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Formulation of an antispasmodic drug as a topical local anesthetic

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Abstract

Mebeverine hydrochloride, a spasmolytic agent on GIT smooth muscles, was reported to have a local anesthetic effect. Thus, it was desired in this study to formulate mebeverine HCl into a gel that could be used locally in the treatment of different oral painful conditions. Poloxamer 407 (P-407) was used as the base for this gel. Different additives were used to enhance drug release from the preparation while others were used to enhance the residence time for the preparation. Different formulae were characterized in terms of drug release and mucoadhesion. The formula which has shown the best compromise between the aforementioned parameters was selected for clinical evaluation in comparison to Lidocaine HCl gel® and rheologically examined. The best drug release enhancer was cetrimide (0.005%, w/w), while hydroxypropylcellulose (0.5%, w/w) as a mucoadhesive additive has shown the best compromise between fast drug release and mucoadhesion. The gel formula (G) has shown a better pain reduction efficiency ($p = 0.0078$) and longer duration ($p = 0.0313$) than Lidocaine HCl gel®. Histopathological examination has shown no change in the inflammatory cells count of rat oral mucosa. Therefore, it could be concluded that (G) is very promising as a local anesthetic preparation for the treatment of different oral painful conditions.

Keywords: Mebeverine HCl; Local anesthetic; Gel; Oral painful conditions; Histopathology

1. Introduction

Mebeverine is a musculotropic antispasmodic agent used for the symptomatic treatment of abdominal pain, bowel disturbances and intestinal discomfort related to irritable bowel syndrome (Ritchie and Truelove, 1980; Subissi et al., 1983). Mebeverine hydrochloride has shown efficacy in the treatment of the irritable bowel syndrome when prepared in different dosage forms including tablets (Inauen and Halter, 1994) and rectal solution (Abdel-Hady et al., 2003). It has also been used successfully in capsule form in reducing the severity of pain in patients with primary dysmenorrhea (Langrick et al., 1989). Mebeverine is also available in the market in other forms such as suspension and effervescent granules. However, mebeverine has never been used before as a local anesthetic.

It has been reported that mebeverine HCl exerts a local anesthetic action by blocking voltage operated sodium channels, an action similar to lidocaine (Den Hertog and Van den Akker, 1987). Moreover, mebeverine was approximately twice as potent and produced a longer duration of local anesthetic activity than did procaine using the mouse-tail local anesthetic test (Czechowicz et al., 1969). Although mebeverine has a strong local anesthetic activity, on the contrary to other local anesthetics it was reported to have non-significant central or peripheral side effects (Connell, 1965; Van Outryve et al., 1995; Gilbody et al., 2000) with LD_{50} 1 g/kg in mice (Radwan et al., 1998). Furthermore, it has shown rapid and facile hydrolysis on reaching circulation (Dickinson et al., 1991).

Compared to other locally applied periodontal solid devices like fibers, tablets or microparticles, gels are easily prepared, highly biocompatible with a lower risk of inflammation or adverse reactions, easily applied and do not need to be removed (Esposito et al., 1996). Furthermore, gels often provide a faster release of drug substance, independent of the water solubility of the drug, as compared to creams and ointments (Nairn, 2000). Also, gel vehicles containing therapeutic agents are especially useful for application to mucous membranes and ulcerated or burned tissues, because their high water content reduces irritancy. Furthermore, gels are easily removed by gentle rinsing

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or natural flushing with body fluids, reducing the propensity for mechanical abrasion (Klech, 1999). Moreover, a main reason behind the selection of hydrophilic polymeric matrices as vehicles for oral mucosal drug delivery systems is the water rich environment of the oral cavity (Amir, 1998). However, gels have very limited residence time that they are quickly washed away by saliva from the treatment site; a problem that could be resolved by the incorporation of mucoadhesive polymers (Park and Robinson, 1984).

Poloxamer 407 (P-407) possesses properties which appear to make it suitable for use in the formulation of topical dosage forms; these include relative low toxicity, high compatibility with other drugs, ability to form clear gels in aqueous media, ease of application, cosmetic appearance (colorless and water-washable) and good drug release characteristics (Miller and Drabik, 1984). Poloxamer 407 (P-407) used in this study as the main base of the gel formula, had been used in vehicles for fluorinated dentifrices, eye applications and contraceptive gels (DiBiase and Rhodes, 1996). A poloxamer 407 (P-407) based dental gel, Protect® (Bulter Brush), has been in use several years for treating patients with sensitive gums and teeth (Zinner et al., 1977). Moreover, P-407 gel has been shown to possess many favorable characteristics for use as a burn dressing. Not only does the gel provide a non-toxic detergent covering to the wound, but specific studies suggest that the pluronic gel itself may have a beneficial action, accelerating wound healing over controls (Nalbandian et al., 1987). This makes P-407 a very suitable vehicle for gels intended to be applied for ulcers and traumatic lesions.

In the development of topical dosage forms, several desirable attributes that contribute to the ultimate patient acceptability and clinical efficacy of the product may be defined. These include drug release, good bioadhesion (to ensure retention at the site of application) as well as acceptable viscosity (Jones et al., 1996).

In the mouth the main associated problem with different oral painful conditions is pain which could affect eating, speech and the patient's life style. Therefore, the objective of this study was to formulate mebeverine HCl into a gel form to be used for the first time as a local anesthetic for symptomatic treatment of different oral painful conditions, e.g. traumatic ulcers, recurrent aphthae or different oral ulcers and erosions associated with different diseases like lichen planus, erythema multiforme, systemic lupus erythematosus or Behcet's syndrome. Gel formulations incorporating mebeverine HCl were developed and investigated in vitro for release properties, mucoadhesion and rheology. Finally the clinical performance of the selected gel formula in the treatment of different oral painful conditions took place as well as histopathological examination of rat buccal mucosa for the assessment of the safety and fitness of this preparation.

2. Materials and methods

2.1. Materials

Mebeverine hydrochloride, hydroxypropylcellulose HPC (Klucel MF), hydroxypropylmethylcellulose HPMC (Methocel E5), polyvinylpyrrolidone PVP (Kollidon K-25) were all kindly supplied by E.I.P.I.Co. (Egypt). Poloxamer 407 (P-407), cetyltrimethylammonium bromide (cetrimide) and crude porcine gastric mucin were purchased from Sigma Chemical Co. (U.S.A.). Hydroxyethylcellulose HEC (Cello size WP-40') was purchased from Fluka (Switzerland). All other chemicals were AnalaR, or equivalent quality. Male and female Sprague-Dawley rats weighing 50–70 g (Animal house, Faculty of Pharmacy, Ain Shams University).

