Carbohydrate Polymers 90 (2012) 1794–1803

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

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

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ARTICLE INFO

Article history:
Received 2 May 2012
Received in revised form 19 July 2012
Accepted 29 July 2012
Available online 3 August 2012

Keywords:
Triclosan
β-Cyclodextrin
Water soluble polymeric β-cyclodextrin
Low methoxy amidated pectin
Carbopol
Buccal delivery

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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http://dx.doi.org/10.1016/j.carbpol.2012.07.074

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.

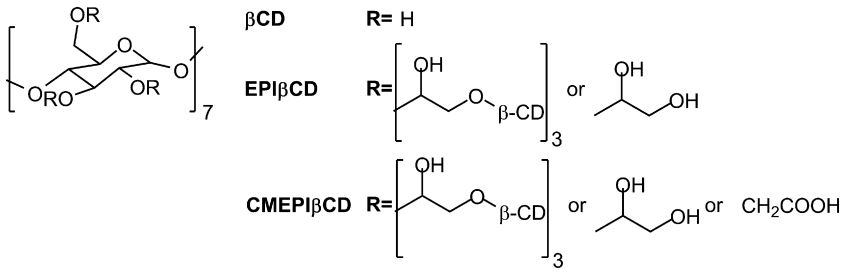

Fig. 1. Structures of βCD, EPIβCD and CMEPIβCD.

Swelling behaviour of the patches in simulated saliva solution at 37 °C was determined gravimetrically according to an experimental setup described by Bertram and Bodmeier (2006). Experiments were performed in triplicate for each sample and the swelling degree was calculated according to Eq. (2):

$$\text{swelling degree}\,(\%) = \left[\frac{W_t - W_0}{W_0}\right] \times 100 \qquad (2)$$

where W_0 and W_t are the weights of dry and swollen patches at predetermined time intervals.

Mucoadhesive properties of the patches were evaluated by measuring the *ex vivo* residence time using porcine buccal mucosa as a model substrate. The porcine buccal mucosa was obtained in a local slaughterhouse from freshly sacrificed 6-month-old animals weighing about 120 kg. The tissue was stored in a simulated saliva solution kept 4 °C, and transferred within 1 h to the laboratory where it was immediately used for experiment. A portion of the porcine buccal tissue (area = 4 cm^2) was fixed with cyanoacrylate glue on a glass support. Prepared patches were put on the porcine buccal tissue by applying a light pressure with a fingertip for 20 s. The supports were then immersed into beakers filled with the simulated saliva solution maintained at 37 °C, under a 150 rpm stirring rate to simulate the movement in the buccal cavity. The experiment was performed up to 8 h, measuring the time necessary for complete detachment of the patch from the mucosal surface (mean of five determinations).

To characterize the mucoadhesive strength of the different formulations, a tensile test was performed, using agar-mucin gel as mucoadhesive substrate. An aqueous solution of agar (1.5%, w/v) and mucin (2%, w/v) was poured on Petri dishes and left to gel at 4 °C for 3 h. Prior to the use, the gel was thermostated at room temperature. For the tensile test, patch samples were attached to a stainless steel support connected to a Sartorius BP 221S balance (Germany), while the Petri dish with the agar-mucin gel was mounted on a mobile support. The patch was brought in contact with the gel and left in contact 5 min. The detachment force was measured as a function of displacement, by lowering the mobile support at a constant rate of 2.5 mm min^{-1} until total patch separation was achieved. The total work of mucoadhesion (TWA) was calculated as the area under the force/distance curve. The measurement for each sample was repeated five times (coefficient of variation, CV < 15%).

Surface topology of patches, prior and after drug release test, was evaluated by scanning electron microscopy. The patches were fixed on a brass stub using a double-sided adhesive tape and observed using an environmental scanning electron microscope XL 30 ESEM FEG (Philips, Netherlands). Elemental analysis of specific structures on the patch surface was performed using energy-dispersive spectroscopy (EDS).

2.6. In vitro drug release studies

To mimic the conditions at the buccal mucosa surface (low aqueous liquid volume and presence of an unstirred water layer), a modification of the procedure described by Mizrahi and Domb (2007) was used. The patch was fixed at the bottom of a 25 mL beaker and exposed to 5 mL of simulated saliva solution. The beaker was sealed with Parafilm, to avoid medium evaporation and mimic the humid environment present in the mouth. Every 10 min the complete volume of dissolution medium was collected and replaced with 5 mL of the fresh one, thermostated at 37 °C. Drug assay was performed spectrophotometrically as described in Section 2.1.

2.7. In vitro microbiological studies

S. mutans was used to evaluate the antibacterial efficacy of prepared patches. Inocula were prepared by dispersing a fresh culture of *S. mutans* ATCC 33402, cultured 48 h at 37 °C on tryptic-soy agar plates (Merck, Germany) containing 5% (v/v) of horse blood in pH 7.4 phosphate buffer up to 0.5 McFarland units (1.5×10^8 CFU mL^{-1}). The McFarland units were adjusted using nephelometer (bioMerieux, France). The prepared inoculum was applied on the surface of Müller–Hinton agar plates. Holes (d = 6 mm) were made at the surface of the inoculated agars, using sterile stainless steel cylinders. To determine the antibacterial efficacy of patches formulations, 50 μL aliquots, withdrawn at selected times during *in vitro* release studies (10, 20, 40, 60, 80, 100, 120 and 240 min), were added into each hole. Plates were incubated at 4 °C for 1 h, followed by incubation at 37 °C for 48 h under aerobic conditions. Results were expressed as diameters of growth inhibition zones (mean of three experiments).

