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Transbuccal permeation, anti-inflammatory activity and clinical efficacy of piroxicam formulated in different gels

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Abstract

In attempts to avoid the systemic side effects of piroxicam (PC) (e.g. gastrotoxicity), several buccal gel formulations containing PC were prepared and their effects on the characteristics of the drug permeation through rabbit buccal mucosa in-vitro were evaluated using a Franz-type diffusion cell. The general rank order of the total flux of 0.5% PC from gels was found to be: hydroxypropylmethylcellulose (HPMC, 2.5%) > hydroxypropylcellulose (HPC, 2.5%) ≥ sodium alginate (Na alg., 7%) > methylcellulose (MC, 3%) > hydroxyethylcellulose (HEC, 1.5%) > carbopol 934 (Carb. 934, 1%) ≥ sodium carboxymethylcellulose (NaCMC, 2%) > pluronic F-127 (PF-127, 20%) > polyvinyl alcohol (PVA, 10%). The effect of various penetration enhancers 1% sodium lauryl sulphate (NaLS), 3% sodium deoxycholate (NaDC), 3% sodium tauroglycocholate (NaTGC) on the rate of permeation across the excised buccal mucosa (of 0.5% PC in gels prepared using 3% MC, 2.5% HPMC or 7% Na alg. base) and histology of the buccal epithelium was also investigated. Pharmacodynamic evaluation of the anti-inflammatory activity of PC in these gel formulations (containing 3% NaDC as an enhancer) was carried out using the kaolin-induced rat paw oedema method. The results obtained indicated that PC administered in 7% Na alg. or 2.5% HPMC gel bases was significantly more effective than the 3% MC gel and oral drug solution in suppressing oedema formation in rats. Comparative clinical studies were conducted in patients with post-operative dental pain and oedema following maxillofacial operations. The results revealed that 7% Na alg. and 2.5% HPMC gel formulations applied to the buccal mucosa were slightly better than or equally effective to the orally administered commercial product (Feldene Flash® tablet) in reducing pain level, swelling and tenderness within a period of 4 days. These findings suggest that PC (0.5%) administered in the buccal gel may present a potential therapeutical use as a strong anti-inflammatory and analgesic agent.

Keywords: Piroxicam; Rabbit buccal mucosa; Buccal permeability; Penetration enhancer; Rat paw oedema

1. Introduction

In oral transmucosal drug delivery, drugs are directly exposed to the oral (buccal and sublingual) mucosa and permeate across the mucosal tissues to reach the systemic circulation. There are many reports (Christrup et al., 1997; Tsutsumi et al., 1998; Lee and Kellaway, 2000) on the buccal drug delivery because it offers many advantages over peroral delivery including abundant blood supply, robustness of the epithelium, facile removal of the dosage form in case of need, satisfactory patient compliance and improved bioavailability due to avoidance of degradation in the gastrointestinal tract (GIT) and hepatic first-pass metabolism (Lee, 1988; Zhang et al., 1994). The use

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of buccal mucosa as a route for drug delivery using mucoadhesive preparations has been reported (Nair and Chien, 1996; Li et al., 1997; Cui and Mumper, 2002). However, most drugs in the term of buccal or sublingual tablets have exhibited low bioavailabilities due largely to the low mucosal membrane permeability and relatively small surface area available for absorption (Rathbone et al., 1994; Lee and Kellaway, 2000).

Semi-solid dosage forms (e.g. gels) seem suitable for the oral cavity, especially for the treatment of inflammatory lesions of the buccal mucosa because they can be spread as a thin pellicle over a large portion of mucosa. Moreover, as a result of their soft structure, they are more able to undergo the stretching and contraction strains of the underlying mucosa than tablets and patches (Jacques et al., 1997). In addition, gels posses a higher biocompatibility, ideal placement characteristics and bioadhesivity allowing adhesion to the mucosa in the dental pocket (in case of periodontitis) and can be rapidly eliminated through normal catabolic pathways (Esposito et al., 1996; Jacques et al., 1997).

The permeation enhancement approach was considered in order to further improve buccal mucosal membrane permeability of various drug delivery systems (Lee and Kellaway, 2000; Shin et al., 2000a,b). However, it is unclear whether they act by transiently altering the mucosa or by damaging it (Zhang et al., 1994). Among a number of penetration enhancers used, bile salts and sodium lauryl sulphate (NaLS) have been found effective as buccal penetration enhancers (Siegel and Gordon, 1985a,b; Steward et al., 1994; Senel et al., 1998). Shin and Kim (2000) and Shin et al. (2000a) used a variety of absorption enhancers including bile salts, glycols and non-ionic surfactants to improve the permeability of a lipophilic drug, triamcinolone acetonide through buccal mucosa and reported that sodium deoxycholate (NaDC) showed the best enhancing effects. Recently, mucoadhesive gels containing 5% NaDC as an enhancer have been also investigated to enhance the drug bioavailability via the buccal epithelium of the rabbit (Shin et al., 2000b).

Piroxicam (PC) is one of the most potent non-steroidal, anti-inflammatory agents which also exhibits anti-pyretic activity in various types of non-rheumatic pains (McMahon and Jain, 1978; Maccagno, 1980). In terms of its anti-inflammatory property, oral PC has been used for the management of post-operative dental pains within a period of 1 week (Cruz, 1984). Although, the drug is well absorbed following oral administration, GIT toxicity is still the most serious adverse effect associated with its oral use (Mueller et al., 1992). The buccal administration route may thus present an alternative for PC delivery. Mueller et al. (1992) revealed that the buccal wafers of PC may not have been absorbed exclusively from the buccal mucosa. On the other hand, Shin et al. (1999) reported that the permeation rate of PC across the rat skin from different poloxamer gel formulations can be increased by the addition of an appropriate enhancer such as surfactants, taurocholic acid, deoxycholic acid and fatty acids. The enhancing effect of various penetration enhancers on the percutaneous absorption of PC from carbopol 940/propylene glycol gel bases was also investigated (Santoyo et al., 1995). Therefore, the use of a gel-type formulation together with permeation enhancers is a logical approach to increase the buccal mucosa absorption of PC.

Aim of the present study was to develop a suitable buccal PC gel formulation and to investigate the efficacy of buccal administration of drug from the prepared gels. The investigation consisted essentially of the following steps: (a) preparation of medicated buccal gels using various polymers of differing chemical structure, (b) testing of the effects of the gel type and concentration on the in-vitro permeation of PC through rabbit buccal mucosa for the initial screening of the suitable buccal drug delivery carrier, (c) evaluation of the effect of different permeation enhancers on the transbuccal delivery of PC from the prepared gels and histology of buccal mucosa, (d) evaluation of the anti-inflammatory activity of the selected PC buccal gels in rats, and (e) a comparative clinical evaluation of the analgesic effectiveness of the buccal dosage form of PC versus its commercial oral product (Feldene Falsh® tablets) in patients with post-operative dental pain and oedema following maxillofacial operations.

2. Materials and methods

2.1. Materials

The following materials were used: piroxicam (Sedico, Egypt), sodium alginate (Na alg.), methyl-

cellulose (MC), sodium carboxymethylcellulose (NaCMC), NaLS (BDH Chemicals, Ltd., Poole, UK), hydroxypropylmethylcellulose E4M (HPMC, Dow Chemical Co., USA), hydroxypropylcellulose MF (HPC, Kolmar Company, CF, USA), polyvinyl alcohol (PVA, Hoechst, Ltd., England), carbopol 934 (B.F. Goodrich Co., USA), pluronic F-127 (PF-127, Sigma Chemical Co., St. Louis, MO, USA), NaDC (Fluka Chemie, Buchs, UK), sodium tauroglycocholate (NaTGC, Winlab, UK). All other chemicals were of analytical reagent grade and were used as received.

2.2. Preparation of buccal gels containing PC

The gels (such as sodium alginate (7, 10%, w/v), hydroxypropyl-methylcellulose (HPMC, 2.5, 5%, w/v), methylcellulose (2, 5%, w/v), hydroxyethylcellulose (HEC, 1.5, 2%, w/v), sodium carboxymethylcellulose (2, 6%, w/v), carbopol 934 (1, 2%, w/v) were prepared by dispersing an appropriate amount of the polymer powder in bicarbonate buffer (pH 9.0) in which PC (0.5%, w/w) and the additives (saccharin sodium: 0.05%, w/v) as a sweeting agent and preservatives (0.2%, w/v methyl paraben and 0.02%, w/v propyl paraben) were previously dissolved. The preparations were brought to final volume with the same buffer and throughly agitated by magnetic stirring (Gallen Kamp, London, UK) until clear, transparent gels were formed. Care is being taken to avoid the formation of air bubbles (Santoyo et al., 1995).

To investigate the effect of drug concentration on the in-vitro release, three different gel bases (3% MC, 2.5% HPMC and 7% Na alg.) were selected and the drug was incorporated into the gel bases at three concentration levels (0.5, 1 and 2%, w/w). On the other hand, for the study of the effect of penetration enhancers 1% NaLS, 3% NaDC and 3% NaTGC on PC permeation rate, they were incorporated in the above-mentioned gels separately with 0.5% (w/w) PC and other additives.

