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Solid state characterisation of silver sulfadiazine loaded on montmorillonite/chitosan nanocomposite for wound healing

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ABSTRACT

Biopolymer chitosan/montmorillonite nanocomposites loaded with silver sulfadiazine for wound healing purposes were prepared via intercalation solution technique. Structure and morphology of loaded nanocomposites were studied and compared with pure components and unloaded nanocomposites. X-ray diffraction, Fourier transformed infrared spectroscopy, high resolution transmission electron microscopy coupled with energy-dispersion X-ray analysis, thermal and elemental analysis were employed for the characterisation. The results confirmed that the drug was effectively loaded in the three-dimensional nanocomposite structures, in which chitosan chains were adsorbed in monolayers into the clay mineral interlayer spaces.

1. Introduction

Bacterial infections are one of the most serious complications in burn wounds [1]. The use of silver sulfadiazine (AgSD) to reduce bacterial burden and promote healing is well documented in the literature [2–5]. Nevertheless, silver derivatives may have important adverse effects, including allergic reactions, cytotoxicity, silver staining, hyperosmolality, methemoglobinemia and haemolysis [6]. These disorders can impair wound healing processes and therefore new strategies are needed to improve drug efficacy. In particular, polymeric silver nanoparticles have shown a great potential in reducing wound infections [7–10], length of stay in hospital and analgesic use by patients [11]. Silver nanoparticles can also be obtained using clay minerals as nanoscale supports. For example, montmorillonite (MT), a laminar phyllosilicate, has been described to provide silver nanoparticles with large surface area and high biocide effectiveness [12]. Otherwise, MT and other clay minerals can be combined with polymeric substances to provide materials (clay polymer nanocomposites, CPNC) that have become key resources in pharmaceutical and biomedical fields as a result of their improved properties and large flexibility [13,14]. Among the polymers that can be used to prepare CPNC, chitosan (CS), a cationic polysaccharide, is able to easily intercalate via ionic exchange into negatively charged MT interlayers [15–17]. Moreover, CS plays an important role in tissue regeneration, as a result of its special characteristics, such as biocompatibility, biodegradability and antimicrobial activity [18]. These properties are maintained when CS is used to prepare nanocomposites with MT or other clays, [19–21]. CS/MT nanocomposites also exhibit wound healing properties over Caco-2 cell cultures, effectively stimulating cell proliferation [22].

Given these premises, aim of the present work was to prepare and characterise MT/CS nanocomposites loaded with AgSD, intended for the treatment of skin wounds. Such systems aim to associate the antimicrobial activity of AgSD with wound healing properties of CS and MT, and reduce drug cytotoxicity. Crystalline pattern of the obtained drug loaded nanocomposites was investigated by X-ray powder diffraction analysis (XRPD). To further detail in nanocomposite structures, high resolution transmission electron microscopy (HTEM) was carried out. Complementary X-EDS (X-ray energy-dispersive spectroscopy) was also performed on selected micrograph areas to localise drug presence and elemental analysis was carried out to corroborate the integrity of the components. Differential scanning calorimetry (DSC), thermogravimetry (TGA) and Fourier transformed infrared (FTIR) analysis were also done to complete the nanocomposite characterisation.

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2. Materials and methods

- MT: Veegum HS©, pharmaceutical-grade clay, kindly gifted by Vanderbilt Company S.A. (USA), with a cation exchange capacity (CEC) of 80.64 meq/100 g [23].
- CS: Viscosity 12 mPa s (90 s^{-1}; 1% w/w concentration in acetic acid) and deacetylation degree of 98% (Giusto Faravelli, I). Glutamic acid (Sigma Aldrich, I) was used to salify CS in stoichiometric amount, to obtained CS glutamate (CSG), considering the deacetylation degree of the polymer. In particular 1 g of chitosan base contains 0.00555 mol of glucosamine or *N*-acetyl glucosamine and the 98% (deacetylation degree) of the moles was salified using a stoichiometric amount glutamic acid; that is 0.82 g of glutamic acid.
- AgSD: MW 357.14 g/mol; purity 98% (Sigma Aldrich, I).

2.1. Preparation of drug loaded-clay polymer nanocomposites (CPNC)

Known amounts of clay powders were added to 1% w/w CSG aqueous solutions containing AgSD (0.02% w/w) following the intercalation solution technique [24]. 0.02% w/w was the amount of AgSD dissolved in presence of chitosan glutamate at 1% w/w, as experimentally determined by solubilisation equilibrium study. The resulting dispersions were shaken for 48 h at 25 °C and centrifuged at 3000 rpm for 10 min to separate AgSD loaded-CPNC in which the clay/polysaccharide weight/weight ratios were 1/0.1 and 1/0.5. These ratios were selected to obtain monolayer intercalated CPNC, in agreement with Darder et al. [15]. These authors found that intercalation of chitosan into a montmorillonite with CEC of 76.4 meq/100 g took place mainly by electrostatic interactions between the $-NH_3^+$ groups in the polysaccharide chain and the negative sites in the clay, resulting in monolayer intercalated structures at clay/chitosan w/w ratios below 1/1. The amount of drug loaded was 1.2% (w/w) for 1/0.1 CPNC (hereafter referred as loaded-CPNC-I) and 4.4% (w/w) for 1/0.5 CPNC (loaded-CPNC-II), respectively. For comparison purposes, unloaded CPNCs (CPNC-I and CPNC-II) and physical mixtures (PM-I and PM-II) having the same weight/weight ratios that the loaded nanocomposites were also prepared.

