. As a result, novel PEG-fluorocarbon-DSPE polymers were designed to increase stability and improve time-release properties of AmB-loaded micelles [25].

The micelles formed by the new polymers showed lower CMCs and higher viscosity cores than those formed by the polymers lacking the fluorocarbon block. The physicochemical properties and *in-vitro* release profile of micelles loaded with AmB were studied and it was concluded that the fluorinated shell in the micelles particularly affected the *in-vitro* release kinetics of AmB, because polymers containing a fluorinated block had significantly longer half-lives than their non-fluorinated analogues [25].

3. LIPOSOMES

Among various versatile drug carriers, liposomes have been extensively investigated. They consist of microscopic vesicles based on lipids, mainly phospholipids, surrounding the aqueous compartment where water-soluble compounds are enclosed while lipophilic and amphiphilic drugs may be associated with the lipid bilayers. According to their size and the number of lipid bilayers, liposomes can be classified as large multilamellar vesicles, small unilamellar vesicles, large unilamellar vesicles or multivesicular vesicles. In general, liposomes present a mean diameter in the range of 80 nm to 100 μm [39-42].

Liposomal formulations of AmB are especially useful for reducing its high toxicity. Furthermore, the use of natural phospholipids to prepare the liposomes provides a lower incidence of toxic side effects [43]. However, after *in vivo* administration, liposomes are very rapidly removed from the bloodstream by monocytes and macrophages, leading to a short circulation half-life for these formulations. However, such property can be used to advantage in the treatment of diseases caused by intracellular obligate parasites affecting phagocytic cells, such as visceral leishmaniasis, candidiasis, aspergillosis, hystoplasmosis, and others [44, 45]. At the moment, Ambisome® is the only liposomal product approved for treatment of visceral leishmaniasis in adults and pediatrics, being considered as first choice for treating patients who are unresponsive to antimonials [40].

Nevertheless, it is possible to avoid the rapid macrophage uptake of liposomal formulations and prolong their blood circulation time by grafting chains of of polyethylene

glycol (PEG) of 2-5kDa on their surface. This strategy of "pegylation" prevents the binding of plasma proteins to the surface of the liposomes and blocks the uptake by macrophages due to an induced hydrodynamic layer on the vesicle surface. As a consequence, the time of residence in blood is increased, accumulation in the organs of the reticuloendothelial system is delayed and the toxicity is reduced [4, 44].

A number of physicochemical and structural studies of AmB-loaded liposomal formulations have helped to understand the interactions between the molecules and their results for the biological properties. By studying AmB-entrapping liposomes containing neutral and charged lipids in various compositions, it was observed that fatty acids resulting from partial decomposition of lipids at high temperature might provide negative charges at the surface of the final formulations. Positively charged formulations may present a higher thermal stability. Furthermore, liposomes with entrapped AmB tend to be smaller and present a higher zeta potential than AmB-free liposomes. The smallest liposomes were those with negative charge; however, larger liposomes could carry higher amounts of drug [46].

X-ray diffraction, small-angle neutron scattering and infrared spectra absorption analysis of liposomes based on egg yolk phosphatidylcholine with different amounts of AmB revealed that a fraction of the drug molecule binds to the hydrophobic core of the membrane. This fraction increases the barrier function of the membrane for transmembrane ion transport [47].

The transfer of AmB present in Ambisome® to the fungal cell membrane was found to depend on the time and the temperature while antifungal activity of free AmB did not change at either 4ºC or 35ºC. The lipid present in liposomal formulations and the temperature also played an important role in the amount of AmB transferred to the fungal membrane [48].

Although liposomal AmB shows lower toxicity, it may present free AmB molecules and aggregates which are highly toxic. Therefore, measuring the amount of free AmB in those formulations is an essential step for pre-formulation and stability studies. However, the limited solubility of AmB and the stability of liposomes may represent complications to several separation and quantification methods. Recently, a practical bioanalytical method based on liquid chromatography–mass spectroscopy was developed to separate and quantify both free and liposomal AmB present in blood after IV administration [49].

Liposomal formulations carry the risk of creating new toxicities. Liposome-entrapped drugs, such as doxorubicin and AmB, have been reported to present an important risk for hypersensitivity reactions due to activation of the complement system (C), the frequency of

which depends on factors related to premedication, patient and product [50]. Because multilamellarity, large size, and the presence of very high amounts of cholesterol in the bilayer membrane are features leading to C activation and C activation-related pseudoallergy, their effects were assessed *in vivo* and *in vitro*, in comparison to Ambisome® [50]. The results showed that Ambisome® was responsible for a massive C activation *in vitro* in normal human serum (NHS), without individual variation among the 20 NHS samples tested of the assay. Furthermore, the *in vivo* study revealed that the administration of Ambisome® was followed by moderate to severe reactions in all of five treated pigs in which a more than threefold rise of pulmonary artery pressure and some 60% decline of systolic artery pressure were detected within 1 minute [50].

The tendency of lipsomes to accumulate in organ rich in phagocytic cells means that hepatotoxicty is always a risk and because of effects reported in animal studies, a retrospective non-randomized study, hepatic autopsy has been performed in order to find histopathologic evidence of abnormalities related to AmB-hepatotoxicity in humans. No significant indication of direct toxicity was observed, although some factors inherent to the study may have compromised its findings, such as its retrospective nature, concomitant use of hepatotoxic drugs by the subjects involved and the lack of data on their dosage [51].

Despite these reports, there is still evidence coming from the clinic to demonstrate the reduced toxicity of L-AmB. Tolerance of L-AmB has been tested in premature neonates and it was discovered that this formulation is well tolerated by very low birth weight infants as prophylaxis against *Candida sp* [52].

A retrospective chart review was conducted at RUSH University Medical Center in Chicago, IL, with 100 consecutive patients receiving L-AmB at doses of 1, 3, and 5 mg/kg [53]**.** Overall nephrotoxicity with L-AmB was common and often multifactorial. Lipid amphotericin B products are associated with lower rates of nephrotoxicity than conventional amphotericin; however, in this analysis, L-AmB was associated with a high incidence of nephrotoxicity. There are also several limitations in the study. It is difficult to truly report adverse drug reactions for L-AmB; however, it appears that both toxic drug effects (i.e., nephrotoxicity and hepatotoxicity) have the potential to develop, and precautions should be taken including pre-medication, hydration (0.9% normal saline), and increased pharmacovigilance. Clinicians are encouraged to be aware of nephrotoxicity and hepatotoxicity with the use of L-AmB therapy.

In Brazil, Ambisome® has been successfully used for the treatment of a 50-year-old male patient with diabetes who had been diagnosed with cutaneous leishmaniasis. He had previously received azythromicin but without success. In contrast, the liposomal AmB formulation healed the lesion after 30 days at doses determined according to the creatinine serum levels [54].

However, it is well-known that despite its high efficacy and reduced toxicity, the high price of Ambisome® represents a substantial disadvantage for its use in developing countries. Therefore, liposome-based formulations have been developed with novel lipids and phospholipids. A new lipid named 1,2-distigmasterylhemisuccinoyl-sn-glycero-3-phosphocholine (DSHemsPC) and formed by two molecules of stigmasterol covalently linked via a succinic acid to glycerophosphocholine, has been successfully used in AmB-entrapping liposomes. Stigmasterol is an expensive unsaturated plant sterol chemically similar to animal cholesterol, and the liposomes derived from DSHemsPC showed excellent colloidal properties, a high IC50 for red blood cells and antifungal and antileishmaniasis activities similar to Ambisome® [55].

Liposomal formulations of AmB open up the possibility of new routes of administration. Thus, surfactant-free liposomal eye drops containing Ambisome® were prepared in order to reduce the ocular irritation and increase the patient compliance. Additionally, their stability was investigated. After storage for 6 months, the physicochemical characteristics of the formulation remained unchanged, such as AmB content, mean diameter of liposomes and polydispersity index. Ultracentrifugation experiments showed that the drug was retained in the liposomes throughout this period, even at room temperature [56].

Several studies have been focused on the use of AmB formulations for the prophylaxis of invasive fungal infections in the lungs [57-60]. Aerosolized L-AmB has been compared to nebulized AmB deoxycholate as regards feasibility, tolerability and results for the prevention of Aspergillus infection in lung transplantation. After the 12 months of study, prophylaxis with both formulations was seem to be well tolerated, although L-AmB appeared to be more convenient and showed no significant plasma concentrations, which reduces the risk of AmB-related nephrotoxicity [58].

Still considering the pulmonary approach, targeted liposomes have been prepared. Multilamellar liposomes containing AmB and coated with alveolar macrophage-specific ligands such as O-palmitoyl mannan and O-palmitoyl pullulan have been analysed by means of a testing apparatus simulating the human trachea and bronchi. The liposomes were found to

be very effective because of the high amount of drug rapidly targeted to the lungs as well as the prolonged delivery of AmB to the alveolar macrophages. However, some physiological factors are responsible for the low accumulation of the drug in the lungs for longer time [61].

4. LIPID-BASED SYSTEMS

The discovery of the complexation of AmB with lipids in the 1990s as a feasible strategy to stabilize the drug molecule in a self-associated state and prevent its interaction with the cholesterol in human cellular membranes resulted in the development of the lipid complexes, such as Abelcet® and Amphocil® or Amphotec® [4, 62].

In Abelcet® (also called AmB lipid complex), AmB forms 2-5 μm ribbon-like complexes with two phospholipids (L-α-dimyristoylphosphatidylcholine and L-α-dimyristoylphosphatidylglycerol in a 7:3 molar ratio) in a 1:1 drug to lipid molar ratio. Amphotec® is the marketed name in the USA of Amphocil® marketed in Europe. This formulation consists of a colloidal dispersion of AmB in an equimolar concentration of cholesterol sulfate forming disc-like structures. It is believed that the lower renal toxicity is due to the higher affinity of AmB to the cholesterol of the formulation, which reduces the presence of free AmB in the bloodstream [4].

Lipid complexes are shown to be not only less cytotoxic and hemolytic but also very effective. In Brazil, after replacing Glutamine® (N-methyl glucamine antimoniate) by Amphocil® to treat the lesion caused by cutaneous leishmaniasis in a 30-year-old male patient, healing could be observed within 20 days after the treatment and persisted for after thirteen months, a longer time than that obtained with the standard treatment with antimonial [54].

A retrospective study on the renal effects of AmB lipid complex investigated over 1,700 patients with systemic fungal infections and observed that therapy with both high and low doses of that formulation at short and long term therapy, respectively, presented good tolerability [63]. The safe and effective use of AmB lipid complex against fungal infections in elderly patients has also been reported [63].

Several case reports worldwide have been published with results that prove the efficient and safe use of AmB lipid complex for a number of conditions, such as cutaneous leishmaniasis, systemic fungal infections, and even rare diseases affecting infants, immunocompromised, elderly, critical renal failure patients, and others [54, 63-67]. This type

of formulation has also been suggested as a suitable approach to fungal prophylaxis by means of intrabronchial instillation in the early postoperative period after lung transplantation (Morales et al., 2009) and as an antifungal lock therapy to treat catheter-associated biofilms and prevent nosocomial bloodstream infections [68].

Although lipid complexes of AmB shows good efficacy and tolerability, some case reports have been published showing that they may cause some infrequent adverse and even fatal effects [69-71], which may suggest the need of improvements in this type of formulation. In 2002, a retrospective study performed in a university hospital confronted the results found by a larger study, which detected a high rate of nephrotoxicity cause by AmB lipid complex. Nonetheless, nephrotoxic effects were also found even in the small group of subjects [69, 71]. Two years later, a case of a fatal embolism was published as the first occurrence caused by IV liposome drug delivery. After receiving a daily 85-mg dose of intravenous AmB, a 41-year-old HIV-positive man developed worsening renal function and started receiving Abelcet®. Within 48 hours, typical fat embolism signs and symptoms were manifested, probably due to the deposition of AmB in capillaries and small arteries, leading to multiorgan failure and death of the patient [70].

The drug content, the lipid composition and the preparation process correspond to important parameters influencing the morphology, size, polydispersity and AmB aggregation during the development of lipid formulations. This fact was proven by absorption and circular dichroism analysis in lipid complexes using dimyristoylphosphatidylcholine and dimyristoylphosphatidilglycerol in different molar ratios [72]. Furthermore, AmB has shown to be better absorbed by cationic amphiphiles, such as dioctadecyl dimethylammonium bromide (DODAB), than in anionic and zwitterionic amphiphilic lipids when forming large unilamellar vesicles [73].

As well as the differences in various aspects, such as lipid composition, morphology, physicochemical properties and pharmacokinetics profiles, the AmB lipid complexes also show different clinical efficacy, but they all less nephrotoxic than conventional AmB [74]. On the other hand, the high cost of those formulations still limit their use and the cost of treatment may be outweighed by the cost of renal toxicity [54, 74].

It is known that elimination of AmB from the body occurs slowly [75]. Marketed L-AmB formulations also have a low rate of elimination and, as a consequence, high doses of administered AmB tend to accumulate in the body [15]. In order to solve these problems, a novel formulation incorporating AmB into thermotropic liquid crystalline phases was

proposed. These systems were assessed for their potential use as a dry powder aerosol. It was hypothesized that the drug efficiency and safety would be improved. The results showed that the minimum inhibitory concentration and minimum fungicidal concentration was lower than that of pure AmB. Thus, it can be inferred that the liquid crystals provided a synergistic effect with AmB to inhibit fungal growth. This may be due to the association of the liquid crystal with the fungal membrane that would facilitate transfer or perhaps induce the formation of ionophores [76]. In a subsequent study, Chuealee and his colleagues evaluated the efficacy and toxicity of cholesteryl carbonate ester formulations of AmB and concluded that these AmB formulations retained the potency of AmB against fungal cells [15] .

5. EMULSIONS, MICROEMULSIONS AND NANOEMULSIONS

Emulsion-based systems are very interesting dispersed systems since they may improve the solubility of both polar and non-polar substances in oil or aqueous phases, respectively. Among this type of systems, microemulsions (MEs) and nanoemulsions are the most frequently studied. Although there are many structural similarities between these two colloidal formulations, there are also some important differences [77].

Emulsions are characterized as kinetically stable systems resulted in the dispersion of two immiscible liquids under manual or mechanical stirring with droplet sizes between 0.1 and 100 μm [78, 79]. These droplets are stabilized by a single layer of a surfactant or a surfactant blend. Nevertheless, emulsions tend to undergo structural modifications over time such as flocculation, coalescence, creaming, gravitational separation, phase inversion, Ostwald ripening and breakdown because the free energy of the oil and water phases, separately, is lower than that of the emulsion itself [79]. A nanoemulsion can be considered as a conventional emulsion that contains very small particles. Nanoemulsions are defined as a thermodynamically unstable colloidal dispersion consisting of two immiscible liquids, with one of the liquids being dispersed as small spherical droplets ($r < 100$ nm) in the other liquid. In principle, a nanoemulsion could be formed from oil and water without using a surfactant. In practice, this system would be highly unstable to droplet coalescence and a surfactant is needed to facilitate the formation of the nanoemulsion and to ensure its kinetic stability during storage [80].

On the other hand, MEs are optically transparent and thermodynamically stable dispersed systems of at least three components: a polar and a non-polar liquid phase (water

and oil, respectively), and a suitable surfactant frequently in combination with a co-surfactant such as an aliphatic alcohol [78]. However, it is important to stress out that MEs are only thermodynamically stable under a given range of compositions and environmental conditions, and are susceptible to breakdown in different conditions (e.g., due to dilution, ingredient addition, or temperature changes) [81].

Egg lecithin, soybean oil and ^{3}H-cholesteryl hexadecyl ether were used to prepare conventional lipid emulsions (droplets of approximately 200 nm) and small lipid emulsions with droplet sizes around 25 and 50 nm by slight variations in the preparation method. After administration of a dose of 1 mg/Kg in mice, rats, dogs and monkeys, a higher plasma concentration of AmB was observed from the small lipid emulsion than from conventional emulsions and AmB-DOC, probably due to slower hydrolysis and uptake by the reticuloendothelial system [82]. Soya lecithin has also been used in a mixture with Tween® 20 to stabilize MEs containing AmB displaying suitable properties from the stability, rheological and physicochemical point of view [83].

It is known that oil-in-water lecithin-based AmB-loaded MEs are 6 times safer than AmB-DOC. When comparing their efficacy, 80% of mice with a very severe infection by *Candida albicans* survived after microemulsion administration while the survival rate was 40% after injection of AmB deoxycholate (1 mg/Kg). In addition, a 3-times higher dose of AmB microemulsion increased the survival rate to 100% [84]. Another example of a lecithin-based microemulsion for the administration of AmB was studied by Moreno and co-workers [85]. A microemulsion based on isopropyl myristate, Polysorbate® 80 and soybean lecithin was prepared and lyophilized in order to avoid hydrolysis reactions of lecithin phosphatide groups and prevent any decomposition during storage. Afterwards, the formulation could be reconstituted in water. This approach was seen to be easy and low-cost in terms of manufacturing and the final products were very stable. Another lyophilized AmB-loaded MEs formulation, based on polyethylene glycol 40 stearate, glyceryl mono-oleate and polyethylene glycol 15 hydroxy stearate, was investigated by Darole and colleagues [86]. These formulations showed an impressive decrease of 92% of hemolysis when compared to AmB-DOC.

Damasceno and colleagues studied the incorporation of AmB into a biocompatible ME and evaluated its physicochemical parameters as well as its *in-vitro* toxicity. The group demonstrated lower leakage of hemoglobin and K^{+} from the RBC when the data was compared with AmB-DOC. The AmB-loaded ME was non toxic up to a concentration of 50

$mg.L^{-1}$. This study revealed that the selectivity for fungal cells was conserved after the incorporation of the drug because, even at low concentrations, the AmB-loaded ME was able to release quite large amounts of K^+ from the fungal cells [87].

Recently Franzini and colleagues developed biocompatible MEs to mimic the negative charge of the anionic emulsions for parenteral nutrition and studied some very important structural parameters with a view to IV administration. The initial diameter of the droplets was seen to increase after the incorporation of AmB. This difference clearly reflects the amphiphilic property of AmB, which was organized at oil-water interface, with more favorable dielectric constant, increasing the local volume of the droplets. The rheological behavior was substantially modified by the AmB incorporation into the system, with an instantaneous increase in the structural organization of the system. Further analysis showed the presence of crystals, which was more evident for the AmB-loaded ME, indicating that the antibiotic strongly influences the crystalline structure formation [88].

Lipid nanoemulsions can also act as successful nanocarrier systems for highly lipophilic drugs like AmB. These systems could be a good alternative to liposomes because of their greater stability. Nasr et al. [88] investigated the potential of two commercially available isotonic nanoemulsions that are used in hospitals for total parenteral nutrition, namely Intralipid® and Clinoleic®, to solubilize AmB for pulmonary inhalation by nebulization. The results suggested that designing aerosol systems that are capable of delivering antifungal agents in sufficient concentrations to the alveolar region is highly desirable not only to target the invading fungi within the lung but also to handle the systemic fungal infection [89].

Isotropic mixtures of drug, lipids and surfactants, usually with one or more hydrophilic cosolvents or coemulsifiers are called self-emulsifying drug delivery systems (SEDDS). They can form fine (oil-in-water) emulsions instantaneously upon mild agitation followed by dilution with aqueous media. SEDDS is a broad term and comprises emulsions with a droplet size ranging from a few nanometers to several microns. More accurately, the term self-microemulsifying drug delivery systems (SMEDDS) refers to formulations forming transparent MEs with oil droplets ranging between 100 and 250 nm while self-nano-emulsifying drug delivery systems is a recent term considering the globule size to be less than 100 nm [90].

SEDDS based on medium chain triglycerides, fatty acids and nonionic surfactants may also play an important role as carriers for AmB, as well as formulations containing glyceryl mono-oleate (Peceol®) with PEG-phospholipids, as described by Wasan and coworkers.

Those systems were shown to allow very good drug solubilization and stability in *in-vitro* studies simulating gastric and intestinal environments. Furthermore, animal studies revealed good antifungal activity with no significant nephrotoxicity after oral administration [91].

6. NANOPARTICLES

Among the newer drug carriers, nanoparticles – either polymeric or lipidic – are known to produce outstanding effects on the reduction of the effective dosage of drugs, improvement of their bioavailability and decrease in their toxicity. Because of their inherent stabilty, they can be administered by several routes. Nanoparticles are very attractive for the delivery of AmB, especially to target deep fungal infections in the brain such as cryptococcal meningitis, which are difficult to cure due to the inability of AmB-DOC to pass through the brain-blood barrier. Xu and coworkers successfully delivered AmB to the brain by using polysorbate 80-coated nanoparticles based on polybutylcyanoacrylate [92]. AmB has been also successfully incorporated into nanoparticles based on the antioxidant L-ascorbyl-6-dipalmitate and distearoylphosphatidylethanolamine-PEG 2000. This surfactant was found to be the most suitable when compared to several others, such as ascorbyl-6-palmitate, ascorbyl-2, 6-dibutyrate, sodium dodecyl sulfate, cetyltrimethyl ammonium bromide, polyoxyethylene stearyl ether, and distearoyl glycerol PEG 2000. Animal studies revealed a higher AmB concentration in plasma and reduced hepatotoxicity and nephrotoxicity compared with the commercial formulation, Fungizone® [93].

Although lipid nanoparticles may be very useful for the delivery of lipophilic drugs, lipid transfer proteins present in the blood stream are able to extract lipophilic drugs from the nanoparticles and transfer them to plasma lipoprotein. Although this could be a drawback to their use, AmB-entrapping lipid emulsions and lipophilic derivatives with cholesterol pro-moiety have been demonstrated to be provide a longer plasma half-life and reduced toxicity for low-dose AmB formulations [75, 94].

Lipid nanoparticles containing AmB with an increased circulation half-life were prepared by using PEG molecules as a coating to improve their pharmacokinetic parameters by proving steric stabilization in the blood stream. Lower toxicity to the kidneys, no hematotoxicity and higher antifungal activity were achieved [95].

Highly efficient solid lipid nanoparticles (SLN) based on stearic acid and containing AmB monomers have been shown to significantly increase the *in-vitro* antifungal activity

when compared with the free drug [96].

Characterization of the interactions between AmB and the excipients is important for understanding the behavior of the system. A thorough study with surface-enhanced resonance Raman scattering (SERRS) on magnetic nanoparticles coated with a bilayer of lauric acid found that the AmB molecules interact with the bilayer specifically by the polyene chain of the macrolactone ring while their hydrophilic heads interact with the cell membrane to form the transmembrane pores [97].

Recently, the combination of a lipid and a polymer into a single system was successfully employed to improve the oral bioavailability of AmB. This formulation is commonly referred to as polymer lipid hybrid nanoparticles (PLNs). To develop PLNs for oral delivery of AmB, lecithin was combined with gelatin to take advantage of the properties of each: the lipid had been shown to enhance the delivery of AmB and the polymer is known for its wide applications in nanopharmaceuticals and its easy availability and low cost. The limitations of lipid or polymer-based systems could be successfully overcome by this single composition [98]. The study presented promising results, including a significant delay in the drug release from the PLNs compared with AmB-DOC. This is a critical factor for reducing the toxicity of AmB owing to the reduced exposure of blood components to the drug and was correlated with the results of the hemolytic toxicity study in which AmB-loaded PLNs induced significantly ($p < 0.001$) lesser hemolysis than AmB-DOC [98].

7. CARBON NANOTUBES

One of the most important classes of material emerging from the recent developments in nanotechnology has been carbon nanotubes (CNTs). They are cylinders of one or several coaxial graphite layers with a catalytic material often present inside and/or at their extremity. A variety of nanoscale carbon tube structures have been prepared and their structure and properties have been discussed in many articles as one of the most promising nano-objects with potential medical applications [99].

Investigations on the potential of CNTs for biomedical applications are rapidly expanding, since functionalized CNTs have been found to be biocompatible and nontoxic at the cellular level. In particular, they could be used as carriers for drug delivery. These systems could solve delivery issues for pharmacologically active compounds that need to be internalized by cells and, for that reason, have not yet found potential therapeutic applications.

In the field of antimicrobial applications, CNTs have been investigated as enhancers to induce pathogen aggregation following an appropriate functionalization with sugar-based ligands recognized by receptors on the surface of the microorganisms [100].

