DEVELOPMENT OF IN SITU GELLING AND MUCOADHESIVE MEBEVERINE HYDROCHLORIDE SOLUTION FOR RECTAL ADMINISTRATION

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من المعروف أن عقار هيدروكلوريد المبيفيرين يتعرض لتأثير العبور الأول، وفي محاولة لتحسين إتاحته الحيوية ولقصر أو تقليل امتصاصه على الجزء السفلي من المستقيم، فقد تم صياغة هيدروكلوريد المبيفيرين على شكل جل موضعي ومحلول هلامي لاصق مستقيمي بواسطة التجلل عند معدل حرارة 30-37°م. وقد تم استخدام خليط من البولوكسامر 407 (P407) والبولوكسامر 188 (P188) للحصول على خاصية التجلل الحساس للحرارة. لتعديل قوة الجل والقوة الهلامية اللاصقة لمحلول البولوكسامر المستقيمي للدواء فقد تم دراسة البوليمرات الهلامية اللاصقة مثل هيدروكسي بروبيل ميثيل السليولوز، وهيدروكسي إيثيل السليولوز وميثيل سليولوز وبولي فاينايل بيروليدون K-25. إن إدخال محلول مستقيمي (10% وزن/وزن) من الدواء زاد من حرارة التجلل لأخلاط البولوكسامر، بينما كان لإضافة البوليمرات الهلامية اللاصقة تأثير عكسي. ومن ناحية أخرى، عززت هذه البوليمرات من قوة الجل والقوة الهلامية اللاصقة للمحلول المحضر. لقد كان التأثير واضحاً مع الميثيل سليولوز. كما أن زيادة التركيز السليولوزي للبوليمرات الهلامية اللاصقة أعاقت إطلاق هيدروكلوريد المبيفيرين من محاليل البولوكسامر بمعدلات متفاوته، وكان الممكن الحفاظ على تأثير الدواء لمدة 8 ساعات. ولم يكن للبولي فاينايل بيروليدون K-25 أي تأثير على إطلاق الدواء. كما أن المعالجة المسبقة لحيوان خنزير غينيا بمحلول الدواء المستقيمي [P188/P407/ ميثيل سليولوز/هيدروكلوريد المبيفيرين (23/7/1.5/10% وزن/وزن)] الذي كان له قوة جل وخواص هلامية لاصقة مُرضية، وخواص إطلاق مستمر معقول ذات حركية من الدرجة صفر قد أظهرت تأثيراً معنوياً ممتداً مضاداً للتقلص في حالات التقلص المستحثه على المعى اللفائفى لخنزير غينيا، ولم تتسبب فى أى تلف نسيجى على أنسجة المستقيم.

Mebeverine hydrochloride (MbHCl) is well known to suffer from extensive first pass effect. In an attempt to improve its bioavailability and possibility restrict its absorption to only the lower rectum, rectal solution with gelation at temperature range of 30-37C°. Mixtures of poloxamer 407 (P407) and poloxamer 188 (P188) were used to confer the temperature-sensitive gelation property. To modulate the gel strength and the mucoadhesive force of MbHCl poloxamer rectal solution, mucoadhesive polymers such as hydroxypropylmethyl cellulose (HPMC), hydroxyethyl cellulose (HEC), methyl cellulose (MC) and polyvinylpyrrolidone K-25 (PVP K-25) were investigated. Incorporation of 10% w/w MbHCl in the rectal solution increased the gelation temperature of the poloxamers mixtures, while the addition of the mucoadhesive polymers had a reverse effect. On the other hand, these polymers reinforced the gel strength and the mucoadhesive force of the prepared solutions. The effect was most pronounced with MC. Increasing the concentration of cellulosic bioadhesive polymers retarded the release of MbHCl from the poloxamer solutions to different extents, and it was possible to sustain the drug effect over a period of 8 hours. PVP K-25 had no effect on drug release. Pretreatment of guinea pigs with MbHCl rectal solution [P407/ P188/ MC/ MbHCl (23/ 7/ 1.5/ 10% w/w)], having satisfactory gel strength, mucoadhesive properties and acceptable sustained release profile with zero order release kinetics, showed a significant extended spasmolytic effect to spasmogens-induced contractions on guinea pig ileum and did not cause any histological damage to the rectal tissues.

Keywords: Rectal solution; mebeverine hydrochloride; poloxamers; mucoadhesive polymers; gelation temperature; dissolution; spasmolytic effect.

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Introduction

The irritable bowel syndrome (IBS) is one of the most frequently encountered disorders of the GIT among young to middle age adults. Little is known about its pathogenesis, meanwhile several studies have proved the efficacy of mebeverine hydrochloride in the treatment of IBS when taken at a dose of 135 mg three to four times daily (1-3). However, MbHCl suffers from extensive first pass metabolism in the gut wall and liver. High plasma concentrations of veratric acid (one of the main inactive metabolites of MbHCl) in addition to negligible amounts of the parent drug were observed in plasma 20-30 minutes after oral administration (4).

