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RESEARCH ARTICLE

Novel cyclodextrin-based film formulation intended for buccal delivery of atenolol

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Abstract

Background: Unknown influence of cyclodextrin on the properties of the film formulation aimed for buccal application. *Aim*: Development and characterization of a novel bioadhesive film formulation for buccal atenolol delivery containing drug/cyclodextrin inclusion. *Method*: Interaction between atenolol and randomly methylated β-cyclodextrin (RAMEB) in solution was studied by phase solubility studies. The complex in solid state was prepared by the freeze-drying method and characterized by differential scanning calorimetry and Fourier-transformed infrared spectroscopy (FTIR). The drug, free or in complex form, was incorporated into polymeric films prepared by the casting method using ethylcellulose (EC), polyvinyl alcohol (PVA), and hydroxypropyl methylcellulose (HPMC). The prepared film formulations were characterized in terms of swelling, bioadhesion, and in vitro drug release. *Results*: The formation of a stabile inclusion complex (K_s = 783.4 ± 21.6 M^{-1}) in 1:1 molar stoichiometry was confirmed in solution and in solid state. The swelling properties of films were predominated by the type of polymer used in the formulation. In vitro bioadhesive properties of the films were well correlated with the swelling properties of the polymers used in the formulation. Although incorporation of the drug, free or in complex form, decreased the bioadhesion of the films, PVA- and HPMC-based formulations retained suitable bioadhesive properties. Higher atenolol solubility upon complexation with RAMEB increased the drug dissolution rate under conditions designed to be similar to those on the buccal mucosa, but it has decreased the drug release rate from the PVA and HPMC film formulation, leading to a sustained drug release pattern. In the case of EC-based films, RAMEB promoted drug release. Other parameters that influenced the drug release rate were associated with the structure of the polymer used in the formulation, swelling characteristics of the films, and the interaction between atenolol and hydrophilic polymers that was demonstrated by FTIR analysis. *Conclusion*: Incorporation of atenolol in the form of an inclusion complex into hydrophilic films may be an appropriate strategy to prepare a suitable formulation for buccal drug delivery.

Key words: *Atenolol; bioadhesion; buccal delivery; polymeric films; randomly methylated β-cyclodextrin*

Introduction

Atenolol is a β-1-selective adrenergic blocking agent widely prescribed in diverse cardiovascular diseases, such as hypertension, angina pectoris, arrhythmias, and myocardial infarction[1]. The drug is frequently indicated in the prophylactic treatment of migraine. Atenolol absorption upon its oral administration to humans is rapid but incomplete[2]. Owing to incomplete intestinal absorption, the systemic bioavailability of atenolol is about 50%–60%. Administration of conventional tablets of atenolol has been reported to exhibit fluctuation in plasma drug levels, resulting either in the manifestation of side effects, such as diarrhea, nausea, ischemic colitis, and mesenteric arterial thrombosis, or in the reduction of the drug concentration on the receptor side[3]. Thus, the development of a suitable drug delivery system for antihypertensives that will maintain a proper blood level for a long period of time without adverse effects connected with frequent oral administration is very important. Cho and Shin[4] have proposed the ethylenevinyl acetate matrix for transdermal delivery of atenolol. Another approach is application of the drug via the buccal mucosa of the oral cavity.

Oral cavity is an attractive site for drug delivery owing to the ease of administration and avoidance of possible drug degradation in the gastrointestinal tract and first-pass

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metabolism. Among potential regions in the oral cavity, buccal drug delivery attracts widespread attention. Buccal drug delivery refers to drug application on the buccal mucosa to affect local and/or systemic pharmacological actions[5]. Buccal regions are very suitable for bioadhesive systems because of their smooth, relatively immobile surface with excellent vascularization, and rapid recovery after exposure to stress and accessibility. Major advantages of bioadhesive systems are the increased residence time in the oral cavity and localization of the drugs in a particular region. The list of bioadhesive polymers employed for the preparation of buccal drug delivery systems includes acrylic acid derivates, different cellulose ethers, naturally occurring resins and gums, chitosan, carrageenan, sodium alginate, different thiomers, and so on[6]. Several delivery systems for buccal application have been developed, and among them bioadhesive films seem to be the most suitable ones. Buccal films are preferred over other devices in terms of flexibility and comfort. Moreover, in the case of local delivery for oral diseases, the films help protect the wound surface and thus reduce pain and treat the disease more efficiently[7].

The development of a dosage form with optimal pharmacokinetic properties is a promising area for continued research and it is enormously important and intellectually challenging. With the right dosage form design, local environment of the mucosa can be controlled and manipulated to optimize the rate of drug dissolution and permeation. To design such an advanced dosage form, suitable carrier materials are used to overcome the undesirable properties of drug molecules. Cyclodextrins, cyclic oligosaccharides, are potential candidates for this role because of their ability to alter physical, chemical, and biological properties of the drug molecule through the formation of inclusion complexes[8]. Furthermore, cyclodextrins, especially methylated β-cyclodextrin derivates, were shown to enhance transmucosal drug absorption probably by transiently changing membrane permeability, overcoming the diffusion barrier, and opening tight junctions[9–11]. Thus, cyclodextrins may be considered as a new class of absorption enhancers that may increase the buccal bioavailability of administered drugs. Methylated cyclodextrins are the most effective absorption enhancers at low concentrations ranging from 2% to 5%. In this concentration range, they do not induce tissue damage even after repeated exposure[12]. Bioadaptability of methylated cyclodextrin derivates has been well documented and they are safe excipients for mucosal drug delivery.

Recently published reports have shown that the incorporation of atenolol into polymeric films may be a suitable strategy to obtain a prolonged drug release at buccal mucosa[13,14]. However, atenolol is a class III drug with high solubility and low permeability across biological membranes; therefore, the presence of an absorption enhancer in the formulation would be beneficial. Although the absorption enhancing effect of methylated cyclodextrins is well known, it is unknown how the presence of cyclodextrins would affect the properties of film formulation important for buccal application, such as bioadhesion, swelling, and drug release rate. Therefore, the aim of this study was development and in vitro characterization of a novel randomly methylated β-cyclodextrin (RAMEB) containing bioadhesive film formulation for buccal delivery, using atenolol as a model drug.

