Development and Mechanical Characterization of Eugenol–Cetalkonium Chloride Sustained Release Mucoadhesive Oral Film

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The aim of this study was to develop and characterize sustained release mucoadhesive films containing eugenol and cetalkonium chloride (HEC) for oral applications. A solvent casting method was used with hydroxypropyl methylcellulose as a film-forming polymer. Physical and mechanical characterization, mucoadhesion, content uniformity, dissolution, and drug permeation were studied on blank film (HB), film containing eugenol (HE), film containing cetalkonium chloride (HC), and films containing eugenol and cetalkonium chloride (HEC) of different casting weight. The tensile strength, viscosity, and pH of HEC films were found to be significantly lower than those of HB films. HEC films with 30 g of casting weight were thicker than HEC films with 25 g of casting weight and drugs were less uniformly distributed in them. It was observed that 30 g casting weight was preferred for film preparation because of higher drug flux and more sustainable drug availability after a period of dissolution. POLYM. COMPOS., 00:000–000, 2015. © 2015 Society of Plastics Engineers

INTRODUCTION

Oral ulcers are painful lesions of the oral mucosa that involve tissue loss in the epithelium and underlying connective tissue [1] and can lead to inflammation and bleeding. Many factors can lead to oral ulcers, and most oral ulcers require specific management in addition to local treatment [2]. Local treatment offers advantages such as protection of the ulcerated site, pain relief, reduction of inflammation, and control of secondary infections. Gels, sprays, mouthwashes, pastes, and lotions are among the formulations available for oral ulcer treatment [3]. However, sustained drug release single dose oral films are considered beneficial because other formulations such as pastes, gels, sprays, and lotions suffer from the problems of dose inaccuracy and uneven drug application at the ulcerated site. Although antiseptic mouthwashes are useful in treating secondary bacterial infections in mucosal ulceration, they need to be used frequently and vigorously to be effective [2], which causes consumer inconvenience. Furthermore, mouthwashes are not convenient to carry and handle. Oral films are the ideal solution to these problems because they are convenient to carry and able to deliver accurate doses to ulcerated sites.

Hydrophilic polymers are generally recognized as mucoadhesive [4]. Mucoadhesive polymers such as hydroxypropyl methylcellulose are used in film preparations permitting the film to remain in contact with the mucosa. Their rapid swelling properties allow them to interact with mucin molecules in the buccal mucosa, in addition to hydrophilic properties that result in rapid disintegration and impart good mouth feel and mechanical properties [5]. The mucoadhesive properties of polymers are affected by factors such as molecular weight, flexibility, hydrogen bonding capacity, cross-linking density, charge, concentration, and polymer hydration [6].

Eugenol and cetalkonium chloride were chosen as model drugs for these studies (structure shown in Fig. 1). Traditionally, eugenol and cetalkonium chloride are used for antiseptic, anti-inflammatory, analgesic, and antibacterial applications in oral healthcare [7–10] and can be considered as potential candidates for oral ulcer treatment.

In this study, mucoadhesive films containing eugenol and cetalkonium chloride were developed and characterized using hydroxypropyl methylcellulose as the polymer. Tests were performed to characterize films with different casting weights (25 and 30 g), apart from mechanical characterization, dissolution, and permeation studies were performed in phosphate buffer saline (PBS) and simulated saliva (SS) solutions.

MATERIALS AND METHODS

Materials

Chemicals used in preparing hydroxypropyl methylcellulose (HPMC) films included HPMC with a molecular

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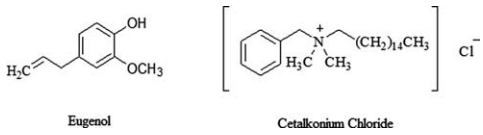

FIG. 1. Molecular structure of Eugenol and Cetalkonium Chloride.

weight of 86,000 (Fisher Scientific, USA), eugenol (Sigma-Aldrich, USA), cetalkonium chloride (Friedemann Schmidt, Australia), propylene glycol (Sigma-Aldrich, USA), and absolute ethanol (Merck, Germany). Disodium hydrogen phosphate anhydrous (R&M Chemicals, UK), potassium dihydrogen phosphate anhydrous (R&M Chemicals), sodium chloride (Sigma-Aldrich, Germany), 85% orthophosphoric acid (R&M Chemicals), sodium hydroxide pellets (Avantor Performance Materials, Sweden), calcium chloride anhydrous powder (R&M Chemicals), and alpha-amylase from *Aspergillus oryzae* (Sigma-Aldrich, Switzerland) were used in preparation of simulated saliva. Cellulose acetate membrane filters with a pore size of 0.45 μm (Membrane Solutions, USA) and type I-S mucin from bovine submaxillary glands (Sigma-Aldrich, USA) were used in characterization tests, whereas *n*-hexane for gas chromatography (Merck KGaA, Germany) was used in the extraction of eugenol.

Preparation

HPMC films were prepared using the solvent casting method. Four HPMC film formulations were prepared and labeled as HB, HE, HC, and HEC (Table 1). HB was the blank film without incorporated drugs (eugenol and cetalkonium chloride). HE contained eugenol, HC contained cetalkonium chloride, and HEC was loaded with both eugenol and cetalkonium chloride.