The HPC gel was prepared as follows: PC (0.5%, w/w) and benzoic acid (0.5%, w/w) were dissolved in ethanol. Other additives were dissolved in bicarbonate buffer (pH 9.0) and glycerine (2%, w/v) and Tween 80 (5%, w/v) were added. Both solutions were then well mixed and finally an appropriate amount of HPMC (2.5 or 5%, w/v) was gradually added while stirring using a magnetic stirrer until a clear gel was formed. The preparation was brought to final volume with buffer, being kept under magnetic stirring for 12 h prior to use.

Pluronic F-127 gels (20 and 25%, w/v) were prepared according to the cold technique (Shin et al., 1999). Briefly, the required amount of PF-127 powder was gradually added into cold bicarbonate buffer (pH 9, 5 °C) containing 0.5% PC and other additives under constant agitation with a magnetic stirrer. The resulting solution was left overnight in a refrigerator at 5 °C to complete polymer desolvation and became a clear liquid. The solution was then brought to volume with cold buffer and well mixed while cold. The gel was found when the solution was brought back to room temperature.

Polyvinyl alcohol (10 and 15%, w/v) gels were prepared as follows: a weighed amount of PVA powder was dissolved in half the amount of bicarbonate buffer (pH 9.0) by heating under constant agitation with a magnetic stirrer. PC (0.5%, w/w) and other additives were dissolved in the other portion of buffer. The resulting solution was then added while stirring to the above-given cold polymer solution and throughly agitated while cold until the gel was formed.

2.3. Preparation of rabbit buccal membrane

Animals (albino rabbits: 2–2.5 kg) were sacrificed immediately before the start of the experiment. The buccal mucosa was freshly excised and stored in isotonic phosphate buffer solution (PBS; pH 7.4) at 4 °C upon removal. The mucosal membrane (epithelium) was separated by removing the remaining muscle and underlying connective tissue with very fine-point forceps and surgical scissors, making sure that the basal membrane was still present (Senel et al., 1998). The tissue was rinsed and then stored in ice-cold PBS until mounted in the diffusion cell (within 1 h upon removal). Slice thickness ranged from 2.2 to 2.5 mm.

2.4. In-vitro permeation study

The rabbit buccal membrane was mounted between the donor and receptor compartments of Franz-type diffusion cells (Senel et al., 1997), with the epithelial side facing the donor compartment. The diffusional surface area was 3.14 cm^2. Isotonic PBS (pH 7.4) was

used for the receptor phase (15 ml) and maintained at 37 ± 1 °C using a circulating water bath. Uniform mixing of the receptor medium was provided by magnetic stirring. After an equilibration period of 30 min with PBS on both sides of the tissue, 1 gm of the gel was applied on the mucosal surface in the donor compartment and 0.5 ml of PBS was then added. At pre-determined time intervals over a 1 h period, samples (2 ml) were withdrawn from the sampling arm of the receiver compartment and replaced immediately with an equivalent volume of fresh PBS at 37 ± 1 °C. The samples were assayed spectrophotometrically (Shimadzu, Double-Beam Spectrophotometer 150-02, Japan) at 353 nm.

The effect of polymer type and concentration, as well as drug concentration (0.5, 1 and 2%) and penetration enhancers (1% NaLS, 3% NaDC and 3% NaTGC) on permeation of PC through rabbit buccal membrane was studied (Tables 1 and 2). The enhancement action of each of the enhancers was separately determined. The effects of drug concentration and enhancers on the permeation rate were studied using the buccal gel bases with the greatest permeation values (7% Na alg., 2.5% HPMC and 3% MC) (Table 2).

In order to investigate the drug accumulation in the tissue after the permeation test, the membrane was placed in 10 ml of PBS for 6 h. At the end of incubation period, the tissue was removed, wiped of with clean tissue paper and washed several times with PBS until a constant concentration of drug was measured. The drug concentration in the buffer and washing solutions was then determined spectrophotometrically at 353 nm to obtain the amount of drug stored in the buccal membrane.

2.5. Permeation data analysis

The cumulative amount of the permeated drug per unit surface area was plotted versus time and the cumulative flux (J_{ss}, $\mu g\,cm^{-2}\,min^{-1}$) was calculated from the steady-state slope of the linear portion of the plot. The steady-state permeability coefficient (P, $cm\,min^{-1}$) was calculated from $P = J_{ss}/C_d$, where C_d is the concentration of drug in the gel added to the membrane in the donor compartment. The lag time

Table 1
Calculated permeation parameters, amounts permeated through the tissue and amounts retained in the tissue of PC delivered from various gel formulations across rabbit buccal mucosa (phosphate buffer, pH 7.4)

Polymer		Permeation parameters			Amounts permeated through the tissue ($\mu g\,cm^{-2}$)	Amounts retained in the tissue ($\mu g\,cm^{-2}$)
Type	Concentration (%)	J ($\mu g\,cm^2\,min^{-1}$)	L_t (min)	$P \times 10^{-4}$ ($cm\,min^{-1}$)		
HEC	1.5	1.58 (±0.01)	4.02 (± 0.2)	3.26 (± 0.07)	91.60 (± 0.8)	178.98 (± 1.6)
HEC	2.0	1.34 (± 0.012)	2.83 (± 0.48)	2.76 (± 0.03)	78.98 (± 1.3)	168.15 (± 1.9)
HPC	2.5	2.99 (± 0.01)	1.40 (± 0.16)	6.10 (± 0.02)	173.00 (± 1.0)	185.35 (± 1.6)
HPC	5.0	2.86 (± 0.01)	4.48 (± 0.15)	5.87 (± 0.022)	156.40 (± 0.98)	172.29 (± 1.6)
HPMC	2.5	3.19 (± 0.008)	1.16 (± 0.13)	6.50 (± 0.016)	185.00 (± 0.97)	178.02 (± 1.68)
HPMC	5.0	1.97 (± 0.011)	3.96 (± 0.20)	3.83 (± 0.02)	108.30 (± 1.0)	174.84 (± 1.27)
MC	3.0	2.84 (± 0.004)	1.42 (± 0.20)	5.90 (± 0.009)	162.20 (± 0.9)	178.34 (± 2.0)
MC	5.0	1.40 (± 0.01)	5.20 (± 0.24)	2.89 (± 0.02)	75.88 (± 1.0)	154.77 (± 0.95)
NaCMC	2.0	1.23 (± 0.007)	1.78 (± 0.30)	2.56 (± 0.015)	71.00 (± 0.78)	136.30 (± 1.68)
NaCMC	6.0	0.95 (± 0.006)	2.89 (± 0.40)	1.99 (± 0.011)	54.24 (± 0.7)	119.74 (± 2.1)
Na alg.	7.0	3.34 (± 0.008)	3.60 (± 0.13)	8.50 (± 0.02)	190.10 (± 0.8)	162.42 (± 1.15)
Na alg.	10.0	2.76 (± 0.01)	5.74 (± 0.18)	6.75 (± 0.02)	152.00 (± 1.0)	153.82 (± 1.37)
PVA	10.0	1.43 (± 0.01)	3.65 (± 0.32)	3.18 (± 0.02)	80.00 (± 0.77)	97.13 (± 1.6)
PVA	15.0	1.05 (± 0.011)	5.13 (± 0.45)	2.38 (± 0.02)	57.50 (± 1.0)	92.04 (± 2.2)
PF-127	20.0	1.52 (± 0.008)	2.20 (± 0.28)	3.39 (± 0.02)	89.47 (± 0.86)	93.31 (± 1.6)
PF-127	25.0	1.35 (± 0.011)	3.46 (± 0.37)	3.02 (± 0.024)	76.00 (± 1.1)	88.85 (± 2.5)
Carb. 934	1.0	1.78 (± 0.005)	1.93 (± 0.34)	3.93 (± 0.011)	100.00 (± 0.8)	114.33 (± 2.5)
Carb. 934	2.0	1.63 (± 0.01)	3.40 (± 0.34)	3.54 (± 0.025)	90.00 (± 1.1)	103.18 (± 1.9)

J is the steady-state flux ($\mu g\,cm^2\,min^{-1}$); L_t, lag time (min); P, permeability-coefficient ($cm\,min^{-1}$). Each gel formulation contains 0.5% PC; values are presented as the mean ± S.D. of three experiments.

