Original Article

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Preparation and evaluation of fenoterol hydrobromide suppositories

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ABSTRACT: Fenoterol HBr is a bronchodilator known to be subject to first pass effect after oral administration. The aim of this study was to prepare and evaluate fenoterol HBr suppositories. Suppositories were prepared by a fusion method using different fatty bases, *viz*. Witepsol H15, Witepsol E75, Suppocire AP, and Suppocire BM, as well as different hydrophilic bases, *viz.* polyethylene glycol and poloxamer bases. *In vitro* release studies revealed a greater release of the drug from hydrophilic bases than from fatty bases. The effect of incorporating different types and concentrations of non-ionic surfactants (Tween 60 and Span 20) on the release rate of the drug from Witepsol H15, as a model fatty base, was investigated. Results showed an enhanced release at low surfactant concentrations. A very fast 100% drug release was achieved when the drug was incorporated as an aqueous solution in Witepsol H15 (F17). This formula was selected to test the effect of fenoterol HBr suppositories on histamine-induced bronchospasms in Guinea pigs. No dyspnea of the animals was recorded for up to 30 min. In addition, thermogel liquid suppositories of different poloxamer 188 and poloxamer 407 proportions in the presence of sodium alginate as a mucoadhesive polymer were prepared. The different formulations behaved similarly concerning sustainment of drug release, however, only the formula containing 15% poloxamer 188 and 25% poloxamer 407 (F20) showed optimal gelation at body temperature. In conclusion, among the studied suppository bases there are bases suitable for fast release of the drug like F17 and hydrophilic bases especially polyethylene glycol, as well as other bases for sustained release applications of fenoterol HBr like fatty and thermogel bases.

Keywords: Suppositories, fenoterol, histamine induced bronchospasm, surfactants, drug in solution form, mucoadhesive liquid suppositories

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1. Introduction

Fenoterol is a direct acting sympathomimetic agent with β-adrenoreceptor stimulant activity. It is used to treat symptoms of asthma, chronic bronchitis, emphysema, and other lung diseases (*1*). Fenoterol is available in the market in the form of syrup, tablet, and inhaler. Fenoterol acts rapidly on inhalation, but is incompletely absorbed from the gastrointestinal tract and is also subject to extensive first-pass metabolism by sulfate conjugation (*2*). Literature review lacks any information concerning the availability of fenoterol HBr in the form of suppositories. The rectal route for drug administration was proven to be advantageous over other routes because of the reduced side effects such as gastrointestinal irritation and the avoidance of both disagreeable taste and first pass effect (*3*). Consequently, rectal administration of fenoterol HBr in suppository form may offer an advantage over its oral administration to increase its bioavailability. Moreover, it offers an advantage over inhalation for pediatrics, which probably finds difficulty in inhaling the drug. Studies have shown that the release properties of many suppositories depend considerably on the physicochemical properties of the drug, suppository base and formulation adjuvants (*4-7*) and a lot of formulation work is normally required to optimize the properties of suppository preparations. Thus, the objective of this investigation was to prepare fenoterol suppositories and to optimize its release characteristics from different suppository bases.

2. Materials and Methods

2.1. *Materials*

The following materials were used: fenoterol HBr (Boehringer Ingelheim, Basel, Switzerland), Polyethylene glycol 400, 1500, and 6000 (Union Carbide, New York, NY, USA), Witepsol H15 and Witepsol E75 (Dynamit Nobel, Leverkusen, Germany), Suppocire BM and Suppocire AP (Gattefossé, Gennevilliers, France), Tween 60 and Span 20 (Atlas Chemical Industries, Wilmington, DE, USA), poloxamer 188 and poloxamer 407 (BASF, Ludwigshafen, Germany), and sodium alginate (Hayashi Pure Chemicals, Tokyo, Japan). All other chemicals were

analytical grade.

2.2. *Preparation of fenoterol HBr suppositories*

Adult 2 g suppositories containing 10 mg of fenoterol HBr were prepared by a cream melting technique (*8*) using different fatty and hydrophilic bases. The suppository base was melted and then the drug was added. Homogeneous dispersions were formed in melted base and then molded in a metal mold (2 g capacity). The selected fatty bases were Witepsol H15, Witepsol E75, Suppocire BM, and Suppocire AP. Hydrophilic bases composed of polyethylene glycol (PEG) mixtures such as PEG 1500/PEG 4000 (3:1) (F1), PEG 1500/PEG 4000 (9:1) (F2), and PEG 1500/PEG 6000 (2:1) (F3) as well as hydrophilic bases consisting of mixtures of poloxamer 188 and propylene glycol in the ratios of 10:0 (F4), 9:1 (F5), and 8:2 (F6) were prepared.

Moreover, suppositories containing additionally 2, 5, and 10% (w/w) Tween 60 and Span 20 were prepared using Witepsol H15 as suppository base. Furthermore, the incorporation of the drug in Witepsol H15 in the form of an aqueous solution (0.2 mL/suppository) instead of the powdered drug form using 2% Span 20 for emulsification was investigated. All suppositories were kept in the refrigerator and were stored in a desiccator at room temperature for 24 h before use.

