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Research Article

Design and Formulation of Mebeverine HCl Semisolid Formulations for Intraorally Administration

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Abstract. Gel formulations of mebeverine hydrochloride (MbHCl) containing hydroxypropylmethylcellulose (HPMC), metolose (MTL), and poloxamer 407 (PLX) were prepared to be used in the treatment of different oral painful conditions. HPMC was used as a mucoadhesive gel base while MTL and PLX were used to prepare sol–gel thermosensitive gels. MTL and PLX formulations showed proper sol–gel transition temperature for intraoral application. Formulations were evaluated in terms of their viscosity, mechanical properties, mucoadhesivity, stability, and *in vitro* drug release. The formulation prepared with 2% of HPMC K100M provided the highest viscosity at room temperature. However, the viscosity of HPMC–PLX mixture showed a significant increase at body temperature. The greatest mucoadhesion was also noted in HPMC–PLX combinations. Texture profile analysis exhibited the differences of the adhesion, hardness, elasticity, cohesiveness, and compressibility of the formulations. The release profiles of MbHCl were obtained, and non-Fickian release was observed from all the formulations. The formulations were stored at different temperature and relative humidity. No significant changes were observed at the end of the 3 months. HPMC–PLX formulation of MbHCl was chosen for *in vivo* studies, and it remained longer than dye solution on the rabbit's intraoral mucosal tissue. It was found worthy of further clinical evaluation.

KEY WORDS: hydroxypropylmethylcellulose; mebeverine hydrochloride; mucoadhesion; poloxamer 407; sol–gel transition.

INTRODUCTION

In dental procedures, topical local anesthetic agents are applied in order to ensure a painless treatment without the distress associated with needle injections for gingival or periodontal therapies. Formulations need to be easy application, remain on the applied tissue, and have sufficient effectiveness and stable storage. Topical anesthetics are used in dentistry to reduce the pain of operative dental procedures, to relieve the pain of superficial mucosal lesions, such as ulcers, to mask the discomfort of injections, and to anesthetize skin prior to vein puncture for general anesthesia or sedation (1). Local anesthetics for topical application can be incorporated into a number of different preparations. The type of preparation can affect efficacy such as film strips, sprays, emulsions, patches, and creams (2–6).

Gels are one step further than the other formulation types, as they correspond to the expectations. The gel formulation must have high viscosity and be mucoadhesive to adhere to the mucosal tissue, prolonging the residence time on the application site. Transmucosal drug delivery systems are designed with mucoadhesive polymers with certain specific characteristics such as high molecular weight, viscosity, long chain length, and flexibility of chain length. There are two broad classes of mucoadhesive polymers: hydrophilic polymers and hydrogels. In the large classes of hydrophilic polymers, those containing carboxylic group exhibit the best mucoadhesive properties, such as cellulose derivatives. Hydrogels, the other class of polymeric biomaterial, exhibit the basic characteristics of the hydrogel by swelling as it absorbs water while interacting by means of adhesion with the mucus that covers epithelia, i.e., polyacrylates and chitosan (7).

In our study, mebeverine hydrochloride (MbHCl) preparations were prepared with different polymers of each group as a preliminary work. However, the members of the anionic and cationic polymers, such as carbopol and chitosan, were discarded as a result of the phase separation after MbHCl was added to the formulation. Thus, MbHCl mucoadhesive gel formulations were prepared with nonionic mucoadhesive polymers and polymer and copolymer mixtures. Hydroxypropylmethylcellulose (HPMC), a well-known cellulose derivative, is used frequently as the gel base to provide sustained release. It is available in a wide range of molecular weights and is classified by the viscosities of their 2% (*w*/*w*) aqueous solution (http://www.dow.com/dowexcipients/products/methocel.htm). Metolose (MTL), consists of methylcellulose and three substitution of hydroxypropylmethylcellulose, is nonionic water soluble cellulose ether. It shows thermoresponsive property which can be characterized by two temperatures (8). Heating causes the viscosity evenly decreases, and above a certain temperature (T1), a sudden fall of viscosity is observed upon

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heating causes rise of viscosity at a certain temperature (T2) (9). In this study, the viscosity fall at T1 was used to provide the formulation spreading over the mucosa easily. It was thought that these kinds of formulations may be more advantageous to eliminate the pain of the ulcerated or burned tissues of the mouth. Poloxamer 407 (PLX), polyoxyethylene–polyoxypropylene–polyoxyethylene triblock copolymer, is used for many pharmaceutical applications. Its polyethylene oxide/polypropylene oxide ratio is 2:1 by weight. It is characterized by a sol–gel transition and shows thermoreversible gelation behavior according to the environmental temperature conditions. Contrary to MTL, it has liquid form at low temperature and is able to revert to gel structure at body temperature. Hence, it is applied easily but remains longer on the mucosal tissue. Moreover, the poloxamer systems can be easily administered by syringe equipped with needles appropriate for intrapocket delivery, becoming semisolid once in the periodontal pocket (10). If it is necessary, sol–gel transition temperature can be changed by adding salts to the solutions of MTL and PLX to provide the transition at the body temperature.