Table 2
Effect of different penetration enhancers on permeation characteristics of PC delivered from various gel formulations across rabbit buccal mucosa (phosphate buffer, pH 7.4)

Polymer		Enhancer		Permeation parameters			
Type	Concentration (%)	Type	Concentration (%)	J ($\mu g\,cm^2\,min^{-1}$)	L_t (min)	$P \times 10^{-4}$ ($cm\,min^{-1}$)	EF (%)
MC	3.0	–	–	2.84 (± 0.004)	1.42 (± 0.2)	5.90 (± 0.001)	–
		NaLS	1.0	3.89 (± 0.004)	1.50 (± 0.17)	8.10 (± 0.01)	140 (± 0.2)
		NaDC	3.0	3.27 (± 0.006)	1.82 (± 0.15)	6.80 (± 0.015)	115 (± 0.3)
		NaTGC	3.0	3.22 (± 0.009)	1.74 (± 0.083)	6.70 (± 0.02)	113 (± 0.4)
HPMC	2.5	–	–	3.19 (± 0.008)	1.16 (± 0.13)	6.50 (± 0.016)	–
		NaLS	1.0	3.97 (± 0.006)	1.30 (± 0.11)	8.06 (± 0.015)	124 (± 0.25)
		NaDC	3.0	3.60 (± 0.01)	1.53 (± 0.33)	7.25 (± 0.01)	112 (± 0.16)
		NaTGC	3.0	3.54 (± 0.005)	1.50 (± 0.25)	7.40 (± 0.02)	114 (± 0.35)
Na alg.	7.0	–	–	3.34 (± 0.008)	3.60 (± 0.13)	8.50 (± 0.02)	–
		NaLS	1.0	4.074 (± 0.006)	3.20 (± 0.17)	10.18 (± 0.015)	120 (± 0.15)
		NaDC	3.0	3.75 (± 0.004)	3.60 (± 0.17)	9.02 (± 0.01)	106 (± 0.1)
		NaTGC	3.0	3.68 (± 0.003)	3.90 (± 0.15)	9.20 (± 0.008)	108 (± 0.1)

NaLS, sodium lauryl sulphate; NaDC, sodium deoxycholate; NaTGC, sodium tauroglycocholate; J, steady-state flux ($\mu g\,cm^2\,min^{-1}$); L_t, lag time (min); P, permeability-coefficient ($cm\,min^{-1}$); EF; enhancement factor. Each gel formulation contains 0.5% PC; values are presented as the mean ± S.D. of three experiments.

values (min) were determined from the x-intercept of the linear portion of the curve at steady-state. The enhancement factor (EF (%)) was calculated as $EF\,(\%) = (P_{enh}/P_{control}) \times 100$, where P_{enh} is the permeability coefficient obtained for the gel containing enhancer and $P_{control}$ is the permeability coefficient for the gel without enhancer (Senel et al., 1998).

2.6. Histological study of the buccal mucosa

After the in-vitro permeation experiments, tissue samples treated with HPMC (2.5%) gel containing 0.5% PC without or with enhancer (3% NaDC, 3% NaTGC or 1% NaLS) were removed from the diffusion cell and examined. An untreated membrane was used as a control, after incubation with phosphate buffer (pH 7.4) on the mucosal and serosal sides for 1 h. Cross-sections from the tissue samples were initially fixed in 2% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) for 2 h followed by a post-fixation performed with 1% osmium tetroxide in phosphate buffer for 2 h. The mucosa was then dehydrated using a graded series of ethanol (30% through absolute) (Senel et al., 1997). After drying, the mucosal surface was coated with gold in a vacuum (SPI Sputter™ coating unit: SPI Supplies, Westchester, PA, USA) and examined using a JEOL scanning electron microscope (SEM) (JSM-5200, Japan). SEM micrographs were taken at 15 kV.

2.7. Anti-inflammatory activity of buccal PC gel formulations

Adult male albino rats (six per group) weighing 200 ± 20 g were used for the test of the anti-inflammatory activity of the selected gels (7% Na alg., 2.5% HPMC and 3% MC) containing 0.5% PC and 3% NaDC by the kaolin-induced paw oedema method. The rats receiving oral doses of PC were fasted with free access to water for 12 h prior to the test. Paw oedema was induced by subcutaneous injection of a 10% kaolin suspension in normal saline into the right subplantar region of the rat hind-paw (Winter, 1964). One hour before induction of oedema (Method A), the first group of rats was given 0.5 ml of PC solution (0.5%, w/v) by oesophageal tube, the second group received 0.5 g of MC gel applied to the buccal area, the third group received 0.5 g of HPMC gel, the fourth group received 0.5 g of Na alg. gel. and the control group was administered only the gel base without the drug by the same mode of application. The thickness of the rat hind-paw was measured by vernier caliper (SMEC, China) before kaolin injection,

immediately after kaolin injection (0 time) and then at various times (30 min interval) for 4 h. The percent swelling of the paw was calculated using the following equation (Chi and Jun, 1990):

$$\text{Percent swelling} = \frac{V - V_i}{V_i} \times 100$$

where V is the paw thickness at each time interval, and V_i, initial paw thickness (before kaolin injection).

The average paw swelling in the group of the drug-treated rats was compared with that of the control rats and the percentage inhibition of the oedema formation was determined using the following equation (Chi and Jun, 1990):

$$\text{Percentage inhibition} = \left(1 - \frac{\text{Percentage swelling of drug-treated group}}{\text{Percentage swelling of control group}}\right) \times 100$$

Statistical differences between the experimental groups were determined by the *t*-test analysis.

In order to investigate the anti-inflammatory effect of the buccal gels on the performed oedema, the test was done by giving the medication 1 h after kaolin injection (Method B). The paw thickness was measured before kaolin injection, immediately after giving the PC gel (i.e. 1 h after kaolin injection) and then after 30 min time intervals for 4 h. The percent swelling and percent inhibition (or reduction) were determined at the designated time intervals as mentioned above and the results were statistically compared with those of Method A.

2.8. Clinical evaluation

Forty patients, 10 non-pregnant females and 30 males (mean age: 27.8 years), referred to the unit of maxillofacial surgery (Assiut University Hospital), for the treatment of post-operative dental pain and oedema following maxillofacial operations were considered. Operations were either minor or major. Minor operations included cyst, simple fracture, cut mucosal wounds, epulides (gum swelling) and cheek polybs. Major operations included fracture mandible, fracture maxilla, glossectomy, mandibulectomy and cleft palate. In these major operations, a single parenteral dose (50 mg) of Pethidine® was given to control the immediate post-operative pain (in the first 2–4 h).

Na alg. (7%), HPMC (2.5%) and MC (3%) were selected as potential vehicles from the previous studies, since all three gels had shown good permeation properties and anti-inflammatory activities. All formulations contained 0.5% PC and 3% NaDC as a penetration enhancer.

Ten patients (who underwent surgery) received a single buccal application of 0.5% PC gel (equivalent to 20 mg of PC) immediately after the operation (day-1), and daily thereafter, for 3 days. PC gel preparations were slowly delivered to the experimental sites by using a disposable single dose tube. For comparison, another group (10 patients) were given the same dose of PC as Feldene Flash® tablets (one tablet) according to the same regimen. Clinical assessments were made on the day of surgery (i.e. 8 h after application of the first dose (day-1)) and then daily at the same time on the second, third and fourth day of treatment.

The following parameters were utilised during the study period:

1. Spontaneous pain: as assessed by the patient using a visual analogue scale (VAS) score (Fig. 1). It ranged from 0 (no pain) to 10 (the worst pain imaginable). The patients were asked by the investigator

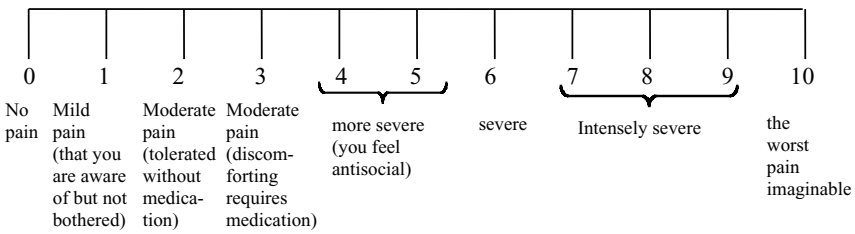

Fig. 1. Visual analogue scale.

to describe the degree of pain they have experienced (Cruz, 1984).

2. Swelling: as assessed by the investigator. The degree of swelling was measured during the four study days and in the pre-operative day (day-0) by measuring the thickness of oedema using a vernier caliper.
3. Tenderness: as assessed by the investigator using a four-point rating scale (none, mild, moderate and severe). Patients were also assessed at each visit as to the occurrence of side effects and the investigator was blinded to the treatment performed. For statistical comparisons between treatments, the means were subjected to the least significant difference (LSD) test to evaluate mean differences in clinical recordings. The analysis of variance (ANOVA) was also applied to test the pain score and swelling variability with time. The level of significance was set at 5%.

3. Results and discussion

3.1. Permeability studies

3.1.1. Effect of polymer type and concentration on the permeation of PC through rabbit buccal mucosa

The desired release rate of the particular drug substance from the base is primarily dependent on drug affinity to the vehicle, the desirability of the base for enhancing the buccal absorption of the drug and the influence of the drug on the consistency or other features of the base.

The in-vitro study applied in this work employed excised rabbit buccal membrane to be mounted in a diffusion cell. One compartment of the diffusion cell contained the PC gel and the other compartment acts as the receiver.

