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International Journal of Pharmaceutics 188 (1999) 1–10

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Transmucosal sustained-delivery of chlorpheniramine maleate in rabbits using a novel, natural mucoadhesive gum as an excipient in buccal tablets

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Received 2 December 1998; received in revised form 2 June 1999; accepted 3 June 1999

Abstract

The objective of this study was to evaluate the gum from *Hakea gibbosa* (Hakea) as a sustained-release and mucoadhesive component in buccal tablets following their application to the buccal mucosa of rabbits. Flat-faced core tablets containing either 22 or 32 mg of Hakea and 40 or 25 mg of chlorpheniramine maleate (CPM) per tablet with either sodium bicarbonate or tartaric acid in a 1:1.5 molar ratio were formulated using a direct compression technique and were coated with Cutina® on all but one face. The resulting plasma CPM concentration versus time profiles were determined following buccal application of the tablets in rabbits. The strength of mucoadhesion of the tablets was also quantitated in terms of the force of detachment as a function of time. Following the application of the mucoadhesive buccal tablets, the following values for several pharmacokinetic parameters were obtained. The force of detachment for the mucoadhesive buccal tablets containing 22 mg of Hakea and either 25 and 40 mg CPM, and 32 mg Hakea and 40 mg CPM increased from 1.64 ± 0.47 to 7.32 ± 0.34 N, 1.67 ± 0.30 to 7.21 ± 0.36 N, and 2.93 ± 0.73 to 7.92 ± 0.60 N, respectively from 5 to 90 min following application to excised intestinal mucosa. Addition of either sodium bicarbonate or tartaric acid, as well as higher amounts of CPM, did not affect the mucoadhesive bond strength. These results demonstrate that the novel, natural gum, *H. gibbosa*, may not only be used to sustain the release of CPM from a unidirectional-release buccal tablet, but also demonstrate that the tablets are sufficiently mucoadhesive for clinical application. The mucoadhesive strength as measured by the force of detachment, can be modulated by altering the amount of Hakea in the tablet. The mucoadhesive buccal tablets evaluated represent an improved transbuccal delivery system for conventional drug substances.

Keywords: Sustained-release; Bioadhesion; Chlorpheniramine maleate; Buccal tablets; Force of detachment

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0378-5173/99/$ - see front matter
PII: S0378-5173(99)00211-2

1. Introduction

Drug delivery by the non-parenteral routes has gained significant attention over the last decade, particularly for the delivery of therapeutically important proteins and peptides. This stems from the significant limitations of traditional routes of drug administration. The limitations include poor absorption, enzymatic degradation, and first-pass metabolism. The non-parenteral routes under investigation include buccal (Dowty et al., 1992; Benes et al., 1997; Li et al., 1997a), sublingual (Cannon et al., 1996), rectal (Watanabe et al., 1996), nasal (Harris et al., 1988), and vaginal (Acarturk and Robinson, 1996). While each non-parenteral route of drug administration has its associated advantages and disadvantages, the buccal route of drug administration has some unique benefits such as easy accessibility, enhanced patient compliance, rapid cellular recovery following local stress (Harris and Robinson, 1992), and the ability to withstand environmental extremes like changes in pH, temperature, etc. A variety of drug substances have been administered by the buccal route. Examples include peptides like TRH (thyrotropin-releasing hormone) (Li et al., 1997b), calcitonin (Heiber et al., 1994), buserelin (Hoogstraate et al., 1996a), oxytocin (Li et al., 1997a), and octreotide (Wolany et al., 1990); steroids such as testosterone and its various esters (Voorspoels et al., 1996); analgesics such as morphine (Hoskin et al., 1989), buprenorphine (Kuhlman et al., 1996); antihypertensives such as nifedipine (Kondo and Sugimoto, 1987); and vasodilators such as nitroglycerin (Dellborg et al., 1991). Buccal drug delivery necessitates the use of mucoadhesive polymers as these dosage forms should ideally adhere to the mucosa and withstand salivation, tongue movement, and swallowing for a significant period of time. Examples of mucoadhesive polymers include sodium carboxymethyl cellulose, Carbopol® 934, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, acacia, gelatin, etc.