To further evaluate the patch antibacterial activity, a modified tablet diffusion method in agar was used. The selected patch formulation was placed directly on the surface of the inoculated Müller–Hinton agar and the plate was incubated as described above. The antibacterial efficacy of the formulation, expressed as a mean diameter of growth inhibition zone, was compared to positive controls (ampicilin discs/10 μg per disc) (BD-Sensy Disk™), and paper discs soaked into 5% (v/v) cetrimide solution (Cetavlon®, Veterina Ltd., Croatia).

2.8. In vitro permeation studies

For *in vitro* permeation studies, the slack connective and adipose tissues were removed from the fresh porcine buccal mucosa (obtained as described in Section 2.5) by using surgical scissors and scalpels. Then, the tissues were soaked in phosphate buffer saline (pH 7.4; PBS) at 60 °C for 1 min, in order to obtain the buccal epithelium, as described by Consuelo, Falson, Guy, and Jacques (2007). The obtained tissues were fixed between the donor and receptor chambers of a horizontal diffusion system equipped with 6 cells (Harvard Apparatus, UK), with the mucosal surface facing up. After buccal mucosa equilibration with PBS, the donor chamber was wetted with 500 μL of simulated saliva solution and loaded with the selected patches, while the receiver chamber was constantly perfused with PBS at 6 ml/h, using a peristaltic pump (Ismatec

SA, Labortechnic-Analytick, Switzerland). Fractions of the effluent were collected during 30 min intervals up to 4 h. The amount of the drug in the collected fractions was analysed spectrophotometrically as described in Section 2.2.

2.9. Statistical analysis

All values are expressed as mean ± SD of *n* separate experiments. Data were compared by one-way ANOVA, followed by Tukey multiple comparison test. The differences were considered statistically significant when $p < 0.05$. Calculations were performed using the GraphPad Prism program (GraphPad Software, Inc., San Diego, CA; www.graphpad.com).

3. Results and discussion

3.1. Phase solubility studies

In order to evaluate the effect of AMP presence on cyclodextrin complexing and solubilizing abilities towards TR, phase solubility studies with both parent βCD and its polymeric derivatives have been performed in the presence or not of a fixed amount (1%, w/v) of the polymer (Fig. 2). The phase solubility curve of TR in the presence of βCD can be classified as Bs type according to Higuchi and Connors (1965), indicative of the formation of an inclusion complex with limited aqueous solubility, in agreement with previously published results (Jug et al., 2011; Loftsson & Masson, 2004; Veiga, Merino, Cirri, Maestrelli, & Mura, 2005). On the contrary, TR solubility increased linearly with increasing EPIβCD or CMEPIβCD concentrations (Fig. 2), indicating the formation of soluble inclusion complexes in both cases. The stability constants of the TR inclusion complexes with EPIβCD and CMEPIβCD, calculated by taking the βCD repeating unit as their molecular weight (Mura, Faucci, Maestrelli, Furlanetto, & Pinzauti, 2002), were, respectively, 4.5 and 1.7 times higher than with parent βCD (Table 1). The solubilizing efficiency, defined as the ratio between TR solubility in the presence and absence of CD, followed the same rank (Table 1). The superior complexing and solubilizing efficiency of polymeric βCD derivatives can be attributed to their higher aqueous solubility than parent βCD, but also to their polymeric structure, which allowed an efficient cooperation of adjacent CD cavities for interaction with the drug (Harada, Furue, & Nozakura, 1987). Since no deviations from linearity were observed in phase solubility diagrams of TR with both polymeric βCDs, the formation of higher order complexes in the CD concentration range studied can be excluded. Also, photon correlation spectroscopy did not provide any evidence of micelle-like structures formation (data not shown), which would explain the high efficient TR solubilization obtained by polymeric CDs. Interestingly, anionic CMEPIβCD formed less stable complexes compared to the non-ionic EPIβCD ($p < 0.001$) and its solubilizing efficiency was 45.6% lower. Probably, the presence of charged carboxymethyl groups near to the CD cavity, reduced its interaction with hydrophobic TR molecules, resulting in lower solubilizing and complexing ability of this derivative towards the drug (Challa, Ahuja, Javed, & Khar, 2005).

The addition of 1% (w/v) AMP in simulated saliva increased TR solubility of about 8.9 times (Table 1, $p < 0.05$), indicating some drug–polymer interaction. Addition of AMP to complexation medium also affected TR complexation with all CD derivatives tested. In the case of βCD, the presence of AMP changed the phase solubility diagram from B_S type, typical of poorly soluble complexes, to A_L type, characteristic of readily soluble complexes. In the same time the polymer presence reduced the slope of the phase solubility curve (Fig. 2), causing a significant decrease of the complex stability constant compared to the system in the absence of AMP

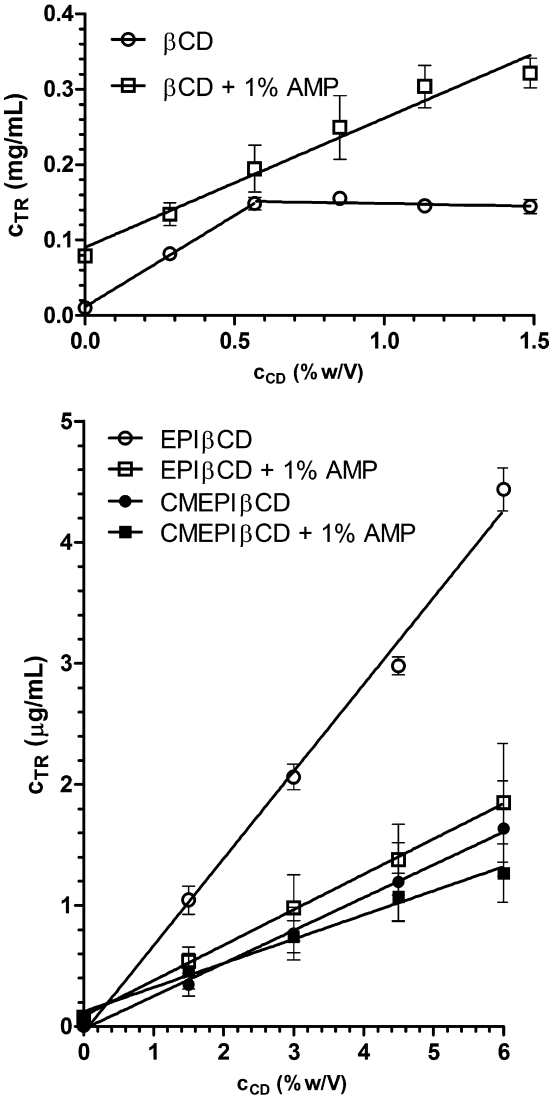

Fig. 2. Phase-solubility diagrams of TR with βCD, EPIβCD and CMEPIβCD in simulated saliva at 37 °C in the presence or not of 1% (w/v) AMP (mean ± SD).

