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# Vaginal inserts based on chitosan and carboxymethylcellulose complexes for local delivery of chlorhexidine: Preparation, characterization and antimicrobial activity

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ABSTRACT

The aim of this work was to prepare vaginal inserts based on chitosan/carboxymethylcellulose polyelectrolyte complexes for local delivery of chlorhexidine digluconate. Complexes were prepared with different chitosan/carboxymethylcellulose molar ratios at a pH value close to $pK_a$ interval of the polymers and were characterized in terms of physico-chemical properties, complexation yield and drug loading. Then complexes were used to prepare inserts as vaginal dosage forms and their physical handling, morphology, water-uptake ability and drug release properties as well as antimicrobial activity toward *Candida albicans* and *Escherichia coli* were evaluated. Results confirmed the ionic interaction between chitosan and carboxymethylcellulose and the influence of the charge amount on the complexation yield. Complexes were characterized by high values of drug loading and showed increasing water-uptake ability with the increase of carboxymethylcellulose amount. The selection of appropriate chitosan/carboxymethylcellulose molar ratios allowed to obtain cone-like shaped solid inserts, easy to handle and able to hydrate releasing the drug over time. Finally, the formulated inserts showed antimicrobial activity against common pathogens responsible for vaginal infections.



## 1. Introduction

Chlorhexidine (CLX), a broad-spectrum antiseptic, is effective against a wide range of both gram-positive and gram-negative bacteria (Salem et al., 1987) and also shows anti-*Candida* properties (Giuliana et al., 1997; Ellepola and Samaranayake, 2001). Actually, CLX is widely used for the prevention and treatment of infections of wounds on skin and of the oral and vaginal cavities (Karki and Cheng, 2012; Ribeiro et al., 2007; Pupe et al., 2011; Juliano et al., 2008; Ambrogi et al., 2009). In particular, for the treatment of vaginal infections, CLX can be widely used thanks to its antimicrobial activity against concomitant bacterial vaginitis and vaginal candidiasis (Molteni et al., 2004). Moreover, several studies demonstrated that vaginal douches based on CLX can reduce peripartum infections during labor and mother-to-child risk of transmission of Group B *Streptococcus* (Rouse and Cliver, 2003). The currently available vaginal formulations containing CLX, such as douches and pessaries, show some problems that can limit their use. In fact, they could be rapidly removed through the washing action of vaginal fluids (Abruzzo et al., 2013) and they are characterized by leakage or messiness that required multiple daily doses, thus leading to poor patient compliance (Dobaria et al., 2007).

In the last years, our research group have developed inserts, based on different chitosan polyelectrolyte complexes as new delivery systems able to be easily applied and deliver a unique dose of drug (Luppi et al., 2009, 2010a; Abruzzo et al., 2013). In this study we investigated the possibility to formulate inserts based on chitosan and carboxymethylcellulose polyelectrolyte complexes loaded with CLX. Chitosan (CH), a *N*-deacetylated product of the polysaccharide chitin, is widely employed for the preparation of different delivery systems (Luppi et al., 2010b) and for the formulation of vaginal mucoadhesive dosage forms (Bonferoni et al., 2008; Perioli et al., 2008; Valenta, 2005; Rossi et al., 2003). It shows interesting properties such as biocompatibility,

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non-toxicity, biodegradability and mucoadhesivity (Kumar Ravi, 2000; Dutta et al., 2004; Muzzarelli, 1997, 2010). Moreover, at pH lower than its p$K_a$ (p$K_a$ = 6.3), CH amino groups are ionized and can interact with anionic polymers thus forming ionically crosslinked hydrogels (Hamman, 2010; Berger et al., 2004; Meshali and Gabr, 1993). Sodium carboxymethylcellulose (NaCMC) is a natural biodegradable and water-soluble molecule derived from cellulose. It is an anionic polymer (p$K_a$ = 4.3) that can interact with the positively charged amino groups of chitosan (Gomez-Burgaz et al., 2008; García et al., 2013; Rosca et al., 2005) and with cationic drugs. The ionic interaction between NaCMC and cationic drugs, such as propranolol hydrochloride, chlorpheniramine maleate, venlafaxine hydrochloride and verapamil hydrochloride, was studied by Palmer et al. (2011, 2013),) in recent years. They observed the formation of a new form/type of active (salt), having more prolonged release compared to the original active as a consequence of ionic bond between anionic polymer and cationic drug. Chlorhexidine digluconate (CLX), the selected drug for this study, has a p$K_a$ = 10.78, so can be ionized at pH lower than its p$K_a$ thanks to the presence of multiple amine and imine groups.

The aim of this work was to prepare vaginal inserts based on chitosan/carboxymethylcellulose polyelectrolyte complexes able to modulate CLX release as a function of their composition. Polyelectrolyte complexes based on CH and NaCMC were prepared with or without the presence of CLX in acetate buffer with a pH in the p$K_a$ range polymers and lower than drug p$K_a$. FT-IR studies and thermogravimetric analysis (TGA) were performed in order to evaluate the interaction between CH and NaCMC and between CLX and the polymers. The physico-chemical properties of loaded drug were studied through X-ray powder diffraction (XRPD). Complexes were characterized in terms of yield, encapsulation efficiency and drug loading. Lyophilized vaginal inserts were prepared with the different loaded complexes and their morphology and friability were evaluated. *In vitro* water-uptake and drug release were performed in order to investigate insert ability to hydrate and to control drug release, respectively. Finally, microbiological assay were conducted to define the antimicrobial activity toward *Candida albicans* and *Escherichia coli*.