The values of the permeation parameters of PC from various gel bases are presented in Table 1. It can be seen from the given data that clear differences in permeation characteristics were found between different types of gel bases. In all instances, a shorter lag time (L_t) in the range of 1.16–5.74 min (depending on base type and concentration) was observed in PC flux across the buccal mucosa. It is also apparent that the L_t increased with increasing the polymer concentration. The highest values of the steady-state flux, J and permeability coefficient, P were obtained from 0.5% PC gel in 7% Na alg. base ($J = 3.34\,\mu g\,cm^2\,min^{-1}$, $P = 8.5 \times 10^{-4}\,cm\,min^{-1}$) and 2.5% HPMC base ($J = 3.19\,\mu g\,cm^2\,min^{-1}$, $P = 6.5 \times 10^{-4}\,cm\,min^{-1}$) and the lowest from 6% NaCMC base ($J = 0.95\,\mu g\,cm^2\,min^{-1}$, $P = 1.99 \times 10^{-4}\,cm\,min^{-1}$). Whilst, for example, the 3% MC gel showed intermediate permeation characteristics ($J = 2.84\,\mu g\,cm^2\,min^{-1}$, $P = 5.9 \times 10^{-4}\,cm\,min^{-1}$). The obtained results could be explained on the basis that 7% Na alg., 2.5% HPMC and 3% MC gels exhibited the lowest viscosity values (values $(\times 10^{-2}) = 105$, 420 and 250 cP, respectively, at 37 °C and a shear rate of 0.73) amongst all the tested formulations. Therefore, a higher P values of these gels was probably due to an increased amount of free water and thus expanding the aqueous channels in the gel (Shin et al., 1999). Also, these polymers are good mucoadhesives and their bioadhesion properties may enhance drug transfer to the biological membrane (Junginger, 1991). Furthermore, as PC is a weakly acidic compound with high protein binding (Florey, 1986), it should have a greater physicochemical attraction to the oral epithelium than to the anionic polymers (e.g. Na alg.) so that the drug can leave the vehicle in favour of the buccal mucosa.

As knowledge of the viscosities of the gel formulations could provide some insight about their permeability characteristics, it remains unclear that gels with higher viscosities such as 20% PF-127 and 1% Carb. 934 (values $\times 10^{-2} = 4200$ and 2530 cP, respectively) had slightly higher permeation than those of a lower viscosity (e.g. 2% NaCMC and 1.5% HEC, viscosity values $(\times 10^{-2}) = 688$ and 890 cP, respectively) (Table 1). These findings can be interpreted in terms of the fact that these polymeric macromolecules are surface active and mucoadhesive agents that may enhance the membrane permeability (Shin et al., 2000a). Generally, the results obtained demonstrate also that an inverse correlation existed between the amount of PC permeated through the buccal mucosa and concentration of gel-forming polymer (Table 1). This tendency was more pronounced in Na alg. (7, 10%, w/v), HPMC (2.5, 5%, w/v) and MC (3, 5%, w/v) gels. Thus, using higher concentrations of these gels resulted in a decrease in the J values by about 17, 38 and 50%, respectively (Table 1). A result that may be attributed to the higher average viscosities of the higher polymer

concentrations which provide higher resistance to drug diffusion. A similar trend was observed on the effect of poloxamer content on diffusivity of PC across a rat abdominal skin (Shin et al., 1999), indicating that the diffusion of PC from the gel formulation is largely dependent on the microviscosity of the water channels of the gel matrix rather than the macroviscosity of the gel and the higher concentration of polymer causes the reduction in the size of water channels, the micellar growth, or greater tortuosity (Hsueh-Ling and Susan, 1990).

In the current study, the cumulative amount of PC that permeated across the buccal membrane over a 1 h period is 2.1–7.6-fold (depending on gel type and concentration) greater than those reported by Santoyo et al. (1995) on the absorption of PC from carbopol aqueous gels in excised rat skin during the initial 6 h. The difference between the values can be related to differences in gel type and concentration, as well as tissue properties in animal models.

Estimation of drug binding in or on the oral epithelium might reflect the rate of drug disappearance from the oral cavity and appearance in the plasma and accordingly the duration of efficacy of drugs after buccal administration (McElnay, 1990). Table 1 summarises the results of the residual amounts of drug within the buccal mucosa following the in-vitro permeation experiments. Obviously, the membrane retention of PC was the highest for cellulose derivative (HEC, HPC, HPMC and MC) and Na alg. gels. In most of the cases, the amount of drug accumulated within the buccal tissue was higher than the amount permeated through the mucosa, which may be attributed to the high protein-binding of PC (>99%) (Florey, 1986). Buccal membrane storage of similar lipophilic compounds was also investigated (Schürmann and Turner, 1978). However, the mucosal constituents responsible for drug binding have not been identified (McElnay, 1990). Table 1 also demonstrates that the amount of PC retained in the buccal mucosa was observed to be related to the PC flux through the membrane. The results obtained indicated similarity with those of Doliwa et al. (2001) on permeation and skin retention of PC from gels containing PC as an inclusion complex with hydroxypropyl-β-cyclodextrin.

From the results of the total flux (amounts permeated through and amounts retained in the tissue) of 0.5% PC from the gel bases prepared at lower polymer concentrations (Table 1), the rank order of permeation appeared to be: HPMC > HPC ≥ Na alg. > MC > HEC > Carb. 934 ≥ NaCMC > PF-127 > PVA. Thus, it seems likely that Na alg., HPMC and MC gel bases may be the vehicles of choice for PC in the consideration of ease of preparation, fast drug release at the target site and membrane retention of the drug, and therefore, were selected for application to further studies.

3.1.2. Effect of drug concentration on the permeation rate of PC

The permeation of PC across the buccal mucosa was studied under conditions of varying drug concentration (0.5–2%, w/w) in the selected gel formulations (3% MC, 2.5% HPMC and 7% Na alg.) (Fig. 2). A plot of the *J* values of PC versus drug concentration in the gels revealed that increasing drug concentration in the gel bases from 0.5 to 2% resulted in a 1.8-fold increase in the flux of PC. These results are entirely relevant to the increase in the driving force of drug in the buccal gel and the changes in vehicle structure with increasing the amount of drug loading. A similar tendency was observed by Shin et al. (1999) on the

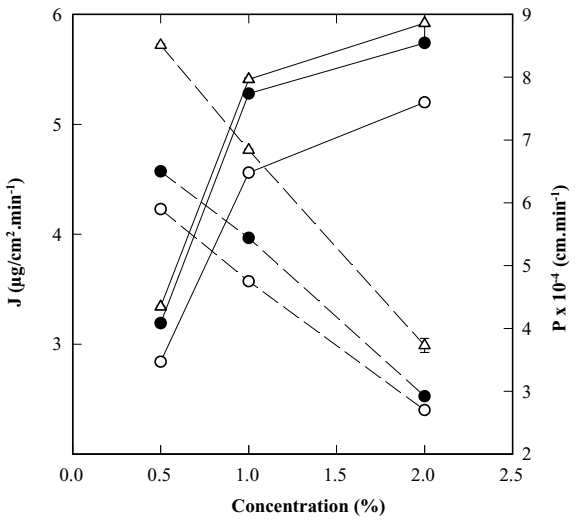

Fig. 2. Effect of initial drug concentration of PC on the mean fluxes, *J* (solid lines) and permeability-coefficients, *P* (dotted lines) of PC delivered form various gel formulations across rabbit buccal mucosa. Gel formulations: (○) 3% MC, (●) 2.5% HPMC, (△) 7% Na alg. Each point represents the mean±S.D. of three experiments.

effect of PC concentration in the poloxamer gel on the drug permeation across rat abdominal skin.

However, the permeation rate from a 2% formulation was only about 8–12% higher than from the 1% formulation. The extent of increase in the permeation rate when the amount of the drug exceeds saturation depends on the relative magnitude of diffusivity of the drug within the gel to the dissolution medium. When the diffusivity becomes the rate-limiting step, the permeation rate does not change significantly with increasing concentration (Shin et al., 1999), as was the case when the concentration of PC exceeded 1%. Also, it can be seen from Fig. 2 that the $P(= (dQ/dt)/\Delta CA)$ values decreased as the concentration of PC was increased. This may be ascribed to the increase in the concentration difference (ΔC) across the buccal mucosa with increasing the drug concentration due to the high buccal membrane retention of PC which retarded the membrane permeability of the drug.

3.1.3. Effect of penetration enhancer on the permeation rate of PC

The effect of various penetration enhancers (1% NaLS, 3% NaDC and 3% NaTGC) on the permeation of 0.5% PC delivered from different gel bases (3% MC, 2.5% HPMC and 7% Na alg.) across rabbit buccal mucosa was investigated (Table 2). Table 2 shows the fluxes, *P*-values and the enhancement factor values of each permeation enhancer tested. Addition of 1% NaLS provided the highest permeation rate with an EF (%) of 120–140 (depending on the type of gel) in comparison with the bile salts which exhibited nearly similar enhancement characteristics and increased the drug permeation only slightly (EF (%) = 106–124). The presence of micelles of NaLS, as a surfactant could be changing the gel structure and strength, as well as the equilibrium of the drug in the gel matrix, thus modifying its release mechanism and rate. In addition, NaLS has a high deep tissue penetrant potential and can enter into blood following the application to the surface of the oral epithelium, thereby facilitating the intercellular as well as transcellular transport of the permeant (Siegel and Gordon, 1985a,b).

