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ORIGINAL ARTICLE

Native and polymeric β-cyclodextrins in performance improvement of chitosan films aimed for buccal delivery of poorly soluble drugs

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Abstract β-Cyclodextrin (βCD) and its soluble polymeric derivative (EPIβCD) were used to improve the effectiveness of chitosan-based bucco-adhesive film formulations containing bupivacaine hydrochloride and triclosan as poorly-soluble model drugs. The film formulations were characterized in terms of swelling, mucoadhesion and in vitro drug release, while possible interactions between the components were investigated by DSC and FTIR analyses. For both drugs EPIβCD showed a higher solubilizing efficiency than βCD; however cyclodextrin effectiveness in improving the release rate from film formulations was influenced by their different interactions with chitosan. Free βCD acted as a channelling agent, favouring the film swelling, while EPIβCD due to interaction with chitosan caused an opposite effect. βCD was the optimal partner for bupivacaine-loaded films in terms of film swelling, mucoadhesion and drug release. Contrariwise, EPIβCD was the best partner for triclosan-loaded films, allowing the highest drug release rate increase, due to its higher solubilizing ability with respect to βCD. Addition of the suitable cyclodextrin enabled formulation of buccal films with suitable drug release properties.

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Introduction

The efficiency of conventional dosage forms for local delivery of active agents to the oral cavity is often hindered by the fast clearance of applied formulation, resulting in limited duration of drug action and need of repeated administrations. On the contrary, flexible and elastic mucoadhesive films are more effective, providing localized and prolonged drug release and presenting a protective role for the wounds on the oral mucosa [1].

Numerous natural and synthetic polymers have been studied as potential candidates for buccal film preparation. Among them, chitosan (CH), the N-deacetylated product of the polysaccharide chitin, attracted widespread attention due to its appealing characteristics such as biodegradability, biocompatibility, low toxicity, and relative low cost [2–6]. From a technological point of view, CH is an effective carrier for sustained drug release, possessing excellent film-forming properties [7]. Moreover, CH has some antibacterial properties, and it is a promising mucoadhesive material at physiological pH values [8, 9]. Finally, CH enhances the function of inflammatory cells, promoting their infiltration and pronouncing granulation phase of wound healing [10]. Therefore, CH based film formulations could be even beneficial in accelerating the wound healing at the oral mucosa.

Drug release from CH films has to be properly modulated, in order to obtain fast onset and prolonged duration of pharmacological action [11]. Due to the limited amount

of dissolution medium present in the mouth (1–2 mL of saliva), achievement of such goals may be especially challenging for drugs with low aqueous solubility. In order to overcome this limitation, the use of cyclodextrins, cyclic oligosaccharides consisting of 6, 7 or 8 α-1,4 linked glucopiranose units, may provide a useful approach. Because of their bioadaptability and multifunctional characteristics, cyclodextrins are able to improve the undesirable properties of drugs intended for various administration routes by inclusion complex formation [12]. Furthermore, incorporation of cyclodextrins into polymeric drug delivery systems results in a greater degree of drug release control [13]. With this purpose, we have evaluated the usefulness of β-cyclodextrin (βCD) and its soluble epichlorohydrin polymer derivative (EPIβCD) in the development of CH-based films aimed for local drug delivery into the oral cavity. The sparingly soluble local anaesthetic bupivacaine hydrochloride (BVP) and triclosan (TR), an antimicrobial compound practically insoluble in water, were used as model drugs. The buccal application of mucoadhesive films containing a local anaesthetic such as BVP may be beneficial in providing sustained pain control in the oral cavity [11], while TR loaded formulations may be suitable for prevention and treatment of dental diseases such as caries and gingivitis [14].

The rationale in development of a film formulation based on the combined use of CH and cyclodextrin would be to simultaneously exploit benefits connected with their inherent properties, namely the good mucoadhesion and film-forming ability of the first and the solubilizing and complexing power of the second one, in order to obtain a sustained local delivery of the poorly soluble drug. Since both CH and the selected cyclodextrins are considered poorly effective as buccal mucosa penetration enhancers [15], limited transmucosal drug absorption can be reasonably presumed from such formulation.

A particular focus of the present study was to evaluate and compare the effectiveness of two different cyclodextrins, i.e. EPIβCD and βCD, taking into account their different physicochemical properties, as well the superior complexing and solubilizing efficiency of the polymeric derivative than the parent one towards BVP and TR [16, 17]. Moreover, incorporation of cyclodextrins into polymeric films would affect not only the drug release rate, but also other important biopharmaceutical properties of the formulation, such as swelling and mucoadhesion, that can have a direct impact on the effectiveness of the formulation in vivo [18]. Therefore, a further aim of this work was to investigate in depth the mechanisms by which cyclodextrins can influence the performance of a buccal film, in order to critically evaluate their utility in this type of formulation.

Materials and methods

Materials

Bupivacaine hydrochloride and TR were kindly donated by S.I.M.S. (Florence, Italy) and by Carlo Erba (Milan, Italy), respectively. The cyclodextrins used were β-cyclodextrin (βCD; MW1135; Kleptose 4PC, Roquette, Lestrem, France) and soluble β-cyclodextrin-epichlorohydrin polymer (EPIβCD; MW 3500–5500; Cyclolab R&D Ltd, Budapest, Hungary). Low molecular weight chitosan with deacetilation degree of 75–85% (CH; Sigma-Aldrich, Milan, Italy) was used as mucoadhesive and film forming component. Simulated saliva solution was prepared by dissolving 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, 8.00 g NaCl in 1000 mL of distilled water and adjusting pH value to 6.75 by the use of orthophosphoric acid. Agar and crude, porcine stomach mucine used for mucoadhesion test were obtained by Sigma-Aldrich (Milan, Italy). All others chemicals and solvents used in this study were of analytical reagent grade.

