Journal of Physics and Chemistry of Solids 81 (2015) 27–33

Contents lists available at ScienceDirect

Journal of Physics and Chemistry of Solids

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

Physicochemical aspects involved in methotrexate release kinetics from biodegradable spray-dried chitosan microparticles

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ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form
12 January 2015
Accepted 30 January 2015
Available online 30 January 2015

Keywords:
Amorphous materials
Polymers
Mechanical properties
Microstructure
Transport properties

ABSTRACT

Spray dried methotrexate (MTX) loaded chitosan microparticles were prepared using different drug/copolymer ratios (9%, 18%, 27% and 45% w/w). The physicochemical aspects were assessed in order to select particles that were able to induce a sustained drug release effect. Particles were successfully produced which exhibited desired physicochemical aspects such as spherical shape and high drug loading. XRD and FT-IR analysis demonstrated that drug is not bound to copolymer and is only homogeneously dispersed in an amorphous state into polymeric matrix. Even the particles with higher drug loading levels presented a sustained drug release profile, which were mathematically modeled using adjusted Higuchi model. The drug release occurred predominantly with drug dissolution and diffusion through swollen polymeric matrix, with the slowest release occurring with particles containing 9% of drug, demonstrating an interesting and promising drug delivery system for MTX.

1. Introduction

Polymeric nanoparticles and microparticles are commonly applied in the chemical, cosmetic, food and pharmaceutical industries as solid dispersed systems. In these particles, substances can be encapsulated, adsorbed or dispersed in order to prevent their degradation, improve specific characteristics or modulate their absorption. In the case of pharmaceuticals, additional advantages can be mucosal penetration, modulation of the time of drug in the organism, improving efficacy and reducing drug toxicity according to physicochemical characteristics of particles [1].

Among different polymers used to produce these particles, chitosan (CH) is a natural copolymer obtained by deacetylation of chitin, a polysaccharide found in the exoskeleton of crustaceans (Fig. 1a). CH is a biodegradable linear copolymer composed of D-glucosamine and N-acetyl-D-glucosamine monomers in different ratios, which gives it interesting properties. In aqueous media this polycationic material can interact with negative charges of mucosal epithelium and enhance bioavailability of drugs limited by a short residence time at the site of absorption [2,3]. Bioadhesion, biocompatibility and biodegradability characteristics allow CH to be used in microencapsulation to obtain new drug delivery systems.

In some cases, the organism eliminates the drug too quickly and this requires the administration of a considerable dose or several doses in a short time interval, causing side effects. This occurs commonly with drugs used for cancer treatment, such as methotrexate (MTX) (Fig. 1b), which is a prototype cytostatic folate antagonist drug used in the treatment of cancer and inflammatory diseases. It is known to be eliminated from organism in a short time and presents side effects and toxicity when high therapeutic doses are administered [4].

Among several microencapsulation methods available to obtain polymeric particles for drug delivery, spray drying is a single step technique that converts a liquid dispersion (solution, emulsion or suspension) into solid particles by exposing the sprayed feed to a heated air flow. Other advantages include total solvent removal, easy scale up and mainly a small and narrow size distribution [5–7]. Some important encapsulation parameters like rate of spraying, the feed rate of drug/polymer solution, nozzle size, inlet and outlet temperatures have previously studied and standardized in our research group to produce efficient drug loaded-spray dried particles which impacted directly on the presence of residual solvent size, shape and surface of particles, as well as the crystallinity, thermal behavior and drug-polymer distribution in particles [8–10]. In this work, different MTX/CH ratios were used to produce

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http://dx.doi.org/10.1016/j.jpcs.2015.01.014
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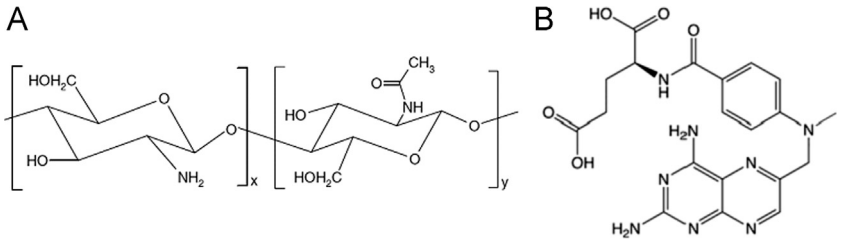

Fig. 1. Schematic representation of chemical structure of chitosan (A) and methotrexate (B).

spray dried microparticles containing a considerable drug amount in particles. Thus, their physicochemical properties were carefully evaluated and correlated with involved drug release kinetics in order to obtain biodegradable microparticles to prolong MTX release and potentially be used in cancer treatment.