Materials and methods

Atenolol was donated by Belupo (Koprivnica, Croatia). RAMEB with an average substitution degree of 1.8 peranhydroglucose unit was used as received (Wacker, Chemie GmbH, Stuttgart, Germany). Ethylcellulose (EC, η = 45 mPas; Sigma Chemical Co., St. Louis, MO, USA), polyvinyl alcohol (PVA, Mowiol 40–80, η = 40 mPas; Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and hydroxypropyl methylcellulose (HPMC, Methocel E5 Premium LV EP, η = 5 mPas; The Dow Chemical Company, Midland, MI, USA) were used as film-forming polymers. Citroflex A4 (Tri-n-butyl Citrate; Pfizer, New york, NY, USA) was used as a plasticizer. All other materials and solvents used were of analytical reagent grade.

Phase solubility study

Phase solubility study was performed according to the method described by Higuchi and Connors[15]. An excess amount of atenolol was added to 20 mL of RAMEB aqueous solution in concentration ranging from 0 to 36 mmol/L. The suspensions were shaken vigorously at 25 ± 1°C for 24 hours until solubility equilibrium was reached. The samples were filtered through 0.22 μm Millipore membrane filter, and drug concentrations in the samples were determined spectrophotometrically at a wavelength of 276 nm (Ultrospec Plus, Pharmacia LKB, Upsalla, Sweden). The apparent stability constant K_s was calculated from the phase solubility diagram using the following equation:

$$K_s = \frac{\text{slope}}{s_0 \times (1 - \text{slope})}, \quad (1)$$

where s_0 is the solubility of the drug in water (intercept).

The complexation efficiency (CE) was calculated using the equation[16]:

$$\text{CE} = \frac{\text{slope}}{(1 - \text{slope})} \quad (2)$$

Preparation of the solid complex

The solid complex was prepared in equimolar drug/cyclodextrin ratio, according to the results of the phase solubility study. RAMEB (4.99 g) was dissolved in 600 mL of purified water. Atenolol (1 g) was added to the prepared solution and stirred (600 rpm) until complete dissolution of the drug occurred. The obtained solution was stirred for next 24 hours at ambient temperature to obtain complexation equilibrium. After that, the solution was filtered, frozen, and subjected to freeze-drying (Freeze Dryer Alpha 1–4 M; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany).

Preparation of the physical mixtures

The corresponding equimolar physical mixture of the substances was prepared by homogeneously blending the weighed amounts of atenolol and RAMEB in a Turbula T2C mixer for 10 minutes.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) thermograms of the solid products were recorded on a DSC7 instrument (PerkinElmer Wallace Inc., Wellesley, MA, USA). The instrument was calibrated with indium and zinc before the analysis of the samples under nitrogen purge. Accurately weighed samples (2–5 mg) were placed in sealed aluminum pans, and scanned at a heating rate of 10°C/min over the temperature range of 25°C–100°C.

Fourier transformed infrared spectroscopic studies

Fourier transformed infrared (FTIR) spectra of the solid products were recorded on a PerkinElmer Spectrum GX spectrometer. The samples were prepared by using the potassium bromide disk method (1%, w/w) and scanned for absorbance in the range of 4500–500 cm^{-1} at 4 cm^{-1} resolution.

Preparation of the film dosage forms

The film dosage forms were prepared by using the casting method. In all formulation, the ratio of the drug (free or as inclusion complex) and polymer was 1:3. To obtain suitable mechanical properties of the films, plasticizer (Citroflex A4) was added in the concentration corresponding to 20% of the polymer amount.

For the film preparation, purified water at 75°C was used as a solvent in the case of PVA- and HPMC-based formulations, while in case of EC-based formulations, the polymer solution was prepared using chloroform as a solvent. The polymers were dissolved in corresponding solvent in 3% (w/v) concentration. The plasticizer was added to the obtained polymer solution. The drug or complex was added into polymer solution as dry powders under magnetic stirring (600 rpm). The prepared dispersions were sonicated (Branson B1210E-DTH, Danbury, CT, USA) to remove incorporated air bubbles and casted on the Teflon-coated Petri dishes. The PVA- and HPMC-based formulations were dried at 45°C while EC-based formulations were dried at room temperature for 24 hours. The samples were stored in a glass container maintained at 25 ± 1°C with a relative humidity of 60 ± 5% until further investigations.

Drug-free films (EC-f_0, PVA-f_0, and HPMC-f_0) were prepared according to the same procedure, omitting the drug (free or as inclusion complex) from the preparation. The composition of drug-loaded films is presented in Table 1.

Table 1. In vitro drug release parameters for inclusion complex and prepared films: correlation coefficient for first-order and Higuchi kinetic models (r^2) and corresponding release constant (k_H).

			r^2		
Sample	Polymer	Drug	First-order	Higuchi	$k_H \times 10/(mg\ min^{-1/2})$
ATE	—	Atenolol	0.8990	0.9866	8.98 ± 0.26
ATE_{RAMEB}		Inclusion complex	0.8797	0.9882	13.40 ± 0.55[a]
EC-f_1	EC	Atenolol	0.8592	0.9866	4.41 ± 0.10
EC-f_2		Inclusion complex	0.8808	0.9882	6.71 ± 0.18[b]
PVA-f_1	PVA	Atenolol	0.8794	0.9678	15.03 ± 1.22
PVA-f_2		Inclusion complex	0.9105	0.9893	13.52 ± 0.36[c]
HPMC-f_1	HPMC	Atenolol	0.9169	0.9903	12.65 ± 0.41
HPMC-f_2		Inclusion complex	0.9097	0.9939	11.66 ± 0.26[d]

Statistically significant differences:
[a]$P < 0.001$ compared to ATE. [b]$P < 0.001$ compared to EC-f_1. [c]$P < 0.001$ compared to PVA-f_1. [d]$P < 0.05$ compared to HPMC-f_1.

Swelling studies

Water uptake of the film was determined gravimetrically in simulated saliva solution, which consisted of 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, and 8.00 g NaCl per liter of distilled water with pH-value adjusted to 6.75 using phosphoric acid. Each film sample with surface area of 18.10 cm^2 was weighed and placed in a preweighed stainless steel wire mesh with sieve opening of approximately 80 μm. The supports containing the film were immersed into swelling medium. At predetermined times, the devices were removed from the media, blotted to remove excess water, and weighted. Water uptake was calculated according to the equation:

$$Q = \frac{W_s - W_d}{W_d} \times 100, \quad (3)$$

where W_d and W_s are the weights of dry and swollen devices, respectively.