Preparation of drug/cyclodextrin interaction products

Solid drug/cyclodextrin interaction products were prepared by co-grinding the corresponding equimolar physical mixtures in a high-energy vibration micromill (Retsch, GmbH, Germany) at 24 Hz, as described previously [17, 19].

Preparation of drug-loaded films

CH films formulations loaded with BVP or TR and their respective interaction products with CDs were prepared by solvent casting method. In all formulations, the mass ratio of the drug (free or as CD complex) to polymer was 1:10 w/w.

For film preparation, a 2% (w/v) CH solution in 3% (v/v) aqueous acetic acid was prepared. A plasticizer, propylene glycol, was added to CH solution, in quantity equal to 20% of dry polymer mass. The obtained viscous solution was gently stirred for 1 h to allow complete hydration of the polymer, followed by addition of the drug or the corresponding amount of drug/CD complex. The prepared homogeneous dispersions were sonicated (Branson B1210E-DTH, Danbury, USA) to remove incorporated air bubbles and casted on Teflon coated Petri dishes. The films were dried in a hot air circulating oven at 45 °C until a constant weight was obtained. Film samples were wrapped in aluminium foil and stored in a desiccator at 25 ± 1 °C and 60 ± 5% relative humidity until further investigations.

To investigate the possible CH/drug and CH/CDs solid-state interactions, as well as their influence on the film

properties, film samples were prepared according to the above described procedure by omitting the plasticizer and the CD (CH/BVP and CH/TR films) or the plasticizer and the drug (CH/βCD and CH/EPIβCD films) from the formulations.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry curves were recorded using a Mettler TA 4000 Star^e apparatus equipped with a DSC 25 cell (Mettler Toledo, Greifensee, Switzerland). The instrument was calibrated with indium and zinc prior to analysis of samples under static air atmosphere. Accurately weighed samples (2–5 mg, Mettler M3 Microbalance) were placed in sealed aluminium pans with pierced lid and scanned at a heating rate of 10 °C min^{-1} over the temperature range of 30–300 °C in case of BVP and 30–200 °C in case of TR. The relative degree of drug crystallinity (*RDC*) in the samples was calculated according to Eq. 1:

$$RDC = \frac{\Delta H_{\text{sample}}}{\Delta H_{\text{drug}}} \times 100\% \quad (1)$$

where ΔH_{sample} and ΔH_{drug} are the drug heat of fusion in the sample (normalised to the drug content) and in the pure drug, respectively. Measurements were carried out in triplicate and the relative standard deviation of crystallinity data was $\pm$ 4%.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded by a Perkin-Elmer Model 1600 spectrometer (Wellesley, USA). Samples were prepared by the potassium bromide disc method (3 mg sample in 297 mg KBr) and scanned in the 4000–400 cm^{-1} range at 2 cm^{-1} resolution.

Swelling studies

Swelling properties of the prepared film samples were investigated by measuring the dynamics of simulated saliva uptake according to the method described by Bertram and Bodmeier [20]. A sponge (5 $\times$ 6.5 $\times$ 3 cm^3, Santex GmbH, Wald-Michelbach, Germany) was fully soaked in simulated saliva and placed in a Petri dish filled with the same medium to a 2 cm height, to keep the sponge soaked during the experiment. Round filter papers (d = 47 mm, Filtros Anoia SA, Barcelona, Spain) used as sample holders, were also soaked in the medium and positioned on the top of the sponge. The Petri dish was covered and the experimental set-up equilibrated 30 min at 37 °C, after which the wet filter papers were weighed (W_0). Accurately weighed film pieces of 18.84 cm^2 (W_1) were then placed on the wet filter papers. At predetermined times the devices were removed from the medium and weighed (W_2). The swelling index *(I),* as a measure of water uptake, was calculated according to Eq. 2:

$$I = \frac{W_2 - W_0}{W_1} \times 100 \quad (2)$$

In vitro drug release studies

To mimic the conditions present at the buccal mucosa surface, characterised by a low aqueous liquid volume and the presence of an unstirred water layer, a modification of the procedure described by Mizrahi and Domb [21] was used. A piece of the film sample, containing 20 mg of drug, was fixed at the bottom of a 25 mL beaker and exposed to 5 mL of simulated saliva solution, which served as dissolution medium. The beaker was sealed with Parafilm® to avoid evaporation of the medium and mimic the humid environment present in the mouth. Every 10 min the complete volume of the dissolution medium was collected and replaced with 5 mL of the fresh one, thermostated at 37 °C. The drug assay was performed spectrophotometrically (Shimadzu 1601 UV–VIS spectrophotometer, Shimadzu Italia S.R.L) at λ = 262.8 nm and λ = 280.4 nm for BVP and TR, respectively. Corresponding drug-free films were also subjected to the same release test, to quantify the contribution of the polymers to the UV-absorption. At each time point, the absorbance value of the placebo was subtracted from the value of the drug loaded film.

In vitro and ex vivo mucoadhesion studies

To evaluate the mucoadhesive properties of prepared buccal films, a tensile strength study was performed using agar-mucin gel as a mucoadhesive substrate [21]. The agar-mucin gel was prepared by dissolving the components in hot water, to obtain final agar and mucin concentrations of 1.5 and 2% w/v, respectively. The obtained solution was poured on Petri dishes and left to gel at 4 °C for 3 h. Prior the use, the agar-mucin gel was thermostated at room temperature. For the tensile test, film samples were cut into circles of 1 cm diameter and attached by cyanoacrylate glue to a stainless steel support connected to a precision balance (Sartorius BP 221S, Goettingen, Germany). The Petri dish with the agar-mucin gel was mounted on a mobile support. The film sample was brought in contact with the gel and left in contact 5 min. The detachment force was then measured as a function of displacement, by lowering the mobile support at a constant rate of 2.5 mm min^{-1} until total film 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%).