Rectal route of administration have been proved beneficial in approaching this problem. In a previous work, our laboratory (5) developed a fast release rectal suppository of MbHCl for the relief of acute gastrointestinal spasms. The development of a rectal formulation of MbHCl with prolonged drug action is expected to be appropriate for the control of IBS. The use of rectal route for sustaining drug action has been successfully reported with a number of drugs such as nifedipine (6), morphine (7,8), metoclopramide HCl (9), zidovudine(10), diclofenac sodium (11) and paracetamol (12). However, conventional suppositories being solid dosage forms which melt or soften in the rectum, may give a feeling of alien, discomfort and refusal to the patients, possibly lowering patient compliance which represents a problem especially when treating a chronic disesase such as IBS. To solve the problem of these conventional solid dosage forms, there have been several attempts to develop a rectal dosage form which exists as liquid at room temperature but gels at physiological one (13). The formed gel is expected to be softer than the conventional suppository.

Poloxamers are surfactant copolymers of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) exhibiting such thermoreversible property. By modulating the gelation temperature of different poloxamer solutions, liquid bases for rectal use can be formulated which form a gel in the rectum at body temperature with suitable gel strength so as not to leak out from the anus after administration (13). In addition, the incorporation of mucoadhesives in these bases would reinforce the drug sustained action, and may help in adhering the formed gel to the lower part of the rectum offering further protection from the first pass effect, hence improving its bioavailability. Mucoadhesive suppositories of propranolol HCl (14) and lidocaine (15) were previously formulated to achieve such protection from the first pass effect.

In this study, we developed *in situ* gelling and mucoadhesive MbHCl rectal solution using poloxamers, P407 and P188, and mucoadhesive polymers. The gelation temperatures of the prepared poloxamer solutions were modulated so as to lie between 30 and 37^{o}C which was reported (13) as being an acceptable range to ensure gelation at physiological temperature without leakage after administration. The gel strength and mucoadhesive force of the prepared rectal solutions were determined. The drug release from the prepared poloxamer solutions and their *ex vivo* antispasmodic activity were investigated and compared with those of a conventional MbHCl suppository. Finally the rectal tissue was examined histopathologically to detect if any irritation or damage had occurred.

Experimental

Materials:

Mebeverine hydrochloride (MbHCl) (MOEHS CANTABRIA S.L. Polgono Industrial Requejada Polanco Cantabria, Spain) was kindly supplied from E.I.P.I.Co., 10th of Ramadan City, Egypt); poloxamers (P407 and P188); polyvinylpyrrolidone K-25 m.wt. 40,000, (PVP); acetylcholine bromide and histamine dihydrochloride were purchased from Sigma Chemical Co., U.S.A.; hydroxypropylmethyl cellulose (Methocel-E4M, m.wt. 86,000, 4000 centipoise (cps)), (HPMC); hydroxyethylcellulose (Natrosol 250 L, m.wt. 80,000, 14 cps), (HEC); methylcellulose (m.wt. 100,000, 20,000 cps), (MC) were from Tama, Japan. Barium chloride was purchased from ADWIC Co, Egypt. All other chemicals were of analytical grade.

Equipment:

Schimadzu UV 240 double beam spectrophotometer (Koyoto, Japan); USP dissolution test apparatus II (Pharma Test, type PTW, Germany); electric balance (Sartorius, GMBH, Gottingen, Germany); digital circulating water bath (Polyscience, type 9101, U.S.A.); magnetic stirrer with heater (Thermolyne Corporation, U.S.A.); cellulose membrane tubes (Spectrapore m.wt. cutoff:

12000-14000, Sigma chemical Co., U.S.A.) and T_3 isotonic myograph transducer attached to strain gauge coupler No. FC117 fitted to an oscillograph (Washington 400 MD2C, Bioscience, Sheerness, Kent, U.K.); Light microscope (Euromex, Netherland).

Methodology:

1. Preparation of the rectal solutions:

Using different concentrations of P407 and/or P188, as shown in Table 1, plain and medicated rectal solutions were prepared using the cold method (16). Briefly, the poloxamers were slowly added to the calculated amount of cold distilled water with continuous agitation using a magnetic stirring bar and the formed dispersions were left at 4°C until clear solutions were obtained. The medicated bases were prepared by the addition of the appropriate amount of MbCHl, 10% w/w, in the calculated amount of distilled water.

The medicated rectal solutions having a base of 23 / 7 and 25 / 5%, w/w P407/P188 were designated as rectal solution A and rectal solution B respectively. The medicated rectal sollutions showing satisfactory gelation temperature (30-37°C) and lower total poloxamer content were mixed with additional amounts of mucoadhesive polymers namely: HPMC, HEC, MC or PVP in concentrations 0.5, 1, 1.5, 2 and 2.5%, w/w.

Table 1. Composition of the rectal Solutions.

Formulations No.	P407%, w/w	P188%, w/w
1	15	-
2	17	-
3	20	-
4	23	-
5	25	-
6	27	-
7	30	-
8	23	5
9	23	7
10	23	10
11	23	15
12	23	20
13	25	5
14	25	7
15	25	10
16	25	15
17	25	20

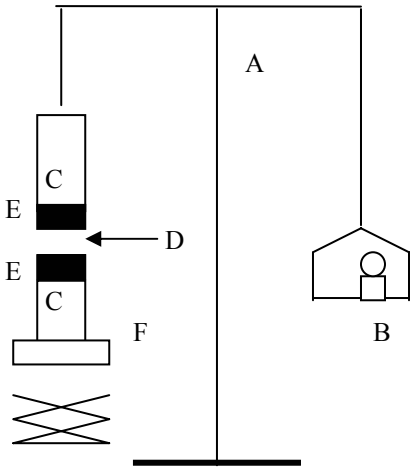

Fig. 1. Bioadhesive Force Measuring Device:(A) modified balance; (B) weights; (C) glass vial; (D) poloxamer gel;(E) rectal tissue; (F) height-adjustable pan.(Ref. 13)