2.2. Preparation of poloxamer oral gels

2.2.1. Preparation of poloxamer gels containing different surfactants and hydrophilizing agents

Medicated gels consisting of mebeverine HCl (20%, w/w) and P-407 (20%, w/w) were prepared with additional amounts of different surfactants; cetrimide and Tween 80 and different hydrophilizing agents; (PG) and (PEG 400). Concentrations were 0.005 and 0.05% (w/w) for cetrimide; 0.01 and 0.05% (w/w) for Tween 80; 5 and 10% (w/w) for both PG and PEG 400. The choice of surfactants concentrations was based on a concentration above critical micelle concentration and a concentration below it. While in case of hydrophilizing agents, the choice of concentrations was based on the most common concentrations used in semisolid preparations. The gels were prepared by dissolving or dispersing each of the mentioned additives in the given concentrations with mebeverine HCl in the calculated amount of distilled water at room temperature, the solutions were cooled to 4 °C, and then P-407 was added with continuous agitation using the magnetic stirring bar. Gels were left overnight at 4 °C until clear solutions were obtained.

2.2.2. Preparation of poloxamer gels containing different mucoadhesive polymers

Medicated gels consisting of P-407 (20%, w/w) and cetrimide (0.005%, w/w) were prepared as mentioned above but with additional amounts of different mucoadhesive polymers namely HEC, HPC and HPMC at the concentrations of 0.5, 1, 2% (w/w).

2.2.3. Preparation of the selected gel formula (G) for rheological, clinical and histopathological assessment

Medicated gels consisting of P-407 (20%, w/w), cetrimide (0.005%, w/w) and HPC (0.5%, w/w) were prepared as mentioned above but with additional amounts of sorbitol (20%, w/w), citric acid (2%, w/w), aspartame (3%, w/w) and orange flavor (1%, w/w). The gel was left overnight at 4 °C. On the next day, the required amount of sorbitol was added and mixed thoroughly with the gel by the aid of a spatula. The preparation was kept at ambient conditions until required.

2.3. In vitro release from the oral gels

Mebeverine HCl release from oral gels was monitored by USP paddle method but with some modifications. Formulations were retained within glass cups anchored to the bottom of the

dissolution vessels, thus ensuring drug release to occur principally from the top of the cups. The cups had a diameter of 1.69 cm and a volume of about 2 cm^3. A 2-g sample of the gel (equivalent to 400 mg mebeverine HCl) was weighed in the cup on an analytical balance and was gently lowered through the 750 ml of the dissolution medium (phosphate buffer saline PBS; pH 6.8) by means of a forceps. The paddle of the modified USP Dissolution Apparatus was centrally positioned 2.5 cm above the rim of the cup. The release study was carried out at 37 ± 0.5 °C and the stirring paddles were rotated at a speed of 50 rpm. Aliquots of 2 ml were withdrawn from the dissolution medium at time intervals of 5, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min. All samples were replaced by the same volume of phosphate buffer saline solution (pH 6.8). Samples were suitably diluted and measured spectrophotometrically at λ_{max} 263.5 nm. The concentration of drug was determined from the previously constructed calibration curve which was linear over the concentration range 1.0–3.0 mg% ($r = 0.9999$, with zero intercept). The presence of different formulation excipients did not interfere with the analysis. The experiments were conducted in triplicates, the results were averaged and blank experiments were carried out at the same time using plain bases.

2.3.1. Statistical analysis of drug release data

Drug release data generated from the dissolution experiments were fitted to the following general release equation (Peppas, 1985) using logarithmic transformations and least squares regression analysis:

$$\frac{M_t}{M_\infty} = kt^n$$

$$\log\frac{M_t}{M_\infty} = \log k + n\log t$$

where M_t/M_∞ is the fraction of released drug at time t; k the release constant incorporating structural and geometrical characteristics of the delivery system; n is the release exponent, a measure of the primary mechanism of drug release.

Statistical analyses were performed on the times required for the release of 10, 30 and 50% ($t_{10\%}$, $t_{30\%}$ and $t_{50\%}$) of the original loading of mebeverine HCl from each formulation (Jones et al., 1997a; O'Hara et al., 1998) using one-way analysis of variance (ANOVA). Post-hoc statistical analyses were performed using Tukey–Kramer test for multiple comparisons. The software employed was Graph Pad Instat® V2.04 and the level of significance was set at 5%.

2.4. In vitro evaluation of the mucoadhesion for the gels

The mucoadhesive force of medicated poloxamer gels containing P-407 (20%, w/w), cetrimide (0.005%, w/w) with the different mucoadhesive polymers mentioned above at variable concentrations was determined using a mucoadhesive force measuring device which is a modified balance that was developed in our lab. This was according to previously reported methods (Yong et al., 2001; Desai and Kumar, 2004). The mucoadhesive strength of the formulations under examination was determined by measuring the force required to detach the formulations from a mucin disc using the mentioned measuring device. Initially, mucin discs were prepared by compression of crude porcine mucin (250 mg) by a single punch tablet press (10 mm diameter die). These discs were horizontally attached to the upper stage of the modified balance by a cynoacrylate adhesive. Prior to mucoadhesion testing, the mucin disc was hydrated by submersion in a freshly prepared 5% (w/w) dispersion of mucin for 60 s. Samples of each gel formulation (0.5 g) were placed on the lower vertically movable stage (containers were removed from the refrigerator and left at room temperature until gelation took place). The lower stage was then elevated till the surface of the sample became contacted to the mucin disc adhered to the upper stage. A constant (preload) downward force of 10 g weight was then applied for 30 s and removed. The sample was left in contact for 5 min with the mucin disc to ensure intimate contact. To the pan on the other side of the used device, water was then added from a glass bottle through an infusion set into a plastic jar at a constant rate of 18–20 drops/min. The addition of water was stopped when mucin disc was detached from the sample. The minimal weight of water required to detach the sample from the mucin disc was noted as the mucoadhesive force, and these experiments were repeated with fresh mucin discs and gel samples in an identical manner ($n = 4$). All detachment tests were carried out at about 34–37 °C in air. Mucoadhesive force or the detachment stress (N/m^2) was determined using the following equation stated by Ch'ng et al. (1985):

$$\text{Detachment stress } (N/m^2) = \frac{mg}{A}$$

where m is the weight of water (g); g the acceleration due to gravity taken as 9.81 m/s^2; A is the area of the mucin disc (area of contact) and is equal to πr^2 (r is the radius of the mucin disc).