2.3. *Preparation of in situ gelling mucoadhesive liquid suppositories*

Liquid suppositories were prepared as previously described (*9*). In brief, fenoterol HBr and sodium alginate (0.6%) were solubilized in distilled water and the solution was cooled down to 4°C. Poloxamer 188 (P188) and poloxamer 407 (P407) in different proportions were then slowly added to the solution with continuous agitation. The liquid suppositories were left at 4°C until a clear solution was obtained.

2.4. *Evaluation of the prepared fenoterol HBr suppositories*

2.4.1. *Weight variation*

The weight variation test was determined according to the British Pharmacopoeia. Briefly, twenty suppositories were weighed individually and the average weights were determined. No suppositories should deviate from average weight by more than 5% except two, which may deviate by not more than 7.5%.

2.4.2. *Content uniformity*

Five suppositories were randomly selected from each base and assayed individually for drug content. The suppository was melted with gentle heating in a water bath in the presence of 25 mL of Sorensen's phosphate buffer solution, pH 7.4. The volume was adjusted to 250 mL with phosphate buffer. The flask was agitated on a shaking water bath (Gallenkamp, Loughborough, UK) at 37°C for 4 h. After centrifugation and filtration, the UV absorbance of the solution was measured spectrophotometrically (Shimadzu UV double beam spectrophtometer, Kyoto, Japan) at λ_{max} 276 nm against a blank solution prepared by treating plain suppositories in the same manner.

2.4.3. *Hardness*

The hardness test was carried out on plain, as well as medicated suppositories. Hardness was determined at room temperature (about 25°C) using a hardness tester (Erweka hardness tester, SBT, Heusenstamm, Germany).

2.4.4. *Measurement of gelation temperature of liquid suppositories*

The gelation temperature was measured according to the method reported (*10*). Briefly, 10 g of each gel were placed in a transparent glass vial with a magnetic stirring bar (15 mm × 6 mm). The preparation was heated starting from 20°C, and was increased 1°C/min with constant stirring at 100 rpm. The temperature at which the magnetic bar stopped moving was taken as the gelation temperature. The evaluation was repeated three times for each formulation.

2.4.5. *In vitro release of fenoterol HBr from solid suppository bases*

The USP rotating basket dissolution apparatus (Pharma Test, Hainburg, Germany) was used at 37°C and 50 rpm for the *in vitro* release study of fenoterol HBr from solid suppository bases. The release was done for 120 min in 300 mL Sorensen's phosphate buffer, pH 7.4. Samples, each of 3 mL, were withdrawn from the release medium at specified time intervals and replaced by fresh buffer. The samples were filtered through Millipore filter (pore size 0.22 μm; Millipore, Billerica, MA, USA) and analyzed spectrophotometrically at 276 nm against a blank of plain suppositories treated by the same method as tested fenoterol HBr suppositories. Each release experiment was performed in triplicate. The extent of drug release was assessed from the total amount of drug present in the dissolution medium at the end of the release experiment.

2.4.6. *In vitro release of fenoterol HBr from liquid suppository bases*

Drug release study of liquid suppositories was performed for 360 min in USP paddle dissolution apparatus (Pharma Test) at 37°C and 50 rpm. Five grams

of liquid suppository were placed into a semi-permeable membrane tube (m.w. cutoff: 6,000-8,000; Spectrum Medical, Fort Mill, SC, USA). Both sides of the tube were tied up with a thread to prevent leakage. The semi-permeable membrane tube was then immersed in 300 mL Sorensen's phosphate buffer, pH 7.4. Sampling and analysis were performed as described above.

2.4.7. *Kinetic analysis of the release data*

In order to describe the release model, the *in vitro* release data from solid suppositories were analyzed according to a zero-order kinetic model (Q *vs*. t), a diffusion controlled model (Q *vs.* square-root of t), and a first-order model (log (Qo – Q) *vs*. t), where Q is the amount of drug released at time t and Qo is the initial amount of the drug. The model that consistently produced the highest correlation among the suppository preparations was used for the assessment of drug release rates (*11*).

To analyze the release mechanism of the drug from liquid suppositories, the release data obtained were fit to the Power Law (*12*):

$$M_t/M_\infty = K \cdot t^n$$

where, M_t/M_∞ is the fraction of drug released at time t and k denotes the constant of the suppository system and n is the diffusion exponent related to the mechanism of the drug release. The n value of 1 corresponds to zero-order dissolution kinetics, $0.5 < n < 1$ means a non-Fickian dissolution model and $n = 0.5$ indicates Fickian diffusion (Higuchi model) (*10*).

2.4.8. *Effect of fenoterol HBr suppository on histamine-induced bronchospasm in Guinea pigs*

Bronchospasm was induced in Guinea pigs by exposing them to a histamine aerosol. Guinea pigs weighing about 350 g were divided into three groups of six animals each. Group I served as control, Group II received selected fenoterol HBr suppositories (5 mg/kg), and Group III received an oral solution of fenoterol HBr (5 mg/kg). Groups 2 and 3 received medication half an hour before exposure to the histamine aerosol. The animals were exposed to 1% histamine aerosol under constant pressure in an aerosol chamber (24 × 14 × 24 cm) made of perplex glass. The end point, preconvulsive dyspnea (PCD), was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsions (*13*). As soon as PCD commenced, the animals were removed from the chamber and exposed to fresh air. The investigations were performed after approval of the local ethical committee at Faculty of Pharmacy, Cairo University.