HPMC powder was weighed (1.5 g) and dispersed in 5 mL of ethanol. Distilled water was added to the dispersion, and the dispersion was stirred well until formation of a gel. Propylene glycol (2 mL) was mixed with the remaining portion of ethanol as a plasticizer. The drugs (eugenol and cetalkonium chloride) were dissolved in the mixture of propylene glycol and ethanol, and later, this solution was added to the HPMC gel and stirred continuously. Blank HPMC gels were also prepared. The blank (HB) and drug-loaded gels (HE, HC, and HEC) were cast onto plastic petri dishes (area of 55.42 cm^2) after air bubbles were removed. Twenty-five- and thirty-gram gels were cast to produce satisfactory films. After casting, the gels were dried in an oven at a temperature of 40°C for 24 h (25 g gels) or 36 h (30 g gels). Formulations were smooth and uniform and were kept in a desiccator containing silica gel beads at room temperature until further analysis.

Film Thickness

Film thickness was measured using a digimatic micrometer (Mitutoyo Corporation, Japan) for all types of films. Five measurements were taken for each film: one at the center and four around the perimeter. The mean and standard deviation of the five measurements were calculated and recorded for each film. The test was repeated three times for each film type, and the average values were calculated. The average of the thickness measurements for each individual film was then used for the mechanical properties tests.

Mechanical Properties

Tensile strength and percentage of elongation at breakpoint were evaluated using an Instron 5567 Universal Testing Machine (Instron Corporation, USA). Films were cut into dumbbell shapes of 30 mm in length and 5 mm in width using the ASTM standard dumbbell shape template. The dumbbell shaped specimens were stretched to breaking at a crosshead speed of 5 mm/min to examine the mechanical properties of the films. The test was run in triplicate for both casting weights of each of the film formulations. The mean and standard deviation of tensile strength and percentage of elongation at breakpoint breakage were calculated and recorded. Tensile strength (MPa) was calculated by dividing the maximum load (N) required to break the film by the cross-sectional area of the film (thickness × width). Percentage of elongation at breakpoint breakage was calculated by dividing the difference in length of the sample at the moment of breakage by the initial length of the sample before stretching (30 mm), and then multiplying this quantity by 100.

In vitro Mucoadhesion Studies

These studies were conducted using a Pro CT3 10K texture analyzer (Brookfield Engineering Laboratories,

TABLE 1. Formulations of hydroxypropyl methylcellulose (HPMC) films.

Formulation code	HPMC (g)	Propylene glycol (ml)	Eugenol (ml)	Cetalkonium chloride (g)	Ethanol (m)	Distilled water (ml)
HB	1.5	2	—	—	10	88.0
HE	1.5	2	0.4	—	10	87.6
HC	1.5	2	—	0.01	10	88.0
HEC	1.5	2	0.4	0.01	10	87.6

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USA) with a 10,000*g* load cell. Film samples were attached to the cylindrical probe (TA4/100) using double-sided adhesive tape. Freshly prepared 2% w/v mucin solution (100 μl) was spread on the fixture base table (TA-DEC). The cylindrical probe (TA4/100) attached to the mobile arm of the texture analyzer was brought into contact with the mucin solution for 5 min and then withdrawn at a speed of 0.5 mm/s. Adhesive force and adhesiveness were calculated from the peak and the area under the curve, respectively, in the force versus distance profile.

pH Values of Hydrogels and Rehydrated Films

After formulation, hydrogel pH was measured using a pH meter (Fisher Scientific, USA). For pH measurements of rehydrated films, a film of each of the two casting weights was dissolved in 5 ml distilled water and the pH of the obtained solution was measured. pH measurements were conducted in triplicate for hydrogels and films of each formulation.

Morphology Studies

Morphology studies of films were conducted using a polarized microscope U-TV1X-2 (Olympus BX41TF with U-TV1X-2, Tokyo, Japan). Films were trimmed to 1.5 × 1.5 cm and observed under 4× and 10× magnifications.

Rheological Studies

Rheological profiles of hydrogels of each of the formulations were measured using a Malvern GEM-200–903 Gemini 11 200 Rheometer (USA) with a cone and plate system of 2°/20 mm. The gap size of the rheometer was set to 70 μm and measurements were conducted at 25°C. The shear rate was increased to 500/s in 3 min followed by a decrease to 0 at a constant rate in the same time interval. Measurements were run three times for each sample. The average of apparent viscosity for each sample was obtained from the flow curve apex at 500/s.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy was conducted by FTIR spectrophotometer (Perkin-Elmer, Fremont, USA). For this purpose of characterization, Eugenol, Cetalkonium chloride, HPMC and the prepared final formulation oral film (HEC) were scanned from 4,000 to 500 cm^{-1}.

Drug Content Uniformity

Film samples of 1.5 × 1.5 cm^2 in size were trimmed from each film at three different random sites using scissors. Each of the film samples was dissolved in 30 ml PBS at pH 6.0 for 12 h at room temperature. The mixture was shaken and the solution was filtered before the drug content was analyzed using a UV-1601 Vis-spectrophotometer (Shimadzu Corporation, Japan). The absorbance was measured at a wavelength of 263.5 nm for cetalkonium chloride and PBS at pH 6.0 was used as the blank solution. The filtered solution was extracted with hexane to allow the measurement of eugenol content. Using hexane as a blank solution, the absorbance of extracted eugenol was measured at a wavelength of 282 nm with a UV-1601 Vis-spectrophotometer. The absorbance values were recorded and the test was repeated using freshly prepared simulated saliva as a dissolution medium. Tests were conducted in triplicate for 25 and 30 g films containing eugenol and cetalkonium chloride (HEC). Average values were calculated and concentrations of eugenol and cetalkonium chloride in the film samples were determined from the calibration curve.