The conjugation of antimicrobial drugs, such as AmB, onto CNTs may bring several benefits, such as the increased solubility of the drug thus avoiding aggregation, an improvement in the selectivity between the target and the host cells, leading to a better therapeutic index, and enhanced efficacy, because of a clustering effect and/or a better capacity of internalization of the CNTs into cells [24]. In 2005, Wu and his group observed that the attachment to CNTs modified the internalization properties of AmB. They also evaluated the antifungal activity of CNTs, functionalized with AmB (f-CNT), against three species of fungi that are either pathogenic or may opportunistically infect humans. AmB was covalently linked to ammonium-functionalized multi- and single-walled carbon nanotubes and it was taken up by mammalian cells without provoking any specific toxic effect. Furthermore, AmB bound to CNTs was shown to preserve its high antifungal activity while f-CNTs were able to enter the cell spontaneously as if they were “nanoneedles” and pass through the cell membrane without causing cell death [101].

Conjugation of AmB to f-CNTs decreased the cytotoxic effects of the drug against mammalian cells, while preserving its high antifungal activity [102]. It has been reported that the various aggregation forms of AmB interact with the sterols present in the membranes in different ways. In fact, AmB induces leakage of K^+ through the mammalian cholesterol-containing membranes only beyond a certain concentration threshold, which corresponds to the formation of self-associated AmB (oligomers). Conversely, the toxicity for the ergosterol-containing membranes, a characteristic feature of fungal cells, is due to the monomeric form of AmB. The conjugation of the drug with CNTs might thus prevent its aggregation and thereby decrease its toxicity toward mammalian cells, while maintaining it in a monomeric form that favors the antifungal activity [4].

The antifungal activity of CNT-AmB-conjugates was tested against a number of fungal reference strains and clinical isolates and compared to that of AmB alone or AmB-DOC [102]. Interestingly, in this study it was found that the multiwalled carbon nanotube also exerts a significant activity against strains that are AmB-resistant, as indicated by the efficacy of this compound against *C. albicans* L21 and ATCC 90029, and *C. famata* M100 and SA550, making this conjugate a very promising hit for future development.

Another research group has demonstrated that AmB attached to CNTs has a beneficial effect on controlled drug delivery, through the oral route, with the potential for eradication of amastigotes from the hamster spleen [103].

8. CONCLUSIONS

AmB is considered as one of the most effective agents in the treatment of systemic fungal infections and is currently used as the secondary treatment of choice against leishmaniasis with 97% of cure rate with no reported resistance [104]. However, the conventional therapy with AmB employs parenteral formulations, whose administration is invasive, demands professionals and suitable facilities, and more importantly, induces a number of side effects. On the other hand, the existing lipid formulations of AmB are very effective, less toxic and require a shorter course of therapy. Notwithstanding, they are still inconvenient due to theneed for IV administration with its associated drawbacks and and are very expensive, which puts them out of reach of the populations of developing countries that are extensively affected by disseminated fungal infections and leishmaniasis. Therefore, the need of oral formulations for AmB is a pivotal concern.

According to this literature review, it is evident that during the last decade many efforts have been exerted and some advances have been made towards the design of suitable AmB formulation for the oral administration. As a result, there are a large variety of strategies and excipients currently available for further studies. Several approaches are aimed at reducing AmB toxicity by modifying the drug formulation. Lipid-based products containing AmB and systems based on “nanotechnology” have been developed in order to improve its bioavailability. The hypothesis that MEs would be a suitable carrier for administration of a poorly soluble drug such as AmB has been addressed [16, 84, 105-109].

The experimental work of our team has shown an increase of 140-fold in the solubility of AmB in water when incorporated into MEs based on esters of caprylic acid. Furthermore, other studies have also revealed an increase in the LD_{50} value over the conventional AmB formulation [86]. These results are promising for the development of a ME-based formulation for the safe use of AmB. However, despite the fact that MEs substantially improve the physicochemical properties of AmB, the most suitable administration route is still to be chosen.

Although the high amount of surfactants in their composition is a potential drawback, improving the AmB solubility profile by means of MEs in order to reach the desired bioavailability is clearly justified by the properties of these systems. In addition, taking into account that the incorporation of AmB into a ME may allow an increase in the administered dose, despite the inherent cytotoxicity due to the high amount of surfactant, to the reduced toxicity suggests that drug release and toxicity studies are merited so that the utility of a ME-based formulation may be confirmed.

10. REFERENCES

[1]. Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: Basic approaches and practical applications. International Journal of Pharmaceutics 2011;420(1):1-10.

[2]. Chen H, Khemtong C, Yang X, Chang X, Gao J. Nanonization strategies for poorly water-soluble drugs. Drug Discov Today 2011;16(7-8):354-60.

[3]. Egito LCM, Medeiros SRB, Medeiros MG, Price JC, Egito EST. Evaluation of the relationship of the molecular aggregation state of amphotericin B in medium to its genotoxic potential. J Pharm Sci 2004;93(6):1557-65.

[4]. Torrado JJ, Espada R, Ballesteros MP, Torrado-Santiago S. Amphotericin B formulations and drug targeting. J Pharm Sci 2008;97(7):2405-25.

[5]. Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: The correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res 1995;12(3):413-20.

[6]. Kagan L, Gershkovich P, Wasan K, Mager D. Physiologically based pharmacokinetic model of amphotericin B disposition in rats following administration of deoxycholate formulation (Fungizone®): Pooled analysis of published data. AAPS J 2011;13(2):255-64.

[7]. Santos CM, Oliveira RB, Arantes VT, Caldeira LR, Oliveira MC, Egito EST, Ferreira LAM. Amphotericin B-loaded nanocarriers for topical treatment of cutaneous leishmaniasis: Development, characterization, and *in vitro* skin permeation studies. J Biomed Nanotechnol 2012;8(1):322-29.

[8]. Barwicz J, Tancrède P. The effect of aggregation state of amphotericin-B on its interactions with cholesterol- or ergosterol-containing phosphatidylcholine monolayers. Chem Phys Lipids 1997;85(2):145-55.

[9]. Espada R, Valdespina S, Alfonso C, Rivas G, Ballesteros MP, Torrado JJ. Effect of aggregation state on the toxicity of different amphotericin B preparations. International Journal of Pharmaceutics 2008;361(1-2):64-69.

[10]. Silva-Filho MA SS, Freire LB, Araújo IB, Silva KGH, Medeiros AC, Araújo-Filho I, Oliveira AG, Egito EST How can micelle systems be rebuilt by a heating process? Int J Nanomed 2012;7(1):141-50.

[11]. Sau K, Mambula SS, Latz E, Henneke P, Golenbock DT, Levitz SM. The antifungal drug amphotericin B promotes inflammatory cytokine release by a toll-like receptor- and CD14-dependent mechanism. J Biol Chem 2003;278(39):37561-68.
[12]. Laniado-Laborín R, Cabrales-Vargas MN. Amphotericin B: side effects and toxicity. Rev Iberoam Micol 2009;26(4):223-27.
[13]. Walker RJ, Endre ZH. Cellular mechanisms of drug nephrotoxicity. In: Alpern RJ, Hebert SC, eds. *Seldin and Giebisch's The Kidney (Fourth Edition)*. San Diego: Academic Press 2008:2507-35.
[14]. Shigemi A, Matsumoto K, Ikawa K, Yaji K, Shimodozono Y, Morikawa N, Takeda Y, Yamada K. Safety analysis of liposomal amphotericin B in adult patients: anaemia, thrombocytopenia, nephrotoxicity, hepatotoxicity and hypokalaemia. Int J Antimicrob Agents 2011;38(5):417-20.
[15]. Chuealee R, Aramwit P, Noipha K, Srichana T. Bioactivity and toxicity studies of amphotericin B incorporated in liquid crystals. Eur J Pharm Sci 2011;43(4):308-17.
[16]. Junghanns JU, Buttle I, Muller RH, Araujo IB, Silva AKA, Egito EST, Damasceno BPGL. SolEmuls® technology: A way to overcome the drawback of parenteral administration of insoluble drugs. Pharm Dev Technol 2007;12(5):437-45.
[17]. Kim YT, Shin BK, Garripelli VK, Kim JK, Davaa E, Jo S, Park JS. A thermosensitive vaginal gel formulation with HPγCD for the pH-dependent release and solubilization of amphotericin B. Eur J Pharm Sci 2010;41(2):399-406.
[18]. Nahar M, Mishra D, Dubey V, Jain NK. Development, characterization, and toxicity evaluation of amphotericin B-loaded gelatin nanoparticles. Nanomedicine 2008;4(3):252-61.
[19]. Nicoletti S, Seifert K, Gilbert IH. N-(2-hydroxypropyl)methacrylamide)-amphotericin B (HPMA-AmB) copolymer conjugates as antileishmanial agents. Int J Antimicrob Agents 2009;33(5):441-48.
[20]. Sedlák M, Pravda M, Kubicová L, Mikulcíková P, Ventura K. Synthesis and characterisation of a new pH-sensitive amphotericin B-poly(ethylene glycol)-b-poly(l-lysine) conjugate. Bioorg Med Chem Lett 2007;17(9):2554-57.
[21]. Tufteland M, Pesavento JB, Bermingham RL, Hoeprich Jr PD, Ryan RO. Peptide stabilized amphotericin B nanodisks. Peptides 2007;28(4):741-46.
[22]. Wang CH, Wang WT, Hsiue GH. Development of polyion complex micelles for encapsulating and delivering amphotericin B. Biomaterials 2009;30(19):3352-58.
[23]. Zhang X, Zhu X, Ke F, Ye L, Chen EQ, Zhang AY, Feng ZG. Preparation and self-assembly of amphiphilic triblock copolymers with polyrotaxane as a middle block and their application as carrier for the controlled release of amphotericin B. Polymer 2009;50(18):4343-51.
[24]. Benincasa M, Pacor S, Wu W, Prato M, Bianco A, Gennaro R. Antifungal activity of amphotericin B conjugated to carbon nanotubes. ACS Nano 2010;5(1):199-208.
[25]. Jee J-P, McCoy A, Mecozzi S. Encapsulation and release of amphotericin B from an ABC triblock fluorous copolymer. Pharm Res 2012;29(1):69-82.
[26]. Starov V, Ivanova N, Rubio RG. Why do aqueous surfactant solutions spread over hydrophobic substrates? Adv Colloid Interface 2010;161(1-2):153-62.

[27]. Shim YH, Kim YC, Lee HJ, Bougard F, Dubois P, Choi KC, Chung CW, Kang DH, Jeong YI. Amphotericin B aggregation inhibition with novel nanoparticles prepared with poly(epsilon-caprolactone)/poly(n,n-dimethylamino-2-ethyl methacrylate) diblock copolymer. J Microbiol Biotechnol 2011;21(1):28-36.
[28]. Jiang L, Yan Y, Huang J. Versatility of cyclodextrins in self-assembly systems of amphiphiles. Adv Colloid Interfac 2011;169(1):13-25.
[29]. Kumar GP, Rajeshwarrao P. Nonionic surfactant vesicular systems for effective drug delivery - An overview. Acta Pharmac Sin B 2011;1(4):208-19.
[30]. Palma S, Manzo R, Lo Nostro P, Allemandi D. Nanostructures from alkyl vitamin C derivatives (ASCn): Properties and potential platform for drug delivery. International Journal of Pharmaceutics 2007;345(1-2):26-34.
[31]. Shunmugaperumal T. Formulation of multifunctional oil-in-water nanosized emulsions for active and passive targeting of drugs to otherwise inaccessible internal organs of the human body. International Journal of Pharmaceutics 2009;381(1):62-76.
[32]. Michael K. What is the current and future status of conventional amphotericin B? Int J Antimicrob Agents 2006;27, Supp 1(0):12-16.
[33]. Froner E, D'Amato E, Adamo R, Prtljaga N, Larcheri S, Pavesi L, Rigo A, Potrich C, Scarpa M. Deoxycholate as an efficient coating agent for hydrophilic silicon nanocrystals. J Colloid Interface Sci 2011;358(1):86-92.
[34]. Jeong Y, Jin GW, Choi E, Jung JH, Park JS. Effect of deoxycholate conjugation on stability of pDNA/polyamidoamine-diethylentriamine (PAM-DET) polyplex against ionic strength. Int J Pharm 2011;420(2):366-70.
[35]. Selvam S, Mishra AK. Disaggregation of amphotericin B by sodium deoxycholate micellar aggregates. J Photochem Photobiol B 2008;93(2):66-70.
[36]. Gaboriau F, Chéron M, Leroy L, Bolard J. Physico-chemical properties of the heat-induced superaggregates of amphotericin B. Biophysical Chemistry 1997;66(1):1-12.
[37]. Shao K, Huang R, Li J, Han L, Ye L, Lou J, Jiang C. Angiopep-2 modified PE-PEG based polymeric micelles for amphotericin B delivery targeted to the brain. J Control Release 2010;147(1):118-26.
[38]. Lemke A, Kiderlen AF, Petri B, Kayser O. Delivery of amphotericin B nanosuspensions to the brain and determination of activity against Balamuthia mandrillaris amebas. Nanomedicine 2010;6(4):597-603.
[39]. Barratt GM. Therapeutic applications of colloidal drug carriers. Pharm Sci Technolo Today 2000;3(5):163-71.
[40]. Date AA, Joshi MD, Patravale VB. Parasitic diseases: Liposomes and polymeric nanoparticles versus lipid nanoparticles. Adv Drug Deliv Rev 2007;59(6):505-21.
[41]. Elizondo E, Moreno E, Cabrera I, Córdoba A, Sala S, Veciana J, Ventosa N. Liposomes and other vesicular systems: Structural characteristics, methods of preparation, and use in nanomedicine. In: Villaverde A, ed. *Progress in Molecular Biology and Translational Science*: Academic Press 2011:1-52.
[42]. Verma RK, Garg S. Current status of drug delivery technologies and future directions. Pharmaceutical Technology On-Line 2001;25(2):1-14.

[43]. Kayser O, Olbrich C, Croft SL, Kiderlen AF. Formulation and biopharmaceutical issues in the development of drug delivery systems for antiparasitic drugs. Parasitol Res 2003;90:S63-S70.
[44]. Briones E, Isabel Colino C, Lanao JM. Delivery systems to increase the selectivity of antibiotics in phagocytic cells. J Control Release 2008;125(3):210-27.
[45]. Solomon M, Baum S, Barzilai A, Scope A, Trau H, Schwartz E. Liposomal amphotericin B in comparison to sodium stibogluconate for cutaneous infection due to Leishmania braziliensis. J Am Acad Dermatol 2007;56(4):612-16.
[46]. Manosroi A, Kongkaneramit L, Manosroi J. Characterization of amphotericin B liposome formulations. Drug Dev Ind Pharm 2004;30(5):535-43.
[47]. Herec M, Islamov A, Kuklin A, Gagos M, Gruszecki WI. Effect of antibiotic amphotericin B on structural and dynamic properties of lipid membranes formed with egg yolk phosphatidylcholine. Chem Phys Lipids 2007;147(2):78-86.
[48]. Shimizu K, Osada M, Takemoto K, Yamamoto Y, Asai T, Oku N. Temperature-dependent transfer of amphotericin B from liposomal membrane of AmBisome to fungal cell membrane. J Control Release 2010;141(2):208-15.
[49]. Deshpande NM, Gangrade MG, Kekare MB, Vaidya VV. Determination of free and liposomal Amphotericin B in human plasma by liquid chromatography-mass spectroscopy with solid phase extraction and protein precipitation techniques. J Chromatogr B 2010;878(3-4):315-26.
[50]. Szebeni J, Bedocs P, Rozsnyay Z, Weiszhár Z, Urbanics R, Rosivall L, Cohen R, Garbuzenko O, Báthori G, Tóth M, Bunger R, Barenholz Y. Liposome-induced complement activation and related cardiopulmonary distress in pigs: factors promoting reactogenicity of Doxil and AmBisome. Nanomedicine 2012;8(2):176-84.
[51]. Chamilos G, Luna M, Lewis RE, Chemaly R, Raad II, Kontoyiannis DP. Effects of liposomal amphotericin B versus an amphotericin B lipid complex on liver histopathology in patients with hematologic malignancies and invasive fungal infections: A retrospective, nonrandomized autopsy study. Clin Ther 2007;29(9):1980-86.
[52]. Arrieta AC, Shea K, Dhar V, Cleary JP, Kukreja S, Morris M, Vargas-Shiraishi OM, Ashouri N, Singh J. Once-weekly liposomal amphotericin B as Candida prophylaxis in very low birth weight premature infants: A prospective, randomized, open-label, placebo-controlled pilot study. Clin Ther 2010;32(2):265-71.
[53]. Patel GP, Crank CW, Leikin JB. An evaluation of hepatotoxicity and nephrotoxicity of liposomal amphotericin B (L-AMB). J Med Toxicol 2011;7(1):12-5.
[54]. Amato VS, Rabello A, Rotondo-Silva A, Kono A, Maldonado TPH, Alves IC, Floeter-Winter LM, Neto VA, Shikanai-Yasuda MA. Successful treatment of cutaneous leishmaniasis with lipid formulations of amphotericin B in two immunocompromised patients. Acta Trop 2004;92(2):127-32.
[55]. Iman M, Huang Z, Szoka Jr FC, Jaafari MR. Characterization of the colloidal properties, *in vitro* antifungal activity, antileishmanial activity and toxicity in mice of a distigmasterylhemisuccinoyl-glycero-phosphocholine liposome-intercalated amphotericin B. Int J Pharm 2011;408(1-2):163-72.

[56]. Morand K, Bartoletti AC, Bochot A, Barratt G, Brandely ML, Chast F. Liposomal amphotericin B eye drops to treat fungal keratitis: Physico-chemical and formulation stability. International Journal of Pharmaceutics 2007;344(1-2):150-53.
[57]. Amparo S. Invasive fungal infections in lung transplantation: Role of aerosolised amphotericin B. Int J Antimicrob Agents 2008;32, Supp 2(0):S161-S65.
[58]. Monforte V, Ussetti P, Gavaldà J, Bravo C, Laporta R, Len O, García-Gallo CL, Tenorio L, Solé J, Román A. Feasibility, tolerability, and outcomes of nebulized liposomal amphotericin B for Aspergillus infection prevention in lung transplantation. J Heart Lung Transplant 2010;29(5):523-30.
[59]. Muñoz P, Guinea J, Narbona MT, Bouza E. Treatment of invasive fungal infections in immunocompromised and transplant patients: AmBiLoad trial and other new data. Int J Antimicrob Agents 2008;32, Supp 2(0):S125-S31.
[60]. Slobbe L, Boersma E, Rijnders BJA. Tolerability of prophylactic aerosolized liposomal amphotericin-B and impact on pulmonary function: Data from a randomized placebo-controlled trial. Pulm Pharmacol Ther 2008;21(6):855-59.
[61]. Vyas SP, Quraishi S, Gupta S, Jaganathan KS. Aerosolized liposome-based delivery of amphotericin B to alveolar macrophages. International Journal of Pharmaceutics 2005;296(1-2):12-25.
[62]. Luke RG, Boyle JA. Renal effects of amphotericin B lipid complex. Am J Kidney Dis 1998;31(5):780-85.
[63]. Hooshmand-Rad R, Reed MD, Chu A, Gotz V, Morris JA, Weinberg J, Dominguez EA. Retrospective study of the renal effects of amphotericin B lipid complex when used at higher-than-recommended dosages and longer durations compared with lower dosages and shorter durations in patients with systemic fungal infections. Clin Ther 2004;26(10):1652-62.
[64]. Bellmann R, Egger P, Djanani A, Wiedermann CJ. Pharmacokinetics of amphotericin B lipid complex in critically ill patients on continuous veno-venous haemofiltration. Int J Antimicrob Agents 2004;23(1):80-83.
[65]. Cone LA, Byrd RG, Potts BE, Wuesthoff M. Diagnosis and treatment of Candida vertebral osteomyelitis: Clinical experience with a short course therapy of amphotericin B lipid complex. Surg Neurol 2004;62(3):234-37.
[66]. Hooshmand-Rad R, Chu A, Gotz V, Morris J, Batty S, Freifeld A. Use of amphotericin B lipid complex in elderly patients. J Infect 2005;50(4):277-87.
[67]. Hourez R, Gillard PH, Martiat P, Aoun M. Disseminated fungemia due to Candida krusei with cutaneous lesions and successful treatment by amphotericin B lipid complex and catheter removal: a case report. Int J Infect Dis 2002;6(4):326-28.
[68]. Mukherjee PK, Long L, Kim HG, Ghannoum MA. Amphotericin B lipid complex is efficacious in the treatment of Candida albicans biofilms using a model of catheter-associated Candida biofilms. Int J Antimicrob Agents 2009;33(2):149-53.
[69]. Slain D, Miller K, Khakoo R, Fisher M, Wierman T, Jozefczyk K. Infrequent occurrence of amphotericin B lipid complex-associated nephrotoxicity in various clinical settings at a university hospital: A retrospective study. Clin Ther 2002;24(10):1636-42.
[70]. Tolentino LF, Tsai SF, Witt MD, French SW. Fatal fat embolism following amphotericin B lipid complex injection. Exp Mol Pathol 2004;77(3):246-48.

[71]. Wingard JR, White MH, Anaissie E, Raffalli J, Goodman J, Arrieta A. A randomized, double-blind comparative trial evaluating the safety of liposomal amphotericin B versus amphotericin B lipid complex in the empirical treatment of febrile neutropenia. Clin Infect Dis 2000;31(5):1155-63.
[72]. Larabi M, Gulik A, Dedieu JP, Legrand P, Barratt G, Cheron M. New lipid formulation of amphotericin B: spectral and microscopic analysis. Biochim Biophys Acta 2004;1664(2):172-81.
[73]. Oliveira TR, Benatti CR, Lamy MT. Structural characterization of the interaction of the polyene antibiotic amphotericin B with DODAB bicelles and vesicles. Biochimica et Biophysica Acta (BBA) - Biomembranes 2011;1808(11):2629-37.
[74]. Antoniadou A, Dupont B. Lipid formulations of amphotericin B: where are we today? J Mycol Med 2005;15(4):230-38.
[75]. Fukui H, Koike T, Saheki A, Sonoke S, Tomii Y, Seki J. Evaluation of the efficacy and toxicity of amphotericin B incorporated in lipid nano-sphere (LNS®). International Journal of Pharmaceutics 2003;263(1-2):51-60.
[76]. Chuealee R, Wiedmann TS, Suedee R, Srichana T. Interaction of Amphotericin B with cholesteryl palmityl carbonate ester. J Pharm Sci 2010;99(11):4593-602.
[77]. McClements DJ. Nanoemulsions versus microemulsions: terminology, differences, and similarities. Soft Matter 2012;8(6):1719-29.
[78]. Burguera JL, Burguera M. Analytical applications of emulsions and microemulsions. Talanta 2012;96(0):11-20.
[79]. McClements DJ. Crystals and crystallization in oil-in-water emulsions: Implications for emulsion-based delivery systems. Advances in Colloid and Interface Science 2012;174(0):1-30.
[80]. McClements DJ. Advances in fabrication of emulsions with enhanced functionality using structural design principles. Curr Opin Colloid Interface Sci 2012;17(5):235-45.
[81]. Rao J, McClements DJ. Food-grade microemulsions and nanoemulsions: Role of oil phase composition on formation and stability. Food Hydrocoll 2012;29(2):326-34.
[82]. Fukui H, Koike T, Saheki A, Sonoke S, Seki J. A novel delivery system for amphotericin B with lipid nano-sphere (LNS¬Æ). Intl J Pharm 2003;265(1-2):37-45.
[83]. Pestana KC, Formariz TP, Franzini CM, Sarmento VHV, Chiavacci LA, Scarpa MV, Egito EST, Oliveira AG. Oil-in-water lecithin-based microemulsions as a potential delivery system for amphotericin B. Colloid Surface B 2008;66(2):253-59.
[84]. Brime B, Molero G, Frutos P, Frutos G. Comparative therapeutic efficacy of a novel lyophilized amphotericin B lecithin-based oil-water microemulsion and deoxycholate-amphotericin B in immunocompetent and neutropenic mice infected with Candida albicans. Eur J Pharm Sci 2004;22(5):451-58.
[85]. Moreno MA, Frutos P, Ballesteros MP. Lyophilized lecithin based oil-water microemulsions as a new and low toxic delivery system for amphotericin B. Pharm Res 2001;18(3):344-51.
[86]. Darole P, Hegde D, Nair H. Formulation and evaluation of microemulsion based delivery system for amphotericin B. AAPS PharmSciTech 2008;9(1):122-28.