In vitro mucoadhesion test

To evaluate the mucoadhesive properties of prepared films, a tensile study was performed using porcine buccal mucosa. Film samples were attached to the stainless steel support connected to a precise balance (Sartorius BP 221S, Goettingen, Germany) using cyanoacrylate glue. The porcine buccal mucosa was fixed to the glass dish mounted on the mobile support. The mucosa surface was wetted with 100 μL of simulated saliva and brought in contact with the sample. The sample and the tissue were left in contact for 5 minutes, allowing the formation of a mucoad-hesive bond. The force of detachment was measured as a function of displacement, by lowering the mobile support at a constant rate of 5 mm/min until total separation of the components was achieved. The total work of bioadhesion (TWA) was calculated as the area under the force/distance curve.

In vitro drug release studies

The drug release experiments employed a standard Franz diffusion cell (PermeGear, Hellertown, PA, USA) with a diffusion area of 10.18 cm^2 and the acceptor compartment volume of 16 mL.

Atenolol, inclusion complex or film samples containing 25 mg of the drug were applied evenly across the prehydrated semipermeable membrane (MWCO 600 Da; Medicell Dialysis Tubing) clamped between the donor and acceptor compartments. The membrane was wetted with 0.1 mL of simulated saliva solution. After the sample was applied, the donor compartment of the Franz diffusion cells was closed with Parafilm® 'M' sealing film (American National Can Company, Chicago, IL) to avoid the evaporation of the release medium and to allow the establishment of constant relative humidity around the sample. Phosphate buffer (pH 7.4) in the acceptor compartment was continuously stirred at 600 rpm using a magnetic stirrer. The cells were thermostated at 37°C. At set time intervals, aliquots of the acceptor phase were removed and assayed spectrophoto-metrically (λ = 276 nm) for the drug content. The removed samples were immediately replaced with an equal quantity of prewarmed receptor medium. Cumulative corrections were made for previously removed samples.

The kinetics of drug release was determined by fitting the best fit ($r > 0.98$) of the dissolution data to distinct models: first-order model and Higuchi kinetic model.

Statistical analysis

All values are expressed as mean ± SD of n separate experiments. Data were compared for single comparison by using Student's t-test and for multiple comparisons by one-way ANOVA, followed by Tukey multiple comparison test. Values of $P < 0.05$ were considered significant. Calculations were performed using the GraphPad Prism program (GraphPad Software, Inc., San Diego, CA, USA; www.graphpad.com).

Results and discussion

Atenolol/RAMEB complexation in solution

The phase solubility diagram obtained for atenolol/RAMEB is presented in Figure 1. The increase in atenolol solubility occurred as a linear function of RAMEB concentration, corresponding to the A_L type

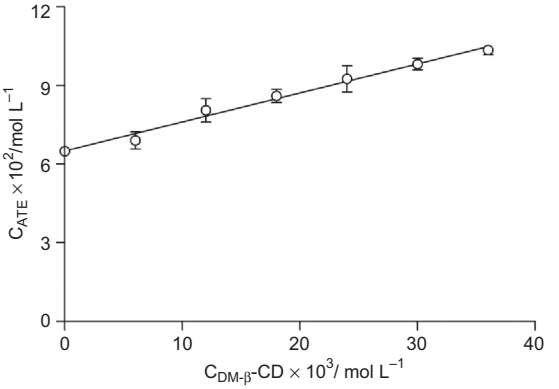


Figure 1. Phase solubility diagram of atenolol in RAMEB aqueous solution at 25°C (mean ± SD; n = 5).

profile defined by Higuchi and Connors[15]. The apparent stability constant and the CE at 25°C were calculated from the parameters of the solubility diagram assuming that a 1:1 complex was initially formed.

The apparent stability constant K_s was calculated to be 783.4 ± 21.6 M^{-1}, indicating a fairly strong affinity of atenolol for complexation with RAMEB. Another parameter that may be used to describe complexation phenomena is the CE. CE represents the ratio between the concentration of cyclodextrin in the complex and free form. CE for the atenolol/RAMEB system was determined to be 54.6 ± 1.5. This value indicates that a majority of cyclodextrin molecules in the atenolol/RAMEB system are in the form of an inclusion complex and the concentration of free cyclodextrin molecules in solution is rather low, thus confirming the high affinity of atenolol for complexation with RAMEB. This result may be explained in terms of the relatively high atenolol solubility in water (14.4 ± 0.8 mg/mL). Hence, a relatively high concentration of dissolved drug molecules is available for the inclusion complex formation with RAMEB. Inclusion complex formation promotes further dissolution of the drug and after equilibrium is achieved in the system, only a low concentration of RAMEB remains in the free form.

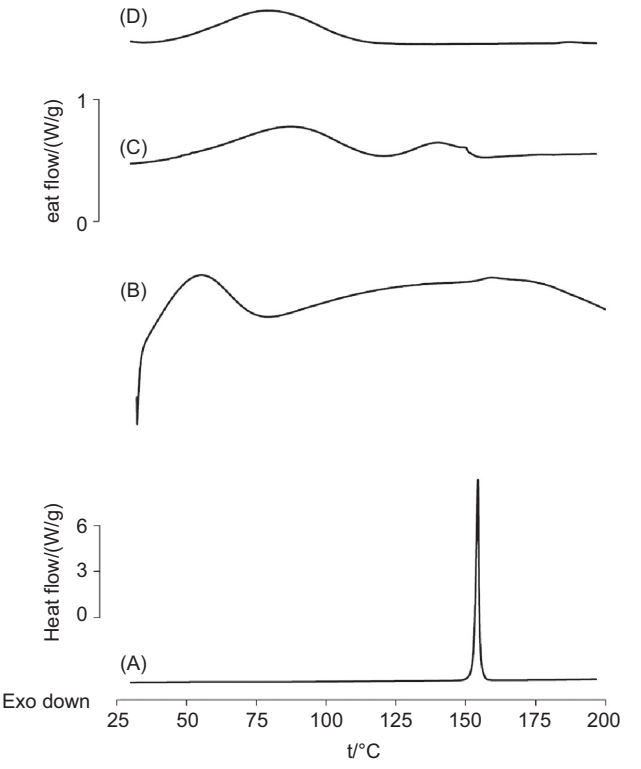


Figure 2. DSC thermograms of atenolol (A), RAMEB (B), equimolar physical mixture (C), and freeze-dried inclusion complex (D).

