

ANNEX

I.

Department of Pharmaceutical Technology, Albert Szent-Györgyi Medical University, Szeged, Hungary

Influence of pH change on drug release from rectal suppositories

S. BERKÓ, G. REGDON JUN. and I. ERŐS

Drugs absorbed from the anal region enter blood circulation bypassing the liver. Thus, their bioavailability can be increased by avoiding the "first-pass-effect" [1, 2]. Therefore the need for a formulation of diuretic rectal suppositories has been expressed [3].

The aim of our research was, on the one hand, to formulate rectal suppositories containing ethacrynic acid in order to improve diuretic therapy. On the other hand, we wanted to study the effect of pH changes of the acceptor phase as the proper pH is essential for simulating physiological conditions. Examinations were performed both in distilled water and in phosphate buffer (pH 7.5) with five suppository bases: Massa Estarinum BC, Suppocire AML, Witepsol S 58, Suppocire AP as lipophilic bases and Macrogolum 1540 as a hydrophilic base. Drug release was determined. Release values obtained with the hydrophilic Macrogolum 1540 base in aqueous medium were manifold higher than those determined with lipophilic bases or powder. This is due to the fact that poorly water-soluble drugs are better released from hydrophilic suppositories. It, however, the results obtained in aqueous medium and buffer medium are compared, it can be seen that drug release from the lipophilic bases was increased about ten-fold in the acceptor phase of pH = 7.5. The lipophilic base Suppocire AML proved to be the best in both acceptor phases. On the other hand, the change of the acceptor phase did not have a significant influence on drug release from the hydrophilic base Macrogolum 1540 (Fig.).

It is obvious that the kinetics of release from lipophilic and hydrophilic bases differ, as drug diffusion from the hydrophilic base showed a considerable increase only after the first hour. This finding is related to the longer disintegration time of hydrophilic bases, which is in fact not disintegration because hydrophilic bases have to be dissolved in the rectal fluid.

It can be concluded that Suppocire AML was the best lipophilic base both in aqueous and buffer phases, and the hydrophilic Macrogolum 1540 was also found to be suitable for the formulation of ethacrynic acid suppositories.

Experimental

1. Materials

Ethacrynic acid was from EGIS (Hungary). Suppocire AML, AP were from Gattefossé (France); Witepsol S58, Massa Estarinum BC were from Condea Chemie GmbH (previously Hüls AG.) (Germany); Macrogolum 1540 is official in the Hungarian Pharmacopoeia Ed. VII.

2. Formulation method

Suppositories were formulated by moulding. Their drug content was 2.5 w/w%, which corresponded to the therapeutic dose: a 2 g adult suppository contains about 50 mg ethacrynic acid. The poorly water-soluble ethacrynic acid was incorporated in various suppository excipients.

3. In vitro release study

Experiments were performed using the method of dynamic membrane diffusion which is a useful method for following the rate of drug release and membrane diffusion from the powder without excipient and from the different suppository compositions as well. The acceptor phases were distilled water and phosphate buffer at a pH 7.5. The suppositories were individually packed in a kidney dialysing membrane (Visking[®]) and placed into distilled water or buffer of body temperature (37 ± 0.5 °C). The samples were exposed to slight shaking and the acceptor phase was replaced after 30, 60, 120, 240 min. The quantity of ethacrynic acid in these samples was measured with a spectrophotometer at $\lambda = 278$ nm. The mean values were calculated from 5 parallel measurements each time.

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Prof. Dr. I. Erős D.Sc.
Department of Pharmaceutical Technology
Eötvös u. 2.
6720 Szeged
Hungary
erős@pharma.szote.u-szeged.hu

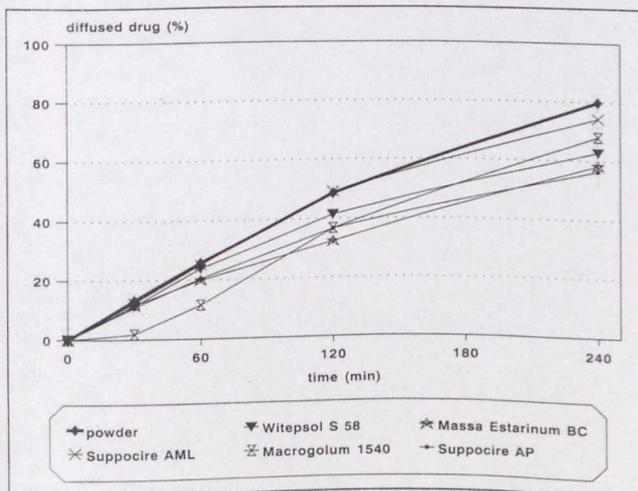


Fig.: Ethacrynic acid release from suppositories (acceptor phase: pH = 7.5 phosphate buffer)

II.

RESEARCH PAPER

Solutol and Cremophor Products as New Additives in Suppository Formulation

Szilvia Berkó,* Géza Regdon Jr., and István Erös

Department of Pharmaceutical Technology, Faculty of Pharmacy,
University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary

ABSTRACT

Our research has a double purpose. On the one hand, doctors have expressed the need to formulate a rectal suppository dosage form from diuretic ethacrynic acid, which would add to the choice of treatment methods and thereby increase the possibilities of individual cure. On the other hand, the liberation and thereby the bioavailability of poorly-soluble ethacrynic acid needs to be enhanced, and for this purpose solubility-increasing additives new to rectal therapy were used. Solutol HS 15, Cremophor RH 40, and Cremophor RH 60 were used as additives in concentrations of 1, 3, 5, and 10%. The quantity of drug released changed as a function of additive concentration. Depending on the acceptor phase, the best results were achieved with an additive concentration of 1–3%, which is related to the optimal additive quantity accumulated on the boundary surface.

Key Words: Cremophor RH 40; Cremophor RH 60; Ethacrynic acid; Solutol HS 15; Suppository

INTRODUCTION

One of the tasks of drug formulation is to develop an already-existing dosage form in such a way that drug release is the best possible under the given circumstances, so increasing the bioavailability. Another important aim is to make a given drug available in as many dosage forms as possible (1–5), which is also confirmed by the concrete therapeutic need expressed by doctors for the

formulation of a rectal preparation containing furosemide (6), followed—after favorable human trials—by the formulation of further diuretic rectal suppositories.

The rectal administration of drugs may have definite advantages in certain cases: the drugs enter the organism bypassing the primary metabolism of the liver. Therefore, despite its disadvantages compared with oral administration, rectal application may be the optimal solution, especially in hepatic patients

*Corresponding author.

for whom this route of administration may offer special therapeutic advantages (7,8).

Diuretic ethacrynic acid belongs to the group of loop diuretics, since its effect is exerted on the ascending loop of Henle (9).

The aim of this research was to formulate a rectal suppository dosage form from a well-known drug, which would add to the choice of existing treatment methods and thus improve the possibilities of individual cure. However, it is a well-known fact that the release of drugs which are easily water-soluble is better from lipophilic suppository bases, so various solubility-increasing additives were used to enhance drug release (10-12).

MATERIALS

Ethacrynic acid was from EGIS (Hungary); Suppocire AML and Suppocire AP were from GATTEFOSSÉ (France); Witepsol H 15, Witepsol W 35, Witepsol S 58, and Massa Estarinum BC were from CONDEA Chemie GmbH (previously HÜLS AG) (Germany); Solutol HS 15, Cremophor RH 40, and Cremophor RH 60 were from BASF (Germany).

METHODS

In Vitro Dissolution Study

The drug content of the rectal suppositories prepared by molding was 2.5%, which corresponded

to the therapeutic dose, that is an adult suppository of 2.00 g contained 50 mg of drug. The method of dynamic membrane diffusion (13) was used to determine the extent of drug liberation and diffusion through the membrane, both from the powder without a suppository base and from drug suppositories of various composition. Distilled water and phosphate buffer of pH 7.5 were used as the acceptor phase. The suppositories packed in membrane (VISKING[®], Germany) were placed one by one into 20 mL of dissolution medium at body temperature ($37 \pm 0.5^\circ\text{C}$). The samples were exposed to slight horizontal shaking and the entire acceptor phase was changed after 30, 60, 120, and 240 min. The quantity of ethacrynic acid contained in the samples was determined spectrophotometrically at a wavelength of $\lambda = 278$ nm from the results of five parallel measurements.

Mathematical Evaluation

Linear regression was used to find a relationship between the process of dissolution and time. The calculations revealed that the process of dissolution could be characterized by a power function, which is also confirmed by the fact that lines were obtained when the logarithm of the quantity of the dissolved drug was plotted against the logarithm of time. The formulae of the lines giving the mathematical description of the relationship and the values of the correlation coefficient are shown in Table 1.