[87]. Damasceno BPGL, Dominici VA, Urbano IA, Silva JA, Araújo IB, Santos-Magalhães NS, Silva AKA, Medeiros AC, Oliveira AG, Egito EST. Amphotericin B microemulsion reduces toxicity and maintains the efficacy as an antifungal product. J Biomed Nanotechnol 2012;8(1):290-300.
[88]. Franzini CM, Pestana KC, Molina EF, Scarpa MV, Egito EST, Oliveira AG. Structural properties induced by the composition of biocompatible phospholipid-based microemulsion and amphotericin B association. J Biomed Nanotechnol 2012;8(1):350-59.
[89]. Nasr M, Nawaz S, Elhissi A. Amphotericin B lipid nanoemulsion aerosols for targeting peripheral respiratory airways via nebulization. Int J Pharm 2012;436(1-2):611-6.
[90]. Kohli K, Chopra S, Dhar D, Arora S, Khar RK. Self-emulsifying drug delivery systems: an approach to enhance oral bioavailability. Drug Discov Today 2010;15(21-22):958-65.
[91]. Wasan EK, Bartlett K, Gershkovich P, Sivak O, Banno B, Wong Z, Gagnon J, Gates B, Leon CG, Wasan KM. Development and characterization of oral lipid-based Amphotericin B formulations with enhanced drug solubility, stability and antifungal activity in rats infected with Aspergillus fumigatus or Candida albicans. International Journal of Pharmaceutics 2009;372(1-2):76-84.
[92]. Xu N, Gu J, Zhu Y, Wen H, Ren Q, Chen J. Efficacy of intravenous amphotericin B-polybutylcyanoacrylate nanoparticles against cryptococcal meningitis in mice. Int J Nanomedicine 2011;6:905-13.
[93]. Moribe K, Maruyama S, Inoue Y, Suzuki T, Fukami T, Tomono K, Higashi K, Tozuka Y, Yamamoto K. Ascorbyl dipalmitate/PEG-lipid nanoparticles as a novel carrier for hydrophobic drugs. International Journal of Pharmaceutics 2010;387(1-2):236-43.
[94]. Seki J, Sonoke S, Saheki A, Koike T, Fukui H, Doi M, Mayumi T. Lipid transfer protein transports compounds from lipid nanoparticles to plasma lipoproteins. International Journal of Pharmaceutics 2004;275(1-2):239-48.
[95]. Jung SH, Lim DH, Jung SH, Lee JE, Jeong KS, Seong H, Shin BC. Amphotericin B-entrapping lipid nanoparticles and their *in vitro* and *in vivo* characteristics. Eur J Pharm Sci 2009;37(3-4):313-20.
[96]. Bianco MA, Gallarate M, Trotta M, Battaglia L. Amphotericin B loaded SLN prepared with the coacervation technique. J Drug Deliv Sci Technol 2010;20(3):187-91.
[97]. Santos CMB, da Silva SW, Guilherme LR, Morais PC. SERRS study of molecular arrangement of amphotericin B adsorbed onto iron oxide nanoparticles precoated with a bilayer of lauric acid. J Phys Chem C 2011;115(42):20442-48.
[98]. Jain S, Valvi PU, Swarnakar NK, Thanki K. Gelatin coated hybrid lipid nanoparticles for oral delivery of amphotericin B. Mol Pharm 2012;9(9):2542-53.
[99]. Jain KK. Advances in use of functionalized carbon nanotubes for drug design and discovery. Expert Opinion on Drug Discovery 2012;7(11):1029-37.
[100]. Luo PG, Wang H, Gu L, Lu F, Lin Y, Christensen KA, Yang S-T, Sun Y-P. Selective interactions of sugar-functionalized single-walled carbon nanotubes with Bacillus spores. ACS Nano 2009;3(12):3909-16.

[101]. Wu W, Wieckowski S, Pastorin G, Benincasa M, Klumpp C, Briand J-P, Gennaro R, Prato M, Bianco A. Targeted Delivery of Amphotericin B to Cells by Using Functionalized Carbon Nanotubes. Angewandte Chemie International Edition 2005;44(39):6358-62.
[102]. Benincasa M, Pacor S, Wu W, Prato M, Bianco A, Gennaro R. Antifungal activity of amphotericin B conjugated to carbon nanotubes. ACS Nano 2011;5(1):199-208.
[103]. Prajapati VK, Awasthi K, Yadav TP, Rai M, Srivastava ON, Sundar S. An oral formulation of amphotericin B attached to functionalized carbon nanotubes is an effective treatment for experimental visceral leishmaniasis. J Infect Dis 2012;205(2):333-6.
[104]. Sachs-Barrable K, Lee SD, Wasan EK, Thornton SJ, Wasan KM. Enhancing drug absorption using lipids: A case study presenting the development and pharmacological evaluation of a novel lipid-based oral amphotericin B formulation for the treatment of systemic fungal infections. Adv Drug Deliv Rev 2008;60(6):692-701.
[105]. Egito EST, Fessi H, Appel M, Barratt G, Legrand P, Bolard J, Devissaguet JP. A morphological study of an amphotericin B emulsion-based delivery system. International Journal of Pharmaceutics 1996;145(1-2):17-27.
[106]. Pestana KC, Formariz TP, Franzini CM, Sarmento VHV, Chiavacci LA, Scarpa MV, Egito EST, Oliveira AG. Oil-in-water lecithin-based microemulsions as a potential delivery system for amphotericin B. Colloids and Surfaces B: Biointerfaces 2008;66(2):253-59.
[107]. Egito EST, Araújo IB, Damasceno BPGL, Price JC. Amphotericin B/emulsion admixture interactions: An approach concerning the reduction of amphotericin B toxicity. Journal of Pharmaceutical Sciences 2002;91(11):2354-66.
[108]. Brime B, Moreno MA, Frutos G, Ballesteros MP, Frutos P. Amphotericin B in oil–water lecithin-based microemulsions: Formulation and toxicity evaluation. Journal of Pharmaceutical Sciences 2002;91(4):1178-85.
[109]. Fanun M. Microemulsions as delivery systems. Current Opinion in Colloid & Interface Science 2012;17(5):306-13.

CHAPTER VI

Article 5: “Development and characterization of oi-in-water microemulsions for the delivery of amphotericin

Le dernier chapitre de cette thèse présente un article intitulé «Development of oil-in-water microemulsions for the oral delivery of amphotericin B» qui sera soumis à « International Journal of Pharmaceutics ».

Les travaux expérimentaux décrits dans cet article ont été réalisés au sein de l'Institut Galien Paris Sud, à l'Université Paris XI Sud, sous la supervision du docteur Gillian Barratt.

L'amphotéricine B (AmB) est un antibiotique polyénique avec de puissantes activités antifongique et leishmanicide. Pourtant, à cause de groupes polaires et apolaires présents dans sa structure chimique, l'AmB est peu soluble dans les milieux aqueux et dans de nombreux solvants organiques, ce qui conduit à une biodisponibilité et une perméabilité de membrane limitées. Par conséquent, le développement de formulations orales représente un défi.

Bien que des formulations parentérales efficaces d'AmB existent sur le marché, elles ont de sérieuses limitations telles que la gêne et la complexité de l'administration par voie intraveineuse, l'incidence des effets secondaires graves aigus liés à la perfusion et leurs coûts élevées. Tout ceci représente un obstacle important pour les patients dans les pays en développement.

Récemment, les formulations à base de lipides ont été largement étudiées en tant qu'approche prometteuse pour améliorer la biodisponibilité orale des médicaments peu solubles. Comme les microémulsions (ME) sont capables d'incorporer une large gamme de principes actifs, de promouvoir leur solubilisation et leur biodisponibilité et réduire leur toxicité, elles sont des systèmes de choix pour l'administration orale de molécules lipophiles telles que l'AmB. L'objectif de cette étude était de mettre au point des MEs huile dans eau (H/E) à base de triglycérides à chaîne longue et moyenne afin d'augmenter la solubilité apparente de l'AmB en milieu aqueux et de permettre son utilisation par voie orale.

Development of oil-in-water microemulsions for the oral delivery of amphotericin B

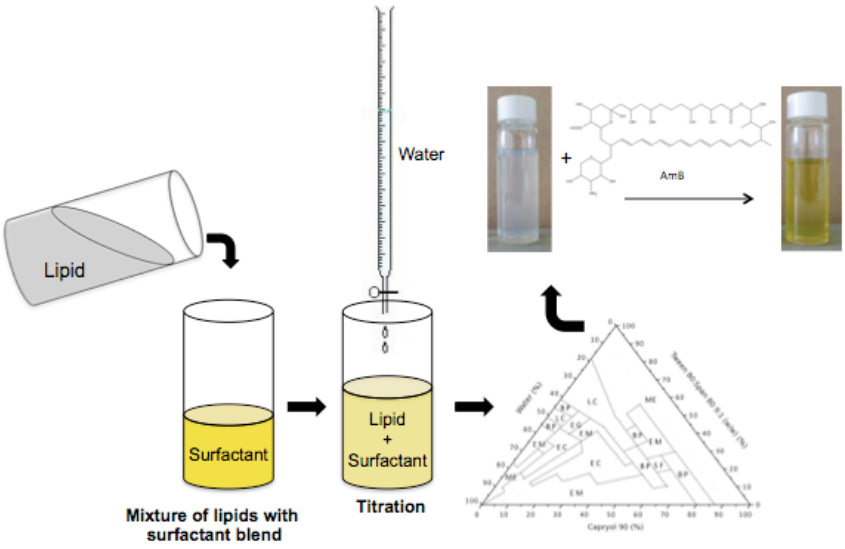
Acarília Eduardo da Silva[a,b], Gillian Barratt[b] and E. Sócrates T. Egito[a]

[a] Universidade Federal do Rio Grande do Norte. Faculdade Farmácia. Laboratório de Sistemas Dispersos (LASID), Rua Gal. Cordeiro de Farias, S/N, Petrópolis, Zip Code 59010-180, Natal-RN, Brazil.

[b] Université Paris Sud XI, Institut Galien Paris Sud, 5 rue Jean-Baptiste Clément, Zip Code 92296, Châtenay-Malabry, France.

* Corresponding author: Dr. Eryvaldo Sócrates Tabosa do Egito
UFRN – CCS – Departamento de Farmácia
Rua Praia de Areia Branca, 8948.
Zip code: 59094-450
Phone: +55 84 94 31 88 16
Fax: + 55 84 33 42 98 17
E-mail address: socratesegito@gmail.com

Keywords: amphotericin B, lipids, microemulsions, surfactants, rheology, *in vitro* toxicity



st serious diseases such as oral bioavailability is limited ceted as high-cost parenteral effects. In this study, oil-in- haracterized with the aim of for AmB. Therefore, different nonionic surfactants from the Tween® and Span® series were tested for their solubilization capacity in combination with several oils. Based on pseudoternary phase diagrams, AmB-loaded MEs with mean droplet sizes about 120 nm were successfully produced. They were able to improve the drug solubility up to 1000-fold. Rheological studies showed the MEs to be low-viscosity formulations with Newtonian behavior. Circular dichroism and absorption spectra revealed that a part of the AmB in the MEs was shown to be aggregated. Cytotoxicity studies revealed limited toxicity to macrophage-like cells that may allow the formulations to be considered as suitable carriers for AmB.

Graphical abstract

1. Introduction

Amphotericin B (AmB) is a polyene antibiotic with potent antifungal and leishmanicidal activities (Ibrahim et al., 2012). Its chemical structure is characterized by a glycosylated lactone with an amphiphilic polyhydroxy region, a conjugated heptane chromophore and an amphoteric ion pair (Figure 1). As a consequence of both apolar and polar sides of its lactone ring and due to the presence of ionizable carboxyl and amine groups, AmB molecule presents both amphoteric and amphiphilic behavior (Damasceno et al., 2012). As a result, AmB is poorly soluble in aqueous media and in many organic solvents (Torrado et al., 2008). This low solubility leads to limited bioavailability and membrane permeability, which hinder the development of formulations for the oral route that is the most convenient and acceptable route for patients.

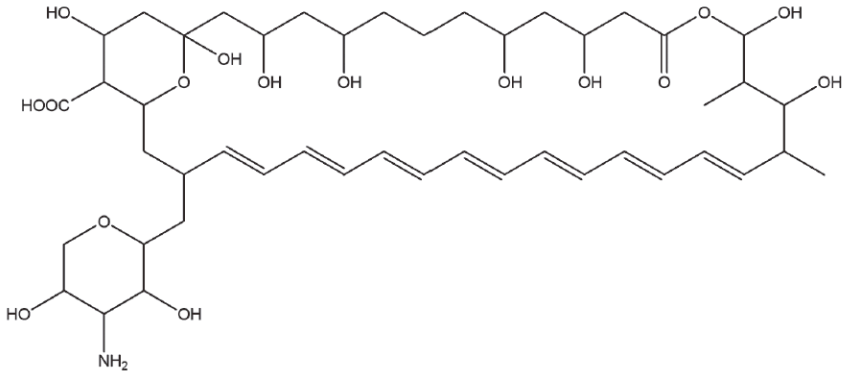


Figure 1. Chemical structure of amphotericin B (Santos et al., 2012).

On the other hand, effective parenteral formulations of AmB have been developed and marketed, but they have some serious limitations such as the inconvenience and complexity of the intravenous administration, the incidence of serious acute infusional side effects and the high cost that poses an important barrier for patients in developing countries (Wasan et al., 2009).

Recently, lipid-based formulations have been extensively investigated as a suitable approach to improve the bioavailability of poorly soluble drugs after oral administration (Han et al., 2009). When incorporated into these systems, the active molecule is believed to remain in solution throughout its period in the gastrointestinal tract (Pouton, 2006). Additionally, the absorption of the drug could be enhanced by the presence of lipids as a result of stimulation of biliary and pancreatic secretions by the gallbladder, an increase in the gastric residence time and others (Dahan and Hoffman, 2008).

Since microemulsions (MEs) are able to incorporate a wide range of drug molecules, increase their solubilization and bioavailability, and reduce their toxicity, they are promising delivery systems for oral administration of lipophilic molecules, such as AmB (Fanun, 2012; Pestana et al., 2008). Therefore, the aim of this work was to develop oil-in-water (O/W) MEs based on long- and medium-chain triglycerides in order to increase the solubility of AmB and enable its use by the oral route.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Sodium hydroxide (NaOH), chloride acid (HCl), amphotericin B (AmB) and HPLC grade methanol were purchased from Sigma Aldrich (Saint Quentin Fallavier, France).

2.1.2. Surfactants

Span® 20, Span® 80, Span® 85, Tween® 20, Tween® 80 and Tween® 85 were purchased from Sigma Aldrich (Saint Quentin Fallavier, France).

2.1.3. Lipids

Capryol® 90 (C90), Capryol® PGMC (CPGMC), Lauroglycol® 90 (L90), Labrafac® lipophile WL 1349 (LWL), Labrafac® PG (LPG) and Peceol® (Pec) were kindly supplied by Gattefossé S. A. (Saint-Priest, France). Corn oil and olive oil were obtained from Sigma Aldrich (Saint Quentin Fallavier, France).

2.2. Methods

2.2.1. Selection of oil and hydrophilic surfactant

Nonionic surfactants of the Tween® series (Table 1) and the lipids mentioned in subsection 2.1.3 were weighed and put into a series of screwcap test tubes in the ratios of 0.1:0.9, 0.2:0.8, 0.3:0.7, 0.4:0.6, and 0.5:0.5 w/w g of 1 g per batch, mixed together, and vortexed thoroughly. Afterwards, 100 µL of distilled water was added to each oil-surfactant mixture in 20–25 µL drops using a micropipette. After each drop of water was added, the system was vortexed for 15 seconds at room temperature.

Visual observations were made, and the clarity or turbidity of each sample was recorded. The isotropy of each system was also observed by light polarized microscopy

through a Nikon E600 Eclipse direct microscope (Champigny/Marne, France) equipped with a long focus objective (LWD 40 x 0.55; 0 - 2mm). A Nikon Coolpix 950 camera was used to record the images with a resolution of 1600 x 1200 pixels. The surfactant forming most clear systems was selected as the hydrophilic surfactant that best matched the tested lipid.

Table 1. Hydrophilic surfactants of the Tween® series and their HLB values (Wang et al., 2004)

Surfactant	Chemical name	General structure*	HLB
Tween® 20	Polyoxyethylene sorbitan monolaurate	$C_{12\text{-}18}S_6E_{20}$	16.7
Tween® 80	Polyoxyethylene sorbitan monooleate	$C_{18}S_6E_{20}$	15.0
Tween® 85	Polyoxyethylene sorbitan trioleate	$(3C_{18})S_6E_{20}$	11.0

*C_n represents a saturated or unsaturated hydrocarbon chain of length n, E_n is n repeating - CH_2CH_2O - groups; S_6 is a sorbitan ring

2.2.2. Selection of surfactant blends

The individual nonionic hydrophilic surfactant chosen in 2.2.1 was blended with the lipophilic surfactants of the Span® series (Table 2) in ratios of 3:2, 7:3, 4:1, and 9:1 (w/w) to produce blends of surfactants with various HLBs in the range of 9.7–14.4 (Table 3). The solubilization capacities of the blends of surfactants were studied using the same method as that used to study the other surfactants individually. The blend of surfactants forming a clear system at most of the ratios was selected as the blend that best matched the HLB of the tested lipid.

Table 2. Lipophilic surfactants of the Span® series and their HLB values (Wang et al., 2004)

Surfactant	Chemical name	General structure*	HLB
Span® 20	Sorbitan laurate	$C_{12}S_6$	8.6
Span® 80	Sorbitan monooleate	$C_{18}S_6$	4.3
Span® 85	Sorbitan trioleate	$(3C_{18})S_6$	1.8

Table 3. Composition of the surfactant blends and their final HLB values

Surfactant blend	Surfactants		Weight ratio	HLB
M1	Tween® 80	Span® 20	3:2	12.4
M2	Tween® 80	Span® 20	7:3	13.1
M3	Tween® 80	Span® 20	4:1	13.7
M4	Tween® 80	Span® 20	9:1	14.4
M5	Tween® 80	Span® 80	3:2	10.7
M6	Tween® 80	Span® 80	7:3	11.8
M7	Tween® 80	Span® 80	4:1	12.9
M8	Tween® 80	Span® 80	9:1	13.9
M9	Tween® 80	Span® 85	3:2	9.7
M10	Tween® 80	Span® 85	7:3	11.0
M11	Tween® 80	Span® 85	4:1	12.4
M12	Tween® 80	Span® 85	9:1	13.7

2.2.3. Construction of pseudoternary phase diagrams

After selection of the most suitable surfactant blend, pseudoternary phase diagrams were constructed based on the types of systems formed when the mixtures of lipids and surfactant blend were serially titrated by water followed by sonication. The systems were characterized by visual observation as described by Mahdi (Mahdi et al., 2011) (Table 4). The systems were also assessed regarding their isotropy by polarized light microscopy as described in subsection 2.2.1.

Table 4: Classification of the systems forming the pseudoternary diagrams

Category	Description
Microemulsions (ME)	Transparent or translucent and can flow easily
Liquid crystal (LC)	Transparent or translucent nonflowable when inverted 90°
Emulsion (EM)	Milky or cloudy and can flow easily
Emollient gel or cream (EG or EC)	Milky or cloudy non flowable when inverted 90°
Bicontinuous phase (BP)	More than one type of dispersion existing in the mixture, as indicated by the presence of more than one abbreviation of dispersions

2.2.4. Preparation of microemulsions

Based on the pseudoternary phase diagrams, the most suitable ratios of oil, surfactant blend and water for the production of O/W microemulsions were selected. The lipid was mixed with the surfactant blend in the weight ratios of 1:9 and 2:8 and 5 mL of water. The mixture was vortexed and subjected to sonication at 140V for 60 seconds.

2.2.5. Drug incorporation

An excess of AmB was added to the blank MEs, and the systems were vortexed for 2 min. After stirring, the mixtures were left for 10, 30 and 60 min under magnetic stirring at pH 11 in order to evaluate the time necessary for the incorporation of AmB into the systems at 25 ± 0.1 °C. After that, the pH was neutralized. The MEs were centrifuged at 10000 × *g* in a Hitachi Himac CP-80 Ultracentrifuge (USA) for 15 min to remove the excess drug. The supernatant was recovered and carefully filtered using a 0.22 μm membrane. The filtrate was diluted and dissolved in methanol for the quantitative analysis of the AmB by HPLC.

2.2.6. Microemulsion characterization

2.2.6.1. Droplet hydrodynamic size, distribution and morphology

The droplet hydrodynamic size and distribution were evaluated by dynamic light scattering (DLS) using a Malvern-Zetasizer Nano ZS (Malvern, Worcestershire, UK). The morphology of the droplets of selected O/W MEs was observed by transmission electron microscopy (TEM) using an electron microscope JEOL 1400 (SamXPlus, France) equipped with a high resolution CCD Gatan digital camera (SC1000 Orius, France) and operated at 60kV as the acceleration voltage.

2.2.6.2. Quantitative analysis of AmB

The incorporation of AmB was determined by high performance liquid chromatography (HPLC) using a Waters 2690 separations module, with a Waters 2487 dual absorbance detector (Waters, Guyancourt, France) and a C18 column (Interchim, 150 × 3 mm, 5 μm). The mobile phase consisted of a solution containing methanol/water

(80:20). An isocratic elution was performed with a flow rate of 0.5 ml min^{-1}. UV detection was performed at a wavelength of 406 nm and 25 μL of sample was injected for each analysis. To determine the linearity of the method, different concentrations of AmB in the range 0.07–4 μg mL^{-1} were prepared and analysed.

2.2.6.3. Rheological behavior

The rheological properties of AmB-loaded and AmB-free MEs were determined using a controlled-stress ARG2 rheometer (TA instruments) with cone-plate geometry. The cone-plate geometry is a 1º Peltier plate aluminum cone with a 40 mm diameter and a truncation gap of 28 μm between the cone and plate. The analyses were carried out with a shear rate in the range of 10^{-3}–10^{5} s^{-1}. All rheological determinations were carried out in triplicate for all samples and at 25.0 ± 0.2 ºC.

2.2.6.4. Absorption and circular dichroism (CD)

Absorbance measurements were made by using a Perkin-Elmer Lambda 11 UV-vis spectrophotometer. The CD spectra were recorded with a Jasco J-810 dichrograph, and $\Delta\epsilon$ ($M^{-1}.cm^{-1}$) is the differential molar absorption dichroic coefficient. These spectroscopic measurements were made at room temperature (around 20ºC) after dilution in water to a final AmB concentration of 0.3 mg/mL (path lengths of quartz cuvette: 1 cm) to evaluate the aggregation state of AmB in the formulations.

2.2.6.5. Toxicity of AmB-loaded microemulsions against macrophages

The cytotoxicity of the AmB-free and AmB-loaded MEs was tested using the MTS [3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] assay. The conversion of this salt by mitochondrial enzymes reflects the number of viable cells. J774.A1 cells (ECACC catalogue number 91051511) were cultivated in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (both from Lonza Sales Ltd., Basel, Switzerland). For experiments, they were seeded into 96-well plates at 5,000 cells/well and incubated for 24 h to allow adhesion. Samples of freshly prepared MEs (50 μL) were added to groups of 6 wells at the following concentrations: 1,

5, 10, 25, 50 and 100 μg/mL. Wells without cells but containing the same concentration of MEs were used to estimate background absorbance due to light scattering. The cells were incubated for 1 and 3h at 37ºC and 0.5% CO_2. After that, 20 μL of MTS was added to the cells and they were incubated for a further 2 h, after which the absorbance was measured using a 492 nm high-pass filter in a Multiskan MS microwell plate reader (Labsystem, Ramat-Gan, Israel). in The mitochondrial enzymatic activity was expressed as a percentage of that of untreated control cells, after correcting for background absorbance.