The purpose of present study was to further evaluate in rabbits a novel, natural gum, *Hakea gibbosa* as a sustained-release and mucoadhesive component in buccal tablets. Chlorpheniramine maleate (CPM), a low molecular weight organic compound, was used as a model drug. CPM has been used extensively as an antihistamine for symptomatic relief of the common cold and allergy (Rumore, 1984). The gum Hakea is a polysaccharide exudate from the plant *H. gibbosa* (Fam: Proteaceae), indigenous to New South Wales, Australia. The gum has a molecular weight of greater than 2×10^6 (g/mol) and is totally water-soluble at a 2% w/v concentration at room temperature. The chemical structure of the gum is described elsewhere (Eagles, 1992; Alur et al., 1999). Previously, we had reported the effect of Hakea on the sustained-release of CPM from buccal tablets in vitro (Alur et al., 1999). In addition, Hakea exhibited excellent mucoadhesive properties (Alur et al., 1999). Based on the previous study (Alur et al., 1999), the CPM-loaded buccal tablets were evaluated in the whole animal. Thus, the aims of the present study were to quantitate the plasma concentrations of CPM following application of a mucoadhesive buccal tablet in rabbits and subsequently estimate the absolute bioavailability. In addition the bioadhesive strength (as reflected by the force of detachment) of these buccal tablets was quantitated in vitro using freshly excised rabbit intestinal mucosa as a model biological interface. The overall goal associated with the present study was not to demonstrate that a conventional drug substance could be administered via the buccal route, but to demonstrate the utility of a new, heretofore untested, natural gum to serve as both a sustained-release and mucoadhesive tablet excipient.

2. Materials and methods

2.1. Materials

Chlorpheniramine maleate (CPM) was obtained from Sigma (St. Louis, MO). All solutions were prepared in deionized water. The Hakea gum was a gift from Dr Peter Eagles of the University of the Western Cape, Cape Town, South Africa. The gum was obtained from the

Kirstenbosch Botanical Gardens. All other materials, except for the gum, were used as received. The gum was purified by filtration by first dissolving it in water and then filtering the 2% solution through muslin cloth. The filtered solution was freeze-dried using a model 10-MR-SA Virtis table top freeze drier (Gardiner, NY). Cutina® (castor oil, hydrogenated) was obtained from Henkel, NJ.

Xylazine (100 mg/ml), ketamine (100 mg/ml), and pentobarbital sodium (50 mg/ml) solutions were provided by the Laboratory Animal Center at the University of Missouri-Kansas City (Kansas City, MO). Heparin sodium injection (10 000 U/ml) was purchased from Elkins-Sinn (Cherry Hill, NJ). I-Cath® Intravenous Placement Units with Stylet attachment (catheter: 22 G × 12 in), (needle: 19 G × 21 in) was purchased from Charter Med (Lakewood, NJ) and tuberculin syringes (1 cc) were obtained from Becton and Dickinson (Sandy, UT).

Blood samples were collected into 1.5-ml Eppendorf tubes containing heparin sodium (100 U/ml) and centrifuged using a Beckman GS-15R centrifuge (Palo Alto, CA). Plasma samples were analyzed by high-performance liquid chromatography (HPLC) using a Waters 600E Systems controller, Rheodyne injector, Nova-pak C_{18} (3.9 × 150 mm) column, and a Waters Model 486 tunable absorbance detector set at a detection wavelength of 262 nm. The peaks of the chromatogram were recorded and integrated automatically by a Waters 746 data module (Milford, MA).

Male New Zealand white rabbits, weighing between 2.0 and 2.5 kg, were purchased from Myrtle's rabbitry (Thompson, TN). The animals were housed individually for at least 1 week prior to experimentation and allowed food and water ad libitum. The average weight of the rabbits at the time of the experiments was 2.70 ± 0.4 kg ($n = 9$).

The bioadhesion experiments were carried out on a model LTC universal tension-compression stand (John Chatillon, Greensboro, NC) equipped with model DFM-10 digital force gauge (John Chatillon, NC).

2.2. Methods

2.2.1. Tablet preparation

Flat-faced core tablets were prepared by direct compression and the tablets were coated with Cutina® on all but one face using a compression coating technique. Release of CPM was unidirectional occurring from only the uncoated tablet face.