Table 1

Formulae of the Lines Describing the Process of Dissolution Mathematically and the Values of the Correlation Coefficient (R^2)

Base	Dissolving Medium: Distilled Water		Dissolving Medium: Phosphate Buffer	
	Line Equation	R^2	Line Equation	R^2
Powder	$y = 0.0272x + 1.2517$	0.9945	$y = 0.313x + 6.6322$	0.9886
Suppocire AML	$y = 0.0284x + 0.6852$	0.9968	$y = 0.2902x + 7.553$	0.9699
Suppocire AP	$y = 0.0071x + 0.493$	0.9882	$y = 0.2126x + 7.157$	0.9775
Massa Estarinum BC	$y = 0.0245x + 0.59$	0.999	$y = 0.2195x + 5.853$	0.9986
Witepsol H 15	$y = 0.0251x + 1.1526$	0.9866	$y = 0.2328x + 9.8609$	0.9294
Witepsol W 35	$y = 0.0201x + 0.6765$	0.9877	$y = 0.1201x + 13.191$	0.9448
Witepsol S 58	$y = 0.0168x + 0.4609$	0.9992	$y = 0.2368x + 8.213$	0.9702

x = log time (min).

y = log diffused drug (%).

RESULTS

The evaluation of the results shows that the changing of the acceptor phase led to about a 10-fold increase in drug release, which can be explained by better drug solubility in a phosphate buffer of pH 7.5 (Figs. 1 and 2).

Compared with the diffusion values of the ethacrynic acid powder without a suppository base, considered as standard, Suppocire AML proved to be the best in both dissolving media. The quantity of ethacrynic acid released was approximately the same as in the case of the Witepsol H 15 base, probably due to the relatively low hydroxyl number of both bases. This is also confirmed by the fact that the worst result was obtained with the Suppocire AP base, the hydroxyl number of which is considerably higher than those of the other bases. The amount of drug released from this base was much smaller

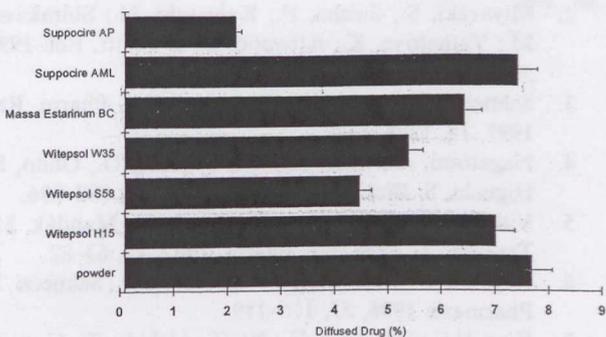


Figure 1. Release of ethacrynic acid from various suppository bases after 240 min. Acceptor phase: distilled water.

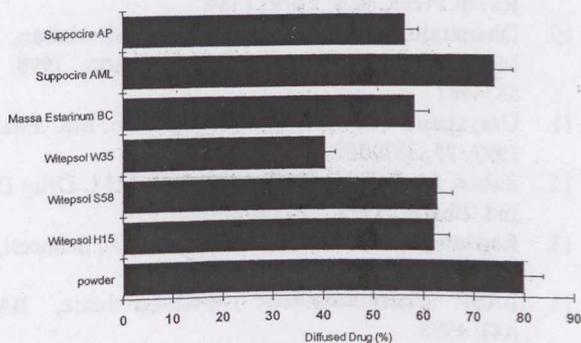


Figure 2. Release of ethacrynic acid from various suppository bases after 240 min. Acceptor phase: phosphate buffer.

than the membrane diffusion of the powder without a suppository base. The relationship between the hydroxyl number and the release of ethacrynic acid is also confirmed by the fact that the hydroxyl numbers of Witepsol S 58 and Witepsol W 35 are similarly high, and the quantity of drug diffused from them is also much less than from other CONDEA (HÜLS) products (Table 2).

Three solubility-increasing additives were tested for enhancing the liberation of poorly water-soluble ethacrynic acid. Solutol HS 15, Cremophor RH 40, and Cremophor RH 60 non-ionic surfactants were added to Witepsol H 15, which had previously proved to be one of the best suppository bases. These are all well-known additives, which had not been used in the dosage form of rectal suppositories before.

These surfactants have good physiological tolerance and considerable efficiency as regards solubilization. Solutol HS 15 is recommended as a non-ionic solubilizing agent to be added to injection solutions, while the use of Cremophor products is proposed to make fat-soluble vitamins, essential oils, hydrophobic drugs, and cosmetics water-soluble (14-16).

When surfactants were used in a concentration of 1, 3, 5, and 10%, the diffusion of the drug was found to vary with their concentration. When distilled water was used as the acceptor phase, a concentration of 3% yielded the best results for all three surfactants. This led to about a twofold increase in liberation. Their use in a concentration of 1-5-10% either did not change or decreased drug liberation (Fig. 3), which can probably be explained by the concentration of surfactants accumulated on the boundary surface as the quantity of the diffused drug is increased by proper saturation. In contrast, a too small or too large amount of surfactant may lead to its decrease.

Table 2

Hydroxyl Number of Bases

Bases	Hydroxyl Number
Suppocire AML	Max. 6
Suppocire AP	30-50
Massa Estarinum BC	30-40
Witepsol H 15	5-15
Witepsol W 35	40-50
Witepsol S 58	60-70

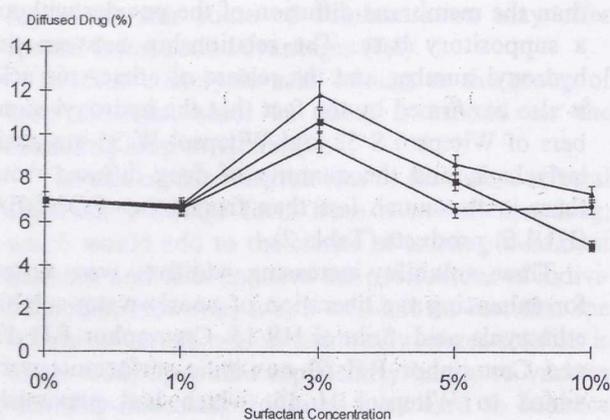


Figure 3. Drug release as a function of additive concentration after 240 min. Acceptor phase: distilled water. ■ Solutol HS 15, ● Cremophor RH 40, ▲ Cremophor RH 60.

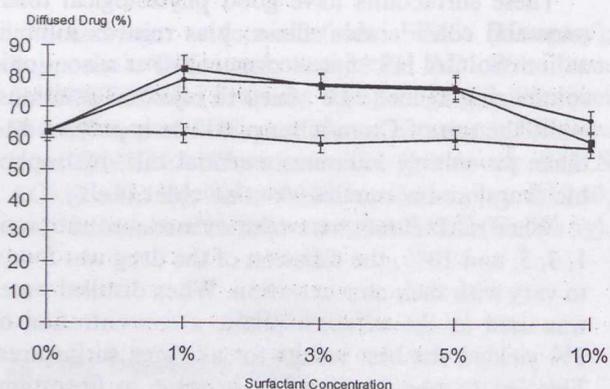


Figure 4. Drug release as a function of additive concentration after 240 min. Acceptor phase: phosphate buffer. ■ Solutol HS 15, ● Cremophor RH 40, ▲ Cremophor RH 60.

When the same examinations were performed in a buffer medium, 1% of Solutol HS 15 and Cremophor RH 40 led to a slight increase in diffusion, while the use of Cremophor RH 60 did not bring about a change in the extent of drug release. Therefore the pH increase is accompanied by a decreasing influence exerted by solubility-increasing additives on the liberation of ethacrynic acid, which is related to the dissolution property of the drug, namely that the solubility of ethacrynic acid increases in phosphate buffer of pH 7.5 (Fig. 4).

CONCLUSION

As a summary, it can be concluded that the use of additives enhanced the release of poorly water-

soluble ethacrynic acid in the medium of distilled water. The increase of liberation was the greatest in a concentration of 3% for all three surfactants. When the pH of the acceptor phase was increased, a surfactant concentration of 1% was found to increase drug release to a slight extent, so the importance of the use of surfactants decreased. This can be explained by the fact that the solubility of ethacrynic acid is better in phosphate buffer of pH 7.5, thus it is released from lipophilic suppository bases more readily. Based on these results, a composition of Witepsol H 15 + 1% Solutol HS 15 is recommended for the formulation of rectal suppositories containing ethacrynic acid, as this composition increased drug release in both dissolving media.

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III.