3. Results and discussion

3.1. Selection of surfactant and surfactant blends

C90, CPGMC, L90, LPG, LWL and Pec are liquid at room temperature and they are mainly composed of mono-, di- and triglycerides of caprylic, capric, lauric and oleic acids. Their exact composition is listed in Table 5, according to information from the supplier and the literature (Varka and Karapantsios, 2011).

Table 5. Qualitative and quantitative composition and HLB values of the lipids tested as the oil phase

Lipid	Composition	HLB
Capryol® 90	Propylene glycol mono- and diesters of caprylic acid (C8), the monoester fraction being predominant (> 90%)	6
Capryol® PGMC	Propylene glycol mono- and diesters of caprylic acid (C8), the monoester fraction being predominant (55 – 80%)	5
Lauroglycol® 90	Propylene glycol mono- and diesters of lauric acid (C12), the monoester fraction being predominant (> 90%)	5
Labrafac® PG	Dipropylene glycol esters of caprylic and capryc (C10) acids	2
Labrafac® lipophile WL 1349	Triglycerides of caprylic and capryc acids	1
Peceol®	Mono-, di- and triglycerides of oleic acid (C18:1), the monoester being predominant (32 - 52%)	3
Corn oil	Triglycerides of linoleic acid (18:2) (60%), oleic acid and linolenic acid (18:3) (<15%).	9
Olive oil	Triglycerides of oleic acid (55 – 85%), linoleic acid (7.5–20%) and palmitic acid (C16:0) (7.5–20%)	8

In order to develop MEs, an important parameter take into account is the

hydrophilic–lipophilic balance (HLB) of the surfactant or surfactant mixture (Feng et al., 2009a). It is related to the contribution of both hydrophilic and hydrophobic fragments of a surfactant molecule. Generally, surfactants with HLB values between 8 and 20 are able to form O/W MEs, while W/O MEs are formed when the HLB range is 4–7 (Lawrence and Rees, 2012).

Tween® 80 was shown to be the hydrophilic surfactant with the highest solubilization capacity when compared with Tween® 20 and Tween® 85. These emulsifiers have the same polar head but different hydrophobic tails (lauric, oleic and oleic acid, respectively in Tween 20, 80 and 85). The length of these hydrophobic chains determines the interactions with the oil phase (Mosca et al., 2013). In our experiments, we obtained clear mixtures of water and oil at the highest weight ratios for C90, CPGMC and L90. For the other oils tested, no clear mixture was obtained. Therefore, these were not used in further studies.

It is possible that the low HLBs of LPG, LWL and Pec, 2, 1 and 3 respectively, were responsible for the incompatibility between these lipids and the hydrophilic surfactants. Furthermore, the interactions between surfactants at an oil–water interphase are known to be highly dependent on the nature of the oil (Mosca et al., 2013). Corn and olive oils are composed of long-chain triglycerides and probably showed a weak interaction with the surfactant from the same fatty acid derivative, as stated in the literature (Mahdi et al., 2011).

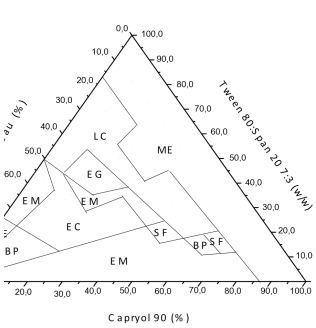
It is known that a single surfactant is not sufficient to form single-phase microemulsions and an adequate mixture of surfactants may be required to optimize the microemulsion formation (Djekic et al., 2011). The use of mixtures of nonionic surfactants is an interesting approach from the pharmaceutical point of view, since such surfactants are generally regarded as their low toxicity and irritancy and considered to be acceptable for oral administration. Additionally, the use of mixtures allows the individual concentration of each surfactant to be decreased, which may increase the biocompatibility of the final formulations (Djekic et al., 2011; Pouton and Porter, 2008). Therefore, Tween® 80 was mixed with the hydrophobic surfactants of the Span® series to provide surfactant blends in order to screen and select the best surfactant mixture to prepare oil-in-water microemulsions.

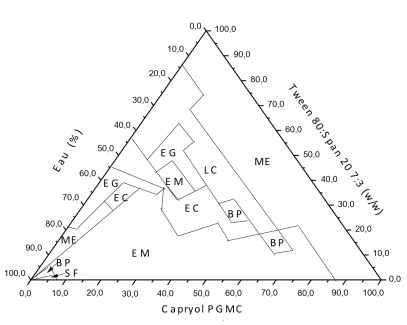
The results obtained for the solubilization power of the surfactant blends revealed two mixtures as having the highest capacities: Tween® 80/Span® 20 7:3 (v/v) (M2) and Tween® 80/Span® 80 9:1 (v/v) (M8), the HLB values of the two blends being 13.1 and 13.9

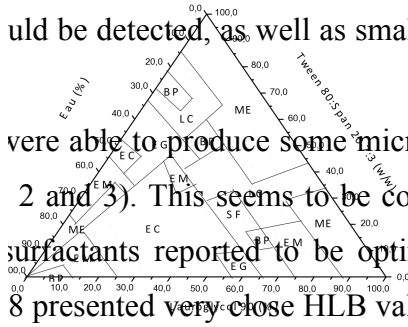
respectively. Thus, these two surfactant blends were selected to study the phase diagram behavior of C90, CPGMC and L90.

3.2. Construction of pseudoternary phase diagrams

According to the pseudoternary phase diagrams, several types of dispersions could be produced by mixing C90, CPGMC and L90 with the surfactant blends M2 and M8 followed by titration with water. For instance, large areas of emulsions, microemulsions







uld be detected, as well as smaller areas of vere able to produce some microemulsion-2 and 3). This seems to be coherent with surfactants reported to be optimal for the 8 presented very close HLB values such as 13.1 and 13.9, respectively. However, it was evident that both C90 and CPGMC were able to produce larger areas of O/W emulsions and O/W microemulsions than L90. Thus, propylene glycol esters of caprylic acid seem to be more appropriate for the preparation of O/W emulsions and microemulsions than propylene glycol esters of lauric acid. Furthermore, the phase diagram behavior of those lipids was not only affected by the HLB value of the surfactant but also by the structure of the cosurfactant.

Figure 2. Pseudoternary phase diagrams formed by a) C90, b) CPGMC and c) L90 as the oil phase and Tween 80:Span 20 7:3 (w/w) as the surfactant blend and water.

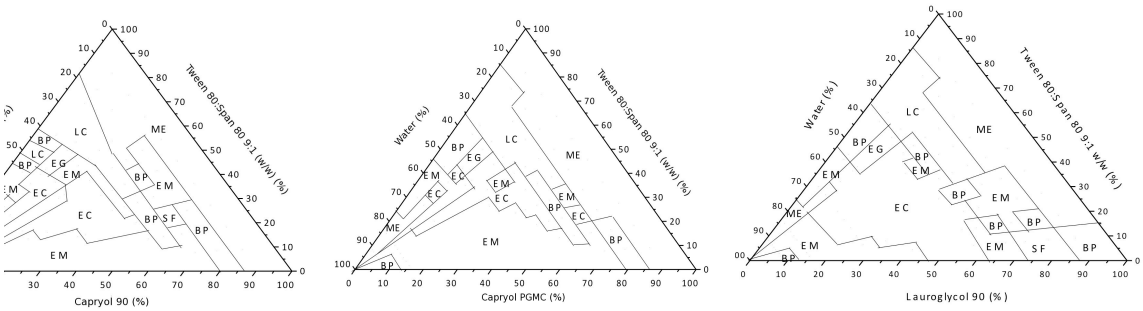


Figure 3. Pseudoternary phase diagrams formed by a) C90, b) CPGMC and c) L90 as the oil phase and Tween® 80:Span® 80 9:1 (w/w) as the surfactant blend and water.

Previous studies have observed that in general the most stable emulsions are formed when the two emulsifying agents have the same hydrocarbon chain length, like the combination between Tween® 80 and Span® 80, because of their similar chemical structure (Schmidts et al., 2009). Mahdi and coworkers stated that high solubilization capacity can be obtained when surfactants with the lowest and highest HLB values are mixed. In our case, we believe that the three chains of oleic acid esters in the molecule of Span® 85 hinder the interaction with Tween® 80. On the other hand, the interaction between Span® 80 and Tween® 80 proved to be more effective in reducing the oil-water interfacial tension and producing MEs. These formulations were used for further studies.

3.3. Microemulsion characterization

Regardless of the oil phase, DLS analysis revealed that drug-free and drug-loaded MEs were composed of spherical non-aggregated droplets with mean sizes around 80 and 120 nm respectively (Fig. 4). This increase in the diameter of the ME droplets after the incorporation of AmB is explained by the fact that AmB has surface properties due to its amphiphilic behavior and is adsorbed at the oil-water interface (Franzini et al., 2012; Santos et al., 2012). The polydispersity index was shown to be around 0.25 and 0.31 for AmB-free MEs and AmB-loaded MEs respectively.

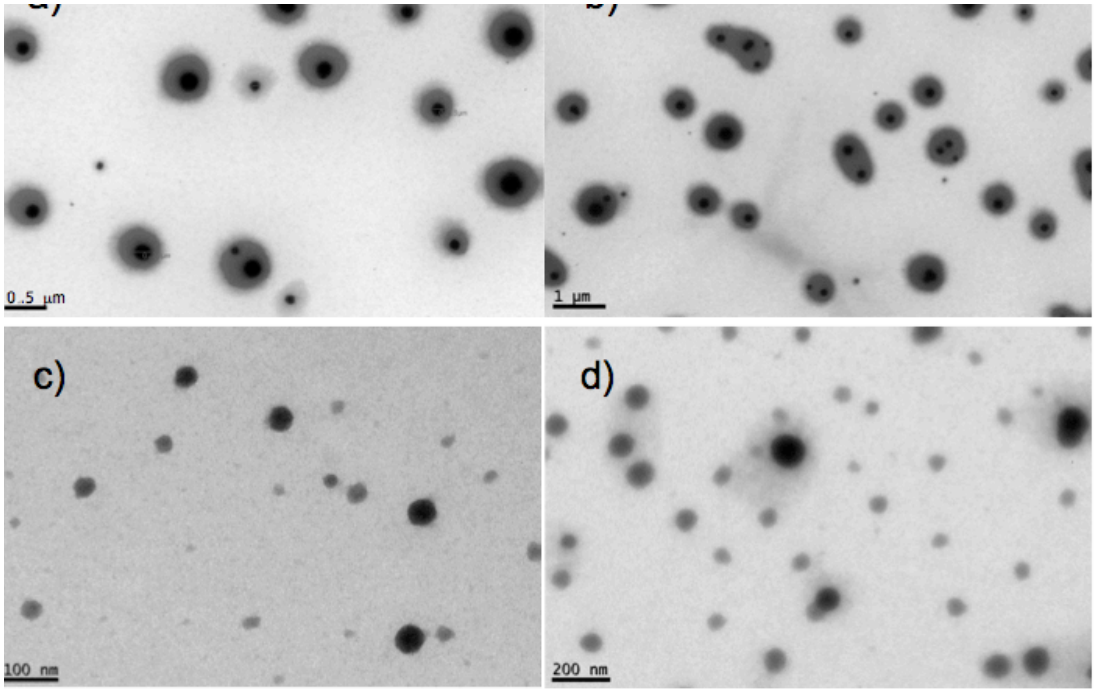


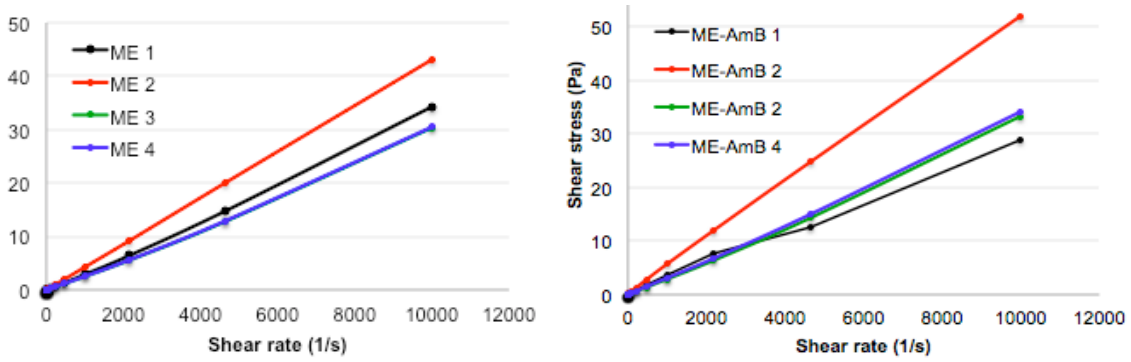
Figure 4. TEM images of AmB-loaded microemulsions based on a, b) C90:M8 1:9 and 2:8 (w/w), respectively; c, d) CPGMC 1:9 and 2:8, respectively, at magnification of 4000, 2500, 30000 and 15000X, respectively.

When the quantity of encapsulated AmB by was measured by HPLC, it was shown that the drug incorporation was dependent on the volume fraction of the dispersed phase and ranged between 70 and 90%. These data highlight the fact that the MEs are able to increase the AmB solubility up to 1000-fold when compared with its solubility in water. It also gives further evidence for the strong interaction between the hydrophobic AmB molecule and the oil present in the ME (Franzini et al., 2012). More precisely, the ME containing C90 as the oil phase at the weight ratio of 2:8 (oil:surfactant) gave the highest rate of incorporation of AmB. The incorporation time did not influence the amount of AmB incorporated, showing that the association occurred rapidly over 10 min.

3.4. Rheological behavior

The physicochemical characterization of delivery systems is an essential step in the pre-formulation process to predict the feasibility of the final products. Among the parameters for the characterization of MEs, rheology is a fundamental approach to investigate structural properties and acquire helpful information not only on the stability of

a) b)



such systems, but also on the handling, storage and pipeline transportation of MEs

very low for all

and 10 s^{-1} and

es revealed that

and shear rate,

. 5a, b). These

tley, 2012) and

suitable for oral delivery due to their low viscosity (Lawrence and Rees, 2012).

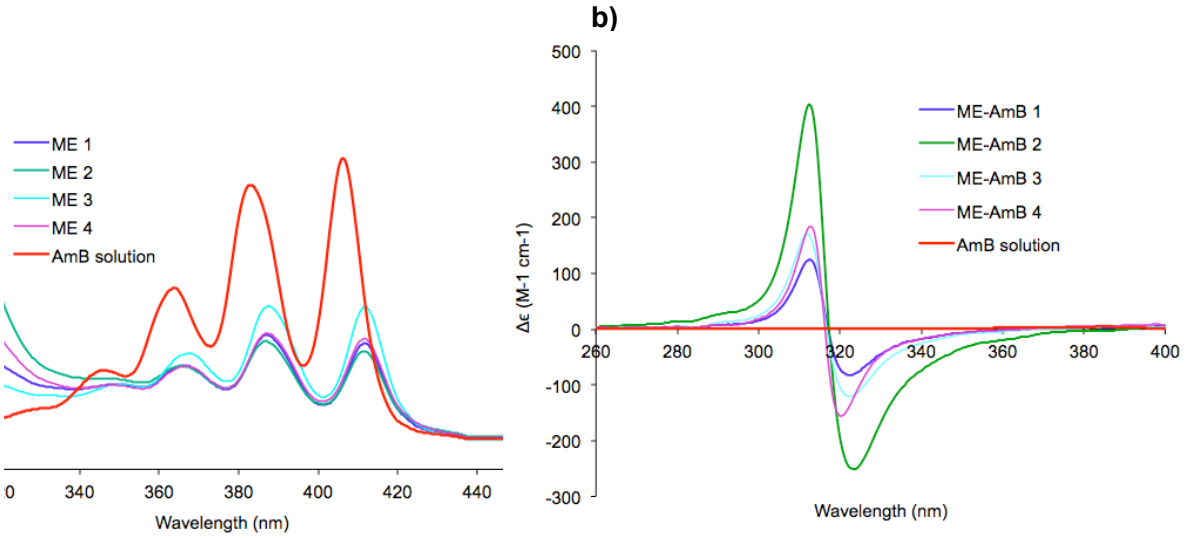
The influence of AmB on the micro-organization of the MEs was investigated. No change in the linear profile of the flow curves was observed (Fig. 5b), indicating that the drug did not influence the flow properties of the system.

Figure 5. Flow curves of a) blank MEs and b) AmB-loaded MEs.

3.5. Aggregate state of AmB

AmB self-associates in aqueous media, forming supramolecular aggregates. Monomers, and soluble and insoluble aggregates present in the dispersing miedium determine the toxicity of AmB (Silva-Filho MA, 2012). In order to study the aggregation state of AmB molecules after incorporation into the MEs, the formulations were analysed by circular dichroism and electronic absorption.

As can be seen in Fig. 6, the absorption spectrum of a stock solution of AmB in methanol after dilution in water to 0.3 mg/mL shows three bands at 410, 385, 365 nm and a smaller band at 344 nm which are characteristic of the monomeric form. The absorption



at the same

ies for each

ules of AmB.

formulation

to surfactant

roportion of

monomers. Its spectrum appears to be very similar to that of AmB in methanol (Fig. 6a).

Figure 6. a) Electronic absorption spectra and b) CD spectra of an aqueous solution of AmB and the AmB-loaded MEs.

These results are corroborated by the CD spectra of the MEs (Figure 6b). They reveal the dichroic doublet, which is characteristic of the aggregation state of AmB, for all the formulations with the center at 317 nm, with different intensities depending on the system.

3.6. Cytotoxicity profile

Figures 7a and b show the viability of J774 macrophages as determined by MTS conversion after contact with AmB-unloaded ME (ME 2), AmB-loaded ME (ME-AmB 2) and a solution of AmB in DMSO (AmB-DMSO) for 1h and 3h, respectively. The chosen

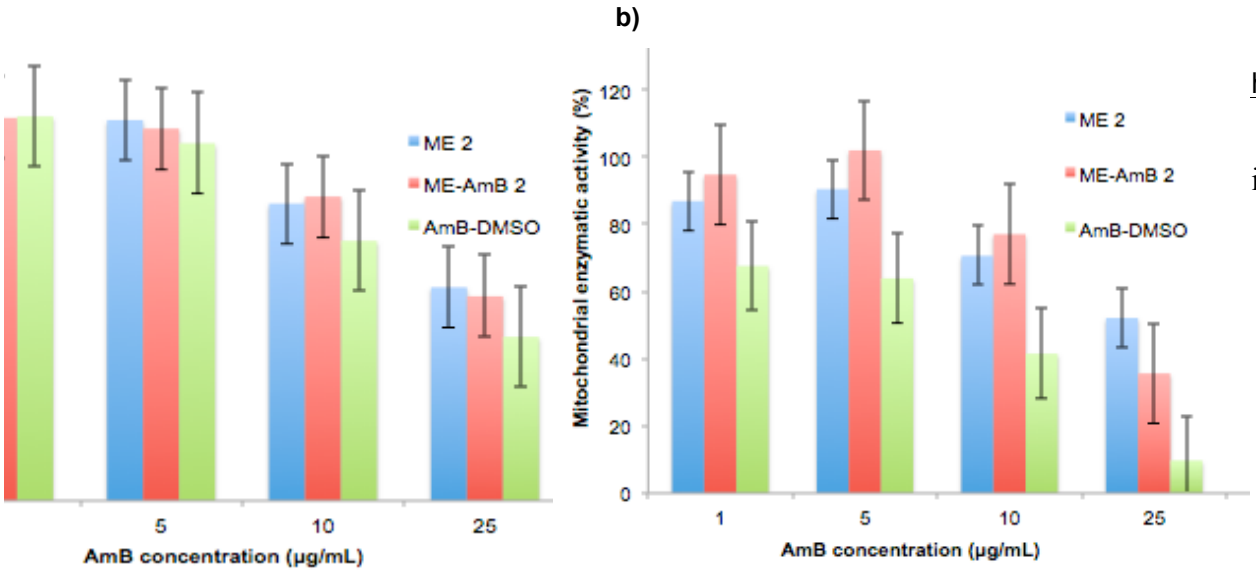


Fig. 7. Dose-response effects of blank and AmB-loaded ME containing C90 as the oil phase in the weight ratio of 2:8 (oil:surfactant) (ME 2 and ME-AmB 2, respectively) and a solution of AmB in DMSO as a control (AmB-DMSO) on the cytotoxicity on mouse macrophages (J774 cell line) after a) 1h and b) 3h of incubation, in the MTS conversion assay. Results are presented as a percentage of the activity of untreated cells.

As can be seen in Fig. 7a, the cell viability exceeded 80% after 1h for the concentrations 1, 5 and 10 μg/mL, but fell to about 60% when the AmB concentration increased to 25 μg/mL, or when the equivalent amount of unloaded MEs were added. This suggests that both blank and drug-loaded ME may be considered as having low acute toxicity. On the other hand, after 3h incubation the cell viability remained high at approximately 85% for the AmB concentrations of 1 and 5 μg/mL while the blank ME and drug-loaded ME exhibited higher toxic effects at higher concentrations of AmB. However, the formulations appeared to affect cell viability less than a solution of AmB in DMSO at the same concentration (Fig. 7b).

The MTS assay revealed that the cytotoxicity of the MEs was time-dependent. There is an indication that the MEs reduce the cytotoxicity at low concentrations, although at AmB concentrations higher than 25 μg/mL no advantage of the MEs could be observed.

4. Conclusions

Our results demonstrate that it is important to select surfactants and mixtures of surfactants with not only a suitable HLB value but also with structural similarity with the chosen oil phase for successful ME formulation. The best surfactant mixture to provide O/W ME with medium chain triglycerides based on caprylic esters as the oil phase was shown to have HLB value around 13. More precisely, Tween® 80:Span® 80 in the weight ratio of 9:1 was the surfactant providing greater solubilization capacity for C90 and CPGMC. They also improved the solubility of AmB up to 1000-fold and showed suitable rheological behavior for the oral administration. However, studies of electron absorption and CD spectra revealed the presence of aggregates of AmB. They are likely to be at the origin of the time-dependent cytotoxicity of our formulations, which were slightly less toxic than a solution of AmB in DMSO, although all formulations were well supported by J774 cells at concentrations up to 25 μg/mL of AmB.

5. References

Acharya, D.P., Hartley, P.G., 2012. Progress in microemulsion characterization. Current Opinion in Colloid & Interface Science 17, 274-280.

Dahan, A., Hoffman, A., 2008. Rationalizing the selection of oral lipid based drug delivery systems by an in vitro dynamic lipolysis model for improved oral bioavailability of poorly water soluble drugs. Journal of Controlled Release 129, 1-10.

Damasceno, B.P.G.L., Dominici, V.A., Urbano, I.A., Silva, J.A., Araújo, I.B., Santos-Magalhães, N.S., Silva, A.K.A., Medeiros, A.C., Oliveira, A.G., Egito, E.S.T., 2012. Amphotericin B microemulsion reduces toxicity and maintains the efficacy as an antifungal product. J Biomed Nanotechnol 8, 290-300.

Djekic, L., Primorac, M., Jockovic, J., 2011. Phase behaviour, microstructure and ibuprofen solubilization capacity of pseudo-ternary nonionic microemulsions. Journal of Molecular Liquids 160, 81-87.

Fanun, M., 2012. Microemulsions as delivery systems. Current Opinion in Colloid & Interface Science 17, 306-313.

Feng, J.-L., Wang, Z.-W., Zhang, J., Wang, Z.-N., Liu, F., 2009a. Study on food-grade vitamin E microemulsions based on nonionic emulsifiers. Colloids and Surfaces A: Physicochemical and Engineering Aspects 339, 1-6.

Formariz, T.P., Chiavacci, L.A., Scarpa, M.V., Silva-Junior, A.A., Egito, E.S.T., Terrugi, C.H.B., Franzini, C.M., Sarmento, V.H.V., Oliveira, A.G., 2010. Structure and viscoelastic behavior of pharmaceutical biocompatible anionic microemulsions containing the antitumoral drug compound doxorubicin. Colloids and Surfaces B: Biointerfaces 77, 47-53.

Franzini, C.M., Pestana, K.C., Molina, E.F., Scarpa, M.V., do Egito, E.S.T., de Oliveira, A.G., 2012. Structural properties induced by the composition of biocompatible phospholipid-based microemulsion and amphotericin B association. J Biomed Nanotechnol 8, 350-359.