2.2.1.1. Direct compression. The directly compressed tablets were prepared by initially mixing the CPM and Hakea for 10 min. Subsequently, lactose and Cab-o-sil® were incorporated and the solid was mixed for an additional 10 min. Finally, magnesium stearate was added and the mixing continued for an additional 5 min. Mixing was performed by mechanical rotation at 225 rpm using a model SER-143 Colton table top coating pan (Vector, Marion, IA). The powders were mixed by both rotation and tumbling. The tablets also contained either sodium bicarbonate or tartaric acid in a 1:1.5 molar ratio with respect to CPM. Sodium bicarbonate was included in the formulation for two reasons. The first reason was to increase the pH of the microenvironment around the tablet/mucosa interface so that the percent of unionized CPM can be increased to theoretically enhance the absorption of CPM across a lipid bilayer membrane. The second reason was to determine whether an increase in the pH of the microenvironment around the tablet/mucosa interface would decrease the mucoadhesive strength of the applied tablet by decreasing the percent of unionized carboxyl groups in the gum. Presumably, if the mechanism of mucoadhesion is by secondary bond formation, then decreasing the percent of the carboxyl groups in the gum which existed in the unionized state should affect secondary (hydrogen) bond formation with the negatively charged sialic acid residues present on the backbone of the mucin molecule. Tartaric acid was included in the formulation to determine whether a decrease in the pH of the microenvironment around the tablet/mucosa interface would increase the mucoadhesive strength of the applied tablet by increasing the percent of carboxyl groups of the polysaccharide which were in the unionized state.

2.2.1.2. Tablet compression. Both core and coated tablets were prepared on a model B, No. 0-24R carver press (Summit, NJ). The core tablets had a diameter of 1 cm, a thickness of 0.1 cm, and were compressed at a force of 5000 psi. The coated tablets were compressed at 2000 psi force to generate a final diameter of 1.2 cm and a thickness of 0.2 cm.

2.2.2. Buccal CPM study

The potential of the mucoadhesive buccal tablets to deliver CPM to the systemic circulation in a sustained fashion was evaluated by conducting the following experiments. There were three groups of rabbits. Animals were lightly anesthetized by an i.m. injection of a 1:5 mixture of xylazine (1.9 mg/kg) and ketamine (9.3 mg/kg). Following induction of anesthesia, a catheter was placed in the marginal ear vein for blood sample collection. A 2-ml blood sample was obtained 5 min before and then at 5, 10, 30, 60, 90, 120, 180, 210, 240, 270, and 300 min following the application of the mucoadhesive buccal tablets. After the collection of each blood sample, the cannula was flushed with 0.2 ml of a 10% (v/v) heparin/normal saline solution to keep the cannula open. The light plane of anesthesia was maintained by an i.m. injection of one-third of the initial dose of xylazine and ketamine mixture as needed. All the blood samples were centrifuged at 3000 rpm for 10 min to separate the plasma and the retrieved plasma was stored at -20°C until the time of analysis. At the end of the experiments the rabbits were euthanized by injecting an overdose of pentobarbital solution (3–5 ml) into the catheter.

2.2.3. Quantitation of plasma CPM

Reversed-phase high-performance liquid chromatography was used to quantitate CPM in the plasma by the method of Athanikar et al. (1979) with several slight modifications. Instead of a double extraction, a single extraction step was performed. Briefly, to 1 ml of plasma, 750 μl of 5% potassium hydroxide and 2 ml of ether were added, the sample vortexed for 5 min, and then centrifuged at 3000 rpm for 15 min. The centrifuged mixture was frozen immediately by placing it on an acetone-dry ice bath. The ether phase was decanted and evaporated to dryness under an N_2 stream. The residue was then dissolved in 100 μl of mobile phase and a 20-μl sample injected onto the chromatograph for analysis.