Solid-state complex characterization

The phase solubility study indicated the feasibility of obtaining the atenolol/RAMEB solid complex in 1:1 molar ratio by the freeze-drying of solubilized atenolol in cyclodextrin solution. Evidence for the complex formation in the solid state was obtained by thermal (DSC) and spectral (FTIR) analyses.

DSC thermograms are shown in Figure 2. The DSC curve of atenolol (A) showed the endothermic event as a melting peak with the onset temperature of 154.5°C (ΔH = 147.5 J/g). The RAMEB (B) thermogram exhibited a very broad endothermic peak from 35°C to 80°C, corresponding to the evaporation of water. The appearance of a small peak corresponding to the atenolol melting peak at 140.3°C was evident in the thermogram of the physical mixture (ΔH = 12.96 J/g) (C). The change of the drug melting peak position and intensity in the physical mixture could be explained by the thermally induced atenolol-RAMEB interaction during the experiment. In the thermogram of the freeze-dried inclusion complex, the atenolol fusion peak was not shown (D). Disappearance of the endothermic peak in the thermogram could be attributed to the amorphous state of the drug. The drug amorphous state may be explained by the inclusion complex formation. Inclusion of atenolol in the RAMEB cavity sterically hinders the formation of the drug crystal lattice during the freeze-drying process, thus stabilizing the drug amorphous state[17].

FTIR spectra of atenolol, RAMEB, equimolar physical mixture of the components, and freeze-dried inclusion complex are presented in Figure 3. FTIR spectrum of the physical mixture (C) shows the superposition of the drug (A) and RAMEB (B) spectra, indicating the absence of any interaction between the components. In the spectrum of the freeze-dried complex (D), the absorption band corresponding to the C=O stretching vibrations shifted from 1638 cm^{-1} (atenolol) to 1675 cm^{-1} (inclusion complex) while the intensity of the band decreased. Also, the absorption band at 1418 cm^{-1}, originating from the C–N bond vibration coupled with the N–H bending vibration present in the atenolol and physical mixture spectra, is completely absent in the FTIR spectrum of the freeze-dried inclusion complex. These spectral changes indirectly confirmed the inclusion complex formation between atenolol and RAMEB in the solid state. The shift in the amide carbonyl absorption band indicated the disruption of intermolecular hydrogen bonds in the crystal lattice of atenolol, probably due to the inclusion complex formation with RAMEB[18]. At the same time, the inclusion complex formation restricted vibrational motions of the included moiety, thereby reducing the absorption band intensity. These changes in the FTIR spectra may be indicative of the drug monomeric dispersion as a consequence of the

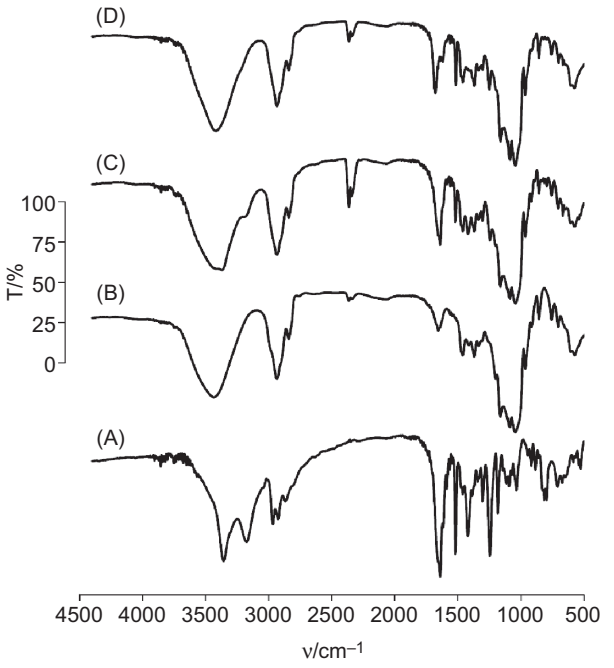


Figure 3. FTIR spectra of atenolol (A), RAMEB (B), equimolar physical mixture (C), and freeze-dried inclusion complex (D).

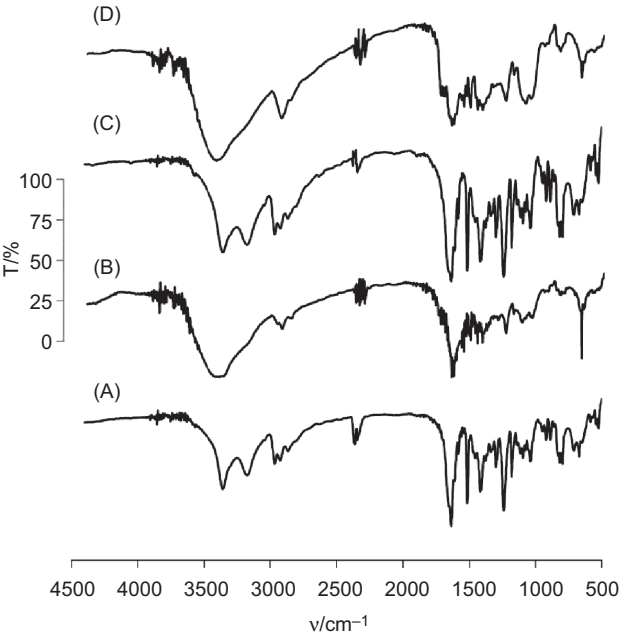


Figure 4. FTIR spectra of atenolol (A), PVA (B), corresponding physical binary mixture (C), and water-treated binary mixture (D).

inclusion complex formation, thus confirming the DSC results.

On the basis of the DSC and FTIR results, it may be concluded that freeze-drying is a suitable method for the preparation of the atenolol/RAMEB inclusion complex in the solid state.

Film dosage form preparation and characterization

Atenolol film dosage forms were prepared using EC, PVA, or HPMC as a film base. Free drug and the atenolol/RAMEB inclusion complex were incorporated into film dosage forms.

FTIR analysis was used to study the possible interactions between atenolol and polymers. FTIR spectra of pure components (atenolol, PVA, and HPMC) and their corresponding binary physical mixtures in 1:1 mass ratio were recorded. As the presence of water may have a significant influence on the drug/polymer interactions, binary physical mixtures were also dissolved/dispersed in water and after removal of the solvent, FTIR spectra of the solid product were recorded. The results are presented in Figures 4 and 5.