Regarding the penetration of PC gels, the absorption of a drug through the buccal mucosa can be determined by the following three steps: (1) the extent of dissolution of the drug in a gel, (2) the rate of penetration of the drug and (3) the rate of penetration of enhancer through the mucosa. Since, the enhancers examined can penetrate mucosa, drug dissolved in gel transports with them (Siegel and Gordon, 1985b; Kitano et al., 1998; Senel et al., 1998). The results obtained are consistent with those of Steward et al. (1994) who found NaLS (1%) to be superior to other types of absorption promoters including sodium taurocholate at 1% concentration in enhancing the buccal absorption of α-interferon. However, Aungst and Rogers (1989) reported that NaLS (5%) was equally effective as bile salts such as NaDC and sodium glycocholate used at 5% concentration in enhancing buccal insulin delivery. Reportedly, the enhancing effect of bile salts is dependent on the concentration of enhancer, where in most cases, concentrations greater than 1% were required, and the type of permeant as well (Aungst and Rogers, 1989; Steward et al., 1994; Shin et al., 2000b).

Fig. 3 shows the representative permeation profile of PC from 7% Na alg. gel containing enhancers (1% NaLS, 3% NaDC or 3% NaTGC) through the buccal mucosa. A linear steady-state phase of the permeating species was obtained when the cumulative amount permeated was plotted against time, indicating that the excised membrane is permeable to the model drug

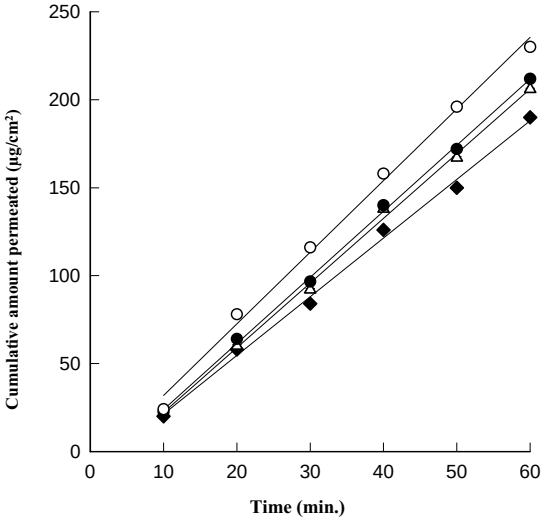

Fig. 3. Effect of different penetration enhancers of the permeation of PC delivered from Na alg. gel formulations across rabbit buccal mucosa. Penetration enhancers: (○) 1% NaLS, (●) 3% NaDC, (△) 3% NaTGC, (◆) control gel. Each gel formulation contains 7% Na alg. and 0.5% PC; each point represents the mean ± S.D. of three experiments.

and the permeation of PC undergoes passive diffusion through the model membrane (Shah et al., 1992).

3.2. Histological studies

SEM micrographs of the control (untreated) buccal epithelium reveal the appearance of the superficial cells of the epithelium which represent the major absorption site in the oral cavity. It is also clear that the stratified squamous cells have intact cell junctions with microriges or micropillee (Fig. 4a, 2000×).

Treatment of the buccal mucosa with the gel formulation containing 0.5% PC (without enhancer) showed that the squamous cells are normal and to some extent similar to those of the control. But, slight histological changes such as shrinkage of superficial cells appeared in some parts of the tissue (Fig. 4b, 2000×). These changes may indicate partial desquamation of the superficial cells when viewed at higher magnification (Fig. 4c, 5000×).

Fig. 4d–f demonstrates SEM micrographs of the buccal mucosa treated with the gel formulation containing 0.5% PC and penetration enhancer (1% NaLS, 3% NaDC or 3% NaTGC). Incorporation of the bile salts in the gel was accompanied by significant morphological changes which include corrugation and desquamation of the superficial cell layers (Fig. 4d, 5000× and Fig. 4e, 5000× for NaDC and NaTGC, respectively). In addition, NaTGC showed obvious signs of structural changes in the superficial epithelial cell layers, such as flattening of the cells and appearance of empty and wider intercellular spaces, indicating the change in membrane permeability (Fig. 4e, 5000×). The effect of bile salts on the permeability barrier has been reported to be reversible and dependent on the concentration of the bile salts, and no gross morphological changes have been observed on buccal epithelium in-vivo (Zhang et al., 1994; Hoogstraate et al., 1996; Shin et al., 2000b). However, Senel et al. (1994) reported that a high concentration (5%, w/w) of bile salts (e.g. glucodeoxycholate) perturbed or resulted in loss of outer epithelial cell layers of porcine buccal epithelium, disruption of the connection between epithelium and the underlying connective tissue, and a disorganisation of the intercellular and membrane lipids. Swelling of the cells and a possible increase in intercellular space were also observed (Senel et al., 1997).

With respect to NaLS, more pronounced histological changes in the superficial buccal epithelium appeared. Thus, the SEM micrographs show severe cell shrinkage and indicate marked corrugation and desquamation of squamous cells (Fig. 4f, 5000×). In addition, an increase in intercellular space and swelling, especially inside the cells, was observed in the micrograph (Fig. 4f, 5000×) which may be due to penetration of NaLS into deep mucosal tissues and its known irritant potential (Siegel and Gordon, 1985a; Bary, 1987). These histological findings correlate well with the permeation results where the permeation of PC through the buccal mucosa was the highest (EF (%) = 120–140) in the presence of NaLS (1%). Similarly, Gandhi and Robinson (1992) depicted that rabbit buccal mucosa treated with 0.1% NaLS or 0.5% NaDC showed loss of surface epithelial layers, which correlated with increased permeability of the permeant. Also, Siegel and Gordon (1985b) reported that application of up to 1% NaLS to the ventral surface of the tongue of dogs caused loss of surface squamous cells and widening and separation of keratin, which could affect the transport of the permeant.

In fact, diffusion of substances through the oral mucosal membrane is principally through the lipid-filled intercellular spaces as the extraction of these lipids and alteration of cellular proteins resulted in a highly permeable tissue (Bary, 1987; Hoogstraate et al., 1996; Senel et al., 1997). We, therefore, suggest that the enhancing effect of the tested enhancers depends on the degree of their membrane irritation potential and the rate of penetration of enhancer through the mucosa and the increase in the fluidity of the intercellular lipids, thereby facilitating diffusion of the drug through the epithelium. Since recovery from potential alterations induced by bile salts exposure is possible only in-vivo (Senel et al., 1998), NaDC as an enhancer was selected for further investigations.

3.3. Anti-inflammatory activity of buccal PC gel formulations

The level of the kaolin-induced paw oedema in rats was chosen as a surrogate measure of anti-inflammatory activity. The anti-inflammatory effect was expressed as the percentage inhibition of oedema compared with vehicle-treated controls. The placebo gels did not modify the response of the paw