2.4.1. Statistical analysis of mucoadhesion data

The results were statistically evaluated using one-way ANOVA. Post-hoc statistical analyses were performed using Tukey–Kramer test for multiple comparisons. The software employed was Graph Pad Instat® V2.04 and the level of significance was set at 5%.

2.5. Rheological measurements

Steady shear measurement was conducted where the rheogram of the selected gel formula (G) was performed at 25 ± 0.1 °C (to examine performance at room temperature) and at 37 ± 0.1 °C (to examine performance at body temperature) using the spindle CP 52 of the Brookfield cone and plate viscometer in the controlled rate mode, with the shear rate ranging from 0.1 to 2 s^{-1}, however the upper limit of the shear rate was extended to 20 s^{-1} to examine the hysteresis of the rheogram as these higher ranges are more representative to spreading and chewing processes. Equilibration of the sample for 5 min was made following loading of the viscometer. Ramp time for

each velocity stage was reading after 30 s. Rheograms were performed in triplicate.

2.5.1. Statistical analysis of rheological data

Rheological data for the selected gel formula (G) was fitted to different models (Bingham, Power law, Casson) to examine the pattern of flow:

- Bingham: $\tau = \tau_0 + \eta\gamma$;
- Power law: $\tau = \eta\gamma^n$;
- Casson: $\tau^{1/2} = \tau_0^{1/2} + \eta^{1/2}\gamma^{1/2}$;

where τ is the shear stress, τ_0 the yield value, η a constant called the apparent viscosity or the consistency index, γ the shear rate and n is the flow index. In case of Newtonian behavior $n = 1$ and $\tau_0 = 0$, whereas in case of pseudoplastic (shear thinning) behavior $0 < n < 1$ and $\tau_0 = 0$; for plastic behavior it is the same as pseudoplastic but with $\tau_0 > 0$, while in case of dilatant flow (shear thickening) $n > 1$.

2.6. Clinical evaluation of the selected gel formula (G)

Twenty-five subjects; 14 males and 11 females aged 18–65 years were randomly chosen from the flow of the outpatient Diagnosis Clinic, Department of Oral Medicine, Diagnosis and Periodontology, Faculty of Dentistry, Ain Shams University, Cairo, Egypt. The protocol of the study was approved by the Ethic Committee of the institute and each patient signed a written consent where the full details of the study were explained. The investigation was designed as a randomized, single blinded clinical trial (split-mouth design). The patients were stratified by a coin flip into one of the following treatment groups:

- Group (1): Included 10 patients with bilateral lesions treated with mebeverine HCl gel (G) versus Lidocaine HCl gel® (L).
- Group (2): Included 15 patients with bilateral lesions treated with mebeverine HCl gel (G) versus a placebo with the same taste.

Patients assessed pain by using a 10-points verbal rating scale, ranging from "no pain" to "unbearable pain" (Jacox et al., 1994). Patients were asked to choose a score (from 0 to 10) according to the degree of their pain sensation before drug application then they were instructed to apply the preparations three to four times daily for 2 days, where they were counseled not to drink or eat for 30 min after application. After 2 days of treatment the patients were asked again to score pain according to the same criteria and to judge the physical properties of the prepared formulae through a scoring questionnaire form, Table 1 (Ukai and Harada, 2003; Tapolsky et al., 2000).

2.6.1. Statistical analysis of clinical data

The results were evaluated statistically by Wilcoxson matched pairs signed test and Mann–Whitney *U*-test using Graph Pad Instat® software V2.04. The level of significance was set at 5%.

Table 1
Scoring questionaire form about physical characters of formula (G) compared to either plain base or market product (L)

Items	Formula (G)	Plain base/market product (L)
Handling (0–3)[a]		
Bitterness (1–5)[b]		
Numbness (1–5)[b]		
Onset of action (s)		
Duration of action (h)		
Overall impression (±)		

[a] Handling (0 = poor, 1 = fair, 2 = good, 3 = very good).
[b] Bitterness/numbness (1 = very bitter/numb, 2 = bitter/numb, 3 = slightly bitter/numb, 4 = dim feeling, 5 = no feeling).

2.7. Histopathological evaluation of the selected gel formula (G)

2.7.1. Preparation of the rats

Application was made only on the right side of the gingiva, leaving the left side as control. Formula (G) was applied three times daily with a 3 h interval between each application for 2 days. Rats were kept away from food and water for only half an hour after each application. On the third day, they were sacrificed where the head of each rat was separated. Dissection, separation and fixation of the gingival tissues were carried out. Four-micrometer thick sections were cut sequentially from each section. Hematoxylin and eosin stain was made for histological examination. Slides were then observed under the microscope (using a color video camera fitted to light microscope and attached to computer).

Inflammatory cells (plasma cells and lymphocytes) in the connective tissue were counted where a total of four adjacent medium power microscopic fields were analyzed at the power of 20×. Counting was made by image analysis software analySIS® version 3.

2.7.2. Statistical analysis of histopathological data

Statistical analysis was made using paired *t*-test at $p < 0.05$ using Graph Pad Instat® V2.04 software.

3. Results

The least concentration of medicated P-407 that could form a gel on application to the oral cavity was (20%, w/w) thus it was selected as the base for the preparation to ensure a fast release.

3.1. Effect of different surfactants and hydrophilizing agents on the release of mebeverine HCl from P-407 (20%, w/w) gel

Different surfactants and hydrophilizing agents were incorporated to P-407 for the aim of enhancing the release rate and to produce a fast onset as well. Table 2 and Fig. 1 show the effect of these additives on the time required for the release of mebeverine HCl from P-407. Where the values of $t_{10\%}$, $t_{30\%}$ and $t_{50\%}$ were significantly the lowest for both of 0.005 and 0.05% (w/w) cetrimide indicating a decrease in drug release

Table 2
Effect of different surfactants and hydrophilizing agents on the time required for release of mebeverine HCl (10, 30 and 50% of original drug loading) from poloxamer 407 (20%, w/w) gel

Formula (%, w/w)	Time (min) required for release of mebeverine hydrochloride (mean ± S.E.)		
	$t_{10\%}$	$t_{30\%}$	$t_{50\%}$
20% P-407 (C)	11.28 ± 1.59	42.19 ± 2.81	78.27 ± 2.38
C + 0.005% cetrimide	4.68 ± 0.68[a]	25.49 ± 1.64[a]	56.30 ± 1.48[a]
C + 0.05% cetrimide	5.56 ± 0.39[a]	28.18 ± 1.28[a]	59.73 ± 12.04[a]
C + 0.01% Tween 80	6.69 ± 0.13	30.90 ± 0.30[a]	62.93 ± 0.34[a]
C + 0.05% Tween 80	7.75 ± 0.50	34.03 ± 1.42	67.71 ± 2.09[a]
C + 5% PEG 400	18.16 ± 1.42[a]	50.85 ± 2.00	82.15 ± 1.77
C + 10% PEG 400	14.83 ± 0.37	47.91 ± 0.93	82.63 ± 1.38
C + 5% PG	10.38 ± 0.80	38.27 ± 1.24	70.28 ± 0.79
C + 10% PG	18.45 ± 1.91[a]	50.60 ± 3.35	80.98 ± 3.94

[a] Considered statistically significant vs. the corresponding values of P-407 (20%, w/w) at $p < 0.05$ using one-way ANOVA followed by Tukey–Kramer test for multiple comparisons.

