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Article

A novel dosage form for buccal administration of bupropion

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Bupropion is an antidepressant used in the treatment of smoking. The purpose of this study was to prepare controlled-release hydrogel films for buccal administration of bupropion and investigate its physicochemical and cytotoxic properties. The films were prepared from ultrapure sodium carboxymethylcellulose, hydroxypropylmethylcellulose K4M, and medium-viscosity chitosan. Evaluation of film physicochemical characteristics was based on scanning electron microscopy, bupropion content, mechanical strength (burst strength, relaxation, resilience, and traction), and cytotoxicity. Bupropion content in bilayer films was 121 mg per 9 cm^2. The presence of bupropion modified film mechanical strength, but did not compromise the use of this pharmaceutical form. As shown by the cytotoxicity results, films containing bupropion did not cause cellular damage. Bupropion administration in the form of hydrogel films is a potentially useful alternative in the treatment of smoking.

Uniterms: Bupropion/buccal administration. Bilayer films/drugs rellease. Drugs/controlled-release. Tobacco/control.

A bupropiona é um antidepressivo utilizado no tratamento do tabagismo. O objetivo deste trabalho foi a preparação de filmes hidrogelatinosos de liberação controlada para administração bucal de bupropiona. Os filmes foram preparados utilizando carboximetilcelulose sódica ultrapurificada, hidroxipropilmetilcelulose K4M e quitosana de média viscosidade. As características físico-químicas dos filmes foram avaliadas por microscopia eletrônica de varredura, teor de bupropiona, resistência mecânica (perfuração, relaxação, resiliência e tração) e citotoxicidade. Os resultados mostraram que os filmes em bicamada apresentaram teor de bupropiona de 121 mg por 9 cm^2 de filme e que a bupropiona modifica a resistência mecânica dos filmes, sem, no entanto, inviabilizar o uso desta forma farmacêutica. Os estudos de citotoxicidade mostraram que as formulações dos filmes contendo bupropiona não causam dano celular. Este estudo mostrou que a bupropiona veiculada na forma de filme hidrogelatinoso pode ser uma alternativa útil no tratamento do tabagismo.

Unitermos: Bupropiona/administração bucal. Filmes em bicamada/liberação de fármacos. Fármacos/liberação controlada. Tabagismo/controle.

INTRODUCTION

Smoking is one of the most important risk factors for developing cardiovascular disease (Jonas *et al*., 1992). Approximately 92% of smokers are aware of the detrimental effects of smoking, and stopping this habit reduces the risk of developing chronic diseases (Jonas *et al*., 1992). About 70% of smokers want to stop smoking, yet only 5% to 10% are successful. Studies on the simultaneous use of nicotine and bupropion have reported smoking cessation within six months (Gold, Rubey, Harvey, 2002; Jorenby *et al.,* 1999).

Bupropion (RS-2-(*tert*-butylamino)-1-(3-chlorophenyl) propan-1-one) has been prescribed as an antidepressant (Cicardo *et al.,* 1986) and was the first non-nicotinic drug used therapeutically against smoking (Paganelli *et al*., 2006). While most antidepressants

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selectively inhibit serotonin reuptake inhibitors or monoamine oxidase activity, bupropion inhibits dopamine uptake and noradrenaline (Ascher *et al*., 1995). Dopamine and catecholamine are involved in the symptoms of withdrawal syndrome (Ascher *et al*., 1995; Gobbi *et al*., 2003). Bupropion has a less potent effect on cardiac function than tricyclic antidepressants, but no anticholinergic or sympathomimetic effects (Soroko, Maxwell, 1983).

Although bupropion effectiveness and safety have been demonstrated (Roose *et al*., 1991; Holt *et al*., 2005), its pharmacological profile, dosage and administration, as well as its tolerability, clinical effectiveness, and safety for some groups of patients have been discussed, particularly when the drug is administered to cardiac smokers (Thompson, Rigotti, 2003) or patients with chronic obstructive pulmonary disease (Tonstad, Johnston, 2004). Paganelli *et al.* (2006) showed that at doses commonly used in humans (3 to 6 mg/kg) the compound caused pulmonary hypertension in normal dogs.

Bupropion is promptly absorbed in the gastrointestinal tract. Plasma concentrations peak in 3 h, remaining elevated in cases of renal failure. Bupropion undergoes extensive hepatic biotransformation by hydroxylation of tert-butyl and/or reduction of carbonyl groups. This hepatic metabolism is mediated by CYP2B6 and cytochrome P45. Its normal half-life of 21 h is extended in hepatic impairment. Approximately 84% of absorbed bupropion binds to plasma proteins, but release is slow (Reichert *et al*., 2008).