3. Results and Discussion

3.1. *Weight variation, content uniformity, and hardness*

The weight variation study for all the suppositories were found to be within the acceptable range of < 5% (Table 1), which indicated perfect calibration of mold. Also, the drug content of five suppositories, from each formulation, did not deviate by more than 10% from the labeled amounts (Table 1). The mechanical strength of all tested suppositories was in the range of 1.50 to 4.00 kg showing optimum hardness for handling and transportation (Table 1).

3.2. *In vitro release of fenoterol HBr from hydrophilic bases*

Table 1. Codes, composition and characterization of tested solid suppository formulations

Code	Suppository composition	Weight variation (g)	Drug content (%)	Hardness (kg)	Extent release at 120 min (%)	Kinetic mechanism of release data
F1	PEG I (PEG 1500/PEG 4000, 3:1)	2.23 ± 0.08	95.43 ± 4.56	3.8 ± 0.11	69.8 ± 2.3	Diffusion
F2	PEG II (PEG 1500/PEG 4000, 9:1)	2.21 ± 0.09	92.27 ± 3.89	3.3 ± 0.06	50.8 ± 1.2	Diffusion
F3	PEG III (PEG 1500/PEG 6000, 2:1)	2.26 ± 0.04	90.09 ± 2.09	4.2 ± 0.04	100.0 ± 0.5	Diffusion
F4	P188/propylene glycol (10:0)	2.23 ± 0.05	96.70 ± 3.87	2.1 ± 0.09	22.7 ± 2.1	Zero
F5	P188/propylene glycol (9:1)	2.24 ± 0.04	94.25 ± 4.65	1.8 ± 0.02	38.9 ± 1.7	Diffusion
F6	P188/propylene glycol (8:2)	2.18 ± 0.06	93.87 ± 2.82	1.5 ± 0.06	57.1 ± 1.3	Diffusion
F7	Witepsol H15 (W_{15})	2.01 ± 0.03	97.58 ± 4.73	2.4 ± 0.04	24.0 ± 3.1	Diffusion
F8	Witepsol E75	2.05 ± 0.00	92.68 ± 1.33	2.6 ± 0.06	13.0 ± 1.9	Zero
F9	Suppocire AP	2.03 ± 0.05	94.37 ± 1.58	2.3 ± 0.04	3.2 ± 0.1	Diffusion
F10	Suppocire BM	1.98 ± 0.06	93.02 ± 2.99	2.6 ± 0.05	17.2 ± 2.5	Diffusion
F11	W_{15} + Tween 60 (2%)	2.06 ± 0.02	92.98 ± 4.45	2.4 ± 0.08	28.4 ± 1.1	Diffusion
F12	W_{15} + Tween 60 (5%)	2.09 ± 0.03	92.49 ± 1.56	2.1 ± 0.07	44.6 ± 1.6	Diffusion
F13	W_{15} + Tween 60 (10%)	2.07 ± 0.05	92.67 ± 3.84	1.8 ± 0.05	34.9 ± 0.7	Diffusion
F14	W_{15} + Span 20 (2%)	2.03 ± 0.06	92.67 ± 3.61	2.4 ± 0.05	73.9 ± 2.4	Diffusion
F15	W_{15} + Span 20 (5%)	2.07 ± 0.05	91.54 ± 2.75	2.1 ± 0.01	61.3 ± 1.6	Zero
F16	W_{15} + Span 20 (10%)	2.09 ± 0.06	90.43 ± 2.90	2.0 ± 0.03	29.1 ± 1.3	Zero
F17	W_{15} + Span 20 (2%) + drug solution	2.06 ± 0.01	93.67 ± 4.56	2.3 ± 0.01	100 in 15 min	--

The results are the mean of three determinations ($n = 3$).
Abbreviations: PEG, polyethylene glycol; P188, poloxamer 188; W_{15}, Witepsol H15; P407, poloxamer 407.

The dissolution profiles of fenoterol HBr from suppositories manufactured using different compositions of PEG, *viz.* F1, F2, and F1 are shown in Figure 1A. The release of fenoterol HBr from the suppositories was relatively high (ranging from 50.8 to 100%). The literature abounds with reports on improvement of dissolution of drugs from a PEG suppository base. This is due to the water absorbing properties of PEG (*7,14,15*), which result in the formation of a hydrophilic matrix with subsequent solubility enhancing effects. This result was in agreement with the kinetic analysis of the results, which revealed a diffusion mechanism for all tested PEG formulations (Table 1). It is known that, as the molecular weights of PEG increase, their water solubility and hygroscopicity decrease (*16*). It is also reported that PEG of different molecular weights can be combined to achieve a suppository base with a specific drug release rate profile (*17*). Ranking the tested combinations in descending order, according to % extent of drug release, was as follows: F3 > F1 > F2 (Table 1). Drug partitioning is a function of the nature of base and the affinity of the drug towards the base, *i.e.* when there is a low affinity between the drug and the base, the release rate of the substances having high solubility in aqueous media was expected to be high. Knowing that fenoterol HBr is freely soluble in water, it is to be noticed that the base with the lowest hydrophilicity, F3, is accompanied by the highest drug release profile (100% extent release). F2 has a high ratio of PEG 1500, which is most likely to have more hydrophilic character, and this was reflected in the slowest drug release (50.8% extent release). It is apparent that this is attributed to the privileged partition of the water soluble fenoterol HBr towards the base. In-between came F1 (69.8% extent release), which has an intermediate hydrophilic character.