(Table 1, $p < 0.05$). Such result excluded the possibility of ternary drug–CD–polymer complex formation, which is usually accompanied by an increase of the complex stability constant (Loftsson & Masson, 2004). On the other hand, a competitive effect between the drug and AMP for the inclusion in the CD cavity can be excluded, considering the structure and hydrophilicity of AMP. Probably, AMP interacted with βCD *via* hydrogen bonds or van der Waals forces, and this interaction reduced the accessibility of the drug to the CD central cavity due to sterical reasons, thus decreasing the complex stability. However, in the same time, the presence of AMP in the medium increased the solubilizing efficiency of βCD towards TR for 57.4% compared to that of βCD alone ($p < 0.001$). This finding may be primary related to the change of the phase solubility diagram from B_S to A_L type in the presence of polymer, which indicated that AMP acted as a solubilizer of the poorly soluble TR/βCD complex.

As in the case of βCD, the presence of AMP in the medium decreased the complexing ability of polymeric CDs towards the drug, causing a 22 and 10 folds reduction of the complex stability constants with EPIβCD and CMEPIβCD, respectively ($p < 0.001$). However, in contrast with that observed for βCD, AMP addition also decreased the solubilizing efficiency of EPIβCD and CMEPIβCD towards TR of 48.2% ($p < 0.001$) and 18.5% ($p < 0.05$) respectively.

Table 1
Apparent stability constants (K_s) of triclosan (TR) complexes with βCD and its polymeric derivatives in simulated saliva at 37 °C, in the presence or not of 1% (w/v) AMP, and relative solubilizing efficiency.

CD type	Without polymer			1% (w/v) AMP		
	TR solubility ($\mu g\,mL^{-1}$)	K_s (M^{-1})	Solubilizing efficiency[a]	TR solubility ($\mu g\,mL^{-1}$)	K_s (M^{-1})	Solubilizing efficiency[a]
βCD	8.5 ± 0.9	2525 ± 38	13.9	75.4 ± 1.5	283 ± 8	32.6
EPIβCD		11,452 ± 40	354.9		520 ± 16	186.8
CMEPIβCD		4213 ± 66	193.2		403 ± 20	157.4

[a] Ratio between TR solubility in simulated saliva in the presence (1.5 and 6%, w/v for parent βCD and polymeric derivatives, respectively) and absence of CDs at 37 °C.

This finding may be attributed to a different affinity of AMP for interaction with βCD and its polymeric derivatives. Nevertheless, in spite of the negative effect of AMP on complexing and solubilizing ability of EPIβCD against TR, this polymeric βCD derivative always showed superior solubilizing performance compared to the other CDs tested and it was considered as the derivative of choice for further formulation development.

3.2. Development of buccal patches

A preliminary step in the development of the new buccal patch formulation was focused on determination of the most suitable polymeric matrix composition of the patch, in order to ensure its integrity during the application, together with good mucoadhesive and controlled release properties. Buccal patches made only of AMP showed rather high erosion degree, which reached 80.15 ± 5.49% after 4 h exposure to simulated saliva (Fig. 3), mainly due to the good aqueous solubility of this polymer, and then they were not suitable for prolonged-release buccal application. In order to rationally select the most suitable polymeric matrix composition to use for drug loading, a series of drug-free buccal patches containing AMP and CAR mixtures in different w/w ratios were prepared and tested for erosion and swelling properties. The anionic polymer was purposely added to improve the mechanical properties of the AMP patches, by *in situ* polyelectrolyte complex formation, thus reducing the matrix erosion rate and, possibly, enhancing the integrity maintenance of the formulation during the application time, allowing a better control of the drug release rate (Saleem et al., 2010).

The erosion rate of the patches decreased significantly ($p < 0.001$) as a function of CAR content in the patch (Fig. 3). Such result may be related to the formation of a more coherent matrix network by interpenetration of AMP polymeric strands with cross-linked CAR chains, but also to the electrostatic interaction between oppositely charged polymers upon swelling (Saleem et al., 2010). Although the patches made of CAR alone showed a very low erosion level of only 2.88 ± 0.45% after 4 h exposure to simulated saliva, probably due to the cross-linked structure of this polymer, they were not suitable for buccal application due to dramatic increase in the patch diameter after swelling (>250%). AMP patches prepared with 20% (w/w) of CAR showed limited erosion (20.60 ± 3.83%) and a negligible change in their diameter, so they were selected as a basis for the further development of the patch formulation. When TR, free or as CD inclusion complex, was incorporated in such a matrix, its erosion rate did not change significantly ($p > 0.05$) (Fig. 3).