Han, S.-f., Yao, T.-t., Zhang, X.-x., Gan, L., Zhu, C., Yu, H.-z., Gan, Y., 2009. Lipid-based formulations to enhance oral bioavailability of the poorly water-soluble drug anethol trithione: Effects of lipid composition and formulation. International Journal of Pharmaceutics 379, 18-24.

Ibrahim, F., Gershkovich, P., Sivak, O., Wasan, E.K., Bartlett, K., Wasan, K.M., 2012. Efficacy and toxicity of a tropically stable lipid-based formulation of amphotericin B (iCo-010) in a rat model of invasive candidiasis. International Journal of Pharmaceutics 436, 318-323.

Lawrence, M.J., Rees, G.D., 2012. Microemulsion-based media as novel drug delivery systems. Advanced Drug Delivery Reviews 64, Supplement, 175-193.

Mahdi, E.S., Sakeena, M.H.F., Abdulkarim, M.F., Abdullah, G.Z., Sattar, M.A., Noor, A.M., 2011. Effect of surfactant and surfactant blends on pseudoternary phase diagram behavior of newly synthesized palm kernel oil esters. Drug Design, Development and Therapy 5, 311-323.

Mosca, M., Cuomo, F., Lopez, F., Ceglie, A., 2013. Role of emulsifier layer, antioxidants and radical initiators in the oxidation of olive oil-in-water emulsions. Food Research International 50, 377-383.

Pal, R., 2011. Rheology of simple and multiple emulsions. Current Opinion in Colloid & Interface Science 16, 41-60.

Pestana, K.C., Formariz, T.P., Franzini, C.M., Sarmento, V.H.V., Chiavacci, L.A., Scarpa, M.V., Egito, E.S.T., Oliveira, A.G., 2008. Oil-in-water lecithin-based microemulsions as a potential delivery system for amphotericin B. Colloids and Surfaces B: Biointerfaces 66, 253-259.

Pouton, C.W., 2006. Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. European Journal of Pharmaceutical Sciences 29, 278-287.

Pouton, C.W., Porter, C.J.H., 2008. Formulation of lipid-based delivery systems for oral administration: Materials, methods and strategies. Advanced Drug Delivery Reviews 60, 625-637.

Santos, C.M., Oliveira, R.B., Arantes, V.T., Caldeira, L.R., Oliveira, M.C., Egito, E.S.T., Ferreira, L.A.M., 2012. Amphotericin B-loaded nanocarriers for topical treatment of cutaneous leishmaniasis: Development, characterization, and in vitro skin permeation studies. J Biomed Nanotechnol 8, 322-329.

Schmidts, T., Dobler, D., Nissing, C., Runkel, F., 2009. Influence of hydrophilic surfactants on the properties of multiple W/O/W emulsions. Journal of Colloid and Interface Science 338, 184-192.

Silva-Filho MA, S.S., Freire LB, Araújo IB, Silva KGH, Medeiros AC, Araújo-Filho I, Oliveira AG, Egito EST 2012. How can micelle systems be rebuilt by a heating process? International Journal of Nanomedicine 7, 141-150.

Torrado, J.J., Espada, R., Ballesteros, M.P., Torrado-Santiago, S., 2008. Amphotericin B formulations and drug targeting. Journal of Pharmaceutical Sciences 97, 2405-2425.

Varka, E.M., Karapantsios, T.D., 2011. Global versus local dynamics during destabilization of eco-friendly cosmetic emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects 391, 195-200.

Wang, Z., Zhao, F., Hao, X., Chen, D., Li, D., 2004. Microbial transformation of hydrophobic compound in cloud point system. Journal of Molecular Catalysis B: Enzymatic 27, 147-153.

Wasan, E.K., Bartlett, K., Gershkovich, P., Sivak, O., Banno, B., Wong, Z., Gagnon, J., Gates, B., Leon, C.G., Wasan, K.M., 2009. Development and characterization of oral lipid-based Amphotericin B formulations with enhanced drug solubility, stability and antifungal activity in rats infected with Aspergillus fumigatus or Candida albicans. International Journal of Pharmaceutics 372, 76-84.

GENERAL DISCUSSION

This thesis was devoted to the development of two delivery systems for the oral administration of two different active molecules. Hence, it is divided in two sections.

The first section addresses the use of a natural polymer abundantly found in cereals and annual plants, namely xylan. It has a great potential for use in the pharmaceutical field as an attractive raw material for the production of colon-specific drug carriers. The experimental work on xylan was carried out at the Laboratory of Dispersed Systems (LASID) at Universidade Federal do Rio Grande (UFRN) in Natal, Brazil, under the supervision of Prof. Sócrates Egito.

Colonic delivery is an extremely useful strategy for the release of fragile molecules susceptible to the degradation in stomach and small intestine and also local delivery of drugs in the colon, for the therapy of conditions such as ulcerative colitis, Crohn's disease, colorectal cancer, constipation, spastic colon and irritable bowel syndrome. In addition, oral delivery systems are preferable for colon delivery, since they can overcome the inconvenience of enemas and suppositories, although the rectal route may also be used.

Due to the fact that is it degraded exclusively by enzymes produced by bacteria present in the human colon, xylan seems to be an interesting polymer for the production of microparticles for colonic delivery of drugs. In our studies, 5-aminosalicylic acid (5-ASA) was chosen as the model drug because it is used for the treatment of inflammatory bowel diseases (IBD).

Xylan as a pharmaceutical additive has been one of the themes of the research group run by Prof. Sócrates Egito at LASID-UFRN since 2001. At that time, the group developed a method of extraction from corn cobs and successfully prepared micro and nanoparticles based on xylan by coacervation (Garcia et al., 2001).

Some years later, our group set up and optimized a new method of microencapsulation to prepare xylan microcapsules. The method, namely interfacial cross-linking polymerization, is comprised of two steps: first, an emulsification process to produce an oil-in-water emulsion, the polymer being dissolved in the aqueous phase; subsequently, the second step consists of the cross-linking of the polymer triggered by terephthaloyl chloride. Different lipophilic phases were tested: a mixture of chloroform:cyclohexane, medium chain triglycerides (Miglyol® 810N) and soybean oil.

First of all, the method was found to be feasible for the preparation of xylan microcapsules and all the lipophilic phases studied were able to produce microparticles. However, the systems prepared with Miglyol® 810N and chloroform:cyclohexane had higher physicochemical stability. Nevertheless, due to the toxicity of organic solvents such

as chloroform and cyclohexane, the mixture of medium chain triglycerides would be much more suitable for a pharmaceutical formulation. This work is thoroughly described in the paper presented in the chapter II of this thesis (Nagashima et al., 2008).

Since xylan may be extracted from several natural sources, such as sorghum, sugar cane, corn stalks and cobs and other cereal and annual plants, it was necessary to characterize precisely the polymer used by our group. Therefore, a comprehensive study was performed in order to define the physicochemical, rheological and toxicological properties of corn cob xylan.

Therefore, we extracted xylan from corn cobs by an optimized methodology described in the third chapter of this thesis (Oliveira et al., 2010). The resulting product was analyzed by electron microscopy and the mean particle size was determined by laser light scattering. The chemical composition and structure of the polymer were also studied by Fourier transform infrared spectroscopy (FT-IR) and NMR spectroscopy (NMR). Finally, the flow properties of the xylan powder were assessed by determining tapped and bulk densities, compressibility index, Hausner ratio and the angle of repose.

The polymer was satisfactorily identified by FT-IR and NMR with chemical bonds typically present in hemicelluloses. The rheological analysis revealed it as a material with low density and poor and non-free flow, which would not favour its use in the manufacturing of solid dosage forms. However, its chemical structure and its site-selective biodegradability in the colon make it a good candidate for colon-specific drug carriers. The complete physicochemical and rheological characterization of corn cob xylan is presented and discussed in detail in chapter III of this thesis.

After the determination of these essential properties, xylan was used for the microencapsulation of 5-aminosalicylic acid (5-ASA) by two techniques: interfacial cross-linking polymerization and spray-drying. In addition, a pH-dependent polymer, Eudragit-S100 (ES100), was applied as a coating for the microparticles.

The final systems were evaluated for their thermal stability, crystallinity, entrapment efficiency, and *in-vitro* drug release. Both methods were able to produce well-defined and stable microparticles. However, thermogravimetry analysis showed that spray-dried microparticles were more stable. This fact was corroborated by the low Arrhenius constant compared with the other systems. Thermal analysis also allowed us to demonstrate the complete entrapment of 5-ASA in the polymer matrix of the spray-dried microparticles because of the absence of any weight loss at the temperature of decomposition of the drug.

The X-ray diffraction studies revealed amorphous systems with low crystallinity probably as a result of the speed of the drying process, which prevented the organization of a crystalline structure. This property leads to faster dissolution due to the low energy required to separate solid amorphous compounds.

In fact, *in-vitro* drug release studies did not give entirely satisfactory results because all the formulations released 5-ASA immediately after contact with the dissolution medium, which consisted of phosphate buffer. This fast release is probably explained by the NaOH remaining in the formulations, which dissolved not only the polymers and the drug before spray-drying but also the microparticles. The pH of the dissolution medium in the presence of the microparticles was found to be around 9.0. In chapter IV of this thesis, the details of this work are discussed in depth.

The second section of this thesis concerns the development and characterization of microemulsions (MEs) containing amphotericin B (AmB). The experimental and analytical studies were carried out at the Institut Galien Paris Sud at Université Paris Sud XI, in Châtenay Malabry, France, under the supervision of Dr. Gillian Barratt.

First of all, a comprehensive literature survey was made of the most interesting findings in pharmaceutical research and development dealing with AmB formulations during the last ten years. This survey was focused on the database compiled by ISI Web of Knowledge and National Center for Biotechnology Information and published between 2002 and 2012 using “nanotechnology”, “amphotericin B”, “colloidal drug delivery system”, “microemulsion”, “nanoemulsion”, “emulsion” “micelles”, “amphiphile”, liposomes”, “nanoparticles”, and “carbon nanotubes” as search terms. The aim of this work was to summarize and provide an update on the most oustanding results on the design and research of carriers for AmB by analyzing previous results in order to envisage future further directions. There is still a need for new strategies to overcome the serious physicochemical limitations of this therapeutically useful molecule.

A number of different formulations for AmB have been investigated and many of them have yielded promising results. Special attention has been directed towards nanotechnological systems, especially lipid and emulsion-based formulations, such as liposomes, nanoparticles, nanoemulsions and microemulsions. Because of their composition, these lipid-based systems are able to incorporate a large amount of lipophilic drugs, thus enhancing the solubility and absorption of poorly soluble drugs, such as AmB, in the gastrointestinal tract. Carbon nanotubes have also been investigated as possible

carriers for AmB and good results have been achieved. Details on the literature survey are found in the chapter V of this thesis.

As a result of the literature survey, the experimental work plan was developed. The initial aim of the research project was to develop solid lipid nanoparticles for the targeting of AmB to macrophages. However, given the importance of finding an effective oral formulation of AmB and the important role of lipids in such formulations, the development of microemulsions for the oral administration of AmB became the aim of the work. The results are described in Chapter VI.

In order to produce an optimized AmB-containing microemulsion, an extensive pre-formulation study was first undertaken. Nine lipids were kindly supplied by Gateffossé S. A. (Saint Priest, France), namely Capryol® 90 (C90), Capryol® PGMC (CPGMC), Lauroglycol® 90 (L90), Labrafac® lipophile WL 1349 (LWL), Labrafac® PG (LPG), Labrasol® (Lab), Peceol® (Pec) and Plurol Oleique® CC 497 (PO). Corn oil and olive oil were also investigated. It was also necessary to choose suitable surfactants and, for this study, non-ionic surfactants were the most appropriate. These have advantages in terms of compatibility, stability, and toxicity when compared to the cationic, anionic, or amphoteric counterparts. In general, they are less toxic, less hemolytic, and less irritating and yield near physiological pH values when in solution (Jiao, 2008). In our work, three hydrophilic compounds of the Tween® series (Tween® 20, Tween® 80 and Tween® 85) and three lipophilic compounds of the Span® series (Span® 20, Span® 80 and Span® 85) were tested in the pre-formulation studies regarding their water solubilization capacity in order to find the suitable excipients to prepare oil-in-water MEs.

Based on the work by Mahdi and colleagues, each hydrophilic surfactant was first screened with each oil to observe whether they were capable of forming isotropic dispersions. (Mahdi et al., 2011). Tween® 80 yielded clear systems at all oil:surfactant weight ratios, and on this basis it was for further studies. The next step was to blend this surfactant with lipophilic nonionic surfactants at the ratios of 3:2, 7:3, 4:1, and 9:1 (w/w) to produce blends of surfactants with various HLBs in the range of 9.7–14.4. Oil and surfactant blends were then mixed and the solubilization capacity in water was tested. As a result of this stage, two surfactant blends producing the most optically clear systems were selected: Tween® 80:Span® 20 7:3 (w/w) and Tween® 80:Span® 80 9:1 (w/w) with HLB values 13.1 and 13.9, respectively.

The fact that the best surfactant blends have HLB close in values indicates the importance of this parameter as a prerequisite for the preparation of microemulsions.

Furthermore, this is also in agreement with with the concept that high solubilization capacity is obtained when surfactants with the lowest and highest HLB values are mixed together (Mahdi et al., 2011).

Among the oils tested, C90, CPGMC and L90 produced better results concerning the solubilization capacity probably due to their chemical structure and composition. These oils are medium-chain triglycerides based on mono- and diesters of caprylic (C8), capric (C10) and lauric (C12) acids, which may interact better with the surfactants than long-chain triglycerides (Mahdi et al., 2011).

Analysis of the pseudoternary phase diagrams yielded by the two surfactant blends selected, showed that both mixtures could produce emulsions and MEs. However, the ME regions were larger for C90 and CPGMC than for the other oils. The blend consisted of 90% of Tween® 80 and 10% of Span® 80 gave larger ME regions than 70% of Tween® 80 and 30% of Span® 20. This may be explained by the similar chemical structure of Tween® 80 and Span® 80, as observed in previous studies on the stability of emulsions (Schmidts et al., 2009).

After defining the composition of the MEs, AmB was successfully incorporated by changing the pH to 11 of the unloaded ME to facilitate the solubilization of AmB and its incorporation to the oil droplets. The final pH of the formulations was adjusted to 7.

The amount of of AmB present in the MEs was determined by a high liquid performance chromatography (HPLC). Before performing the assays, it was necessary to optimize the method. After testing several mobile phases and flow rates, mobile phase of a mixture of methanol: water 80:20 (v/v) used isocratically at 0.5mL/min for 15 min with peak detection at 406 nm was shown to be suitable for the determination of AmB in the MEs. AmB concentrations between 0.07 and 4.5 µg/mL could be quantified by this method. Drug incorporation in the ME to be dependent on the amount of oil phase and ranged from 70 to 90% of the added AmB, leading to concentrations of 0.9 to 1.2 mg/mL in the final formulation.. The ME with an oil:surfactant ratio of 2:8 incorporated the highest amount of AmB when the concentration added was 1.2 mg/mL. These results demonstrate the high capacity for AMB solubilization provided by MEs, since these values are 1000-fold higher than the solubility of the drug in water. This can be explained by the strong interaction between the hydrophobic AmB molecule and the oil present in the ME (Franzini et al., 2012).

The incorporation of AmB into the MEs did not show to have any influence on the rheological behaviour of the systems. They were shown to have low viscosity and to be Newtonian fluids.

The aggregation state of AmB within a formulation is a very important parameter because it allows the toxicity towards mammalian cells to be predicted (Egito et al., 1996; Gaboriau et al., 1997; Larabi et al., 2004). Therefore, circular dichroism (CD) was used to investigate this property in the optimized MEs. CD analysis revealed that a part of AmB in the MEs was shown to be aggregated and the formulation containing CPGMC as the oil phase at the weight ratio of 1:9 between oil to surfactant mixture showed the lowest proportion of aggregates and the highest proportion of monomers.

As a first approach to determining the biocompatibility of these formulations, a standard cytotoxicity assay was employed after exposure of a macrophage-like cell line, J774.A1 to the optimized MEs. This cell type was chosen because of their capacity to take up colloidal drug carrier systems by phagocytosis and because they are the host cells for leishmaniasis, a parasitic disease which is treated by AmB. In an acute toxicity study, it was observed that the formulations appeared to be less toxic than the solution of AmB in DMSO for short exposure times. The cell viability as measured by the capacity of mitochondrial enzymes to metabolize MTS remained at 80% of untreated controls after 1h for the concentrations 1, 5 and 10 μg/mL and when the AmB concentration increased to 25 μg/mL, the cell viability dropped about 60%. However, after incubation for 3h the cell viability was approximately 85% for the AmB concentrations of 1 and 5 μg/mL while the blank ME and drug-loaded ME exhibited stronger toxic effects at higher concentrations of AmB. This slight reduction in toxicity can be correlated with the proportion of AmB monomers observed in CD studies. However, the results need to be extended by studies at longer exposure times, and using different end-points, such as Trypan blue exclusion and LDH release. We also plan to perform similar experiments with Caco-2 cells as a model of intestinal epithelial cells, to predict the biocompatibility after oral administration.

The two parts of this thesis can be linked by the desire to use non parenteral routes to administer drugs with proven therapeutic activity in a more convenient way. Oral administration is a preferred route because of better patient compliance and less reliance of sophisticated medical facilities. However, innovative formulations are required to avoid drawbacks of drug instability, local toxicity and poor bioavailability. Cost is also an issue, and the two systems proposed in this thesis both use cheap and readily available starting materials. The important of the pre-formulation step, to study the interactions between the

components of the formulations using a variety of physico-chemical techniques, is also highlighted. In this way, the properties of the system can be tailored to optimize its therapeutic efficiency.

References

Egito, E.S.T., Fessi, H., Appel, M., Barratt, G., Legrand, P., Bolard, J., Devissaguet, J.P., 1996. A morphological study of an amphotericin B emulsion-based delivery system. International Journal of Pharmaceutics 145, 17-27.

Franzini, C.M., Pestana, K.C., Molina, E.F., Scarpa, M.V., do Egito, E.S.T., de Oliveira, A.G., 2012. Structural properties induced by the composition of biocompatible phospholipid-based microemulsion and amphotericin B association. J Biomed Nanotechnol 8, 350-359.

Gaboriau, F., Chéron, M., Leroy, L., Bolard, J., 1997. Physico-chemical properties of the heat-induced superaggregates of amphotericin B. Biophysical Chemistry 66, 1-12.

Garcia, R.B., Nagashima Jr, T., Praxedes, A.K.C., Raffin, F.N., Moura, T.F.A.L., do Egito, E.S.T., 2001. Preparation of micro and nanoparticles from corn cobs xylan. Polymer Bulletin 46, 371-379.

Jiao, J., 2008. Polyoxyethylated nonionic surfactants and their applications in topical ocular drug delivery. Advanced Drug Delivery Reviews 60, 1663-1673.

Larabi, M., Gulik, A., Dedieu, J.-P., Legrand, P., Barratt, G., Cheron, M., 2004. New lipid formulation of amphotericin B: spectral and microscopic analysis. Biochimica et Biophysica Acta (BBA) - Biomembranes 1664, 172-181.

Mahdi, E.S., Sakeena, M.H.F., Abdulkarim, M.F., Abdullah, G.Z., Sattar, M.A., Noor, A.M., 2011. Effect of surfactant and surfactant blends on pseudoternary phase diagram behavior of newly synthesized palm kernel oil esters. Drug Design, Development and Therapy 5, 311-323.

Nagashima, T., Oliveira, E.E., Silva, A.E., Marcelino, H.R., Gomes, M.C., Aguiar, L.M., de Araujo, I.B., Soares, L.A., Oliveira, A.G., do Egito, E.S., 2008. Influence of the lipophilic external phase composition on the preparation and characterization of xylan microcapsules - A technical note. AAPS PharmSciTech 9, 814-817.

Oliveira, E.E., Silva, A.E., Nagashima Jr, T., Gomes, M.C.S., Aguiar, L.M., Marcelino, H.R., Araujo, I.B., Bayer, M.P., Ricardo, N.M.P.S., Oliveira, A.G., Egito, E.S.T., 2010. Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization. Bioresource Technology 101, 5402-5406.

Schmidts, T., Dobler, D., Nissing, C., Runkel, F., 2009. Influence of hydrophilic surfactants on the properties of multiple W/O/W emulsions. Journal of Colloid and Interface Science 338, 184-192.

CONCLUSIONS

The need for drug delivery systems that improve the therapeutic action of a drug by enhancing its pharmacokinetics and biopharmaceutical properties has stimulated interesting advances in the pharmaceutical research field. Nanotechnology has been one of the scientific disciplines to contribute extensively with the development of novel drug delivery systems that may increase the solubility and membrane permeability of lipophilic drugs. It has also been the basis for site-specific and controlled delivery systems. Furthermore, recent interest in the use of renewable materials has directed attention to natural and biodegradable polymers as raw materials for drug carriers.

The aim of this thesis was to study two drug delivery systems: i) microparticles (MPs) based on xylan and Eudragit® S100 for the colon delivery of 5-ASA and ii) microemulsions (MEs) based on medium-chain triglycerides to improve the solubility of amphotericin B (AmB). In general, the main subject of this thesis was the recycling of i) xylan, a biopolymer abundantly found in hardwood and annual plants and normally discarded as agricultural waste products, and ii) AmB, a very potent antifungal and leishmanicidal drug whose lipophilic character hinders its solubility and membrane permeability and, consequently, oral bioavailability.

It was shown that xylan is indeed a promising material for the production of MPs by two methods: interfacial cross-linking polymerization and spray-drying. MPs with suitable physical characteristics and satisfactory yields were prepared by both methods, although the spray-dried systems showed higher thermal stability. This thermal stability and the absence of toxic agents in the preparation methods make spray-dried MPs preferable as drug delivery systems.

Concerning the extension of the use of AmB by its incorporation in MEs based on medium chain triglycerides as oil phase and non-ionic surfactants, it was shown that is was possible to increase its solubility 1000-fold when compared to that in water.

However, more work needs to be done on these systems: for example to optimize the *in-vitro* drug release rate from the MPs and improve the biocompatibility of MEs. Both systems must be evaluated *in vivo*.

During these 4 years of PhD studies a great deal of scientific information has been acquired. The opportunity of working in two distinct laboratories with different routines, staff and cultural environment was of outstanding importance for my scientific, professional and personal background.

As far as the scientific environment is concerned, I was able to learn and apply several manufacturing and analytical techniques including methods of microencapsulation

(coacervation, spray-drying, interfacial cross-linking polymerization), construction and analysis of pseudoternary phase diagrams, the calibration of drug assay methods by UV/Vis spectrophotometry and HPLC, the determination of the critical micellar concentration by spectrofluorimetry, analysis by circular dichroism and transmission electron microscopy, study of the rheological behaviour of fluids and solids, cellular culture and cytotoxicity assay in different cell lines.

Furthermore, having the opportunity of collaborating with acknowledged experts at UFRN and Université Paris Sud XI has been absolutely very rich experience, which I will benefit for my entire professional career.

REFERENCES

ABDULLAH, E. C. & GELDART, D. 1999. The use of bulk density measurements as flowability indicators. *Powder Technology,* 102**,** 151-165.

ACHARYA, D. P. & HARTLEY, P. G. 2012. Progress in microemulsion characterization. *Current Opinion in Colloid & Interface Science,* 17**,** 274-280.

AGNIHOTRI, S. A. & AMINABHAVI, T. M. 2004. Controlled release of clozapine through chitosan microparticles prepared by a novel method. *Journal of Controlled Release,* 96**,** 245-259.

AGRAWALA, P. 1990. Pharmaceutical preformulation: The physicochemical properties of drug substances. By James I. Wells. Ellis Horwood: Chichester, U.K. 1988. 227 pages. ISBN 0–7458–0276–1. *Journal of Pharmaceutical Sciences,* 79**,** 553-553.

AI, Z., JIANG, Z., LI, L., DENG, W., KUSAKABE, I. & LI, H. 2005. Immobilization of Streptomyces olivaceoviridis E-86 xylanase on Eudragit S-100 for xylo-oligosaccharide production. *Process Biochemistry,* 40**,** 2707-2714.

AL-THYABAT, S., MILES, N. J. & KOH, T. S. 2007. Estimation of the size distribution of particles moving on a conveyor belt. *Minerals Engineering,* 20**,** 72-83.