Research paper

In vitro and in vivo study in rats of rectal suppositories containing furosemide

Szilvia Berkó^a, Géza Regdon Jr.^a, Eszter Ducza^b, George Falkay^b, István Erős^{a,*}

^aDepartment of Pharmaceutical Technology, University of Szeged, Szeged, Hungary

^bDepartment of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, Hungary

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Abstract

The aim of our experimental work was to formulate furosemide-containing rectal suppositories, to study drug release with in vitro membrane diffusion examinations and to increase drug liberation with the use of non-ionic surfactants (Solutol HS 15, Cremophor RH 60, Montanox 60 DF), which were incorporated in the suppository base in various concentrations. Suppocire AS₂X proved to be the best suppository base (diffused drug: 69.78%). The use of 1% Cremophor RH 60 additive with the Witepsol H 15 base increased the quantity of the diffused drug from 62 to 75%. The membrane diffusion examinations were followed by studying the influence of suppository bases and additives exerted on the actual diuretic effect in Sprague–Dawley male rats. Once again the Suppocire AS₂X suppository base gave the best results compared to the control; the quantity of the animals' urine showed a fourfold increase. Used with the Witepsol H 15 base, even 1% of all the three additives resulted in a considerable increase of diuretic effect, so their use proved to be advantageous. The comparison of the membrane diffusion examinations with the in vivo diuretic effect reveals that in vitro drug release and the pharmacological effect usually showed the same tendency, that is a greater extent of in vitro furosemide release was associated with a greater quantity of rat urine. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Furosemide; Suppository; Solutol HS 15; Cremophor RH 60; Montanox 60 DF; Rat; Diuretic effect

1. Introduction

Furosemide, which belongs to the group of loop-diuretics, is very effective in draining all kinds of oedemas (of cardiac, hepatic or renal origin), in mild or moderate hypertension (in itself or combined with other antihypertensive drugs), or used in greater doses in acute and chronic renal failure, in oliguria. Currently it is available as oral solution, capsules or granules for oral and parenteral administration [1].

Nowadays one of the basic tasks of drug formulation is to develop an already existing dosage form in a way which makes drug release the best possible under the given circumstances, that is to enhance bioavailability in this way [2–4]. The other important aim is to widen the choice of products with respect to dosage, that is to make a given drug available in as many dosage forms as possible [5–9]. Furosemide is a weak acid (pK_a = 3.9) which is absorbed incompletely from the gastrointestinal tract after oral

administration. There have been reports of considerable intra- and interindividual variability in its bioavailability. This is due to the fact that absorption of furosemide depends on pH, food intake and dosage form [10,11].

In view of the above the future objective of research can be to formulate a furosemide-containing rectal suppository of proper biological effectiveness, which is currently missing from the pharmaceutical trade in spite of the fact that internists expressed a concrete therapeutic need for the formulation of a rectal preparation containing furosemide. Regdon et al. were the first to deal with this task [12]. The formulation of this dosage form would add to the choice of existing treatment methods and would also improve the possibilities of individual cure in cases when the oral and intravenous administration of furosemide should be avoided (vomiting, shock, patient with bad compliance, patient with parenteral nutrition). The results of our in vitro examinations were confirmed with in vivo trials carried out in rats, and these served as the basis for choosing the ideal suppository composition concerning the given drug from among the bases and additives examined [13].

* Corresponding author. University of Szeged, Faculty of Pharmacy, Department of Pharmaceutical Technology, Szeged-6720, Eötvös u. 6, Hungary. Tel./fax: +36-62-545-571.

E-mail address: eros@pharma.szote.u-szeged.hu (I. Erős).

Table 1
Properties of suppository bases

Name of base	Chemical description	Melting range (°C)	Hydroxyl value	Function
Witepsol [®] H 15	Triglycerides (C10–C18)	33.5–35.5	5–15	Lipophilic base
Witepsol [®] W 35	Higher proportion of mono- and di-glycerides (C10–C18)	33.5–35.5	40–50	Lipophilic base
Massa Estarinum [®] B	Higher proportion of mono- and di-glycerides (C12–C18)	33.5–35.5	20–30	Lipophilic base
Massa Estarinum [®] BC	Higher proportion of mono- and di-glycerides (C12–C18)	33.5–35.5	30–40	Lipophilic base
Suppocire [®] AML	Triglycerides (C8–C18) with the presence of a phospholipid	35–36.5	Max. 6	Lipophilic base
Suppocire [®] AP	Saturated polyglycolized glycerides	33–35	30–50	Amphiphilic base
Suppocire [®] AS ₂ X	Higher proportion of mono- and di-glycerides (C8–C18) with the presence of a non-ionic emulsifying additive	35–36.5	15–25	Lipophilic base

2. Materials and methods

2.1. Materials

Furosemide (CHINOIN, Hungary) was used as drug. Suppocire[®] AML, AP, AS₂X (GATTEFOSSÉ, France), Witepsol[®] W 35, H 15, and Massa Estarinum[®] B and BC (CONDEA Chemie GmbH, Germany) were used as suppository bases. Cremophor[®] RH 60, Solutol[®] HS 15 (BASF, Germany), and Montanox[®] 60 DF (SEPPIC, France) were used as surfactants.

2.2. Formulation method

During the experiments seven suppository bases with various properties and three surfactants were used to formulate furosemide-containing suppositories (Tables 1 and 2). Suppositories were formulated by moulding. In the case of *in vitro* experiments the drug content was 2.5 w/w%, which corresponded to the therapeutic dose, that is a 2 g adult suppository contained about 50 mg furosemide. Suppositories (0.3 g) were prepared for the animal experiments and adjusted to the anatomical size of rats; the drug content was 15 mg/suppository. The additives were incorporated in the suppository base in a concentration of 1, 3, 5 or 10%.

2.3. *In vitro* release study

Experiments were performed with the method of dynamic membrane diffusion, which is a useful method for following the rate of drug release and membrane diffusion from the powder without excipient and from the different suppository compositions, too. The acceptor phase was phosphate buffer at pH 7.5 (modelling the rectal pH). The suppositories were individually packed in a kidney dialysing membrane (VISKING[®] Dialysis Tubing 36/32 SERVA, Germany) and placed into buffer of body temperature (37 ± 0.5 °C).

The samples were exposed to slight shaking and the acceptor phase was replaced after 30, 60, 120, and 240 min. The quantity of furosemide in these samples was measured with a spectrophotometer at λ = 274 nm, using the absorbance value. The mean values were calculated from five parallel measurements each time (±SEM) [14].

2.4. *In vivo* study

Animal investigations were carried out with the approval of the Ethical Committee for Animal Research, University of Szeged (Registration number: 23/1999).

The animal studies were performed with Sprague–Dawley male rats of 280–300 g. After 6 h of fasting the oral administration was done with an oral tube and the suppository was placed in the animals in ether anaesthesia; then they received 20 ml/kg water per rat. They were placed in special cages where urine was collected every 10 min for 150 min. The control rats received only 20 ml/kg water.

2.5. Mathematical evaluation

The results were evaluated and analyzed statistically with the Prism 2.01 (GraphPad Software, USA) computer program. For statistical evaluations, data were analyzed by ANOVA with the Newman–Keuls test.

3. Results and discussion

The membrane diffusion of the powder without a suppository base was regarded as the control during the *in vitro* experiments. It can be stated that drug diffusion from Suppocire AS₂X (***P* < 0.001), Massa Estarinum B (***P* < 0.01) and Witepsol H 15 (**P* < 0.05) was about the same as from the powder without a suppository base. Suppocire AML (***P* < 0.001), Massa Estarinum BC

Table 2
Properties of surfactants

Name of surfactant	Chemical description	Solidification point (°C)	Hydroxyl value	Function
Solutol [®] HS 15	Macrogol-15 hydroxystearate	25–30	90–110	Nonionic solubilizer
Cremophor [®] RH 60	Polyoxyl 60 hydrogenated castor oil	20–28	60–75	Nonionic solubilizer
Montanox [®] 60 DF	Polyoxyethylated sorbitan monostearate	–	81–96	Nonionic emulsifier

(** $P < 0.01$) and Suppocire AP (** $P < 0.001$) decreased drug release to a smaller extent, while Witepsol W 35 (** $P < 0.001$), which has a relatively high hydroxyl value, decreased drug release with orders of magnitude (Fig. 1a). The different physical parameters of suppository bases (melting range, viscosity) and their chemical properties (chemical nature, acid value, hydroxyl value, presence of additive) may influence drug liberation [15–17]. This is contradicted by the fact that the hydroxyl value of Suppocire AP is approximately the same as that of Witepsol W 35; nevertheless, furosemide liberation shows a significant difference. This is probably due to the amphiphilic properties of Suppocire AP, which for most drugs lead to increased bioavailability compared to traditional lipophilic suppository bases.