Figure 1B shows the release profiles of fenoterol HBr from poloxamer/propylene glycol bases. It is to be noticed that, PEG-based suppositories had significantly higher dissolution rates of fenoterol HBr than any poloxamer-based suppositories (Figures 1A and 1B). These results suggested that PEG was soluble in the dissolution medium, while poloxamer was not soluble but rather gelled (*18*). The formed gel neither absorbed water nor was it hydrophilic. This may be due to the hindrance of the hydrophobic polyoxypropylene segment of the molecule by the hydrophilic polyoxyethylene moiety of the molecule (*19*). The mechanism of drug release was therefore found to be zero-order kinetics (Table 1).

The rank of poloxamer/propylene glycol bases in descending order, according to % extent of drug release, was as follows: F6 > F5 > F4 (Table 1). It is obvious that increasing the amount of propylene glycol was accompanied by increased dissolution rates of fenoterol HBr. This finding is probably due to the hygroscopic properties of propylene glycol (*20*), which caused increased water absorption and the formation of a hydrophilic polymer matrix. This effect changed the mechanism of release of fenoterol HBr from zero order in the case of poloxamer alone (F4) into diffusion through the hydrophilic matrix of poloxamer/propylene glycol bases (F5 and F6).

3.3. *Release of fenoterol HBr from fatty bases*

Release of fenoterol HBr from four different types of semi synthetic fatty bases was studied (Figure 2). Ranking them in descending order according to percent extent drug release was as follows: Witepsol H15 > Suppocire BM > Witepsol E75 > Suppocire AP (Table 1).

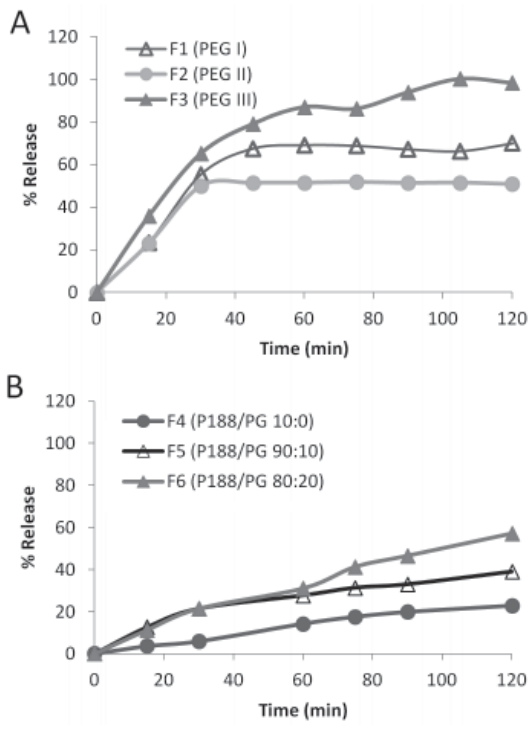

Figure 1. Release of fenoterol HBr from various formulations tested. (A) Hydrophilic PEG bases. **(B)** Hydrophilic poloxamer (P188) bases with different propylene glycol (PG) proportions. The results are the mean of three determinations ($n = 3$).

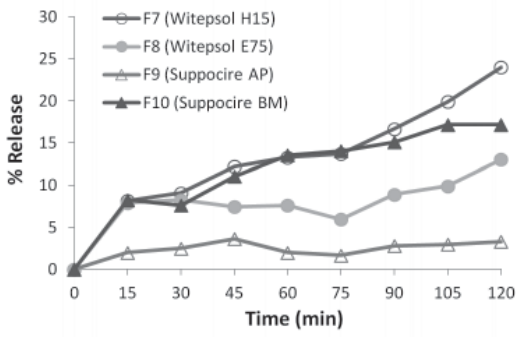

Figure 2. Release of fenoterol HBr from fatty bases. The results are the mean of three determinations ($n = 3$).

Semi-synthetic suppository bases are mixtures of fatty acids and esters with certain amounts of glycerides. The hydroxyl values reported for specific bases represent the proportions of free mono- and di-glycerides, *i.e.* free hydroxyl functional groups that are available for interaction. A high hydroxyl value is an indication of the potential for a base to adsorb water. The presence of a high hydroxyl value in fatty bases could thus favor the formation of a water-in-oil emulsion, which will generally result in a very slow transfer of drug molecules from the inner aqueous phase, *i.e.* retarded drug release (*3*). In this respect, drug release from formulations manufactured with Suppocire AP, which has a high hydroxyl value of 30-50, was slower than that from formulations manufactured using Suppocire BM (hydroxyl value < 6) or from either type of Witepsol (hydroxyl value 5-15) (*21*).