Ex vivo mucoadhesion time measurements of buccal films were performed using pig buccal mucosa as biological substrate, according to a previously developed procedure [22]. Briefly, a portion of the porcine buccal tissue (area = 4 cm^2) was fixed with cyanoacrylate glue on the internal side of a beaker. Each film was cut in portions of 3.0 cm^2; a side of each film was hydrated with 50 μL of simulated saliva solution and put on the porcine buccal tissue by applying a light pressure with the fingertip for 20 s. The beaker was then filled with the simulated saliva solution and maintained at 37 °C; after 2 min, a 150 rpm stirring rate was applied in order to simulate the movement in the buccal cavity and film adhesion was checked during 4 h, which is considered the usual duration for buccal drug delivery. The time necessary for complete detachment of the film from the mucosal surface was recorded (mean of five determinations).

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).

Results and discussion

Study of the interactions between drugs and chitosan

The possible solid-state interactions between BVP or TR and CH were investigated by DSC and FTIR analyses, since their existence may have a significant impact on swelling, mucoadhesion and drug release behaviour of the films.

The DSC curves of BVP, CH, their physical mixture and corresponding films are presented in Fig. 1. The thermal curve of BVP is characterised by a broad endothermic peak at 128.33 °C, due to transition of the monohydrate to the anhydrous form, followed by a sharp endothermic peak at 253.19 °C (ΔH = 93.4 J/g), due to melting of the anhydrous drug [19].

In the CH thermal curve (Fig. 1), beside the wide endothermic band corresponding to the moisture evaporation, no thermal events were observed up to 250 °C, where thermally induced degradation of the sample took place. No glass-transition temperature (T_g) was observed for the tested polymer, probably due to the moisture presence,

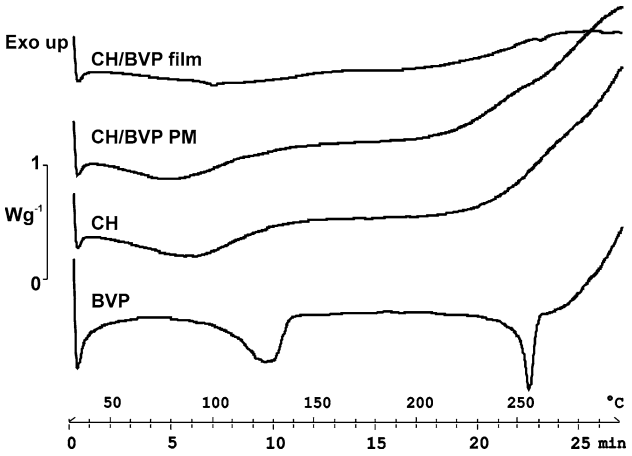

Fig. 1 DSC curves of BVP, CH, drug/polymer physical mixture (PM) in 1:10 w/w ratio and corresponding film sample

whose evaporation covered the glass-transition process. No endothermic effect corresponding to the drug melting was observed in polymer/drug physical mixtures, indicating a thermally-induced interaction between the components. The reported T_g value for CH, obtained by dynamic mechanical thermal analysis (DMTA), is between 140 and 150 °C [23]. Therefore, CH transition from glassy to rubbery state occurred below the drug melting temperature, leading to formation of almost molecular drug dispersion in the rubbery polymer, resulting in the disappearance of the drug fusion peak. The absence of additional thermal events or significant modifications in the DSC curve of the drug/polymer film with respect to the physical mixture, would be indicative of the lack of interactions between the components induced during film formation. Due to thermally-induced interaction between BVP and CH, the determination of the relative drug crystallinity degree (RDC) in the films by DSC analysis was not possible.

Different results were observed in case of TR. The thermal curve of pure drug (Fig. 2) presented a sharp endothermic fusion peak with onset temperature of 55.40 °C and fusion enthalpy of 81.93 J/g, which corresponds to the melting of anhydrous drug [17]. The TR fusion peak is still present in the DSC curve of 1:10 (w/w) TR/CH physical mixture, as well as in that of the corresponding film, but, in the second case, its intensity is significantly reduced compared to the physical mixture, and shifted to lower temperature (T_{onset} 52.12 °C vs. 53.65 °C), indicating some interaction between CH and TR occurred during film preparation. The corresponding drug RDC values were 98.22 ± 1.51% for the physical mixture, confirming the lack of thermally induced interaction, and 44.36 ± 1.65% for the film, indicating the presence of drug microcrystalline areas inside the polymeric matrix [24], as well as significant reduction of drug crystallinity degree probably attributable to the anti-nucleant action of the polymer during film preparation [25]. On the contrary,

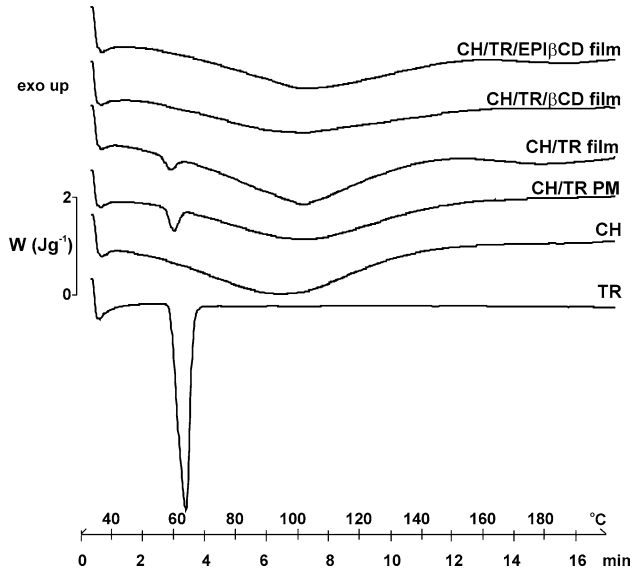

Fig. 2 DSC curves of TR, CH, drug/polymer physical mixture (PM) in 1:10 w/w ratio, corresponding film (CH/TR film) and film formulations loaded with TR/βCD or TR/EPIβCD complexes (CH/TR/βCD film and CH/TR/EPIβCD film, respectively)

the thermal curves of CH/TR/β-CD and CH/TR/EPI-β-CD films did not present the drug fusion peak, indicating its completely amorphous status inside the CH matrix, caused by TR complexation with cyclodextrins. Furthermore, these data confirmed that the film preparation procedure did not compromise the stability of TR-CD complexes.