MbHCl is a potent direct antispasmotic, acting mainly on the smooth muscles of the gastrointestinal tract (11). It has been also reported that MbHCl exerts a local anesthetic action, which is similar to lidocaine (12). Although MbHC1 has a strong local anesthetic activity, it has nonsignificant side effects (13). The purpose of the present work was to evaluate different mucoadhesive oral formulations of MbHCl. The three gel formulations of MbHCl were prepared with HPMC. The fourth formulation, consisting of MTL, reverts to a liquid form due to the body temperature. The fifth formulation is prepared with PLX as a solution, which turns into a gel after administration.

MATERIALS–METHODS

Materials

MbHCl was gifted from Solvay Pharma Pharmaceutical Company (Turkey). Hydroxypropylmethylcellulose (HPMC E50 (40–60) and K100M (80,000–120,000 cps)), metolose 60SH 4000, and poloxamer 407 were obtained from Colorcon Ltd. (USA), Shin-Etsu Chemical Co., Ltd. (Japan), and BASF Chemical Company (Germany), respectively. All other materials were of analytical grade.

Methods

Preparation of Mebeverine Hydrochloride Mucoadhesive Intraoral Gels with HPMC

HPMC K100M as a ratio of 1.5 or 2% and HPMC E50 as a ratio of 10%, which have different viscosity values, were used. HPMC was swelled with an adequate amount of distilled water for 24 h. MbHCl was dissolved with the remaining amount of distilled water in the ultrasonic water bath. Then, it was added to the swelled polymer and was mixed completely.

Preparation of Mebeverine Hydrochloride Mucoadhesive Intraoral Formulation with Metolose SH 4000

A concentration of 2% (*w*/*w*) MTL solution was used to ensure the most optimal gel structure (14). Half the amount of distilled water was heated up to 70°C, and MTL was gradually added while stirring. MbHCl was dissolved in the rest of the amount of distilled water. Both of them were combined and then stirred homogeneously. The mixture was cooled in ice water until it became transparent.

Preparation of Mebeverine Hydrochloride Mucoadhesive Intraoral Gels with HPMC K100M and Poloxamer 407 Mixture

To prepare MbHCl, both a mucoadhesive and thermosensitive formulation of MbHCl, PLX, and HPMC were used together.

Twenty percent PLX and 2% HPMC K100M were mixed with an adequate amount of distilled water in 50 mL volumetric flask, and the mixture was left in the refrigerator at 4°C for 24 h. MbHCl was added into the gel base by stirring regularly after it was dissolved with distilled water in the ultrasonic water bath. Table I shows the composition of MbHCl gel formulations. Each mucoadhesive formulation consisted of 20% of MbHCl.

Drug Content

A 0.5 g of formulation was weighed and mixed with 60 mL of pH 6.8 buffered solutions in 100 mL of volumetric flask. It was left in the ultrasonic bath for 15 min and then adjusted to 150 mL with the same buffered solution. Ten microliters of the sample solution was withdrawn and diluted to 10 mL. After the filtration of the solution, the absorbance of the sample was measured spectrophotometrically at 264 nm using the assay method validated according to International Conference on Harmonisation (15).

pH Measurements

pH values of the formulations were measured with pH meter (Nel Mod 821) at room temperature after 24 h from the preparation to discard the air bubbles.

Viscosity Studies

The viscosity of the samples was measured using a AND SV-10 Vibro Viscometer (A&D Company Ltd., Japan). Sample volumes of 35 mL were applied to the sample container at both 25±1°C and 37±1°C. The Vibro viscometer measures the driving electric current to vibrate the sensor plates with a uniform frequency and amplitude, and then the

Table I. The Composition of the Formulations of MbHCl

Codes	MbHCl (%)	HPMC E50 (%)	HPMC K100M (%)	MTL (%)	PLX (%)
F1	20		1.5		
F2	20		2		
F3	20	10			
F4	20			2	
F5	20		2		20

MbHCl mebeverine hydrochloride, *HPMC* hydroxypropylmethylcellulose, *MTL* metolose, *PLX* poloxamer

viscosity is given by the positive correlation between the driving electric current and the viscosity. Viscosity measurement studies were carried out in triplicate.

