Materials Science and Engineering C 79 (2017) 140–150

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Formulation and *in-vitro* efficacy of antifungal mucoadhesive polymeric matrices for the delivery of miconazole nitrate

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ARTICLE INFO

Article history:
Received 31 January 2017
Received in revised form 25 April 2017
Accepted 7 May 2017
Available online 8 May 2017

ABSTRACT

Oral candidiasis is the most common opportunistic infection affecting patients with the human immunodeficiency virus. Miconazole buccal tablets or miconazole gel are approved for the treatment of oropharyngeal candidiasis. However, buccal films present more flexibility and also offer protection for the wounded mucosa, reducing pain. Due to their small size and thickness, buccal films may improve patients' compliance, compared to tablets. Additionally, they may increase the relatively short residence time on the mucosa of oral gels, which are easily removed by saliva. Polymeric films loaded with miconazole nitrate were prepared by a casting/solvent evaporation methodology using chitosan, carbopol, gelatin, gum arabic, and alginate to form the polymeric matrices. The morphology of films was investigated by scanning electron microscopy; interactions between polymers were analyzed by infrared spectroscopy and drug crystallinity by differential thermal analysis and X-ray diffraction. Films were characterized in terms of thickness, folding endurance, tensile properties, swelling, adhesiveness, and drug release. Finally, the antifungal activity against cultures of the five most important fungal opportunistic pathogens belonging to *Candida* genus was investigated. The more appropriate formulations were those based on chitosan-gelatin and chitosan-carbopol which showed good mechanical properties and adhesiveness, a relative low swelling index, improved drug release, and showed better *in vitro* activity against *Candida* cultures than miconazole nitrate raw material. Thus, it will be possible to produce a new pharmaceutical form based on polymeric films containing chitosan and miconazole nitrate, which could be loaded with low drug concentration producing the same therapeutic effect against *Candida* cultures.

1. Introduction

Candidiasis is a well-known fungal infection caused by several species of *Candida*, including *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei*. *Candida* strains reside as a commensal in the gastrointestinal and genitourinary tracts and in the oral and conjunctival flora. Despite the fact that these yeasts are harmless to healthy individuals, most people with weakened immune systems are at risk of developing such fungal infections. Particularly, oral candidiasis is an opportunistic infection encountered in the population of patients with acquired immunodeficiency syndrome (AIDS) as well as in patients with hematological malignancies [1]. Even though antifungal drugs used in clinical treatments appear to be diverse and numerous, only a few are currently available to treat mucosal or systemic infections caused by *Candida* spp. Currently, azole antifungal agents including miconazole, econazole, clotrimazole, and ketoconazole are widely prescribed for antifungal therapy [1]. Recently, miconazole buccal tablets have been approved by the FDA for the treatment of oropharyngeal candidiasis. As reported, this formulation showed non-inferiority in the treatment of such infection compared with miconazole gel [2].

Although buccal devices containing antifungal compounds such as, buccal gel [3], bioadhesive buccal tablets [4], nanofiber mats [5] and patches [6–8] have been previously developed, buccal films present more flexibility and are able to protect the wounded mucosa, reducing pain. Furthermore, due to their small size and thickness, buccal films may improve patients' compliance if compared to tablets. Additionally, they may increase the relatively short residence time on the mucosa of oral gels, which are easily removed by saliva [9]. An ideal buccal film should possess good bioadhesive strength, flexibility, elasticity, softness and should also be adequately strong to withstand breakage due to stress from mouth activities. In general, buccal films are

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http://dx.doi.org/10.1016/j.msec.2017.05.034
0928-4931/