To further investigate the existence of drug/polymer interactions, FTIR analysis of the plain components, drug/polymer physical mixtures and corresponding films was performed. The obtained FTIR spectra for CH/BVP and CH/TR samples are presented in Figs. 3 and 4, respectively.

The FTIR spectrum of BVP is characteristic of the monohydrate drug form [26], while the FTIR spectrum of CH/BVP physical mixture was the simple sum of the spectra of each component. Moreover, the absorption bands at 3512 cm^{-1} (O–H stretching) and 3246 cm^{-1} (stretching of the hydrogen bonded N–H) which are characteristic for monohydrate form of BVP as well as doublet at 1686 and 1650 cm^{-1} (stretching of intermolecularly hydrogen-bonded and free C=O), appeared at the same wavenumbers as in the pure BVP spectrum. All this indicates the absence of interactions between the components, confirming that the lack of the drug fusion peak in the corresponding DSC curve was simply the consequence of the thermally-induced CH/BVP interaction, as discussed above.

On the contrary, the comparison of FTIR spectra of drug/polymer physical mixture and corresponding film reveals some changes that could indicate the existence of some interaction induced during film formation. In particular, the shape and position of the peaks corresponding to the O–H/N–H vibrations have been changed: the CH band

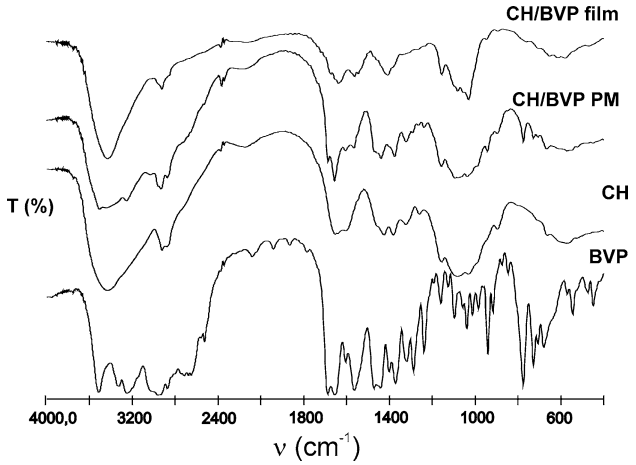

Fig. 3 FTIR spectra of BVP, CH, drug/polymer physical mixture (PM) in 1:10 w/w ratio and corresponding film

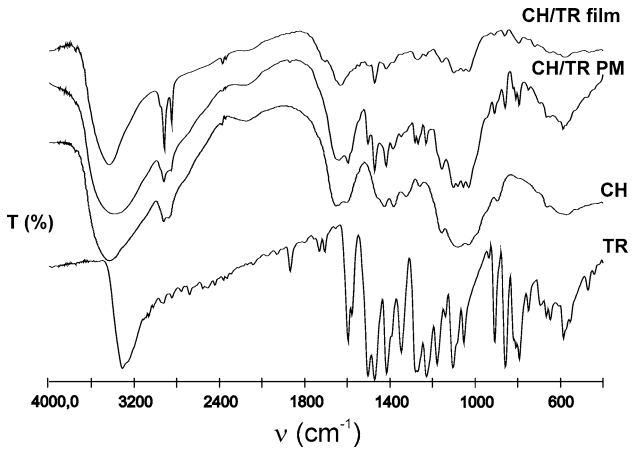

Fig. 4 FTIR spectra of TR, CH, drug/polymer physical mixture (PM) in 1:10 w/w ratio and corresponding film

was shifted from 3504 cm^{-1} (physical mixture) to 3447 cm^{-1} (film) and the characteristic doublet due to the C=O stretching vibrations of the amide carbonyl of the drug molecule almost disappeared. This may suggest that the drug-polymer interaction involved hydrogen bonds formation between the BVP amide carbonyl and the CH hydroxyl/amino groups.

Similar findings were observed for CH/TR samples. The FTIR spectrum of CH/TR physical mixture (Fig. 4) was the superposition of those of pure components, confirming the absence of solid state interaction. On the contrary, the characteristic drug absorption bands at 1596 and 1578 cm^{-1} (doublet), 1504 and 1471 cm^{-1} (doublet) and 1417 and 1391 cm^{-1} (doublet), that are the consequence of skeletal C–C vibrations inside the benzene ring, as well as several intense peaks in 1420–1330 cm^{-1} region (O–H in plane bending), are almost completely absent in the CH/TR film spectrum. This is indicative of interactions between the components brought about by the sample treatment, in

agreement with DSC results. Probably, such interactions restricted the vibrational motions of the benzene ring, thus resulting in disappearance of TR characteristic absorption bands. Since the absorption band of CH carbonyl group at 1654 cm^{-1} was significantly reduced, it may be speculated that hydrogen bonding formation between CH carbonyl groups and TR hydroxyl groups plays an important role in their interaction.