Determination of Transition Temperatures of Thermoresponsive Formulations

Measurement of the Gelation Temperature. A 20-mL transparent vial containing a magnetic bar and 10 g of F5 formulation was placed in a water bath at 4°C. The temperature was increased gradually while the sample was stirred at a constant rate (200 rpm). When the magnetic bar stopped moving due to the gelation, the temperature displayed on the immersed thermometer was recorded and accepted as a gelation temperature of F5 (16).

Measurement of T1 for F4. The similar process was carried out as mentioned above to determine T1 which the viscosity falls dramatically. T1 was taken as the formulation moved freely due to the reverting solution. Both of the measurements were performed in triplicate.

Texture Profile Analysis

Evaluation of the mechanical properties of the formulations was performed using TA-XT Plus Texture Analyzer (Stable Microsystems, Halsmere, UK) in texture profile analysis mode. Beakers filled with the formulations were placed in the ultrasonic water bath for 20 min prior to experiment to remove any air bubbles. The tubular probe (10:150 mm, diameter/length) was inserted twice into each sample to a depth of 15 mm at a rate of 2.0 mm/s, with 15-s delay between insertions. Experiments were done triplicate at 25°C for all the formulations. F4 and F5 were also evaluated at 37°C because of their thermosensitive sol–gel transition properties. Hardness, compressibility, adhesiveness, cohesiveness, and elasticity were determined (17).

These variables were evaluated with the variance analysis in terms of the means of the individual groups. Variance homogeneity was controlled with the Levene test. When the differences between the groups were significant, either Tukey's honestly significant difference test or Dunnett's T3 was used for homogeny or heterogeneous groups for binary analysis, respectively. All the controls of hypothesis were studied at 0.05 of significant level (α). All analyses were performed in triplicate.

Mucoadhesion Studies

The mucoadhesive strength of the formulations was evaluated by measuring the force required to detach the formulation from a mucin disk using a TA-XT plus Texture Analyser (Stable Micro Systems) equipped with a 5-kg load cell in tension mode. Mucin disks were prepared by compression of a known weight of crude porcine mucin (250 mg) using a single punch tablet machine (Carver Laboratory Press, USA) with flat-faced punches. The disks were then horizontally attached to the lower end of the cylindrical probe (length 5 cm, diameter 1 cm) using double-sided adhesive tape. The samples, packed into the small cylindrical vessels, were placed under the upper probe. After the mucin disk made contact with the sample for 120 s, the probe was then moved at a constant speed of 0.1 mm/s. The peak value in the force–time plot determined the result. The work of mucoadhesion was also calculated using according to Eq. 1.

$$\text{Work of mucoadhesion}\left(\text{mJ/cm}^2\right) = \text{AUC}_{1-2}/\pi.\text{r}^2 \qquad (1)$$

All the above three experiments from the formulations were conducted in triplicates at 37°C.

Stability Studies

The stability of the formulations was studied by storing samples at three different temperatures and relative humidity (25±2°C, 60%±5; 30±2°C, 65%±5; 40±2°C, 65%±5). They were inspected visually and evaluated every 15 days for their viscosity, pH, and the amount of the active substance.

In Vitro Release Studies

A diffusion system was employed for *in vitro* release studies. An adequate amount of MbHCl gel was placed on the cellulose acetate membrane which was mounted on the bottom of the glass tube opened from both ends (1.3 cm diameter, 5.0 cm length). It was fixed to provide a contact with the receptor phase containing 15 mL of phosphate buffer at 6.8 (18). The receptor phase was stirred at approximately 600 rpm in water bath at 37±0.5°C. The samples were withdrawn at certain time intervals for 24 h and immediately replaced by the same volume of fresh medium to maintain the sink condition. Then they were analyzed by UV spectrometer at 264 nm.

To investigate the mechanism of drug release, the data generated from the study were fitted to the Eq. 2 using logarithmic transformations and least squares regression analysis (19).

$$M_t/M_\infty = kt^n \qquad (2)$$

M_t is the amount of the drug released at time t, M_∞ is the total drug content, k is the constant, incorporating structural and geometric characteristics of the delivery system, and n is the release exponent which indicates the mechanism. The experiments were conducted in triplicate.