2. Evaluation of In Situ gelling rectal solutions:

2.1. Measurement of the gelation temperature:

The gelation temperatures of plain and medicated bases were measured according to the technique described by Gilbert *et al*. (17). Accordingly, a 2ml aliquot of the rectal solution was transferred to a test tube sealed with a parafilm and put in a water bath at 4°C. The temperature of the bath was increased in increments of 3°C (or 1°C in the region of sol-gel transition temperature) and left to equilibrate for 15 minutes at each new setting. The samples were then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting through 90°. The maximum temperature tested was 50°C.

2.2. Determination of the mucoadhesive force:

The mucoadhesive forces of the MbHCl rectal solutions were determined by means of the mucoadhesive force-measuring device shown in Fig. 1 and according to the previously reported methods (13,18), using tissues cut from the fundus of rabbit (New Zealand white) rectum. The pieces of tissues were stored frozen in Sorensen's phosphate buffer pH 7.4 and thawed to room temperature before use (19). At the time of testing a section of tissue (E) was secured, keeping the mucosal side out, on to each glass vial (C) using a rubber band and an aluminum cap. The diameter of each exposed mucosal membrane was 1.1cm. The vials with the

rectal tissue were stored at 37°C for 10 min. Next, one vial with a section of tissue (E) was connected to the balance (A) and the other vial was fixed on a height-adjustable pan (F). To the exposed tissue on this vial, a constant amount of 0.1g poloxamer gel (D) was applied. The height of the vial was adjusted so that the gel could adhere to the mucosal tissues of both vials. A constant (preload $\simeq$ 0.5 Newton) force was placed on the upper vial and applied for 2 minutes (20), after which it is removed and the upper vial was then connected to the balance. Weights (B) were added at a constant rate to the pan on the other side of the modified balance of the used device until the two vials were separated. The bioadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights that detached the two vials using the following equation (21):

$$\text{Detachment stress (dyne/cm}^2) = \frac{m.g}{A} \qquad \text{Eq (1)}$$

where,

m: the weight added to the balance in gram.

g: acceleration due to gravity taken as 980 cm/sec^2.

A: area of tissue exposed and is equal to πr^2 (r-the radius of the circular hole in the aluminium cap)

2.3.Measurement of Gel Strength:

The gel strength was determined according to a previously adopted method (12,13,22). A sample of 50g of the rectal solution was put in a 100-ml graduated cylinder and gelled in a thermostatically controlled water bath at 37°C. A weight of 35g was then placed onto the gelled solution. The gel strength, which is an indication for the viscosity of the rectal solutions at physiological temperature, was determined by the time in seconds the weight took to penetrate 5cm down through the gel. A range of 10-50 sec. is accepted, because less than 10 seconds the rectal solution leaked out from the rectum and more than 50 seconds it was difficult to administer the rectal preparation (13).

All the above three experiments (gelation temperature, mucoadhesive force and gel strength) were conducted in triplicates.

2.4. In vitro release of MbHCl from the rectal solutions:

The drug release from the prepared rectal solutions was monitored by the USP paddle method (12, 22). An amount of rectal solution or a conventional PEG suppository (PEG400:PEG 6000:H_2O in 1:3:1) each equivalent to 200 mg MbHCl was inserted into a semipermeable cellulose membrane tube tied from both ends. The membrane tube was then connected to the paddle of the dissolution tester and immersed in 500 ml of Sorensen's phosphate buffer pH 7.4 maintained at 37 ± 0.5°C. The speed of rotation of the paddle was adjusted at 50 rpm. Aliquots of 5ml were withdrawn from the release medium at appropriate time intervals and assayed spectrophotometrically for MbHCl at 263/nm. The experiments were conducted in triplicates.

The mechanism of release of MbHCl was analyzed using the following equations (23):

$$\frac{M_t}{M_\infty} = Kt^n \qquad \text{Eq (2)}$$

$$\text{Log}\ \frac{M_t}{M_\infty} = \log K + n \log t \qquad \text{Eq (3)}$$

where,

$\frac{M_t}{M_\infty}$: the fraction of released drug at time t

k: release characteristic constant.

n: release exponent indicative of the release mechanism.

When n is equal to 0.5, the drug is released from the polymer with a Fickian diffusion mechanism (Higuchi model). If 0.5< n <1 this indicates anomalous or non-Fickian release, while if n=1 this indicates zero-order release (23).

2.5. Spasmolytic activity of mucoadhesive MbHCl rectal solutions:

The MbHCl rectal solutions composed of 23, 7, 1.5, and 10% w/w of P407, P188, MC and MbHCl in water was tested for its spasmolytic activity on spasmogens-induced contractions on guinea pig ileum.

A total of 48 adult male guinea pigs (each weighing 450-500 g) were kept fasted for 24 hours prior to the experiment but were allowed free access to water and were divided into 8 groups each consisting of at least 6 animals. Group I (control group), groups II, III, IV, V and VI received, by means of a micropipette, an amount of rectal solutions equivalent to 16 mg MbHCl and were killed after 2, 4, 6, 8, and 10 hrs. respectively. Group VII and VIII received conventional PEG suppositories containing the same dose of MbHCl and were killed after 2 and 4 hours respectively.