Study of the interactions between chitosan and cyclodextrins

Interactions between CDs and both the examined drugs, TR and BVP, have been previously investigated, and their effect on drug solubility and other physicochemical properties has been already evaluated in depth [16, 17, 19]. However, it is also important to check the possible existence of solid-state interactions between the other formulation components, i.e. CDs and CH. With this aim, DSC and FTIR analyses of their physical mixtures and films were performed and compared with those of pure components. The polymer/CD ratio was calculated according to the drug/polymer ratio 1:10 (w/w) and the equimolar drug/CD ratio used to prepare the films.

The DSC curves of CH, CDs and their physical mixtures and films are presented in Fig. 5a.

The comparison between DSC curves of CH/βCD physical mixture and corresponding film revealed some greater thermal stability of the film that may also reflect the existence of some CH/βCD interactions. In case of CH/EPIβCD physical mixture, an exothermic peak at 220.44 °C was observed, attributable to a thermally induced interaction between the components, followed by thermal degradation. This exothermic event was not observed in the corresponding film, where, as in the case of CH/βCD film, a greater thermal stability was noticed with respect to the physical mixture, indicative of possible interactions between the components.

FTIR analysis was used to investigate more in depth the possibility of interactions between CH and CDs. The FTIR spectra of plain components and their mixtures and corresponding films are presented in Fig. 5b. The comparison of FTIR spectra of CH/βCD and CH/EPIβCD physical mixtures and respective films revealed changes in position and shape of the absorption bands corresponding to the intermolecular hydrogen bonded O–H/N–H groups that may be attributed to hydrogen bonds reorganization due to polymer/CD interactions. Also, the band at 2923 cm^{-1}, corresponding to the C–H stretching was significantly reduced and its position varied, suggesting the existence of non-polar interactions. Moreover, the absorption bands in the 1221–915 cm^{-1} range, characteristic of the glucopiranose units, has been changed in the film formulation, further supporting the presence of non-polar interactions between the components. All these observations confirmed the assumption made on the basis of DSC results.

Swelling studies of film formulations

Swelling index was used as a measure of water uptake, representing the amount of simulated saliva adsorbed per

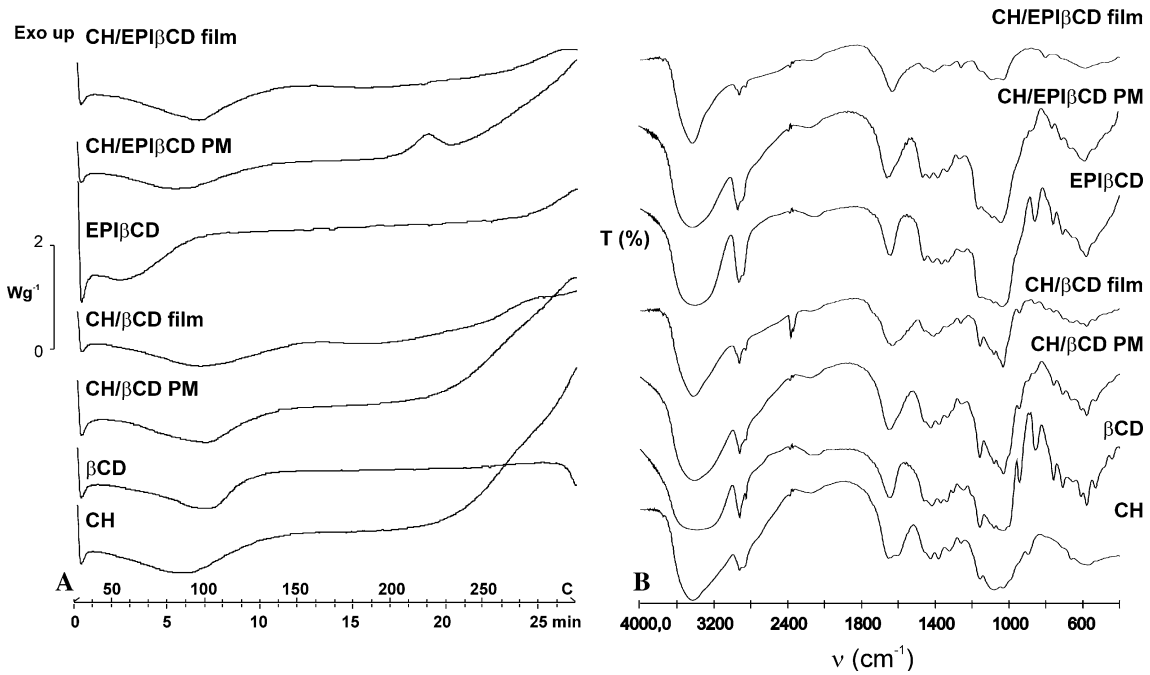

Fig. 5 DSC curves (**a**) and FTIR spectra (**b**) of chitosan and cyclodextrins investigated, their physical mixtures (PM) and films

gram of dry polymeric matrix, expressed as percentage. The swelling curves of the samples tested are shown in Fig. 6.

A relatively fast swelling of plain CH film was observed, resulting in an increase of the swelling index up to 471% in first 60 min, while after 240 min its value was 926%. CH is practically insoluble in simulated saliva (pH 6.75) and its amino groups are manly unionized (pKa value of CH is about 6.3–6.5 [27]), resulting in decreased hydration ability and reduced mobility of its polymeric chains, which remained in the coiled formation, restricting the water transport inside the matrix.

The presence of βCD significantly improved the swelling characteristics of CH films (Fig. 6a), resulting in a 90% increase of the swelling index in first 60 min ($p < 0.001$), followed by a plateau after 120 min, with a swelling index value of 1413% ($p < 0.001$). Probably, the hydrophilic βCD molecules, only loosely bonded to CH chains, acted as a channelling agent, thus promoting the matrix water uptake and polymer chains hydration.