The extent of drug release from formulations manufactured with Witepsol H15 was higher than that from formulations manufactured using Witepsol E75 despite having similar hydroxyl values. This finding is related to the melting characteristic of the bases. Witepsol H15 has a melting point value of 33.5-35.5°C while Witepsol E75 has a melting point of 37-39°C (*20*). A complete melting of a suppository in the dissolution medium is certainly required for the drug to have the potential to be completely released. These results explain the kinetic analysis of the release data, which revealed a diffusion model for Witepsol H15 and a zero-order mechanism for Witepsol E75.

From the results it is evident that, PEG and poloxamer formulations released fenoterol HBr to a greater extent than those from fatty base suppositories. This is in accordance with previous reports (*22*,*23*), which stated that lipophilic bases for conventional suppositories led to much slower release than the hydrophilic bases. Nevertheless, the research conducted focused on improving the rate and extent of release of fenoterol HBr from fatty bases, since PEG bases have been reported to cause some irritation to mucosal tissues (*24*,*25*).

3.4. *Effect of surfactants on fenoterol HBr release*

In this study, the possibility of increasing the release of fenoterol HBr from fatty base suppositories was evaluated by the incorporation of non-ionic surfactants namely, Tween 60 as an example of a hydrophilic surfactant (hydrophilic-lipophilic balance (HLB) = 14.9) and Span 20 as an example of a lipophilic surfactant (HLB = 8.6) into a Witepsol H15 suppository base. Witepsol H15 was selected for further studies since it has an intermediate hydroxyl value, an optimal melting range and the greatest release profile among the tested fatty bases. The incorporation of non-ionic surfactants affected the rate of medicament release depending on nature and concentration of surfactant (*26*).

3.4.1. *Effect of Tween 60*

The release data for suppositories containing 2, 5, and 10% (w/w) Tween 60 are presented in Figure 3A. Percent drug release increased significantly upon incorporating up to 5% Tween 60. It is probably due to the fact that Tween 60 lowered the interfacial tension, and hence increased dispersibility of the suppository base with the dissolution fluid (*27*). However, further addition of Tween 60 up to a concentration of 10% increased the release rate of the drug to a smaller extent than that achieved with 5%. At higher concentrations, the surfactant might have exceeded its critical micellar concentration (CMC), and thus retarded drug release, as a result of micellar entrapment of the drug (*3*). The addition of Tween 60 to Witepsol H15 did not change the mechanism of drug release, which was found to be a diffusion model.

3.4.2. *Effect of Span 20*

The release data for suppositories containing 2, 5, and 10% (w/w) Span 20 are presented in Figure 3B. Two percent of Span 20 increased drug release significantly, which may be attributed to enhanced

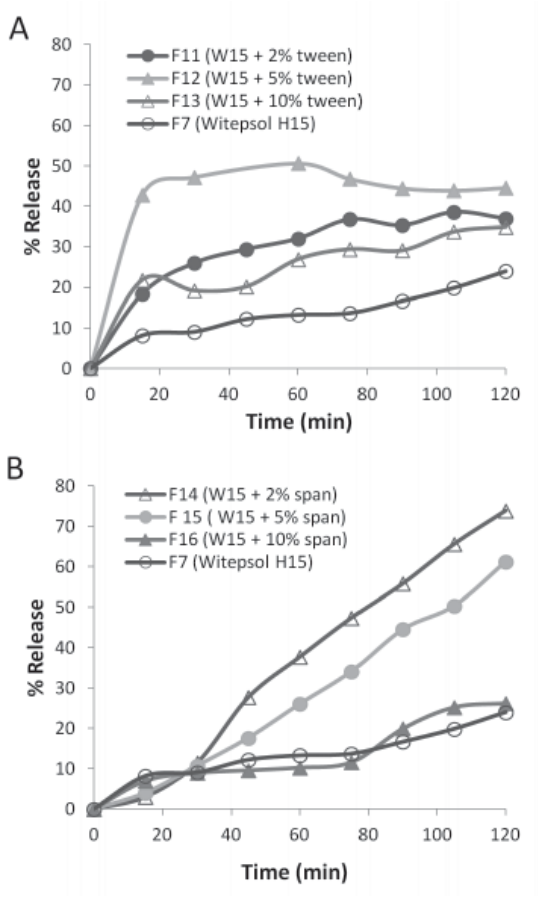

Figure 3. Release of fenoterol HBr from Witepsol H15 containing Tween 60 (A) or Span 20 (B). The results are the mean of three determinations (n = 3).

wetting of the matrix (*27*) and subsequently facilitated dissolution of the drug in the suppository and in the dissolution medium. Further addition of Span 20 up to a concentration of 10% led to a lesser improvement in rate and extent of fenoterol HBr release. Span 20 at higher concentrations may lead to a decrease in drug release due to formation of a water-in-oil emulsion (*28*). Other studies have reported that incorporation of surfactants may increase or decrease drug release from suppositories (*23*,*29*). It is worthy to note that for each surfactant there is an optimum concentration at which the drug exhibits maximum release rate.

