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Influence of chitosan and carboxymethylchitosan on the polymorphism and solubilisation of diflunisal

David Lucio, Arantza Zornoza, María Cristina Martínez-Ohárriz *

Departamento de Química y Edafología, Facultad de Ciencias, University of Navarra, Irunlarrea s/n, Pamplona 31080, Navarra, Spain

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ABSTRACT

The interactions of diflunisal (DF) with chitosans (CS) of different molecular weights and carboxymethylchitosan (CMCS), a water-soluble derivative, have been investigated. The interactions in solution have been studied by solubility assays in which the highest solubilisation (13-fold) was obtained with CMCS. Solid dispersions were prepared by coevaporation and kneading methods. Solid state characterisation was performed by X-ray diffraction analysis, scanning electron microscopy, thermomicroscopy, differential thermal analysis and infrared spectroscopy. Drug–polymer electrostatic interactions and hydrogen bonds are the main binding forces in these systems. The kneading method gave rise to amorphous systems regardless of the polymer employed. However, coevaporation resulted in the formation of different polymorphs of diflunisal (form II or III) depending on the type of polymer used. Therefore, it seems that drug–polymer interactions determine the crystallization pattern of the drug. Finally, diflunisal release from these systems improved markedly with CMCS and significantly in the presence of low molecular weight CS.

1. Introduction

Chitosan [(1-4)-2-amino-2-deoxy-β-D-glucan] is a linear cationic polysaccharide comprising glucosamine and N-acetyl glucosamine units. Chitosan is obtained by alkaline deacetylation of chitin, which is a polysaccharide widely distributed in nature and the second most abundant after cellulose. Research with chitosan is not as advanced as that carried out with cellulose, however, recent studies have attracted attention in fields such as biology, chemistry, pharmacology and medicine. A significant advantage of this polymeric material over celluloses is its functionality, as being an aminopolysaccharide it is susceptible to many reactions and further modifications, thereby extending its range of different applications (Kurita, 1986; Rinaudo, 2006).

Chitosan and its derivatives are receiving increasing attention in the pharmaceutical domain due to their special properties, such as non-toxicity, biocompatibility, biodegradability, bioadhesiveness and dissolution and transmucosal penetration enhancer, together with its abundance and low cost (He et al., 2009; Pillai et al., 2009; Sinha et al., 2004; Zerrouk et al., 2004). In addition, its antiacid and antiulcer activities (Hillyard et al., 1964) can be used to prevent or reduce gastric irritation induced by anti-inflammatory drugs (Acikgoz et al., 1995; Imai et al., 1991; Mura et al., 2003). Over the last decade, chitosan has been extensively used in the development of potentially innovative drug delivery systems (Bernkop-Schnürch and Dünnhaupt, 2012).

Some structural characteristics, namely the degree of acetylation and molecular weight, can greatly influence various properties such as solubility, physiological activities, chemical reactivity and biodegradability (Kato et al., 2003; Majeti and Ravi Kumar, 2000). The solubility of chitosan is critical for the potential applications of the polymer. Protonation of the amino groups in acid media results in the formation of salts whose solubilities depend on the nature of the anion involved, the degree of deacetylation, the molecular weight of the polymer and temperature (Uragami and Tokura, 2006). This dependence of the solubility on various parameters gives rise to possible complications when working in solution, for this reason, water soluble derivatives have been synthesized (Prabaharan, 2008; Zhou et al., 2009).

Different water-soluble derivatives have been prepared by introducing hydrophilic groups such as carboxymethyl (Sugimoto et al., 1998; Tong and Chen, 2013). Carboxymethylchitosan is one of the most studied and can be obtained by direct or reductive alkylation of chitosan; three types of derivatives of chitosan can be obtained by carboxymethylation depending on the substituted

* Corresponding author. Tel.: +34 948425600x6378; fax: +34 948425649.
E-mail addresses: dlucio@alumni.unav.es (D. Lucio), azornoza@unav.es (A. Zornoza), moharriz@unav.es (M.C. Martínez-Ohárriz).

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group: O-carboxymethylchitosan, N-carboxymethylchitosan and N,O-carboxymethylchitosan (de Abreu and Campana-Filho, 2009; Mourya et al., 2010).

The biological properties of carboxymethylchitosan, such as bioadhesion together with antioxidant and antimicrobial activities, are influenced by the structural characteristics of the polymer and the substitution degree of amino or hydroxyl groups, in addition to molecular weight and degree of deacetylation, as in the case of non-modified chitosan (Feng et al., 2008; Lu et al., 2007).

A variety of applications are emerging because of the advantages provided by the carboxymethylated derivative of chitosan. Controlled drug release has been achieved using several strategies as the formation of aggregates, cross-linked hydrogels and nanoparticles (Aiping et al., 2007; Feng et al., 2013; Zhu et al., 2013). Moreover, the $-NH_2$ and $-COOH$ ionisable groups determine the state of the CMCS gel as a function of pH.

In the present study, the interactions of diflunisal with different types of chitosans which differ in the molecular weight and a chitosan derivative, carboxymethylchitosan, have been analyzed. Diflunisal is a non-steroidal antiinflammatory drug that exhibits low bioavailability after oral administration as a consequence of its poor water solubility at acidic pH. The most common side effects of diflunisal involve the gastrointestinal system, mucosal ulcerations and stomach pain. These problems could be solved by the use of gastro-resistant preparations and probably the interaction with chitosan could provide solutions as well.