In Vivo Studies

Study Design

The experimental protocol was reviewed and approved by the Ethics Committee for Animal Research at Ege University, Faculty of Pharmacy in İzmir. Eighteen male New Zealand rabbits (2.5–3 kg) were used for each formulation; 0.02% of toluidine blue solution as a dye solution was added to the gel formulation. No anesthesia was applied to avoid changing the amount of the secretion in oral cavity. The test animals were divided into three groups. G1 was a reference group where the 0.02% of toluidine blue solution was applied as a control. In group G2, subjects were applied with the gel base only consisting of toluidine blue. Finally, the MbHCl gel formulation, contained 0.02% of toluidine blue, was applied on the labial gingival mucosa of the members of

G3. The syringe without a needle was used for the equal application of the formulation. The formulations were applied at room temperature. The photographs of the application area were taken at certain time intervals, i.e., 5th, 10th, 15th, 20th, 25th, 30th, 45th, and 60th minutes. The distance and the angle of the camera were fixed, and the same light direction was used. The observations were also recorded. The digital image and the observations were compared to provide the correct comments. The extent of the coloring was recorded for each examination. A grading scale from 0 (no coverage) to 3 (complete coverage) was used to evaluate the area covered by each formulation. In addition, the visual assessment of color intensity was performed and described as "no color", "poor", "moderate", or "good" (20,21). The experiment was repeated five times (n=5) for each formulation.

RESULTS AND DISCUSSION

The calibration curve for the quantitative estimation of MbHCl was found to be linear. Absorbance was measured at 264 nm, and the coefficient of determination was r^2=0.999, indicating good linearity. Calculated regression variations of plotted standard curves were y=47.804x−2.311 in phosphate buffer at pH=6.8. y was the concentration (micrograms per milliliter), and x was the absorbance. The sensitivity of the standard calibration curve in both media is 5–40 μg/mL. The results of the drug content and pH studies were presented at Table II.

Viscosity Studies

Table II shows the results of the drug content and their viscosity values of the formulations. The highest viscosity value was observed in the F5 formulation which contained 2% of HPMC K100M and 20% PLX at room temperature. To compare the viscosity changes of F5 formulation due to the temperature with the other formulations was found significant. The 1,533.04 cP of viscosity value of F5 at room temperature increased to 11,786.10 cP at body temperature. The highest viscosity after jellifying at 37°C makes the formulation important for local administrations. F4, prepared with MTL, was in a gel at room temperature while its viscosity decreased at body temperature from 1,244.06 to 1,078.33 cP. The differences among the viscosity values of the HPMC formulations were not found significant due to the temperature change. It was also obtained that the higher amount of the HPMC K100M provided higher viscosity values when compared with the formulation containing 1.5% of HPMC K100M. It was an expected result and in accordance with the literature (22,23).

Texture Profile Analysis

A gel strength determination is one of the important techniques to define the physical characterization of pharmaceutical semisolids. Knowledge of the physical properties of such products is of value for the predictive performance of the product under the following conditions: during product filling, while spreading over and adhering to mucosal sites, and easily removing the product from the packaging system (24).

Texture profile analysis defines the mechanical parameters in terms of hardness, adhesiveness, cohesiveness, compressibility, and elasticity. These mechanical parameters of each formulation are presented in Table III. The higher concentration of HPMC K100M increased all the mechanical properties of the gel formulation when F1 and F2 were compared. The hardness introduces the necessary force to provide the deformation of gels. This parameter expresses the applicability of the gel to the desired site. The gels should have low hardness value to be administered to the mucosa easily and an obvious relation between the viscosity and the hardness of the formulations (25). The results showed that F2 had both the highest hardness value (0.074±0.001) and the highest viscosity value (9509±1.73) among the other formulations at the room temperature. At body temperature, the highest hardness and compressibility belonged to F5 formulation. It is an advantage of F5 that it resists compression and reflects the alterations in product viscosity after the intraoral application (16). The compressibility defines the required work for the compactibility of the product along a definite distance (23). This parameter expresses taking the prepared gel from the container and the simplicity of the spreadability on the application site. The compressibility value should be low to take the prepared gel from the container and to be easily spread on the mucosal epithelia (26). The compressibility of the gels increased when the polymer concentration in the gel formulations also increased (24,27). When we examined the compressibility value of F5, it increased from 0.022 to 1.254 according to the temperature. It was an advantage of this formulation because it means it was easily applicable at room temperature.