In vitro Dissolution Test

Film samples of 1.5 × 1.5 cm^2 in size were cut and attached to the bottoms of beakers with the aid of PBS. Ten milliliters of PBS at pH 6.0 was added to the beakers. pH 6.0 was chosen as a compromise between the optimum reaction pH (pH 6.0–6.5) and the pH of the oral cavity (pH 5.8–7.6) [11, 12]. Beakers were shaken horizontally at 50 rpm in a water bath (Julabo SW22, Germany) which was maintained at 37°C. Beakers were sealed with parafilm to avoid evaporation of the dissolution medium and to mimic the humid environment of the mouth. A non-agitated system was selected to perform the dissolution test to eliminate any effect of turbulence on drug release rate. Samples of 4 ml were withdrawn and replaced with 4 ml of fresh PBS at regular time intervals for 40 min. Samples were filtered before being subjected to analysis using a UV-1601 Vis-spectrophotometer (Shimadzu Corporation, Japan). Absorbance values were measured at a wavelength of 263.5 nm for cetalkonium chloride and PBS at pH 6.0 was used as the blank solution. To measure eugenol content, the filtered samples were extracted with hexane and measured at a wavelength of 282 nm. Hexane was used as a blank solution. The absorbance values were recorded and the test was repeated using freshly prepared simulated saliva as a dissolution medium. Tests were conducted in triplicate for 25 and 30 g films containing eugenol and cetalkonium chloride (HEC).

Franz Cell Drug Release

The Franz cell drug release test was performed on 25 and 30 g films containing both eugenol and cetalkonium chloride. A Franz diffusion cell with a 3.5-ml receptor volume and cellulose acetate membrane with a pore size of 0.45 μm (Membrane Solutions, USA) were used in this study. PBS at pH 6.0 or simulated saliva (SS) were the receptor mediums in this study.

A beaker containing 500 ml of PBS at pH 6.0 was maintained at a temperature of 37°C by placing it into a 37°C water bath. The receptor chamber was filled with PBS until it reached the sampling port. A cellulose acetate membrane was mounted on top of the receptor chamber. Film samples of 1.5 × 1.5 cm^2 in size were cut and placed above the cellulose acetate membrane. A flat ground joint was placed above the film. The donor and receptor compartments of the Franz diffusion cell were assembled together with a clamp. The temperature of the system was maintained at 37°C throughout the experiment to mimic the human body temperature. The receptor chamber was continuously stirred at 150 rpm using a magnetic bar.

Samples of 1 ml were withdrawn from the receptor medium every 30 min for 6 h and replaced with an equal volume (1 ml) of fresh PBS equilibrated at the same temperature to maintain conditions. The samples were diluted with 2.5 mL of PBS, filtered, and analyzed using a UV-1601 spectrophotometer at a wavelength of 263.5 nm for cetalkonium chloride and a wavelength of 282 nm for eugenol. Eugenol was extracted from the filtered samples using hexane. The blank solutions were PBS at pH 6.0 for cetalkonium chloride and hexane for eugenol. The absorbance values of the samples were recorded. Concentrations of eugenol and cetalkonium chloride were calculated from the calibration curves. The cumulative amount of permeated eugenol and cetalkonium chloride per square centimeter of membrane surface area was calculated and plotted against time (in hours). Drug flux was also calculated with the help of Fick's diffusion equations [13].

$$J = -D[\mathrm{dc}/\mathrm{dx}] \quad (1)$$

$$\text{Permeability coefficient } (K_p) = J/C \quad (2)$$

Where J is equal to mass flux, D is equal to diffusion constant dc/dx is concentration gradient which can be calculated from the slope, and C is the concentration of drug in the donor compartment. To further confirm the release mechanism, the percentage cumulative release data have been fitted to the empirical equation. The technique is used in many other release studies as well [14–16].

$$M_t/M_\infty = \mathrm{kt}^n \quad (3)$$

Here, M_t/M_∞ is fractional drug release at time t, k is a kinetic parameter that represents drug–polymer interaction, and n is an empirical parameter characterizing the nature of the release mechanism. Experiments were performed in triplicate and repeated using simulated saliva (SS) as the receptor medium.

Statistical Analysis

Experimental data were expressed as mean ± standard deviation. Microsoft Excel 2007 was used to tabulate data. All of the data were analyzed using independent-samples *t*-tests and one-way analysis of variance (ANOVA) with SPSS statistical software (version 21). pH tests for hydrogel and rehydrated films were analyzed using paired-samples *t*-test. For independent-samples *t*-test and paired-samples *t*-test, the significance threshold was $p < 0.05$. For one-way ANOVA, the difference between the tested groups was considered to be significant if the *p*-value was less than 0.05, and Tukey's HSD multiple comparison *post hoc* tests were used to determine whether the differences between the groups were statistically significant.