The method followed was previously reported by Hosny *et al.* (24). Briefly, contractions were induced in guinea pig ileum using histamine (250-1250 ng/ml), acetylcholine (100-400 ng/ml) and barium chloride (100-400µg/ml) separately. The mean height of contractions in the control group was determined and the percentage of inhibition induced by each treatment at the submaximal dose of the used spasmogens was calculated according to the following equation:

Percentage inhibition at submaximal dose =

$$\frac{\text{[Mean height of control (mm)– height of treated animals (mm)] X 100}}{\text{Mean height of control (mm)}}$$

The mean percentage inhibition was then calculated for each group and data were statistically treated using one-way analysis of variance ANOVA at $P < 0.05$.

2.6. Histopathological examination of the rectal tissues:

Eight hours after inserting the rectal solutions into the rectum of the guinea pigs, animals were sacrificed and the rectum was isolated, rinsed with a saline solution, fixed in Bouin's solution (25) then embedded in paraffin wax and cut into slices. The slices were stained with hematoxylin- eosin and observed under a light microscope (15,26,27).

Results and Discussion

1.Gelation temperature:

As previously mentioned an accepted rectal solution must have a gelation temperature in the range of 30-37^{o}C so as to be in a liquid form at room temperature and to form a gel phase instantly in the rectum (13).

Poloxamer solutions are known to exhibit this thermoreversible gelation, depending on the polymer grade, concentration and other included formulation components. The thermoreversibility of poloxamers could be explained on the basis that they are more soluble in cold than hot water with an increased solvation and hydrogen bonding at low temperatures. Being surfactant in nature, the temperature-dependent gelation of poloxamer solutions could be explained as being a change in their micellar properties by temperature increase. Raising the temperature of poloxamer solution will be accompanied by an increase in the micellar aggregation number and a decrease of critical micelle concentration, allowing the formation of a more closely packed and a more viscous gel. Moreover, conformational changes in the orientation of the methyl groups in the side chains of poly(oxypropylene) polymer chains, constituting the core of the micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation phenomenon (28,29).

Table 2 shows that plain rectal solutions of less than 20% w/w P407 did not gel over the temperature range tested (up to 50^{o}C) and that increasing P407 concentration, by an increment of 2-3% decreased, the gelation temperature of its solution. On the other hand, P188 in the tested concentrations (20-30%) failed to give the suitable range of gelation temperature, where all the recorded temperature values were >50^{o}C and a gelation started to be observed at 47.3^{o}C with a 35% solution of P188 (results not shown). This means that increasing the P188 concentration decreases the gelation temperature. The previous findings indicate that neither P407 nor P188 alone could provide gelation at the physiological temperature. A modulation of the gelation temperature to reach the desired range (30-37 ^{o}C) could be achieved through the use of a combination of the two poloxamer grades.

Table 2. Gelation Temperatures of Different Mebeverine Hydrochloride Rectal Solutions.

Poloxamer	Concentration (%,w/w)	Mean Gelation Temperature (^{o}C) ± S.D.	
		Plain	Medicated
P407	15	>50	>50
	17	>50	>50
	20	27 ± 1	48.3 ± 0.58
	23	23.3 ± 0.58	42 ± 0.0
	25	19.7 ± 0.58	38 ± 1
	27	18 ± 0.0	28 ± 1
	30	15 ± 1	25.7 ± 0.58
P407/P188	23/5	21 ± 0.0	39 ± 0.0
	23/7	20 ± 1	37 ± 0.0
	23/10	18.3 ± 0.58	36 ± 0.0
	23/15	16.7 ± 0.58	34.3 ± 0.58
	23/20	14 ± 1	32 ± 1
	25/5	18 ± 1	35 ± 0.0
	25/7	17.7 ± 0.58	34 ± 0.0
	25/10	16 ± 0.0	33 ± 0.0
	25/15	15.3 ± 0.58	30 ± 1
	25/20	13 ± 0.0	25.7 ± 0.58

The incorporation of 10% w/w MbHCl greatly increased the gelation temperature. This finding is in agreement with that obtained by Gilbert *et al.* (17) and Yun et *al.* (30) who stated that water soluble substances cause modification of the process of micellar association of poloxamer solutions leading to an increase in gelation temperature.

It is to be noted that MbHCl rectal solutions composed of 23% and 25% P407 showed intermediate gelation temperatures (42 and 38°C respectively) so they are good candidates for gelation temperature modulation using P188. The medicated rectal solutions prepared with other concentrations of P407 exhibited either high (48.3 and >50°C) or low (28 and 25.7°C) gelation temperatures compared to the reported accepted range (30-37°C). From the same table it is obvious that it was possible to obtain several formulations gelling at the required temperature range (30-37°C) by the use of P407/P188 blend. In addition, with both concentrations of P407 (23 and 25%), it was found that the gradual increase in P188 concentration was accompanied by a concomitant decrease in the gelation temperature of the prepared rectal solutions. It was also noticed that, with all the concentrations of P188, mixtures containing lower percentage of P407 (23%) gelled at a higher temperature than those containing the higher percentage (25%). This revealed that P407 is the main polymer determining the gelation temperature of the solution and might be explained on the basis of its higher molecular weight (average M.Wt. 12000) compared to that of P188 (average M.Wt. 8350) (31) and its higher amount in the formulation.

