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Development of low methoxy amidated pectin-based mucoadhesive patches for buccal delivery of triclosan: Effect of cyclodextrin complexation

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ABSTRACT

A novel mucoadhesive buccal patch formulation of triclosan (TR), a broad spectrum antibacterial agent, was developed using low methoxy amidated pectin (AMP). The integrity of AMP matrix was improved by addition of 20% (w/w) Carbopol (CAR). The efficiency of β-cyclodextrin-epichlorohydrin polymer (EPIβCD) and anionic carboxymethylated β-cyclodextrin-epichlorohydrin polymer (CMEPIβCD) in optimization of TR solubility and release from such a matrix was investigated and confronted to that of parent β-cyclodextrin (βCD). Loading of TR/βCD co-ground complex into AMP/CAR matrix resulted in a biphasic release profile which was sensitive upon the hydration degree of the matrix, due to lower solubilizing efficiency of βCD, while the drug release from patches loaded with TR/EPIβCD complex was significantly faster with a constant release rate. Microbiological studies evidenced faster onset and more pronounced antibacterial action of TR/EPIβCD loaded patches, clearly demonstrating their good therapeutic potential in eradication of *Streptococcus mutans*, a cariogenic bacteria, from the oral cavity.

1. Introduction

The formation of a biofilm on the teeth is a primary step leading to caries formation, gum inflammation (gingivitis) or gums and proximal bone degradation (periodontitis). Furthermore, it has been shown that oral infections are connected with development of systemic diseases, such as cardiovascular diseases, diabetes, rheumatoid arthritis, and osteoporosis, representing an emerging problem in medicine (Gilbert, McBain, & Sreenivasan, 2007; Rautemaa, Lauhio, Cullinan, & Seymour 2007; Sreenivasan, & Gaffar, 2008). Therefore, elimination of cariogenic bacteria from the dental film is an important step in prevention and treatment of dental conditions as well as of related systemic diseases. Besides regular brushing, such a goal could be achieved by local application of different antimicrobial agents to the oral cavity (Gilbert et al., 2007).

Triclosan (TR), a broad spectrum antibacterial agent is a possible candidate for such a role. In fact, it has a long history of safe use in consumer products and its local application to the oral cavity is lacking of significant side-effects (Bhargava & Leonard, 1996; Rautemaa et al., 2007). Dual antibacterial and anti-inflammatory action of TR offers advantages in the routine oral hygiene as well as in management of periodontal diseases and other oral conditions (Sreenivasan & Gaffar, 2008). Clinical studies have demonstrated the effectiveness of TR in reducing plaque and gingivitis and slowing down progression of periodontal diseases (Rautemaa et al., 2007). Usually, TR is delivered in the mouth by toothpastes and mouthwashes, resulting in an immediate high drug concentration on oral surfaces, followed by its rapid clearance by salivary flow and swallowing. Approximately 25% of TR dose is retained in the oral cavity immediately after brushing, with a clearance half-life of 20 min (Marsh, 2003). However, sustained delivery of TR is crucial to obtain complete eradication of *Streptococcus mutans*, a common causative of caries and other dental diseases (Jug, Kosalec, Maestrelli, & Mura, 2011). Thus, to obtain effective TR levels in the oral cavity over an extended period of time, there is the need of developing a suitable formulation which would allow both a prolonged *in situ* residence and a controlled drug release.

The design of such a formulation for a poorly soluble drugs such as TR is a challenging issue, especially taking into account the limited amount of saliva present in the mouth, which serves as a dissolution medium (Azarmi, Roa, & Löbenberg, 2007; Bruschi & Freitas, 2005). In this regard, cyclodextrins emerged as an effective tool for increasing drug release of sparingly soluble drugs from different polymeric matrices (Bibby, Davies, & Tucker, 2000; Cappello et al., 2006; Mura et al., 2010). Water-soluble polymeric β-cyclodextrin derivatives often show superior solubilizing and complexing ability towards different guest molecules compared to parent β-cyclodextrin (Jug, Maestrelli, Bragagni, & Mura, 2010; Jug

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et al., 2011; Layre, Gosselet, Renard, Sebille, & Amiel, 2002; Volkova et al., 1999), and, moreover, exhibited a better potential to obtain sustained drug release profiles from different polymeric matrices (Arakawa, Kawakarni, Yamashita, & Hashida, 2005; Xin et al., 2010; Zugasti et al., 2009).

Pectins, a mixture of polysaccharides containing α-(1,4)-linked D-galacturonic acid residues, are widely used as excipients in food and pharmaceutical fields mainly as gelling agent (Thakur, Singh, & Honda, 1997; Watts & Smith, 2009). In nature, carboxyl groups of galacturonic acid are partially esterified with methanol; depending on the methylation degree, pectins are classified as high (<50%) and low (>50%) methoxyl pectins. De-esterification with ammonia will generate low methoxy amidated pectin (AMP). Such pectin derivatives, due to their better mucoadhesion properties, have shown good potential in nasal (Watts & Smith, 2009) and other mucosal drug delivery (Liu, Fishman, & Hicks, 2007).

Carbopol (CAR), an anionic polymer derivative of polyacrylic acid, is widely employed in pharmaceutical field due to its excellent mucoadhesive properties and low toxicity (Andrews, Laverty, & Jones, 2009). Moreover, it has been shown that blends of anionic and cationic polymers can be used for improving, by electrostatic interactions, the mechanical and physicochemical properties of polymeric matrices and their ability to control drug release rate (Saleem, Azharuddin, Ali, & Patil, 2010).