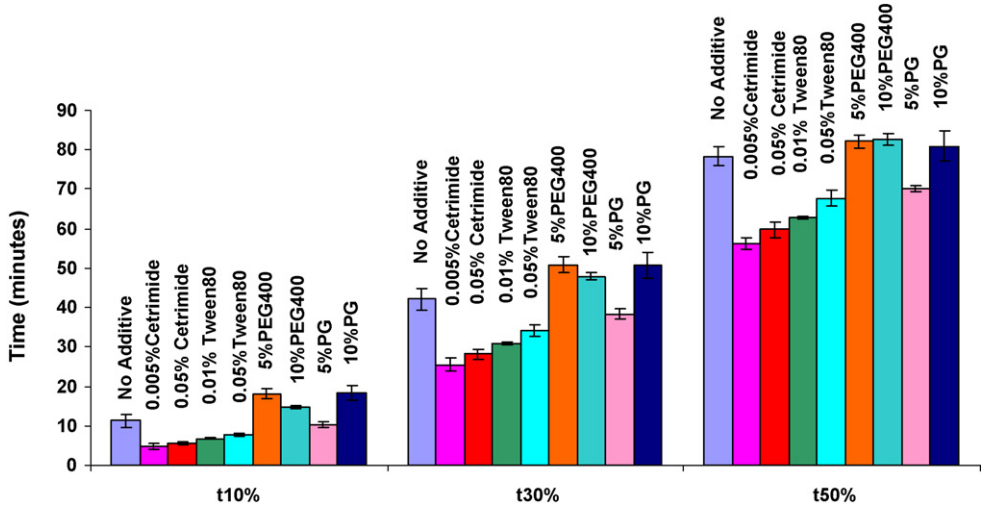

Fig. 1. Effect of different surfactants and hydrophilizing agents on the time required for the release of mebeverine HCl from P-407 (20%, w/w) gel.

time in comparison to other additives and to the base without any additive ($p < 0.05$). Though there was no significant difference between 0.005 and 0.05% (w/w) cetrimide on enhancing the rate of release, the former concentration was conveniently selected as the release enhancer for the formula for economic reasons.

Release kinetics data for P-407 (20%, w/w) without and with different surfactants and hydrophilizing agents are shown in Table 3 where it is clear that the gel containing 0.005 and 0.05% (w/w) cetrimide have the highest values of the kinetic constant k (i.e. the highest release rates). Although 0.01 and 0.05% (w/w) Tween 80 have shown a significant enhancement for the drug release versus control (base without additives), their overall enhancement effect is still less than that of cetrimide concentrations. On the other hand, the concentrations of PEG 400 and PG have shown a non-significant effect on drug release except at $t_{10\%}$ where (5%, w/w) PEG 400 and (10%, w/w) PG have shown to retard the release significantly versus the control. It is also shown from the values of the exponential constant (n) that the main mechanism of release from (20%, w/w) P-407 base with these additives is mostly anomalous diffusion ($0.5 < n < 1$).

3.2. Effect of different mucoadhesive polymers on the release of mebeverine HCl from P-407 (20%, w/w) gel

Mucoadhesive polymers such as HEC, HPC and HPMC were added in variable concentrations to the medicated P-407 (20%, w/w) gel containing cetrimide (0.005%, w/w) as a release enhancer in order to prolong the contact time of the prepara-

Table 3
Kinetic data of mebeverine HCl released from poloxamer 407 (20%, w/w) gels using different surfactants and hydrophilizing agents at different concentrations

Formula (%, w/w)	Release exponent (n)	Kinetic constant (k, %/min^n)	Regression coefficient (r^2)
20% P-407 (C)	0.825	0.0139	0.9751
C + 0.005% cetrimide	0.640	0.0378	0.9730
C + 0.05% cetrimide	0.675	0.0315	0.9755
C + 0.01% Tween 80	0.714	0.0261	0.9750
C + 0.05% Tween 80	0.741	0.022	0.9755
C + 5% PEG 400	1.056	0.0048	0.9590
C + 10% PEG 400	0.937	0.008	0.9838
C + 5% PG	0.845	0.0137	0.9661
C + 10% PG	1.078	0.0044	0.9598

Table 4
Effect of different mucoadhesive polymers on the time required for the release of mebeverine HCl (10, 30 and 50% of original drug loading) from poloxamer 407 (20%, w/w) gels using cetrimide (0.005%, w/w) as a release enhancer

Formula (%, w/w)	Time (min) required for release of mebeverine hydrochloride (mean ± S.E.)		
	$t_{10\%}$	$t_{30\%}$	$t_{50\%}$
20% P-407 + 0.005% cetrimide (E)[x]	4.68 ± 0.68	25.49 ± 1.64	56.30 ± 1.48
E + 0.5% HEC[a]	6.58 ± 0.46	30.70 ± 1.08	62.85 ± 1.16
E + 1% HEC[b]	13.58 ± 0.07	44.36 ± 0.39	76.90 ± 0.80
E + 2% HEC	19.71 ± 0.35	55.49 ± 0.27	89.79 ± 0.82
E + 0.5% HPC[c]	10.57 ± 0.23	40.08 ± 0.59	74.50 ± 0.96
E + 1% HPC	23.38 ± 0.17	63.75 ± 0.24	101.64 ± 0.46
E + 2% HPC	31.99 ± 1.54	81.74 ± 1.40	128.99 ± 2.61
E + 0.5% HPMC[d]	7.98 ± 0.89	35.73 ± 2.36	71.82 ± 3.24
E + 1% HPMC[e]	13.22 ± 1.32	40.60 ± 2.80	75.68 ± 2.90
E + 2% HPMC	19.32 ± 1.00	57.86 ± 1.56	96.39 ± 1.53

Using one-way ANOVA followed by Tukey–Kramer test for multiple comparisons at $p < 0.05$, there was no significant difference at $t_{10\%}$, $t_{30\%}$, $t_{50\%}$ for the following: (1) a vs. x; (2) a vs. d; (3) b vs. c–e; (4) c vs. d and e; (5) d vs. e.