To reinforce the gel strength and bioadhesive force of the rectal solutions, mucoadhesive polymers were further incorporated. The rectal solutions A and B were selected for the purpose. The chosen formulations not only had a gelation temperature in the desirable range (30-37°C), but also represented the least total poloxamer concentration, each in its group.

The different mucoadhesive polymers used in this study are either swellable or water soluble but differ in their nature and charge: the cellulosic derivatives, HPMC, HEC and MC, as neutral and swellable polymers and PVP as a synthetic, cationic and water-soluble one. It is to be noted that anionic bioadhesive polymers were excluded from the study because of the previously reported interaction between them and MbHCl (32).

Table 3 shows that the addition of any of the used mucoadhesive polymers lowered the gelation temperature of the rectal solutions.

The impact of bioadhesive polymers on the gelation temperature was found to depend on their nature and concentration. Increasing the concentration of any of the used bioadhesives from 0.5 to 2.5% produced a gradual decrease in the gelation temperature of the corresponding rectal solutions. In comparison with the preparations containing no bioadhesive, the mean average decrease in the gelation temperature noticed with both rectal solutions A and B, containing 2.5% of different bioadhesives used, can be arranged in the following descending order: MC > HPMC > HEC > PVP where the values of decrease were of 7° , 6.5° , 4.35° and 3.85°C respectively. It is to be noted that the difference in the gelation temperature between MC and HPMC was non significant as well as between HEC and PVP (ANOVA followed Least Square Difference at $p < 0.05$). This order of arrangement correlated well with the viscosities of the used polymers being of 20000, 4000 and 14 centipoise (cps) respectively for 2% aqueous cellulose solutions and 4.5 cps for a 10% aqueous PVP solution. The direct relation between the gelation temperature and the viscosity of the polymer solutions has been previously reported by Jeong *et al.* (33).

The gelation temperature-lowering effect of mucoadhesive polymers could be explained by their ability to bind to the polyoxyethylene chains present in the poloxamer molecules. This will promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding which will lead to gelation at lower temperature (14,17).

2. Mucoadhesive Force:

The bioadhesive force is an important physicochemical parameter for in situ gelling rectal solutions since it prevents the gelled solutions from reaching the upper part of the rectum, the pathway for the first-pass effect.

The addition of different mucoadhesive polymers reinforced the mucoadhesive force of rectal solutions A and B, as shown in Table 3. The bioadhesive force has significantly increased as the concentration of mucoadhesive polymers increased over the range of 0.5-2.5%. The mucoadhesive polymers could be arranged according to their

mucoadhesive force-enhancing effect at 2.5% concentration, for both rectal solutions A and B, as follows: MC > HPMC > HEC > PVP. The differences in the mucoadhesive force between all the compared mucoadhesives were significant in case of rectal solution A, as well as for the rectal solution B except for the difference between HEC and PVP (ANOVA followed Least Square Difference at $p < 0.05$). Similarly, it was reported that MC has a higher bioadhesive force than HPMC and PVP polymers (34-35). Furthermore, Ahuja *et al.* (37) stated that high molecular weight is important to maximize adhesion through entanglements and Van der Waal forces. The previously mentioned order of arrangement correlated with the molecular weights of the used polymers, thus MC polymer with the highest m.wt. (~ 100,000) showed the highest bioadhesive force compared to HPMC, HEC and PVP polymers with m.wt. of 86000, 80000 and 40000 respectively. The reinforcement of the mucoadhesive forces of rectal solutions A and B by the used polymers could be explained by the fact that secondary bond forming groups (e.g. hydroxyl, ether oxygen and amine) are the principle source of mucoadhesion. The cellulosic polymers have an abundance of hydroxyl and ether groups along their length while PVP polymer has amine groups (38).

3. Release of Mb HCl from the rectal solutions:

The time for 100% drug release (T_{100}) was selected for comparison between the PEG conventional suppository (5) and the prepared rectal solutions, as 100% drug release was achieved after 8 hours with some of the prepared sustained release formulations which is an advantage. Table 4 shows that the release of MbHCl from the prepared rectal solutions A and B, formulated either with or without mucoadhesives, was retarded when compared with the conventional PEG suppository. (T_{100}) was 30 minutes in case of the conventional formula, and the T_{100} determined for the rectal solutions A and B, in absence of mucoadhesive, was 120 and 180 minutes respectively. The higher T_{100} value of the rectal solution B might be attributed to its higher content of P407. These results were also found when the release from P407 gel was studied with benzoic acid and related compounds (39), diclofenac and hydrocortisone (40) and lidocaine (41). The workers suggested that increasing the concentration of P407 forms a gel structure with an increased yield strength or rigidity. This gel functions as resistant barrier to drug diffusion due to reductions in the numbers and dimensions of the aqueous channels through which solutes diffuse.