DSC analysis was performed to investigate the solid state interaction between AMP and CAR (Fig. 4). The thermal curve of AMP presented a wide endothermal effect, ranged between 70 and 110 °C, attributable to a dehydration process, followed by a sharp endothermic melting peak at 152.7 °C and finally by a decomposition process at temperatures higher than 230 °C. The DSC curve of CAR was a characteristic of an amorphous compound, showing a broad dehydration band between 50 and 100 °C and an intense endothermal event at temperatures above 180 °C, attributable to thermal decomposition of the polymer through sequential melting and decomposition (Sakeer, Al-Zein, Hassan, Martin, & Nokhodchi, 2010). The thermal curve of the AMP/CAR 80:20 (w/w) physical mixture, exhibited a small endothermic peak at 87.5 °C, which emerged from the large dehydration band, probably due to a thermally induced interaction between the components, followed by the melting peak of AMP and the characteristic decomposition pattern of CAR. This indicated a lack of interaction between components in the mixture. On the contrary, after its exposure to simulated saliva and consequent drying, only a wide endothermal effect peaked at about 105 °C was present, and no thermal events between 130 and 220 °C were observed. This change in DSC pattern in AMP/CAR mixture upon exposure to simulated saliva may be taken as a confirmation of *in situ* interaction between polymers.

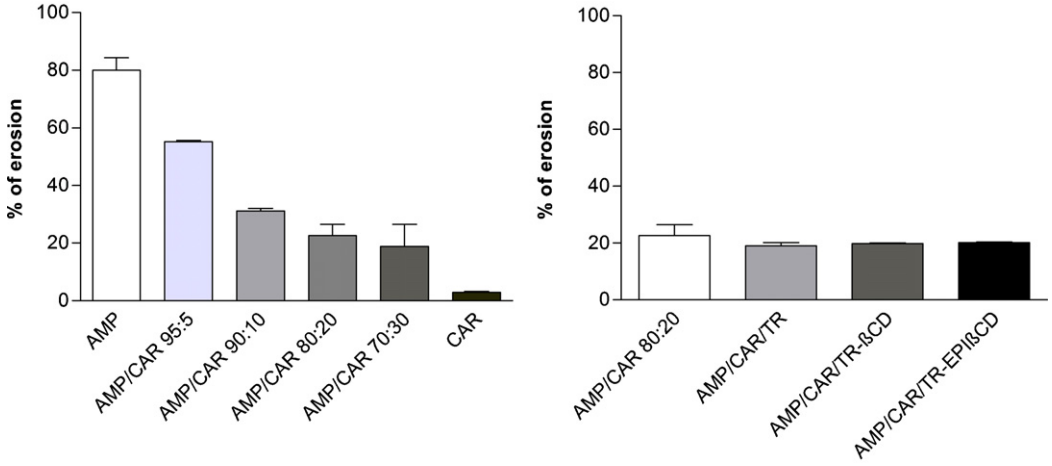

Fig. 3. Effect of polymeric matrix composition and drug loading on the erosion patterns of buccal patches in simulated saliva at 37 °C (mean ± SD; $n = 5$).

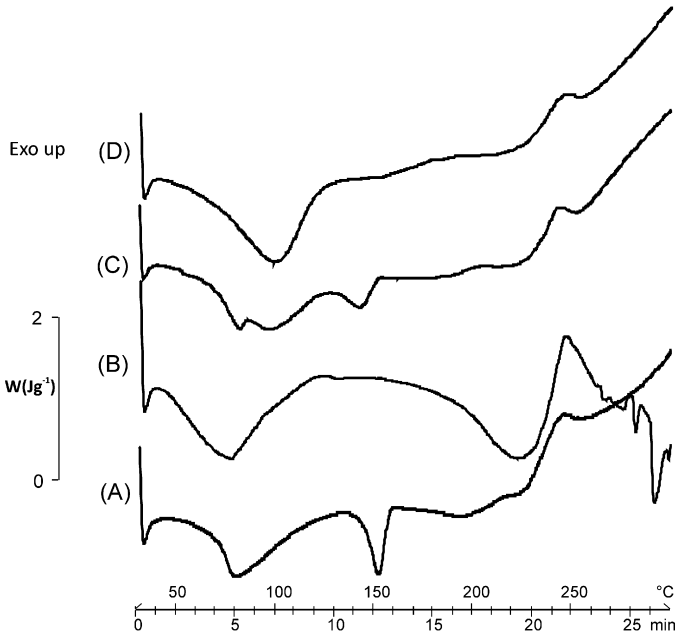

Fig. 4. DSC curves of pure AMP (A), CAR (B), their 80:20 (w/w) mixture before (C) and after (D) exposure to simulated saliva and consequent drying.

The degree of polymeric matrix hydration is one of the key factors determining the extent and rate of drug release from swellable matrices and it is also important for their mucoadhesion properties (Andrews et al., 2009; Miranda, Millan, & Caraballo, 2006). Therefore, the swelling behaviour of all patches was carefully investigated (Fig. 5). Patches prepared with only AMP showed a significantly ($p < 0.05$) superior swelling capacity compared to plain CAR patches, resulting in water binding capacity, after 4 h exposure to the simulated saliva solution, of 4.45 ± 0.24 and 3.85 ± 0.28 g of water per g of dry polymer, respectively. The ionization of AMP amino groups gave rise to an osmotic pressure inside the matrix, which facilitated the water uptake. In fact, it can be reasonably supposed that the osmotic pressure caused a relaxation of the polymeric network, followed by water absorption. The matrix swelling took place until the osmotic pressure equals the forces of cross-linking bonds that maintain the structure of the polymer network stable (Andrews et al., 2009). Although CAR is also ionized at the simulated saliva pH, the lower swelling degree of patches formulated with this polymer compared to AMP patches can be related to its cross-linked nature that restricted the free movement of polymer strands, restraining their hydration capability and also resulting in formation of a more coherent polymeric network.