Buccal administration has been used for compounds that undergo extensive hepatic first-pass metabolism or that are poorly stable in the gastrointestinal environment. Hydroxybupropion, a metabolite of bupropion, is less effective than its parent compound, despite having similar potency (Hardman, Limbird, 2003; Rang, Dale, 2007). Hydrogel films provide a more effective manner of controlling drug dosages for buccal administration than other pharmaceutical forms (Semalty, Semalty, Nautiyal, 2010; Nappinnai, Chandanbala, Balaijirajan, 2008). Buccal administration can promote rapid, yet prolonged, responses, ensuring drug delivery to patients with swallowing difficulties (Nerkar, Gattani, 2012; Park *et al*., 2012).

In mucoadhesive hydrogel films, fast drug release is ensured by prompt hydrogel dissolution, while the slow erosion of polymers facilitate controlled release (Cid *et al*., 2012; Giovino *et al*., 2012; Wu *et al*., 2012; Yuan *et al.,* 2011).

Mucoadhesive films have been widely studied for oral drug absorption and can be potentially employed in the treatment of diabetes (glipizide and insulin carriers), hypertension, and angina pectoris (enalapril maleate, nitrendipine), oral candidiasis (fluconazole, clotrimazole), asthma (salbutamol), and Alzheimer's disease (donepezil) (Semalty, Semalty, Kumar, 2008; Sahni *et al*., 2008; Semalty, Semalty, Nautiyal, 2010; Nappinnai, Chandanbala, Balaijirajan, 2008; Singh *et al*., 2008, 2010; Yehia, El-Gazayerly, Basalious, 2009).

Preparing hydrogel bilayer films is a strategy to promote peak concentration within minutes while ensuring prolonged effect. This allows bupropion (with a half-life of 21 h) and its metabolites (20-37 h half-lives) to be administered only once daily.

The purpose of this study was to develop a suitable dosage form for buccal administration of bupropion. The mechanical properties, drug content, and cytotoxicity of hydrogel films containing bupropion, ultrapure sodium carboxymethylcellulose (CMC), hydroxypropylmethylcellulose K4M (HPMC), and medium-viscosity chitosan (MVC) were evaluated.

MATERIAL AND METHODS

All compounds employed—namely, Highly Purified sodium carboxymethylcellulose (CPKelco, Limeira, Brazil), hydroxypropylmethylcellulose (Methocel K4M®, Colorcon, Cotia, Brazil), Medium-viscosity chitosan (Sigma-Aldrich, São Paulo, Brazil), and bupropion hydrochloride (Dipharma Francis, Italy), were of pharmaceutical purity.

Preparation of hydrogels

Hydrogels compositions are shown in Table I. Formulations F1, F3, and F4 were prepared by dispersing the polymer and other components in purified water. The mixture was homogenized, mechanically stirred at 7000 rpm (T-25D Ultra Turrax disperser, IKA) for 5 min or until polymer lumps disappeared, and left to stand at 10 °C for 24 h for spontaneous elimination of air bubbles. Formulation F2, containing MVC, was prepared by dispersing this polymer in 0.1 M acetic acid. The dispersion was subjected to orbital stirring at 150 rpm for 48 h. For formulations containing bupropion (F3, F4), the drug was previously dissolved in purified water and incorporated into the HPMC K4M hydrogel.

To achieve the desired physical and chemical characteristics, F1, F2, and F3A were blended at a 1.5:4.5:15.0 (m/m) ratio, respectively, for preparation of the drug-amended hydrogel (mixture A). F1, F2, and F3B were blended at a 1.5:4.5:15.0 (m/m) ratio, respectively, to yield the placebo hydrogel (mixture B). The mixtures thus prepared were left to rest at 10 °C for 24 h for spontaneous elimination of air bubbles.

Characterization of hydrogels with and without bupropion

The resulting hydrogels underwent hydrogen ion concentration (pH) and viscosity measurements. For pH measurements (performed on a model 300 pH-meter, Analyzer, São Paulo, Brazil), they were dispersed at 10% in previously neutralized water. All measurements were performed in triplicate and recorded as log values. Viscosity was measured using a digital viscometer (I RDV Prime, Brookfield, São Paulo) equipped with an adapter for small samples. A coaxial spindle (SC4-28, Brookfield, São Paulo) was employed, and viscosity was measured at a constant temperature of 25 °C in a thermostatic bath (TC-550, Brookfield, São Paulo).

Preparation of hydrogel films

Mixtures A and B were separately used to prepare the films, employing a 12 cm–long, 3 cm–high acrylic dispenser (working volume: 30 cm³) with a 3 mm–wide slit on the lower face, from which the hydrogel was dispensed onto a degreased glass plate while the dispenser was moved against it at constant speed.