tion. Generally, these mucoadhesive polymers decreased drug release from the formula by different extents depending on the molecular weight as well as concentration of the polymer added. By increasing the concentration of any polymer, drug release was reduced. HEC and HPMC were of low molecular weight, thus they have shown faster drug release from their formulations in comparison to HPC which had a higher molecular weight (850 000 Da). However, the aim was to select the mucoadhesive polymers which convey a satisfactory retention time for the gel at application site with the least effect on the time required for drug release as local anesthetic preparations should produce a rapid onset of action. Regarding this aim, HEC (0.5%, w/w) had no significant effect on drug release versus the formula without this additive. Furthermore, 0.5, 1% (w/w) HEC; 0.5% (w/w) HPC and 0.5, 1% (w/w) HPMC have also shown non-significant differences among themselves on drug release and had a minimum effect on retarding drug release from their formulae, Table 4. It could be seen that by increasing the concentration of the mucoadhesive polymer or using a polymer of a high molecular weight as HPC, $t_{10\%}$, $t_{30\%}$ and $t_{50\%}$ were increased. Release kinetics data for the formulae containing these mucoadhesive polymers are listed in Table 5. It is clear that kinetic constant (k) values decrease by increasing the concentration for every polymer indicating a decreasing effect on the release. It is also shown that the values of the release exponent (n) move from anomalous diffusion to zero order ($n = 1$) release by increasing the concentration of the mucoadhesive polymer.

Table 5
Kinetic data of mebeverine HCl released from poloxamer 407 (20%, w/w) gels using cetrimide (0.005%, w/w) as a release enhancer at different mucoadhesive polymers

Formula (%, w/w)	Release exponent (n)	Kinetic constant (k, %/min^n)	Regression coefficient (r^2)
20% P-407 + 0.005% cetrimide (E)	0.640	0.0378	0.9730
E + 0.5% HEC	0.712	0.0262	0.9647
E + 1% HEC	0.928	0.0089	0.9555
E + 2% HEC	1.060	0.0043	0.9660
E + 0.5% HPC	0.824	0.0143	0.9558
E + 1% HPC	1.094	0.0032	0.9769
E + 2% HPC	1.100	0.0023	0.9978
E + 0.5% HPMC	0.729	0.0222	0.9714
E + 1% HPMC	0.813	0.0149	0.9510
E + 2% HPMC	0.999	0.0052	0.9664

3.3. Effect of polymer type and concentration on mucoadhesion

On the addition of the different mucoadhesive polymers to P-407, the mucoadhesive strength (expressed in terms of detachment force) was highest in case of HPC followed by HPMC and HEC. However, only formulae containing HPC (0.5, 1, 2%, w/w) as well as the formula containing HPMC (2%, w/w) have shown a significantly stronger mucoadhesion in comparison to poloxamer base without any mucoadhesive polymer, Table 6. The effect of the added polymer type and

Table 6
Mucoadhesive strength for poloxamer P-407 gels using different mucoadhesive polymers

Mucoadhesive polymer	Concentration (%, w/w)	Detachment force ($\times 10$ N/m^2) (mean ± S.E.)
No mucoadhesive polymer		55.90 ± 5.93
HEC	0.5	58.11 ± 5.56
	1	59.57 ± 2.53
	2	74.44 ± 4.77
HPMC	0.5	65.85 ± 6.37
	1	74.52 ± 4.07
	2	83.81 ± 5.24[a]
HPC	0.5	89.85 ± 3.82[a]
	1	100.01 ± 3.83[a]
	2	109.26 ± 0.522[a]

[a] Considered statistically significant vs. the corresponding values of P-407 without mucoadhesive polymers at $p < 0.01$ using one-way ANOVA followed by Tukey–Kramer test for multiple comparisons.

Table 7
Effect of the taste improving additives on the time required for the release of mebeverine HCl (10, 30 and 50% of original drug loading) from the selected gel formula (G)

Formula (%, w/w)	Time (min) required for release of mebeverine hydrochloride (mean ± S.E.)		
	$t_{10\%}$	$t_{30\%}$	$t_{50\%}$
(G) without taste improving additives	10.54 ± 1.09	38.41 ± 2.28	70.05 ± 2.75
(G)	10.57 ± 0.23[a]	40.08 ± 0.59[a]	74.50 ± 0.96[a]

[a]Values of $t_{10\%}$, $t_{30\%}$ and $t_{50\%}$ in the presence of taste improving additives were non-significantly different from those in the absence of these additives at $p < 0.05$ using Student's t-test.

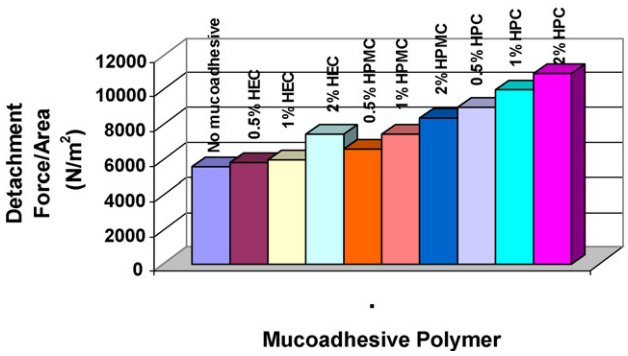

Fig. 2. Effect of polymer type and concentration on the mucoadhesive strength of poloxamer P-407 gels using different mucoadhesive polymers.

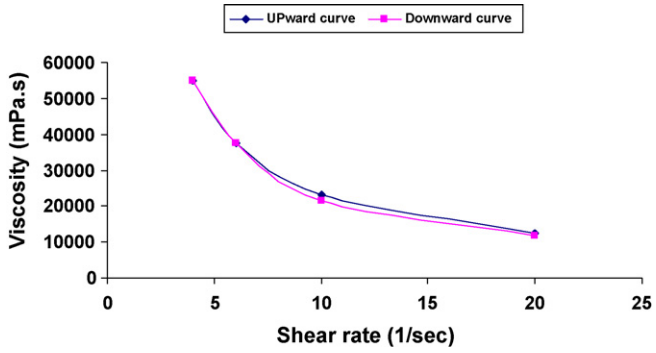

Fig. 3. Rheogram of the selected gel formula (G) showing up and down curves.

concentration on mucoadhesive strength for P-407 based gels is shown in Fig. 2. From the above fore mentioned results of release and mucoadhesion, it could be seen that the P-407 based gel formula containing HPC (0.5%, w/w) as a mucoadhesive additive has shown the optimal compromise of a relatively fast release and an acceptable mucoadhesive strength. Thus, it was selected as the mucoadhesive additive for the gel formula.