In the course of the *in vivo* trials the dose–effect relationship was examined after the administration of furosemide orally and rectally (suppository with the Witepsol H 15 base) (Fig. 2). The ED50 value was calculated from the figure in both cases ($ED_{50_{\text{supp}}} = 15.39$ mg, $ED_{50_{\text{per os}}} = 19.03$ mg), which revealed that rectal administration is slightly more effective than oral administration. In the case of furosemide the hepatic first-pass effect is almost negligible; the major site for the first-pass metabolism of the drug in rats is probably the GI tract. Gastrointestinal and intestinal first-pass effect has been described in rats concerning furosemide,

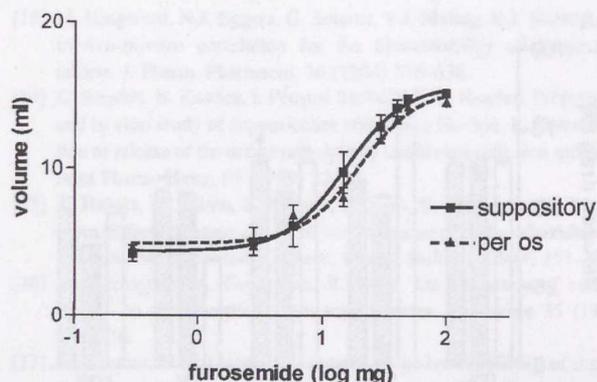


Fig. 2. Dose-dependent effect of furosemide. After suppository and *per os* application 20 ml/kg water was given *per rat*. The urine was collected for 150 min. The error bars represent the SEM.

where 20–40% of the administered drug is metabolized [18]. Further examinations were carried out with the ED50 value calculated from the dose–effect examinations.

Furosemide was incorporated in suppository bases, and after application in rats urine was collected for 150 min. Compared to the control, a significant increase was observed in the quantity of urine when Suppocire AP ($*P < 0.05$), Witepsol H 15 ($*P < 0.05$), Witepsol W 35 (** $P < 0.01$), Massa Esterinum B (** $P < 0.001$) and Suppocire AS₂X (** $P < 0.001$) suppository bases were used. The use of Suppocire AML and Massa Esterinum BC did not bring about a significant difference in urine quantity compared to the control (Fig. 1b). The effectiveness of Suppocire AS₂X and Massa Esterinum B is clearly shown by the fact that the amount of urine collected for 150 min came near to the 24 h urine quantity of rats [19].

Three non-ionic surfactants were also tested for increasing furosemide liberation. The disintegration time of suppositories is usually shortened by surfactants; the lipophilic base is made lipohydrophilic by their moistening effect, which usually enhances the release and absorption of drugs incorporated in the suppositories. Solutol HS 15, Cremophor RH 60 and Montanox 60 DF are additives, which are well-known in the pharmaceutical industry but had not been used in the dosage form of rectal suppositories before; they have good physiological tolerance and considerable efficiency as regards solubilization and emulsification [20–22]. The surfactants were incorporated in the Witepsol H 15 base in a concentration of 1, 3, 5 and 10%. The Witepsol H 15 suppository base was chosen because it did not yield maximum results either during the *in vitro* or mainly in the *in vivo* examinations, so the use of additives was expected to enhance drug liberation and diuretic effect. During the *in vitro* examinations only the 1% concentration of Cremophor RH 60 led to a significant increase; in the other cases no significant differences were observed, and furosemide diffusion even decreased with the increase of the surfactant concentration (Fig. 3a). The decrease in drug diffusion through the membrane is due to two causes: (1) the additive,

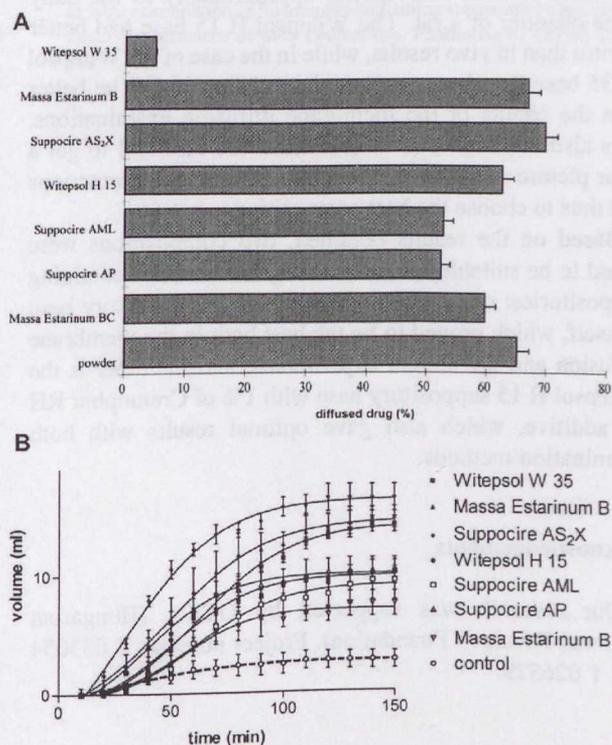


Fig. 1. (a) Furosemide release from different suppository bases after 240 min. (b) Diuretic effect of different suppository bases containing furosemide in rats. The data are the averages of the results of six experiments (\pm SEM).

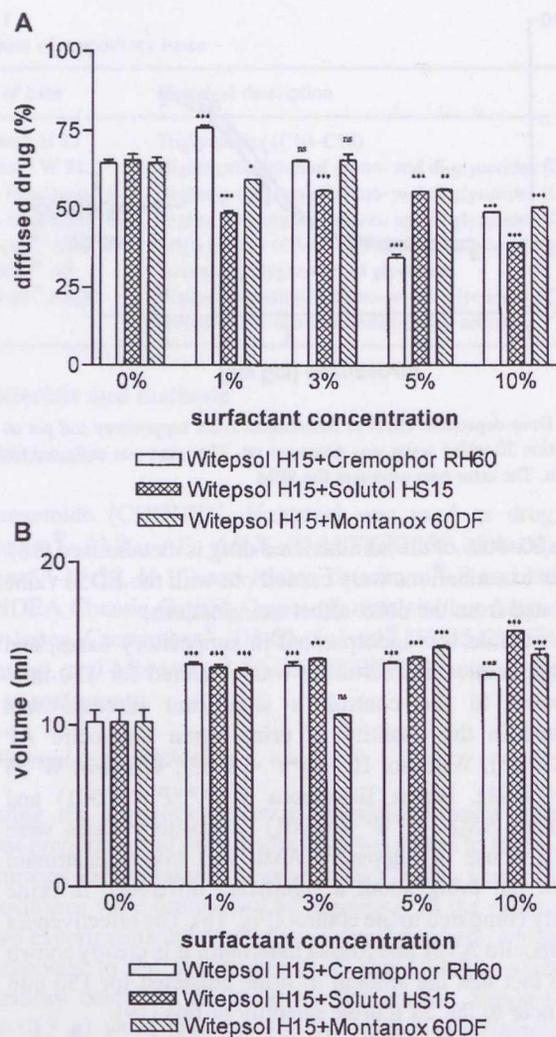


Fig. 3. (a) Influence of additives on drug release after 240 min ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus control, analysis of variance Newman-Keuls test). (b) Influence of additives on diuretic effect in rats. The data are the averages of the results of six experiments (\pm SEM) ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus control, analysis of variance Newman-Keuls test).

drug and base formed a stable complex, or the conditions of dissociation were influenced unfavourably by the additive; (2) although the drug was released from the suppository base, a certain extent of increase in the surfactant concentration resulted in the formation of micelles of colloidal size, so it is possible that the drug molecules closed in the micelles were unable to pass through the dialyzing membrane which had a pore size of 25 Å. This latter supposition is confirmed by the results of the *in vivo* experiments, in which the diuretic effect was definitely enhanced by the surfactants, and in the case of Cremophor RH 60 the critical micellar concentration was probably over 1% so no aggregate was formed and the drug could diffuse through the membrane.

In the *in vivo* examinations the use of surfactants led to a significant increase in the amount of urine collected (Fig. 3b).

Their effect is composed of several factors: they moisten the drug, they denature the proteins found on the intestinal mucosa thereby disrupting the integrity of the membrane, and furthermore they increase the number of adsorption places by cleaning the membrane surface. Nerurkar et al. suggest that surfactants, which are commonly added to pharmaceutical formulation, may enhance the intestinal absorption of some drugs by inhibiting an apically polarized efflux system [23]. In the animal experiments performed with rats all three additives increased the quantity of the excreted urine approximately to the same extent, which indicates increased drug liberation. Fig. 3b also shows that the increase of the surfactant concentration was not accompanied by significant changes, so a concentration of 1% is enough to achieve the desired effect.

4. Conclusion

The comparison of the membrane diffusion examinations with the actual diuretic effect shows that drug release and the pharmacological effect had the same tendency in 70% of cases, so a greater extent of furosemide release was associated with a greater quantity of rat urine. The best results were achieved in both cases with the Suppocire AS₂X base, which means that drug release was about 70% and the animal produced about 15 ml of urine in 150 min, and according to the literature this corresponds approximately to the daily urine quantity of a rat. The Witepsol H 15 base had better *in vitro* than *in vivo* results, while in the case of the Witepsol W 35 base the pharmacological results proved to be better than the results of the membrane diffusion examinations. This also confirms that *in vivo* trials are essential to get a clear picture of the drug–base–living organism interactions and thus to choose the best composition.