Unexpectedly, EPIβCD gave rise to an opposite effect with respect to that obtained for βCD (Fig. 6a). In fact, polymeric βCD, despite of its higher aqueous solubility, decreased the water uptake of CH films, resulting in swelling index values at 60 and 240 min of only 329 and 661%, respectively ($p < 0.001$). This result could be directly related to the more intense interactions between this CD derivate and CH (as pointed out by DSC and FTIR studies), which limited their respective tendency to interact with water molecules, resulting in a decreased swelling power of the sample. Furthermore, these interactions could produce an entangled structure which mechanically hindered the mobility of the polymer chains, thus additionally reducing their hydration ability.

The swelling profiles of CH films loaded with plain BVP and its complexes with βCD and EPIβCD (Fig. 6b) were rather similar to those obtained for the corresponding drug-free films ($p > 0.05$). This finding indicated that the presence of a slightly soluble drug such as BVP within the CH film does not influence its swelling behaviour, while it is the type of CD used to complex the drug which plays the mayor role in determining the film swelling profile.

Different results were instead observed in TR-loaded CH films (Fig. 6c). TR, being a very hydrophobic molecule, strongly restricted the water transport inside the polymeric matrix. In fact, the swelling index of CH/TR film was only about 253% after 240 min, with a reduction of more than 70% with respect to the original CH film ($p < 0.001$). Furthermore, in this case, the positive influence of βCD on the swelling properties of CH films was completely absent, due to the limited aqueous solubility of the TR/βCD complex [17]. On the other hand, also the film loaded with TR/EPIβCD complex, in spite of its higher aqueous solubility with respect to the TR/βCD complex [17], showed a swelling behaviour similar to those of the other TR-loaded films, pointing to the prevalent effect of the presence of the strongly hydrophobic drug on the film swelling process.

In vitro drug release studies

The release profiles of BVP and TR from CH film formulations are presented in Fig. 7. All formulations retained their integrity during the whole release test and film dissolution was not observed. The release of BVP was characterised by a burst effect, resulting in the release of approximately 55% of the incorporated drug after 20 min of the experiment. This may be related to the geometry of the polymeric matrix, characterised by a large surface available for drug release, and to the relatively fast initial swelling process of CH films containing BVP. Such observed burst effect may be beneficial in rapidly

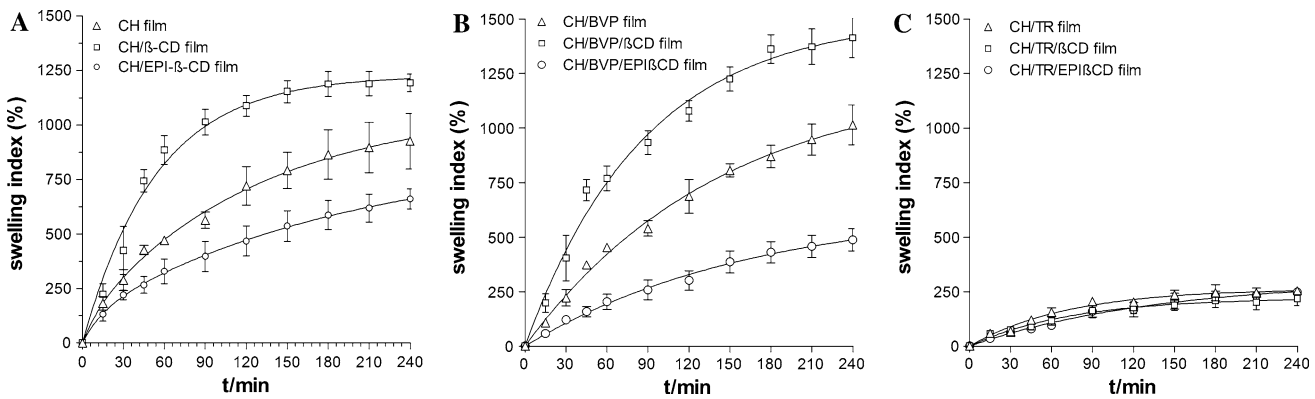

Fig. 6 Swelling profiles in simulated saliva at 37 °C of CH films (**a**), CH films loaded with BVP or its CD complexes (**b**) and CH films loaded with TR or its CD complexes (**c**; mean ± SD, $n = 5$)

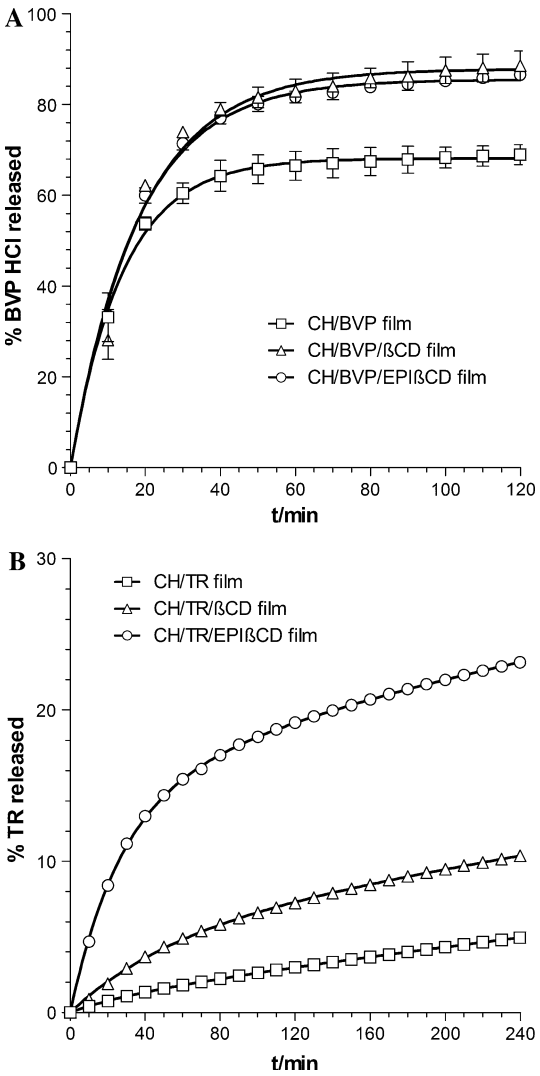

Fig. 7 *In vitro* release profiles of BVP (**a**) and TR (**b**) in simulated saliva at 37 °C from CH film formulations with and without cyclodextrins tested (mean ± SD, $n = 6$)