Based on all these premises, in this study we considered worthy of interest to develop a novel mucoadhesive buccal formulation of TR, by using AMP as matrix forming polymer of the patch, and exploiting cyclodextrin complexation for improving drug solubility and optimizing its release rate from the matrix. With this aim we investigated drug interactions with two different water-soluble polymeric β-cyclodextrin derivatives, comparing their effectiveness to that of parent β-cyclodextrin, in order to select the most suitable partner for TR. As for the polymeric matrix formulation, a series of patches containing AMP alone or in different (w/w) mixtures with CAR, were prepared and evaluated for swelling and erosion properties. Optimal matrix formulations were then loaded with selected TR-cyclodextrin complexes. A comprehensive characterization of the final formulations was then performed, by carefully simulating the conditions present on the buccal mucosa, in order to determine swelling, erosion and mucoadhesive properties of patches as well as the *in vitro* drug release kinetic. Changes on surface topology of the patches prior and after drug release were investigated by scanning electron microscopy. Finally, the *in vitro* antibacterial activity of the developed patch formulations on *S. mutans*, a common causative of dental plague and caries (Takahashi & Nyvad, 2008), was evaluated.

2. Materials and methods

2.1. Materials

Triclosan (TR) was kindly donated by Carlo Erba, Italy. β-Cyclodextrin (βCD; Kleptose 4PC) was a gift from Roquette, France. β-Cyclodextrin-epichlorohydrin polymer (EPIβCD, mean MW 4500) and anionic carboxymethylated β-cyclodextrin-epichlorohydrin polymer (CMEPIβCD, mean MW 5300, 4.7% free carboxylated groups) were purchased from Cyclolab Ltd., Hungary. The structures of cyclodextrins used are presented in Fig. 1. Mucoadhesive polymers used were low methoxy amidated pectin (AMP; Amid CF 020, Herbstreith & Fox, Germany), and Carbopol 71G NF (CAR, Lubrizol Anvanced Materials, Inc., Belgium). Simulated saliva solution was prepared by dissolving 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, 8.00 g NaCl in 1 l of distilled water and adjusting pH to 6.75 by the use of orthophosphoric acid. These components were obtained from Sigma (St. Louis, USA). All other chemicals and solvents were of analytical reagent grade.

2.2. Phase solubility studies

Phase solubility studies were performed by adding 50 mg of TR to 10 mL of simulated saliva containing increasing amounts of βCD (0–1.5%, w/v) or EPIβCD or CMEPIβCD (0–6%, w/v), in the presence or not of 1% (w/v) of AMP. Samples, in sealed glass containers, were sonicated 60 min in an ultrasonic bath (Eurosonic 44, Wilten Woltil, de Meern, The Netherlands) and then magnetically stirred at constant temperature (37 ± 0.5 °C) until complexation equilibrium was reached (72 h). An aliquot of the samples was centrifuged 20 min at 6000 rpm, filtered through 0.45 μm Millipore membrane filters, and spectrophotometrically assayed for drug content (1601 UV-VIS spectrophotometer, Shimadzu Italia s.r.l.) at 280.4 nm. Each experiment was performed in triplicate (coefficient of variation, CV < 5%). The apparent stability constants of TR-CD complexes were calculated from the slope of the straight line of phase solubility diagrams and TR solubility in the absence of CDs (s_0) (Higuchi & Connors, 1965).

2.3. Preparation of drug/cyclodextrin complexes

TR/cyclodextrin complexes were prepared by co-grinding the corresponding equimolar physical mixtures in a high-energy vibration micromill (Retsch, GmbH, Germany) at 24 Hz for 80 min and complex formation was verified as described previously (Jug et al., 2011).

2.4. Preparation of buccal patches

Drug-free patches were prepared by directly compressing AMP–CAR mixtures in different (w/w) ratios, at a compression force of 49,100 N for 5 s, using a hydraulic press with a flat faced punch. The accurately weighed components were sieved through 170 μm sieve, mixed 10 min in a Turbula mixer (Willy A. Backhofen Maschinenfabrik, Switzerland) and then compressed.

Drug-loaded patches were prepared by mixing TR, free or as co-ground complex with β-CD or EPI-βCD, with selected AMP–CAR mixtures, using the same procedure as described above. In the case of patches loaded with plain drug, an amount of lactose was added, corresponding to the amount of CD present in formulations with co-ground complexes. Mg-stearate (1%, w/w) was used as lubricant. The final dose of TR in all formulations was 4 mg. All patches had a diameter of 13 mm, mean thickness of 0.7 mm and weight of 120 mg.

2.5. Characterization of buccal patches

For erosion studies, patches were weighed (W_0) and exposed to simulated saliva thermostated at 37 °C for 4 h. After that, patches were removed from the simulated saliva, and dried in a circulating air oven thermostated at 40 °C until to constant mass (W_{4h}). Percentage of erosion was calculated according to Eq. (1):

$$\%\ \text{of erosion} = \left[\frac{W_0 - W_{4\,h}}{W_0}\right] \times 100 \quad (1)$$

The results are the mean of five separate experiments.

Solid-state interactions between polymeric components of the patch (AMP and CAR) were investigated by differential scanning calorimetry (DSC), using a Mettler TA 4000 Star^e apparatus equipped with a DSC 25 cell (Mettler Toledo, Switzerland). AMP, CAR and their mixtures in preselected (w/w) ratios, prior and after exposure to water followed by drying in a circulating air oven at 40 °C until a constant mass, were accurately weighed (2–5 mg, Mettler M3 Microbalance), placed in sealed aluminium pans with pierced lid and scanned at 10 °C min^{-1} over the 30–300 °C temperature range.