Table II. Results of Drug Content, Viscosity Studies, pH, and Transition Temperatures of the Formulations

Code	MbHCl (%)±SD	Viscosity (cP)±SD	pH	Transition temperature
F1	99.11±0.24	769.87±0.22	5.38±0.20	–
F1 (37°C)		892.34±0.34		
F2	99.27±0.28	1,128.33±0.57	5.09±0.01	–
F2 (37°C)		1,226.51±0.41		
F3	99.35±0.24	881.50±0.37	5.48±0.37	–
F3 (37°C)		715.34±0.24		
F4	99.11±0.24	1,244.06±0.45	5.34±0.14	>37.03°C±0.06
F4 (37°C)	–	1,078.33±0.36		
F5	99.51±0.14	1,533.04±0.98	5.61±0.02	>32.00°C±0.01
F5 (37°C)	–	1,178.61±1.40		

MbHCl mebeverine hydrochloride

Table III. The Mechanical Data of MbHCl Formulations

Code	Hardness[c] (N)±SD	Adhesiveness[d] (N mm)±SD	Cohesiveness[e]±SD	Compressibility[f] (N mm)±SD	Elasticity[g]±SD
F1[a]	0.036±0.001	0.204±0.050	1.093±0.540	0.079±0.000	1.223±1.624
F1[b]	0.034±0.003	0.155±0.010	1.015±0.006	0.090±0.004	0.766±0.005
F2[a]	0.074±0.001	0.301±0.005	1.245±0.581	0.141±0.001	1.424±1.534
F2[b]	0.055±0.004	0.259±0.001	1.268±0.004	0.099±0.007	1.250±0.009
F3[a]	0.049±0.010	0.230±0.038	1.105±0.550	0.094±0.039	1.234±1.112
F3[b]	0.039±0.009	0.165±0.041	1.096±0.012	0.106±0.005	0.886±0.048
F4[a]	0.027±0.002	0.130±0.008	1.027±0.480	0.079±0.008	1.040±2.340
F4[b]	0.019±0.001	0.112±0.007	0.972±0.517	0.061±0.001	0.969±2.302
F5[a]	0.017±0.008	0.012±0.001	0.911±0.478	0.022±0.006	0.910±1.014
F5[b]	0.553±0.035	1.138±0.175	0.984±0.112	1.254±0.185	1.196±1.580

[a] At room temperature
[b] At body temperature
[c] Difference is meaningful between F2 and the others except F3 ($p<0.001$)
[d] Difference is meaningful between F2 and F4 ($p<0.001$), F2 and F5 ($p<0.001$), F3 and F5 ($p=0.37$), F4 and F5 ($p=0.005$)
[e] Difference is meaningful between F2 and the others; also F1 and F4, F1 and F5 ($p<0.001$), F3 and F4, F3 and F5 ($p<0.001$)
[f] Difference is meaningful between F2 and F4 ($p<0.021$), F2 and F5 ($p<0.021$), F3 and F5 ($p<0.010$)
[g] Difference is meaningful between F1 and F5 ($p=0.07$), F2 and F4 ($p=0.002$), F2 and F5 ($p<0.001$), F3 and F5 ($p<0.006$)

The cohesion introduces the measure of the reconstruction of the gel after application. Cohesiveness increases the performance of the product on the application site. The high value of cohesiveness provides full structural recovery following gel application (23,26–28). HPMC formulations showed higher cohesiveness than other formulations at room temperature. The cohesiveness value F5 increased at body temperature. It was related with the solidifying of the formulation and the increasing of the viscosity at the body temperature.

Elasticity is defined as the direction of reconstruction of the gel after its deformation by compression in the means of time. The increase in the quantitative value of elasticity obtained during texture profile analysis shows the decrease in the elasticity of the gel (27). It was reported that the tissue adhesion of gels which included high elasticity components increased. The basic physical mechanism of bioadhesion is related to the elasticity of the polymer chains. The elastic polymer chains form stronger adhesive bonds by inclusion between polymer and mucus (29). It was observed that the elasticity of all the gel formulations was acceptable, and F2 coded formulations showed the highest elasticity (1.424± 1.534). When the elasticity was evaluated, the relation with adhesion was observed for all the formulations. More elasticity provided more adhesion, and the difference was found significant among the formulations ($P<0.001$). The result was in accordance with the literature (30).