2. Experimental

2.1. Materials

Methotrexate (MTX) was purchased from DEG (Brazil); Chitosan (CH) with a degree of 85% deacetylation from Sigma Co (Saint Louis, USA) and acetic acid from Merck S.A. (Brazil). All other reagents were analytical grade. The purified water (1.3 μS) was prepared from reverse osmosis purification equipment; model OS50 LX, Gehaka (Brazil).

2.2. Sample preparation

Suitable amounts of drug and copolymer were dissolved in 0.1 M acetic acid solution in order to obtain MTX-loaded CH microparticles with different MTX/CH ratios (9%, 18%, 27% and 45% w/w), these were then dried in a mini spray-dryer Buchi-191 with a 0.7 mm nozzle using an inlet temperature of 140 °C and outlet temperature of 90 °C. An air flow of 500 Nl h^{-1}; spray feed rate of 3 ml min^{-1} and aspirator efficiency of about 90% were selected during all experiments. Dried microparticles were collected and stored under vacuum at room temperature. Physical mixtures were used as control for analysis, which were prepared by mixing MTX and CH in a mortar, using the same mass ratios of MTX-loaded CH microparticles (9%, 18%, 27% and 45% w/w). The drug/polymer ratios were established according to experimental results of drug-loading efficiency.

2.3. Physicochemical aspects

The shape and surface aspect of microparticles were accessed using scanning electronic microscopy SEM (SSX 550, Shimadzu). The volume-based mean diameter and the size distribution of the microparticles were determined by using Dynamic Light Scattering, in a Nanotrac NPA252 (Microtrac Inc., City, USA). The particles were suspended in 0.5% of tween 80 aqueous solution. The particle size distribution was characterized by mean diameter (D10, D50, D90), and SPAN was calculated as [(D90 − D10)/D50]. For drug loading analysis, samples were dissolved in 0.1 M acetic acid and analyzed by using UV spectrophotometry in a 1 cm path-length cuvette, which had been previously validated. The analyses were carried out in triplicate and drug concentration was calculated using the equation from the standard curve fitted plot. Drug-loading efficiency was determined as the ratio between the analytical and theoretical drug content. FT-IR spectra were recorded by KBr disk method (prepared with 2 mg of samples mixed with 300 mg of KBr) using Perkin Elmer FT-IR Spectrum. The X-ray diffraction (Rikugu diffractometer model Dmax 2500PC) was determined using Cu-Kα radiation ($\lambda = 1.54056$ Å).

2.4. In vitro drug release

The drug release profile was monitored using an amount of biodegradable microparticles containing 2 mg of drug, which was incubated in 4 mL of phosphate buffer medium (KH_2PO_4 0.05 mol l^{-1}, pH = 7.4) at 37 °C ± 0.2 °C. At specific intervals, the flasks were centrifuged at 3500 rpm and supernatant analyzed by UV spectrophotometry at 303 nm. Cumulative percentage of released MTX was plotted versus time and different mathematical models: zero order, first order and adjusted Higuchi model were used to fit the experimental data.

3. Results and discussion

SEM images (Fig. 2) indicating that particles were successfully produced with a predominant spherical smooth shape for different drug-loaded microparticles. The droplet residence time and droplet diameter are important, but mainly the characteristics of products are fundamental to produce regular particles. A smaller amount of particles present some irregularity in shape like toroid cenospheres, mainly for large particles and for samples with lower drug loading. This kind of particle is typically formed after evaporation in two stages. At the first, the instantaneous contact of spray droplets with drying air led to the quick formation of a saturated vapor film around the particle surface, which evaporates at constant rate until reaching a critical point sufficient to form a dried shell. On the other hand, the second stage of evaporation is dependent on the diffusion rate of moisture through this dried shell, which increases causing a continuous reduction in the evaporation rate according to the diameter of the droplet, as was observed in large particles [11,12]. Furthermore, the used inlet temperature during all experiments was superior to the boiling point of the solvent mixture, contributing to vapor formation inside the particles. The rapid crust formation in large particles increases the internal pressure and depending on the nature of the crust, a rupture or collapse can occur, as observed in particles with lower drug loading [13]. Although this effect appeared in a smaller portion of the particles and the majority of particles presented spherical and smooth particles for all studied compositions. In previous studies, Oliveira et al. prepared MTX-loaded poly (D,L-lactide-co-glycolide) (PLGA) spray dried microparticles. However, the presence aggregate or agglomerates of particles were observed, mainly for particles with higher amount of drug loading. This was attributed to particles' composition because biodegradable polyesters exhibit low Tg, which make it prone to aggregation during spray drying [10]. Thus, the selected parameters and composition were fundamental to obtain small droplets during drying to produce regular spherical chitosan microparticles, which also showed a dependence on used drug ratio.

The biological activity of drug loaded particles is directly connected with particle aspects such as surface and shape, due to precision and accuracy of drug release rate that the system can