ENALAPRIL MALEATE LOADED PULLULAN FILM FOR MUCOADHESIVE BUCCAL DRUG DELIVERY APPLICATIONS

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Pullulan, a water soluble, bioadhesive and biocompatible biopolymer is used as an efficient matrix for enalapril maleate film preparation. The obtained film contains as much as 67% of enalapril maleate, relative to the initial amount used for experiments. The drug content was evaluated by using ^{1}H-NMR and UV-vis spectroscopy. The pullulan-enalaprilmaleate blends exhibit good physicochemical properties, as well as a high dissolution rate, which makes these formulations usable as mucoadhesivebuccal film.

Keywords: pullulan, enalapril maleate, buccal film, drug delivery

INTRODUCTION

Fast-dissolving drug-delivery systems were developed in the late 70s and were rapidly introduced as an alternative to tablets, capsules and syrups.[1-3]The delivery systems of fast dissolving oral films (FDOFs) are more flexible and easier to administrateto pediatric, geriatric, mentally ill patients, that experience difficulties swallowing oral solid dosage forms.[2,4,5] Due to their size and thickness, theyare easy to handle, administer, maintain and keep in convenient packaging, improving patient compliance; moreover, these dosage forms are economically effective.[1,2,6,7] Usually, the thin film is placed on top, or under the tongue, or on any mucosal tissue and is dissolved, or disintegrated in the saliva in a few seconds, without the need for water or chewing. The oral mucosa offers some distinct advantages, such as faster uptake of the drug into the systemic circulation and enhanced bioavailability, leading to rapid therapeutic efficacy. The drug is absorbed from the mouth, pharynx and esophagus as the saliva passes down into the stomach. In such cases, the bioavailability of the drug is significantly greater than that observed in the case of conventional tablet dosage forms.[1,3,8,9]

Typically, buccal films are designed and formulated using mucoadhesive polymers, which are generally of high molecular weight, high viscosity grades, with greater flexibility and optimum chain length.[6,10,11] One of the best candidates for such purposes are natural polymers, due to their availability, abundance and biocompatibility. Moreover, due to their composition, various chemical modifications are possible in order to achieve the desired structure, for further application. Also, they form strong intermolecular hydrogen bonding, without any change in the physical properties of the delivery matrix.[12-15] Among all the biopolymers used, pullulan is a water-soluble microbial polysaccharide produced extracellularly by the fungus-like yeast called *Aureobasidium pullulans*. Pullulan can be used as carrier for oral delivery of drugs, due to its bioadhesive, non-toxic, non-immunogenic, non-mutagenic and non-

carcinogenic nature; also, pullulan is impermeable to oxygen and immune to the digestive enzymes from the human gut. Pullulan has a considerable mechanical strength and good properties of film formability.[16-19]

Enalapril maleate (1-{N-[(s)-1-carboxyl-3-phenylpropyl]-L-alanyl-}-L-proline 1-ethyl ester maleate) is an angiotensin converting enzyme inhibitor (ACE-Inh), used clinically mainly in the treatment of hypertension, angina pectoris and postinfarction.[20,21] It is a pro-drug without direct biological activity, which is rapidly adsorbed after oral administration and deesterified *in vivo* by esterase enzymes, which are present in the blood and liver.[22,23]

The aim of this study was to develop and characterize a new mucoadhesive buccal film of enalapril maleate based on pullulan, using the solvent casting technique, which would provide rapid therapeutic efficacy and increase the bioavailability of the drug. The prepared film was characterized by FTIR and ^{1}H-NMR spectroscopy and the content of the enalapril maleate incorporated into the pullulan film was assessed by^{1}H-NMR and UV-vis spectroscopy. Moreover, the film was evaluated in terms of physicochemical properties, such as solubility, thickness, pH, disintegration time and folding endurance.