TABLE 2. Mechanical properties for film formulations with 25 and 30 g casting weights (data expressed as mean ± SD).

Formulation code	Thickness (mm)	Tensile strength (MPa)	Percentage of elongation at break (%)
HB (25 g)	0.059 ± 0.009	26.85 ± 1.86	59.57 ± 2.35
HB (30 g)	0.069 ± 0.007	29.33 ± 1.94	57.43 ± 5.09
HE (25 g)	0.045 ± 0.006	16.59 ± 1.13	49.99 ± 3.15
HE (30 g)	0.066 ± 0.010	11.11 ± 0.68	42.95 ± 1.23
HC (25 g)	0.059 ± 0.009	28.67 ± 2.43	49.21 ± 7.77
HC (30 g)	0.072 ± 0.009	11.01 ± 0.80	55.83 ± 1.31
HEC (25 g)	0.059 ± 0.005	17.59 ± 1.55[a]	63.10 ± 4.90
HEC (30 g)	0.068 ± 0.009[a]	17.56 ± 1.03[c]	67.39 ± 0.46

[a,c]Significantly different ($p < 0.05$) compared with 25 and 30 g HB films.

[a]Significantly different ($p < 0.05$) compared with 25 g HB and HEC films.

RESULTS

Film Thickness

The film thickness in this study was consistent for each of the film formulations and increased as casting weight increased. Thus, 30 g films were thicker than 25 g films, as shown in Table 2. We found that 30 and 25 g casting weight films produced thicknesses in the range from 45 to 72 μm.

Mechanical Properties

In tensile strength measurements, significant differences ($p < 0.05$) were observed between the blank films and the HEC films. The tensile strength of blank films was higher than that of HEC films (Table 2).

In vitro Mucoadhesion Studies

Adhesive force (N) and adhesiveness (mJ) were the parameters used to study the mucoadhesion of films in this study. In general, blank films exhibited higher adhesive force and adhesiveness than HEC films (Table 3). In adhesive force measurements, no significant differences were observed between blank films and HEC films. In

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TABLE 3. Texture analysis of blank films and films containing eugenol and cetalkonium chloride (HEC) with 25 and 30 g casting weights (data expressed as mean ± SD).

Formulation code	Adhesive force (N)	Adhesiveness (mJ)
HB (25 g)	2.93 ± 0.72	3.37 ± 1.10
HB (30 g)	3.47 ± 0.82	3.97 ± 0.55
HEC (25 g)	1.99 ± 0.39	1.63 ± 0.31
HEC (30 g)	1.96 ± 0.64	1.40 ± 0.44[a]

[a]Significantly different ($p < 0.05$) compared with 25 g HB films.

adhesiveness measurements, 30 g HB films (3.97 ± 0.55 mJ) had significantly higher ($p < 0.05$) adhesiveness values than 30 g HEC films (1.40 ± 0.44 mJ), indicating that drug loading onto the blank films slightly reduced the mucoadhesivity of the HEC films.

pH Value of Hydrogels and Rehydrated Films

The pH values of films containing eugenol and cetalkonium chloride (HEC) were within the pH range of the oral cavity (pH values of 5.84 ± 0.18 and 6.05 ± 0.29, respectively, for 25 and 30 g rehydrated HEC films) as shown in Table 4.

Morphology Studies

All of the films showed even, homogenous and nonporous surfaces and no interfaces were seen in blank films (Fig. 2). Blank films (HB) had the smoothest texture of the films, with few surface agglomerates, whereas eugenol-loaded films (HE) showed more agglomerates than cetalkonium chloride-loaded films (HC) and films containing eugenol and cetalkonium chloride (HEC). Crystals were observed with the aid of a polarized lens in eugenol-loaded films (HE), cetalkonium chloride-loaded films (HC), and films containing eugenol and cetalkonium chloride (HEC). In contrast, no crystals were observed in blank films.

Rheological Studies

All of the hydrogel formulations showed pseudoplastic flow curves (Fig. 3). At a shear rate of 500/s, both of the formulations containing eugenol only (HE) and eugenol with cetalkonium chloride (HEC) showed significantly lower ($p < 0.05$) viscosities (0.18 ± 0.00 Pa.s for both HE and HEC) than the blank formulations (HB) and formulations containing cetalkonium chloride only (HC)(0.30 ± 0.01 Pa.s for both HB and HC). Generally, all of the formulations in this study showed pseudoplastic flow, which indicated that hydrogels decreased in viscosity as shear rate increased.

TABLE 4. pH values of hydrogels and rehydrated films (data expressed as mean ± SD)..

Formulation code	Hydrogels	Rehydrated films	
		25 g	30 g
HB	6.55 ± 0.02	7.11 ± 0.19	7.15 ± 0.15
HE	6.12 ± 0.01	6.78 ± 0.08	6.58 ± 0.10
HC	6.29 ± 0.08	6.32 ± 0.02	6.33 ± 0.03
HEC	6.04 ± 0.20*	5.84 ± 0.18*	6.05 ± 0.29[a]

[a]Significantly different ($p < 0.05$) compared with hydrogel and 25 and 30 g HB formulations.