For thermosensitive formulation, the results showed variation. The mechanical property values of F5 formulation decreased by the decreasing temperature due to the reverting liquid form at low temperature. At 37°C, the hardness, the compressibility, and the adhesiveness of the formulation significantly increased. On the contrary, a decrease was obtained for F4 formulation prepared with metolose at body temperature. Table III shows the results and the statistically data of the texture profile analysis of the formulations

Mucoadhesion Studies

The gel formulations were prepared with HPMC and examined to determine adhesiveness; it was found that a higher polymer concentration increased the adhesiveness of the gels. While the lowest adhesiveness was seen for F4 (0.086±0.001 Nmm) and F5 (0.264±0.004 Nmm), formulation exhibited the highest adhesiveness at body temperature (Fig. 1). It was concluded that one formulation, F5, consisting of both HPMC and PLX creates a synergism. It is known that HPMC exhibits a mucoadhesive property. Although PLX is not as mucoadhesive as HPMC, its sol–gel transition ability increases the viscosity of the solution at physiological temperature. Hence, combination of HPMC and PLX showed the highest mucoadhesiveness at 37°C. The work of mucoadhesion was calculated for each formulation and was found as 0.009±0.017, 0.013±0.029, 0.010±0.005, 0.007±0.001, and 0.021±0.004 mJ/cm^2±SD for F1, F2, F3, F4, and F5 formulation, respectively.

Determination of Transition Temperatures of Thermoresponsive Formulations

Sol–gel transition temperature was found 32°C±0.00 for F5 formulation whereas gel–sol temperature was 37.03°C± 0.06 for F4. Although MTL has higher T1 values than body temperature in the literature, it was thought that consisting 10% MbHCl in the metolose 60SH 4000 gel (F4) provided that T1 shifted to the body temperature. The possible explanation could be that the salts dehydrate the Metolose®

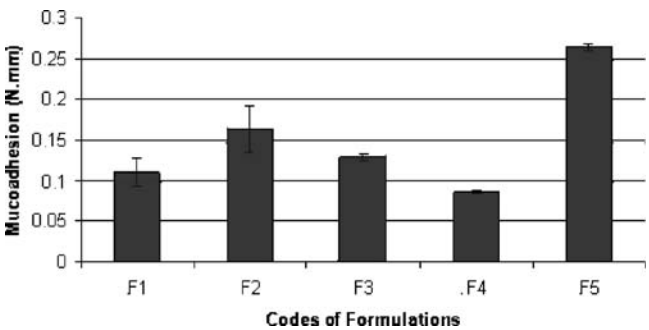

Fig. 1. Result of mucoadhesion studies at 37°C

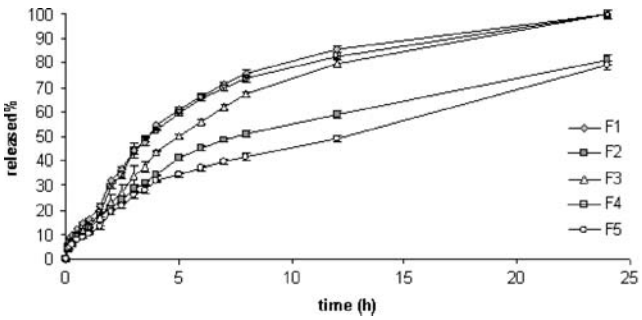

Fig. 2. *In vitro* release profiles of MbHCl from gel formulations

gel, thus resulting in gel–sol transition. The higher the concentration of the salt in the gel system, the lower the temperature needed to the transition (9). In the oral cavity, the temperature is generally around 37.8°C (31). F4 takes a gel form at room temperature and can liquefy after administration onto the intraoral mucosa, so it will be able to cover the application area extensively. However, the F5 formulation can be applied both over the mucosa and into the periodontal pocket. This is possible because F5 becomes a solution at room temperature and solidifies after the administration, whereas a sol–gel transition temperature of 32°C is sufficient. Therefore, it is not necessary to add any agent to increase the sol–gel temperature. Usually, the gelation temperatures are considered to be suitable if in the range of 25–37°C. If the gelation temperature of a thermosensitive formulation is lower than 25°C, a gel might be formed at room temperature leading to difficult manufacturing, handling, and administering. If the gelation temperature is higher than 37°C, the formulation does not solidify and remains a liquid dosage form which can result in leakage from the administered site (32). F4 and F5 showed proper sol–gel transition temperatures to meet the expectations.

Stability Studies

The stability tests were performed with all the formulations. General appearance and organoleptic properties did not change significantly in 3 months in the formulations submitted to accelerated stability testing. Drug contents of F1, F2, F3, F4, and F5, which were kept at 40°C±2 and 65%± 5 relative humidity for 3 months, were changed from 99.11± 0.24%, 99.27±0.28%, 99.85±0.24%, 99.11±0.24%, and 99.51±0.14% to 98.24±0.28%, 98.80±0.28%, 98.33±1.04%, 98.91±0.41%, and 98.87±0.41, respectively. The lower decrease was observed with other two different test conditions. There was no significant decrease at pH values of the formulations. The pH values of the prepared formulations were found as 5.38± 0.2, 5.18±0.08, 5.48±0.37, 5.34±0.14, and 5.61±0.02 at the beginning for F1, F2, F3, F4, and F5 formulations, respectively. It was observed 5.21±0.01, 5.09±0.01, 5.45±0.01, 5.49±0.01, and 5.49±0.01 for the sequential formulations which were kept at 40°C±2 and 65±5% relative humidity. Viscosity was also followed during the stability studies, and the same viscosity values were almost measured for all the formulations. The standard deviation (SD) was found ±0.6 as minimum for F1, F3, F4, and ±1.0 for F2 as maximum. In brief, no significant changes of the investigated properties of the formulations were obtained under accelerated stability test conditions.