EXPERIMENTAL

Material

Pullulan (Mw = 150 kDa) purchased from TCI Europe was dried under vacuum at 100°C overnight prior to use. Pure enalapril maleate drug (99%) was provided by Zhejiang Huahai Pharmaceutical. All other chemicals and solvents were of analytical grade and purchased from Sigma-Aldrich and Fluka. The dissolution tests were carried out at pH 6.8 in aqueous buffers.

Drug loaded pullulan buccal film preparation

A fast-dissolving film with enalapril maleate was prepared by the solvent casting technique, using 1% (w/v) aqueous solution of pullulan (PU) and 0.1% (w/v) enalapril maleate (EM) solution in dimethyl sulfoxide (DMSO). PU was dissolved in Milli Q water and gently stirred to a clear and homogeneous solution. EM was dissolved in DMSO and, after the complete dissolution, it was added to the PU solution. The resulted mixture was stirred for 24 h at room temperature, and then precipitated with ethanol. The precipitate was separated by centrifugation for 60 minutes at 5000 rpm. The recovered solid was washed several times with ethanol and then was redissolved in 50 mL Milli Q water. 20 mL of the final solution was cast into a Petri dish and dried at 40°C for 48 h. The film was packed in aluminum foil and stored in a desiccator at 18°C for further studies.

Physicochemical evaluation and compatibility study between drug and polymer

The prepared film was evaluated as to its physicochemical properties, such as solubility, uniformity of thickness and weight. Spectroscopic (FTIR, ^{1}H-NMR) and thermal (TG) analyses were performed in order to evaluate the compatibility between the drug and the polymer. Disintegration film studies were performed *in vitro*.

FTIR Spectroscopy

IR spectra were obtained on a Fourier Transform Infrared spectrophotometer (Bruker VERTEX 70) over the 4000-400 cm^{-1} region, using a KBr pellet (12 mm disc), compressed in a hydraulic press at 10 tons for 30 seconds.

^{1}H-NMR Spectroscopy

The ^{1}H-NMR spectra (δ, ppm) of pullulan, enalapril maleate and film (PU+EM) were recorded in DMSO-d_6 on a Bruker Avance DRX 400 MHz machine. The percentage of enalapril maleate embedded in the polymer was calculated by ^{1}H-NMR analysis.

Thermogravimetric analysis (TGA)

AnSTA 449 F1 Jupiter thermal analyzer (Netzsch, Germany) was used to determine the weight loss % of the drug loaded pullulan film. Samples (about 10 mg) were placed in Al2O3 crucibles and heated under nitrogen from 30 to 700°C, with a 10 °C min-1 heating rate. TG and DTG curves, recorded with a ±0.5°C precision were analyzed with Netzsch Proteus analysis software.

Film solubility

To determine the solubility of the film, different film surfaces of 10×10 mm^2 were analyzed in various solvents, such as water, dimethyl sulfoxide, methanol, dichloromethane and chloroform.

Film thickness and weight

The thickness was measured using a micrometer screw gauge at different positions of the film and the average thickness was calculated. This established the uniformity of the film thickness, which is directly related to the accuracy of the dose in the strip. For the weight test, pieces of 1 cm^2 were cut from three different places of the film and were weighed individually on an analytical balance; then, the average weight was calculated.

Surface pH

The surface pH of the fast-dissolving film was determined in order to find out anypossible *in vivo* side effects. The film was placed in a Petri dish and was

moistened with 0.5 mL of distilled water and kept for 1 hour. The pH was recorded after bringing the electrode of the pH meter in contact with the surface of the film, and then allowing it for 1 min to reach equilibrium. The procedure was performed in triplicate and the average was determined with standard deviation.

Disintegration time

Disintegration time is defined at the time (second) when a film breaks when brought into contact with water or saliva. The test was carried out visually by introducing 1 cm^2 film into a glass dish with 25 mL distilled water and swirling every 10 seconds.[24]

Folding endurance

To determine folding endurance, a strip of film was cut and repeatedly folded in the same place until it broke. The number of times the film could be folded in the same place without breaking gives the value of the folding endurance.