2.2.4. Bioadhesion study

The potential of Hakea for use as a bioadhesive polymer in buccal tablets has been reported previously (Alur et al., 1999). The aim of this experiment was to quantitate the force of detachment (bioadhesive strength) of CPM buccal tablets applied to freshly excised rabbit intestinal mucosa. Rabbit small intestine was used as a model membrane since the intestine provided a flat and uniform surface. A 2-cm long piece of intestinal mucosa was mounted on the platform of the tension-compression stand. The tablet was applied using super glue to the bottom face of a stainless steel disk attached to the force gauge. The mucosal surface was hydrated by placing 20 μl of distilled water and the tablet and the mucosal surfaces were brought in contact at a constant force of 20 N and the tablet pulled off the tissue surface at 5, 10, 20, 30, 45, 60, and 90 min following application. The value for the force of detachment was measured in Newtons by lowering the platform of the tension-compression stand at a constant rate.

2.2.5. Data and statistical analysis

The area under the plasma CPM concentration versus time curve was calculated using the trapezoidal rule (Gibaldi and Perrier, 1982) within the time periods of 0–5 h for tablets with 25 mg CPM and 22 mg Hakea, 0–4.5 h for tablets with 40 mg CPM and 22 mg Hakea, and 0–4.5 h for tablets with 40 mg CPM and 32 mg Hakea. The AUC following intravenous administration of CPM was calculated from the data of Huang and Chiou (1981). The dose administered in their work was 3 mg/kg.

The following equations (Gibaldi and Perrier, 1982; Hoogstraate et al., 1996a,b; Li et al., 1997b) were used to estimate the absolute bioavailability and clearance of CPM following buccal administration. The C_{max}, C_{min}, and t_{max} were estimated directly from the plasma CPM versus time profiles following buccal administration. The bioavailabil-

ity and clearance were calculated using Eqs. (1) and (2), respectively,

$$F = \frac{(\text{Dose}_{\text{i.v.}}) \times (\text{AUC}_{0-t_{\text{buccal}}})}{(\text{Dose}_{\text{buccal}}) \times (\text{AUC}_{0-t_{\text{i.v.}}})} \quad (1)$$

$$CL = \frac{F \times D}{\text{AUC}_{0-t_{\text{buccal}}}} \quad (2)$$

F denotes the absolute bioavailability, D is the dose of CPM in the buccal tablets, and $\text{AUC}_{\text{buccal}}$ represents the area under the plasma CPM concentration versus time curve following buccal administration.

All data was expressed as the mean value ± S.D. Mean values were compared for statistical significance at the 5% level using Student's one-tailed t-test.

3. Results

3.1. Buccal CPM study

The plasma CPM concentration versus time profiles following administration of the buccal tablets with 22 mg of Hakea and 25 mg of CPM, 22 mg of Hakea and 40 mg of CPM, and 32 mg of Hakea and 40 mg of CPM are shown in Fig. 1. The relevant pharmacokinetic parameters are listed in Table 1. From Fig. 1, it is apparent that

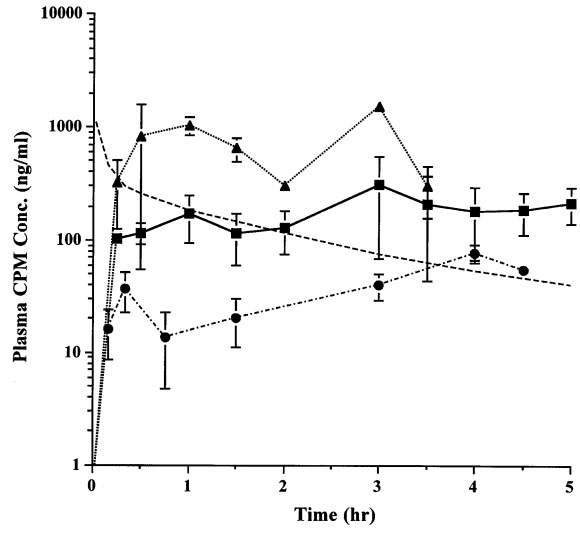

Fig. 1. Plasma profiles of CPM in rabbits following buccal administration of directly compressed tablets which contained 40 mg CPM and 22 mg Hakea (---▲---), 25 mg CPM and 22 mg Hakea (—■—), and 40 mg CPM and 32 mg Hakea (-·-●-·-). All data points represent the mean value ± S.D. of three experiments. (----) represents the plasma CPM profile observed by Chiou et al. (23) following an intravenous injection of a dose of 3 mg/kg of CPM to rabbits. Lines through mean values are included to visualize the trend and do not represent a mathematical fit of the data.