3.4. Studying the effect of taste improving ingredients

As mebeverine HCl has a bitter taste, different taste improving ingredients (liquid sorbitol, 70%, w/v), citric acid, aspartame, and orange flavor were added to the formula before rheological evaluation as this step was the final before clinical evaluation. The addition of sorbitol to P-407 has lead to permanent gel formation and abolished any thermoreversibility exhibition for this polymer. However, these additives did not show any significant change in neither the release profile nor the mucoadhesive strength of the formula, Tables 7 and 8.

Table 8
Effect of taste improving additives on the mucoadhesive strength of the selected gel formula (G)

Formula	Detachment force ($\times 10\ N/m^2$) (mean ± S.E.)
(G) without taste improving additives	89.85 ± 3.82
(G)	87.12 ± 2.52[a]

[a]Values of detachment force in the presence of taste improving additives were non-significantly different from those in the absence of these additives at $p < 0.05$ using Student's t-test.

Table 9
Fitting data of the selected gel formula (G) to different rheological models

Model	Equation	Regression coefficient (r^2)
Power law	$y = 1843.9x^{0.3788}$	0.9607
Bingham	$y = 856.7x + 760.3$	0.8073
Casson	$y = 20.532x + 21.176$	0.8953

3.5. Rheological flow pattern exerted by the gel formula (G) selected for clinical evaluation

The gel formula (G) that was selected for clinical evaluation has shown no hysteresis where the up and down curves coincide or overlay as shown in Fig. 3, which indicates a non-thixotropic behavior. The data was best fitted to Power law model, Table 9 and the gel formula has shown a shear thinning (pseudoplastic) behavior at 25 °C as the flow index (n) is less than 1. Shear thinning behavior was also revealed at both room (25 °C) and body temperatures (37 °C) as shown in Fig. 4 where there is a decrease in viscosity by increasing the shearing rate.

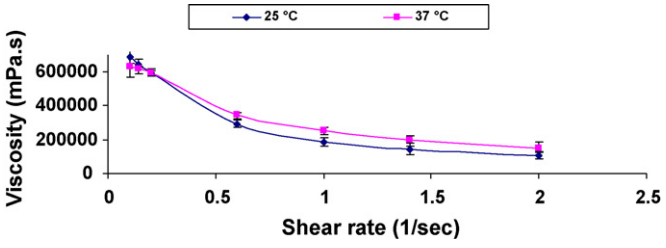

Fig. 4. Rheogram of the selected gel formula (G) at room (25 °C) and body (37 °C) temperatures.

Table 10
Pain scores of (G) vs. that of placebo and market product (L)

Formula	Mean ± S.E.	Median	Median reduction in pain scores (%)	*p*-Value
G ($n=15$)	1.80 ± 0.28	2	–	<0.0001[a]
Placebo ($n=15$)	7.53 ± 0.57	8	–	
G ($n=10$)	1.60 ± 0.40	2	75	0.0078[a]
L ($n=10$)	4.60 ± 0.85	4	50	

[a] Statistically significant at $p<0.05$ using Wilcoxson-signed ranks test.

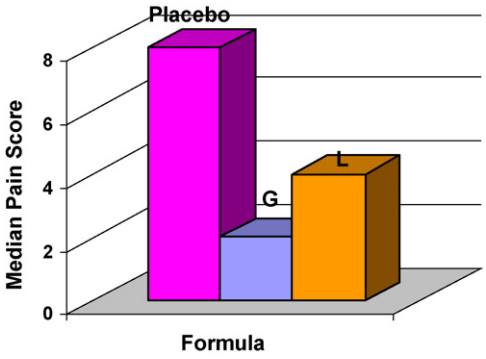

Fig. 5. Pain score of (G) vs. (L). 0 score = no pain while 10 score = unbearable pain. (Score for pain increases by increasing the degree of pain.)

3.6. Clinical evaluation of the selected gel formula (G)

In case of pain reduction efficiency, anesthesia obtained by (G) was statistically significant than placebo. (G) had proven to be more effective in pain reduction when compared to the market product (L), as shown in Table 10 and Fig. 5 where many patients declared that (G) improved their life style in eating, talking and sleeping. Interestingly, some patients on (G) had reported that the course of ulcer duration was unusually reduced from 10 days to only 4 days.

Table 11
Scores of physical parameters of (G) vs. that of market product (L)

Physical parameter	Formula ($n=10$)	Mean ± S.E.	Median	*p*-Value
Handling	G	2.70 ± 0.15	3	0.8125
	L	2.60 ± 0.26	3	
Bitterness	G	2.40 ± 0.40	2	0.0078[a]
	L	4.10 ± 0.34	4.5	
Numbness	G	2.70 ± 0.42	3	0.0156[a]
	L	4.10 ± 0.43	5	

[a] Statistically significant at $p<0.05$ using Wilcoxson-signed ranks test.

In case of the physical parameters, Table 11, there was no significant difference in the ease of handling of (G) versus the market product (L), where it was very good for both preparations. Despite of using flavors and sweeteners (G) was bitterer than the market product (L) ($p=0.0078$). However, patients had stated that this bitterness lasted only for 1–2 min and they were used to it in a way that did not affect their compliance. (G) has shown a higher degree of numbness when compared to (L) ($p=0.0156$), which reflects its greater anesthetic efficiency when compared to the market product. This feeling of numbness which could be annoying to some patients did not last long and the lesion was still painless.

The median onset of action for (G) was not quite significant from the market product (L) ($p=0.5$), where it was 10 s for (G) versus 60 s for (L), while the duration of action of (G) was more significant when compared to (L) ($p=0.0313$), where it was 2.5 h for (G) versus 0.75 h for (L).