The films thus obtained (film A: bupropion-amended monolayer; film B: placebo monolayer) were weighed and kept at 23-25 °C in a dry atmosphere (60-70% RH) protected from light and environmental impurities. Upon reaching constant weight, as confirmed by three consecutive measurements at 60 min intervals, the films were removed from the glass plates, cut into 9 cm^2 pieces, and tightly sealed in laminated packaging material.

Preparation of bilayer films

To prepare the bilayer films, the hydrogel mixtures (MA and MB) were blended and spread on a degreased glass using the same equipment described for the monolayer films. The plate was kept at room temperature (23-25 °C) in a dry place (60-70% RH) protected from light and environmental impurities, until reaching constant weight. Once the films had dried completely, formulation F4A (Table I) was spread on film A (Figure 4) and Formulation 4B (Table I) on film B (Figure 4) using a 18 cm³ dispenser with a 1 mm–wide slit on the lower face. The glass plate was maintained at room temperature (23-25 °C) in a dry place (60-70% RH), protected from light and environmental impurities, until reaching constant weight. The bilayer film was removed from the plate and cut into 9 cm² pieces for evaluation of mechanical resistance and cytotoxicity, pH measurement, and content quantification.

Characterization of films with and without bupropion

Because discontinuous films are not resistant to handling, the evaluation of physicochemical and cytotoxic properties was preceded by selection of film samples, based on macroscopic appearance. Samples containing air bubbles, thickness variability exceeding 5%, or small superficial incisions were discarded.

TABLE I - Composition of hydrogels

Components	F1	F2	F3A	F3B	F4A	F4B
Sodium carboxymethylcellulose	2%	-	-	-	-	-
Hydroxypropylmethylcellulose K4M	-	-	3.75%	3.75%	3.75%	3.75%
Medium-viscosity chitosan	-	2%	-	-	-	-
Acetic acid, 0.1 M	-	50 mL	-	-	-	-
Sorbitol	0.5%	-	0.5%	0.5%	0.5%	0.5%
Propylene glycol	0.5%	-	0.5%	0.5%	0.5%	0.5%
Hydrogenated castor oil	0.5%	-	0.5%	0.5%	0.5%	0.5%
Sodium cyclamate	0.5%		0.5%	0.5%	0.5%	0.5%
Sodium chloride	0.5%	-	0.5%	0.5%	0.5%	0.5%
Citric acid	0.5%	-	0.5%	0.5%	0.5%	0.5%
Dye	10 drops	-	10 drops	10 drops	10 drops	10 drops
Flavor	30 drops	-	30 drops	30 drops	30 drops	30 drops
Bupropion hydrochloride	-	-	9g	-	27g	-
Purified water	100 mL	-	100 mL	100 mL	100 mL	100 mL

Measurement of weight and thickness of bilayer films containing bupropion

The samples of films containing bupropion were weighed on an analytical balance (DV215CD, Ohaus, São Paulo). The samples were cut into 9 cm² pieces and weighed. Film thickness was measured with calipers (150 mm, stainless steel, Lee Tools, São Paulo) at five points, one at each corner of the piece and one at its center. Weight and thickness were measured in triplicate.

Measurement of pH of bilayer films containing bupropion

For quantification of hydrogen ion concentrations, the film pieces were dissolved in 10 mL of purified water previously neutralized. The procedure was performed in triplicate and the results were recorded as log values.

Mechanical properties

The mechanical properties (burst strength, relaxation, resilience and traction) of mono- and bilayer placebo films and mono- and bilayer bupropion-amended films were evaluated in triplicate using a texturometer (TA-TX Plus, Stable Micro Systems, UK; Extralab, Brazil). The parameters adopted to evaluate the mechanical properties are listed in Table II.

To evaluate tensile strength, the ends of the 9 cm² film pieces were fixed by clamps (mini tensile grips) with brackets positioned 3 cm apart. The internal surfaces of the tabs covered with double-face adhesive tape to minimize the effect of the tab grooves on film resistance.

The films were tested for burst strength, resistance, resilience, and relaxation against a spherical probe with a 0.25 mm diameter. For this purpose, a film piece was placed between two perforated plates firmly attached to the equipment base. In the burst strength test, compressive strength was recorded at film rupture. In the resilience test, resilience was calculated (as percentage) by the equipment's software, which also calculated retained strength (as percentage) in the relaxation test.

Film morphology

The placebo films were evaluated both macroscopically and by SEM, using a 6390LV device (JEOL USA). SEM images were captured for the top, bottom, and lateral surfaces. To obtain the lateral views the films were cross-sectioned. The film samples were fixed on one side of a double-face adhesive tape set against an aluminum support. The carrier containing the film was coated with gold ions, the top layer of which was deposited in a vacuum at 3 mA electrical conductivity for 3 min, to a total thickness of ~150 Å.