In the FTIR spectrum of the atenolol/PVA physical mixture (Figure 4C), the position of all absorption bands remained the same as in the spectrum of each component, indicating that in the absence of water no interaction between the drug and PVA could be detected. The same conclusion could be made for the atenolol/HPMC physical mixture (Figure 5C). FTIR

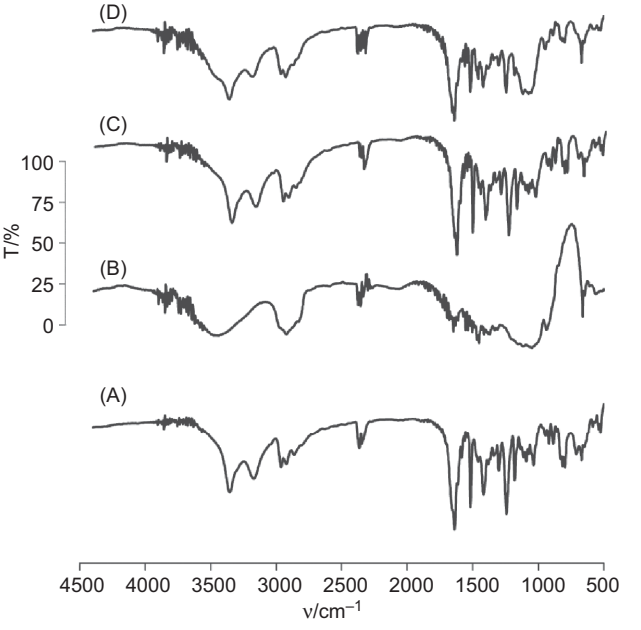


Figure 5. FTIR spectra of atenolol (A), HPMC (B), corresponding physical binary mixture (C), and water-treated binary mixture (D).

spectrum of the water-treated binary mixture (Figure 4D) differs from the spectrum of the atenolol/PVA physical mixture, indicating the drug/polymer interaction upon exposure to water. Absorption bands corresponding to asymmetrical and symmetrical stretching vibrations of the N–H bond at 3355 and 3173 cm^{-1} completely

disappeared in the FTIR spectrum of the water-treated binary mixture (Figure 4D), while they were still present in the FTIR spectrum of the physical mixture (Figure 4C). Also, the intensity of the amide carbonyl absorption band was remarkably reduced and the peak was moved from 1638 cm^{-1} in the case of atenolol to 1654 cm^{-1} in the case of the water-treated binary mixture. These data indicated the existence of interaction between atenolol and PVA, probably by hydrogen bonding. The interaction involves NH and C=O groups of the drug molecule. The presence of water is necessary for interactions between the components. In solid state, polymer chains are in coiled conformation and thus functional groups involved in the interaction are hindered. In contact with water, hydration of the polymer occurred, followed by relaxation of polymer chains. Functional groups of the polymer then become available for interactions with the drug molecule. In the case of the water-treated atenolol/HPMC binary mixture, FTIR spectrum (Figure 5D) showed a shift of absorption bands corresponding to asymmetrical and symmetrical stretching vibrations of the N–H bond in the drug molecule from 3356 and 3174 cm^{-1} (atenolol and physical mixture) to 3364 and 3185 cm^{-1} (water-treated binary mixture), while the intensity of the bands decreased. This indicated the existence of interactions between atenolol and HPMC, which included the amino groups of the drug. Changes of drug absorption bands in the FTIR spectrum of atenolol/HPMC system were less pronounced than in the case of atenolol/PVA. This may point to the conclusion that the affinity of atenolol for interactions with HPMC is less pronounced than in the case of PVA. This fact may be explained by different chemical structures of the polymers. PVA possesses numerous OH groups in the structure, which are good proton donors, while the number of OH groups in HPMC is greatly reduced.

Swelling properties of the polymer matrix have a strong impact on the drug release pattern and on bioadhesive properties of the matrices. Swelling analysis was, therefore, conducted and swelling profiles of polymeric films in simulated saliva solution are shown in Figure 6.

A considerable difference in swelling behavior was observed between the polymers used. PVA and HPMC films hydrated very quickly within the first 30 minutes and showed remarkable swelling. The hydrosolubility of HPMC and PVA promoted water entry and its entrapment in the polymer network. Maximum hydration was achieved with formulations containing PVA, whereas films containing HPMC showed slightly lower hydration. These results suggested that, as expected, PVA films exhibited a higher capacity of water uptake than HPMC films because of higher water solubility of PVA. In the case of HPMC, the swelling index increased from 0% to 200% in 15 minutes. The matrix was completely hydrated after 30 minutes and hence supported rapid

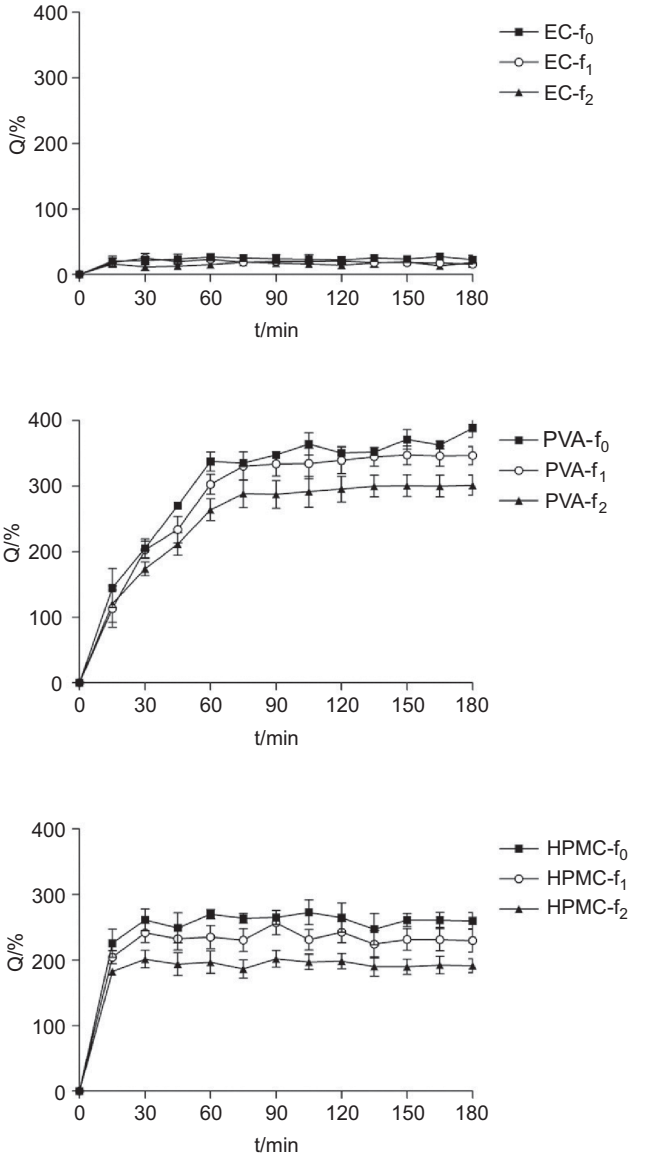


Figure 6. Swelling profiles of the films composed of plain polymers (EC-f_0, PVA-f_0, HPMC-f_0), films prepared with atenolol (EC-f_1, PVA-f_1, HPMC-f_1), or the atenolol-RAMEB complex (EC-f_2, PVA-f_2, HPMC-f_2) in simulated saliva (mean ± SD; n = 5).