Based on the results obtained, two compositions were found to be suitable for formulating furosemide-containing suppositories: one is the Suppocire AS₂X suppository base in itself, which proved to be the best both in the membrane diffusion and the animal experiments, and the other is the Witepsol H 15 suppository base with 1% of Cremophor RH 60 additive, which also gave optimal results with both examination methods.

Acknowledgements

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IV.

Investigation of Ethacrynic Acid and Random-methyl- β -cyclodextrin Binary Complexes

ZOLTÁN AIGNER¹, ERZSÉBET SZEPESI¹, SZILVIA BERKÓ¹, CSABA NOVÁK², GÉZA REGDON JR.¹ and MIHÁLY KATA¹

¹University of Szeged, Department of Pharmaceutical Technology, H-6720 Szeged, Eötvös u. 6. Hungary; ²Budapest University of Technology and Economics, Institute of General and Analytical Chemistry, H-1111 Budapest, Gellért tér 4. Hungary

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Key words: cyclodextrins, DSC, ethacrynic acid, inclusion complexes, kneading, membrane diffusion, mixing, random-methyl- β -cyclodextrin, solubility, suppository

Abstract

Cyclodextrin complexation was applied to achieve better aqueous solubility of the drug and to formulate suppositories. Binary products were prepared in several mole ratios by two different methods. The dissolution profiles and in vitro membrane diffusion behaviour of the compositions were investigated. Thermoanalytical studies were performed in order to confirm inclusion complex formation. Compositions were selected for further detailed investigations and for incorporation into suppository dosage form.

Introduction

As a consequence of the wide-ranging therapeutic considerations for the use of diuretics, these preparations comprise a very important group of medicines. Ethacrynic acid belongs in the “loop” diuretic group and is effective in all types of oedema (heart, liver or renal) [1, 2]. It is official in tablet and injection dosage form in several pharmacopoeias [1, 3–5]. It is registered in Hungary as Uregyt[®] (tablet and injection) for oral and parenteral administration. Its oral dose is 50–200 mg [6, 7]. Its pharmacology was described by Beyer [8], and its chemical structure (Figure 1) was investigated by Lamotte [9].

The side-effects include hypochloaemia, hypokalaemia, hypovolaemia and metabolic alkalosis. There are adverse gastrointestinal reactions, such as nausea, vomiting, and dysphagia, and in some cases allergic reactions and acute pancreatitis. Ethacrynic acid selectively acts renally, and its side-effects are therefore fairly rare.

The rectal administration of pharmacocon has the advantage of avoiding the liver first-pass effect, which means an optimal solution in spite of the inconvenience of this route as compared to the oral route, especially in the event of liver problems [10–12].

It is well known that the liberation of a water-soluble drug from lipophilic suppository bases is favoured. Accordingly, the goal of the authors was to improve the aqueous solubility of ethacrynic acid.

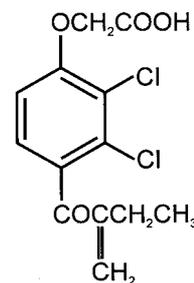


Figure 1. Chemical structure of ethacrynic acid.

Experimental

Materials

Ethacrynic acid (**E**) [2,3-dichloro-4-(2-methylene-1-oxobutyl)phenoxy]acetic acid, (EGIS, Budapest, Hungary); α -, β - and γ -cyclodextrin (CD), dimethyl- β -CD (DIMEB), random-methyl- β -CD (RAMEB), hydroxypropyl- β -CD (HP- β -CD) (Cyclolab R&D. Laboratory, Budapest, Hungary); hydroxyethyl- β -CD (HE- β -CD), methyl- β -CD (ME- β -CD) (Wacker-Chemie GmbH, Munich, Germany); Witepsol H15, Witepsol W35, Massa Estarinum 299 (CONDEA Chemie GmbH, Hamburg, Germany); Suppocire AML, Suppocire AP (Gattefossé, Saint-Priest, France); sodium chlorate, potassium dihydrogenphosphate, disodium hydrogenphosphate, glycolcol, hydrochloric acid, sodium hydroxide (Reanal, Budapest, Hungary). Ethanol is official in *Pharmacopoeia Hungarica VII* [13].

Table 1. Solubility increasing effect of CD derivatives (%)

1. Ethacrynic acid	100
2. E + α -CD	177
3. E + γ -CD	338
4. E + HP- β -CD	697
5. E + HE- β -CD	718
6. E + β -CD	795
7. E + RAMEB	933
8. E + ME- β -CD	1,010
9. E + DIMEB	1,113

Apparatus

USP rotating-basket dissolution apparatus (Erweka DT, Erweka Apparatebau GmbH, Heusenstamm, Germany); kneading mixer (Erweka LK5, Erweka Apparatebau GmbH, Heusenstamm, Germany), Unicam UV2 UV/Vis Spectrometer (Unicam, Cambridge, England); Sartorius membrane apparatus (Sartorius-Membranfilter GmbH, Göttingen, Germany), STD 2960 Simultaneous DTA-TGA and DSC 2920 Modulated DSC instruments, Vibrotherm shaking-waterbath, type 609/A (MTA KUTESZ, Budapest, Hungary).

Preliminary experiments

The effects of the different CD derivatives on the solubility of the active agent were determined: a mixture of 0.10 g of ethacrynic acid and 0.50 g of CD derivative was mixed with water to 20.0 g and stirred for 10 min with a magnetic mixer. The suspension was filtered through filter paper and, after suitable dilution, the UV spectra were recorded. A system without CD was used as a control. DIMEB, methyl- β -CD and RAMEB had the highest influence on the solubility of the active agent (Table 1). RAMEB was chosen for further examinations on the basis of the costs and the solubility-increasing effect: the solubility was increased by a factor of 9.33.

The absorption maximum of the active agent was determined (280 nm). The calibration plot revealed that the absorption obeys the Bouguer–Lambert–Beer law in the concentration interval 0–70 $\mu\text{g mL}^{-1}$. The molar extinction coefficient (ϵ) was 13,045.

Preparation of products

The two-component products were prepared in four different mole ratios (drug : CD mole ratio = 2 : 1, 1 : 1, 1 : 2 and 1 : 3).

Physical mixtures (PM): The ground components were mixed in a mortar and sieved through a 100 μm sieve.

Kneaded products (KP): Physical mixtures of the drug and RAMEB were mixed (Erweka LK5) in the same quantity of ethanol + water (1 : 1). They were kneaded until the bulk of the solvent mixture had evaporated. After this, they were dried at room temperature and then at 105 °C, and were next pulverized and sieved through a 100 μm sieve.

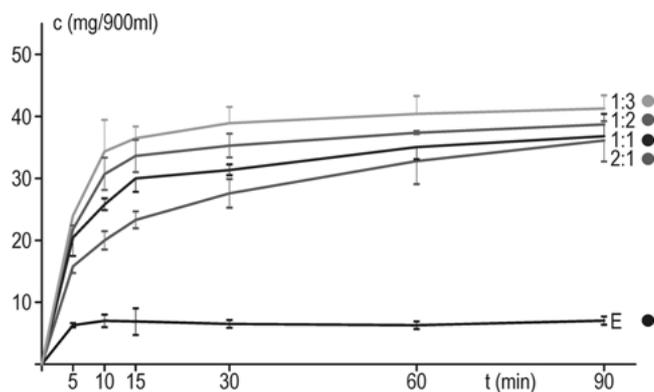


Figure 2. Dissolution of ethacrynic acid from physical mixtures (artificial gastric juice).

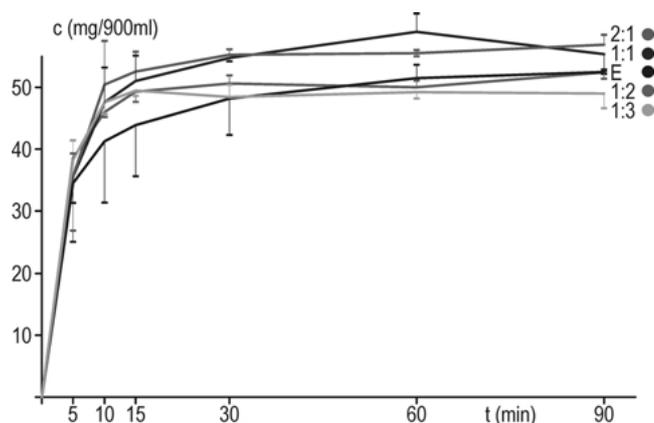


Figure 3. Dissolution of ethacrynic acid from physical mixtures (artificial intestinal juice).

Products were stored under normal conditions at room temperature in closed glass containers.