The release profiles are consistent with the kinetic analysis of the data. The release rate followed diffusion only in the case of 2% Span 20 but changed to zero-order at higher concentrations of Span 20 which may be due to the formation of the water-in-oil emulsion.

From the previous results it is concluded that, low concentrations of surfactant are more effective in enhancing drug release than higher concentrations. This finding makes surfactant incorporation in suppository bases applicable as it has been demonstrated by Nakanishi *et al.* (*30*) that higher concentrations of nonionic surfactants are associated with adverse histological changes in the rectal tissue of rats. The enhancement of release rate produced by Span 20 was greater than that produced by Tween 60 (Table 1). This may be attributed to the much higher hydroxyl value of Span 20 (330-358) relative to Tween 60 (81-96) (*31*), which may increase the ability of the surfactant in wetting the matrices and producing a greater number of channels for the dissolution fluid to leach out the drug (*32*). However, this high hydroxyl value is probably the reason why at higher concentrations of Span 20 a water-in-oil emulsion is formed and drug release rate is decreased.

3.5. *Effect of incorporating fenoterol-HBr in a solution form in the suppository base on its release*

All previous trials to enhance drug release from Witepsol H15 succeeded only to a limited extent, because the highly hydrophilic drug fenoterol HBr is present in the base in a suspended form rather than a dissolved one. In this case, drug release is the result of a series of successive steps that involve melting of the base, migration of drug particles to the interface between the melted excipients and the dissolution medium, and finally the passage of the particles through this interface to be released in a molecular form (*3*).

The drug was thus incorporated in the base in the form of an aqueous solution instead of the powder form using Span 20 for emulsification. A 100% release was achieved in the first 15 min, because once the base was melted, the drug which was already dissolved was released all at once into the medium.

3.6. *Evaluation of liquid suppository*

Conventional suppositories are solid at room temperature and melt or soften at body temperature. Due to the characteristics of the solid, patients feel discomfort and, as a result, prefer oral administration of drugs, rather than *via* conventional suppositories.

In an attempt to solve this problem, suppositories that are liquid at room temperature and gel at body temperature have been developed and have been proposed as alternatives to conventional solid suppositories for the administration of drugs. Liquid suppositories are usually prepared from poloxamers since an aqueous solution of poloxamers, at high concentration, exhibits reversible thermal gelation (*33-36*). Hence, liquid suppositories are converted to solids in the rectum by thermal gelation following rectal administration. Since they can be administered as liquids, they would be expected to be more acceptable to patients and to cause less irritation to rectal mucosa compared to conventional suppositories. The problem associated with liquid suppositories is the migration of drugs that may undergo first-pass metabolism, up to the colon (*17*,*36*). This problem could be solved by the incorporation of a mucoadhesive polymer to the preparation. In the present study, sodium alginate was chosen as a mucoadhesive polymer because it causes no irritation to rectal mucosa and it exhibits a large mucoadhesive force (*37*).

3.6.1. *Gelation temperature*

The gelation temperature of liquid suppositories was dependent on the concentration of poloxamers (P188 and P407). F18 (15% (wt) each of P188 and P407) exhibited a gelation temperature of 39 ± 1°C (Table 2). Increasing the percentage of P407 to 20% (F19) and 25% (F20) was accompanied with a reduction in the gelation temperature to 37 ± 0.5°C and 36 ± 0.3°C, respectively (Table 2). F20 revealed therefore an optimal gelation temperature for the *in situ* gelling of the liquid suppository.

Table 2. Codes, composition and characterization of tested liquid suppositories

Code	Suppository composition	Extent release at 120 min (%)	Diffusion exponent (n)	Kinetic mechanism of release data	Gelation temperature (°C)
F18	Thermogel: 15% P188, 15% P407, 0.6% sodium alginate	22.9 ± 2.5	0.704	Non-Fickian	39 ± 1
F19	Thermogel: 15% P188, 20% P407, 0.6% sodium alginate	19.7 ± 1.9	0.748	Non-Fickian	37 ± 0.5
F20	Thermogel: 15% P188, 25% P407, 0.6% sodium alginate	21.6 ± 1.5	0.745	Non-Fickian	36 ± 0.3

The results are the mean of three determinations (n = 3).

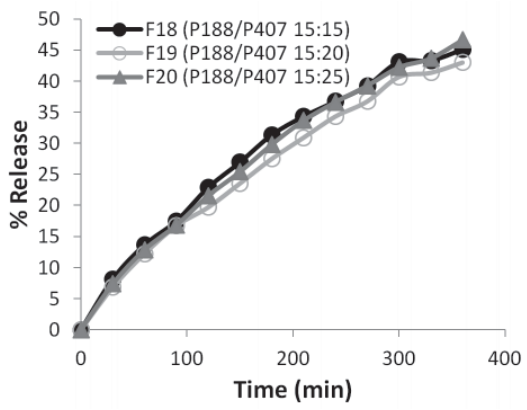

Figure 4. Release of fenoterol HBr from liquid suppositories composed of different proportions of P188 and P407. The results are the mean of three determinations ($n = 3$).