The swelling degree of the AMP/CAR patches decreased with increasing the CAR content (Fig. 5). Patches formulated with AMP/CAR 70:30 (w/w) showed practically the same swelling behaviour as CAR samples. Such a finding might be attributed to the formation of more entangled system, due to interactions between the amino groups of AMP and the carboxylic groups of CAR, which exhibits a greater resistance to the osmotic pressure. In the same time, the interactions between the oppositely charged polymers reduced the number of ionisable groups inside the polymeric network, resulting in decrease of osmotic pressure formed. Interplay of these two factors resulted in slower swelling of AMP/CAR mixtures patches. Similar swelling behaviour was also observed in the case of alginate-chitosan mixed beads (Pasparakis & Bouropoulos, 2006) or of chitosan-carboxymethylcellulose inter-polymer complex matrices (Gomez-Burgaz, Garcia-Ochoa, & Torrado-Santiago, 2008).

The addition of plain TR to the selected AMP/CAR 80:20 (w/w) patch had no effect on the matrix swelling ($p > 0.05$), while when the drug was loaded as CD complex, a slight decrease in patch swelling was observed (Fig. 5). This result might be related to the competition between CD and polymer strands for available water molecules for hydration (Jug, Bećirević-Laćan, & Bengez, 2009).

3.3. Characterization of selected patch formulations: mucoadhesion and in vitro release studies

The mucoadhesion properties of the selected buccal patch matrix formulation (AMP/CAR 80:20, w/w) were determined by measuring the *ex vivo* residence time on the porcine buccal mucosa used as model substrate. Under the given conditions, the patch detachment from the mucosa was not observed up to 8 h of the experiment, regardless the patch composition (drug-free or loaded with TR, TR-βCD or TR-EPIβCD complex; Fig. 6A). In order to get a deeper insight into effects that TR and CDs used may have on the mucoadhesion strength of the patches, the total work of adhesion (TWA) required to break the adhesive bond

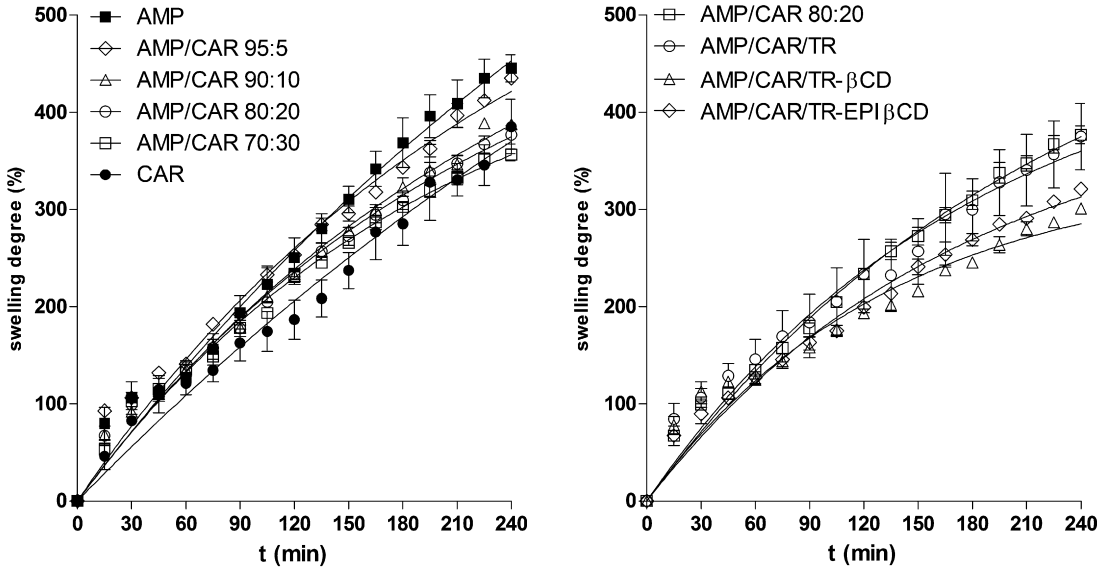

Fig. 5. Effect of polymeric matrix composition and drug loading on the swelling patterns of buccal patches in simulated saliva at 37 °C (mean ± SD; $n = 5$).

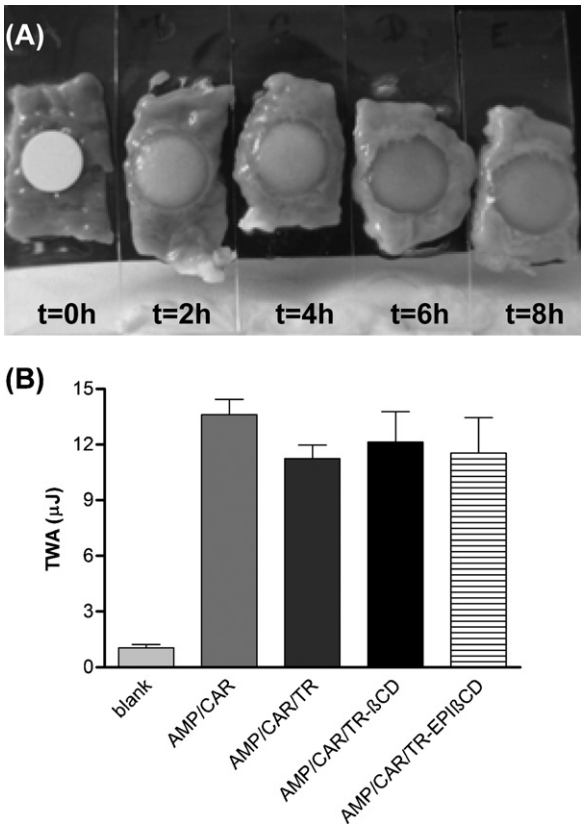

Fig. 6. Mucoadhesion properties of AMP/CAR 80:20 (w/w) buccal patches evaluated as *ex vivo* residence time on excised porcine buccal mucosa (A), and as *in vitro* mucoadhesion strength (B), expressed as total work of adhesion (TWA) on agar-mucin gel substrate (mean ± SD; $n = 9$).