In Vitro Release Studies

Figure 2 illustrates the *in vitro* release of MbHCl from the formulations at 37°C. The fraction of the diffused amount of the drug was plotted against the time. As it was expected, F4 yielded the highest release because it turned into a liquid form at 37°C. Although F1 and F4 were prepared with different polymers, they showed similar diffusion profiles and provided faster release than other formulations. The significant difference between the diffusion profiles of F1 and F2 was found. This difference pointed to the fact that the concentration of the polymer affected the release rate as was previously reported (33). In addition, the type of the HPMC affected the released amount of MbHCl from the formulations. Although a higher amount of (10%) HPMC E50 was used to prepare F3, the release of MbHCl from F2, containing 2% of HPMC K100M, was found lower than F3. The result could be explained by the structural difference between the types of HPMC; 2% of HPMC E50 solution in water shows 40–60 cps of viscosity while K100M's is 80,000–120,000 cps (http://www.dow.com/dowexcipients/products/methocel.htm).

The F5 formulation, containing HPMC K100M and PLX, was prepared in order to combine both mucoadhesion and sol–gel transition characteristics into one formulation. At 37°C, F5 jellified and made the diffusion of the active substance difficult. Hence, the release from the F5 formulation decreased. It was clear that its high viscosity was the cause of the decrease. The result was found in accordance with the literature, which explained that the PLX consists of a large population of micelles in an aqueous phase at body temperature. Drug release can be affected by the viscosity of the gel, the size of the aqueous channels, and the distribution of drug between the micelles and the aqueous phase (34). Also the presence of HPMC K100M was to fortify the gel strength and affected the diffusion rate. It could be an advantage for the formulation to be used as a long-acting single-dose administration in the treatment of different orally painful conditions

Table IV. Characteristics of *In Vitro* MbHCl Release Studies for 5 h from the Formulations

Code	Drug released per unit area (mg/cm^2)±SD	Jss (mg/cm^2/h)±SD	$D\times10^{-11}$ (cm^2/h)±SD	r^2	n	t_{30} (h)
F1	43.262±1.348	8.186±0.284	4±0.1	0.981	0.658	2.53
F2	28.848±8.110	6.233±2.019	3±0.6	0.987	0.616	2.88
F3	36.268±5.763	6.777±1.113	5±1.2	0.990	0.656	2.84
F4	48.997±1.813	10.065±0.432	6±0.1	0.977	0.690	2.11
F5	27.691±4.539	5.130±0.665	6±0.1	0.993	0.627	3.41

Jss steady-state influx, *D* diffusion coefficient, r^2 correlation coefficient

Table V. Coverage of Mucosal Tissue and Intensity of Their Coloring after Administration of the MbHCl Formulations

Formulation	Time (min)											
	0		10		15		20		45		60	
Dye solution	2^a	3^b	1^a	1^b	0^a	0^b	0^a	0^b	0^a	0^b	0^a	0^b
F1	2^a	3^b	2^a	3^b	2^a	3^b	2^a	2^b	1^a	0^b	0^a	0^b
F2	2^a	3^b	2^a	3^b	2^a	2^b	1^a	1^b	0^a	0^b	0^a	0^b
F3	2^a	3^b	2^a	3^b	2^a	3^b	2^a	3^b	2^a	1^b	0^a	0^b
F4	2^a	3^b	1^a	2^b	0^a	0^b	0^a	0^b	0^a	0^b	0^a	0^b
F5	2^a	3^b	2^a	3^b	2^a	2^b	2^a	2^b	2^a	2^b	1^a	1^b

[a] Coverage ability (0—no coverage, 1—moderate, 2—completely)
[b] Visual evaluation (0—no color, 1—poor, 2—moderate, 3—good)

or for the periodontal pocket applications. Also, its high adhesiveness, containing HPMC as a mucoadhesive polymer, increased its efficacy by preventing any leakage with saliva.

