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A novel gel based on an ionic complex from a dendronized polymer and ciprofloxacin: Evaluation of its use for controlled topical drug release

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ABSTRACT

The development and characterization of a novel, gel-type material based on a dendronized polymer (DP) loaded with ciprofloxacin (CIP), and the evaluation of its possible use for controlled drug release, are presented in this work. DP showed biocompatible and non-toxic behaviors in cultured cells, both of which are considered optimal properties for the design of a final material for biomedical applications. These results were encouraging for the use of the polymer loaded with CIP (as a drug model), under gel form, in the development of a new controlled-release system to be evaluated for topical administration. First, DP-CIP ionic complexes were obtained by an acid-base reaction using the high density of carboxylic acid groups of the DP and the amine groups of the CIP. The complexes obtained in the solid state were broadly characterized using FTIR spectroscopy, XRP diffraction, DSC-TG analysis and optical microscopy techniques. Gels based on the DP-CIP complexes were easily prepared and presented excellent mechanical behaviors. In addition, optimal properties for application on mucosal membranes and skin were achieved due to their high biocompatibility and acute skin non-irritation. Slow and sustained release of CIP toward simulated physiological fluids was observed in the assays (*in vitro*), attributed to ion exchange phenomenon and to the drug reservoir effect. An *in vitro* bacterial growth inhibition assay showed significant CIP activity, corresponding to 38 and 58% of that exhibited by a CIP hydrochloride solution at similar CIP concentrations, against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively. However, CIP delivery was appropriate, both in terms of magnitude and velocity to allow for a bactericidal effect. In conclusion, the final product showed promising behavior, which could be exploited for the treatment of topical and mucosal opportunistic infections in human or veterinary applications.

1. Introduction

Hydrogels are usually defined as a crosslinked polymeric network having the capacity to hold a large amount of water within its porous structure. The water holding capacity of hydrogels is mostly ascribed to the presence of hydrophilic groups, *e.g.* amino, carboxyl and hydroxyl groups, in the polymer network [1]. Hydrogels comprise an important class of biomaterials specially used for drug delivery applications, due to their biocompatibility, good rheological and bioadhesive properties, high capacity for drug loading and modified-release behaviors. As a result, these kinds of materials are potentially suitable as drug carriers for therapeutic uses. The use of synthetic hydrogels as carriers for drug delivery has rapidly developed over the last few decades [2]. Versatile, reproducible and organic solvent-free synthesis procedures and finely tunable mechanical features turn hydrogels into ideal candidates for use as biomaterials in drug delivery applications [3,4].

Polyelectrolytes (PE) in the form of ionic hydrophilic polymers have been widely used in pharmaceutical systems. Significant progress has been achieved in the development of new pharmaceutical technology platforms, based on ionic condensation between anionic or cationic PE with an ionizable drug (D) of opposite charge [5]. The acid-base interaction between the PE and the acidic or basic D is a valuable approach to yield new materials with physicochemical, pharmaceutical and biopharmaceutical properties different from those of their precursors. Ionic PE-D complexes can be obtained in a wide variety of qualitative

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and quantitative compositions such as aqueous dispersions, gels or in the solid state. Eq. (1) shows the schematic representation of the acid-base interaction of an anionic PE and a basic drug, where *P-COOH* represents an acid PE, *D* is a basic drug, $P\text{-}COO^-$ is an ionized PE, DH^+ represents a protonated basic drug and $P\text{-}COO^-DH^+$ represents an ionic pair.

$$P\text{-}COOH + D \rightleftarrows P\text{-}COO^- + DH^+ \rightleftarrows \left[P\text{-}COO^-DH^+\right] \quad (1)$$

Previous reports proved that high proportions of *D* form ionic pairs with an oppositely charged PE, acting as a reservoir of D. According to the properties of the newly formed chemical entity, PE-D complexes can improve some unfavorable physicochemical or biopharmaceutical properties of recognized as safe and effective drugs such as low chemical stability in solution [6–8], low apparent solubility in the vehicle [9,10], low permeability through biological membranes [11,12], intracellular targeting [13], bacterial uptake [14], or modulation of drug release [15–17]. Therefore, these products are useful for the development of new drug delivery systems (DDS) [5,13,18–20].

Drug delivery technologies are always in need of new carrier materials with innovative properties that can enhance or improve control over the release profiles of any active pharmaceutical ingredient. Hence, stimuli-sensitive hydrogels (also known as smart hydrogels) are currently viewed as the new generation of carriers for biomedical applications [21–25].

In addition, the design of hydrogels based on highly functionalized or dendronized polymers is considered a novel alternative to smart carriers for controlled drug release due to their multivalent properties [26]. The development of hydrogels based on dendronized polymers that have acid groups is of particular interest to the pharmaceutical field. The acid functionality offers improved bioadhesion properties and confers the capacity to easily incorporate active pharmaceutical ingredients such as drugs, dyes and peptides into the hydrogel structures through ionic interaction or hydrolysable chemical bonds. Besides, the presence of acid groups offers the possibility of changing the macroscopic volume of their network structure with a change in pH [26].