Dissolution studies

In the USP rotating-basket dissolution apparatus, 50 mg of pure ethacrynic acid or binary products containing 50 mg of drug were examined in 900.0 g of artificial gastric juice or intestinal juice. The basket was rotated at 100 rpm. Sampling was performed after 5, 10, 15, 30, 60 and 90 min. The volume of the sample was 5.0 mL. The ethacrynic acid con-

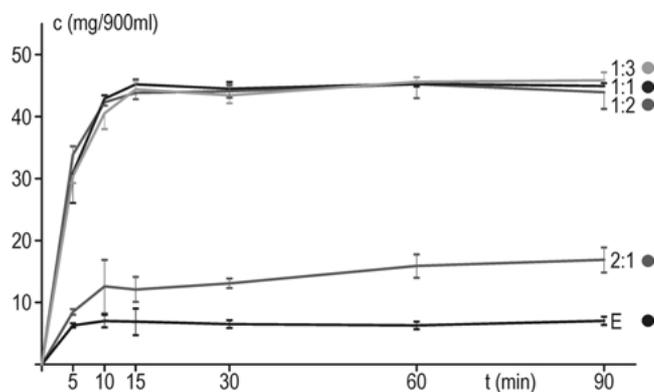


Figure 4. Dissolution of ethacrynic acid from kneaded products (artificial gastric juice).

Table 2. Composition of artificial juices

		Gastric juice	Intestinal juice	Plasma	Phosphate buffer
		1.1	7.0	7.5	7.5
pH (± 0.1)					
1N HCl	(g)	94.0	–	–	–
NaCl	(g)	0.35	–	–	–
Glycine	(g)	0.50	–	–	–
Na ₂ HPO ₄ ·2H ₂ O	(g)	–	14.4	20.5	–
KH ₂ PO ₄	(g)	–	7.1	2.8	10.57
NaOH	(g)	–	–	–	2.44
Distilled water	to		1000 mL		

Table 3. Membrane diffusion examinations of physical mixtures

Products	From gastric juice			From intestinal juice		
	K _d (10 ⁻⁴)	S	Diff.	K _d (10 ⁻⁴)	S	Diff.
	[cm/min]		%	[cm/min]		%
E	4.51	0.89	15.04	2.41	1.25	8.02
2:1	6.96	3.48	23.19	1.67	1.10	5.58
1:1	7.58	4.28	25.27	2.00	0.86	6.67
1:2	8.42	4.81	28.07	1.67	1.19	5.55
1:3	8.46	4.60	28.20	2.21	1.16	7.35

S = Standard deviation.

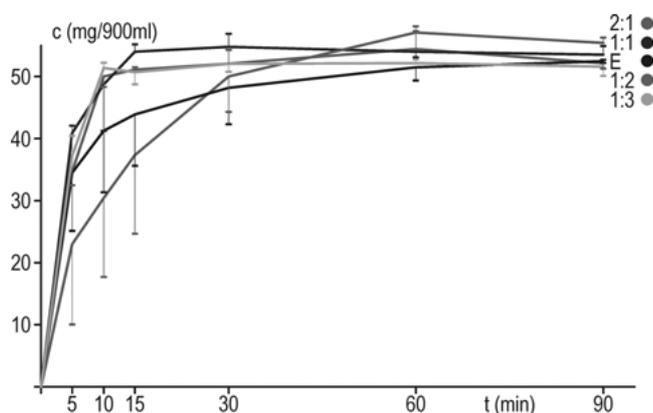


Figure 5. Dissolution of ethacrynic acid from kneaded products (artificial intestinal juice).

tents of the samples were determined spectrophotometrically after filtration and dilution.

Membrane diffusion experiments

Stricker's Sartorius instrument was used [3, 4]. Measurements were performed from 100.0 mL of artificial gastric juice or artificial intestinal juice into artificial plasma (Table 2). 50 mg of active agent, or product containing 50 mg of ethacrynic acid, was in the donor phase in all cases. The temperature was 37.5 ± 1.5 °C. During the examination, 5.0 mL samples were taken five times (after 30, 60, 90, 120 and 150 min) and their active agent contents were determined spectrophotometrically after filtration and dilution. The amount of diffused active agent and the diffusion constant K_d were calculated:

$$K_d = \frac{c_{II2} - c_{II1}}{T_2 - T_1} \cdot \frac{1}{c_{I0}} \cdot \frac{V_{II0}}{F} [\text{cm min}^{-1}]$$

where c_{IIx} is the corrected drug concentration in phase II at time T_x (mg mL^{-1}); V_{II0} is the volume of aqueous phase II at time T_0 (100 mL); F is the surface area of the membrane (cm^2); T_x is the time (min); and c_{I0} is the theoretical initial drug concentration in phase I (mg mL^{-1}) [3].

Thermoanalytical methods

Thermogravimetry (TG), derivative thermogravimetry (DTG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) were used as thermoanalytical methods to confirm the presence of inclusion complexes.

CDs generally lose water below 100 °C, and decompose above 250 °C. The DSC method therefore can be used if the crystallized drug melts in the temperature range between the temperature of water loss from the CD and the temperature of its decomposition (120–250 °C).

A distinction can be made between surface adsorption and inclusion complex formation by thermoanalytical methods. The presence of an inclusion complex is shown indirectly: changes (e.g., in evaporation, thermal decomposition, oxidation, melting or polymorphism) relative to the non-complexed free drug are recorded [14].

Approximately 2–5 mg of active agent or product containing 2–5 mg of ethacrynic acid was examined between 25 °C and 300 °C. The heating rate was 5 °C min^{-1} . The rate of argon flow was 10 L hour^{-1} .

Table 4. Membrane diffusion examinations of kneaded products

Products	From gastric juice			From intestinal juice		
	K_d (10^{-4}) [cm/min]	<i>S</i>	Diff. %	K_d (10^{-4}) [cm/min]	<i>S</i>	Diff. %
E	4.51	0.89	15.04	2.41	1.25	8.02
2:1	2.77	0.83	9.24	1.94	1.28	6.47
1:1	9.65	4.96	32.16	1.78	0.82	5.92
1:2	8.99	5.31	29.97	1.85	1.05	6.15
1:3	8.64	4.91	28.81	2.48	1.06	8.27

S = Standard deviation.

Sample: Ethacrynic acid
Size: 0.9700 mg
Method: Heating rate: 5 C/min
Comment: Atm.: Argon 10 l/h

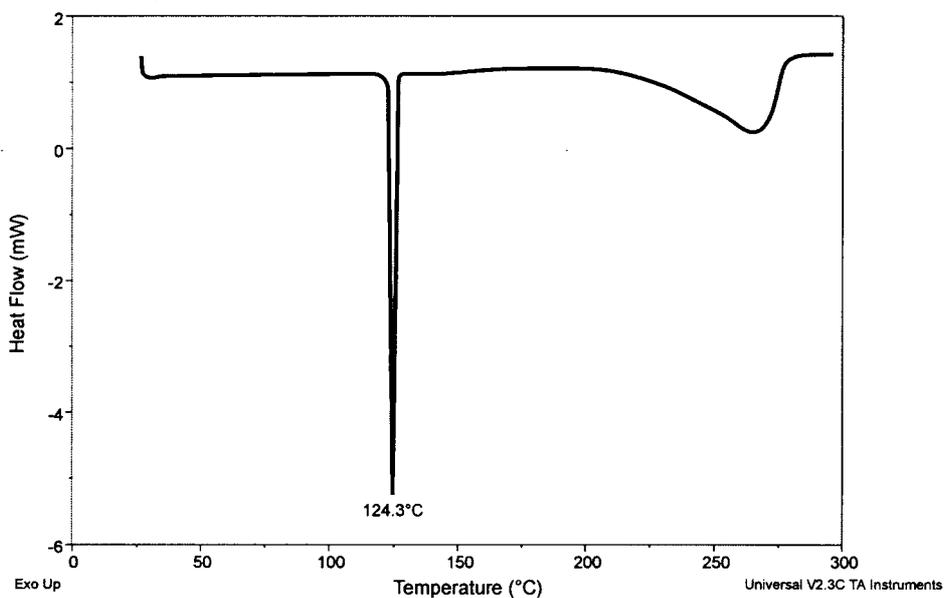


Figure 6. DSC curve of ethacrynic acid.