3.7. Histopathological evaluation of the selected gel formula (G)

Comparison of the photomicrographs of control gingival tissues against the photomicrographs of gingival tissues which have received treatment with formula (G), Fig. 6, has shown

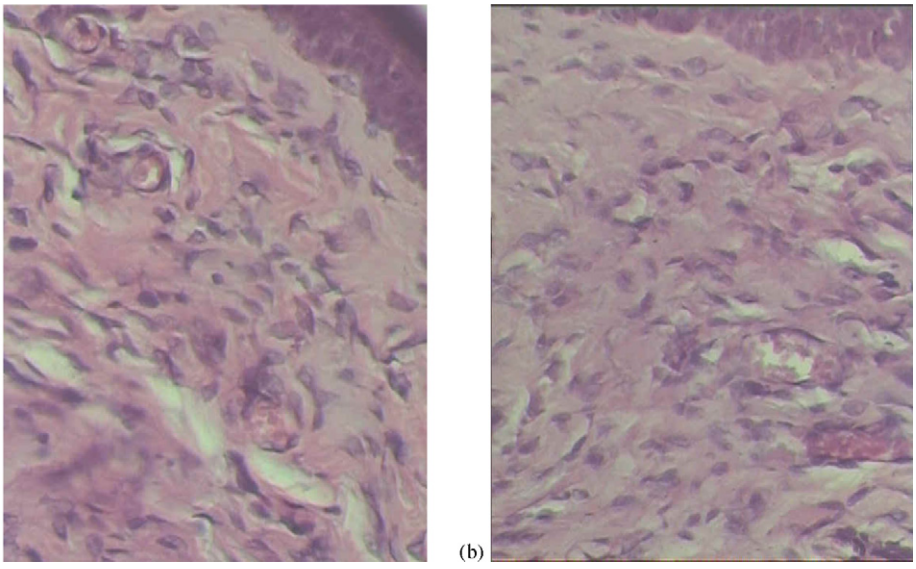

Fig. 6. Photomicrographs of rat gingiva stained with hematoxylin and eosin showing connective tissue infiltrated with few inflammatory cells (20×) for (a) control; (b) formula (G).

Table 12
Mean number of inflammatory cells count

Formula	Mean ± S.E.	*p*-Value
(G)	40.50 ± 2.26[a]	0.6121
Control	38.92 ± 1.80	

[a]Considered non-significant vs. control at $p < 0.05$ using paired Student's *t*-test.

no detectable tissue damage resulting from the exposure to (G). Moreover, the count of inflammatory cells infiltration in connective tissue, e.g. plasma cells and lymphocytes, Table 12 has shown non-significant difference in the inflammatory cells count between (G) versus its control ($p = 0.6121$).

4. Discussion

The addition of hydrophilic or lipophilic additives into pharmaceutical formulations in order to modulate the release of drugs is mostly common. Polarity produced by the additives is an important factor that affects drug release. The higher the water solubility of the additive, the greater the enhancement of dissolution for the formulation it would do and thereby the more the rate of drug release and vice versa (Desai and Blanchard, 1998). In previous studies, it has been reported that the incorporation of PEG or PG (in concentrations ranging from 2.5 to 10%) into different polymer bases including poloxamer has significantly resulted in enhancing drug release rate. This was related to the high water solubility of glycols where they readily absorb water and therefore speeds up the hydration rate and so the dissolution rate of the polymer into which these hydophilizers are incorporated (Buckton and Tawburic, 1992; DiBiase and Rhodes, 1996). However, in this study both PEG and PG did not have a significant effect on improving drug release from P-407 which could be explained by their low solubilizing effect when compared to that of poloxamer. Moreover, the cause of retarding drug release by 5% PEG 400 and 10% PG at $t_{10\%}$ may be attributed to increasing the apparent viscosity of P-407 at the beginning of release which could be related to desolvation, enhanced polymer entanglement and more extensive hydrogen bonding.

In addition to the effects on the bulk properties of the gel, it is foreseeable that polymer–surfactant interactions dramatically alter the microenvironment in which the solute diffusion occurs. The formation of polymer–surfactant aggregates or free micelles could modify the size of the water filled regions or the mobility of the polymer chains (Amsden, 1998). The polyethylene oxide block (PEO) of poloxamer has been found to interact with and bind to cationic surfactants (although weakly) and this possibly affects the solubilization, where by introducing charges on the polymer backbone the solvency of the polymer effectively improves and hence better release is observed (Scherlund et al., 2000). This could explain the enhancing effect of the cationic surfactant cetrimide on the release rate of mebeverine HCl from the P-407 based gel. On the other hand, the addition of the non-ionic surfactant Tween 80 to P-407 based gels has also enhanced the release rate which could be attributed to increasing the number and dimensions of the aqueous channels available for drug diffusion, thus increasing the effective porosity of the gel matrix. Also, Tween 80 has emulsifying properties and decreases the gel viscosity due to increasing the formation of intra-polymeric micelles while inter-polymeric connections decrease which enables the drug to be easily released. This was in accordance with what was reported by Hansson and Lindman (1996).

Cetrimide (Siegel and Gordon, 1985), PG (Aungst and Rogers, 1989), Tween 80 (Steward et al., 1994) were all used as oral permeation enhancers which could be another advantage for using these additives besides using them as release enhancers.

In attempt to improve the bioadhesive properties of poloxamer, which is known to be poorly or moderately bioadhesive with short retention time (Huang et al., 2002), polymers with good mucoadhesive characters were always added to improve the residence time for poloxamer. Among these additive polymers cellulose derivatives such as MC, HPMC, HPC and CMC have been used to increase the retention time for poloxamer gels (Choi et al., 1998; Bhardwaj and Blanchard, 1996). While the length and size of the diffusional paths between poloxamer micelles are prominent in controlling drug release, changes in the release kinetics are likely to result from additive compounds. The presence of mucoadhesive polymers as additives in poloxamer gels could distort or squeeze the diffusion channels and delay the release process (Choi et al., 1998). Hydrophilic polymers like cellulose derivatives would decrease the amount of free water in the water channels and affect molecular orientation of the gel matrix. It was reported by Miller and Drabik (1984) that on the addition of HPMC to poloxamer gel, tightly gel structures are formed within poloxamer micelle pathways by HPMC-molecule entanglement and extensive hydrogen bonding. This would lead to a decrease in the release rate. This was also in accordance with what have been reported by Paavola et al. (1998), who stated that cellulose additives such as carboxymethyl cellulose (CMC) and HPMC significantly prolonged ibuprofen release from poloxamer gels. Moreover, the lower aqueous solubility of cellulose derivatives and the increased viscosity of P-407 gel formulations containing these cellulose polymers probably slowed the dissolution of P-407 gel formulations and decreased the rate of penetration of the aqueous release medium into the gel network. This, in turn, may have reduced the leaching action by the release medium for the drug. These results were in accordance with those reported by Guzman et al. (1992) and Kumar et al. (1994).