Bupropion analytical curve

The analytical curve was obtained from aqueous solutions of bupropion at 20, 60, 100, 140, and 180 µg/mL. Bupropion concentrations in the solutions were determined by UV spectroscopy (800XI UV/Vis, Femto, São Paulo) at $\lambda = 252$ nm (BRAZIL, 2010a). The average absorbance ($n = 3$) for each concentration was calculated and employed to evaluate linearity and obtain the curve equation.

TABLE II – Parameters adopted to evaluate the mechanical properties of films

Parameters	Mechanical properties of resistance			
	Traction	**Burst strength**	**Resilience**	**Relaxation**
Apparatus	Mini tensile grip (part code A/MTG; batch 13101)	Film support rig (part code HDP/FSR; batch 13085)	Film support rig (part code HDP/FSR; batch 13085)	Film support rig (part code HDP/FSR; batch 13085)
Test mode	Tension	Compression	Compression	Compression
Pre-test speed	1 mm/s	2 mm/s	1 mm/s	1 mm/s
Test speed	2 mm/s	1 mm/s	0.5 mm/s	0.5 mm/s
Post-test speed	10 mm/s	10 mm/s	0.5 mm/s	10 mm/s
Target mode	Distance	Distance	Distance	Distance
Distance	10 mm	5 mm	2 mm	2 mm
Trigger type	Auto	Auto	Auto	Auto
Trigger force	5 *g*	5 *g*	5 *g*	5 *g*

Bupropion content of bilayer films

Bupropion content in the films was calculated by applying the curve equation, after determining bupropion concentrations by UV ($\lambda = 252$ nm). Briefly, a 9 cm^2 sample of film was dissolved in purified water to a theoretical bupropion concentration of 83 μg/mL (Brazil, 2010). The samples were randomly selected and the procedure was performed in triplicate.

Evaluation of cell viability

Cell viability was evaluated in human bone marrow lymphoblasts (cell line K-562). The cells were thawed and placed in culture flasks containing RPMI medium supplemented with 10% fetal bovine serum, required for replication. After 24 h the cells were plated in 6-well culture plates at 1105 cells/mL. The cells were then exposed to placebo monolayer, placebo bilayer, bupropion monolayer, or bupropion bilayer films for 24 h. Negative controls were cell not exposed to any films. Cell viability tests were performed at 6 and 24 h of exposure. Viability was evaluated in a 150 µL sample using a Tali image-based cytometer (Life Technologies). The sample was centrifuged for 5 min at 1500 rpm, the supernatant discarded, and the precipitate treated using a Tali apoptosis kit. For image reading, 25 µL of treatment material was dispensed onto specific plates and cell viability was assessed by green/red fluorescence.

RESULTS AND DISCUSSION

Preparation and characterization of hydrogels with and without bupropion

While preparing the hydrogels, ensuring a 24 h rest time at 10 °C was critical for full removal of air bubbles formed during the hydration of polymers and mixing of components. Table III shows hydrogel pH and viscosity values, expressed as means ($n = 3$). Formulations F1, F2, F3, and F4 are described in Table I. Formulation 4A and Mixture A correspond, respectively, to the apical layer and the basal layer of the bilayer films. The other formulations were used as placebos in the composition of films, to allow the influence of bupropion on the physicochemical features of hydrogels and films to be investigated, as well as their cytotoxicity.

Bupropion changed hydrogel pH values when used at a concentration of 27% (F4A), but did not significantly alter pH values when employed at 9% (F3A and Mixture A). The formulations containing bupropion (F3A, F4A, and Mixture A) exhibited reduced viscosity, probably due to their acidic character.

Preparation and characterization of hydrogel films

The technique employed for obtaining hydrogel films using acrylic dispensers on a glass plate proved suitable for bilayer films, ensuring homogeneous physical characteristics and appearance. Mean weight and thickness of 9 cm^2 film pieces were 236.25 mg ± 0.5 mm and 0.08 mg ± 0.05 mm, respectively.

Mechanical strength properties of bilayer films

Mechanical strength data are shown in Table IV and Figures 1a-d. Burst strength, relaxation, and resilience tests measure film ability to resist compression, while traction strength test measures the ability to resist elongation.