EDAX analysis

The morphological structure of the film (PU+EM) was observed using a scanning electron microscope (Quanta-200) with an X-ray scattering system, operating at 5 kV under low vacuum. The samples were fixed on a copper support.

Estimation of drug content uniformity in the film

The wavelength of maximum absorption for enalapril maleate was determined by scanning a dilute aqueous solution of the drug in the wavelength region 200-400 nm, by a UV-vis spectrophotometer (Hewlett Packard 8453). A standard graph was prepared by measuring the absorption of a series of drug solutions of known concentrations. In order to ascertain the distribution of the drug in the polymeric film, the content uniformity test was performed. Specimens of 1 cm^2 were cut from the film in three different places and dissolved separately in 100 mL of water. The content of the drug in the polymer film was determined spectrophotometrically and the average drug content of three replicate samples was presented.

***In vitro* dissolution studies**

In vitro dissolution of the pullulan-enalapril maleate fast-dissolving film and enalapril maleate tablets was carried out in an SR 8 plus Series apparatus. The dissolution mediaused were phosphate buffer (pH 6.8) and distilled water (100 mL). The temperature was maintained at 37±0.5°C, throughout the experiment. A sample (3 mL) of the solution was withdrawn from the dissolution apparatus at predetermined time intervals. The samples were replaced with the same quantity of fresh dissolution medium (3 mL). The cumulative percentage of the drug released was determined using a UV-visspectrophotometer.

RESULTS AND DISCUSSION

FTIR Spectroscopy

Fig. 1 presents the FTIR spectra of pure enalapril maleate (EM), pullulan (PU) and the resulted mixture film (PU+EM). The FTIR spectra of enalapril maleate show bands at 1751 cm^{-1}corresponding to the vibration of the carbonyl group of ester and at 1727 cm^{-1}corresponding to carbonyl stretching of carboxylic acid.[25,26]

The spectra of pullulan exhibit a broad peak centered around 3434 cm^{-1} assigned to O-H stretching, due to the inter-molecular or/and intra-molecular hydrogen bonds. The peak around 2900 cm^{-1} corresponds to the C-H stretching, while the peak between 1200 and 1300 $cm^{-1}$is assigned to the C-O stretching vibration. On the other hand, the peak located at 1150 cm^{-1} appears to be the polysaccharide (1→4) glycosidic bond stretching vibration. The pullulan spectrum is similar to those described in previous reports.[27-29]

The FTIR spectra of the film (PU+EM) show that the characteristic frequency of the OH group is shifted to lower wavenumbers, from 3434 cm^{-1} (the hydroxyl group in pullulan) to 3312 cm^{-1} (in PU+EM film), which suggests the formation of an additional hydrogen bond between pullulan and enalapril.

^{1}H-NMR Spectroscopy

The recorded ^{1}H-NMR spectra for enalapril maleate and pullulan are in accordance with those reported in the literature.[30,31]For pullulan, there are peaks between chemical shifts of δ=5.40 ppm and δ=5.36 ppm, corresponding to anomeric protons of α-(1→4) linkagesandalso, chemical shifts of δ=4.92 ppm, showing the presence of the anomeric protons of α-(1→6) linkages.[30,31] The typical spectra of enalapril maleate (Fig. 2) show the presence of the aromatic ring, which is detectable at δ=7.32 ppm for the orto and para protons, and at δ=7.24 ppm for the meta protons.[32] Other proton signals lie in the aliphatic region. The ^{1}H-NMR spectra of the PU+EM film show signals characteristic of the pullulan as the main constituent, but also there are small signals originating from the aromatic ring of enalapril maleate, confirming the incorporation of the drug in thepolymer. Fortunately, the proton signal region from 5.36-5.40 ppm is characteristic only of the pullulan, whereas those located between 7.24-7.32 ppm belong only to EM. In this way, it becomes possible to calculate the percentage of the drug incorporated into the polymer, by the ratio of these two peak regions. Based on this

calculation, 67% EM (relative to the initial quantity of the EM used for the experiment) has been found in the pullulan film.

