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Chitosan/alginate complexes for vaginal delivery of chlorhexidine digluconate

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ABSTRACT

Chitosan/alginate complexes were prepared at different polycation/polyanion molar ratios and freeze-dried vaginal inserts were obtained for chlorhexidine digluconate local delivery in genital infections. Complex yield, FT-IR spectra, and TGA thermograms were studied to confirm the interaction between the two polyions. The influence of different complexes on physical handling, morphology, and drug distribution in the samples were evaluated by friability test, scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDS), respectively. In vitro water-uptake, mucoadhesion and release tests were performed as well as microbiological tests toward pathogenic vaginal microorganisms. The results showed that the selection of suitable chitosan/alginate molar ratio and drug loading allowed modulate insert ability to hydrate, adhere to the mucosa, and release chlorhexidine digluconate. The insert containing an excess of alginate was found to be the best performing formulation and showed good antimicrobial activity toward the pathogens *Candida albicans* and *Escherichia coli*.

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1. Introduction

Disturbances in the vaginal environment due to abnormal vaginal flora and vaginal infections are highly prevalent among reproductive-aged women. Vaginal candidiasis is ranked as one of the most common gynecological infections, and it has been estimated that about 75% of women experience an acute episode once in their lifetime. It has been reported that 30–35% of vaginitis episodes are due to *Candida albicans* (Das Neves et al., 2008; Nyririesy, Weitz, Grody, & Lorber, 2001; Sobel, 1988). Aerobic vaginitis is another frequent form of abnormal vaginal flora which has been considered an important cause of pregnancy complications, such as ascending chorioamnionitis, preterm rupture of the membranes, and preterm delivery. Aerobic vaginitis is defined as a disruption of the lactobacillary flora, accompanied by signs of inflammation and the presence of a predominantly aerobic microflora, composed of enteric commensals or pathogens, especially *Escherichia coli* and *Streptococcus agalactiae* (Donders, Bellen, & Rezeberga, 2011; Donders et al., 2002).

Topical imidazoles are considered standard treatments of candidiasis, while kanamycin or quinolones are a good choice for the therapy of aerobic vaginitis (Tempera & Furneri, 2010). In the case of mixed vaginitis, the use of a monotherapy becomes ineffective, whereas treatment with a wide-spectrum antibacterial and antifungal substance, such as chlorhexidine digluconate, may be promising for a more rapid healing (Molteni et al., 2004).

Several drug delivery systems are used for treatment of vaginal infections (Alamdar & Fakhrul, 2005). Indeed, conventional vaginal formulations (suspensions, pessaries, cream, and solutions) are characterized by short residence time at the site of administration, due to washing action of physiological secretions of vaginal fluids. Bioadhesive vaginal drug delivery systems, such as tablet, inserts, and gels, may adhere to vaginal mucosa in order to bring drug in contact with target tissues for sufficient period of time and prevent expulsion of formulation (Ceschel, Maffei, Borgia, Ronchi, & Rossi, 2001; Dobaria, Mashru, & Vadia, 2007; Dobaria, Badhan, & Mashru, 2009; Kast, Valenta, Leopold, & Bernkop-Schnürch, 2002; Valenta, 2005; Woodley, 2001). Tablets and some gel-based vaginal delivery systems are associated with problems like messiness and leakage of formulations causing inconvenience to users and leading to poor patient compliance (Dobaria et al., 2007). For this reason, in this study we focused the attention on the possibility to formulate a new suitable delivery system, able to overcome these limitations and characterized by a convenient application and easy handling. To achieve this goal, the vaginal insert was chosen as final dosage form, easily applicable and able to deliver a unique dose of drug in the vaginal cavity, while chitosan and sodium alginate were selected in order to obtain good insert mucoadhesion ability. Furthermore, different chitosan/alginate molar ratios were tested in

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order to obtain a system releasing the suitable chlorhexidine digluconate amount, accordingly to the therapeutic needs and providing the complete inhibition of pathogens, such as *C. albicans* and *E. coli*.

Chitosan, a N-deacetylated product of the polysaccharide chitin, shows interesting biological properties, including biocompatibility, non-toxicity, biodegradability, and mucoadhesivity (Dutta, Dutta, & Tripathi, 2004; Koga, 1998; Muzzarelli, 1997, 2010; Ravi Kumar, 2000). It was also widely used for different type drug delivery systems (Dodane & Vinod, 1998; Luppi, Bigucci, Cerchiara, & Zecchi, 2010) and largely employed to prepare vaginal mucoadhesive dosage forms (Bonferoni et al., 2008; Perioli et al., 2008; Rossi, Sandri, Ferrari, Bonferoni, & Caramella, 2003; Valenta, 2005). Chitosan can also interact with anionic polymers in order to prepare ionically crosslinked hydrogels (Berger, Reist, Mayer, Felt, Peppas, & Gurny, 2004; Hamman, 2010; Meshali & Gabr, 1993; Remuñán-López & Bodmeier, 1996). Sodium alginate, an anionic, biocompatible, hydrophilic, and biodegradable polymer, derived primarily from brown seaweed and bacteria, is a linear polysaccharide that consists of β-D-mannuronic acid and α-L-guluronic acid repeating units in various ratios (Hanne & Jan, 2002; Tønnesen & Karlsen, 2002).