swelling due to the high diffusibility of the HPMC polymer. PVA matrix was completely hydrated after 60 minutes, and swelling index increased from 0% to over 300%. EC showed reduced swelling capacity compared with PVA and HPMC (Figure 6). Hydrophobicity and weak gel forming capacity in saliva were responsible for the weak swelling characteristics of EC.

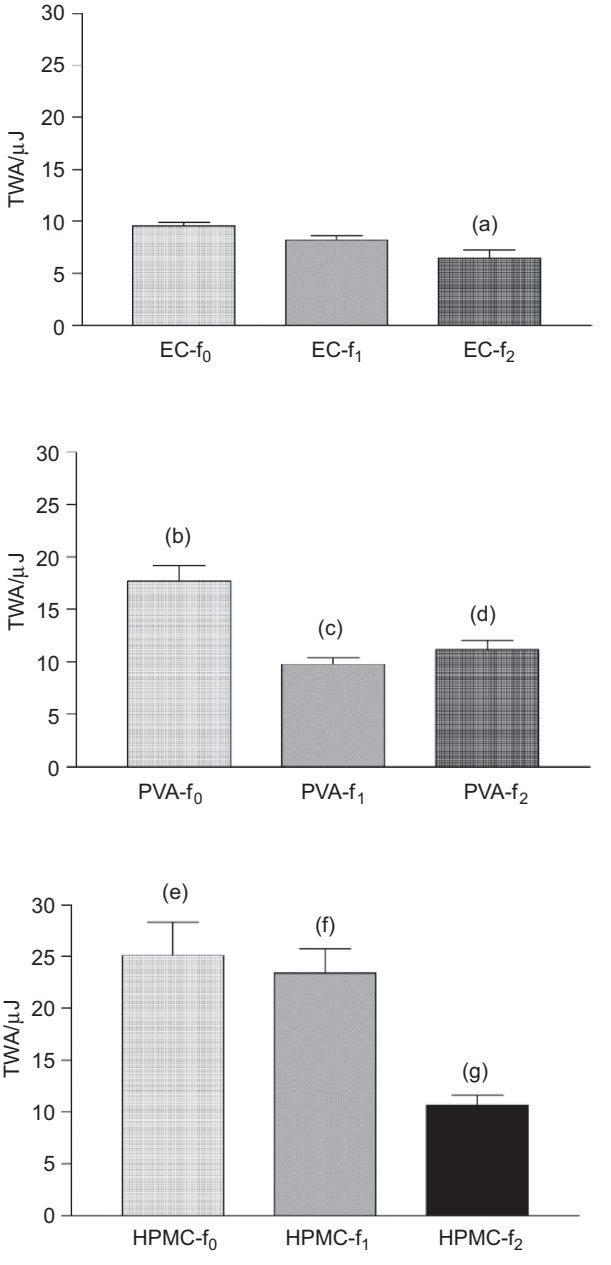
Atenolol incorporation into polymer films only slightly reduced their swelling properties, probably due to the lipophilic character of the drug. The presence of RAMEB additionally decreased the swelling properties of polymer films. In the investigated systems, the hydration and

fast dissolution of the inclusion complex modified the solvent structure in the vicinity of polymer chains and thereby affected the polymerwater interaction. Cyclodextrin molecules are extensively hydrated and water molecules bound to RAMEB are not active in the process of polymer swelling. Therefore, the competition between cyclodextrin and polymer strands for hydration water may explain the observed reduced swelling properties of the films that contained RAMEB. Similar changes in swelling behavior were already observed in the case of polymeric matrices that contained highly hydrated compounds[19,20].

To evaluate the bioadhesive properties of prepared films, the total work of adhesion necessary for the breakdown of the bioadhesive bond between the film and mucosal surface was measured. Figure 7 illustrates the bioadhesive properties of films composed of plain polymers (EC-f_0, PVA-f_0, HPMC-f_0) and films prepared with atenolol (EC-f_1, PVA-f_1, HPMC-f_1) or the atenolol-RAMEB complex (EC-f_2, PVA-f_2, HPMC-f_2).

The type of polymer used in the formulation had a significant influence on the bioadhesive properties of films. The films composed of PVA and HPMC showed remarkably higher bioadhesion compared with EC films (Figure 7). The swelling behavior of a polymer was related to its bioadhesive performance. The initial water uptake enables relaxation of the originally stretched or entangled chains, resulting in exposure of all the bioadhesive sites of the polymer available for bonding. Therefore, faster swelling of HPMC and formation of the entangled interface resulted in faster initiation of bioadhesion. According to Jacques and Bruni[21], materials having the highest initial rate of hydration reach the highest mucoadhesive strength. HPMC films showed a faster hydration rate than PVA or EC and achieved maximum swelling in a shorter period, which could promote interpenetration of the polymer chain with mucin. It is thus anticipated that its in vitro bioadhesion should be greater than PVA, which hydrated at a slower rate. The bioadhesion of EC films was limited by its hydrophobicity.