Hakea effectively sustained and controlled the release of CPM from the buccal tablets and also maintained an elevated plasma CPM concentra-

Table 1
Pharmacokinetic parameters of CPM after buccal administration in New Zealand white rabbits[a]

Pharmacokinetic parameters	25 mg CPM and 22 mg Hakea	40 mg CPM and 22 mg Hakea	40 mg CPM and 32 mg Hakea
AUC (ng h/ml)[a]	867 ± 472	1857 ± 1080	133 ± 16
CL (l/kg h)	4.2 ± 0.002	4.3 ± 0.069	4.5 ± 0.003
F	0.45 ± 0.13*	0.70 ± 0.12 *, †	0.04 ± 0.005
C_{max} (ng/ml)	312 ± 245	1339 ± 460	77 ± 12
C_{min} (ng/ml)	83 ± 14	310 ± 155	12 ± 6
t_{max} (h)	0.5	0.5	0.5

[a] Values are mean ± S.D., $n = 3$.

* Indicates a statistically significant increase in the mean value of absolute bioavailability when a tablet containing 22 mg of Hakea with either 25 and 40 mg of CPM is compared with a tablet containing 32 mg of Hakea and 40 mg of CPM at $P < 0.05$ using Student's one-tailed t-test.

† Indicates a statistically significant increase in the mean value of absolute bioavailability when a tablet containing 22 mg of Hakea with 40 mg of CPM is compared with a tablet containing 22 mg of Hakea and 25 mg of CPM at $P < 0.05$ using Student's one-tailed t-test.

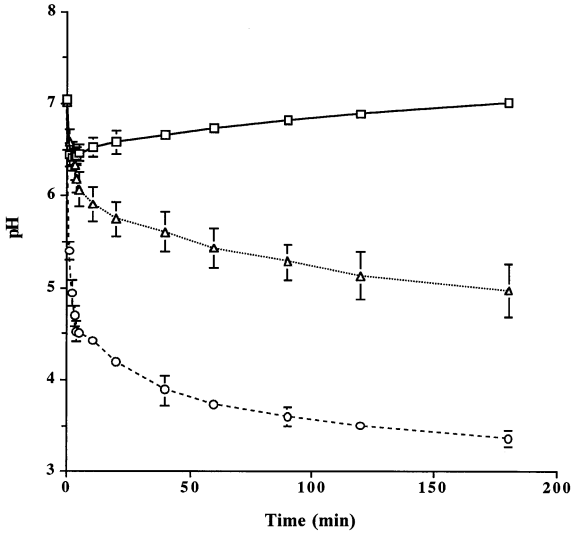

Fig. 2. pH-time profile of the aqueous bulk phase when directly compressed buccal tablets which contained 40 mg CPM, 22 mg Hakea, and either sodium bicarbonate (—□—), tartaric acid (---○---), or neither excipient (···△···) were immersed in the aqueous dissolution medium. All data points represent the mean value ± S.D. of three experiments. Lines through mean values are included to visualize the trend and do not represent a mathematical fit of the data.

tion during the entire application period. The absolute bioavailability (F) for the tablets with 22 mg of Hakea and 25 mg of CPM was significantly ($P < 0.05$) less than the tablets with 22 mg of Hakea and 40 mg of CPM. The absolute bioavailability (F) for the tablets with 32 mg of Hakea and 40 mg of CPM was significantly ($P < 0.05$) less than the tablets containing 22 mg of Hakea with either 25 or 40 mg of CPM. As mentioned previously, sodium bicarbonate was incorporated into the buccal tablets in an attempt to alter the pH of the microenvironment (tablet/mucosa interface) and thereby raise the fraction of CPM in the unionized state and hence increase the amount of CPM that would ultimately be transported across the buccal mucosa. Moreover, sodium bicarbonate was incorporated into the tablet to lower the percent of unionized carboxylate in the Hakea in order to elucidate a potential mechanism associated with mucoadhesion (secondary hydrogen bond formation). The in vitro pH profiles of tablets with or without sodium bicarbonate and tartaric acid are shown in Fig. 2. It is apparent from Fig. 2 that both sodium bicarbonate and tartaric acid effectively altered the pH of the microenvironment at the exposed solid-liquid interface.