In addition, it was obvious from Fig. 2 that the release of MbHCl was not only affected by the grade of poloxamer but also by the type of mucoadhesive used. So, all the cellulosic polymers retarded the drug release from rectal solutions A and B while PVP had no effect in comparison with those devoid from mucoadhesive polymers. The null effect of PVP on MbHCl release may be attributed partly to the low viscosity of the K-25 grade used in this study, and partly due to its water soluble nature which allowed more rapid penetration of dissolution fluid into the product initiating product surface dissolution/erosion (42). These findings coincide with those obtained by Bhardwaj and Blanchard (43) studying the release of Melanotan-I from P407 formulations containing PVP, MC or HPMC and with those obtained by Ryu *et al.* (14) who reported that PVP did not retard the release of propranolol HCl from its thermally gelling liquid suppository. Accordingly, the release data of the formula containing PVP was not subjected to release kinetic studies and further studies. The retarding effect of the cellulosic mucoadhesive polymers could be attributed to their ability to increase the overall product viscosity (44) as well as their ability to distort or squeeze the extra-micellar aqueous channels of poloxamer micelles through which the drug diffuses thereby delaying the release process (22). These mucoadhesives can be arranged according to their release-retarding effect as follows: MC > HPMC > HEC. This order of arrangement correlated well with the viscosities and the gelation temperature-lowering effect of these polymers as previously mentioned.

According to the release data shown in Table 4, it was possible to modulate the release of MbHCl by adjusting the concentration of the polymer to obtain a sustained drug release profile for 8 hours as it has been studied by other workers on other drugs (13,14,22). This was achieved by using mucoadhesive polymer concentration strarting from 2% of each of HPMC or HEC and 1.5% of MC for both formulea A and B. It is to be noted that an almost 100% drug release was obtained after 8hrs. in case of 1.5% MC, with either rectal solution A or B where the respective percent drug release measured were 98.9 and 96.85%.

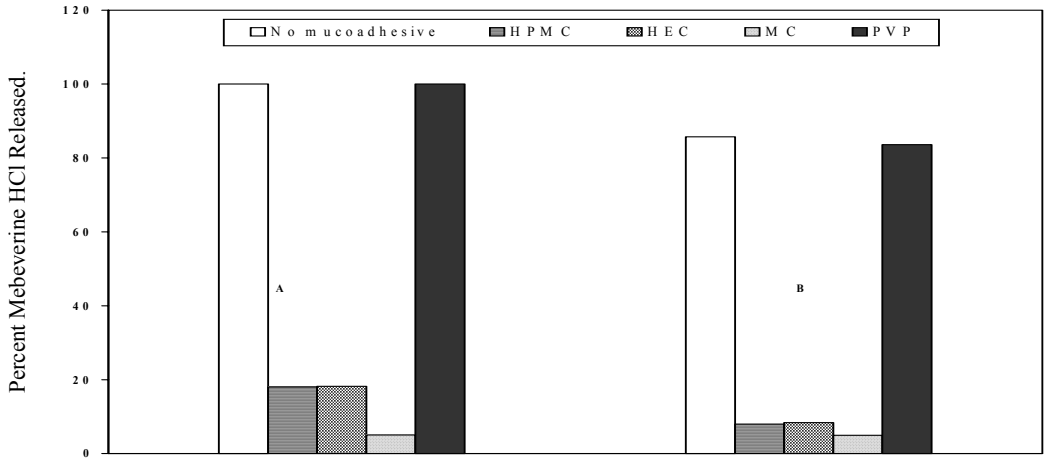

Fig. 2. Effect of 2.5 %, w/w of Different Mucoadhesive Polymers on the Release of Mebeverine Hydrochloride from Different Rectal Solutions after 120 Minutes in Sorensen's Phosphate Buffer pH 7.4 .
A:Rectal solution composed of [23/7/10 %,w/w P407/P188/MbHCl].
B:Rectal solution composed of [25/5/10 %,w/w P407/P188/MbHCl].

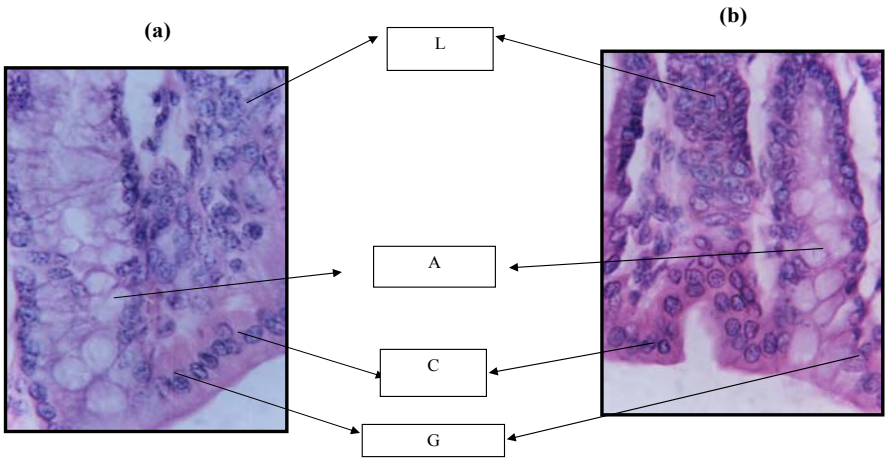

Fig. 3. Photomicrographs of Rectal Mucous Membrane of Guinea pig (a) Control and (b) 8 Hours after Administration of Mucoadhesive Mebeverine Hydrochloride Liquid Suppositories. Lymphocytes (L), Areolar layer(A), Columnar cells(C), Goblet cells (G).

By reviewing the release kinetic data in Table 4, it could be deduced that, at absence of and at low mucoadhesive polymers concentration, the formulations exhibited n values between 0.5-1 indicating an anomalous or non-Fickian release suggesting a coupled erosion-diffusion mechanism. On the other hand, Fickian diffusion (Higuchi model) was evident in case of rectal solution A containing 2.5% MC. In case of rectal solution B the Fickian diffusion was observed at a concentration of 2 or 2.5 % of either HPMC or MC and at 2.5% HEC.