AMATO, V. S., RABELLO, A., ROTONDO-SILVA, A., KONO, A., MALDONADO, T. P. H., ALVES, I. C., FLOETER-WINTER, L. M., NETO, V. A. & SHIKANAI-YASUDA, M. A. 2004. Successful treatment of cutaneous leishmaniasis with lipid formulations of amphotericin B in two immunocompromised patients. *Acta Tropica,* 92**,** 127-132.

AMIDON, G. L., LENNERNAS, H., SHAH, V. P. & CRISON, J. R. 1995. A theoretical basis for a biopharmaceutic drug classification: The correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharmaceutical Research,* 12**,** 413-420.

AMPARO, S. 2008. Invasive fungal infections in lung transplantation: Role of aerosolised amphotericin B. *International Journal of Antimicrobial Agents,* 32, Supp 2**,** S161-S165.

ANDRY, M. C., EDWARDS-LÉVY, F. & LÉVY, M. C. 1996. Free amino group content of serum albumin microcapsules. III. A study at low pH values. *International Journal of Pharmaceutics,* 128**,** 197-202.

ANDRY, M. C. & LEVY, M. C. 1997. In vitro degradation of serum albumin microcapsules: Effect of process variables. *International Journal of Pharmaceutics,* 152**,** 145-151.

ANTON, N., BENOIT, J. P. & SAULNIER, P. 2008. Design and production of nanoparticles formulated from nano-emulsion templates - A review. *Journal of Controlled Release,* 128**,** 185-199.

ANTONIADOU, A. & DUPONT, B. 2005. Lipid formulations of amphotericin B: where are we today? *Journal de Mycologie Médicale / Journal of Medical Mycology,* 15**,** 230-238.

ARRIETA, A. C., SHEA, K., DHAR, V., CLEARY, J. P., KUKREJA, S., MORRIS, M., VARGAS-SHIRAISHI, O. M., ASHOURI, N. & SINGH, J. 2010. Once-

weekly liposomal amphotericin B as Candida prophylaxis in very low birth weight premature infants: A prospective, randomized, open-label, placebo-controlled pilot study. *Clinical Therapeutics,* 32**,** 265-271.

BARRATT, G. M. 2000. Therapeutic applications of colloidal drug carriers. *Pharmaceutical Science & Technology Today,* 3**,** 163-171.

BARWICZ, J. & TANCRÈDE, P. 1997. The effect of aggregation state of amphotericin-B on its interactions with cholesterol- or ergosterol-containing phosphatidylcholine monolayers. *Chemistry and Physics of Lipids,* 85**,** 145-155.

BELGACEM, M. N. & GANDINI, A. (eds.) 2008. *Monomers, polymers and composites from renewable resources,* Oxford: Elsevier.

BELLMANN, R., EGGER, P., DJANANI, A. & WIEDERMANN, C. J. 2004. Pharmacokinetics of amphotericin B lipid complex in critically ill patients on continuous veno-venous haemofiltration. *International Journal of Antimicrobial Agents,* 23**,** 80-83.

BENINCASA, M., PACOR, S., WU, W., PRATO, M., BIANCO, A. & GENNARO, R. 2010. Antifungal activity of amphotericin B conjugated to carbon nanotubes. *ACS Nano,* 5**,** 199-208.

BIANCO, M. A., GALLARATE, M., TROTTA, M. & BATTAGLIA, L. 2010. Amphotericin B loaded SLN prepared with the coacervation technique. *Journal of Drug Delivery Science and Technology,* 20**,** 187-191.

BILLON, A., CHABAUD, L., GOUYETTE, A., BOULER, J.-M. & MERLE, C. 2005. Vancomycin biodegradable poly(lactide-co-glycolide) microparticles for bone implantation. Influence of the formulation parameters on the size, morphology, drug loading and in vitro release. *Journal of Microencapsulation,* 22**,** 841-852.

BOSCH, F. & ROSICH, L. 2008. The Contributions of Paul Ehrlich to Pharmacology: A Tribute on the Occasion of the Centenary of His Nobel Prize. *Pharmacology,* 82**,** 171-179.

BRIME, B., MOLERO, G., FRUTOS, P. & FRUTOS, G. 2004. Comparative therapeutic efficacy of a novel lyophilized amphotericin B lecithin-based oil-water microemulsion and deoxycholate-amphotericin B in immunocompetent and neutropenic mice infected with Candida albicans. *European Journal of Pharmaceutical Sciences,* 22**,** 451-458.

BRIONES, E., ISABEL COLINO, C. & LANAO, J. M. 2008. Delivery systems to increase the selectivity of antibiotics in phagocytic cells. *Journal of Controlled Release,* 125**,** 210-227.

BRUCE, L. D., SHAH, N. H., WASEEM MALICK, A., INFELD, M. H. & MCGINITY, J. W. 2005. Properties of hot-melt extruded tablet formulations for the colonic delivery of 5-aminosalicylic acid. *European Journal of Pharmaceutics and Biopharmaceutics,* 59**,** 85-97.

BURGUERA, J. L. & BURGUERA, M. 2012. Analytical applications of emulsions and microemulsions. *Talanta,* 96**,** 11-20.

CARR, L. L. 1995. Classifying flow properties of solids. *Chem Eng.***,** 2091-2111.

CHAKRABORTY, S., SHUKLA, D., MISHRA, B. & SINGH, S. 2009. Lipid - An emerging platform for oral delivery of drugs with poor bioavailability. *European Journal of Pharmaceutics and Biopharmaceutics,* 73**,** 1-15.

CHAMILOS, G., LUNA, M., LEWIS, R. E., CHEMALY, R., RAAD, I. I. & KONTOYIANNIS, D. P. 2007. Effects of liposomal amphotericin B versus an amphotericin B lipid complex on liver histopathology in patients with hematologic malignancies and invasive fungal infections: A retrospective, nonrandomized autopsy study. *Clinical Therapeutics,* 29**,** 1980-1986.

CHANG, T. M. S. 1964. Semipermeable microcapsules. *Science,* 146**,** 524-&.

CHEN, H., KHEMTONG, C., YANG, X., CHANG, X. & GAO, J. 2011. Nanonization strategies for poorly water-soluble drugs. *Drug Discovery Today,* 16**,** 354-360.

CHEOW, W. S. & HADINOTO, K. 2012. Self-assembled amorphous drug-polyelectrolyte nanoparticle complex with enhanced dissolution rate and saturation solubility. *Journal of Colloid and Interface Science,* 367**,** 518-526.

CHUEALEE, R., ARAMWIT, P., NOIPHA, K. & SRICHANA, T. 2011. Bioactivity and toxicity studies of amphotericin B incorporated in liquid crystals. *European Journal of Pharmaceutical Sciences,* 43**,** 308-317.

CHUEALEE, R., WIEDMANN, T. S., SUEDEE, R. & SRICHANA, T. 2010. Interaction of Amphotericin B with cholesteryl palmityl carbonate ester. *Journal of Pharmaceutical Sciences,* 99**,** 4593-4602.

CILURZO, F., MINGHETTI, P., SELMIN, F., CASIRAGHI, A. & MONTANARI, L. 2003. Polymethacrylate salts as new low-swellable mucoadhesive materials. *Journal of Controlled Release,* 88**,** 43-53.

COLIN W, P. 2000. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and self-microemulsifying drug delivery systems. *European Journal of Pharmaceutical Sciences,* 11, Supplement 2**,** S93-S98.

COLLINS, T., GERDAY, C. & FELLER, G. 2005. Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiology Reviews,* 29**,** 3-23.

COLLNOT, E.-M., ALI, H. & LEHR, C.-M. 2012. Nano- and microparticulate drug carriers for targeting of the inflamed intestinal mucosa. *Journal of Controlled Release,* 161**,** 235-246.

COMAS, D. I., WAGNER, J. R. & TOMAS, M. C. 2006. Creaming stability of oil in water (O/W) emulsions: Influence of pH on soybean protein-lecithin interaction. *Food Hydrocolloids,* 20**,** 990 996.

CONE, L. A., BYRD, R. G., POTTS, B. E. & WUESTHOFF, M. 2004. Diagnosis and treatment of Candida vertebral osteomyelitis: Clinical experience with a short course therapy of amphotericin B lipid complex. *Surgical Neurology,* 62**,** 234-237.

CONTI, B., GIUNCHEDI, P. & CONTE, U. 1997. Cellulose microparticles in drug delivery. *STP Pharma Sciences,* 7**,** 331 342.

COURNARIE, F., SAVELLI, M. P., ROSILIO, W., BRETEZ, F., VAUTHIER, C., GROSSIORD, J. L. & SEILLER, M. 2004. Insulin-loaded W/O/W multiple

emulsions: comparison of the performances of systems prepared with medium-chain-triglycerides and fish oil. *European Journal of Pharmaceutics and Biopharmaceutics,* 58**,** 477-482.

COUVREUR, P., BARRATT, G., FATTAL, E., LEGRAND, P. & VAUTHIER, C. 2002. Nanocapsule technology: A review. *Critical Reviews in Therapeutic Drug Carrier Systems,* 19**,** 99-134.

CRCAREVSKA, M. S., DODOV, M. G. & GORACINOVA, K. 2008. Chitosan coated Ca-alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa. *European Journal of Pharmaceutics and Biopharmaceutics,* 68**,** 565-578.

DAGGUPATI, V. N., NATERER, G. F., GABRIEL, K. S., GRAVELSINS, R. J. & WANG, Z. L. 2011. Effects of atomization conditions and flow rates on spray drying for cupric chloride particle formation. *International Journal of Hydrogen Energy,* 36**,** 11353-11359.

DAHAN, A. & HOFFMAN, A. 2008. Rationalizing the selection of oral lipid based drug delivery systems by an in vitro dynamic lipolysis model for improved oral bioavailability of poorly water soluble drugs. *Journal of Controlled Release,* 129**,** 1-10.

DAMASCENO, B. P. G. L., DOMINICI, V. A., URBANO, I. A., SILVA, J. A., ARAÚJO, I. B., SANTOS-MAGALHÃES, N. S., SILVA, A. K. A., MEDEIROS, A. C., OLIVEIRA, A. G. & EGITO, E. S. T. 2012. Amphotericin B microemulsion reduces toxicity and maintains the efficacy as an antifungal product. *Journal of Biomedical Nanotechnology,* 8**,** 290-300.

DAROLE, P., HEGDE, D. & NAIR, H. 2008. Formulation and evaluation of microemulsion based delivery system for amphotericin B. *AAPS PharmSciTech,* 9**,** 122-128.

DATE, A. A., JOSHI, M. D. & PATRAVALE, V. B. 2007. Parasitic diseases: Liposomes and polymeric nanoparticles versus lipid nanoparticles. *Advanced Drug Delivery Reviews,* 59**,** 505-521.

DAUS, S. & HEINZE, T. 2010. Xylan-based nanoparticles: Prodrugs for ibuprofen release. *Macromolecular Bioscience,* 10**,** 211-220.

DE SOUZA, K. C. B., PETROVICK, P. R., BASSANI, V. L. & ORTEGA, G. G. 2000. The adjuvants Aerosil 200 and Gelita-Sol-P influence on the technological characteristics of spray-dryed powders from Passiflora edulis var. flavicarpa. *Drug Development and Industrial Pharmacy,* 26**,** 331 336.

DEN HAAN, R. & VAN ZYL, W. H. 2003. Enhanced xylan degradation and utilisation by Pichia stipitis overproducing fungal xylanolytic enzymes. *Enzyme and Microbial Technology,* 33**,** 620-628.

DESAI, K. G. H. & PARK, H. J. 2005a. Encapsulation of vitamin C in tripolyphosphate cross-linked chitosan microspheres by spray drying. *Journal of Microencapsulation,* 22**,** 179-192.

DESAI, K. G. H. & PARK, H. J. 2005b. Preparation of cross-linked chitosan microspheres by spray drying: Effect of cross-linking agent on the properties of spray dried microspheres. *Journal of Microencapsulation,* 22**,** 377-395.

DESHPANDE, N. M., GANGRADE, M. G., KEKARE, M. B. & VAIDYA, V. V. 2010. Determination of free and liposomal Amphotericin B in human plasma by liquid chromatography-mass spectroscopy with solid phase extraction and protein precipitation techniques. *Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences,* 878**,** 315-326.

DEVY, J., BALASSE, E., KAPLAN, H., MADOULET, C. & ANDRY, M. C. 2006. Hydroxyethylstarch microcapsules: A preliminary study for tumor immunotherapy application. *International Journal of Pharmaceutics,* 307**,** 194-200.

DEY, R., SINGH, A. K. & PANDEY, J. D. 2008. A temperature dependent viscometric study of binary liquid mixtures. *Journal of Molecular Liquids,* 137**,** 88-91.

DIN66141 1997. Darstellung von Korn-(teilchen-)grössenverteilungen - Grundlagen. *DINTaschenbuch 133 - Particlemeßtechnik Normen.* 3 ed. Berlin: Beuth.

DIN66145 1997. Darstellung von Korn-(teilchen-)grössenverteilungen - RRSB-Netz. *DINTaschenbuch 133 - Particlemeßtechnik Normen.* 3 ed. Berlin: Beuth.

DJEKIC, L., PRIMORAC, M. & JOCKOVIC, J. 2011. Phase behaviour, microstructure and ibuprofen solubilization capacity of pseudo-ternary nonionic microemulsions. *Journal of Molecular Liquids,* 160**,** 81-87.

DROTLEFF, S., LUNGWITZ, U., BREUNIG, M., DENNIS, A., BLUNK, T., TESSMAR, J. & GOPFERICH, A. 2004. Biomimetic polymers in pharmaceutical and biomedical sciences. *European Journal of Pharmaceutics and Biopharmaceutics,* 58**,** 385-407.

DURRIGL, M., KWOKAL, A., HAFNER, A., SEGVIC KLARIC, M., DUMINIC, A., CETINA-CIZMEK, B. & FILIPOVIC-GRCIC, J. 2011. Spray dried microparticles for controlled delivery of mupirocin calcium: Process--tailored modulation of drug release. *Journal of Microencapsulation,* 28**,** 108-121.

EBRINGEROV√˚ , A., KARDO≈°OV√˚ , A., HROM√˚ DKOV√˚ , Z., MALOVƑ±ÃÅKOV√˚ , A. & H≈ÔƑ±ÃÅBALOV√˚ , V. 2002. Immunomodulatory activity of acidic xylans in relation to their structural and molecular properties. *International Journal of Biological Macromolecules,* 30**,** 1-6.

EBRINGEROVÁ, A. 2005. Structural diversity and application potential of hemicelluloses. *Macromolecular Symposia,* 232**,** 1-12.

EBRINGEROVA, A. & HEINZE, T. 2000. Xylan and xylan derivatives - biopolymers with valuable properties, 1 - Naturally occurring xylans structures, procedures and properties. *Macromolecular Rapid Communications,* 21**,** 542-556.

EBRINGEROVA, A. & HROMADKOVA, Z. 1997. The effect of ultrasound on the structure and properties of the water-soluble corn hull heteroxylan. *Ultrasonics Sonochemistry,* 4**,** 305-309.

EBRINGEROVA, A. & HROMADKOVA, Z. 1999. Xylans of industrial and biomedical importance. *In:* HARDING, S. E. (ed.) *Biotechnology and Genetic*

Engineering Reviews. Andover: Intercept Ltd Scientific, Technical & Medical Publishers.

EBRINGEROVA, A., HROMADKOVA, Z., ALFODI, J. & HRIBALOVA, V. 1998. The immunologically active xylan from ultrasound-treated corn cobs: extractability, structure and properties. *Carbohydrate Polymers,* 37**,** 231-239.

EBRINGEROVA, A., HROMADKOVA, Z. & HRIBALOVA, V. 1995. Structure and mitogenic activities of corn cob heteroxylans. *International Journal of Biological Macromolecules,* 17**,** 327-331.

EBRINGEROVA, A., HROMADKOVA, Z., KACURAKOVA, M. & ANTAL, M. 1994. Quaternized xylans: Synthesis and structural characterization. *Carbohydrate Polymers,* 24**,** 301-308.

EDWARDS-LÉVY, F., ANDRY, M. C. & LEVY, M. C. 1994. Determination of free amino group content of serum-albumin microcapsules. II. Effect of variations in reaction-time and terephthaloyl chloride concentration. *International Journal of Pharmaceutics,* 103**,** 253-257.

EGITO, L. C. M., MEDEIROS, S. R. B., MEDEIROS, M. G., PRICE, J. C. & EGITO, E. S. T. 2004. Evaluation of the relationship of the molecular aggregation state of amphotericin B in medium to its genotoxic potential. *Journal of Pharmaceutical Sciences,* 93**,** 1557-1565.

ELIZONDO, E., MORENO, E., CABRERA, I., CÓRDOBA, A., SALA, S., VECIANA, J. & VENTOSA, N. 2011. Liposomes and other vesicular systems: Structural characteristics, methods of preparation, and use in nanomedicine. *In:* VILLAVERDE, A. (ed.) *Progress in Molecular Biology and Translational Science.* Academic Press.

ESPADA, R., VALDESPINA, S., ALFONSO, C., RIVAS, G., BALLESTEROS, M. P. & TORRADO, J. J. 2008. Effect of aggregation state on the toxicity of different amphotericin B preparations. *International Journal of Pharmaceutics,* 361**,** 64-69.

FANUN, M. 2012. Microemulsions as delivery systems. *Current Opinion in Colloid & Interface Science,* 17**,** 306-313.

FENG, J.-L., WANG, Z.-W., ZHANG, J., WANG, Z.-N. & LIU, F. 2009. Study on food-grade vitamin E microemulsions based on nonionic emulsifiers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 339**,** 1-6.

FORD, A. C., KHAN, K. J., SANDBORN, W. J., HANAUER, S. B. & MOAYYEDI, P. 2012. Efficacy of topical 5-aminosalicylates in preventing relapse of quiescent ulcerative colitis: A meta-analysis. *Clinical Gastroenterology and Hepatology,* 10**,** 513-519.

FORMARIZ, T. P., CHIAVACCI, L. A., SCARPA, M. V., SILVA-JUNIOR, A. A., EGITO, E. S. T., TERRUGI, C. H. B., FRANZINI, C. M., SARMENTO, V. H. V. & OLIVEIRA, A. G. 2010. Structure and viscoelastic behavior of pharmaceutical biocompatible anionic microemulsions containing the antitumoral drug compound doxorubicin. *Colloids and Surfaces B: Biointerfaces,* 77**,** 47-53.

FOSTER, T. P. & LEATHERMAN, M. W. 1995. Powder Characteristics of Proteins Spray-Dried from Different Spray-Dryers. *Drug Development and Industrial Pharmacy,* 21**,** 1705-1723.

FRANZINI, C. M., PESTANA, K. C., MOLINA, E. F., SCARPA, M. V., DO EGITO, E. S. T. & DE OLIVEIRA, A. G. 2012a. Structural properties induced by the composition of biocompatible phospholipid-based microemulsion and amphotericin B association. *Journal of Biomedical Nanotechnology,* 8**,** 350-9.

FRANZINI, C. M., PESTANA, K. C., MOLINA, E. F., SCARPA, M. V., EGITO, E. S. T. & OLIVEIRA, A. G. 2012b. Structural properties induced by the composition of biocompatible phospholipid-based microemulsion and amphotericin B association. *Journal of Biomedical Nanotechnology,* 8**,** 350-359.

FREIBERG, S. & ZHU, X. X. 2004. Polymer microspheres for controlled drug release. *International Journal of Pharmaceutics,* 282**,** 1 18.

FRIEND, D. R. 2005. New oral delivery systems for treatment of inflammatory bowel disease. *Advanced Drug Delivery Reviews,* 57**,** 247-265.

FRONER, E., D'AMATO, E., ADAMO, R., PRTLJAGA, N., LARCHERI, S., PAVESI, L., RIGO, A., POTRICH, C. & SCARPA, M. 2011. Deoxycholate as an efficient coating agent for hydrophilic silicon nanocrystals. *Journal of Colloid and Interface Science,* 358**,** 86-92.

FU, Z.-Q., WANG, L.-J., LI, D. & ADHIKARI, B. 2012. Effects of partial gelatinization on structure and thermal properties of corn starch after spray drying. *Carbohydrate Polymers.*

FUKUI, H., KOIKE, T., SAHEKI, A., SONOKE, S. & SEKI, J. 2003a. A novel delivery system for amphotericin B with lipid nano-sphere (LNS¬Æ). *International Journal of Pharmaceutics,* 265**,** 37-45.

FUKUI, H., KOIKE, T., SAHEKI, A., SONOKE, S., TOMII, Y. & SEKI, J. 2003b. Evaluation of the efficacy and toxicity of amphotericin B incorporated in lipid nano-sphere (LNS®). *International Journal of Pharmaceutics,* 263**,** 51-60.

GABORIAU, F., CHÉRON, M., LEROY, L. & BOLARD, J. 1997. Physico-chemical properties of the heat-induced superaggregates of amphotericin B. *Biophysical Chemistry,* 66**,** 1-12.

GALLO, M. A. 2010. History and scope of toxicology. *In:* SHANAHAN, J. & NAGLIERI, C. (eds.) *Casarett & Doull's Essentials of Toxicology.* 2 ed.: The McGraw Hill Companies.

GARCIA, R. B., GANTER, J. & CARVALHO, R. R. 2000. Solution properties of D-xylans from corn cobs. *European Polymer Journal,* 36**,** 783-787.

GARCIA, R. B., NAGASHIMA JR, T., PRAXEDES, A. K. C., RAFFIN, F. N., MOURA, T. F. A. L. & DO EGITO, E. S. T. 2001. Preparation of micro and nanoparticles from corn cobs xylan. *Polymer Bulletin,* 46**,** 371-379.

GELDART, D., ABDULLAH, E. C., HASSANPOUR, A., NWOKE, L. C. & WOUTERS, I. 2006. Characterization of powder flowability using measurement of angle of repose. *China Particuology,* 4**,** 104-107.

GOUIN, S. 2004. Microencapsulation: Industrial appraisal of existing technologies and trends. *Trends in Food Science & Technology,* 15**,** 330-347.

GROOTAERT, C., DELCOUR, J. A., COURTIN, C. M., BROEKAERT, W. F., VERSTRAETE, W. & VAN DE WIELE, T. 2007. Microbial metabolism and prebiotic potency of arabinoxylan oligosaccharides in the human intestine. *Trends in Food Science & Technology,* 18**,** 64-71.

HABIBI, Y. & VIGNON, M. R. 2005. Isolation and characterization of xylans from seed pericarp of Argania spinosa fruit. *Carbohydrate Research,* 340**,** 1431-1436.

HAJDU, S. I. 2005. Two pioneering chemists, three hundred years apart. *Annals of Clinical and Laboratory Science,* 35**,** 105-107.

HAN, S.-F., YAO, T.-T., ZHANG, X.-X., GAN, L., ZHU, C., YU, H.-Z. & GAN, Y. 2009. Lipid-based formulations to enhance oral bioavailability of the poorly water-soluble drug anethol trithione: Effects of lipid composition and formulation. *International Journal of Nanomedicine,* 379**,** 18-24.

HARTSEL, S. & BOLARD, J. 1996. Amphotericin B: new life for an old drug. *Trends in Pharmacological Sciences,* 17**,** 445-449.

HAUSNER, H. H. 1967. Friction conditions in a mass of metal powders. *International Journal of Powder Metallurgy***,** 7-13.

HAUSS, D. J. 2007. Oral lipid-based formulations. *Advanced Drug Delivery Reviews,* 59**,** 667-676.

HEINZE, T., PETZOLD, K. & HORNIG, S. 2007. Novel nanoparticles based on xylan. *Cellulose Chemistry and Technology,* 41**,** 13-18.

HEREC, M., ISLAMOV, A., KUKLIN, A., GAGOS, M. & GRUSZECKI, W. I. 2007. Effect of antibiotic amphotericin B on structural and dynamic properties of lipid membranes formed with egg yolk phosphatidylcholine. *Chemistry and Physics of Lipids,* 147**,** 78-86.

HEURTAULT, B., SAULNIER, P., PECH, B., PROUST, J.-E. & BENOIT, J.-P. 2003. Physico-chemical stability of colloidal lipid particles. *Biomaterials,* 24**,** 4283-4300.