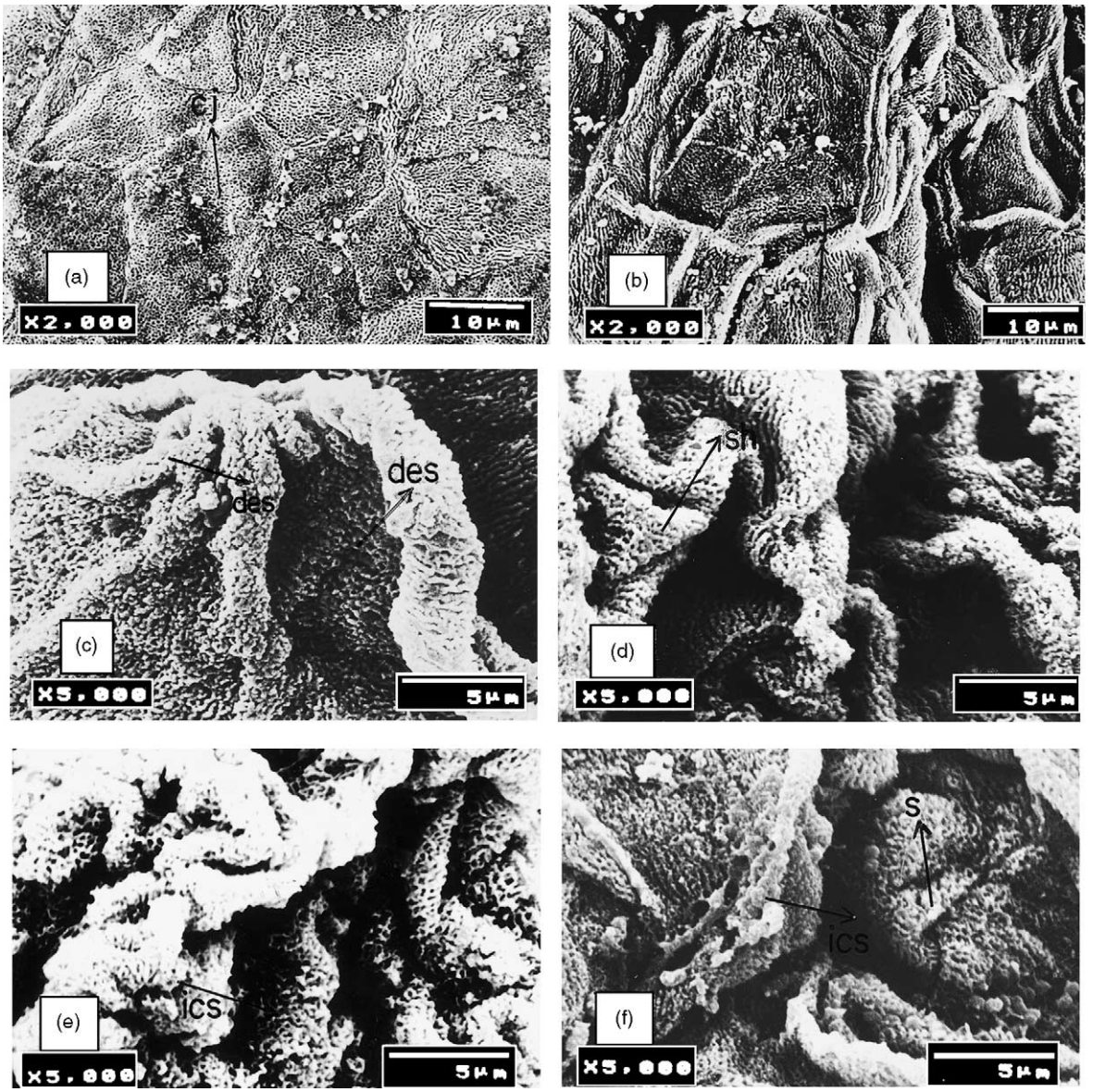

Fig. 4. Scanning electron micrographs of rabbit buccal mucosa: (a) before treatment, (b and c) after 1 h treatment with 2.5% HPMC gel containing 0.5% PC, (d–f) after 1 h treatment with 2.5% HPMC gel containing 0.5% PC and penetration enhancers (3% NaDC, 3% NaTGC or 1% NaLS, respectively). Abbreviations: cj is the cell junction; des, desquamation of superficial cells; sh, shrinkage of superficial cells; ics, intercellular space and s, swelling of cells are illustrated by arrows.

to the kaolin-induced oedema and the percentage swelling ranged from 119.3 ± 1.5 to 192.0 ± 3.2; for the time course of 0.5–4 h after the injection of kaolin, respectively.

Fig. 5A shows the percent inhibition of the oedema formation by the application of doses (equivalent to 20 mg PC kg^{-1}) of the 0.5% PC gel in different gel bases (3% MC, 2.5% HPMC and 7% Na alg.)

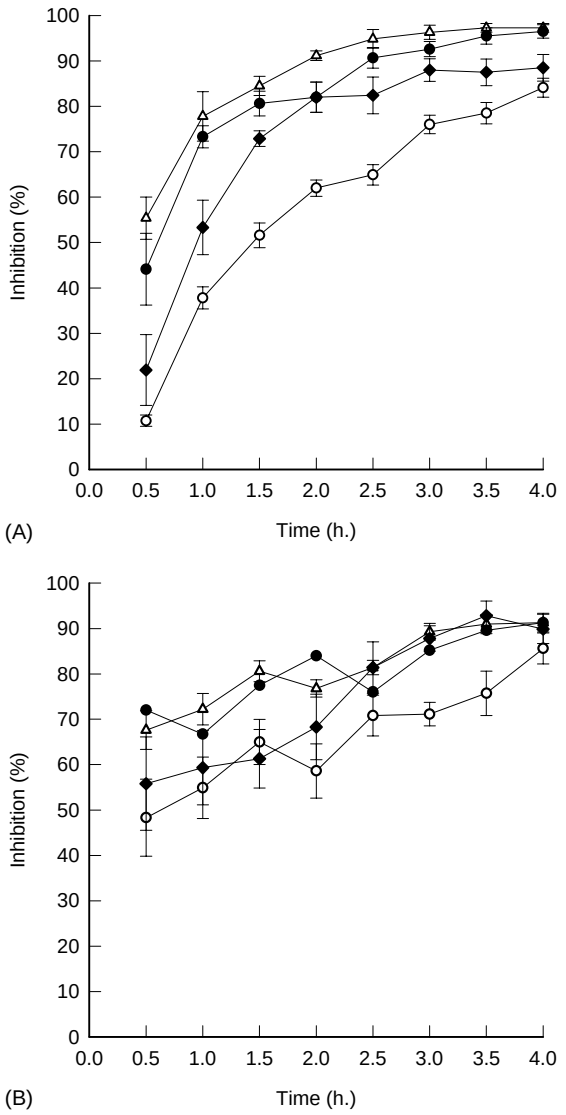

Fig. 5. A comparison of the anti-inflammatory effects of various PC gel formulations applied to the buccal mucosa of rats: (A) 1 h before the induction of oedema in rats and (B) 1 h after the induction of oedema in rats. Gel formulations: (○) 3% MC, (●) 2.5% HPMC, (△) 7% Na alg., (◆) 0.5% oral PC solution. Each gel formulation contains 0.5% oral 3% NaDC as an enhancer, each point represents the mean ± S.E. of rats.

1 h prior to the injection of kaolin (Method A). The anti-inflammatory activity of a 0.5% PC solution after the oral administration according to the same dose regimen was also shown (Fig. 5A). In general, all preparations reduced swelling significantly ($P < 0.001$), but to a variable extent (inhbition (%) ≈ 38–98), over the time course studied (1–4 h) as compared with the control. At the first 30 min of the observation, the 0.5% PC gel in the Na alg. and HPMC bases produced significant inhibitory effects (44 and 55% inhibition of the kaolin-induced oedema formation, respectively) as compared with about 11 and 22% inhibitions obtained with 0.5% PC gel in a MC base or a 0.5% oral PC solution, respectively ($P < 0.05$). The fast inhibitory action of a gel formulation at the receptor sites may be a desirable feature for anti-inflammatory drugs to induce maximal pharmacological action without being prematurely wiped off from the applied site. It is also clear that the reference 0.5% PC solution was slightly more effective between 1 and 3 h (inhibition (%) ≈ 53–88, $P < 0.05$) against the inflammatory response than the MC gel (inhibition (%) = 38–76). At the end of the 4-h study period, at least a 12% greater anti-inflammatory effect was achieved with both Na alg. and HPMC gels (inhibition (%) ≈ 98) than the MC gel or the oral PC solution. This could be attributed to a higher release of the drug from the Na alg. and HPMC gel bases (Table 1) and the difference in the gel structure and the bioadhesion properties of the bases. Similar results were also obtained by Chi and Jun (1990) on studying the anti-inflammatory activity of ketoprofen gel on carrageenan-induced paw oedema in rats.

The percent inhibition of oedema formation by PC after the administration of the 0.5% PC buccal gel or solution at 1 h after the kaolin injection (Method B) was also investigated and compared with that of Method (A). The duration of action of the drug was determined by studying the effect of the 0.5% PC preparations on swelling over 4 h (Fig. 5B). Obviously, the percent inhibition of oedema appeared to be minimal for a 0.5% PC incorporated in a MC base or an aqueous solution of the drug as compared to the PC gel in the Na alg. and HPMC bases. In almost all the cases, higher inhibitory effects were observed with Method B (compared to Method A) at the initial stage of experiments. However, the differences did not reach statistical significance over the time course studied (0–4 h, $P > 0.1$).

In general, the rank order of the oedema inhibition using both methods was noticed to be: Na alg. ($P > 0.1$) ≥ HPMC ($P < 0.05$) > MC. The higher anti-inflammatory activity of the Na alg. and HPMC

gels prepared in this study (compared to oral PC solution) suggests that the inhibitory effects of these gels can not be explained entirely on the basis of systemic absorption and that the drug may enter the circulation through the buccal tissue. This indicates that there would be good practical benefit in using the buccal method of administration for PC.

3.4. Clinical evaluation

Previous multiple-dosing studies have evaluated oral PC (Feldene Flash®) at 20 mg per day in the treatment of patients with post-operative dental pain and showed that the oral preparation effected a significant improvement of clinical parameters (such as pain level, swelling and tenderness) of the patients from the initial to the last (fifth) day of therapy (Cruz, 1984).