3.2. Bioadhesion study

3.2.1. Time-dependent mucoadhesion study

A profile showing the mean values of the force of detachment of the buccal tablets following their application to excised rabbit intestinal mucosa is shown in Fig. 3. It can be noted that the mean values of the force of detachment increased with time and were significantly ($P < 0.05$) greater for tablets containing 32 mg of Hakea as compared to the tablets with 22 mg Hakea.

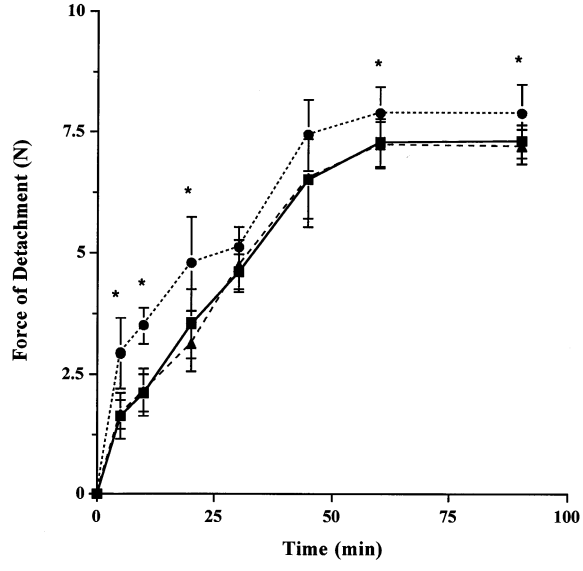

Fig. 3. Force of detachment from excised rabbit intestinal mucosa for directly compressed buccal tablets which contained 25 mg CPM and 22 mg Hakea (—■—), 40 mg CPM and 22 mg Hakea (---▲---), 40 mg CPM and 32 mg Hakea (···●···). All tablets contained 1.5 mol of sodium bicarbonate. All data points represent the mean value ± S.D. of five experiments. Lines through mean values are included to visualize the trend and do not represent a mathematical fit of the data. * Indicates a statistically significant increase in the mean value of the force of detachment of a tablet at $P < 0.05$ using the one-tailed Student's t-test.

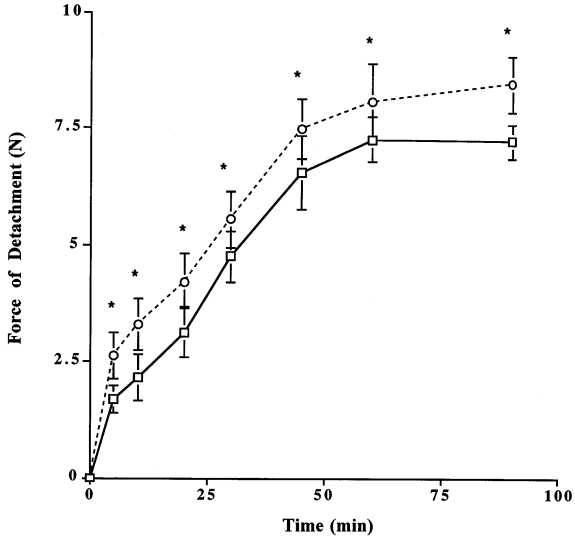

Fig. 4. Force of detachment from excised rabbit intestinal mucosa for directly compressed buccal tablets which contained 40 mg CPM, 22 mg Hakea, and either sodium bicarbonate (—□—) or tartaric acid (---○---). All data points represent the mean value ± S.D. of five experiments. Lines through mean values are included to visualize the trend and do not represent a mathematical fit of the data. * Indicates a statistically significant increase in the mean value of the force of detachment of a tablet at $P < 0.05$ using the one-tailed Student's t-test.

3.2.2. Effect of diffusion layer pH on mucoadhesive strength

The pH of the bulk solution for a typical dissolution experiment using the tablets formulated with 40 mg CPM, 22 mg Hakea, and either 1.5 mol of sodium bicarbonate or tartaric acid is shown in Fig. 2. Fig. 4 shows the mean values of the force of detachment of tablets containing 40 mg CPM, 22 mg Hakea, and either 1.5 mol of sodium bicarbonate or tartaric acid. As mentioned previously acid/base was incorporated in the formulation to investigate the effect of a change in the pH of the microenvironment around the tablet/mucosa interface on the bioadhesive strength. Fig. 4 clearly depicts that the force of detachment increased with time for both tablets which contained either sodium bicarbonate or tartaric acid. The tablets which were formulated with sodium bicarbonate resulted in mean values of the force of detachment which were significantly ($P < 0.05$) less at every time point than corresponding values for tablets which contained tartaric acid.