providing high drug concentration at the action site, ensuring fast onset of its pharmacological effect. After the initial fast phase, BVP release profile was characterised by the achievement of a plateau, corresponding to a release of approximately 70% of the incorporated drug. The incomplete release may be related to the limited swelling properties of CH film at the simulated saliva pH value, but also to the poor BVP solubility. Both CDs tested significantly increased ($p < 0.05$) BVP release from the film, in virtue of the improved drug solubility upon complexation, leading to almost complete drug release after 60 min of the experiment. Interestingly, although βCD and EPIβCD presented opposite effects on the swelling properties of CH films, the BVP release profile was practically the same ($p > 0.05$). This may be explained by the different solubilizing ability of the examined CDs towards BVP. In fact, it has been demonstrated that EPIβCD is 1.6 times more efficient than βCD in BVP solubilization [19]. Therefore, the higher solubilizing power of EPIβCD counterbalanced its negative influence on the film swelling. In the same way, the lower solubilizing efficacy of βCD was counterbalanced by its positive effect on the water uptake of CH matrix.

The in vitro release of TR from CH film (Fig. 7b), was slow and incomplete, resulting in a release of only 5% of incorporated drug after 4 h of the experiment. TR is practically insoluble in simulated saliva [17] and, it significantly reduced the water uptake of CH matrix, as demonstrated earlier. All this contributed to the observed low drug release rate, giving drug concentrations in simulated saliva that are probably below the level necessary to present any significant pharmacological effect. Incorporation of TR as complex with βCD slightly but significantly ($p < 0.05$) increased its in vitro release rate, resulting in a release of approximately 10% of the incorporated drug after 4 h of the experiments. As can be noticed, the drug release profile showed a biphasic pattern: after an initial exponential phase tooking place up to 90 min ($r^2 = 0.9937$), a zero order release kinetic ($r^2 = 0.9941$) was observed, which is a desirable property for a controlled release formulation. The CH films containing TR as complex with EPIβCD presented a significant improvement in drug release rate ($p < 0.01$) compared to both CH/TR and CH/TR/βCD films, enabling a release of approximately 25% of the incorporated drug at the end of the experiment. Also in this case a biphasic release profile was obtained, presenting the exponential phase up to first 60 min ($r^2 = 0.9985$), followed by the zero order release pattern ($r^2 = 0.9920$). The initial exponential release phase is reasonably due to the drug release from the film surface layer, while the zero-order phase probably corresponds to the drug release from the deeper layers. The higher drug release from CH films containing the complex with EPIβCD may be explained by its about 30 times higher solubilizing power than βCD towards TR [17].

Moreover, the presence of solid-state interactions between CH and both drugs and CDs, as evidenced by DSC and FTIR analyses, do not influence negatively the release rate of the drugs from the films. It is expected that the effect of each CD on drug release rate from CH films would be directly related to its solubilizing power towards the drug. This was true in the case of CH films loaded with TR complexes with βCD and EPIβCD, whose different drug release rate was actually related to the very different solubilizing efficiency of such CDs towards the drug [17]. On the contrary, in the case of BVP, where the difference in solubilizing power between βCD and EPIβCD was considerably more limited [19], the influence of CD on the film swelling properties became important, and it competed with its solubilizing effect in determining the drug release rate.

Mucoadhesive properties of CH films

Measurements of ex vivo mucoadhesion time of buccal films, performed using pig buccal mucosa as biological substrate, indicated that all the examined film formulations had satisfying mucoadhesion properties. In fact, no detachment of the film from the mucosal surface was recorded within 240 min. This should allow in all cases a sufficient resident time of the film on the application site.

The mucoadhesive power of CH film formulations was further investigated in depth by in vitro measurements of the total work of adhesion (TWA) required to break the adhesive bond with the agar-mucine substrate. TWA values of CH films were compared to that obtained for plain stainless steel support, which was taken as a non-adhesive control. The obtained results are presented in Fig. 8.

All film formulations, irrespective of the drug type and CD presence, exhibited TWA values which were significantly higher than those of the control ($p < 0.001$), confirming their mucoadhesive characteristics. Loading of the drug into CH films slightly reduced their mucoadhesivity in comparison to drug-free films. This effect was more pronounced for BVP ($p < 0.01$) than for TR-loaded films, where the decrease in mucoadhesivity was not statistically significant ($p > 0.05$). This finding may be explained by the different chemical properties of the tested drugs. The mechanism of CH mucoadhesion mainly involves the mechanical interpenetration of flexible CH chains within the mucin network, followed by ionic interactions between CH amino groups with sialic acid substructures of mucus [9]. Probably, BVP as cationic compound ($pK_a = 8.1$ [28]), competed with CH chains for interaction with sialic acids of mucin, thus reducing the mucoadhesivity of CH/BVP films. In case of TR (pKa = 7.8 [29]), at pH of simulated saliva (pH = 6.75), the concentration of undissociated drug form is approximately 11 times higher than that of the anionic one, which might interact with the positively charged CH strands. Due to the very low TR solubility in simulated saliva, the concentration of TR anionic form is negligible, so its interaction with CH did not influence the formulation mucoadhesivity.