Drug Content Uniformity

Drug content uniformity was tested for films containing eugenol and cetalkonium chloride (HEC). No significant difference was observed for eugenol content and cetalkonium chloride content in 25 and 30 g films when they were dissolved in 30 ml PBS or simulated saliva (SS), as shown in Table 5.

Fourier Transform Infrared Spectroscopy

For this purpose of characterization, Eugenol, Cetalkonium chloride, HPMC and the prepared final formulation oral film (HEC) were scanned from 4000 to 500 cm^{-1} in FTIR. In HEC films, many peaks representing eugenol structure was observed (3,500–3,200 $cm^{-1,}$ O–H stretch, 1,500–1,400 cm^{-1} C–C stretch aromatics and 1,470–1,450cm^{-1} C–H bend alkanes). HPMC polymer peak of 1100-1,000 Stretching vibration of C–O–C group was also observed in the HEC FTIR spectra (as shown in Fig. 4).

In vitro Dissolution Test

Dissolution profiles of eugenol and cetalkonium chloride were shown in term of percentage dissolution of drugs over a time period of 40 min (Fig. 5). All of the films had lost their integrity at the end of dissolution test. For 25 and 30 g HEC films, the percentage of eugenol dissolution showed an increasing trend for 20 min, after which it decreased. However, when 25 g HEC films were dissolved in simulated saliva (SS), maximum percentage of eugenol dissolution was reached after 15 min, this is because of alpha amylase present in SS which resulted into quicker digestion of the oral films. When using PBS as the dissolution medium, 25 g HEC films showed a significantly higher percentage of eugenol dissolution (69.06 ± 1.15%) than 30 g HEC films (58.18 ± 2.06%).

In cetalkonium chloride dissolution tests, 25 g HEC films showed the highest percentage of dissolution at 15 min when PBS or SS were used as dissolution mediums. In contrast, 30 g HEC films showed the highest percentage of cetalkonium chloride dissolution at 20 min under the same conditions. Furthermore, 25 g HEC films had a significantly higher percentage of cetalkonium chloride dissolution than 30 g HEC films. In PBS, the maximum percentages of cetalkonium chloride dissolution for 25

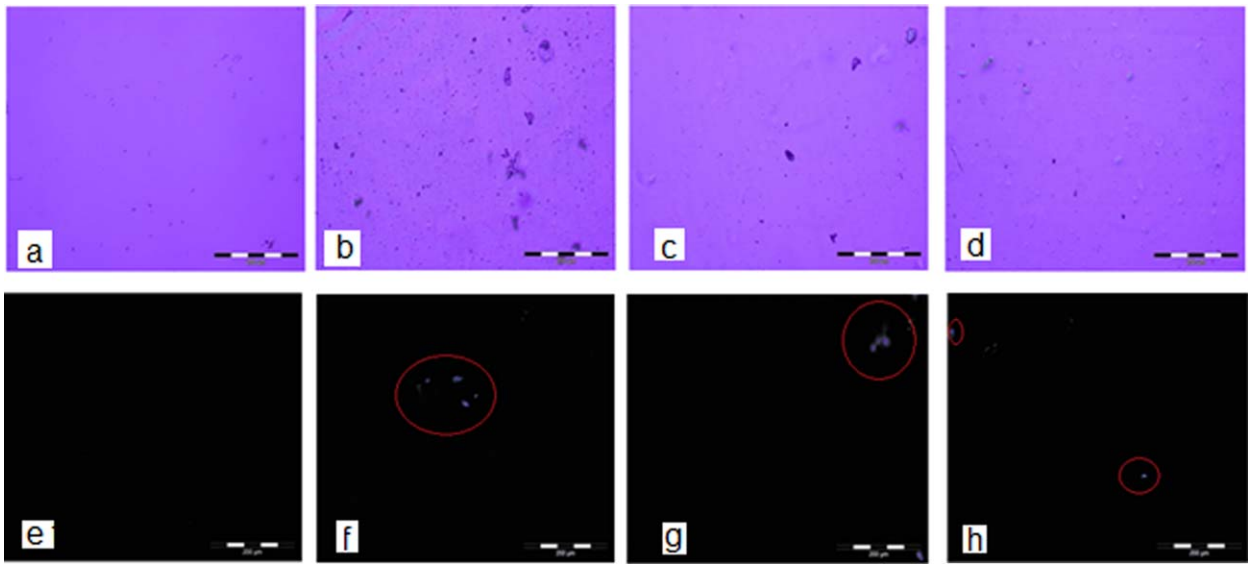

FIG. 2. Morphology of films under 10× magnification: (a) blank film (HB), (b) eugenol-loaded film (HE), (c) cetalkonium chloride-loaded film (HC), and (d) film containing eugenol and cetalkonium chloride (HEC). Morphology of films under 10× magnification with polarized lens: (e) blank film (HB), (f) eugenol-loaded film (HE), (g) cetalkonium chloride-loaded film (HC), and (h) film containing eugenol and cetalkonium chloride (HEC). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and 30 g HEC films were $85.54 \pm 9.62\%$ and $61.86 \pm 3.84\%$, respectively. In SS, the maximum percentage of cetalkonium chloride dissolution for 25 and 30 g HEC films were $83.07 \pm 8.21\%$ and $63.27 \pm 0.58\%$, respectively.