HILLERY, A. M. 1997. Supramolecular lipidic drug delivery systems: From laboratory to clinic A review of the recently introduced commercial liposomal and lipid-based formulations of amphotericin B. *Advanced Drug Delivery Reviews,* 24**,** 345-363.

HODGSON, E. 2004. Introduction to toxicology. *In:* HODGSON, E. (ed.) *A textbook of modern toxicology.* 3 ed. Hoboken, NJ: John Wiley & Sons.

HOOSHMAND-RAD, R., CHU, A., GOTZ, V., MORRIS, J., BATTY, S. & FREIFELD, A. 2005. Use of amphotericin B lipid complex in elderly patients. *Journal of Infection,* 50**,** 277-287.

HOOSHMAND-RAD, R., REED, M. D., CHU, A., GOTZ, V., MORRIS, J. A., WEINBERG, J. & DOMINGUEZ, E. A. 2004. Retrospective study of the renal effects of amphotericin B lipid complex when used at higher-than-recommended dosages and longer durations compared with lower dosages and

shorter durations in patients with systemic fungal infections. *Clinical Therapeutics,* 26**,** 1652-1662.

HOUREZ, R., GILLARD, P. H., MARTIAT, P. & AOUN, M. 2002. Disseminated fungemia due to Candida krusei with cutaneous lesions and successful treatment by amphotericin B lipid complex and catheter removal: a case report. *International Journal of Infectious Diseases,* 6**,** 326-328.

HROMADKOVA, Z., KOVACIKOVA, J. & EBRINGEROVA, A. 1999. Study of the classical and ultrasound-assisted extraction of the corn cob xylan. *Industrial Crops and Products,* 9**,** 101-109.

HUGHES, G. A. 2005. Nanostructure-mediated drug delivery. *Nanomedicine: Nanotechnology, Biology and Medicine,* 1**,** 22-30.

IBRAHIM, F., GERSHKOVICH, P., SIVAK, O., WASAN, E. K., BARTLETT, K. & WASAN, K. M. 2012a. Efficacy and toxicity of a tropically stable lipid-based formulation of amphotericin B (iCo-010) in a rat model of invasive candidiasis. *International Journal of Pharmaceutics,* 436**,** 318-323.

IBRAHIM, F., GERSHKOVICH, P., SIVAK, O., WASAN, E. K. & WASAN, K. M. 2012b. Assessment of novel oral lipid-based formulations of amphotericin B using an in vitro lipolysis model. *European Journal of Pharmaceutical Sciences,* 46**,** 323-328.

IMAN, M., HUANG, Z., SZOKA JR, F. C. & JAAFARI, M. R. 2011. Characterization of the colloidal properties, *in vitro* antifungal activity, antileishmanial activity and toxicity in mice of a distigmasterylhemisuccinoyl-glycero-phosphocholine liposome-intercalated amphotericin B. *International Journal of Pharmaceutics,* 408**,** 163-172.

JAIN, K. K. 2012. Advances in use of functionalized carbon nanotubes for drug design and discovery. *Expert Opinion on Drug Discovery,* 7**,** 1029-1037.

JAIN, S., VALVI, P. U., SWARNAKAR, N. K. & THANKI, K. 2012. Gelatin coated hybrid lipid nanoparticles for oral delivery of amphotericin B. *Molecular Pharmaceutics,* 9**,** 2542-53.

JEE, J.-P., MCCOY, A. & MECOZZI, S. 2012. Encapsulation and release of amphotericin B from an ABC triblock fluorous copolymer. *Pharmaceutical Research,* 29**,** 69-82.

JENSEN, G. M., BUNCH, T. H., WOLF, S. & LAYBOURNE, S. 2010. Erroneous determination of hyperphosphatemia ('pseudohyperphosphatemia') in sera of patients that have been treated with liposomal amphotericin B (AmBisome). *Clinica Chimica Acta,* 411**,** 1900-1905.

JEONG, Y., JIN, G. W., CHOI, E., JUNG, J. H. & PARK, J. S. 2011. Effect of deoxycholate conjugation on stability of pDNA/polyamidoamine-diethylentriamine (PAM-DET) polyplex against ionic strength. *International Journal of Pharmaceutics,* 420**,** 366-370.

JIANG, B. B., HU, L., GAO, C. Y. & SHEN, J. C. 2006. Cross-linked polysaccharide nanocapsules: Preparation and drug release properties. *Acta Biomaterialia,* 2**,** 9-18.

JIANG, L., YAN, Y. & HUANG, J. 2011. Versatility of cyclodextrins in self-assembly systems of amphiphiles. *Adv Colloid Interfac,* 169**,** 13-25.

JUNG, S. H., LIM, D. H., JUNG, S. H., LEE, J. E., JEONG, K. S., SEONG, H. & SHIN, B. C. 2009. Amphotericin B-entrapping lipid nanoparticles and their *in vitro* and *in vivo* characteristics. *European Journal of Pharmaceutical Sciences,* 37**,** 313-320.

JUNGHANNS, J. U., BUTTLE, I., MULLER, R. H., ARAUJO, I. B., SILVA, A. K. A., EGITO, E. S. T. & DAMASCENO, B. P. G. L. 2007. SolEmuls® technology: A way to overcome the drawback of parenteral administration of insoluble drugs. *Pharmaceutical Development and Technology,* 12**,** 437-445.

JYOTHI, N. V. N., PRASANNA, P. M., SAKARKAR, S. N., PRABHA, K. S., RAMAIAH, P. S. & SRAWAN, G. Y. 2010. Microencapsulation techniques, factors influencing encapsulation efficiency. *Journal of Microencapsulation,* 27**,** 187-197.

KAČURÁKOVÁ, M., BELTON, P. S., WILSON, R. H., HIRSCH, J. & EBRINGEROVÁ, A. 1998. Hydration properties of xylan-type structures: an FTIR study of xylooligosaccharides. *Journal of the Science of Food and Agriculture,* 77**,** 38-44.

KACURÁKOVÁ, M., CAPEK, P., SASINKOVÁ, V., WELLNER, N. & EBRINGEROVÁ, A. 2000. FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers,* 43**,** 195-203.

KAČURÁKOVÁ, M., EBRINGEROVÁ, A., HIRSCH, J. & HROMÁDKOVÁ, Z. 1994. Infrared study of arabinoxylans. *Journal of the Science of Food and Agriculture,* 66**,** 423-427.

KAGAN, L., GERSHKOVICH, P., WASAN, K. & MAGER, D. 2011. Physiologically based pharmacokinetic model of amphotericin B disposition in rats following administration of deoxycholate formulation (Fungizone®): Pooled analysis of published data. *The AAPS Journal,* 13**,** 255-264.

KAWABATA, Y., WADA, K., NAKATANI, M., YAMADA, S. & ONOUE, S. 2011. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: Basic approaches and practical applications. *International Journal of Pharmaceutics,* 420**,** 1-10.

KAYSER, O., OLBRICH, C., CROFT, S. L. & KIDERLEN, A. F. 2003. Formulation and biopharmaceutical issues in the development of drug delivery systems for antiparasitic drugs. *Parasitology research,* 90**,** S63-S70.

KAYSERILIOĮÜLU, B. L. Û., BAKIR, U., YILMAZ, L. & AKKA≈Ü, N. 2003. Use of xylan, an agricultural by-product, in wheat gluten based biodegradable films: mechanical, solubility and water vapor transfer rate properties. *Bioresource Technology,* 87**,** 239-246.

KAYSERILIOGLU, B. S., BAKIR, U., YILMAZ, L. & AKKAS, N. 2003. Use of xylan, an agricultural by-product, in wheat gluten based biodegradable films: mechanical, solubility and water vapor transfer rate properties. *Bioresource Technology,* 87**,** 239-246.

KIM, B.-Y., KANG, H.-S. & KIM, J.-D. 2002. Thermo-sensitive microparticles of PNIPAM-grafted ethylcellulose by spray-drying method. *Journal of Microencapsulation,* 19**,** 661-669.

KIM, Y. T., SHIN, B. K., GARRIPELLI, V. K., KIM, J. K., DAVAA, E., JO, S. & PARK, J. S. 2010. A thermosensitive vaginal gel formulation with HPγCD for the pH-dependent release and solubilization of amphotericin B. *European Journal of Pharmaceutical Sciences,* 41**,** 399-406.

KOHLI, K., CHOPRA, S., DHAR, D., ARORA, S. & KHAR, R. K. 2010. Self-emulsifying drug delivery systems: an approach to enhance oral bioavailability. *Drug Discovery Today,* 15**,** 958-965.

KOST, J. & LANGER, R. 2012. Responsive polymeric delivery systems. *Advanced Drug Delivery Reviews,* 64, Supplement**,** 327-341.

KREUTER, J. 1996. Nanoparticles and microparticles for drug and vaccine delivery. *Journal of Anatomy,* 189 (Pt 3)**,** 503-505.

KULKARNI, N., SHENDYE, A. & RAO, M. 1999. Molecular and biotechnological aspects of xylanases. *FEMS Microbiology Reviews,* 23**,** 411-456.

KUMAR, G. P. & RAJESHWARRAO, P. 2011. Nonionic surfactant vesicular systems for effective drug delivery - An overview. *Acta Pharmaceutica Sinica B,* 1**,** 208-219.

KUMAR, M. 2000. Nano and microparticles as controlled drug delivery devices. *Journal of Pharmacy and Pharmaceutical Sciences,* 3**,** 234-258.

KUMAR, V., DE LA LUZ REUS-MEDINA, M. & YANG, D. 2002. Preparation, characterization, and tabletting properties of a new cellulose-based pharmaceutical aid. *International Journal of Pharmaceutics,* 235**,** 129-140.

LANE, J. W., REHAK, N. N., HORTIN, G. L., ZAOUTIS, T., KRAUSE, P. R. & WALSH, T. J. 2008. Pseudohyperphosphatemia associated with high-dose liposomal amphotericin B therapy. *Clinica Chimica Acta,* 387**,** 145-149.

LANIADO-LABORÍN, R. & CABRALES-VARGAS, M. N. 2009. Amphotericin B: side effects and toxicity. *Revista Iberoamericana de Micología,* 26**,** 223-227.

LARABI, M., GULIK, A., DEDIEU, J. P., LEGRAND, P., BARRATT, G. & CHERON, M. 2004. New lipid formulation of amphotericin B: spectral and microscopic analysis. *Biochim Biophys Acta,* 1664**,** 172-181.

LARIONOVA, N. V., KAZANSKAYA, N. F., LARIONOVA, N. I., PONCHEL, G. & DUCHENE, D. 1999. Preparation and characterization of microencapsulated proteinase inhibitor aprotinin. *Biochemistry,* 64**,** 857-862.

LAWRENCE, M. J. & REES, G. D. 2012. Microemulsion-based media as novel drug delivery systems. *Advanced Drug Delivery Reviews,* 64, Supplement**,** 175-193.

LEE, H. J., MCAULEY, A., SCHILKE, K. F. & MCGUIRE, J. 2011. Molecular origins of surfactant-mediated stabilization of protein drugs. *Advanced Drug Delivery Reviews,* 63**,** 1160-1171.

LEMKE, A., KIDERLEN, A. F., PETRI, B. & KAYSER, O. 2010. Delivery of amphotericin B nanosuspensions to the brain and determination of activity against Balamuthia mandrillaris amebas. *Nanomedicine: Nanotechnology, Biology and Medicine,* 6**,** 597-603.

LEONARDI, D., SALOMÓN, C. J., LAMAS, M. C. & OLIVIERI, A. C. 2009. Development of novel formulations for Chagas' disease: Optimization of benznidazole chitosan microparticles based on artificial neural networks. *International Journal of Pharmaceutics,* 367**,** 140-147.

LEVY, M. C. & ANDRY, M. C. 1990. Microcapsules Prepared through Interfacial Cross-Linking of Starch Derivatives. *International Journal of Pharmaceutics,* 62**,** 27-35.

LÉVY, M. C. & ANDRY, M. C. 1990. Microcapsules prepared through interfacial cross-linking of starch derivatives. *International Journal of Pharmaceutics,* 62**,** 27-35.

LI, B.-Z., WANG, L.-J., LI, D., CHIU, Y. L., ZHANG, Z.-J., SHI, J., CHEN, X. D. & MAO, Z.-H. 2009. Physical properties and loading capacity of starch-based microparticles crosslinked with trisodium trimetaphosphate. *Journal of Food Engineering,* 92**,** 255-260.

LI, X., SHI, X., WANG, M. & DU, Y. 2011. Xylan chitosan conjugate - A potential food preservative. *Food Chemistry,* 126**,** 520-525.

LIU, Z., JIAO, Y., WANG, Y., ZHOU, C. & ZHANG, Z. 2008. Polysaccharides-based nanoparticles as drug delivery systems. *Advanced Drug Delivery Reviews,* 60**,** 1650-1662.

LUKE, R. G. & BOYLE, J. A. 1998. Renal effects of amphotericin B lipid complex. *American Journal of Kidney Diseases,* 31**,** 780-785.

LUO, J.-Y., ZHONG, Y., CAO, J.-C. & CUI, H.-F. 2011. Efficacy of oral colon-specific delivery capsule of low-molecular-weight heparin on ulcerative colitis. *Biomedicine & Pharmacotherapy,* 65**,** 111-117.

LUO, P. G., WANG, H., GU, L., LU, F., LIN, Y., CHRISTENSEN, K. A., YANG, S.-T. & SUN, Y.-P. 2009. Selective interactions of sugar-functionalized single-walled carbon nanotubes with Bacillus spores. *ACS Nano,* 3**,** 3909-3916.

M. D. L. MORETTI, E. G., C. JULIANO, G. PIRISINO, P. GIUNCHEDI 2001. Spray-dried microspheres containing ketoprofen formulated into capsules and tablets. *Journal of Microencapsulation,* 18**,** 111-121.

MACEDO, J. P. F., FERNANDES, L. L., FORMIGA, F. R., REIS, M. F., NAGASHIMA, T., SOARES, L. A. L. & EGITO, E. S. T. 2006. Micro-emultocrit technique: A valuable tool for determination of critical HLB value of emulsions. *Aaps Pharmscitech,* 7.

MAHDI, E. S., SAKEENA, M. H. F., ABDULKARIM, M. F., ABDULLAH, G. Z., SATTAR, M. A. & NOOR, A. M. 2011. Effect of surfactant and surfactant blends on pseudoternary phase diagram behavior of newly synthesized palm kernel oil esters. *Drug Design, Development and Therapy,* 5**,** 311-323.

MANOSROI, A., KONGKANERAMIT, L. & MANOSROI, J. 2004. Characterization of amphotericin B liposome formulations. *Drug Development and Industrial Pharmacy,* 30**,** 535-543.

MARKOWITZ, J. 2008. Current treatment of inflammatory bowel disease in children. *Digestive and Liver Disease,* 40**,** 16-21.

MARQUES, A. P. C., H. R.; COUTINHO, O. P.; REIS, R. L. 2005. Effect of starch-based biomaterials on the in vitro proliferation and viability of osteoblast-like cells. *Journal of Materials Science: Materials in Medicine,* 16**,** 833-842.

MCCLEMENTS, D. J. 2012a. Advances in fabrication of emulsions with enhanced functionality using structural design principles. *Current Opinion in Colloid & Interface Science,* 17**,** 235-245.

MCCLEMENTS, D. J. 2012b. Crystals and crystallization in oil-in-water emulsions: Implications for emulsion-based delivery systems. *Adv Colloid Interface Sci,* 174**,** 1-30.

MCCLEMENTS, D. J. 2012c. Nanoemulsions versus microemulsions: terminology, differences, and similarities. *Soft Matter,* 8**,** 1719-1729.

MENDOZA, D., CONNORS, S., LANE, C. & STEHNACH, S. 2008. Liposomal amphotericin B as a cause of pseudohyperphosphatemia. *Clinical Infectious Diseases,* 46**,** 645-646.

MICHAEL, K. 2006. What is the current and future status of conventional amphotericin B? *International Journal of Antimicrobial Agents,* 27, Supp 1**,** 12-16.

MISRA, A., HICKEY, A. J., ROSSI, C., BORCHARD, G., TERADA, H., MAKINO, K., FOURIE, P. B. & COLOMBO, P. 2011. Inhaled drug therapy for treatment of tuberculosis. *Tuberculosis,* 91**,** 71-81.

MLADENOVSKA, K., CRUAUD, O., RICHOMME, P., BELAMIE, E., RAICKI, R. S., VENIER-JULIENNE, M. C., POPOVSKI, E., BENOIT, J. P. & GORACINOVA, K. 2007. 5-ASA loaded chitosan-Ca-alginate microparticles: Preparation and physicochemical characterization. *International Journal of Pharmaceutics,* 345**,** 59-69.

MÖBUS, K., SIEPMANN, J. R. & BODMEIER, R. 2012. Zinc-alginate microparticles for controlled pulmonary delivery of proteins prepared by spray-drying. *European Journal of Pharmaceutics and Biopharmaceutics,* 81**,** 121-130.

MONFORTE, V., USSETTI, P., GAVALDÀ, J., BRAVO, C., LAPORTA, R., LEN, O., GARCÍA-GALLO, C. L., TENORIO, L., SOLÉ, J. & ROMÁN, A. 2010. Feasibility, tolerability, and outcomes of nebulized liposomal amphotericin B for Aspergillus infection prevention in lung transplantation. *The Journal of Heart and Lung Transplantation,* 29**,** 523-530.

MORALES, P., GALÁN, G., SANMARTÍN, E., MONTE, E., TARRAZONA, V. & SANTOS, M. 2009. Intrabronchial instillation of amphotericin B lipid complex: A case report. *Transplantation Proceedings,* 41**,** 2223-2224.

MORAND, K., BARTOLETTI, A. C., BOCHOT, A., BARRATT, G., BRANDELY, M. L. & CHAST, F. 2007. Liposomal amphotericin B eye drops to treat fungal keratitis: Physico-chemical and formulation stability. *International Journal of Pharmaceutics,* 344**,** 150-153.

MORENO, M. A., FRUTOS, P. & BALLESTEROS, M. P. 2001. Lyophilized lecithin based oil-water microemulsions as a new and low toxic delivery system for amphotericin B. *Pharmaceutical Research,* 18**,** 344-351.

MORIBE, K., MARUYAMA, S., INOUE, Y., SUZUKI, T., FUKAMI, T., TOMONO, K., HIGASHI, K., TOZUKA, Y. & YAMAMOTO, K. 2010. Ascorbyl dipalmitate/PEG-lipid nanoparticles as a novel carrier for hydrophobic drugs. *International Journal of Pharmaceutics,* 387**,** 236-243.

MORRIS, K. R., NAIL, S. L., PECK, G. E., BYRN, S. R., GRIESSER, U. J., STOWELL, J. G., HWANG, S.-J. & PARK, K. 1998. Advances in pharmaceutical materials and processing. *Pharmaceutical Science & Technology Today,* 1**,** 235-245.

MOSCA, M., CUOMO, F., LOPEZ, F. & CEGLIE, A. 2013. Role of emulsifier layer, antioxidants and radical initiators in the oxidation of olive oil-in-water emulsions. *Food Research International,* 50**,** 377-383.

MUKHERJEE, P. K., LONG, L., KIM, H. G. & GHANNOUM, M. A. 2009. Amphotericin B lipid complex is efficacious in the treatment of Candida albicans biofilms using a model of catheter-associated Candida biofilms. *International Journal of Antimicrobial Agents,* 33**,** 149-153.

MULLER, R. H., GOHLA, S. & KECK, C. M. 2011. State of the art of nanocrystals - Special features, production, nanotoxicology aspects and intracellular delivery. *European Journal of Pharmaceutics and Biopharmaceutics,* 78**,** 1-9.

MUÑOZ, P., GUINEA, J., NARBONA, M. T. & BOUZA, E. 2008. Treatment of invasive fungal infections in immunocompromised and transplant patients: AmBiLoad trial and other new data. *International Journal of Antimicrobial Agents,* 32, Supp 2**,** S125-S131.

MURA, C., NÁCHER, A., MERINO, V., MERINO-SANJUÁN, M., MANCONI, M., LOY, G., FADDA, A. M. & DÍEZ-SALES, O. 2012. Design, characterization and in vitro evaluation of 5-aminosalicylic acid loaded N-succinyl-chitosan microparticles for colon specific delivery. *Colloids and Surfaces B: Biointerfaces,* 94**,** 199-205.

MURO, S. 2012. Challenges in design and characterization of ligand-targeted drug delivery systems. *Journal of Controlled Release,* 164**,** 125-137.

NAGASHIMA, T., OLIVEIRA, E. E., SILVA, A. E., MARCELINO, H. R., GOMES, M. C. S., AGUIAR, L. M., ARAUJO, I. B., SOARES, L. A. L., OLIVEIRA, A. G. & EGITO, E. S. T. 2008. Influence of the lipophilic external phase composition on the preparation and characterization of xylan microcapsules - A technical note. *AAPS PharmSciTech,* 9**,** 814-817.

NAHAR, M., MISHRA, D., DUBEY, V. & JAIN, N. K. 2008. Development, characterization, and toxicity evaluation of amphotericin B-loaded gelatin nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine,* 4**,** 252-261.

NARAYAN, P. & HANCOCK, B. C. 2005. *The influence of particle size on the surface roughness of pharmaceutical excipient compacts,* Kidlington, UK, Elsevier.

NASR, M., NAWAZ, S. & ELHISSI, A. 2012. Amphotericin B lipid nanoemulsion aerosols for targeting peripheral respiratory airways via nebulization. *International Journal of Pharmaceutics,* 436**,** 611-6.

NESLIHAN GURSOY, R. & BENITA, S. 2004. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomedicine & Pharmacotherapy,* 58**,** 173-182.

NICOLETTI, S., SEIFERT, K. & GILBERT, I. H. 2009. N-(2-hydroxypropyl)methacrylamide)-amphotericin B (HPMA-AmB) copolymer conjugates as antileishmanial agents. *International Journal of Antimicrobial Agents,* 33**,** 441-448.

NUNTHANID, J., LAUNGTANA-ANAN, A., SRIAMORNSAK, P., LIMMATVAPIRAT, S., PUTTIPIPATKHACHORN, S., LIM, L. Y. & KHOR, E. 2004a. Characterization of chitosan acetate as a binder for sustained release tablets. *Journal of Controlled Release,* 99**,** 15-26.

NUNTHANID, J., LAUNGTANA-ANAN, M., SRIAMORNSAK, P., LIMMATVAPIRAT, S., PUTTIPIPATKHACHORN, S., LIM, L. Y. & KHOR, E. 2004b. Characterization of chitosan acetate as a binder for sustained release tablets. *Journal of Controlled Release,* 99**,** 15-26.

OCHEKPE, N. A., OLORUNFEMI, P. O. & NGWULUKA, N. C. 2009. Nanotechnology and drug delivery Part 1: Background and applications. *Tropical Journal of Pharmaceutical Research,* 8**,** 265-274.

OLIVEIRA, E. E., SILVA, A. E., NAGASHIMA JR, T., GOMES, M. C. S., AGUIAR, L. M., MARCELINO, H. R., ARAUJO, I. B., BAYER, M. P., RICARDO, N. M. P. S., OLIVEIRA, A. G. & EGITO, E. S. T. 2010. Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization. *Bioresource Technology,* 101**,** 5402-5406.

OLIVEIRA, T. R., BENATTI, C. R. & LAMY, M. T. 2011. Structural characterization of the interaction of the polyene antibiotic amphotericin B with DODAB bicelles and vesicles. *Biochim Biophys Acta,* 1808**,** 2629-2637.

PAL, R. 2011. Rheology of simple and multiple emulsions. *Current Opinion in Colloid & Interface Science,* 16**,** 41-60.

PALMA, S., MANZO, R., LO NOSTRO, P. & ALLEMANDI, D. 2007. Nanostructures from alkyl vitamin C derivatives (ASCn): Properties and potential platform for drug delivery. *International Journal of Pharmaceutics,* 345**,** 26-34.

PATEL, G. P., CRANK, C. W. & LEIKIN, J. B. 2011. An evaluation of hepatotoxicity and nephrotoxicity of liposomal amphotericin B (L-AMB). *Journal of Medical Toxicology,* 7**,** 12-5.