In the current study, a time-series analysis was undertaken to determine the efficacy and safety of the analgesic and anti-inflammatory activity of 0.5% PC formulated in three different buccal gel bases (3% MC: Formulation #1, 2.5% HPMC: Formulation #2 and 7% Na alg.: Formulation #3) versus the commercial oral PC (Feldene Flash® tablet) among patients with post-operative pain and oedema following maxillofacial operations. Table 3 shows the mean visual analogue scale scores over the study period starting from day-1, i.e. the night of the operation day, to day-4 after the administration of buccal gels and the classical oral preparation in patients. In general, there has been a progressive decline in the level of pain within the days of therapy for both gels and oral preparation. At the end of the observation period, pain scores (PS) were reduced by about 85, 86 and 80% for Formulations #2 and 3 and the tablets, respectively. When the means were subjected to the least significant difference test, results were significant between the first day scores and those obtained on the next 3 days and even between the third and fourth day of treatment (Table 3). Application of the analysis of variance (ANOVA) to test the PS variability with time yielded a significant F value at the 5% level ($F = 2.85$) for Formulation #1 and at the 1% level for Formulation #2 ($F = 25.1$), Formulation #3 ($F = 78.3$) and the tablet ($F = 23.7$). Comparison among the results in Table 3 revealed also that subjects treated with Formulations #2 and 3 and the tablets showed a trend towards a greater PS reduction (at the end of the follow-up period) compared with those treated with the MC gel (Formulation #1) ($P < 0.01$). The LSD test indicated that differences between Formulations #2 and 3 and the tablets did not reach statistical significance, whereas Formulation #1 differs significantly from these formulations (Table 4). This indicates that Formulations #2 and 3 were

Table 3
Statistical analysis of the effect of various buccal PC gel formulations and Feldene Flash® tablets on average pain score and mean swelling thickness reduction in patients

Formulation type	Clinical parameter	Score level					Least significant difference test				
		Days					First vs. second day	First vs. third day	First vs. fourth day	Third vs. fourth day	LSD test
		0	1	2	3	4					
Formulation #1	PS	–	4.5	4.0	3.2	2.8	0.30	1.2	1.5	0.30	0.29
	ST (cm)	1.13	1.89	1.82	1.65	1.4	–	–	–	–	–
Formulation #2	PS	–	4.7	3.1	1.3	0.70	1.6	3.4	4.0	0.60	1.58
	ST (cm)	0.76	1.00	0.80	0.68	0.62	–	–	–	–	–
Formulation #3	PS	–	4.9	3.1	1.4	0.70	1.8	3.5	4.2	0.70	0.54
	ST (cm)	0.94	1.26	1.02	0.84	0.70	0.24	0.42	0.56	0.14	0.21
Feldene Flash® tablet	PS	–	5.5	3.6	2.3	1.1	1.9	3.2	4.4	1.2	1.81
	ST (cm)	1.02	1.23	1.01	0.81	0.60	0.22	0.42	0.63	0.21	0.060

Formulation #1: 3% MC gel, Formulation #2: 2.5% HPMC gel, Formulation #3: 7% Na alg. gel, each gel formulation contains 0.5% PC and 3% NaDC as an enhancer. PS, pain score; ST, mean swelling thickness (cm). Day-0 is the pre-operative day; day-1 is the day of operation starting 8 h after the application of gel.

Table 4
Comparative statistical analysis of the effect of various buccal PC gel formulations and Feldene Flash® tablets on average pain score reduction in patients

Pairs of comparison	LSD test
Formulation #1 vs. Feldene Falsh®	2.7*
Formulation #2 vs. Feldene Falsh®	0.4
Formulation #3 vs. Feldene Falsh®	0.2
Formulation #1 vs. Formulation #2	2.3*
Formulation #1 vs. Formulation #3	2.5*
Formulation #2 vs. Formulation #3	0.2 (LSD = 0.86)

Formulation #1: 3% MC gel, Formulation #2: 2.5% HPMC gel, Formulation #3: 7% Na alg. gel. Each gel formulation contains 0.5% PC and 3% NaDC as an enhancer.
* Significant value.

slightly better than or equally effective to the tablets in alleviating the pain symptoms and level in all the tested cases (Table 4).

Table 3 also shows the mean values for swelling thickness (ST) measured at baseline (day-0) and at the 4 days following operation. Results revealed that Formulations #2 and 3 and the tablets were equally effective in reduction of the swelling on the day of operation and post-operative day as indicated by the insignificant difference in their swelling scores ($P > 0.05$, $F = 0.212$). During the 4-day period of observation, swelling was significantly improved after treatment with Formulation #3 ($P < 0.05$, $F = 3.35$) and the tablets ($P < 0.01$, $F = 14.5$). The ST values were also subjected to the LSD test (Table 3) and it was found that the differences were significant between the first day and each of the following 3 days (for each formulation), indicating effective reduction of oedema with time progression. On the other hand, no significant changes in the mean ST scores were observed for Formulation #2 from the second to the fourth day of treatment. As the initial swelling level (ST value = 0.76 cm (day-0)) was already low in this case, it is thus expected that the swelling change would be insignificant if the initial level was lower (Cruz, 1984).

On the basis of the number of major and ultramajor operations, it can be considered that Formulation #3 (six major cases) was equally effective to Formulation #2 (seven major and ultramajor cases), but more effective than the tablets (four major cases) in reduction of the first follow-up day swelling. Illustrative cases in Figs. 6 and 7 show that treatment with Formulation #3 inhibited post-operative swelling in patients with oedema following maxillofacial operations. However, application of Formulation #1 in patients with oedema did not result in a significant improvement of the

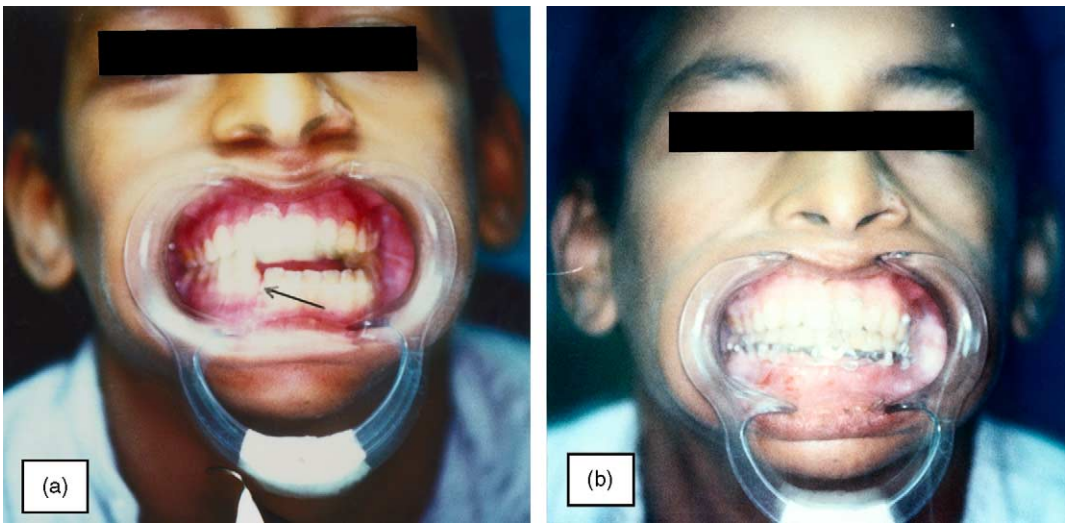

Fig. 6. Illustrative case I indicates: (a) preoperative photograph of fracture mandible (arrow) with mild local swelling due to fracture and (b) photograph showing absence of post-operative swelling 1 day after operation and application of Formulation #3 to the patient. Formulation #3: 7% Na alg. gel containing 0.5% PC and 3% NaDC as an enhancer.