with the agar-mucine substrate has been measured. TWA values of AMP/CAR patches were compared to those of an inert lactose support, used as blank control (Fig. 6B). All AMP/CAR patches showed significantly higher TWA values compared to lactose control ($p < 0.001$), confirming their good mucoadhesion power, in agreement with previously published results (Thakur et al., 1997; Thirawong, Nunthanid, Puttipipatkhachorn, & Siriamornsak, 2007) and with results obtained by *ex vivo* residence time measurements. Although drug loading into the patches as CD complex somewhat reduced their swelling properties (Fig. 5), it had no significant impact on the formulations mucoadhesion power ($p > 0.05$). Such a finding might be explained with the fact that the mucoadhesion is a complex interfacial phenomenon (Andrews et al., 2009), and thus the swelling properties of the whole matrix had a minor impact on the overall mucoadhesion process. *Ex vivo* residence time measurements clearly demonstrated that, after application to the buccal mucosa, the patches prepared would be able to remain at the application site for a prolonged time period.

In vitro release profiles of TR from the selected buccal patch matrix formulation are presented in Fig. 7. The release rate from patches loaded with pure drug was very slow and followed a zero order kinetic ($k_{TR} = 1.09 \pm 0.08\ \mu g\ min^{-1}$; $r^2 = 0.9937$). Incorporation of TR as complex with both βCD and EPIβCD increased significantly ($p < 0.001$) the drug release rate from the patch, but the release kinetic model was dependent on the type of CD used. Patches loaded with the βCD complex showed a two-phase zero-order release pattern, with a slow initial phase up to 60 min ($k_{\beta CD\ 0-60} = 1.46 \pm 0.11\ \mu g\ min^{-1}$; $r^2 = 0.9711$), followed by a second faster release phase ($k_{\beta CD\ 60-240} = 4.46 \pm 0.08\ \mu g\ min^{-1}$;

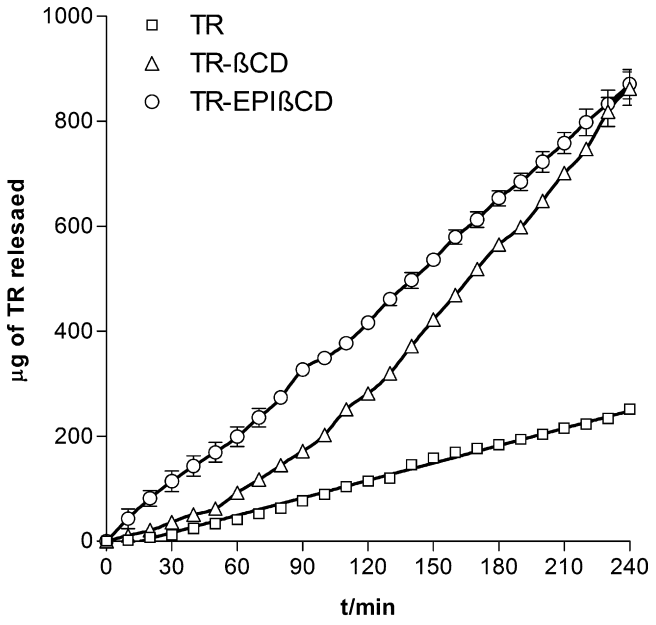

Fig. 7. *In vitro* release profiles of TR from AMP/CAR 80:20 (w/w) patches in simulated saliva at 37 °C (mean ± SD, $n = 5$).

$r^2 = 0.9943$). On the other hand, drug release from patches containing the polymeric βCD derivative followed a zero-order kinetic ($k_{EPI\beta CD} = 3.36 \pm 0.03\ \mu g\ min^{-1}$; $r^2 = 0.9990$). However, in spite of the different release kinetic, the amount of drug released after 240 min was practically the same ($p > 0.05$). The different release patterns from patches formulated with parent βCD or its polymeric derivative might be related to the different solubility of their complexes with TR, but also to the difference in their molecular dimensions.

In the case of the patch loaded with the TR-βCD complex, in the first phase of release test, due to the low solubility of the complex and the limited amount of water in the matrix, the dissolution of the complex was the limiting step controlling the overall drug release rate (Fig. 7). As the patch swelled and the amount of water in the matrix passed a critical level, the low solubility of TR-βCD complex was no more the step limiting the release rate. Also, interaction between AMP and the complex, as observed in phase solubility studies, additionally increased the solubility of the complex, thus contributing to the faster TR release rate in the second phase. Furthermore, the molecular weight of βCD is lower than that of its polymeric derivative, allowing a faster diffusion of the dissolved complex through the swollen polymeric matrix. On the other hand, polymeric βCD showed superior solubilizing efficiency toward TR (Fig. 2), and the dissolution of TR-EPIβCD complex in simulated saliva was much faster compared to that of TR-βCD complex (Jug et al., 2011). Thus, the release rate from patches loaded with the TR-EPIβCD complex was less sensitive to the matrix swelling degree, resulting in a quicker drug release at a constant rate. The absence of burst effect, in spite the high solubility of TR-EPIβCD complex, might be related to the high molecular weight of polymeric βCD (MW 4500 g mol^{-1}), which restricted the diffusion of the complex across the swollen polymeric matrix. In such system, the complex dissociation controlled the overall release kinetic, resulting in a zero-order release profile.