EDAX analysis

The elemental composition of the loaded film was determined by EDAX. The incorporation of EM into the PU matrix is confirmed by the presence of the N atom signal, while other atom signals (C, O) are derived from the polysaccharide matrix. The spatial distribution of the nitrogen atoms, which is characteristic of EM, is presented in Fig. 3.

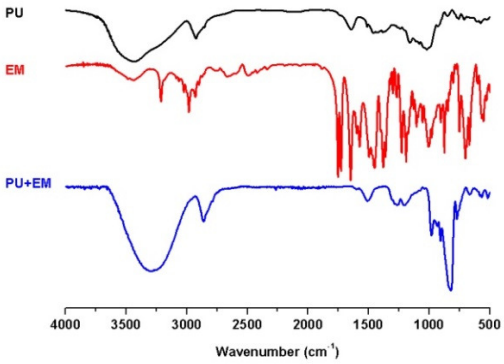

Figure 1: FTIR spectra of enalapril maleate, pullulan and blend film (PU+EM)

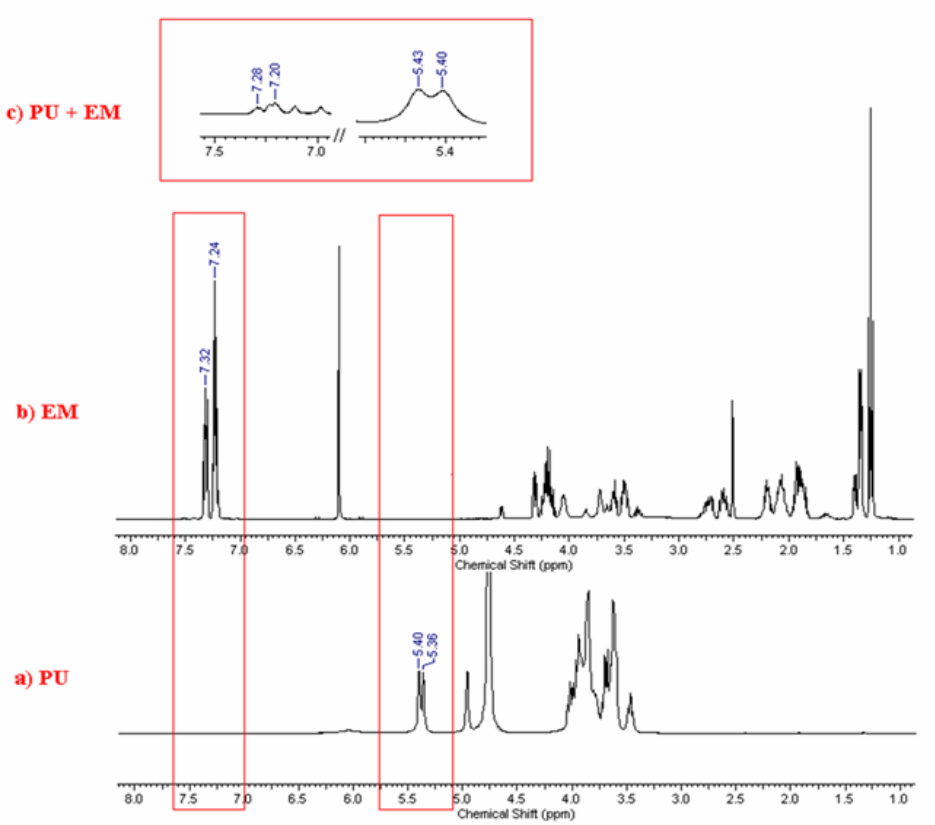

Figure 2: ^{1}H-NMR spectra of (a) pullulan in D_2O, (b) enalapril maleate in DMSO-d_6, (c) PU+EM blend film in D_2O