4. Discussion

In the present study, controlled release of CPM in rabbits following application of mucoadhesive buccal tablets was successfully demonstrated using the natural gum excipient Hakea. Although the t_{max} was the same for all tablets irrespective of the amount of Hakea incorporated, the tablets which contained 32 mg of Hakea demonstrated a significantly ($P < 0.05$) lower mean value of the absolute bioavailability (F) compared to tablets which contained 22 mg Hakea. This result would suggest that Hakea effectively retarded the rate of release of CPM from the dosage form (Fig. 1 and Table 1). The values of F calculated in the present study were 45, 70, and 4% for tablets with 25 mg CPM and 22 mg Hakea, 40 mg CPM and 22 mg Hakea, and 40 mg CPM and 32 mg Hakea, respectively. Athanikar and Chiou (1979) have reported a mean bioavailability of CPM in dogs of 9.4, 35.7 and 39.4% following oral administration of 50, 100, and 200 mg, respectively. The oral bioavailability of CPM in humans ranges between 25 and 50% (Rumore, 1984).

Estimates of absolute bioavailability for other compounds following buccal administration of oxytocin in rabbits (Li et al., 1997a), fluorescein isothiocynate-dextran 4400 in pigs (Hoogstraate et al., 1996b), buserelin in pigs (Hoogstraate et al., 1996a), butorphanol in humans (Shyu et al., 1993) and testosterone in dogs (Voorspoels et al., 1996) are 0.1, 1.8, 1, 29, and 14%, respectively. Previously, we reported that tablets coated with Cutina® on all sides released negligible amounts of CPM within 12 h of dissolution demonstrating that the Cutina® coating was water-impermeable (Alur et al., 1999). In the present study, the drug-releasing, uncoated surface adhered to the buccal mucosa during the entire application period. This mucosal binding possibly minimized the loss of drug into the surrounding oral cavity and the gastrointestinal tract in the event that CPM dissolved in saliva was potentially swallowed.

CPM, an alkylamine has a molecular weight of 390.5 and a pK_a of 9.2. According to the pH-par-

tition theory, approximately 0.006% of CPM exists as the unionized species at pH 5 (the pH of a 15-ml aqueous sample 90 min after immersing a tablet containing 40 mg CPM and 22 mg Hakea) determined using the Henderson-Hesselbach equation (Block, 1991; Martin, 1993). Therefore, in an attempt to increase the fraction of CPM in the unionized form and thereby the transport of CPM across buccal mucosa, sodium bicarbonate was included in the tablets to increase the pH of the tablet diffusion layer/mucus interface. Incorporation of sodium bicarbonate (1.5 mol) into the tablets increased the pH to 7 (the pH of a 15-ml aqueous sample 90 min after immersing a tablet containing 40 mg CPM, 22 mg Hakea, and 1.5 mol of sodium bicarbonate) and increased the fraction of unionized CPM to 0.63%. While the fraction of CPM was still less than 1%, its absorption was nevertheless observed which resulted in a value of F equal to 70 and 45% with 40 and 25 mg doses.

A wide variety of drug substances have been delivered across the buccal mucosa. Sustained-release, mucoadhesive dosage forms have the advantage of not only adhering to the mucus membrane for the required length of time, but also sustaining the release of drug substances. In the present study, the amount of Hakea incorporated into the buccal tablet was observed to be a critical factor in defining the resulting bioadhesive strength. A potential reason for an increase in mucoadhesive bond strength with increasing Hakea content (Fig. 3) may be due to enhanced water uptake by the gum which resulted in tablet swelling and mobilization of flexible polysaccharide chains. The amount of CPM present per tablet did not appear to affect the bioadhesive bond strength as observed with tablets containing either 25 and 40 mg of CPM and 22 mg of Hakea (Fig. 3). However, Voorspoels et al. found that the force of detachment from a porcine gingival mucosa decreased gradually with an increase in the amount of active drug substance in the tablets (Voorspoels et al., 1996). These authors used a combination of Carbopol® 974P and a drum-dried waxy maize as a bioadhesive polymer in their buccal tablets (Voorspoels et al., 1996). In the present study, CPM being a hydrophilic molecule, may facilitate hydration of the Hakea and mobilize all flexible polymer chains for interpenetration and physical entanglement with the mucus.