Franz Cell Drug Release

Drug release profiles of eugenol and cetalkonium chloride were shown in terms of the cumulative amount of drug that crossed the cellulose acetate membrane within 6 h (Fig. 6). Overall, there were no significant differences in eugenol and cetalkonium chloride release that were caused by the choice of PBS or SS as the receptor medium. When PBS was used as the receptor medium, 30 g HEC films exhibited higher drug fluxes of eugenol and cetalkonium chloride than 25 g HEC films. When PBS was used as the receptor medium, 25 and 30 g HEC films had eugenol fluxes of 0.070 ± 0.016 mg/cm^2/h and 0.092 ± 0.016 mg/cm^2/h, respectively. For cetalkonium chloride, 25 and 30 g HEC films had fluxes of 1.734 ± 0.226 mg/cm^2/h and 2.182 ± 0.372 mg/cm^2/h, respectively. From the drug release profiles of eugenol and cetalkonium chloride, it was also observed that cetalkonium chloride had a higher drug flux than eugenol (Table 5). Release results were also analyzed using the empirical equation. The initial 60% drug release data (i.e., linear region of the plots) were fitted to *Eq. 3*. Values of n increased with increasing crosslink density. These values for cetalkonium chloride ranged between 0.75 and 0.80, indicating anomalous drug release mechanism, and eugenol n value was greater than 0.89 indicating super case II transport [14, 15]. Case-II relaxational release is the drug transport mechanism associated with stresses and state-transition in hydrophilic glassy polymers which swell in water or biological fluids [16, 17]. The r^2 values for the cetalkonium chloride and eugenol release are shown in Table 5.

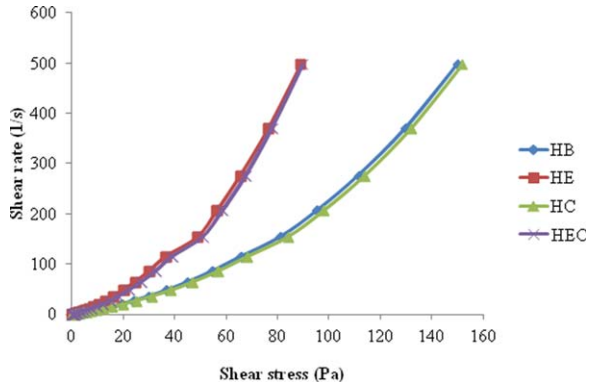

FIG. 3. Rheology profile of hydrogels of each of the formulations: blank formulation (HB), eugenol-loaded formulation (HE), cetalkonium chloride-loaded formulation (HC), and formulation containing eugenol and cetalkonium chloride (HEC). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

Film Thickness

Film thickness is important in assuring the dose accuracy of a particular film. If the thickness of a film is consistent across all of the films produced, then good dose

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TABLE 5. Drug content (mg/cm^2) and drug flux (mg/cm^2/h) of films containing eugenol and cetalkonium chloride (HEC) with 25 and 30 g casting weights using phosphate-buffered saline (PBS) and simulated saliva (SS) as dissolution mediums (data expressed as mean ± SD)..

	Drug content (mg/cm^2)		Drug flux (mg/cm^2/h)	
Formulation code	Eugenol	Cetalkonium chloride	Eugenol	Cetalkonium chloride
HEC (25 g)[a]	0.27 ± 0.03	4.77 ± 0.45	0.070 ± 0.016	1.734 ± 0.226
HEC (30 g)[a]	0.27 ± 0.07	4.54 ± 0.60	0.092 ± 0.016	2.182 ± 0.372
HEC (25 g)[b]	0.25 ± 0.02	4.56 ± 0.41	0.061 ± 0.009	2.173 ± 0.142
HEC (30 g)[b]	0.25 ± 0.10	4.73 ± 1.02	0.065 ± 0.010	1.751 ± 0.128

	$M_t/M_\infty = kt^n$	
Formulation code	Eugenol	Cetalkonium chloride
HEC (25 g)[a]	0.9661	0.9921
HEC (30 g)[a]	0.9838	0.9819
HEC (25 g)[b]	0.9930	0.9842
HEC (30 g)[b]	0.9794	0.9948

[a]PBS as dissolution medium.
[b]SS as dissolution medium.

accuracy can be expected. The film thicknesses in this study ranged from 45 to 72 μm which is generally considered to be in the ideal thickness range for buccal films [18].

Mechanical Properties

An ideal buccal film is flexible, elastic, soft, and strong enough to prevent breakage because of stress from mouth activities [19]. Thus, mechanical properties are important in assuring the ability of films to withstand stress imposed during manufacturing, handling, and administration [20].

Eugenol and cetalkonium chloride incorporation might cause HEC films to exhibit lower tensile strength than blank films. Indeed, differences in casting weight of HEC films did not affect the tensile strength of films of either formulation significantly. There is a significant difference between the blank films and HEC films in terms of percentage elongation at breakpoint.