Table 3. Onset of dyspnea recorded for three groups of Guinea pigs with histamine-induced bronchospasm

Groups	Medication	Pre-convulsion dyspnea (min)
Gp I	Control	1.5 ± 1.2
Gp II	Oral solution of fenoterol HBr (5 mg/kg)	12.0 ± 2.3
Gp III	Fenoterol HBr suppository (F17) (5 mg/kg)	no reaction (animals were removed after 30 min from the chamber)

Values are mean ± S.D., $n = 6$ in each group. $p < 0.05$ as compared to control (unpaired Student's *t*-test).

3.6.2. *Release of fenoterol HBr from liquid suppositories*

The release profile of the drug from the tested formulations (F18, F19, and F20) was very similar, *i.e.* the change in proportions of P188 to P407 did not significantly influence drug release (Figure 4, Table 2). Drug release was generally retarded probably due to the mucoadhesive polymer. It was reported that, among many tested mucoadhesive polymers, sodium alginate exhibited the highest release retardation (*37*). Sodium alginate seems to affect the release rates by influencing the physicochemical properties of gel matrix. The polymer may have distorted or squeezed the diffusion channels, thereby delaying the release process. F20 with optimal gelation temperature thus presents a promising sustained release formulation for fenoterol HBr.

Kinetic analysis of release data revealed n values between 0.5 and 1.0 (Table 2), the mechanism of drug release is defined as anomalous (non-Fickian), where the release is controlled by a combination of diffusion and polymer relaxation (*38*).

3.7. *Histamine-induced bronchospasm in Guinea pigs*

All groups of Guinea pigs were subjected to histamine aerosols in order to induce bronchospasm, which was demonstrated by dyspnea followed by convulsions. The onset of dyspnea was recorded for all animals and the results are shown in Table 3.

The oral solution of fenoterol HBr succeeded in delaying the onset of dyspnea about 8 times (12 ± 2.3 min). For the study the suppository formula, in which the drug was incorporated in Witepsol H15 as aqueous solution (F17), was selected because of its very fast drug release. For the group of animals, that received the suppositories no dyspnea was recorded up to 30 min (after 30 min the animals were removed from the chamber to prevent their suffocation due to depletion of oxygen). This result implies that suppositories possess greater bioavailability than oral solutions probably due to avoidance of first pass effect.

4. Conclusion

In conclusion, hydrophilic bases were superior to lipophilic bases in terms of their ability to release fenoterol HBr from the suppository formulations. Incorporation of non-ionic surfactants to Witepsol H15 at lower concentrations improved drug release. A very fast release of the drug was achieved by incorporating the drug as an aqueous solution in Witepsol H15. This formula succeeded in preventing histamine-induced bronchospasm in Guinea pigs for up to 30 min. Liquid suppositories composed of various proportions of P188 and P407 in addition to the mucoadhesive polymer, sodium alginate, have shown remarkable retarded release of the drug. The formula that contained 15% P188 and 25% P407 exhibited optimal gelation temperature for the *in-situ* gelling of the liquid suppository. This formula could be regarded as a promising sustained release formulation suitable for further investigation.

References

1. Heel RC, Brogden RN, Speight TM, Avery GS. Fenoterol: A review of its pharmacological properties and therapeutic efficacy in asthma. Drugs. 1978; 15:3-32.
2. Sweetman SC. Martindale: The Complete Drug Reference. Pharmaceutical Press, London, UK, 2009; p. 1121.
3. Tukker J. Rectal and vaginal drug delivery. In: Pharmaceutics, The Science of Dosage Form Design (Aulton ME, ed.). Churchill Livingstone, Edinburgh, UK, 2009; pp. 534-543.
4. Zuber M, Pellion B, Arnaud P, Chaumeil JC. Kinetics of theophyline release from suppositories *in vitro*: Influence of physicochemical parameters. Int J Pharm. 1988; 47:31-36.
5. Onyeji CO, Adebayo AS, Babalola CP. Effects of absorption enchancers in chloroquine suppository formulations: I. *In vitro* release characteristics. Eur J Pharm Sci. 1999; 9:131-136.
6. Realdon N, Ragazzi E, Ragazzi E. Effects of drug solubility on *in vitro* availability rate from suppositories with lipophilic excipients. Pharmazie. 2000; 55:372-377.
7. Realdon N, Ragazzi E, Ragazzi E. Effect of drug solubility on *in vitro* availability rate from suppositories