The work of adhesion gradually decreased with the addition of the drug or the complex to the films (Figure 7). This may be associated with the reduced swelling properties of the samples. Also, reduction in film mucoadhesion after the drug or drug complex incorporation could be explained by the prevention of the formation of the electrostatic or hydrogen bond responsible for the stabilization of mucoadhesive bonds. In the presence of water, atenolol interacts with the functional groups of polymers, as it has been demonstrated using FTIR analysis. This interaction caused the formation of a hydration sheath surrounding the polymer, with a consequent reduction in swelling and relaxation of polymer chains[22]. This restricted the possibility of interpenetration between



Statistically significant differences:

(a) $P < 0.01$ compared to drug free EC film
(b) $P < 0.05$ compared to drug free EC film
(c) $P < 0.01$ compared to drug free PVA film
(d) $P < 0.05$ compared to atenolol loaded PVA film
(e) $P < 0.01$ compared to drug free EC film
(f) $P < 0.05$ compared to drug free HPMC film
(g) $P < 0.05$ compared to atenolol loaded HPMC film

Figure 7. In vitro bioadhesive properties of films composed of plain polymers (EC-f_0, PVA-f_0, HPMC-f_0), films prepared with atenolol (EC-f_1, PVA-f_1, HPMC-f_1), or the atenolol-RAMEB complex (EC-f_2, PVA-f_2, HPMC-f_2) expressed as total work of adhesion (TWA; mean ± SD, $n = 5$).

polymer and mucin strands, thus reducing the bioadhesivity. Atenolol showed higher affinity for interaction with PVA than with HPMC. This is probably the reason why the drug presence in the PVA film caused a more pronounced decrease of bioadhesion than in the case of the HPMC-based film.

In case of the HPMC formulation, the presence of the drug complex caused a relatively marked reduction in bioadhesion compared with PVA- or EC-containing films. The highly soluble RAMEB may compete with the polymer for available water, thereby decreasing the hydration degree of polymer strands. This reduced their mobility and thereby their bonding with mucin. At the same time, the cyclodextrin molecule has the ability to form hydrogen bonds. It has already been demonstrated that the hydrogen bonding ability of some molecules allows them to interfere with the formation of the bioadhesive bond[23]. Also, cyclodextrins are able to form noninclusion complexes with polymers and drug-cyclodextrin-polymer ternary complexes, especially with HPMC[24]. This interaction may also contribute to the decrease of the observed bioadhesion in the case of HPMC films.

In vitro drug release

In vivo dissolution/release of the drug on the buccal mucosa is limited by the amount of saliva presented within the mouth. As a result, dissolution tests using standard USP apparatus and large volumes of the dissolution media might not produce results that reflect the in vivo dissolution[25]. Therefore, modifications of the dissolution tests are required to mimic in vivo conditions for accurate analysis of buccal film formulations. In this study, in vitro drug release study was performed by the use of Franz diffusion cells, according to Han et al.[26] The obtained drug release profiles from the complex or polymer films prepared are presented in Figures 8 and 9.

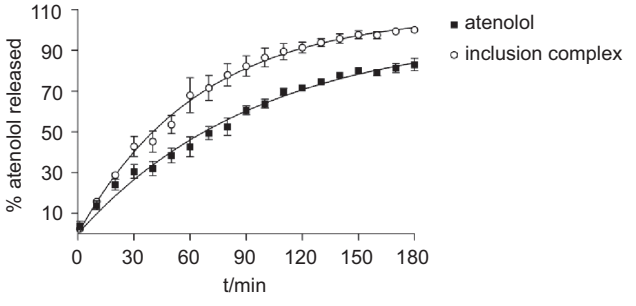


Figure 8. In vitro dissolution profiles of atenolol and RAMEB inclusion complex (mean ± SD; n = 5).

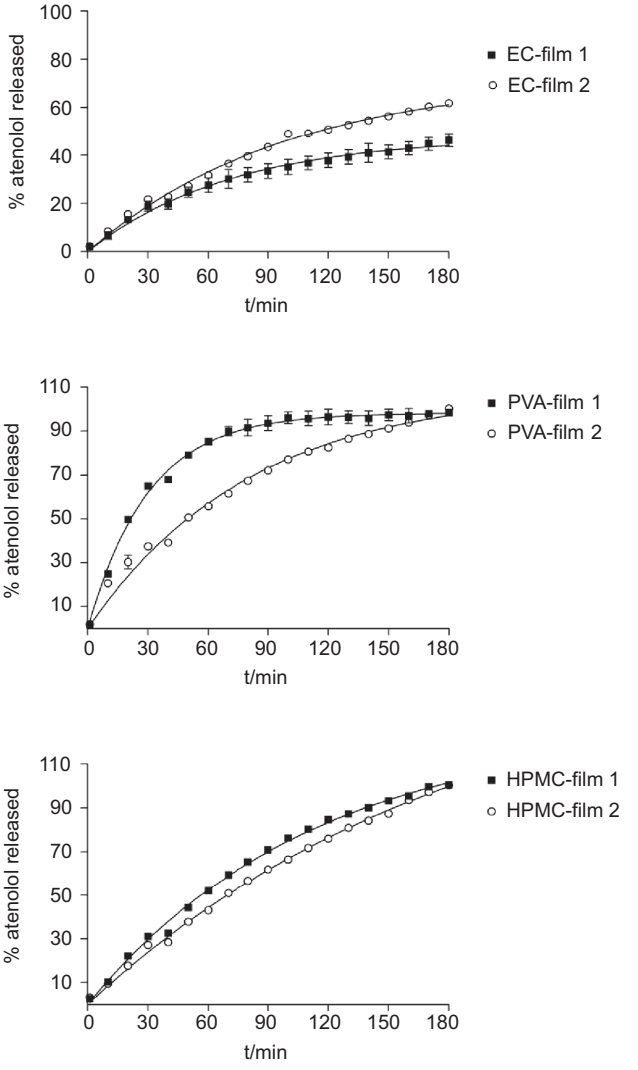


Figure 9. In vitro drug release profiles from films prepared with atenolol (EC-f_1, PVA-f_1, HPMC-f_1) or the atenolol-RAMEB complex (EC-f_2, PVA-f_2, HPMC-f_2) (mean ± SD; n = 5).

To determine atenolol release kinetics from the complex or polymer films, experimental data were fitted to the first-order and Higuchi kinetic models. The data are presented in Table 1. The correlation coefficient values (r^2) indicate the release kinetics mechanism. According to the correlation coefficient values, drug release could be described by the Higuchi kinetic model.

In the experiment, after dissolution/release, the drug molecules had to diffuse across the unstirred aqueous layer on the membrane surface in the donor compartment, followed by diffusion across the semipermeable membrane to the acceptor compartment of the Franz diffusion cell. The semipermeable membrane had a molecular weight cutoff value of 600 Da and so atenolol could pass through freely. Thus, the observed release kinetics indicated that the diffusion of the drug across

the hydrodynamic layer on the membrane surface is one of the major rate-limiting steps in the overall drug release process. Also, in the case of film formulations, the polymer swelled in contact with the simulated saliva solution to an extent determined by the type of polymer used, thus creating an additional diffusional pathway for drug molecules. The diffusion of atenolol, free or in complexed form, across the formed gel layer controlled the drug release rate.