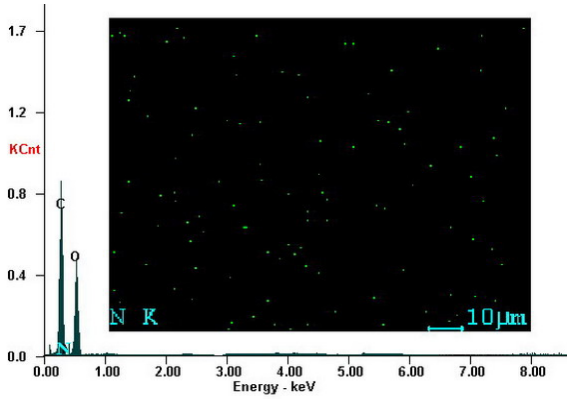

Figure 3: EDAX analysis of PU+EM blend film

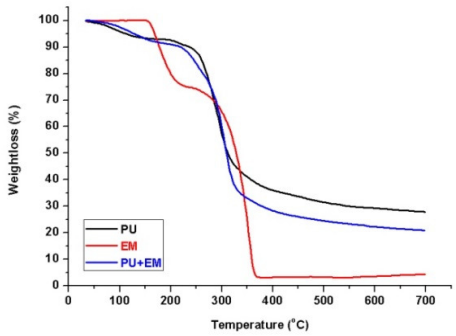

Figure 4: TG curves for enalapril maleate, pullulan and PU+EM blend film

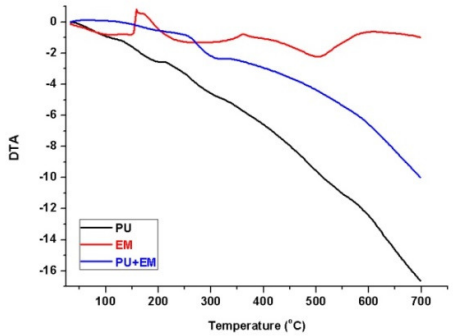

Figure 5: DTA curves for enalapril maleate, pullulan and PU+EM blend film

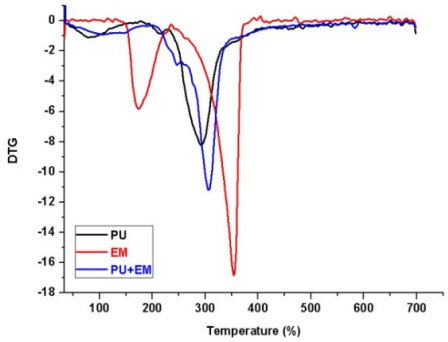

Figure 6: DTG curves for enalapril maleate, pullulan and PU+EM blend film

Thermogravimetric analysis

The characterization of the thermal behavior of the blend film (PU+EM) and of the two initial components (enalapril maleate and pullulan) was performed by using thermogravimetric analysis (TG), differential thermal analysis (DTA) and derivative thermogravimetric analysis (DTG). The TG, DTA and DTG curves of all investigated substances (enalapril maleate, pullulan and blend film (PU+EM)) are shown in Figs. 4, 5 and 6, respectively.

The data obtained from the thermal studies are presented in Table 1:the initial temperature at which thermal decomposition started T_{onset}, the temperature at which maximal degradation was recorded T_{peak}, the temperature at which the investigated process ended T_{endset}, the percentage of mass loss in each step W%, the quantity of residue left and the DTA characteristics.

Table 1
Thermogravimetric characteristics of enalapril maleate, pullulan and PU+EM blend film

Material	Thermal degradation step	T_{onset} (°C)	T_{peak} (°C)	T_{endset} (°C)	W (%)	Residue	DTA characteristic
Enalapril maleate	I	157	174	214	25.14	3.99	Endothermic
	II	254	354	363	70.97		Endothermic
Pullulan	I	85	106	157	8.27	20.65	Exothermic
	II	216	246	272	14.25		Exothermic
	III	272	306	329	56.38		Exothermic
Film (PU+EM)	I	54	79	125	6.27	27.49	Exothermic
	II	203	213	317	46.48		Exothermic
	III	317	292	383	18.84		Exothermic

As can be seen from the results presented in Table 1 and Figs. 4-6, the blend film (PU+EM) shows an entirely dissimilar thermal behavior to those of enalapril maleate and pullulan.