CH film containing BVP as βCD complex presented the highest TWA value among all formulations tested (Fig. 8a), exhibiting a significant increase of mucoadhesive properties compared to CH films with or without the drug ($p < 0.001$). βCD alone does not possess mucoadhesive characteristics, so the observed effect probably may be attributed to the enhanced swelling properties of CH/BVP/βCD film in comparison with the other formulations (Fig. 6b). Although extensive hydration can lead to a weakening of the mucoadhesive bond, probably as a result of dilution of functional groups available for adhesive interaction at the interface between mucoadhesive film and

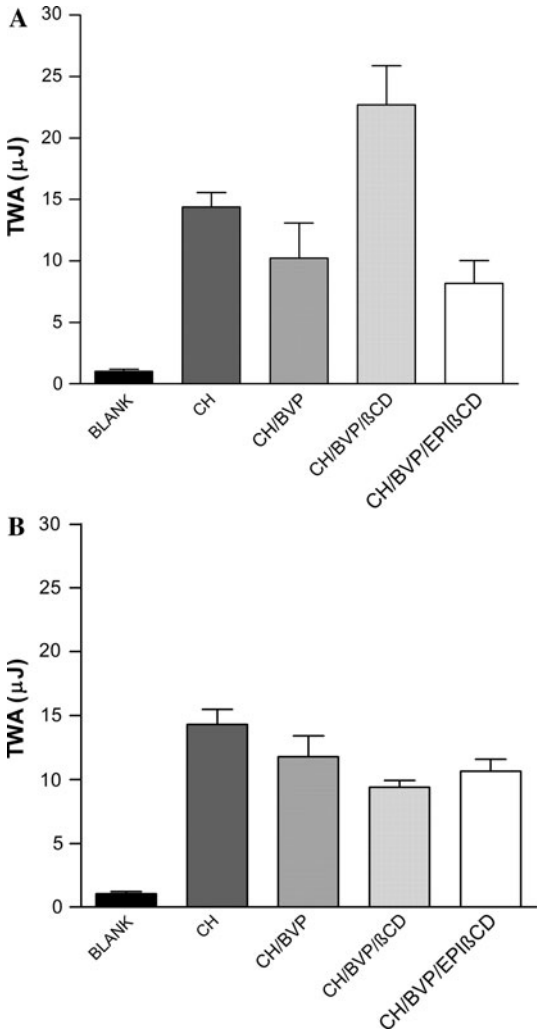

Fig. 8 *In vitro* mucoadhesive properties of CH films loaded with BVP (**a**) or TR (**b**), free or as complexes with βCD and EPIβCD, expressed as total work of adhesion (TWA; mean ± SD, $n = 5$)

the mucus [30], this effect was not observed for CH/BVP/βCD film. Probably, the improved hydration of CH films caused upon addition of βCD did not pass the critical level, but only additionally exposed CH adhesive sites and facilitated their interpenetration with the mucoadhesive substrate to a sufficient depth in order to create stronger adhesive bonds. Better mucoadhesion was not observed in case of CH films loaded with TR/βCD (Fig. 8b), probably because the limited aqueous solubility of this complex significantly restricted the film swelling (Fig. 6c).

When the complex of BVP with EPIβCD was loaded into CH films (Fig. 8a), their mucoadhesion decreased in comparison to other formulations ($p < 0.01$). This may be related to the reduced swelling properties of these films (Fig. 6b). Moreover, the interaction between CH and EPIβCD, demonstrated by DSC and FTIR analyses, may further concur to decrease the strength of mucoadhesive bonds. On the contrary, the TWA value of films loaded

with TR/EPIβCD complex (Fig. 8b) was not significantly different from those of CH/TR and CH/TRβCD films ($p > 0.05$), indicating that, in this case, the presence and type of CD used did not have an important impact on the mucoadhesive properties of films. Moreover, although loading into CH films of TR, both free and as CD complex with βCD and EPIβCD, dramatically reduced the swelling properties of these formulations (Fig. 6c), its effect on mucoadhesion was not so pronounced.

From these results, it appears evident that the mucoadhesion process occurring at the film formulation/mucin interface is a very complex phenomenon, which was influenced by several factors, and not only by the swelling properties of the polymeric matrix. Actually, it has been recognized that mucoadhesion is the result of a series of supplementary processes involved in the different stages of the mucus-substrate interaction, including wetting, electrostatic, adsorption, and diffusion-interlocking phenomena [9]. In particular, our results clearly demonstrated that, beside the presence of solid-state interactions between the formulation components and their possible influence on the film swelling characteristics, other parameters, such as the chemical properties of the loaded drug and the presence and type of CD used to modulate the drug release rate, as well as possible relations among all these variables, must be carefully evaluated, since they would have an important impact on the mucoadhesion properties of the final formulation. Moreover, the superior discriminating power of TWA method than the ex vivo mucoadhesion time measurement, probably attributable to the different experimental conditions of the two series of experiments, pointed out the importance of using different methods for a more exhaustive determination and comparison of mucoadhesive properties of formulations.

Conclusion

In the present research we demonstrated that incorporation of drug/CD complexes into CH films may be a suitable strategy to obtain an effective formulation for buccal delivery of drugs with low aqueous solubility, such as BVP and TR. The type of CD used, i.e. βCD or EPIβCD, differently affected the film biopharmaceutical properties and drug release rate, as a consequence of their different affinities for interaction with both CH and the examined drugs. In case of the sparingly soluble BVP, βCD was more effective than EPIβCD, allowing relatively fast and complete drug release, which may be beneficial for buccal application of local anaesthetics. βCD also contributed to the mucoadhesion of the formulation, showing a positive influence on the film swelling. On the contrary, in case of TR, its high hydrophobicity had a predominant effect in strongly reducing the swelling and mucoadhesion of CH films, irrespective on the CD presence and type in the matrix. However, EPIβCD provided superior solubilizing power towards the drug than βCD, which resulted in faster TR release, making CH films loaded with TR/EPIβCD complex the formulation of choice for further testing and development.

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