4. Gel Strength:

It has been previously reported that the optimal in situ gelling rectal solution must have suitable gel strength, in the range of 10 to 50 seconds. This would allow ease of administration for the rectal preparation and no leakage from the anus (12). The two formulations composed of 23, 7, 1.5 and 10 % w/w and 25, 5, 1.5 and 10 % w/w of P407, P188, MC and MbHCl respectively which provided desirable sustained drug release profile, were subjected to gel strength measurements. The measured gel strength of the former rectal solution was 57.17 seconds while that of the latter one was 40.8 seconds.

5. Spasmolytic activity:

The rectal solution composed of 23, 7, 1.5 and 10 % w/w of P407, P188, MC and Mb HCl respectively, with optimal physical parameters including a gelation temperature (33°C), gel strength (40.8 sec.) and mucoadhesive force (29056 dyne/cm^2) and a satisfactory drug sustainment was selected to study its ex vivo spasmolytic effect and compared with a conventional PEG suppository.

From table (5), it is clear that the selected MbHCl rectal solution had a significant spasmolytic effect as revealed by a great percentage of inhibition of guinea pig ileum contractions induced by different spasmogens viz: histamine, acetylcholine and barium chloride. Data in Table 5 show that the maximum spasmolytic activity was observed 8 hrs. following the administration of the rectal solution after which it began to decline. This represents an extended action as compared to the conventional suppository that shows a rapid decline of spasmolytic activity after 2 hrs. only. Furthermore, this observation would confirm the role of mucoadhesives in the reinforcement of the drug sustained action.

6. Histopathological examination of the rectal tissues:

Fig. 3a shows the histopathological structure of the normal rectal mucosa of guinea pigs where a series of small longitudinal folds are found large and broad. The rectal mucous membrane is lined with simple columnar epithelial cells containing goblet cells. Concerning the rectal mucosa of the same animals, 8 hours after the administration of MbHCL rectally, shown in Fig. 3b it was obvious that no change had occurred in the structure of the mucous membrane of the rectal tissue. This was manifested by the appearance of the numerous nearly flat folds with the mucosa lined with simple columnar epithelium containing goblet cells. In addition it is to be noted that in both the control and treated animals, the outer part of the mucous membrane is very areolar so as to give a wide space for the muscular layer to contract. Furthermore, invading lymphocytes between columnar cells were present in adequate number in both cases. Similar observations were reported by other investigators (14,15,30).

Visual examination of the rectum of dissected guinea pig at the end of the experiment revealed that no migration had occurred for the inserted dose. This adhesion to the site of application (the lower part of the rectum) would avoid the first pass effect.

Conclusion

Mebeverine HCl formulated as mucoadhesive thermoreversible poloxamer solution for rectal administrsation could have potential as a safe and sustained release rectal delivery system to control the chronic irritable bowel syndrome. Mucoadhesion that allows stagnation of the gel in the lower region of the rectum may be an advantage to protect the drug from extensive first-pass effect.

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Table 3. : Effect of Mucoadhesive Polymers on the Gelation Temperature and Mucoadhesive Force of Mebeverine Hydrochloride Rectal Solution.

Mucoadhesive Polymer	Concentration (%,w/w)	Mean Gelation Temperature (^oC) ± S.D. of Mb HCl Liquid Suppositories		Mean Mucoadhesive Force (dyne/cm^2 x 10^3) ± S.D. of Mb HCl Liquid Suppositories	
		A*	B**	A	B
No Mucoadhesive Polymer		37 ± 0.0	35 ± 0.0	1.719 ± 0.298	1.547 ± 0.0
	0.5	37.3 ± 0.58	35 ± 0.0	8.941 ± 0.596	5.33 ± 0.298
	1	36.3 ± 0.58	34.3 ± 0.58	20.632 ± 1.032	26.579 ± 1.032
HPMC	**1.5**	36 ± 0.0	34 ± 0.0	28.885 ± 1.032	25.79 ± 0.0
	2	35 ± 0.0	33.3 ± 0.58	30.26 ± 0.597	29.916 ± 0.1
	2.5	30 ± 1	29 ± 0.0	30.948 ± 1.032	34.043 ± 0.0
	0.5	36.3 ± 0.58	35 ± 0.0	4.47 ± 0.596	2.923 ± 0.298
	1	36.3 ± 0.58	34.3 ± 0.58	11.348 ± 1.032	13.411 ± 1.033
HEC	**1.5**	36 ± 0.0	33 ± 1	18.569 ± 0.0	19.941 ± 0.596
	2	34 ± 1	32 ± 0.0	24.408 ± 0.596	23.727 ± 0.0
	2.5	32 ± 0.0	31.3 ± 0.58	25.79 ± 1.032	26.994 ± 0.298
	0.5	36 ± 1	35 ± 0.0	16.505 ± 0.0	15.818 ± 0.596
	1	34.3 ± 0.58	33 ± 1	24.408 ± 0.596	25.79 ± 1.032
MC	**1.5**	33 ± 1	32 ± 0.0	29.057 ± 0.298	30.26 ± 0.596
	2	32 ± 0.0	29.3 ± 0.58	42.296 ± 0.1	41.605 ± 0.596
	2.5	30 ± 1	28 ± 0.0	51.58 ± 1.032	47.626 ± 0.298
	0.5	37 ± 0.0	35 ± 0.0	4.47 ± 0.596	5.33 ± 0.298
	1	36 ± 1	35 ± 0.0	8.941 ± 0.596	7.737 ± 0.516
PVP	**1.5**	36 ± 0.0	33.3 ± 0.58	15.818 ± 0.596	14.752 ± 0.596
	2	34 ± 1	32 ± 0.0	20.941 ± 0.596	20.632 ± 1.032
	2.5	33 ± 0.0	31.3 ± 0.58	24.408 ± 0.596	27.165 ± 0.596