The results of the burst strength test (Figure 1a), which measures compressive strength as a function of time, revealed, as expected, that bilayer films are mechanically stronger than monolayer films. In the presence of bupropion, however, mechanical strength was

TABLE III – Mean pH and viscosity values of hydrogels

Formulation	pH	Viscosity (cP)
F1	4.24 ± 0.02	6833 ± 0042
F2	4.18 ± 0.19	2210 ± 0084
F3A	2.92 ± 0.10	6225 ± 0169
F3B	2.78 ± 0.05	7175 ± 0074
F4A	2.55 ± 0.12	6850 ± 0183
F4B	2.78 ± 0.07	7175 ± 0046
Mixture A (F1, F2, and F3A)	3.75 ± 0.09	6600 ± 0130
Mixture B (F1, F2, and F3B)	3.56 ± 0.15	7725 ± 0042

TABLE IV – Values (means ± SD) obtained for bilayer films (with and without bupropion) and monolayer (with and without bupropion), subjected to strength test

Films	Burst strength (kg)	Relaxation (kg)	Resilience (kg)	Traction (kg)
Mixture A + F4A	2.878 ± 0.215	1.757 ± 0.023	1.740 ± 0.106	4.992 ± 0.072
Mixture B + F4B	7.720 ± 0.422	1.910 ± 0.015	1.456 ± 0.076	4.142 ± 0.212
Mixture A	1.698 ± 0.179	0.958 ± 0.108	0.878 ± 0.049	2.348 ± 0.319
Mixture B	4.722 ± 0.624	1.053± 0.097	0.894 ± 0.022	2.360 ± 1.639

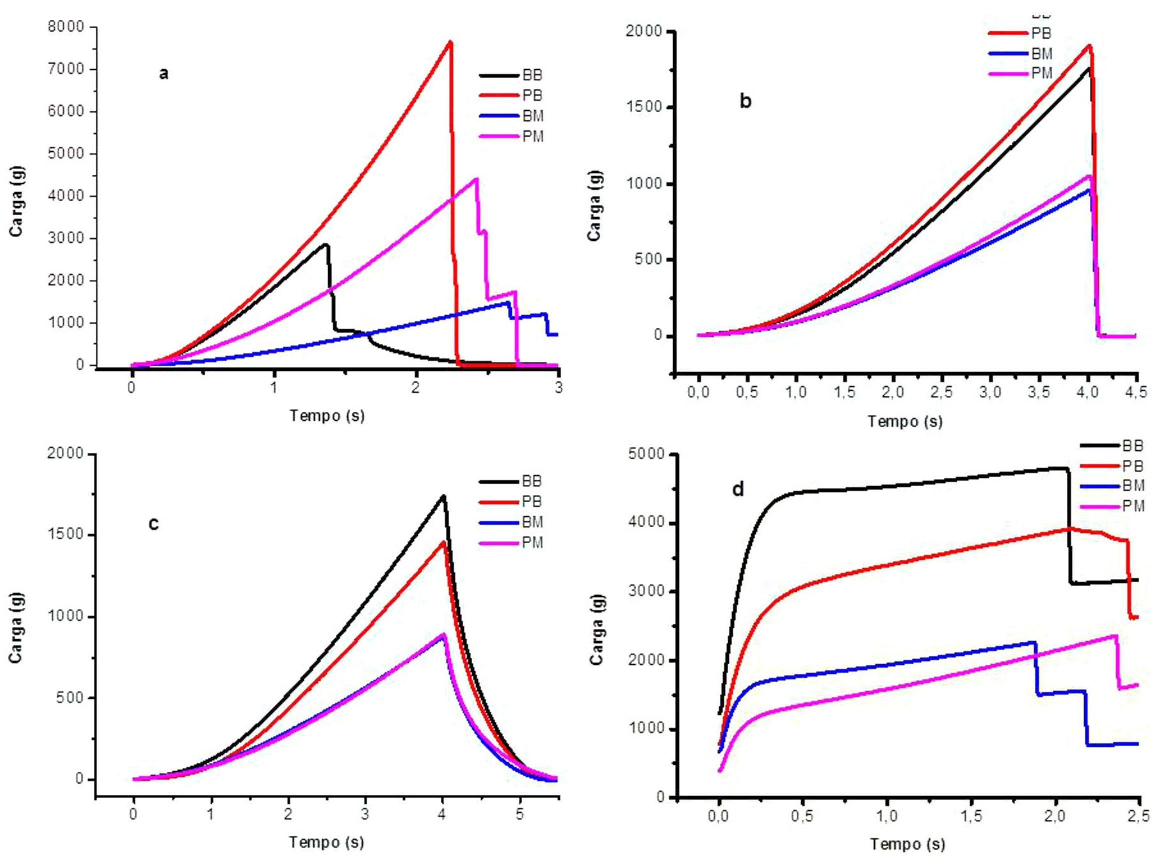

FIGURE 1 - Mechanical strength (a) Burst strength; (b) Relaxation; (c) Resilience; (d) Traction. BB (bilayer bupropion); PB (placebo bilayer); BM (bupropion monolayer); PM (placebo monolayer).

reduced, for both types of film. Presence of bupropion in crystalline or amorphous form dispersed in the polymer matrix decreased polymer reticulation affecting, negatively, the mechanical strength of films. Presence of bupropion decreased burst strength resistance by 62.72% and 64.04% in monolayer and bilayer films, respectively. In the absence of bupropion, bilayer films were 63.04% more resistant than monolayer films. Presence of bupropion caused the resistance of bilayer films to be 69.49% higher than in monolayer films.