2.2. Solid state characterisation

2.2.1. XRPD analysis

X-Ray powder diffraction (XRPD) was done by using a Philips® X-Pert diffractometer with Cu K_α radiation. The diffraction data were analyzed using the XPOWDER® software [25]. The experiments were run in triplicate (experimental error ± 5%).

2.2.2. HRTEM and X-EDS analysis

High resolution TEM study was carried out using a Philips CM-20 microscope fitted with an ultrathin window at 200 keV. Energy dispersive X-ray (EDAX) spectra were recorded using solid-state Si (Li) detector. (CIC, University of Granada).

2.2.3. FTIR

FTIR spectra were recorded on a FTIR spectrophotometer (JASCO 6200, with software SPECTRA MANAGER v2). Measurements were carried out using KBr pellets from 600 to 4000 cm^{-1} at 0.5 cm^{-1} resolution. The experiments were run in triplicate (experimental error ± 0.2%).

2.2.4. Thermal analysis

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were carried out by using a METTLER TOLEDO mod. TGA/DSC1 with FRS5 sensor and a microbalance (precision 0.1 μg) (Mettler-Toledo GMBH). Samples were heated in air atmosphere at 5 °C/min. The experiments were run in triplicate and standard deviations of temperature and mass loss were calculated.

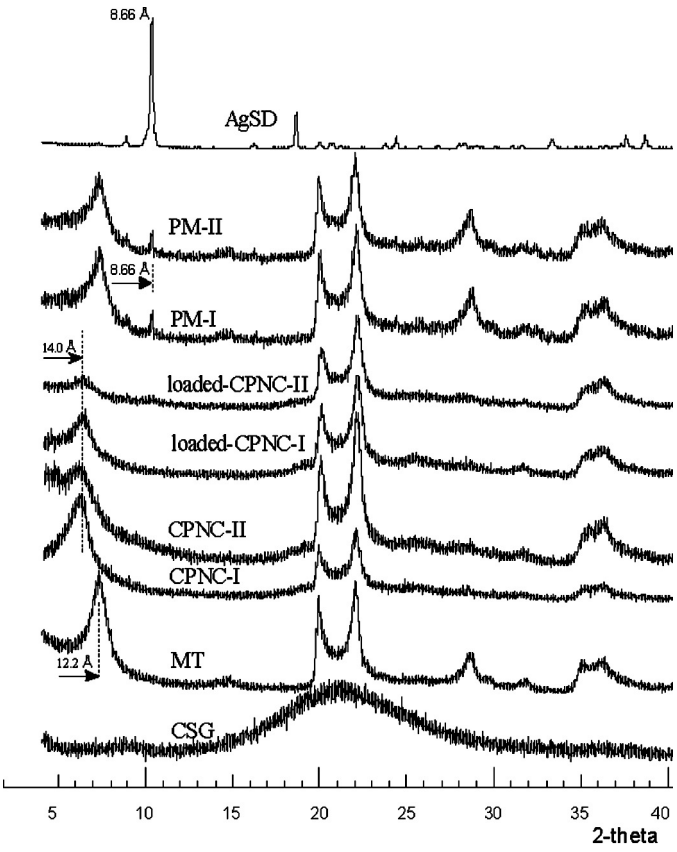

Fig. 1. XRPD patterns of loaded and unloaded nanocomposites, physical mixtures and their pure components.

2.2.5. Elemental analysis

To corroborate the composition of the nanocomposites, contents of C, N and S were determined by using a CHNS/O analyzer, FLASH 2000 model (Thermo Scientific). Ag content was assessed by an ICP-OES Spectrometer, Optima 8300 model (Perkin Elmer).

3. Results and discussion

3.1. XRPD analysis

Fig. 1 shows XRPD patterns of nanocomposites and their pure components. AgSD showed a crystalline pattern, with the main reflection around 10.2° 2θ (8.66 Å) as described in literature [26]. This peak was also observed in physical mixtures (PM-I and PM-II) but not in loaded nanocomposites (loaded-CPNC-I and II), suggesting that no free crystalline drug remained in nanocomposite matrices. MT exhibited a peak at about 7° 2θ due to the d_{001} basal reflection of the clay mineral. On the basis of Bragg's law, the calculated basal spacing (12.2 Å) is typical of a predominantly Na^+ smectite. In loaded-CPNC, this reflection was shifted to 6° 2θ (14.0 Å) approximately, revealing that intercalated nanocomposites were formed by insertion of drug and/or polymer molecules into MT layers. The d_{001} reflection of loaded-CPNC-II also showed broadening and intensity reduction compared to loaded-CPNC-I, indicating a greater disorder degree in nanocomposite structure, suggesting possible exfoliation of clay sheets as a result of higher