Powder Technology 255 (2014) 109–119

Contents lists available at ScienceDirect

Powder Technology

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

Preparation and evaluation of chitosan–hydrophobic silica composite microspheres: Role of hydrophobic silica in modifying their properties

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ARTICLE INFO

Available online 10 November 2013

Keywords:
Chitosan
Spray drying
Nanocomposite
Drug delivery

ABSTRACT

Biodegradable microspheres used as controlled release systems are important in pharmaceutics. Chitosan biopolymer represents an attractive alternative to other biomaterials because of its significant physicochemical and biological behaviors. Chitosan microspheres are expected to become promising carrier systems for drug and vaccine delivery, especially via oral, mucosal and transdermal routes. Controlling the swelling rate and swelling capacity of the hydrogel and improving the fragile nature of microspheres under acidic conditions are the key challenges that need to be overcome to allow the use of chitosan microspheres for controlled or sustained release specially via these non-invasive administration routes.

There have been many studies on the modification of chitosan microsphere structures with cross-linkers, blends with various kinds of polymers and new organic–inorganic hybrid systems in order to obtain some improved properties. In this work, microspheres composed of chitosan and nanosized hydrophobic silica commercialized under the name Aerosil R972 were generated by a method consisting of two steps: first, preparation of a macroscopically homogeneous chitosan–hydrophobic silica dispersion by an optimized procedure, and then drying. Spray drying was the technique used here. FTIR spectroscopy, X-ray powder diffraction, differential scanning calorimetry, thermal gravimetric analysis, Scanning Electron Microscopy (SEM) and high resolution Transmission Electron Microscopy (TEM) were used to characterize the microspheres, besides acid stability, moisture sorption capacity, release properties and biological assays.

The chitosan–hydrophobic silica composite microspheres showed improved thermal degradation, lower water affinity, better acid stability and ability to retard rifampicin (drug model) release under simulated gastric conditions. In vitro biocompatibility studies indicated low cytotoxicity and low capacity to activate cell production of the pro-inflammatory mediator nitric oxide, encouraging further studies on the use of the new chitosan–hydrophobic silica composite microspheres as drug carrier systems via oral or nasal routes.

1. Introduction

Chitosan is a biopolymer derived from chitin, a natural polysaccharide usually obtained from carapaces of marine crustaceans such as crabs and shrimps [1]. Due to its natural origin, chitosan cannot be defined as a unique chemical structure, but as a family of polymers of different molecular weights and degrees of deacetylation, defined in terms of the percentage of primary amino groups in the polymer backbone [2].

Chitosan has many physicochemical (reactive hydroxyl and amino groups, high positive charge in acidic conditions, good film formation) and biological (biocompatible, biodegradable, lack of toxicity, antimicrobial effects, mucoadhesive character) properties, that make it an attractive material for use as a new functional material of high potential in fields as different as biomedical [3–5], food [6–9], water treatment [5,10–12], and agricultural [9,12,13], among others. Chitosan has, for example, the ability to bind to particular materials including proteins, metal ions, and even tumor cells. Moreover, chitosan can be incorporated into hydrogels and microspheres which demonstrate large potential in delivery systems for drugs via oral, nasal or transdermal routes [14–18].

Chitosan has many advantages, particularly for developing nano/microspheres as carrier systems. These include its ability to control the release of active agents and to avoid the use of hazardous organic solvents while fabricating particulate systems, since it is soluble in aqueous acidic solution. However, the stability of the chitosan microspheres under different physiological conditions is a prerequisite for their successful application. For example, microspheres intended for nasal administration have to be optimized in terms of particle size and surface charges necessary to induce mucoadhesiveness. Chitosan has been

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http://dx.doi.org/10.1016/j.powtec.2013.10.046

shown to possess mucoadhesive properties owing to the electrostatic interaction between its positive charges and negatively charged mucosal surfaces. However, chitosan microspheres absorb water, swell and become too fragile after being swollen, losing their rigidity and shape, which can clog the nostrils and hampers intranasal delivery. Another example is the considerable limitation of chitosan microspheres as sustained release carrier systems for oral administration. Chitosan rapidly adsorbs water, swells and dissolves under gastric conditions, leading to fast drug release.

In order to overcome these problems, it is possible to modify the chitosan structure by introducing cross-linking structure, blending chitosan with synthetic polymers such as poly(vinyl alcohol) (PVA) [19], and developing organic–inorganic hybrid membranes [20].

Cross-linking is a common way to improve the controlled-release properties and mechanical strength by introducing a three-dimensional network structure [21]. Consequently, the motion of solutes across cross-linked polymer membranes can be controlled by precisely controlling this network structure. The available amino and hydroxyl groups on chitosan are active sites capable of forming a number of linkages. To date, the most common cross-linkers used involving bonds with chitosan amine groups are aldehydes, such as glutaraldehyde [22–24], formaldehyde [25,26] or glyoxal [27,28]. Epichlorohydrin, a bifunctional molecule which contains two functional groups, is another cross-linker reagent highly reactive with the hydroxyl groups of chitosan [29,30].