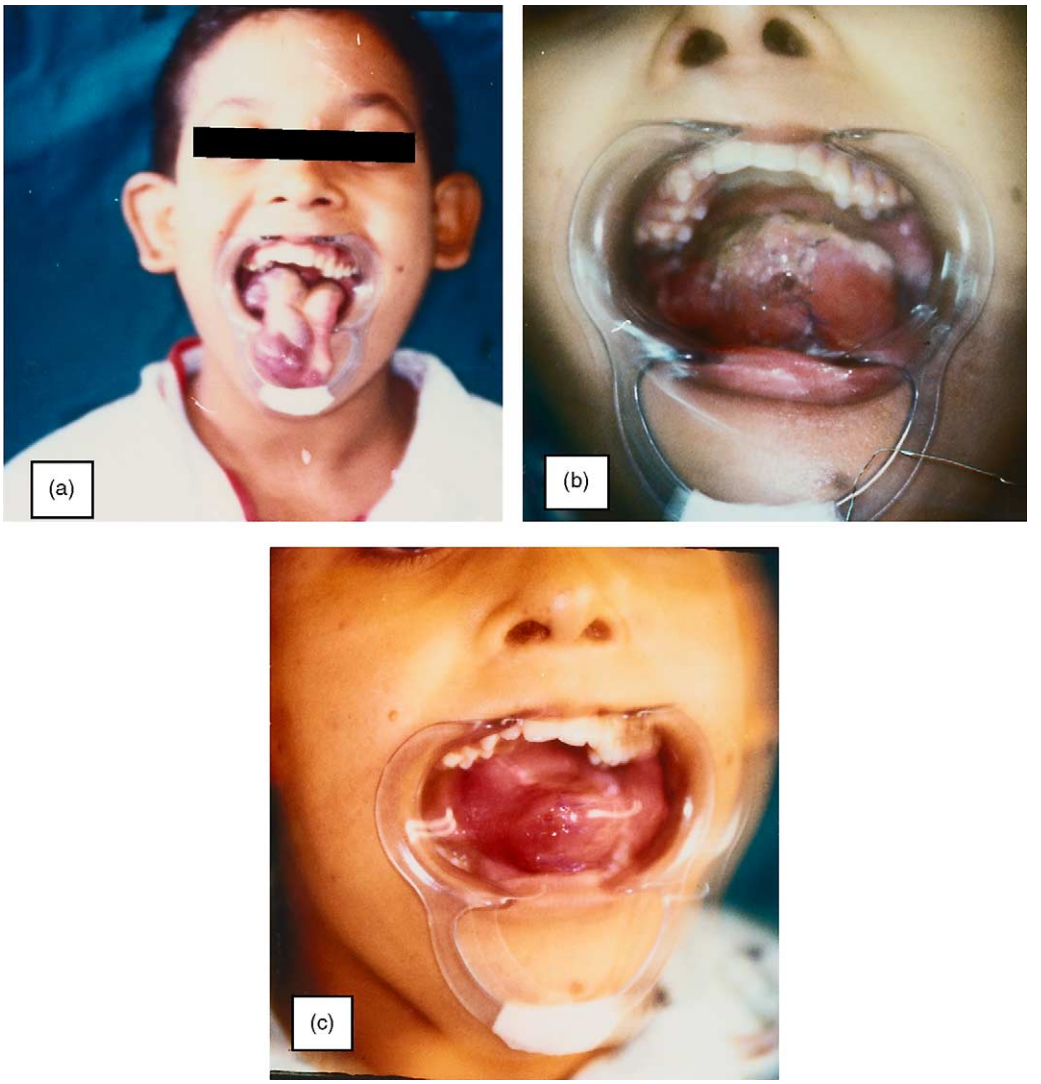

Fig. 7. Illustrative case II indicates: (a) pre-operative photograph of a 14-year-old boy with hamangioma of the tongue (macroglossia), (b) photograph showing reduction of the bulbous tongue and post-operative oedema swelling 1 day after operation and application of Formulation #3 to the patient and (c) photograph showing a marked reduction of post-operative swelling 4 days after operation and application of Formulation #3 to the patient. Formulation #3: 7% Na alg. gel containing 0.5% PC and 3% NaDC as an enhancer.

clinical parameter from the second to the fourth day of therapy as significant swelling was recorded on the first day of operation ($P < 0.05$, $F = 8.4$) compared with day-0.

Fig. 8 shows the tenderness level with time for the tested formulations. Each formulation was tested on 10 patients. At the initial stage (day-1), 70% of patients treated with Formulation #1 experienced severe tenderness (which was sustained until the second day), whereas 30, 20 and 40% of patients were recorded for Formulations #2 and 3 and the tablets, respectively. By day-2, no patient of those receiving the buccal gels or the tablets reported severe tenderness. With time progression, the shift from the moderate to mild level was notable for both gels and tablets (except for Formulation #1) such that, by the third day, no patient had moderate tenderness, which is reflective of a high rate of improvement. At the end of the follow-up period,

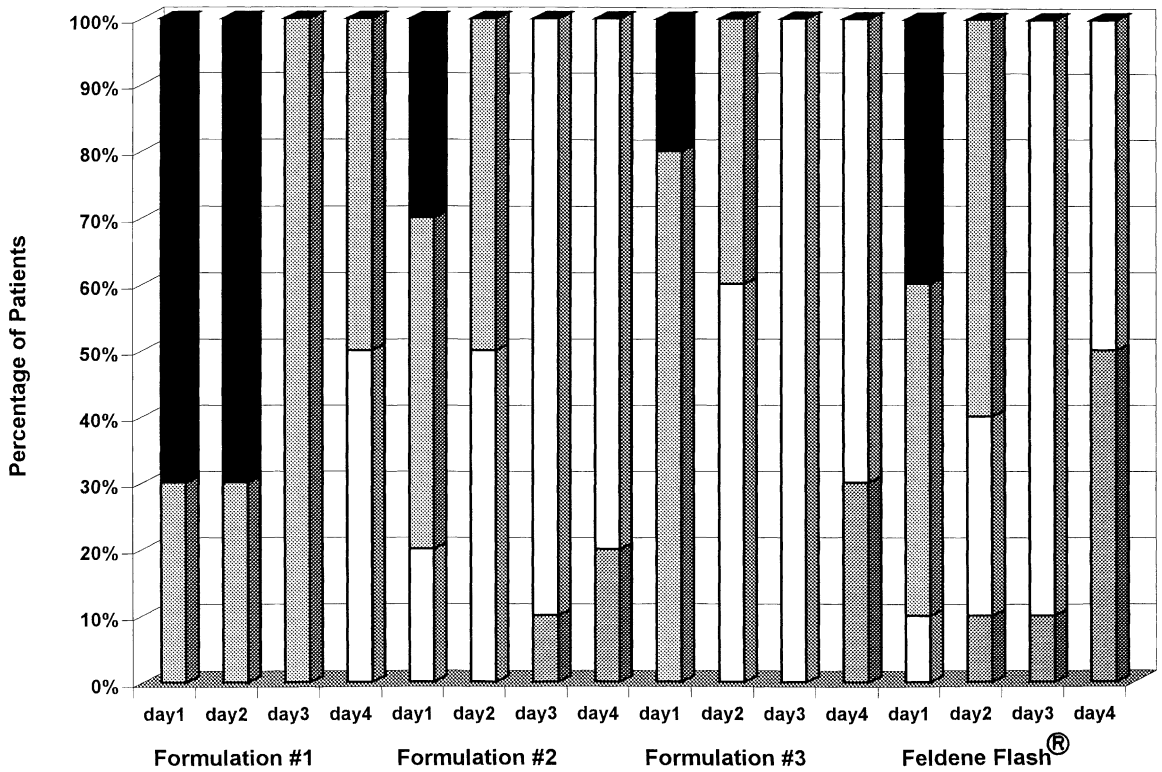

Fig. 8. Tenderness levels after application of various buccal PC gel formulations and Feldene Flash® tablets to patients. Tenderness level: (■) severe, (▨) moderate, (□) mild, (▨) none. Formulation #1: 3% MC gel; Formulation #2: 2.5% HPMC gel; Formulation #3: 7% Na alg. gel, each gel formulation contains 0.5% PC and 3% NaDC as an enhancer.

the global rating of pain control with Formulation #3 was judged mild for 70% of patients, none for 30% (compared with 80 and 20% for mild and none levels, for Formulation #2, respectively); whereas it was judged mild for 50% of patients and none for 50% for the tablets. However, Formulation #1 resulted in lower improvement of the clinical parameter at the end of the study (i.e. 50% of patients suffered from moderate tenderness and 50% complained of mild tenderness).

In the above-mentioned studies, the percentage of patients showing effective reduction in tenderness level by two grades (i.e. from severe to mild or from moderate to none) from each group were compared by the Student's *t*-test. Patients who had mild tenderness (on day-1) were excluded from the total number of patients in each group. The findings revealed no significant differences ($P > 0.1$) in the tenderness reduction levels of Formulations #2 and 3 and the tablets indicating that, these buccal gels are equally effective to the oral tablets in improving tenderness level. However, the difference in change of tenderness levels during the observation interval was statistically significant between Formulation #1 and each of the other formulations ($P < 0.01$ for Formulatoin #1 versus Formulation #2 or the tablet, $P < 0.05$ for Formulation #1 versus Formulation #3), implying that Formulation #1 was less effective in relieving the pain symptoms.

The clinical observations also indicated that most common side-effect reported over 4 day after the first dose administered of PC buccal gel was mild dryness of buccal mucosa in 10% of patients. No complaints of GIT complications which are usually related to the oral administration of the drug were reported. Overall, drug toleration was excellent in 90% of the patients, which is indicative of a high response to the drug by the buccal route.

4. Conclusions

The higher flux of 0.5% PC in 7% Na alg. and 2.5% HPMC gels than in other gel formulations suggests that these gels may be the vehicle of choice for fast release and higher anti-inflammatory effects of PC after buccal application. The permeation rate of PC across rabbit buccal mucosa can be satisfactorily controlled by changing the polymer and drug concentration in the gel and/or by the addition of an appropriate enhancer. Histological SEM investigations showed that interaction of enhancers with the tissue appears to be more pronounced with NaLS compared to bile salts, thereby indicating the higher enhancing effect of NaLS. The results of the clinical studies recommend that the buccal route of administration of PC formulated especially, in Na alg. gel is a promising, safe alternative to the orally administered commercial product (Feldene Flash® tablets) for the management of post-operative dental pain and oedema following maxillofacial operations and prevention of the serious untoward effects of the oral drug administration. Overall, the results obtained from the in-vitro permeation study and anti-inflammatory activity in rats were in accordance with the clinical responses in patients.

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