Sample: RAMEB
Size: 4.5580 mg
Method: Heating rate: 5 C/min
Comment: Atm.: Argon 10 l/h

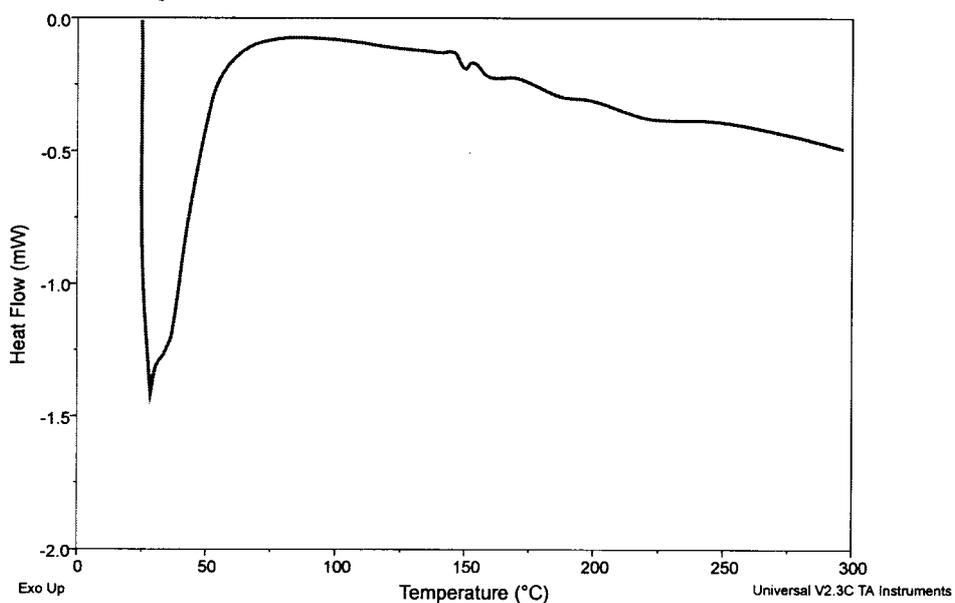


Figure 7. DSC curve of RAMEB.

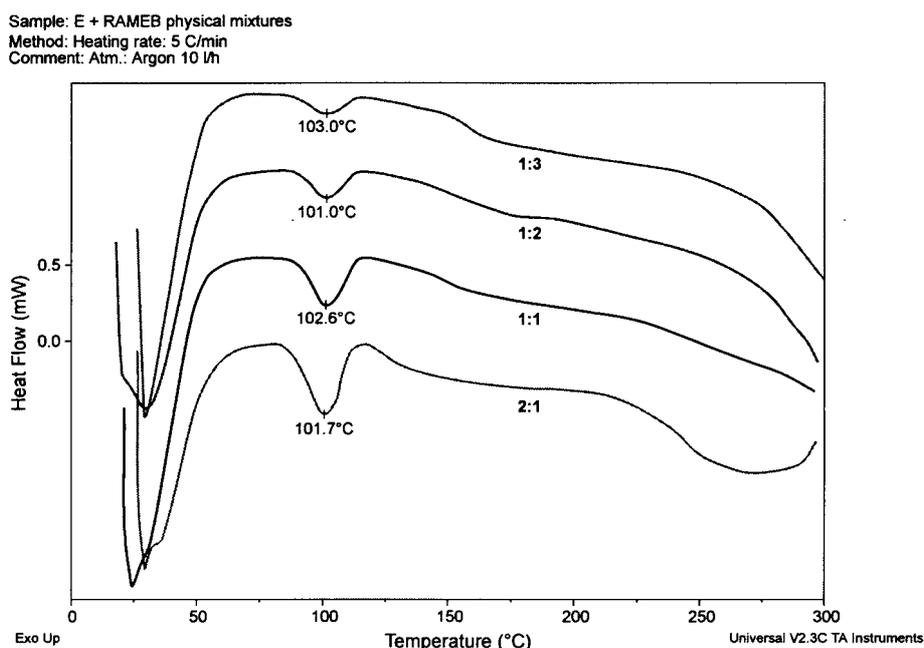


Figure 8. DSC curves of physical mixtures.

In vitro study of release from rectal suppositories

The drug content of the rectal suppositories prepared by moulding was 2.5%, which corresponded to the therapeutic dose, an adult suppository of 2.00 g containing 50 mg of ethacrynic acid. The method of dynamic membrane diffusion was used to determine the extent of drug liberation and diffusion through the membrane from the powder without a suppository base, from suppositories containing ethacrynic acid and from suppositories containing the kneaded product of ethacrynic acid + RAMEB in a mole ratio of 1 : 1. Distilled water and pH=7.5 phosphate buffer were used as acceptor phases (Table 2). The suppositories packed in membrane (Visking®) were placed one by one into 20 mL of acceptor phase at body temperature (37 ± 0.5 °C). The samples were exposed to slight horizontal shaking and the entire acceptor phase was changed after 30, 60, 120 and 240 min. The ethacrynic acid contents in the samples were determined spectrophotometrically from the results of five parallel measurements.

Results

Dissolution studies

The pure drug dissolved better in artificial intestinal juice (approximately 50 mg into 900 mL in 90 min) than in artificial gastric juice (7 mg into 900 mL in 90 min). The solubility in artificial gastric juice was increased 5.1–5.8-fold when physical mixtures of CD and the active agent were used (Figure 2).

All the solubility data relating to the preparations were better than those for the pure drug, but no significant differences were observed between the individual preparations. Only slight increases in solubility and dissolution rate were

measured when the CD ratio in the preparations was increased. The highest values were attained for the product containing the drug and the CD molecule in a ratio of 1 : 3.

Increase of the pH of the acceptor phase increased the solubility of the active agent in the case of artificial intestinal juice. Salt formation occurred resulting in a better solubility as compared to that of the acid. This solubility could not be further improved by the presence of CD. Inclusion complex formation can have the opposite effect, the solubility slightly decreasing with increasing CD ratio, as the presence of the inclusion complex hinders salt formation from the drug. This was observed in comparison with the situation experienced with the physical mixtures in artificial gastric juice (Figure 3).

The dissolution of the pure drug from the kneaded products into artificial gastric juice was poor, because of its acidic character. The solubility of the 2 : 1 drug : CD product was only slightly higher than that of the pure pharmac. The solubility was significantly improved and similar for the 1 : 1, 1 : 2 and 1 : 3 products. The highest solubility was measured for the 1 : 3 drug : CD composition, where a 6.4-fold increase was observed. It can be concluded that this method slightly increases the rate of dissolution of the pharmac. The maximum concentration was reached after 15 min for the CD-containing kneaded products, but somewhat later for the physical mixtures. The dissolved drug concentration increased continuously during the experiment (Figure 4).

The dissolution profiles of the four different kneaded products in artificial intestinal juice were quite similar, and did not differ significantly from the values for the pure drug (Figure 5).

It can be stated, therefore, that the solubility-increasing effect of CD depends on the pH of the acceptor phase. The dissolution profile measured in artificial gastric juice leads

Sample: E + RAMEB kneaded products
 Method: Heating rate: 5 C/min
 Comment: Atm.: Argon 10 l/h

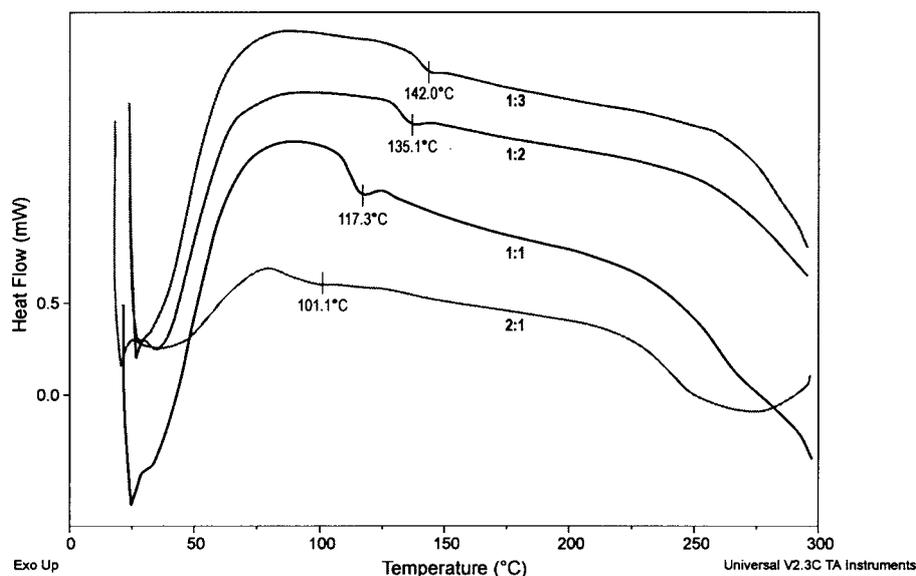


Figure 9. DSC curves of kneaded products.

us to the conclusion that both the preparation method and the product composition influence the amount of drug dissolved and the dissolution rate. The pH of artificial intestinal juice makes salt formation possible, and a further solubility increase by the addition of CD is therefore not possible.

Membrane diffusion examinations

Tables 3 and 4 list the diffusion rate constants, and the diffused drug amounts measured at 150 min. The *in-vitro* diffusion rate of the active agent was 4.513×10^{-3} cm min^{-1} and only 15% of the incorporated drug was diffused after 150 min.

The CD concentration in the physical mixtures significantly changed the diffusion rate in artificial gastric juice: both K_d and the diffused drug amount increased with increasing CD concentration (Table 3, column 1). The best results were observed for the 1:3 composition, where the diffused drug amount had doubled after 2.5 hours.