PAULY, M. & KEEGSTRA, K. 2010. Plant cell wall polymers as precursors for biofuels. *Current Opinion in Plant Biology,* 13**,** 304-311.

PESTANA, K. C., FORMARIZ, T. P., FRANZINI, C. M., SARMENTO, V. H. V., CHIAVACCI, L. A., SCARPA, M. V., EGITO, E. S. T. & OLIVEIRA, A. G. 2008. Oil-in-water lecithin-based microemulsions as a potential delivery system for amphotericin B. *Colloid Surface B,* 66**,** 253-259.

PETZOLD-WELCKE, K., SCHWIKAL, K., DAUS, S. & HEINZE, T. 2012. Xylan derivatives and their application potential Mini-review of own results. *Carbohydrate Polymers,* In Press, Corrected Proof.

POUTON, C. W. 2006. Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. *European Journal of Pharmaceutical Sciences,* 29**,** 278-287.

POUTON, C. W. & PORTER, C. J. H. 2008. Formulation of lipid-based delivery systems for oral administration: Materials, methods and strategies. *Advanced Drug Delivery Reviews,* 60**,** 625-637.

QV, X.-Y., ZENG, Z.-P. & JIANG, J.-G. 2011. Preparation of lutein microencapsulation by complex coacervation method and its physicochemical properties and stability. *Food Hydrocolloids,* 25**,** 1596-1603.

RAFFIN, R. P., COLOMÉ, L. M., POHLMANN, A. R. & GUTERRES, S. S. 2006a. Preparation, characterization, and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles. *European Journal of Pharmaceutics and Biopharmaceutics,* 63**,** 198-204.

RAFFIN, R. P., COLOMÉ, L. M., SCHAPOVAL, E. E. S., POHLMANN, A. R. & GUTERRES, S. S. 2008. Increasing sodium pantoprazole photostability by microencapsulation: Effect of the polymer and the preparation technique. *European Journal of Pharmaceutics and Biopharmaceutics,* 69**,** 1014-1018.

RAFFIN, R. P., JORNADA, D. S., RÉ, M. I., POHLMANN, A. R. & GUTERRES, S. S. 2006b. Sodium pantoprazole-loaded enteric microparticles prepared by spray drying: Effect of the scale of production and process validation. *International Journal of Pharmaceutics,* 324**,** 10-18.

RAO, J. & MCCLEMENTS, D. J. 2012. Food-grade microemulsions and nanoemulsions: Role of oil phase composition on formation and stability. *Food Hydrocolloids,* 29**,** 326-334.

RAO, J. P. & GECKELER, K. E. 2011. Polymer nanoparticles: Preparation techniques and size-control parameters. *Progress in Polymer Science,* 36**,** 887-913.

RIBEIRO, A. J., NEUFELD, R. J., ARNAUD, P. & CHAUMEIL, J. C. 1999. Microencapsulation of lipophilic drugs in chitosan-coated alginate microspheres. *International Journal of Pharmaceutics,* 187**,** 115-123.

RODRIGUES, A. & EMEJE, M. 2012. Recent applications of starch derivatives in nanodrug delivery. *Carbohydrate Polymers,* 87**,** 987-994.

ROUSSEAU, D., RAFANAN, R. R. & YADA, R. 2011. Microemulsions as nanoscale delivery systems. *In:* MURRAY, M.-Y. (ed.) *Comprehensive Biotechnology.* 2 ed. Burlington: Academic Press.

RUBINSTEIN, A. 1995. Approaches and opportunities in colon-specific drug delivery. *Critical Reviews in Therapeutic Drug Carrier Systems,* 12**,** 101-149.

SACHS-BARRABLE, K., LEE, S. D., WASAN, E. K., THORNTON, S. J. & WASAN, K. M. 2008. Enhancing drug absorption using lipids: A case study presenting the development and pharmacological evaluation of a novel lipid-based oral amphotericin B formulation for the treatment of systemic fungal infections. *Advanced Drug Delivery Reviews,* 60**,** 692-701.

SAHA, B. C. 2000. Alpha-L-arabinofuranosidases: Biochemistry, molecular biology and application in biotechnology. *Biotechnology Advances,* 18**,** 403-423.

SALAUN, F., BEDEK, G., DEVAUX, E., DUPONT, D. & GENGEMBRE, L. 2011. Microencapsulation of a cooling agent by interfacial polymerization: Influence of the parameters of encapsulation on poly(urethane-urea) microparticles characteristics. *Journal of Membrane Science,* 370**,** 23-33.

SANTOS, C. M., OLIVEIRA, R. B., ARANTES, V. T., CALDEIRA, L. R., OLIVEIRA, M. C., EGITO, E. S. T. & FERREIRA, L. A. M. 2012. Amphotericin B-loaded nanocarriers for topical treatment of cutaneous leishmaniasis: Development, characterization, and *in vitro* skin permeation studies. *Journal of Biomedical Nanotechnology,* 8**,** 322-329.

SANTOS, C. M. B., DA SILVA, S. W., GUILHERME, L. R. & MORAIS, P. C. 2011. SERRS study of molecular arrangement of amphotericin B adsorbed onto iron oxide nanoparticles precoated with a bilayer of lauric acid. *Journal of Physical Chemistry C,* 115**,** 20442-20448.

SAU, K., MAMBULA, S. S., LATZ, E., HENNEKE, P., GOLENBOCK, D. T. & LEVITZ, S. M. 2003. The antifungal drug amphotericin B promotes inflammatory cytokine release by a toll-like receptor- and CD14-dependent mechanism. *Journal of Biological Chemistry,* 278**,** 37561-37568.

SCHMIDTS, T., DOBLER, D., NISSING, C. & RUNKEL, F. 2009. Influence of hydrophilic surfactants on the properties of multiple W/O/W emulsions. *Journal of Colloid and Interface Science,* 338**,** 184-192.

SEDLÁK, M., PRAVDA, M., KUBICOVÁ, L., MIKULCÍKOVÁ, P. & VENTURA, K. 2007. Synthesis and characterisation of a new pH-sensitive amphotericin B-poly(ethylene glycol)-b-poly(l-lysine) conjugate. *Bioorganic & Medicinal Chemistry Letters,* 17**,** 2554-2557.

SEDLMEYER, F. B. 2011. Xylan as by-product of biorefineries: Characteristics and potential use for food applications. *Food Hydrocolloids,* In Press, Corrected Proof.

SEKI, J., SONOKE, S., SAHEKI, A., KOIKE, T., FUKUI, H., DOI, M. & MAYUMI, T. 2004. Lipid transfer protein transports compounds from lipid nanoparticles to plasma lipoproteins. *International Journal of Pharmaceutics,* 275**,** 239-248.

SELVAM, S. & MISHRA, A. K. 2008. Disaggregation of amphotericin B by sodium deoxycholate micellar aggregates. *Journal of Photochemistry and Photobiology B: Biology,* 93**,** 66-70.

SHALLOM, D. & SHOHAM, Y. 2003. Microbial hemicellulases. *Current Opinion in Microbiology,* 6**,** 219-228.

SHAO, K., HUANG, R., LI, J., HAN, L., YE, L., LOU, J. & JIANG, C. 2010. Angiopep-2 modified PE-PEG based polymeric micelles for amphotericin B delivery targeted to the brain. *Journal of Controlled Release,* 147**,** 118-126.

SHIGEMI, A., MATSUMOTO, K., IKAWA, K., YAJI, K., SHIMODOZONO, Y., MORIKAWA, N., TAKEDA, Y. & YAMADA, K. 2011. Safety analysis of liposomal amphotericin B in adult patients: anaemia, thrombocytopenia,

nephrotoxicity, hepatotoxicity and hypokalaemia. *International Journal of Antimicrobial Agents,* 38**,** 417-420.

SHIMIZU, K., OSADA, M., TAKEMOTO, K., YAMAMOTO, Y., ASAI, T. & OKU, N. 2010. Temperature-dependent transfer of amphotericin B from liposomal membrane of AmBisome to fungal cell membrane. *Journal of Controlled Release,* 141**,** 208-215.

SHOUKRI, R. A., AHMED, I. S. & SHAMMA, R. N. 2009. In vitro and in vivo evaluation of nimesulide lyophilized orally disintegrating tablets. *European Journal of Pharmaceutics and Biopharmaceutics,* 73**,** 162-171.

SHUNMUGAPERUMAL, T. 2009. Formulation of multifunctional oil-in-water nanosized emulsions for active and passive targeting of drugs to otherwise inaccessible internal organs of the human body. *International Journal of Pharmaceutics,* 381**,** 62-76.

SILVA, A. E., MARCELINO, H. R., GOMES, M. C. S., OLIVEIRA, E. E., JR, T. N. & EGITO, E. S. T. 2012. Xylan, a promising hemicellulose for pharmaceutical use *In:* JOHANNES, C. & VERBEEK, R. (eds.) *Products and Applications of Biopolymers.* InTech.

SILVA, A. K. A., DA SILVA, E. L., OLIVEIRA, E. E., NAGASHIMA, J. T., SOARES, L. A. L., MEDEIROS, A. C., ARAUJO, J. H., ARAUJO, I. B., CARRICO, A. S. & EGITO, E. S. T. 2007. Synthesis and characterization of xylan-coated magnetite microparticles. *International Journal of Pharmaceutics,* 334**,** 42-47.

SILVA, S. S., CARVALHO, R. R., FONSECA, J. L. C. & GARCIA, R. B. 1998. Extração e caracterização de xilanas de sabugo de milho. *Polímeros: Ciência e Tecnologia,* 2**,** 1-9.

SILVA-FILHO MA, S. S., FREIRE LB, ARAÚJO IB, SILVA KGH, MEDEIROS AC, ARAÚJO-FILHO I, OLIVEIRA AG, EGITO EST 2012. How can micelle systems be rebuilt by a heating process? *International Journal of Nanomedicine,* 7**,** 141-150.

SILVA-JUNIOR, A. A., DE MATOS, J. R., FORMARIZ, T. P., ROSSANEZI, G., SCARPA, M. V., EGITO, E. S. T. & OLIVEIRA, A. G. 2009. Thermal behavior and stability of biodegradable spray-dried microparticles containing triamcinolone. *International Journal of Pharmaceutics,* 368**,** 45-55.

SILVA-JUNIOR, A. A., SCARPA, M. V., PESTANA, K. C., MERCURI, L. P., MATOS, J. R. & OLIVEIRA, A. G. 2008. Thermal analysis of biodegradable microparticles containing ciprofloxacin hydrochloride obtained by spray drying technique. *Thermochimica Acta,* 467**,** 91-98.

SIMÓ, C., CIFUENTES, A. & GALLARDO, A. 2003. Drug delivery systems: Polymers and drugs monitored by capillary electromigration methods. *Journal of Chromatography. B, Analytical technologies in the biomedical and life sciences,* 797**,** 37-49.

SINHA, V. R. & KUMRIA, R. 2001. Polysaccharides in colon-specific drug delivery. *International Journal of Pharmaceutics,* 224**,** 19 38.

SINHA, V. R., MITTAL, B. R., BHUTANI, K. K. & KUMRIA, R. 2004. Colonic drug delivery of 5-fluorouracil: An in vitro evaluation. *International Journal of Pharmaceutics,* 269**,** 101-108.

SLAIN, D., MILLER, K., KHAKOO, R., FISHER, M., WIERMAN, T. & JOZEFCZYK, K. 2002. Infrequent occurrence of amphotericin B lipid complex-associated nephrotoxicity in various clinical settings at a university hospital: A retrospective study. *Clinical Therapeutics,* 24**,** 1636-1642.

SLOBBE, L., BOERSMA, E. & RIJNDERS, B. J. A. 2008. Tolerability of prophylactic aerosolized liposomal amphotericin-B and impact on pulmonary function: Data from a randomized placebo-controlled trial. *Pulmonary Pharmacology & Therapeutics,* 21**,** 855-859.

SOLOMON, M., BAUM, S., BARZILAI, A., SCOPE, A., TRAU, H. & SCHWARTZ, E. 2007. Liposomal amphotericin B in comparison to sodium stibogluconate for cutaneous infection due to Leishmania braziliensis. *Journal of the American Academy of Dermatology,* 56**,** 612-616.

SONU, I., LIN, M. V., BLONSKI, W. & LICHTENSTEIN, G. R. 2010. Clinical pharmacology of 5-ASA compounds in inflammatory bowel disease. *Gastroenterology Clinics of North America,* 39**,** 559-599.

STAROV, V., IVANOVA, N. & RUBIO, R. G. 2010. Why do aqueous surfactant solutions spread over hydrophobic substrates? *Adv Colloid Interface,* 161**,** 153-162.

STRICKLEY, R. 2004. Solubilizing excipients in oral and injectable formulations. *Pharmaceutical Research,* 21**,** 201-230.

STRICKLEY, R. G. 2007. Currently marketed oral lipid-based dosage forms: Drug products and excipients. *Oral Lipid-Based Formulations.*

STUART, M. A. C. 2008. Supramolecular perspectives in colloid science. *Colloid and Polymer Science,* 286**,** 855-864.

STULZER, H. K., TAGLIARI, M. P., PARIZE, A. L., SILVA, M. A. S. & LARANJEIRA, M. C. M. 2009. Evaluation of cross-linked chitosan microparticles containing acyclovir obtained by spray-drying. *Materials Science and Engineering: C,* 29**,** 387-392.

SUN, R. C., FANG, J. M., GOODWIN, A., LAWTHER, J. M. & BOLTON, A. J. 1998. Fractionation and characterization of polysaccharides from abaca fibre. *Carbohydrate Polymers,* 37**,** 351-359.

SUN, X.-F., WANG, H.-H., JING, Z.-X. & MOHANATHAS, R. 2013. Hemicellulose-based pH-sensitive and biodegradable hydrogel for controlled drug delivery. *Carbohydrate Polymers,* 92**,** 1357-1366.

SUN, X. F., XU, F., SUN, R. C., GENG, Z. C., FOWLER, P. & BAIRD, M. S. 2005a. Characteristics of degraded hemicellulosic polymers obtained from steam exploded wheat straw. *Carbohydrate Polymers,* 60**,** 15-26.

SUN, X. F., XU, F., ZHAO, H., SUN, R. C., FOWLER, P. & BAIRD, M. S. 2005b. Physicochemical characterisation of residual hemicelluloses isolated with cyanamide-activated hydrogen peroxide from organosolv pre-treated wheat straw. *Bioresource Technology,* 96**,** 1342-1349.

SZEBENI, J., BEDOCS, P., ROZSNYAY, Z., WEISZHÁR, Z., URBANICS, R., ROSIVALL, L., COHEN, R., GARBUZENKO, O., BÁTHORI, G., TÓTH, M., BUNGER, R. & BARENHOLZ, Y. 2012. Liposome-induced complement activation and related cardiopulmonary distress in pigs: factors promoting reactogenicity of Doxil and AmBisome. *Nanomedicine: Nanotechnology, Biology and Medicine,* 8**,** 176-184.

TAN, M. L., CHOONG, P. F. M. & DASS, C. R. 2010. Recent developments in liposomes, microparticles and nanoparticles for protein and peptide drug delivery. *Peptides,* 31**,** 184-193.

TEWA-TAGNE, P., BRIANÇON, S. & FESSI, H. 2006. Spray-dried microparticles containing polymeric nanocapsules: Formulation aspects, liquid phase interactions and particles characteristics. *International Journal of Pharmaceutics,* 325**,** 63-74.

TEWA-TAGNE, P., BRIANÇON, S. & FESSI, H. 2007. Preparation of redispersible dry nanocapsules by means of spray-drying: Development and characterisation. *European Journal of Pharmaceutical Sciences,* 30**,** 124-135.

TIAN, H., TANG, Z., ZHUANG, X., CHEN, X. & JING, X. 2012. Biodegradable synthetic polymers: Preparation, functionalization and biomedical application. *Progress in Polymer Science,* 37**,** 237-280.

TIYABOONCHAI, W. & LIMPEANCHOB, N. 2007. Formulation and characterization of amphotericin B chitosan-dextran sulfate nanoparticles. *International Journal of Pharmaceutics,* 329**,** 142-149.

TOLENTINO, L. F., TSAI, S. F., WITT, M. D. & FRENCH, S. W. 2004. Fatal fat embolism following amphotericin B lipid complex injection. *Experimental and Molecular Pathology,* 77**,** 246-248.

TORCHILIN, V. P. 2006. Multifunctional nanocarriers. *Advanced Drug Delivery Reviews,* 58**,** 1532-1555.

TORRADO, J. J., ESPADA, R., BALLESTEROS, M. P. & TORRADO-SANTIAGO, S. 2008. Amphotericin B formulations and drug targeting. *Journal of Pharmaceutical Sciences,* 97**,** 2405-2425.

TRAN, V. T., BENOÎT, J. P. & VENIER-JULIENNE, M. C. 2011. Why and how to prepare biodegradable, monodispersed, polymeric microparticles in the field of pharmacy? *International Journal of Pharmaceutics,* 407**,** 1-11.

TUFTELAND, M., PESAVENTO, J. B., BERMINGHAM, R. L., HOEPRICH JR, P. D. & RYAN, R. O. 2007. Peptide stabilized amphotericin B nanodisks. *Peptides,* 28**,** 741-746.

ÜNLU, C. H., GÜNISTER, E. & ATICI, O. 2009. Synthesis and characterization of NaMt biocomposites with corn cob xylan in aqueous media. *Carbohydrate Polymers,* 76**,** 585-592.

VAN DE VEN, H., PAULUSSEN, C., FEIJENS, P. B., MATHEEUSSEN, A., ROMBAUT, P., KAYAERT, P., VAN DEN MOOTER, G., WEYENBERG, W., COS, P., MAES, L. & LUDWIG, A. 2012. PLGA nanoparticles and nanosuspensions with amphotericin B: Potent *in vitro* and *in vivo* alternatives to Fungizone and AmBisome. *Journal of Controlled Release,* 161**,** 795-803.

VAN DEN MOOTER, G. 2012. The use of amorphous solid dispersions: A formulation strategy to overcome poor solubility and dissolution rate. *Drug Discovery Today: Technologies,* 9**,** e79-e85.

VARKA, E. M. & KARAPANTSIOS, T. D. 2011. Global versus local dynamics during destabilization of eco-friendly cosmetic emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 391**,** 195-200.

VARSHOSAZ, J., AHMADI, F., EMAMI, J., TAVAKOLI, N., MINAIYAN, M., MAHZOUNI, P. & DORKOOSH, F. 2011. Microencapsulation of budesonide with dextran by spray drying technique for colon-targeted delivery: an in vitro/ in vivo evaluation in induced colitis in rat. *Journal of Microencapsulation,* 28**,** 62-73.

VEHRING, R. 2008. Pharmaceutical particle engineering via spray-drying. *Pharmaceutical Research,* 25**,** 999-1022.

VERMA, R. K. & GARG, S. 2001. Current status of drug delivery technologies and future directions. *Pharmaceutical Technology On-Line,* 25**,** 1-14.

VILLANOVA, J. C. O., OREFICE, R. L. & CUNHA, A. S. 2010. Aplicações farmacêuticas de polímeros. *Polímeros,* 20**,** 51-64.

VYAS, S. P., QURAISHI, S., GUPTA, S. & JAGANATHAN, K. S. 2005. Aerosolized liposome-based delivery of amphotericin B to alveolar macrophages. *International Journal of Pharmaceutics,* 296**,** 12-25.

WALKER, R. J. & ENDRE, Z. H. 2008. Cellular mechanisms of drug nephrotoxicity. *Seldin and Giebisch's The Kidney (Fourth Edition)*. San Diego: Academic Press.

WANG, C. H., WANG, W. T. & HSIUE, G. H. 2009. Development of polyion complex micelles for encapsulating and delivering amphotericin B. *Biomaterials,* 30**,** 3352-3358.

WANG, Y. & ZHANG, J. 2006. A novel hybrid process, enhanced by ultrasonication, for xylan extraction from corncobs and hydrolysis of xylan to xylose by xylanase. *Journal of Food Engineering,* 77**,** 140-145.

WASAN, E. K., BARTLETT, K., GERSHKOVICH, P., SIVAK, O., BANNO, B., WONG, Z., GAGNON, J., GATES, B., LEON, C. G. & WASAN, K. M. 2009. Development and characterization of oral lipid-based Amphotericin B formulations with enhanced drug solubility, stability and antifungal activity in rats infected with Aspergillus fumigatus or Candida albicans. *International Journal of Pharmaceutics,* 372**,** 76-84.

WASHINGTON, C. 1996. Stability of lipid emulsions for drug delivery. *Advanced Drug Delivery Reviews,* 20**,** 131 145.

WATANABE, A., MATSUMOTO, K., IGARI, H., UESATO, M., YOSHIDA, S., NAKAMURA, Y., MORITA, K., SHIBUYA, K., MATSUBARA, H., YOSHINO, I. & KAMEI, K. 2010. Comparison between concentrations of amphotericin B in infected lung lesion and in uninfected lung tissue in a patient treated with liposomal amphotericin B (AmBisome). *International Journal of Infectious Diseases,* 14, Supp 3**,** e220-e223.

WAUGH, C. D. 2007. Amphotericin B. *xPharm: The Comprehensive Pharmacology Reference*, 1-5.

WHISTLER, R. L., SMART, C.L. 1953. *Polysaccharide Chemistry,* New York, Academic Press Inc.

WILSON, B., SAMANTA, M. K., SANTHI, K., SAMPATH KUMAR, K. P., RAMASAMY, M. & SURESH, B. 2009. Significant delivery of tacrine into the brain using magnetic chitosan microparticles for treating Alzheimer's disease. *Journal of Neuroscience Methods,* 177, 427-433.

WINGARD, J. R., WHITE, M. H., ANAISSIE, E., RAFFALLI, J., GOODMAN, J. & ARRIETA, A. 2000. A randomized, double-blind comparative trial evaluating the safety of liposomal amphotericin B versus amphotericin B lipid complex in the empirical treatment of febrile neutropenia. *Clinical Infectious Diseases,* 31, 1155-1163.

WU, W., WIECKOWSKI, S., PASTORIN, G., BENINCASA, M., KLUMPP, C., BRIAND, J.-P., GENNARO, R., PRATO, M. & BIANCO, A. 2005. Targeted delivery of amphotericin B to cells by using functionalized carbon nanotubes. *Angewandte Chemie International Edition,* 44, 6358-6362.

XU, F., SUN, R. C., SUN, X. F., GENG, Z., XIAO, B. & SUN, J. 2004. Analysis and characterization of acetylated sugarcane bagasse hemicelluloses. *International Journal of Polymer Analysis and Characterization,* 9, 229-244.

XU, N., GU, J., ZHU, Y., WEN, H., REN, Q. & CHEN, J. 2011. Efficacy of intravenous amphotericin B-polybutylcyanoacrylate nanoparticles against cryptococcal meningitis in mice. *International Journal of Nanomedicine,* 6, 905-13.

YAMASHITA, F. & HASHIDA, M. 2012. Pharmacokinetic considerations for targeted drug delivery. *Advanced Drug Delivery Reviews*.

YANG, L., CHU, J. S. & FIX, J. A. 2002. Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. *International Journal of Pharmaceutics,* 235, 1-15.

YANG, R., XU, S., WANG, Z. & YANG, W. 2005. Aqueous extraction of corncob xylan and production of xylooligosaccharides. *Lwt-Food Science and Technology,* 38, 677-682.

YOO, J.-W., GIRI, N. & LEE, C. H. 2011. pH-sensitive Eudragit nanoparticles for mucosal drug delivery. *International Journal of Pharmaceutics,* 403, 262-267.

YORK, W. S. & O'NEILL, M. A. 2008. Biochemical control of xylan biosynthesis - Which end is up? *Current Opinion in Plant Biology,* 11, 258-265.

ZHANG, X., ZHU, X., KE, F., YE, L., CHEN, E. Q., ZHANG, A. Y. & FENG, Z. G. 2009. Preparation and self-assembly of amphiphilic triblock copolymers with polyrotaxane as a middle block and their application as carrier for the controlled release of amphotericin B. *Polymer,* 50, 4343-4351.

ZHANG, Y. & ROCHEFORT, D. 2010. Comparison of emulsion and vibration nozzle methods for microencapsulation of laccase and glucose oxidase by interfacial reticulation of poly(ethyleneimine). *Journal of Microencapsulation,* 27, 703-713.