The thermogravimetric curve of enalapril maleate (Fig. 4) indicates twosteps of degradation. The first step of degradation corresponds to the loss of maleic acid together with water. Water is eliminated in an intramolecular cyclization reaction of the enalapril maleate molecule, which leads to the formation of diketopiperazine. In the second step, the total amount of the organic matter is decomposed to volatile compounds. The final temperature of degradation, indicated on the DTG curve,(354°C) corresponds to a weight loss of 95% of the initial substance(TG curve). The DTA analysis indicates a two-step endothermic thermal decomposition, the first at 157°C with a heat of fusion of 368.8 J/g and the second at 360°C with a heat of fusion of 127.9 J/g. These results are in agreement with the data reported in the literature.[25,33,34]

For pullulan, the thermogravimetric behavior also indicates the presence of two steps of degradation.[28,35] The second step of degradation is larger, with a weight loss of over 56% at about 330°C. The DTA curve shows a significant thermal degradation without effect. The thermogravimetric analysis of the enalapril maleate-pullulan film reveals that the most significantstep of degradation occurs at 292°C, because the weight loss was of 46.48%. For the two components, the most important step of degradation occurred at 306°C for pullulan and at 354°C for enalapril maleate. This difference suggests the presence of hydrogen bonds between the two constituents.The first step of degradation of the enalapril maleate-pullulan film, with a weight loss of 6.27% at 79°C, may be due either to the decomposition of proline nucleus or to the presence of solvent traces in the film. These statements are also supported by the IR analysis, which indicate shifts of the absorption bands caused by the hydroxide group in pullulan, and a considerable decrease of the peaks due to carboxyl and amide type groups in enalapril maleate.

Physicochemical evaluation of the film

The prepared film was evaluated as to its physicochemical properties, such as solubility, uniformity of thickness and weight, surface pH and folding endurance. The obtained values are presented in Table 2.

The results of the disintegration test highlighted an 18 seconds time interval, necessary to disintegrate the polymeric film. According to the recommendations provided by the Food and Drug Administration (FDA), this time interval corresponds with the requirements for muchoadhesive buccal films, which must disintegrate within 30 seconds.[24,25]

Estimation of drug content uniformity in the film

The wavelength of the maximum absorption for enalapril maleate was determined by scanning a drug solution with the concentration of 10 μg/mL in the region 200-400 nm and was found to be 208 nm. The first stage of this determination consisted in measuring the absorption for three series of drug solutions in the concentration range of 2 to 16 μg/mL. The results shown in Table 3 are found to comply with Beer's law. Fig. 7 shows the calibration curve of enalapril maleate with a regression value of 0.9995 and slope of 0.0638. The calculationsof the drug content and its uniformity are based on the calibration curve.

Table 2
Evaluation of physicochemical parameters ofenalapril maleate, pullulan and PU+EM blend film

Property	EM	PU	Film (PU+EM)
Physical appearance	White powder	Almost white powder	Uniform surface and transparency
Solubility	Freely soluble in methanol and dimethylsulfoxide, slightly solublein water	Freely soluble in water	Soluble in water, insoluble in methanol
Melting point (°C)	144-147	Not applicable	252-255
Thickness (mm)	-	-	0.17±0.02
Weight (mg)	-	-	14±0.08
Surface pH	2.6	-	6.85±0.03
Folding endurance	-	-	181±3.51
Disintegrationtime (s)	-	-	18±0.12