Among the mechanical properties of polymer films, mechanical relaxation is the least investigated. Relaxation curves (Figure 1b) depict film viscoelasticity, an important property that provides information directly related to the conformation of macromolecules and molecular relaxation phenomenon (Ferry, 1980; Chandra, Sobral, 2000). The results shown in Figure 1b reveal significant differences between monolayer and bilayer films. Compared with monolayer films, bilayer films promote changes in the macromolecular conformation of polymers, increasing film resistance by roughly 55%. Irrespective of bupropion content, however, the difference between monolayer and bilayer films was of only 10%. In Figure 1b, the ascending curve is a result of the deformation constant. After 4 s, the force applied was not sufficient to maintain deformation. This behavior is characteristic of viscoelastic materials.

In the presence of bupropion, elastic deformation of the bilayer films was 50.46% higher than in monolayer films. In bilayer films containing bupropion, elastic deformation was 26.32% higher than in bilayer films devoid of bupropion. Elastic deformation was 0,894 kg s^{-1} in monolayer films containing bupropion and 0,878 kg s^{-1} in those devoid of drug.

Figure 1d shows the mechanical tensile strengths of monolayer and bilayer films with and without bupropion. Bilayer films, as expected, exhibited greater resistance to rupture than monolayer films. The presence of bupropion in bilayer films decreased tensile strength by 17.03%. In monolayer films, the yield stress of films containing bupropion was higher than in those devoid of drug. However, the yield strength of mono- and bilayer films containing bupropion was 0.51% lower than for monolayer films devoid of drug. The results obtained for tensile yield strength are characteristic of ductile materials. Malleability and flexibility are a desirable characteristic of films intended for buccal application, particularly on the hard palate.

Morphology of hydrogel films

The SEM images selected for Figure 2 show the morphology of a bilayer film (panel A, apical surface (MA); panel B, basal surface (F4A); panels C-F, lateral surface at 500, 1500, 2500, and 4500× magnification)

The apical surface (MA, panel A) is deposited on the glass plate surface, the porosity of which makes the film rougher. The basal surface (F4A, panel B) is smooth, and the stains, invisible macroscopically, may be due to the mixture of polymers (CMC, HPMC K4M, MVC).

In cross-section images (CF), the division between layers is clearly visible. The position of the image corresponds to that of film placement in the oral cavity. The upper, lighter layer of polymer (MA) adheres to the palate epithelium; the lower layer consists of F4A. Placed in the oral cavity, the basal surface becomes prone to rapid erosion, allowing faster release of the drug contained in it.

In the SEM images, the apical and basal layers have distinctive features, confirming the characteristics observed macroscopically. This allows oral appliances in the form of bilayer films to be tested for their drug delivery ability.