Chitosan/alginate complexes were obtained by mixing polymeric solutions with different molar ratios of chitosan and alginate and then freeze-drying the precipitates. Complex yield, FT-IR analysis, TGA thermograms were studied to investigate the interaction between the two polyions. The complexes were used to prepare vaginal inserts loaded with chlorhexidine digluconate. Physical handling, morphology, and drug distribution in the samples were studied by friability test, scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDS) analysis. In vitro water-uptake, mucoadhesion, release and microbiological tests were performed in order to investigate the polyelectrolyte complexes ability to adhere to mucosa, to release chlorhexidine digluconate and to study the antimicrobial activity toward *C. albicans* and *E. coli.*

2. Materials and methods

2.1. Materials

Sodium alginate low viscosity ($M_w \approx 140{,}000$ Da, viscosity 100–300 cP, 2%), chitosan low molecular weight ($M_w \approx 150{,}000$ Da, viscosity 20–300 cP, $T = 20\,^{\circ}C$, 1% in 1% acetic acid; deacetylation degree 97%) and chlorhexidine digluconate used for this study were obtained commercially from Sigma–Aldrich (Milan, Italy). All other chemicals and solvents were of analytical grade and purchased from Carlo Erba (Milan, Italy). Complex preparation, water-uptake, mucoadhesion, and release studies were carried out in aqueous buffers with the following compositions per liter of distilled water: 8.99 ml CH_3COOH 2 N and 2.62 g CH_3COONa for acetate buffer at pH 5.0; 13.61 g KH_2PO_4, adjusted with hydrochloric acid to pH 4.5, for buffer simulating vaginal secretions.

2.2. Preparation of chitosan/alginate complex and solid complex weight measurement

Chitosan/alginate was prepared according to a method reported in a previous work (Bigucci et al., 2008) with some modifications. Briefly, chitosan (1.50 mmol of monomer in 200 ml) and alginate (1.50 mmol of monomer in 200 ml) were separately dissolved in acetate buffers at pH 5.0 at the same ionic strength (50 mM). Different volumes of chitosan solutions were added to alginate solutions and stirred at room temperature for 24 h, in order to obtain different chitosan/alginate molar ratios (1:9, 3:7, 1:1, 7:3, and 9:1).

The precipitate was separated by ultracentrifugation at 10,000 rpm for 10 min (ALC 4239R Centrifuge; Milan, Italy). Then it was washed with deionized water and homogenized at 17,500 rev min^{-1} for 5 min (Ultra-Turrax, T 25 basic homogenizer; IKA, Dresden, Germany) for three times in order to eliminate sodium acetate. Finally, the precipitate was suspended again in deionized water and freeze-dried (Christ Freeze Dryer ALPHA 1-2, Milan, Italy), obtaining five different chitosan/alginate complexes:

CH/ALG(1:9), CH/ALG(3:7), CH/ALG(1:1), CH/ALG(7:3), and CH/ALG(9:1).

Each precipitate was weighted for the determination of solid complex weight.

2.3. FT-IR spectroscopy and thermogravimetric analysis (TGA)

To verify interactions between chitosan and alginate, FT-IR spectroscopy (FT-IR-4100 spectrophotometer recorded with a Jasco, 650–4000 cm^{-1}) and TGA (STA 409 PC Luxx® Netzsch, temperature range: 5–1700 °C, heating and cooling rates: 0.01–50 K/min, inert atmospheres) of unloaded complex, chitosan and alginate powders and their physical mixture were performed. The IR spectra for the test samples were obtained using KBr disk method. Measurements were carried out at least in triplicate (relative standard deviation ±5%).

2.4. Preparation of chitosan/alginate complex vaginal inserts

The freeze-dried chitosan/alginate complexes were used to prepare vaginal inserts. For unloaded inserts (average diameter 0.6 cm, height 1.0 cm) 200 μl of phosphate buffer at pH 4.5 were added to 20 mg of different complex/mannitol mixtures (9:1; w/w). Mannitol was added, as a bulking agent in order to improve mechanical strength of lyophilized vaginal inserts when handled (Luppi et al., 2009; McInnes et al., 2005). Loaded inserts were prepared in the same way adding 200 μl of chlorhexidine digluconate solutions (in phosphate buffer at pH 4.5) at different concentration in order to obtain three different complex/drug weight ratios (2:0.5, 2:1, and 2:2) for every type of complex. The resultant suspensions, filled into polypropylene microcentrifuge tubes, were allowed to settle to swell and remove air and finally lyophilized, obtaining cone-like shaped solid inserts. The inserts were stored in a desiccator until use (Luppi, Bigucci, Abruzzo, et al., 2010).

Moreover, control formulations were prepared, without chitosan/alginate complexes, using 20 mg of mannitol and 200 μl of chlorhexidine digluconate solutions at different concentration (mannitol/drug weight ratio, 2:0.5, 2:1, and 2:2).

2.5. Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS)

The morphology of vaginal inserts was studied by SEM analysis. Inserts were cut with a razor blade to expose the inner structure, fixed on supports and coated with gold–palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with LEO 420 (LEO Electron Microscopy Ltd., England) using secondary electron imaging at 15 kV in order to examine the surface morphology and structure of the inserts.

Moreover, drug distribution in the samples was evaluated by energy dispersive X-ray spectroscopy (EDS).

2.6. Friability studies

Friability tests were conducted by subjecting at least 10 inserts to repeat revolutions using a friability tester. Inserts were weighted before and after the testing and % friability was measured as a percentage of weight lost during a standardized abrasion.

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