Cross-linked chitosan networks are highly useful in the pharmaceutical field for the formulation of various novel drug delivery systems like microspheres, nanospheres and films/membranes. However, acceptance of such cross-linked products depends upon the amount of cross-linking agent present in the final products. In that sense, the toxicity of aldehydes has enormously limited the exploitation of the cross-linked chitosan microparticles in the pharmaceutical field [31,32]. To overcome toxicity, Ré and co-workers have also long been studying the potential use of d,l-glyceraldehyde as a biocompatible cross-linking agent, since it is present in the human organism as a metabolic product of fructose. The morphology, particle size, surface charge and water-uptake capacity properties of the cross-linked chitosan microspheres have been characterized and published [33,34].

In another way, chitosan–clay nanocomposites have been synthesized as coating materials for tablets to retard acid swelling and improvement of film stability in gastric fluid. Clays are composed of silicate layers. The silicate layers can be separated and form three-dimensional structures when they are hydrated in water. They have negative charge and can interact with chitosan. It was found that chitosan reacts with several types of clays such as montmorillonite [35–38], magadiite [39], rectorite [20] or magnesium aluminum silicate [40] for modified-release tablets. Montmorillonite chitosan nanocomposites have been proposed as new drug delivery for oral administration combining mucoadhesive properties with low solubility in acidic environment for modified drug delivery formulations [37].

Silica has already been combined with chitosan to synthesize silica–chitosan hybrid materials by sol–gel process under acid conditions in various structures such as hierarchical porous materials and composite membranes [16,40–43]. We proposed here an alternative way to control chitosan microsphere properties such as moisture sorption capacity, acid stability, surface charge and release properties by combining chitosan with nanosized organic silica oxide named Aerosil R972 into chitosan composite microspheres. The objective of this work was to produce chitosan–Aerosil R972 composite microspheres by spray drying and to examine the role of the nanosized modified silica in modifying their properties.

2. Materials

Low molecular weight chitosan (75–85% deacetylated chitosan) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrophobic silica named Aerosil R972 (primary particle size of 16 nm, specific surface area of 110 ± 20 $m^2\ g^{-1}$) was supplied by Degussa-Huls Corporation, Brazil. Analytical grades of absolute ethanol, anhydrous acetic acid, dimethyl sulfoxide, hydrochloric acid and sodium hydroxide used in this study were provided by Synth (Brazil).

Cell culture reagents: Dulbecco Modified Minimum Essential Medium (DMEM) and heat inactivated fetal calf serum were obtained from Cultilab, Brazil; lipopolysaccharide, Triton X-100 and the Griess reagents were from Sigma Aldrich. The lactate dehydrogenase kit was purchased from Doles, Brazil. Rifampicin, $C_{43}H_{58}N_4O_{12}$ (structural formula given in Fig. 1), was used as drug model. The raw material (purity >99%) was obtained from Luohe Nanjiecun Pharmaceutical (Group Pharmacy China) and used without further purification.

3. Methods

3.1. Measurements of the contact angle of ethanol–water solutions on Aerosil R972

The first problem to be solved in this study was how to properly disperse a hydrophobic nanosized material like Aerosil R972 into an aqueous solution of chitosan. We proposed the introduction of ethanol in the mixture to facilitate the silica wetting and dispersion. To define the concentration values of ethanol/water mixtures to be used, the Aerosil R972 powder was pressed in tablets and the wetting of water/ethanol mixtures on the silica tablet surfaces was analyzed by the sessile drop method coupled with digital image analysis. Briefly, a liquid drop of water/ethanol mixture was gently deposited on the solid substrate and the profile of the drop was captured digitally by using an optical microscope.

3.2. Preparation of chitosan–Aerosil R972 microspheres

The chitosan solution (1 g) was prepared by dissolving the polymer in deionized water containing 3% w/v acetic acid (approximately 80 ml). The mixture was maintained under mechanical stirring for 30 min. After complete dissolution the pH was adjusted to 5.5–6.0 with a 0.5 M NaOH solution.

In order to facilitate the dispersion of the nanosized particles of Aerosil R972 in the aqueous chitosan solution, they were first wetted with absolute ethanol (approximately 25 ml). Next, the ethanolic dispersion was added to the chitosan solution under stirring (200 rpm) to give, after this dilution, a dispersion containing 20% w/w ethanol, able to ensure silica wetting in the hydroalcoholic dispersion. We then homogenized the dispersion in order to break potential particle aggregates, using a high-pressure homogenizer (model APV-2000, Stansted Fluid Power, APV, USA) operated at 800 bar for five homogenization cycles (15 min).

Chitosan–Aerosil R972 microspheres were then produced with the subsequent removal of the solvent of the homogenized suspensions.

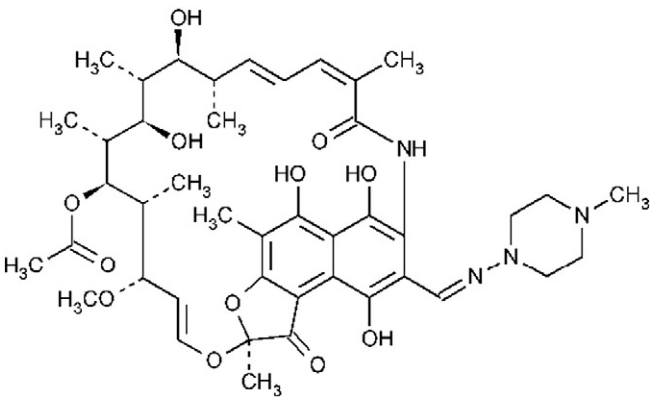

Fig. 1. Chemical structure of the drug model (rifampicin) used in this study.