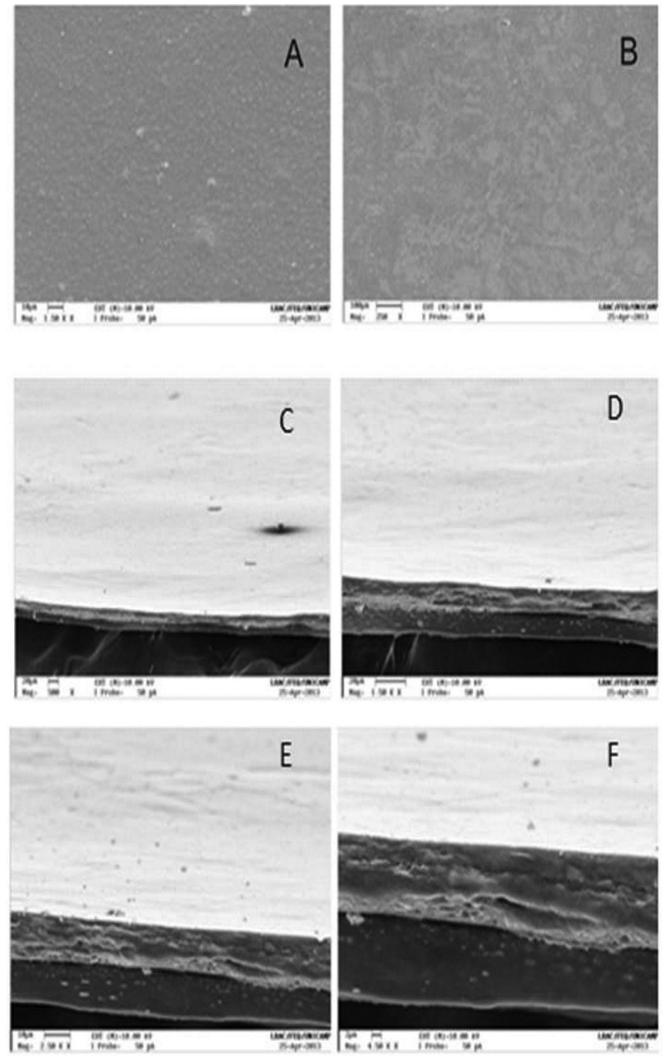

FIGURE 2 – SEM images of bilayer films: (A) upper surface; (B) lower surface; (C-F) cross-sections, at different magnifications.

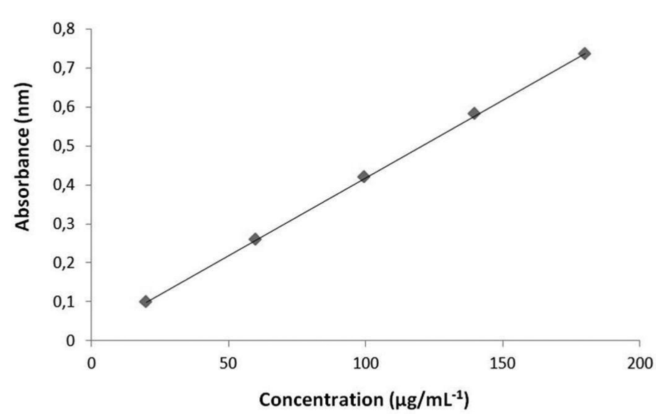

FIGURE 3 – Analytical curve constructed from the absorbance values of samples.

Evaluation of bupropion content in bilayer films

Bupropion content in bilayer films was calculated from the straight-line equation (Table V). Figure 3 shows the calibration curve obtained by UV spectroscopy (λ = 252 nm). A linear correlation (R^2 = 0.9999) was observed between absorbance at 252 nm and bupropion concentration in the range of 20 to 180 µg/mL. Bupropion concentration in a 9 cm^2 film piece was 121 mg, which corresponds to 80.67% of the expected content (150 mg/film).

TABLE V – Analytical parameters of the calibration curve

Parameters	Values
Angle of inclination with respect to the X axis	0.004
Point of intersection with the Y axis	0.0181
Coefficient of linearity (R^2)	0.9999
Equation of the slope	y = 0.004x + 0.0181

Cell viability

The percentages of living, apoptotic, and dead cells (Figure 4) showed that at 6 h of exposure the viability of K-562 cells dropped slightly. Cell cultures containing placebo monolayer films (MCP), bupropion monolayer films (MCB), placebo bilayer films (BCP), and bupropion bilayer films (BCB) exhibited vitality rates of 76%, 81%, 67%, and 47%, respectively, while for controls the rate was 79%. At 6 h of exposure (Figure 5) the rates of cell death were 20% (controls), 7% (MCP), 11% (MCB), 30% (BCP), and 10% (BCB).

Low cell death rates are expected at the beginning of treatment, since the cells are adapting to the new conditions. In this study, cell death rates for cells receiving MCP, MCB and BCB were lower than for controls. For those receiving BCB, death rate at 6 h was 50% higher than for controls. The results show that bupropion contents of up to 150 mg per 9 cm^2 of film (BCB) failed to induce cell death.

At 24 h of exposure, the cell vitality rate increased, while death and apoptosis rates decreased (Figure 4), revealing that the cells recovered vitality after the initial 6 h of exposure. Based on the vitality of controls (94%), if can be concluded that cells exposed to MCB (96%) and BCP (92%) behaved similarly and that polymer concentrations in the monolayer and bilayer films do not influence cell behavior. The lower growth rate observed in cells exposed to BCB can be explained by a higher concentration of residues from the cell-growth medium. For cells exposed to MCP, the viability index at 24 h of exposure (79%) cannot be explained, since no cell death occurred (Figure 4).

Death rates for controls and cells exposed to MCB, BCP, and BCB were 5%, 7%, 9%, and 17%, respectively (Figure 4), with no significant differences between controls and cells exposed to MCB or BCP. In contrast, the death rate of cells exposed to BCB was roughly 3 times as high as for controls.