*:Liquid Suppository composed of [P407/P188/MbHCl (23/7/10 %,w/w)].
**:Liquid Suppository composed of [P407/P188/MbHCl (25/5/10 %,w/w)].

Table 4.: Release and Kinetic Data of Mebeverine Hydrochloride Liquid Suppositories

Suppository	Mucoadhesive Polymer	Concentration (%,w/w)	Time for 100% release (min.)	Percent drug release after 8 hrs	Release Exponent (n)	Kinetic Constant ($k,\%/min.^n$)	Correlation Coefficient (r)
Conventional *	**-**	**-**	30	100	****	****	****
A**	**No mucoadhesive polymer**		120	100	0.8429	1.883	0.9993
	HPMC	**0.5**	180	100	0.8256	1.492	0.9976
		1	240	100	0.8864	0.894	0.9954
		1.5	300	100	0.867	0.715	0.9935
		2	>480	74.93	0.7953	0.546	0.9929
		2.5	>480	57.45	0.7836	0.411	0.9964
	HEC	**0.5**	180	100	0.8209	1.5396	0.9939
		1	240	100	0.796	1.342	0.9986
		1.5	300	100	0.7895	1.087	0.9944
		2	>480	79.97	0.7296	0.913	0.9977
		2.5	>480	58.32	0.7528	0.509	0.9964
	MC	**0.5**	180	100	0.8698	1.257	0.9913
		1	300	100	0.8578	1.092	0.9856
		1.5	>480	98.9	0.7454	1.085	0.9938
		2	>480	73.82	0.6926	0.937	0.982
		2.5	>480	10.49	0.3904	0.843	0.9905
B***	**No mucoadhesive polymer**		180	100	0.8177	1.767	0.9935
	HPMC	**0.5**	240	100	0.8119	1.38	0.9931
		1	300	100	0.774	1.21	0.9931
		1.5	480	100	0.7372	1.094	0.994
		2	>480	33.29	0.5281	1.189	0.9951
		2.5	>480	15.08	0.4485	0.91	0.9937
	HEC	**0.5**	180	100	0.8263	1.478	0.9934
		1	240	100	0.8254	1.21	0.9974
		1.5	420	100	0.7912	0.906	0.9946
		2	>480	68.35	0.7111	0.779	0.9857
		2.5	>480	19.03	0.502	0.771	0.9968
	MC	**0.5**	240	100	0.8052	1.408	0.9957
		1	300	100	0.7865	1.227	0.9985
		1.5	>480	96.85	0.7316	0.961	0.9928
		2	>480	21.44	0.4746	0.901	0.9861
		2.5	>480	9.35	0.3721	0.871	0.9961

*: Conventional suppository: PEG 400:PEG 6000: water (1:3:1) containing 200 mg Mb HCl. This was the formula showing the fastest release of MbHCl from conventional suppositories studied in our laboratory (Ref. 5).
**: Liquid Suppositttory:P407/P188/MbHCl (23/7/10 %,w/w).
***: Liquid Suppository: P407/P188/MbHCl (25/5/10 %,w/w).
****: Not studied using equation (1)

Table 5. : Comparative *Ex Vivo* Spasmolytic Effect of Mebeverine Hydrochloride Conventional and Liquid Suppositories on Spasmogens-Induced Contractions on Guinea-pig Ileum.

Spasmogen	Conventional Suppository*		Liquid Suppository**				
	Mean Percentage Inhibition at Different Time Intervals (hrs.) ± S.E.						
	2	4	2	4	6	8	10
Histamine (250ng/ml)* **	98.3 ± 1.13	57.1 ± 1.359	77.53 ± 0.974	80.08 ± 1.31	89.02 ± 0.832	96.43 ± 0.942	72.42 ± 1.589
Acetylcholine (200ng/ml)* **	84.26 ± 2.98	58.2 ± 1.765	74.91 ± 1.145	83.01 ± 1.63	85.36 ± 0.774	90.01 ± 0.756	71.77 ± 1.328
Barium chloride (100ug/ml)* **	82.42 ± 2.2	59.7 ± 1.568	70.64 ± 1.34	78.59 ± 1.128	79.82 ± 0.821	82.57 ± 1.32	66.97 ± 1.563
Pooled Data	88.33 ± 8.69	58.33 ± 1.301	74.36 ± 3.48	80.56 ± 2.25	84.73 ± 4.63	87.2 ± 5.9	70.39 ± 2.98

*Conventional suppository: PEG 400:PEG 6000: water (1:3:1) containing 16 mg MbHCl
** Liquid suppository: P407/P188/MC/Mb HCl (23/7/1.5/10 %,w/w).
*** Submaximal doses

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