Application of the Eq.1 enabled calculation of n and hence the mechanism of release from the formulations. In this context, n=0.5 indicates release controlled only by Fickian diffusion, and n=1 indicates released controlled only by relaxation of polymer chains (19). It was observed that the release exponent (n) ranged from 0.5 to 1.0, indicating non-Fickian drug transport (Table IV). These results have a very good fit with the studies of Grassi *et al.*, where a non-Fickian release from scleroglucan gel was reported, and Karavana *et al.* which observed non-Fickian release from benzydamine hydrochloride gel (35,36). The times required for 30% release of MbHCl from the gel, t_{30}, were calculated. t_{30} values were observed as 2.53, 2.88, 2.84, 2.11, and 4.14 h for F1, F2, F3, F4, and F5 formulations, respectively. t_{30} of F5 which contained both HPMC and PLX was significantly greater than the other formulations. F4, prepared with MTL and liquefied at body temperature, had the smallest t_{30} as was expected.

In Vivo Studies

The fraction of the formulation remaining on the mucosal tissue decreased as a function of time. The dye solution was completely removed from the mucosa after 5 min which indicated that the dye solution cannot stain the mucosal tissue by itself. No significant difference was observed between the formulations with or without MbHCl. F4 formulation was readily removed from the mucosa. However, F5 formulation showed more mucoadhesive property and coverage ability than other formulations during the first 30 min. At the end of the 60 min, no difference was observed among the formulations. Table V and Fig. 3 show the coverage ability of the formulations and the intensity of their color.

CONCLUSION

In this study, the design and the development of the mucoadhesive semisolid formulations of MbHCl for intraoral application and their physicochemical properties both *in vitro* and *in vivo* were examined. The results showed that MbHCl could be released from the F5 formulation over a prolonged period of time. Its mechanical properties especially both its adhesiveness and mucoadhesiveness are important properties to resist a leakage of the applied formulation due to the intraoral conditions. As a result, the evaluation of the entire candidate formulations indicated that the F5 formulation, which was prepared with HPMC and PLX, had a potentially advantageous role in the therapy of periodontal disease when accompanied with different orally painful conditions and was found worthy of clinical evaluation.

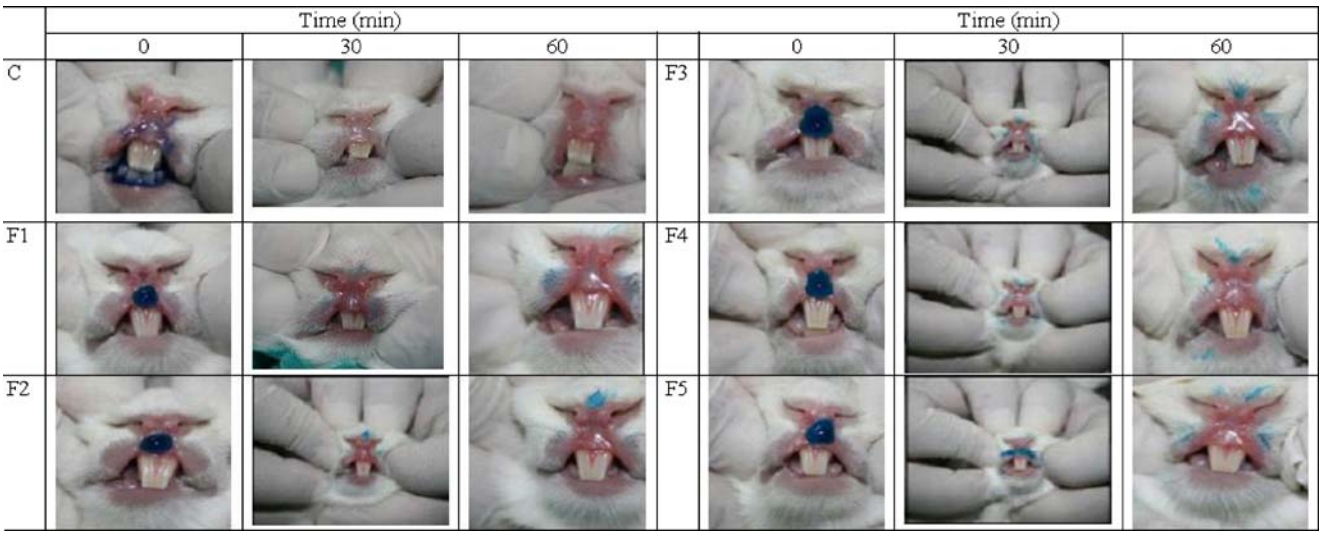

Fig. 3. Appearances of oral mucosa at the application and after 30 and 60 min

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