The bioadhesive force is known to be dependent on the nature and the concentration of bioadhesive polymers (Shin et al., 2000). Cellulose derivatives HEC, HPC and HPMC were selected as mucoadhesive additives to P-407 in this study due to their compatibility with mebeverine HCl in comparison to other charged polymers. Moreover, Choi et al. (1998) reported that the charges of bioadhesive polymers seem not to play a major role in affecting the physico-chemical properties of poloxamer-based liquid suppository. This was rather confirmed by Peh and Wong (1999) where they reported that HPMC films exhibited greater in vivo bioadhesion although their in vitro bioadhesive strength was lower than that of Na CMC films. It is likely that the number of hydrogen bonding groups rather than fixed charge that is important (Jimenez-Castellanos et al., 1993). In this study,

HPC has shown the greatest mucoadhesive force due to its high molecular weight (850 000 Da) which is together with its linear structure gives it a good spatial conformation for adhesion (Gurny et al., 1984). Moreover, the increase of mucoadhesive strength associated with increased concentrations of each polymer were a result of the greater concentration of polymer chains at the formulation surface and, subsequently, the greater number of entanglements/interactions with mucus glycoproteins and this explains the increase in detachment force by increasing polymer concentration. This accorded with what was reported by Jones et al. (2000).

Sorbitol (20%, w/w) and aspartame (3%, w/w) were added as sweeteners, besides sorbitol has a cooling action, non-cariogenic and could act as a humectant. Citric acid (2%, w/w) was added as a flavor enhancer beside its action in increasing the solubility of aspartame by lowering the pH and its stabilizing effect as antioxidant and sequestering agent (Kibbe, 2000). Orange flavor was reported to be among the best flavors for masking bitter taste (Lipari and Reiland, 2002). The abolishment of P-407 thermoreversibility exhibited by sorbitol was explained by its dehydrating effect for P-407 thus promoting extensive chains interaction leading to permanent gel formation. This was in accordance by what reported by Mitchell et al. (1990) about the gel point depressing effect exhibited by sorbitol. Sorbitol addition affected the macroviscosity rather than microviscosity of the gel and this explains why the release profile was not affected. Gallagher et al. (2003) have reported that the macroviscosity or bulk viscosity on its own does not account for interactions between active and excipient and it is the microviscosity, in which solute diffusion occurs, that is responsible for any change in drug release. This conclusion was also confirmed by Barreiro-Iglesias et al. (2001).

Formulation viscosity contributes to product adhesiveness, reflecting the importance of product rheology on this parameter (Hägerström and Edsman, 2003; Bromberg et al., 2004). It was also reported that formulation viscosity contributed to product mechanical properties (sensorial parameters) such as compressibility and hardness which are in turn are related to ease of product removal from container, ease of application onto a substrate and product comfort within the oral cavity (Jones et al., 1997a). Mathematical models provide a mean of representing a large quantity of rheological data in terms of a simple mathematical expression. These equations are called "constitutive equations" and are useful to predict the flow behavior in complex systems in a certain shear rate range (Laba, 1993). The Bingham, Power's law and Casson mathematical models describing viscoplastic systems were applied in this study. The Bingham and Casson models describe plastic systems with a yield value while the Power's law is the most suitable model for pseudoplastic (shear thinning) systems. The best fitting model for (G) was the Power's law revealing that this formula showed no apparent yield value, indicating the limited resistance to flow at low stress values characteristic of pseudoplastic flow. This shear thinning behavior is a desirable property for topical preparations, as they should be thin during application and thick otherwise (Pena et al., 1994). Furthermore, the loss of consistency on application of shear which was shown by (G) is an advantageous property for formulations intended to be applied to the oral mucosa. This was in accordance with what reported by Jones et al. (1997b). The low flow index (n) exhibited by the gel formula is desirable as it facilitates processing the gel during manufacture and spreading the gel during application as reported by Ramachandran et al. (1999). Also increased pseudoplasticity invariably increases the preparation stability (Vaughan, 1993). As the area between the up and down curves (hysteresis loop) is zero, this indicates a time independent flow; a property that helps the retention of gels on the buccal mucosa. Thixotropic (time dependent) materials may not have sufficient time to reform again after application (Eouani et al., 2001).

In the present study the physical parameters of the prepared formula (G) was evaluated to investigate the extent of patient acceptance and comfortability to the formula and his overall impression about it. This could reflect patient compliance. Assessment of physical characters for preparations intended to be applied locally on the mucous membrane of the oral cavity has been performed before through certain questionnaires asked to volunteers (Irwin et al., 2003; Perioli et al., 2004).

The non-significant difference in the onset of action between (G) and the market product (L) could be explained by the generally fast onset of action for gels. The longer duration of (G) in comparison to (L) could be explained by the better residence of (G) due to its high mucoadhesive power against rapid washing out by saliva.

Although local anesthetic potency could be evaluated in vitro (Kitagawa et al., 1990), pain assessment should be determined by patient self-report (in his own words) as he/she is the one who is most aware about his/her condition where pain perception is subjective.

Availability of pain scores will provide an important index for monitoring improvement in the pain management. The numeric rating scale (NRS) used in this study to assess pain is easy to administer and demonstrates high compliance rates on the contrary of the usual visual analog scales (VAS) which have been difficult to understand, especially for elder people (Jensen et al., 1992). A split-mouth design was applied to overcome any personal variability (pain threshold) and to enable within-subject comparison of the different formulae.

There were no previous full investigations about the use of different local anesthetics in the treatment of variable oral painful conditions. In most studies, they were mainly used for periodontal scaling and root planning or before infiltration anesthesia to reduce pain associated with needle prick (Friskopp et al., 2001; Primosch and Rolland-Asensi, 2001). Furthermore, mebeverine HCl in this study was used for the first time as a local anesthetic for the treatment of different oral painful conditions.

Histological examinations for the oral cavity has been always dependent on taking observations for the application site only by naked eye and photomicrographs using light and electron microscopes (Şenel et al., 1998) to notice any changes in the tissue after application of the dosage form. Moreover, mucosal irritation was evaluated by giving scores for erythema and edema formation (Ikinci et al., 2000) or by asking the patient about the feeling of irritancy (Nagai and Konishi, 1987) which appears to

be highly subjective when compared to the technique performed in this study which is counting the inflammatory cells at the application site.

5. Conclusion

The gel formula (G): mebeverine HCl (20%, w/w), P-407 (20%, w/w), HPC (0.5%, w/w), cetrimide (0.005%, w/w), sorbitol (20%, w/w), citric acid (2%, w/w), aspartame (3%, w/w), orange flavor (1%, w/w) has shown a compromise between a relatively fast drug release profile and good mucoadhesion, suitable rheological properties as well which made it suitable to be examined clinically. The formula has shown to be a highly effective local anesthetic preparation that could be used for different oral painful conditions. Moreover, it has significantly shown superiority on the market product Lidocaine HCl gel® in pain reduction and duration of action where it could increase patient compliance by reducing application times and decreasing pain course together with its safety as a topical preparation for oral use on the buccal mucosa.

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