To gain a deeper insight into phenomena that might occur during drug release, environmental electron microscopy was used to analyse the microstructure and surface topography of the patches prior and after 60 min of release test (Fig. 8). Detailed examination of the surface of all patches prior the release test revealed the presence of cracks, formed due to the analysis performance under high

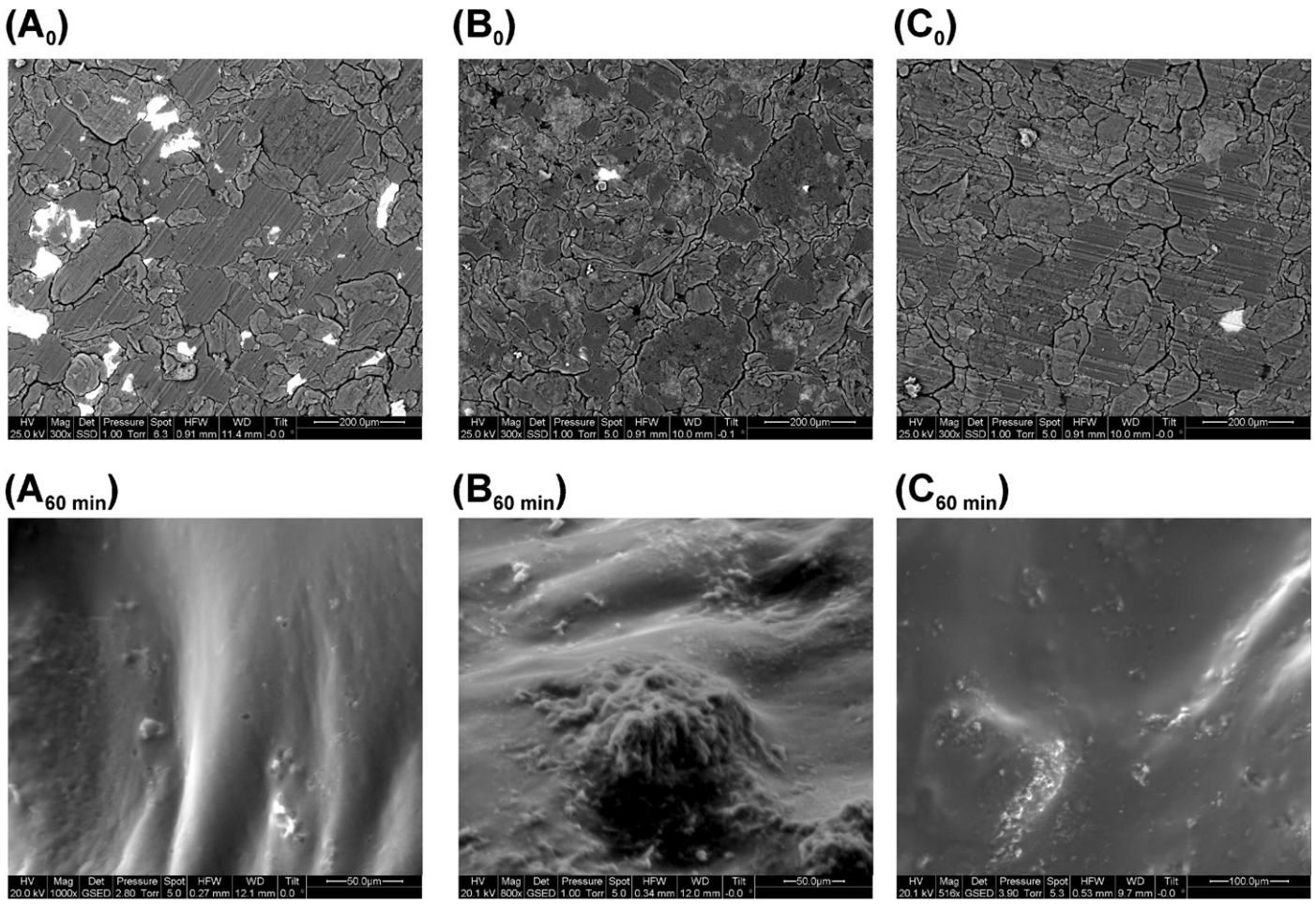

Fig. 8. ESEM micrographs of the AMP/CAR 80:20 (w/w) patches loaded with TR (A), TR-βCD (B) or TR-EPIβCD complexes prior and after 60 min of release test in simulated saliva at 37 °C.

vacuum. Elemental analysis carried out by energy-dispersive spectroscopy (EDS) showed that the bright zones that might be observed in all dry samples contained high amount of chlorine, while its content was very low in darker areas. Since TR is the only compound in the formulation containing chlorine in its structure, the bright zones at the surface of the patches can correspond to drug rich areas. After 60 min of release test, patches loaded with plain TR (Fig. 8$A_{60\,min}$) showed a smooth surface with small holes, probably formed as a consequence of lactose dissolution, which might facilitate the water intake into the matrix. On the contrary, patches loaded with TR-βCD complex showed the presence of wrinkled formations emerging from a smooth background (Fig. 8$B_{60\,min}$). EDS analysis showed that such formations are located only in drug rich areas. It might be assumed that interaction between TR-βCD complex and AMP caused some changes in the matrix swelling at microscopic level, not detectable during the swelling studies. Probably, this interaction caused small eruptions that additionally facilitated TR release from the matrix, thus contributing to the faster drug release observed in the second phase of release test. Interestingly, such particular formations were not observed in patches loaded with TR-EPIβCD complex, which showed an overall smooth surface.