In vitro Mucoadhesion Studies

Buccal films need to remain in contact with the mucosa for as long as drug delivery is ongoing [19]. Adhesive force (N) and adhesiveness (mJ) were the parameters used to study the mucoadhesion of films in this study. In general, blank films exhibited higher adhesive force and adhesiveness than HEC films (Table 3). In adhesive force measurements, no significant differences were observed between blank films and HEC films. The

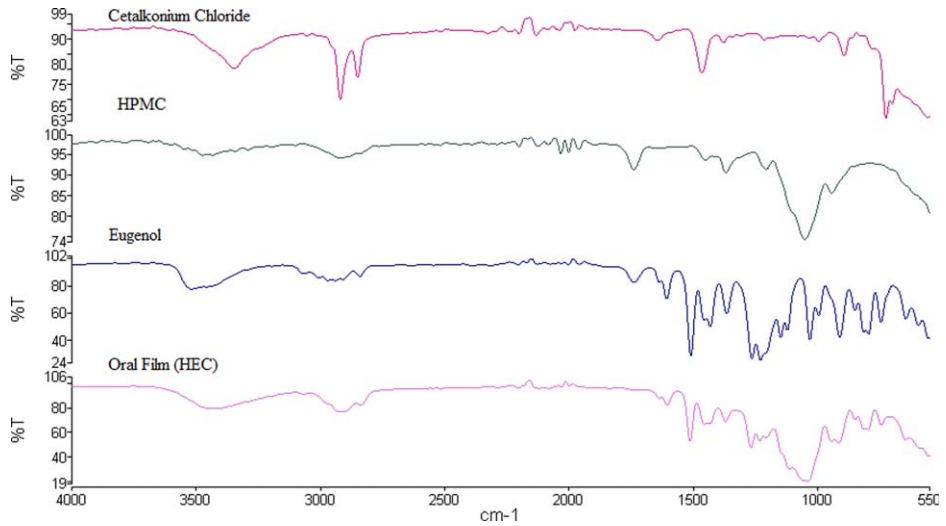

FIG. 4. FTIR characterization of the HEC oral film. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

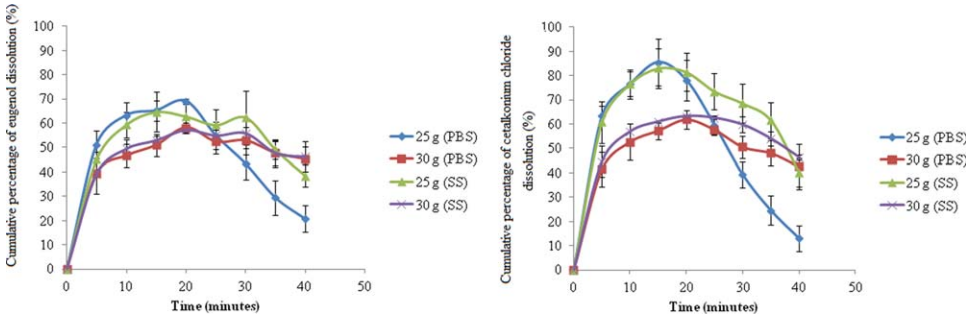

FIG. 5. (a) Drug dissolution profiles for 25 and 30 g films containing eugenol and (b) cetalkonium chloride (HEC) dissolved in phosphate-buffered saline (PBS) or simulated saliva (SS) (c) Franz cell diffusion cell. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

higher adhesiveness of blank films indicated that the drug loading slightly reduces mucoadhesivity of the oral films.

pH Value of Hydrogels and Rehydrated Films

In this study, films with eugenol and cetalkonium chloride had a lower pH (as shown in Table 4) than blank films, which might be due to the incorporation of drugs. Casting weight and drying process did not significantly alter the pH values of films, confirming that drug incorporation plays a primary role in determining the pH of films.

Morphology Studies

Eugenol, which is water insoluble, crystallized during incorporation into hydrophilic polymeric dispersions. The larger agglomerates observed in films containing eugenol may have been due to the aggregation of eugenol crystals. Although cetalkonium chloride is water soluble, small crystals were still observed under a polarized microscope, the water solubility of cetalkonium chloride resulted into reduction of agglomerates. This is of great importance because crystals are undesirable in films due to their negative effects on solubility and penetration. Crystallization of eugenol can be reduced by high speed stirring and slowly adding the alcoholic dispersion into the polymeric dispersion during film production process.

Rheological Studies

Generally, all of the formulations in this study showed pseudoplastic flow, which indicated that hydrogels decreased in viscosity as shear rate increased. In other words, films were able to revert to a hydrogel state without compromising rheological characteristics [21]. Formulations containing cetalkonium chloride had the same viscosity as blank formulations, whereas formulations containing eugenol had the same viscosity as HEC formulations. These results suggest that the low viscosity of the HEC formulation can be primarily attributed to the presence of eugenol in the formulation. The addition of eugenol can affect the interconnected network of polymers, resulting in markedly decreased hydrogel viscosity. Because the resulting HEC formulation is less viscous, homogenization is easier to achieve and drugs are more likely to be uniformly distributed throughout the film.

Drug Content Uniformity

In this study, eugenol and cetalkonium chloride were uniformly distributed throughout the entire 25 g film, as indicated by the smaller standard deviation value.