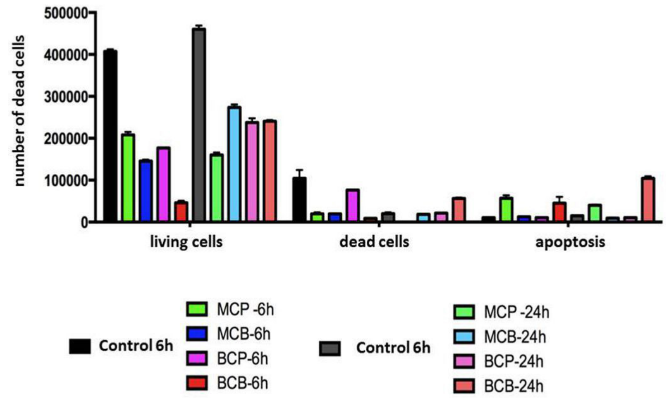

FIGURE 4 – Results obtained with image cytometer at 6 and 24 h of exposure.

Apoptosis rates of cells exposed to MCB (5%) and BCP (6%) were similar to those of controls (4%), while those of cells treated with MCP (20%) and BCB (27%) were 5 to 7 times as high as for other cells. The results obtained for films devoid of bupropion (MCP and BCP) cannot be explained by the residual concentration of polymers in the culture medium, since the mass of the monolayer placebo films (MCP) was less than 10% that of bilayer placebo films (BCP). Figure 5 shows values of cell viability as a function of time, revealing steady growth, equivalent to that of controls.

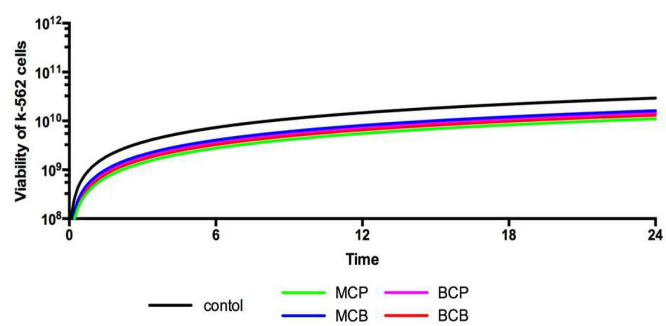

FIGURE 5 – Cell viability *vs*. time, for different treatments.

The higher death rates found among cells exposed to bupropion and the viability rates along time suggest the presence of a stimulus for cell division, because despite the higher death rate among treated cells, the number of cells per milliliter remained high. Cell morphology was evaluated at 24 h of treatment, revealing no morphological differences between controls and cells exposed to BCP or BCB (Figure 6). Presence of cell divisions was indicative of a normal division process.

The cytotoxicity results showed that monolayer and bilayer films containing bupropion are safe for human use, as they did not cause cell damage. The results for cell

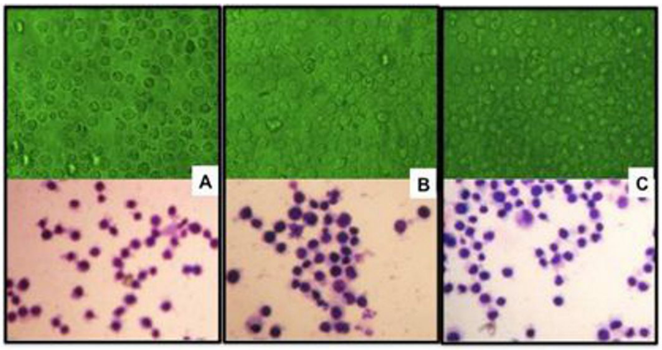

FIGURE 6 – K-562 cells at 24 h of exposure. Top row: living cells. Bottom row: cells fixed and stained with Giemsa after test completion. (A) Controls. (B) Placebo bilayer film. (C) Bilayer film containing bupropion.

death and apoptosis at 6 and 24 h were irrelevant, since film permanence times in the oral cavity are necessarily shorter than those evaluated in the present study.

CONCLUSIONS

Hydrogel films for oral administration of drugs have low cost of production, and their physicochemical and biological quality control costs are also low. Easy to carry and to administer, they constitute a useful resource to improve adherence to smoking cessation treatments. The technique employed in the present study to prepare the films proved practical, reproducible, and scalable. The macroscopic characteristics of films were satisfactory, both physically and in sensory terms. The values obtained for the mechanical properties show that the films can be easily handled during cutting and packaging. The cytotoxicity tests demonstrated the biological safety of the product. Further studies are necessary to evaluate release profiles and mucoadhesive strength values, for biopharmaceutical characterization of the films.

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