Chitosan has been selected because of its above mentioned interesting properties. In addition, chitosan is an excellent direct compression adjuvant, an important characteristic for a potential pharmaceutical formulation (García Mir et al., 2010). Diflunisal has been chosen because polymorphic forms of the drug were previously reported by our group (Martínez-Ohárriz et al., 1994) and changes in the crystalline state of the drug were evidenced upon interaction with different polymers, furthermore, the physico-chemical properties of chitosan and its derivative appear to be appropriate to achieve the crystallization of different polymorphic forms of diflunisal. In the past, our group also explored the use of different polymers such as PEG (Martínez-Ohárriz et al., 1999), PVP (Martínez-Ohárriz et al., 2002) and cyclodextrins (Zornoza et al., 1999; Zugasti et al., 2009) to obtain more soluble and bioavailable systems of diflunisal. In a recent study our group has reported the optimization of chitosan coated nanoparticles of diflunisal (Lucio et al., 2013).

The aim of this paper was to investigate the interactions of different chitosan polymers with diflunisal, as well as the influence of the type of polymer and the preparation method of the solid dispersions on the polymorphism, solubility and release profiles of diflunisal.

2. Materials and methods

2.1. Materials

Diflunisal (DF) polymorph II was kindly supplied by Merck Sharp and Dohme (Spain). Chitosan of low (CS_L), medium (CS_M) and high molecular weight (CS_H) was supplied by Aldrich. The molecular weights for the three polymers were 50–190, 190–310 and >375 kDa respectively. The degree of deacetylation for the three polymers was 75–85%, and the viscosity of a 1% solution of CS_L, CS_M and CS_H in 1% acetic acid at 25 °C (Brookfield) was 20–300, 200–800 and 800–2000 cP respectively. Carboxymethylchitosan (CMCS) was supplied by Hepppe Medical Chitosan GMBH. This polymer presented 80–95% degree of deacetylation and a molecular weight of 51 kDa.

The reagents ethanol (Scharlau, P.A.) and hydrochloric acid (Panreac, analytical grade) were used as received. All aqueous solutions were prepared with deionized water obtained from a commercial Millipore Elix 3 system. (0.1 μS/cm conductivity).

2.2. Phase solubility studies

Phase solubility studies of the diflunisal-chitosan systems were carried out by addition of 5 mg in cases of non-modified chitosan and 15 mg in case of carboxymethylchitosan to 50 mL of aqueous solution of polymer (0.25–1.5%, w/v). The amount of DF was different for each polymer in order to ensure the presence of an excess of drug to achieve the solubility equilibrium. The pH of the solutions at equilibrium ranged from 5.3 to 5.5 due to the presence of chitosan in solution, water was used as solution medium in order to avoid interactions between the polymers and ions present in buffer solutions. Sealed glass containers were magnetically stirred at constant temperature (25 °C) until equilibrium was reached (24 h). After equilibrium, an aliquot of solution (3 mL) was simultaneously withdrawn and filtered with a syringe filter (pore size 0.45 μm) and diflunisal concentration was determined immediately at 252 nm by UV–vis spectrophotometry (Hewlet Packard 8452A diode-array spectrophotometer).

2.3. Preparation of solid dispersions

Diflunisal-chitosan solid dispersions at 30:70 drug/polymer ratio were prepared using two methods: kneading and coevaporation. This drug/polymer ratio was chosen because it was found to exhibit different crystalline states depending on the preparation method and the solubility enhancement was adequate.

Kneading method: amounts of diflunisal (150 mg) and either chitosan or carboxymethylchitosan (350 mg) with a 30:70 ratio were wetted in a mortar with a minimum volume of an ethanol/water solution (50%, v/v) and kneaded thoroughly with a pestle for 30 min until a dense paste was formed. The pastes were dried in an oven at 40 °C for 24 h and stored in a desiccator.

Coevaporation method: amounts of diflunisal (150 mg) and either chitosan or carboxymethylchitosan (350 mg) with a 30:70 ratio were accurately weighed and separately dissolved. Firstly, the chitosan polymer was dissolved in 50 mL of an aqueous solution of hydrochloric acid (pH 1.2), then 10 mL of an ethanolic solution of diflunisal was added. Both solutions were mixed with constant stirring and the solvent was eliminated under vacuum in a rotary evaporator (Buchi R-3000) at 60 °C. The samples were dried in an oven at 40 °C for 24 h to complete dryness.

Physical mixtures were prepared (for comparison purposes) by a very careful mixing of the components with a spatula (2 min) in order to avoid the establishment of undesired interactions between DF and the polymers. All samples were stored in a desiccator.

These techniques were applied also to the pure drug in order to evaluate any possible effect of the sample preparation method on the physicochemical characteristics of the drug

2.4. Characterization of solid dispersions

The solid dispersions obtained were analysed by X-ray powder diffraction, FTIR spectroscopy, differential thermal analysis (DTA) and hot stage microscopy. The microscopic structure of the samples was observed by scanning electron microscopy (SEM).

X-ray powder diffraction patterns were collected on a X Bruker axs D8 Advance diffractometer (Karlsruhe, Germany) using a Ni filter, CuK_α radiation, a voltage of 40 kV and a current of 30 mA. The scanning rate was 1°/min, the time constant 3 s/step over a 2θ interval of 2–40°.

The infrared spectra were recorder on a Nicolet- FTIR Avatar 360 spectrometer (WI, USA) using a MKII Golden Gate ATR device, with