3.4. Antimicrobial efficiency evaluation of the patches

The efficiency of the developed buccal patches against *S. mutans*, a Gram-positive bacterium involved in pathogenesis of caries and other periodontal diseases (Catuogno & Jones, 2003), was assessed by measuring the zone of inhibition obtained after addition of aliquots of dissolution medium collected during the *in vitro* release test to the bacterium cultures on agar plates (Fig. 9). The amount of TR released in the first 60 min was not sufficient to exhibit any antibacterial effect against *S. mutans*. After 80 min, only patches containing TR-CD complexes showed an antimicrobial effect, while those loaded with plain TR were still ineffective ($p < 0.001$). At all other time points both formulations loaded with TR-CD complexes

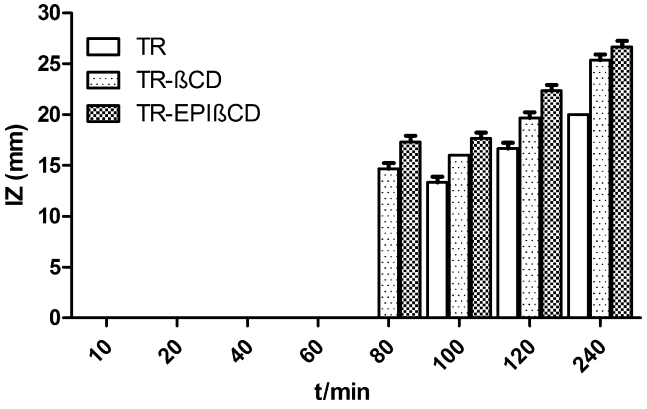

Fig. 9. Antibacterial activity of aliquots collected at given times of *in vitro* release test from patches loaded with pure drug (TR) and its complexes with βCD and EPIβCD, expressed as diameters of inhibition zones (IZ) of growth of *Streptococcus mutans* cultures on the agar plates.

showed superior antibacterial activity against *S. mutans* than those with TR alone ($p < 0.001$), clearly confirming their better therapeutic efficacy, and demonstrating the importance of CD complexation in improving the formulation performance. Moreover, the antibacterial activity of TR-EPIβCD-loaded formulation was significantly higher than that with TR-βCD complex ($p < 0.05$), in agreement with the results of *in vitro* release test (Fig. 7).

To confirm the high therapeutic efficacy of the patch loaded with TR-EPIβCD, another antibacterial test was performed, by placing the patch formulation directly on the inoculated agar plate and comparing the observed inhibition zone with that given by positive controls, consisting in ampicilin discs (10 μg per disc) and paper disc soaked into 5% (v/v) cetavlon solution. To test the possible contribution of the polymers used for patch formulation to the effect against *S. mutans*, drug-free AMP/CAR 80:20 (w/w) patches were also tested. In this kind of test, the amount of dissolution medium was highly restricted, which simulated well with the actual *in vivo* situation in the mouth. Under such experimental conditions, TR-EPIβCD loaded patch exhibited an inhibition zone (IZ) with a diameter of 51.0 ± 1.3 mm, showing an antibacterial effect against *S. mutans* similar to that of ampicilin control (IZ = 48.1 ± 5.3 mm, $p > 0.05$) and better than cetavlon control (IZ = 36.8 ± 2.7 mm, $p < 0.01$). The results clearly confirmed the good therapeutic potential of the developed patch formulation in eradication of *S. mutans* from the oral cavity. Under the same conditions, polymers used for patch formulation did not show any antibacterial activity against *S. mutans* (IZ = 0 mm).

3.5. In vitro permeability of triclosan from the patches

Permeation experiments were performed only on AMP/CAR patches loaded with TR-EPIβCD complex, owing to their superior drug release and antimicrobial properties compared to other examined formulations. However, the drug concentration in all fractions of the receptor medium collected during the experiment was below the detection and quantification limits (LOD and LOQ, respectively) of the spectrophotometric method. Taking into account that LOD of the method was 0.39 μg/ml (*i.e.* 0.009% of the applied drug dose), while LOQ was 1.3 μg/ml (*i.e.* 0.033% of the applied drug dose), it might be rationally presumed that this patch formulation would provide only local delivery of triclosan to the oral cavity, while transbuccal absorption would be negligible.

4. Conclusion

Solubilizing and complexing abilities of tested CDs towards TR followed the rank βCD < CMEPIβCD < EPIβCD, clearly demonstrating the superior efficiency of polymeric derivatives as complexing agents for the drug. AMP, used for patch formulation, affected CD complexation of TR, increasing the solubilizing efficiency of parent βCD, by acting as a solubilizer of the poorly soluble TR/βCD complex. On the contrary, in case of polymeric CD derivatives, AMP decreased solubilizing and complexing affinity of both CMEPIβCD and EPIβCD. However, despite such a negative effect, EPIβCD retained superior solubilizing efficiency towards TR, compared to the other tested CDs. Preliminary experiment showed that patch formulations containing only AMP were unable to assure prolonged drug release conditions, due to their high erosion rate. On the contrary, AMP/CAR 80:20 (w/w) mixtures showed optimal swelling and erosion properties, due to *in situ* inter-polymer complex formation, and thus they were selected as the polymeric matrix base for the buccal patch development. Loading of the drug, free or as CD complex did not change significantly the good mucoadhesive properties of patch formulations. CD complexation significantly enhanced the *in vitro* drug release rate from buccal patches. However, in the case of TR/βCD complex, due to its limited aqueous solubility, the drug release rate was sensitive upon the hydration degree of the matrix, showing a biphasic release profile. Oppositely, the drug release rate from patches loaded with TR/EPIβCD complex was almost constant, followed a zero-order release kinetic, which is a desirable property for a controlled release formulation. Microbiological study of the formulated patches on *S. mutans* showed faster onset and more pronounced antibacterial action of patches loaded with both TR-CD complexes, with respect to those containing the plain drug, further demonstrating the actual effectiveness of CD complexation in improving the drug therapeutic efficacy. Moreover, EPIβCD containing formulations showed superior performance than patches loaded with TR/βCD complex. The antibacterial efficacy of patches formulated with TR/EPIβCD complex was comparable to that of ampicilin control, clearly demonstrating their good therapeutic potential in eradication of *S. mutans* from the oral cavity. *In vitro* permeation studies indicated that transbuccal drug absorption was negligible, thus additionally confirming the suitability of the developed patch formulation for local triclosan delivery to the oral cavity.

Acknowledgments

Financial support from MIUR and a personal Post doctoral grant to M. Jug from the National Foundation for Science, Higher Education and Technological Development of the Republic of Croatia are greatly acknowledged.

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