Atenolol release rate was significantly increased by its complexation with RAMEB (Table 1). The ability of forming a complex with RAMEB led to an increase in apparent drug solubility and a marked increase in the dissolution rate. At the same time, complex formation facilitated the diffusion of the drug across the hydrodynamic layer on the membrane surface. Though the complex could not penetrate the membrane, the drug in the complex was in rapid dynamic equilibrium with the uncomplexed drug, thus providing a high concentration of atenolol molecules in diffusible form. Cyclodextrin acted as a carrier of drug molecules across the unstirred water layer, thereby additionally facilitating the drug release.

Incorporation of atenolol, free or as cyclodextrin complex, into polymer films influenced the drug release (Table 1). The type of polymer used in film formulation affected the drug release rate. The observed variation in the drug release rate could be attributed to the physicochemical properties of the films and to the changes that they undergo in the dissolution media. The drug release rate from EC films was lower than that of PVA and HPMC films. This was well correlated with the swelling study. Hydrophobic properties and low water permeation into EC-based films restricted the drug release from the film formulation, contributing to the lowest release rate compared with PVA and HPMC films.

PVA- and HPMC-based films showed an initial burst release of the drug (Figure 9). The cumulative release of atenolol from PVA films reached almost 90% within 1 h, while the drug release from HPMC films exceeded 50% after 1 hour. After the initial burst release, a steady state in drug release was observed. The burst effect of PVA- and HPMC-based formulations may be explained by the large surface area and fast hydration of the films observed during the swelling analysis. As shown in Table 1, the drug release rate from PVA films was higher than that of HPMC films. Higher water solubility and consequently higher swelling of PVA films might be the reasons for such results. Also, the interaction of atenolol with PVA and HPMC may additionally contribute to faster drug release. The FTIR analysis showed a shift of the amide carbonyl absorption band to a higher wave number in the case of the water-treated drug/PVA mixture. This change may be explained by the disruption of hydrogen bonds in the atenolol crystal lattice and probably transition of the drug from crystalline to amorphous state. It is well known that several polymers act as antinucleating agents and inhibit crystal growth[27]. Atenolol was probably associated with PVA by hydrogen bonding. This interaction resulted in the formation of a hydrodynamic layer around drug molecules, which sterically hindered the nucleation process and collision between the drug molecules, thereby inhibiting crystal growth. Interaction of the drug with the polymer also increased the glass transition temperature (T_g) of the system formed[28]. This means that the polymer may inhibit or slow down the crystallization process by increasing the apparent viscosity of the systems and decreasing the diffusion of drug molecules required for crystal lattice formation[29]. Thus, the fast release of atenolol from PVA-based films may be attributed to the amorphization of the drug due to its interaction with the polymer. Amorphous drug state and drug interaction with hydrophilic polymers facilitated to some extent the wetting of drug particles, providing a lower energy pathway for drug dissolution. The drug release rate from the PVA-based film is therefore higher than that of the free drug and even the atenolol/RAMEB inclusion complex (Table 1). As the FTIR analysis indicated that the affinity of atenolol for interaction with HPMC is less pronounced, probably more crystalline drug areas existed for the HPMC films, contributing to slower drug release.

Incorporation of the atenolol/RAMEB inclusion complex into the EC polymer film led to a slight increase in the drug release rate (Table 1). Although the presence of RAMEB did not facilitate the swelling of EC films, the increased drug release rate may be explained by higher drug solubility, which facilitated the drug dissolution process upon its contact with dissolution media, thereby contributing to the overall release rate.

In contrast, atenolol complexation slightly decreased the drug release rate from PVA- and HPMC-based films (Table 1), providing a sustained drug release pattern. Uptake of saliva into the PVA and HPMC films resulted in film swelling, followed by dissolution of the complex. Drug diffusion in the swollen gel layer controlled the drug release. The complexing ability of RAMEB decreased the drug release rate in films formulated with PVA and HPMC, probably due to restricted diffusion of the complex over the hydrated swollen polymer. When the RAMEB complex was present in the swollen film, a partition of drug molecules was assumed to occur between free and complexed states. Thus, the total amount of atenolol was present in two forms, free and complexed, with different diffusivities. The stability constant value for the atenolol/RAMEB complex was $783.4 \pm 21.6\ M^{-1}$, indicating a stabile association of atenolol and cyclodextrin. At the same time, high CE indicated that after equilibrium was attained, most of

the drug molecules were in complexed form. Thus, RAMEB complexation reduced the concentration of free atenolol molecules that may diffuse more freely across the swollen polymer matrix. Diffusivity of a drug molecule within the polymer matrix would be considerably lowered by the drug-cyclodextrin complex formation. This effect is associated with a significant increase in the molecular weight of the diffusant upon complexation with RAMEB, resulting in a decrease of the average mobility of the drug in the swollen films[30]. Thus, the observed reduction in drug release may be attributed to the difference in the diffusion coefficient between the free and complexed drug.

In determining the release kinetics, drug diffusivity, swelling kinetics, polymer structure, and polymer-drug interaction play an important role.

Conclusion

The present study has showed that the incorporation of atenolol in the form of an inclusion complex into hydrophilic films may be an appropriate strategy to prepare a suitable formulation for buccal drug delivery. Atenolol formed a stabile inclusion complex with RAMEB in solution and in solid state. The inclusion complex formation decreased the drug release rate from PVA and HPMC film formulations, leading to a sustained drug release pattern. In the case of EC-based films, RAMEB promoted drug release. Other parameters that influenced the drug release rate were related to the structure of the polymer used in formulation, swelling characteristics of the films, and the interaction between atenolol and hydrophilic polymers, which was demonstrated by using FTIR analysis. Swelling characteristics of films were predominately determined by the type of the polymer used in the formulation. The presence of the drug, free or in complexed form, decreased the swelling rate of the polymer films investigated, consequently affecting the bioadhesive properties of the samples. Despite that, PVA- and HPMC-based formulations showed good bioadhesive properties. The strategy involving the addition of an inclusion complex into hydrophilic polymer films may be a suitable technology for obtaining a delivery system with sustained release of an active compound suitable for buccal application.

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