## 2. Experimental

### 2.1. Materials

NaCMC (viscosity 850 mPa, 2%; substitution degree 0.78) was purchased from ACEF (Piacenza, Italy). CH low molecular weight (Mw ≈ 150 kDa; viscosity 20–300 cP, $T$ = 20 °C, 1% in 1% acetic acid; deacetylation degree 97%) and chlorexidine digluconate (CLX) aqueous solution (20% p/v) were obtained commercially from Sigma–Aldrich (Milan, Italy). All other chemicals and solvents were of analytical grade and purchased from Carlo Erba (Milan, Italy). Luria-Bertani (LB) and Sabouraud dextrose (SD) media, used for microbiological assays, were purchased from Difco (Detroit, MI).

### 2.2. Preparation of chitosan/carboxymethylcellulose complexes with chlorhexidine digluconate

CH and NaCMC were separately dissolved in acetate buffer at pH 5.0 ($CH_3COOH$ 18 mM, $CH_3COONa$ 32 mM; 50 mM ionic strength) at the same concentration (5 μmol of monomer per ml). Then different volumes of NaCMC solution were added to CH solution in order to obtain a final volume (200 ml) with different CH/CMC molar ratios (50:50, 30:70 and 10:90). The obtained suspensions were stirred at room temperature for 24 h and centrifuged at 12.000 rpm for 30 min (ALC 4239R ultracentrifuge, Milan, Italy). The precipitate was suspended in deionized water and homogenized at 6.500 rev/min for 2 min (Ultra-Turrax, T 25 basic homogenizer, IKA, Dresden, Germany). Finally, it was freeze-dried (Christ Freeze Dryer ALPHA 1-2, Milan, Italy) and weighted for the determination of solid complex yield. For loaded complex preparation, CLX was added to chitosan phase prior the mixing with NaCMC solution in order to obtain a final drug concentration of 1 mg/ml. Loaded complexes (50:50, 30:70, 10:90 and 0:100; CH/CMC molar ratios) were isolated as described before and weighted.

Different polymeric complexes were named in this work as follows: CH:$CMC_{(50:50)}$, CH:$CMC_{(30:70)}$, CH:$CMC_{(10:90)}$ and CH:$CMC_{(0:100)}$ for 50:50, 30:70, 10:90 and 0:100 CH/CMC molar ratios, respectively.

#### 2.2.1. FT-IR spectroscopy, TGA and XRPD

To verify the interaction between CH and CMC, FT-IR studies (PerkinElmer Spectrum One recorded with MIRacle™ ATR, Pike Technologies, Madison, WI, US) and TGA (Mettler Toledo, Novate Milanese, Italy; temperature range: 5–500 °C, heating and cooling rates: 0.01–50 K/min, inert atmospheres) of CH:$CMC_{(50:50)}$ unloaded complex and CH and NaCMC powders were performed. Moreover, FT-IR spectra of CLX and CH:$CMC_{(0:100)}$ loaded complex were obtained.

Finally, X-ray powder diffractograms of loaded complexes (CH:$CMC_{(30:70)}$, CH:$CMC_{(50:50)}$, CH:$CMC_{(10:90)}$, CH:$CMC_{(0:100)}$) were obtained using an X'PERT PRO PANalytical diffractometer (Almelo, The Netherlands) with a graphite monochromator (radiation used: CuKa, λ = 1.542 A, excitation voltage: 40 kV, anode current: 40 mA, measured range: 3–40° 2$\theta$).

#### 2.2.2. Drug content determination

The determination of drug content was performed by dissolving 18 mg of complex into 15 ml of phosphate buffer ($KH_2PO_4$ 0.1 M adjusted with ortophosphoric acid to pH 4.5), simulating vaginal conditions. After 48 h, aliquots were centrifuged at 3.000 rpm for 5 min and the supernatant was analyzed through HPLC. The chromatographic system was composed of a Shimadzu (Milan, Italy) LC-10ATVP chromatographic pump and a Shimadzu SPD-10AVP UV–vis detector set at 260 nm. Separation was obtained on a Phenomenex (Torrance, CA, USA) Sinergy Fusion-RP 80A (150 × 4.6 mm I.D., 5 μm) coupled to a Phenomenex (Torrance, CA, USA) C18 guard cartridge (4 × 3.0 mm I.D., 5 μm). The mobile phase was composed of a mixture of acetate buffer ($CH_3COONa$ 30 mM and adjusted with glacial acetic acid to pH 3.3): acetonitrile (50:50 v/v) (Padpois et al., 2012). The flow rate was 0.4 ml/min and manual injections were made using a Rheodyne 7125 injector with a 20 μl sample loop. Data processing was handled by means of a CromatoPlus computerized integration system (Shimadzu Italia, Milan, Italy). A calibration curve was set up in the 8–80 μg/ml range and a good linearity was found ($r^2$ = 0.995).

The drug encapsulation efficiency (EE) and loading capacity (LC) were calculated as follow:

$$EE\% = \frac{Drugamountinthecomplex}{Theoreticaldrugamount} \times 100$$

$$LC\% = \frac{Drugamountinthecomplex}{Loadedcomplexweight} \times 100$$

#### 2.2.3. Water-uptake ability

Water-uptake studies were performed in phosphate buffer at pH 4.5, following the procedure reported in our previous work (Luppi et al., 2010a). Briefly, 18 mg of complex were placed on filter paper ($d$ = 40 mm) soaked with phosphate buffer at pH 4.5 and positioned on top of a sponge (5 cm × 5 cm × 2 cm), previously placed in a Petri dish filled with the same buffer to a height of