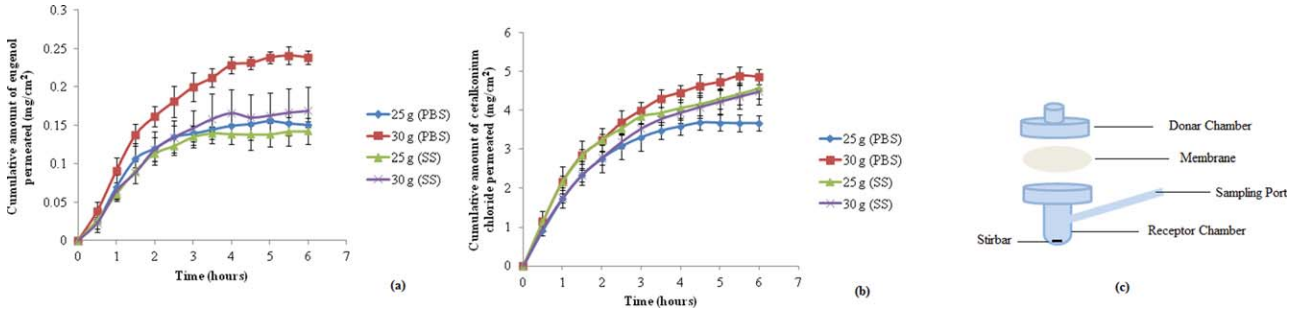

FIG. 6. Drug permeation profiles for 25 and 30 g films containing eugenol and cetalkonium chloride (HEC) using phosphate-buffered saline (PBS) or simulated saliva (SS) as receptor medium. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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Indirectly, it also demonstrated that the film preparation method was reproducible. However, drugs were less uniformly distributed in the 30 g HEC films, which showed a larger SD value than 25 g films. Self-aggregation has been identified as a primary reason for poor drug content uniformity in films. It has been suggested that a long drying process causes intermolecular attractive and cohesive forces to be favored, which leads to self-aggregation [22]. Thus, the longer drying times of the 30 g films may have caused their poor uniformity.

Fourier Transform Infrared Spectrophotometer

The FTIR characterization of HEC oral film showed a clear presence of eugenol inside the oral film formulation. The intensity and presence of peaks of eugenol in HEC film indicated the stability of eugenol inside the formulation as well. Although there was cetalkonium chloride added inside the formulation as well but perhaps due to the lesser content and higher aqueous solubility the cetalkonium chloride was not clearly observed inside the HEC FTIR spectra.

In vitro Dissolution Test

In vitro dissolution tests were performed to compare the dissolution rates of 25 and 30 g HEC films and to investigate the effects of PBS and SS on dissolution rates. The results clearly showed that PBS and SS did not significantly affect film dissolution. Eugenol and cetalkonium chloride films of 25 and 30 g casting weights achieved their maximum percentages of drug released at the same time (15 min for 25 g HEC films and 20 min for 30 g HEC films) except for the 25 g HEC films dissolved in PBS. Overall, cetalkonium chloride had a higher maximum percentage of dissolution than eugenol. Eugenol and cetalkonium chloride dissolution did not reach 100%, which may have been caused by poor drug solubility and the limited swelling properties of HEC films at a pH of 6.0 [23]. Films with a casting weight of 25 g had a higher maximum dissolution percentage than those with casting weights of 30 g, which showed that the percentage of drug dissolution is governed by the polymer content of the film. The films with 30 g casting weights had higher polymer contents than those with 25 g casting weights, which resulted in decreased drug dissolution and inhibited drug diffusion [24].

Franz Cell Drug Release

Franz cell drug release studies were performed to compare eugenol and cetalkonium chloride release from 25 and 30 g HEC films dissolved in PBS and SS. Results from this study revealed that the drug flux (eugenol and cetalkonium chloride) of 30 g HEC films was higher than 25 g HEC films when PBS was used as the receptor medium. Thus, an increase in casting weight is associated with increases in drug content and polymer content, and 30 g HEC films are expected to contain more drug. The release of poorly water soluble drugs is primarily governed by surface erosion, whereas the release of highly water soluble drugs is primarily governed by diffusion (depending on polymer molecular weight and concentration) [25]. Eugenol is water insoluble and thus uses surface erosion as its main route of drug release, whereas cetalkonium chloride is water soluble and is believed to be released through diffusion. Drug release from films can be initiated by hydration of dried films with fluid, which is followed by film swelling and gel formation, after which drugs diffuse through the swollen gel. Eventually, erosion of the polymer gel and diffusion will occur, which determine the rates of drug release [26].

CONCLUSIONS

Oral mucoadhesive sustained release films containing eugenol and cetalkonium chloride were successfully developed using hydroxypropyl methylcellulose polymer. The casting weights 25 and 35 g of films showed significant variation in mechanical properties, mucoadhesion, pH, and drug content uniformity. All oral films exhibited pseudoplastic behavior, which indicated that films were able to revert to a hydrogel state without compromising rheological properties. The 30 g films showed higher drug flux (0.092 ± 0.016 mg/cm^2/h and 2.182 ± 0.372 mg/cm^2/h for eugenol and cetalkonium chloride, respectively) and sustainable drug availability after a period of dissolution. Drug release during dissolution exhibited a burst in the first 5 min, as evidenced by the steep slope of the dissolution profiles of eugenol and cetalkonium chloride. Burst release of eugenol and cetalkonium chloride can be beneficial by producing high drug concentrations at the action site within a short period of time, which leads to fast onset of pharmacological effects.

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