Data = Means±SD (n = 3); SD – standard deviation

Table 3
Absorbance values for calibration curve of enalapril maleate at 208 nm

EM concentration (µg/mL)	Absorbance				SD
	I	II	III	Average	
2	0.1192	0.1221	0.1187	0.1200	0.0018
4	0.2397	0.2383	0.2402	0.2394	0.0010
6	0.3541	0.3495	0.3521	0.3519	0.0023
8	0.4907	0.4985	0.5085	0.4992	0.0089
10	0.6257	0.6312	0.6224	0.6264	0.0044
12	0.7507	0.7524	0.7486	0.7506	0.0019
14	0.8792	0.8821	0.8725	0.8779	0.0049
16	1.0092	1.0012	1.0102	1.0069	0.0049

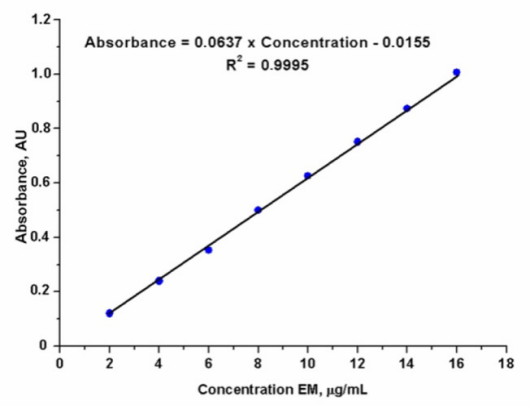

Figure 7: Calibration curve of enalapril maleate

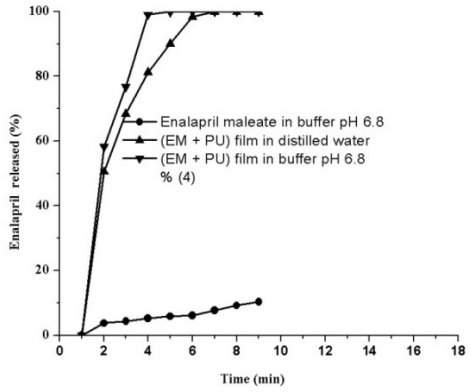

Figure 8: Comparative study of dissolution profiles of enalapril maleate and PU+EM blend film

The second stage of this determination consisted in measuring the absorption of each solution that contained 1cm^2 of film. On the basis of the calibration curve, the concentration of enalapril maleate was calculated. The amount of drug present in 1 cm^2 film is shown in Table 4.The results presented in Table 4 show that, from 100 mg of enalapril maleate, only 67.71 mg were loaded into the polymer and the drug content uploaded to 1 cm^2 film was 1.185 mg.

***In vitro* dissolution studies**

As shown in Fig. 8, a comparison was made between the release profile of enalapril maleate fast-dissolving film and enalapril maleate tablets, in buffer solution (pH 6.8) and water. It was found that after the first 5 minutes of the dissolution test, the polymeric film released 100.30% of the enalapril maleate into the buffer solution and 98.31% into water. Under the same conditions, the enalapril maleate from tablets

presented a release of 5.1% into the buffer solution and 4.8% into water. These results validate both the experimental conditions of performing the *in vitro* dissolution test, and the recommendation to use these mucoadhesive buccal films for enalapril maleate administration.

Table 4
Drug content in PU+EM blend film

Absorbance (AU)	Calculated concentration (μg/mL)	Average concentration±SD	Calculated concentration (mg/1cm^2)	Calculated concentration (mg/57cm^2)	Theoretical concentration (mg)
0.7321	11.72				
0.7496	11.99	11.85±0.14	1.185	67.71	100
0.7395	11.83				

CONCLUSION

The results presented in this study show that the solvent casting technique enables the use of enalapril maleate and pullulan in the preparation of fast-dissolving oral films. The physicochemical analyses have shown no interactions between the drug and the polymer. The *in vitro* dissolution tests highlighted the opportunity of using the pullulan-enalapril maleate film as a mucoadhesivebuccal film for enalapril maleate administration.

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