with polyethylene glycol excipients. Pharmazie. 2001; 56:163-167.
8. Jones DS. Pharmaceutics – Dosage Form and Design. Pharmaceutical Press, London, UK, 2008; pp. 157-179.
9. Choi HG, Oh YK, Kim CK. *In situ* gelling and mucoadhesive liquid suppository containing acetaminophen: Enhanced bioavailability. Int J Pharm. 1998; 165:23-32.
10. Yun MO, Choi HG, Jung JH, Kim CK. Development of a thermo-reversible insulin liquid suppository with bioavailability enhancement. Int J Pharm. 1999; 189:137-145.
11. Dangprasirt P, Pongwai S. Development of diclofinac sodium controlled release solid dispersion powders and capsules by freeze drying technique using ethylcellulose and chitosan as carriers. Drug Dev Ind Pharm. 1998; 24:947-953.
12. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. Pharm Acta Helv. 1985; 60:110-111.
13. Armitage AK, Boswood J, Large BJ. Thioxanthines with potent bronchodilator and coronary dilator properties. Br J Pharmacol Chemother. 1961; 16:59-76.
14. Khoo SM, Porter CJ, Charman WN. The formulation of halofantrine as either non-solubilizing PEG 6000 or solubilizing lipidbased solid dispersions: Physical stability and absolute bioavailability assessment. Int J Pharm. 2000; 205:65-78.
15. Emara LH, Badr RM, Elbary AA. Improving the dissolution and bioavailability of nifedipine using solid dispersions and solubilizers. Drug Dev Ind Pharm. 2002; 28:795-807.
16. Rowe RC, Sheskey PJ, Weller PJ. Handbook of Pharmaceutical Excipients. 6th Edition, Pharmaceutical Press, London, UK, 2009; pp. 518-519.
17. Coben LJ, Lieberman HA. Suppositories. In: The Theory and Practices of Industrial Pharmacy (Lachman L, Lieberman HA, Kanig JL, eds.). Lea and Febiger, Philadelphia, PA, USA, 1986; pp. 564-588.
18. Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh YK, Kim CK. Development of *in situ*-gelling and mucoadhesive acetaminophen liquid suppository. Int J Pharm. 1998; 165:33-44.
19. Pandit NK, Wang D. Salt effects on the diffusion and release rate of propranolol from poloxamer 407 gels. Int J Pharm. 1998; 167:183-189.
20. Rowe RC, Sheskey PJ, Weller PJ. Handbook of Pharmaceutical Excipients. 6th Edition, Pharmaceutical Press, London, UK, 2009; p. 592.
21. Rowe RC, Sheskey PJ, Weller PJ. Handbook of Pharmaceutical Excipients. 6th Edition, Pharmaceutical Press, London, UK, 2009; pp. 722-726.
22. Ermic D, Tarimci N. Ketoprofen sustained-release suppositories containing hydroxypropylmethylcellulose phthalate in polyethylene glycol bases. Int J Pharm. 1995; 113:65-71.
23. Oladimeji FA, Omoruyi SI, Onyeji CO. Preparation and *in vitro* evaluation of suppositories of halofantrine hydrochloride. Afr J Biotechnol. 2006; 5:1775-1780.
24. Young C, Palin KJ, Reid AS, Thomas NW, Gould PL. Formulation of fenbufen suppositories. II. Selection of a suppository base using dissolution studies and histological studies in rats. Int J Pharm. 1987; 40:187-191.
25. Reid AS, Thomas NW, Palin KJ, Gould PL. Formulation of fenbufen suppositories. I. Quantitative histological assessment of the rectal mucosa of rats following treatment with suppository bases. Int J Pharm. 1987; 40:181-185.
26. Realdon N, Dal zotto M, Morpurgo M, Franceschinis E. Effects of surfactant characteristics on drug availability from suppositories. Pharmazie. 2008; 63:459-463.
27. Hanaee J, Javadzadeh Y, Taftachi S, Farid D, Nokhodchi A. The role of various surfactants on the release of salbutamol from suppositories. Farmaco. 2004; 59:903-906.
28. Abd el-Gawad AH, el-Din EZ, Abd el-Alim HA. Effect of surfactant incorporation techniques on sulphamethoxazole suppository formulations. Pharmazie. 1988; 43:624-627.
29. Lee DK, Wang DP. Formulation development of allopurinol suppositories and injectables. Drug Dev Ind Pharm. 1999; 25:1205-1208.
30. Nakanishi K, Masada M, Nadai T. Effect of pharmaceutical adjuvants on the rectal permeability of drugs. II. Effect of Tween-type of surfactants on the permeability of drugs in the rat rectum. Chem Pharm Bull. 1983; 31:3255-3263.
31. Rowe RC, Sheskey PJ, Weller PJ. Handbook of Pharmaceutical Excipients. 6th Edition, Pharmaceutical Press, London, UK, 2009; pp. 551-678.
32. Bolourtchian N, Javid FS, Dadashzadeh S. The effect of various surfactants on release behavior of procainamide HCl from ethylcellulose based matrices. Iran J Pharm Res. 2005; 1:13-19.
33. Hadgraft J, Howard JR. Drug release from pluronic gels. J Pharm Pharmacol. 1982; 34:3P.
34. Miller SC, Donovan MD. Effect of poloxamer 407 gel on the miotic activity of pilocarpine nitrate in rabbits. Int J Pharm. 1982; 12:147-152.
35. Tomida H, Shinohara M, Kuwada N, Kiryu S. *In vitro* release characteristics of diclofenac and hydrocortisone from pluronic F-127 gels. Acta Pharm Suec. 1987; 24:263-272.
36. Huang CH, Tokumura T, Machida Y, Nagai T. Formulation of double-layered suppository for prolonged stay in lower rectum. Yakuzaigaku. 1987; 47:42-48.
37. Ryu JM, Chung SJ, Lee MH, Kim CK, Shim CK. Increased bioavailability of propranolol in rats by retaining thermally gelling liquid suppositories in the rectum. J Control Release. 1999; 59:163-172.
38. Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliv Rev. 2001; 48:139-157.

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