Hakea possesses both hydroxyl and carboxyl terminal groups which can contribute to bioadhesion. Both these functional groups have to be in the unionized form in order to optimally interact with the negatively charged mucin molecule (under neutral or slightly acidic conditions). According to the Henderson-Hesselbach equation, the percent of unionized carboxyl groups would increase as the pH of the aqueous gum solution is decreased. The pH of the tablet diffusion layer/mucus interface was modulated in these experiments by adding either sodium bicarbonate or tartaric acid (Fig. 2). Based on calculations using the Henderson-Hesselbach equation and the pH versus time profiles for an aqueous solution in which a buccal tablet formulated with either sodium bicarbonate or tartaric acid had been immersed, it can be calculated that the percent of unionized carboxyl groups contained in Hakea remained constant at 0.5% throughout the 90-min test period for the tablets which contained sodium bicarbonate whereas the percent of unionized carboxyl groups for the gum containing tartaric acid increased from 0.5% (0 min) to approximately 93% at 90 min. Although it can be predicted based on pH values that most ($\sim 93\%$) of the carboxyl groups were in the unionized form when tartaric acid was included in the tablet, a corresponding increase in the force of detachment was not observed. However, the mean values of the force of detachment for tablets with sodium bicarbonate were significantly ($P < 0.05$) less than the mean values of the force of detachment of tablets which contained tartaric acid. These results are in agreement with the findings of Park and Robinson (1989) and contrary to the findings of Bouckaert and Remon (1993). Bouckaert and Remon (1993) using a combination of a drum-dried waxy maize and polycarbophil attempted to evaluate the influence of pH on in vitro bioadhesion using an isotonic phosphate buffer solution over a pH range of 5–7.4. The study did not report a significant difference in the force of detachment of tablets at pH 5 versus 7.4 (Bouckaert and Remon, 1993). Park and Robinson (1989) demonstrated the ability to modify the in vitro mucoadhesive properties of custom synthesized

polycarbophil hydrogels as a function of pH. These authors reported that the force of detachment at pH 2 was seven times greater than the force of detachment measured at pH 7. This report also noted that the force of detachment decreased as the pH of the medium increased (Park and Robinson, 1989). In the present study, the bioadhesive bond strength increased over time and (with both sodium bicarbonate and tartaric acid as excipients) suggesting that the mechanism of bioadhesion is likely due to chain interpenetration and physical entanglement of Hakea with mucus rather than secondary bond formation (e.g. hydrogen-bonding).

In conclusion, the ability of the novel gum, Hakea, to sustain the release of a small organic molecule, namely CPM, when applied to the buccal mucosa of rabbits has been demonstrated. Moreover, in vitro bioadhesive strength versus time measurements demonstrated that the gum possessed excellent mucoadhesive properties allowing for the convenient application and removal of the tablets from the buccal mucosa. The mechanism of bioadhesion may potentially result from chain interpenetration and physical entanglement of Hakea with the mucus layer. The rate of release of the drug substance as well as the bioadhesive bond strength of the formulation can be modulated by varying the amount of Hakea included in the tablet. The mucoadhesive buccal tablets evaluated in the present study were easy to formulate, inexpensive, provide easy application and convenient removal from the mucosal surface, and did not irreversibly damage the underlying tissue. Therefore, such tablet formulations containing a polysaccharide bioadhesive gum, Hakea, may represent an improved buccal delivery system for a variety of water-soluble, low molecular weight drug substances.

Acknowledgements

This work was supported by grants from Hoechst-Marion-Roussel, Inc. (Ashim K. Mitra) and the University of Missouri Research Board (Thomas P. Johnston). The authors are grateful to Dr Peter Eagles and Yusuf Alexander of the University of the Western Cape, South Africa for the generous supply of the Hakea gum.

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