Behera's amine (BA) is a commercially available dendritic amine developed by Raijani Behera of Newkome's research group [27]. The preparation of hydrogels based on this BA-derivative dendritic monomer (DM) was performed in our labs [26]. In a first step, the dendritic monomer (DM) was synthesized by reaction of amidation between Behera's amine (BA) and acryloyl chloride. Then, the *tert*-butyl ester groups were hydrolyzed with formic acid to yield the tri-acidic dendritic monomer, DM. Posterior, the synthesis of the hydrogels was performed from DM and the crosslinking agent, *N*,*N*-diallyltartardiamide (DAT). The chemical characterization, swelling behavior, rheology, fibroblast cytotoxicity and ionic drug load-release capacity of the new materials were studied in order to analyze the properties and the possibilities of biomedical application in drug delivery formulations. The influence of the pH environment on the swelling properties of the dendronized hydrogels was studied. It was clearly shown that the pH of the medium has considerable importance in the swelling properties of these ionic gels since %ESR is different depending on pHs. As example, in the case of one of the yielded hydrogels (HG 1.00/2) [26], the swelling capacity increases 6.3 times between pH 3 and 7. At pH 3, lower than pKa of the monomer, the carboxylic groups of the material are protonated. However, at pH 5 or 7, the acid groups are partially or totally deprotonated; consequently, the swelling is greater than at pH 3 because the electrostatic repulsion between the chains increases the water absorption capacity [26]. There are few reports on this subject.

Ciprofloxacin (CIP) is a fluoroquinolone antimicrobial agent with broad-spectrum antibacterial activity that is approved for the treatment of several infections by both, oral and topical administration [28]. In particular, topical pharmaceutical products, containing CIP between 0.2 and 0.3% w/w, are available as aqueous solutions, ointments or hydrogel forms. In aqueous solution, CIP exists mainly in their zwitterionic form owing to the acid/base interaction between the basic nitrogen of the piperazine and the carboxylic acid group. Such interaction also determines the low aqueous solubility of CIP at pH close to 7 [29].

In this context, the aim of this work was to explore the use of a dendronized polymer (DP) as a CIP carrier prepared under gel form, and to evaluate the physicochemical and *in vitro* drug release from DP-CIP complexes. Furthermore, the evaluation of biocompatibility and antimicrobial activity was conducted in order to define the potential use of these systems in the design of topical DDS.

2. Materials and methods

2.1. Materials

The following chemicals were used as purchased: ammonium persulfate (APS, Aldrich), tetramethylethylenediamine (TEMED, Aldrich) and (+)-*N*,*N*′-diallyltartramide (DAT, Aldrich), KH_2PO_4 p.a. (Anedra®, Bs.As., Arg.), NaCl p.a. (Parafarm®, Bs.As., Arg.), 1 N NaOH and HCl solutions (Anedra®, Bs.As., Arg.), and Carbopol® 934NF and 974P polymers (Lubrizol Adv. Mat. Inc., OH). The dendritic monomer (DM) BA-derivative, di-*tert*-butyl-4-acryloylamine-4-(2-*tert*-butoxycarbonylethyl) heptanoate, was synthesized according to a previous report [30]. Ciprofloxacin (CIP) free base was obtained by neutralization of CIP hydrochloride salt (Parafarm®, USP grade, Bs.As., Arg.) with 1.0 N NaOH solution, after which the precipitate was washed, filtered and dried at 100 °C until a constant weight was achieved.

2.2. Preparation and characterization of dendronized polymers

The general procedure for the synthesis of DP was previously reported by Cuggino et al. [26] and is represented in Fig. 1. The principal experimental descriptors of the DP^X synthesized for this work are reported in Table 1. Regarding the nomenclature of DP^X, the superscript "X" indicates that the hydrogel was prepared using an appropriate amount of DAT in relation to the total dendritic monomer (DM), expressed as mol%. For this particular work, DP containing 0.5, 2.0 or 4.0% crosslinking agent was synthesized following the general procedure. Thus, appropriate amounts of DM (1.0 M), DAT (0.5, 2.0 or 4.0 mol% with respect to DM) and APS (2 mol% with respect to DM) were dissolved in distilled water (4 mL), using a glass tube with a septum. This solution was then bubbled using N_2 for 2 min, and an appropriate volume of 0.32 M TEMED aqueous solution was added (2 mol% with respect to DM). Immediately, this solution mixture was transferred to a 5 mL disposable polypropylene syringe, which served as a reactor. The closed syringe was placed in a water bath at 25 °C for 24 h. After the reaction, the tips of syringe was carefully cut, the rigid rod-shaped form semisolid product was retired from the syringe barrel and then regular discs of about 3–4 mm thickness (about 1 cm in diameter) were cut. The discs were exhaustively washed in 500 mL of distilled water for 48 h (the solvent was changed every 12 h) to remove all of the unreacted monomer and then dried at 25 °C until a constant weight was achieved. The synthetic yield was calculated by gravimetry after polymer purification (Table 1), considering the percent of polymer mass recovery (%PMR) with respect to that of the mass of initial reagents (monomer and crosslinker). Finally, the dry discs were pulverized for the next studies.

For ^1H NMR characterization, approximately 5 mg of fine polymer powder was swelled in 0.8 mL of D_2O for 24 h before each measurement in order to facilitate water incorporation.

The swelling behavior of the DP^X products was determined by a gravimetric method. Approximately 35 mg of fine powder DP^X was weighed (m_d) in a pre-weighed tube. Then, 1.0 mL of ultrapure water was added to achieve swelling equilibrium at 25 °C for 24 h. The excess water was removed using a graduated pipette and the hydrogels inside the tubes were weighed (m_e). The percentage of