composed of polymeric matrices. Several bioadhesive polymers including chitosan (CH), cellulose derivatives, pectins, poly (vinyl alcohol), and poly (vinyl pyrrolidone) are commonly used to prepare thin films. In many cases, the desired film properties may be achieved by mixing two or more polymers [10–12]. In this context, films based on CH-hydroxypropyl methylcellulose (HPMC) and CH-pectin (PC) for the delivery of miconazole nitrate were developed by our group [13]. It was observed that CH in combination with the non-ionic polymer showed smoother morphology, higher elongation to break, swelling index, and release of miconazole from the matrix than CH in combination with pectin [13]. These differences were mainly based on the matrix interactions due to the combination of "charged/non-charged" or "charged/charged" polymers. In addition, it has been postulated that the interaction between two oppositely charged polymers results in the formation of a complex, termed as polyelectrolyte complex. Different matrices of CH, a cationic polymer, with anionic polymers including carbopol (CB), gelatin (GEL), gum arabic (GA) and alginate (ALG) have been extensively studied, and some of them, used as potential carriers for the delivery of therapeutic agents [12,14–20]. For instance, matrices of CH and CB have been developed to facilitate the vaginal delivery of econazole nitrate [12], to prepare an extended-release matrix tablet containing theophylline [21], and to be used as potential skin drug delivery systems [22]. Matrices of CH and GEL have been developed for buccal delivery of propranolol hydrochloride [23], and have been evaluated as a postoperative adhesion barrier in rat cecum model [24]. They have also been formulated as bi-layer films with potential antimicrobial activity [25], and used to release tramadol hydrochloride [26]. Matrices of CH and GA have been developed and its formation analyzed according to the pH and ionic strength [27], and applied to produce nanoparticles loaded with insulin [18]. Moreover, matrices of CH and ALG have been extensively used as bioadhesive vaginal tablets [19] and for the implant delivery of vancomycin [20], among others. Those polymers have good mucoadhesive properties, which makes them suitable candidates for the design of bioadhesive buccal dosage forms [28]. By modifying the composition of the polyelectrolyte complexes, different charge densities may interact in various degrees, producing films with different mechanical properties, adhesiveness, swelling degree, morphology, drug release, and *in vitro* activity. Thus, according to the particular properties of CH, CB, GEL, GA and ALG, the main objective of this work was to develop buccal mucoadhesive films for the treatment of oral candidiasis, based on the formation of polylectrolyte complexes. The factors affecting the formulation of miconazole nitrate films, including bioadhesive and mechanical properties, swelling degree, dissolution rate and solid-state characterization, were analyzed. Additionally, the antifungal efficacy of these formulations based on CH and anionic polymers with different charge density, was investigated against cultures of the five most important fungal opportunistic pathogens belonging to *Candida* genus.

2. Materials and methods

2.1. Materials

CH (230 KDa average molecular weight and 80.6% of *N*-deacetylation) was supplied by Aldrich Chemical Co. (Milwaukee, WI, USA), GA (Mw = 250KDa), GEL (type A from pork skin, 125 Bloom value) and MN pharmaceutical grade were purchased from Parafarm, (Buenos Aires, Argentina). Sodium ALG (Sigma-Aldrich Co. Buenos Aires, Argentina), CB (Carbopol ® 971NF) by Lubrizol Advanced Materials, Inc. (Cleveland, OH, USA). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Film formulation

Formulation of films was based on the ionic interaction between CH and four anionic polymers: CB, GEL, GA and ALG.

CH solutions (3% w/v) were prepared dispersing CH in a solution of 10% v/v lactic acid (pH = 2.67) [29]. Aqueous solutions of CB (1.5% w/v), ALG (1.5% w/v), GEL (3.0% *w/v*), and GA (3.0% w/v) were prepared, stirred overnight and filtered through Miracloth® (Calbiochem-Novabiochem Corp., San Diego, CA). Then, CH solutions were dripped over each polymeric solution under magnetic stirring (Boecco stirrer, Germany) at 80 °C to avoid precipitation. MN (2% w/w) was solubilized in PEG 400, employed as a plasticizer (30% w/w) [30] and added to the polymeric solutions. The mixtures were stirred at 200 rpm for 2 h. Then, the solutions were cast on 9 cm diameter Petri dishes and dried (72 h at 40 °C, and 58% relative humidity (RH)). After being dried, films were neutralized in casting by addition of a phosphate buffer pH = 6.8 solution, washed with distilled water, and dried again [31]. Dried films were removed from the Petri dishes and conditioned in a chamber (72 h at 25 °C and 58% RH). The films used in the different tests were selected based on the lack of physical defects such as cracks, bubbles, and holes. The formulated films are described in Table 1.

2.2.2. Film characterization

2.2.2.1. Film thickness and folding endurance. For each film, six thickness measurements were made (around and in the center of the film) with a digital micrometer (Schwyz, China) [31]. Folding endurance was determined by repeatedly folding the films at the same place until they broke or were folded 300 times [32].

2.2.2.2. Mechanical properties. The mechanical strength of the films was evaluated by using an Universal Testing Machine Instron, single column, Series 3340 (Instron, Norwood, MA, United States) with a 10 N load cell. Films for each mechanical test were conditioned (24 h at 25 °C and 58% RH) and cut into strips (7 mm wide and 60 mm long) to evaluate tensile properties. The strip ends were mounted with double-sided tape and squares of 30 mm of cardstock to prevent tearing and slippage in the testing device (the exposed film strip length between cardstock ends was 30 mm). The initial grip distance was 30 mm and the crosshead speed was 0.05 mm/s. The parameters obtained from stress/strain curves were tensile strength (calculated by dividing the peak load by the cross-sectional area of the initial film), and elongation (calculated as the percentile of the change in the length of film with respect to the original distance between the grips). For each mechanical probe, three replicate measurements were performed.

Table 1
Film composition.

Composition					
CH	CB	GEL	GA	ALG	MN
100%					2% w/w
50%	50%				2% w/w
	100%				2% w/w
50%		50%			2% w/w
		100%			2% w/w
50%			50%		2% w/w
			100%		2% w/w
50%				50%	2% w/w
				100	2% w/w
100%					–
50%	50%				–
	100%				–
50%		50%			–
		100%			–
50%			50%		–
			100%		–
50%				50%	–
				100%	–