Artificial intestinal juice slightly reduced the diffusion of the pharmacon; hence, the improved solubility did not mean an increased diffusivity (Table 3, column 2).

The *in vitro* membrane diffusion results on the kneaded products were similar. Increase of the CD content (except for the 2:1 product) led to increases in the diffusion rate constant and in the diffused drug amount in artificial gastric juice (Table 4, column 1).

The diffusion of the CD-containing products into artificial intestinal juice was less than that for the drug itself; only the 1:3 composition gave a better result after 2 hours (Table 4, column 2).

Thermoanalytical investigations

The peak temperature in the DSC plot of ethacrynic acid is the melting point of the drug. On further increase of the temperature, a sustained endothermic peak appeared between 180 and 280°C, which relates to the evaporation and decomposition of the pharmacon (Figure 6). Mass loss started at 180°C, as seen in the TG plot, followed by evaporation and decomposition of the drug. Mass loss was registered up to 340°C.

The water content of RAMEB was revealed by TG to be 2%. No further mass loss was detected in the TG and DSC curves between the water loss and the decomposition. This is an amorphous material; the glass-like state melts on increase of the temperature. Signs indicative of melting are seen at different sites on the DSC, as a consequence of the different degrees of substitution of the molecules (Figure 7). Decomposition started at 250°C, and around 9% of the material had decomposed by 340°C. The water loss from RAMEB took place from room temperature up to 70°C.

An endothermic peak appeared after the water loss in the DSC curve of the 2:1 physical mixture, which is lower by more than 20°C as compared to the peak for pure ethacrynic acid. The background of the 101.7°C peak may be eutectic formation. The situation is similar with other physical mixtures. The water content of the system increased with increasing RAMEB content. The peaks were observed between 90 and 130°C, the peak temperatures varying within ± 1 °C. The area under the peak is proportional to the enthalpy change. The active agent content of the products is proportional to the area under the peak (Figure 8).

The DSC plots of the kneaded products are shown in Figure 9. The second endothermic peak shifts towards higher temperatures with increasing CD content.

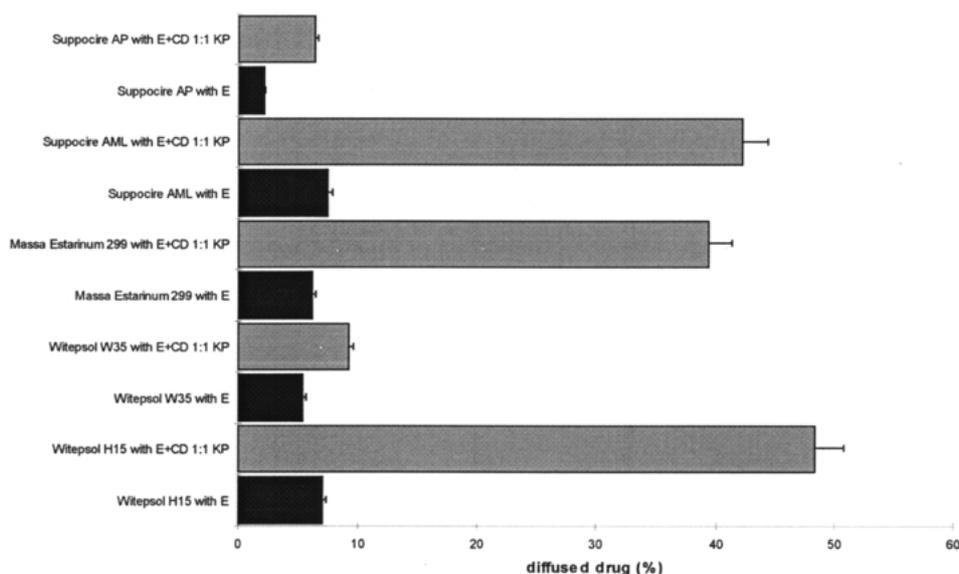


Figure 10. Ethacrynic acid release from different suppository compositions after 240 minutes. Acceptor phase: distilled water.

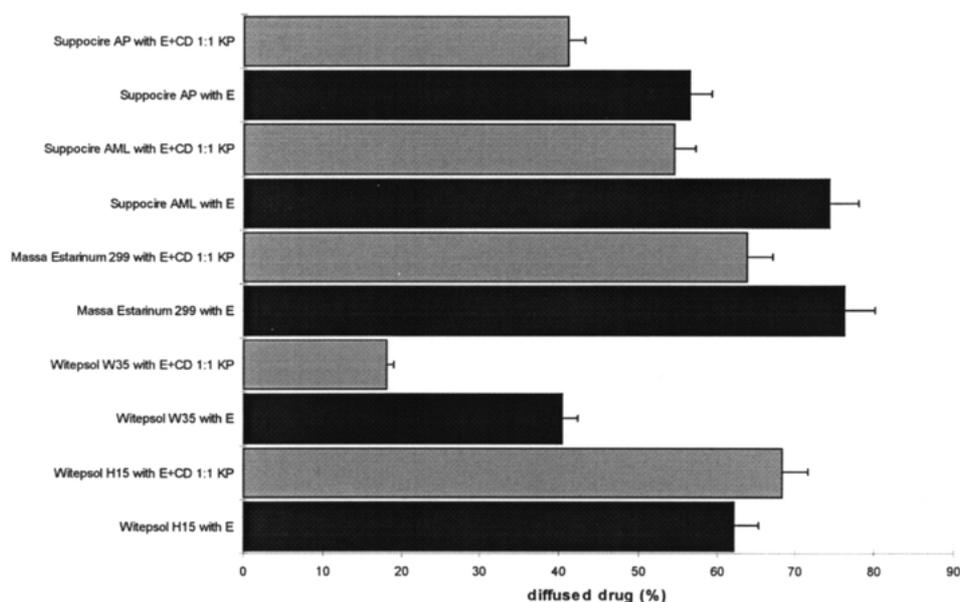


Figure 11. Ethacrynic acid release from different suppository compositions after 240 minutes. Acceptor phase: phosphate buffer (pH = 7.5).

Further compositions (e.g., drug: CD ratios of 4 : 1, 3 : 1, 1 : 4 and 1 : 5) are to be tested for a detailed discussion of this phenomenon. A relationship is proposed between the active agent and CD on the basis of these results. This can be thermally induced complex formation, as a 40 °C peak temperature difference was experienced for the investigated compositions. The mass change starts at 170 °C for the 2 : 1 kneaded product and physical mixture, and their decomposition starts earlier.

Drug release from suppository compositions

Ethacrynic acid and the previously selected ethacrynic acid+RAMEB 1 : 1 kneaded product were incorporated into 5 different lipophilic suppository bases.

The amount of ethacrynic acid released in distilled water was under 10% (Figure 10). This can be explained by the aqueous solubility of the active agent, resulting in an unsatisfactory liberation from lipophilic suppository bases. Witepsol H 15 and Suppocire AML afforded the best results as concerns the investigated suppository bases. The diffusion of the pharmacion from all the suppository bases was higher when the CD complex of ethacrynic acid was used. A 10-fold increase in liberation was experienced in the cases of Witepsol H 15, Suppocire AML and Massa Estarinum 299.

The solubility of ethacrynic acid increased with increase of pH of the acceptor phase, and so did the diffusibility through the membrane (Figure 11). The best suppository bases in the distilled water experiments (Witepsol H15, Suppocire AML and Massa Estarinum 299) were also the best in the phosphate buffer medium. The diffusion results for

the suppositories containing CD complex were poorer than those for the suppository containing pure ethacrynic acid, which can be explained by the higher solubility of ethacrynic acid in the phosphate buffer. The rectal pH range is 6.8–8. As the liberation and diffusion of the active agent are pH-dependent processes, the diuretic effect can fail if the rectal pH lies out of the physiological range. The CD complex of ethacrynic acid was found to be appropriate for the production of suppositories. Witepsol H15 containing the CD complex gave the best results, which was independent of the pH of the surrounding media.

Conclusions

The results of our investigations may be summarized as follows:

1. The ethacrynic acid solubility-increasing efficiencies of the different CD derivatives were determined; RAMEB was selected for further experiments.
2. Drug CD mole ratio compositions of 2 : 1, 1 : 1, 1 : 2 and 1 : 3 were prepared with the selected CD derivative by kneading and physical mixing.
3. The dissolution profiles of the products were measured by the rotating basket method. A relationship was found between the amount of drug dissolved, the dissolution rate and the pH of the acceptor medium, and the preparation methods. The highest solubility increase was achieved for the kneaded products in artificial gastric juice, where the amount of drug dissolved from the 1 : 3 composition was 6.4-fold as compared to that for the pure pharmacon. The composition of the products had no significant influence on the amount of drug dissolved in artificial intestinal juice.
4. Significant increases in the diffusion rate constant and the diffused drug amount were measured under in vitro conditions for artificial gastric juice, with either physical mixtures or kneaded products.
5. The membrane diffusion rate varied only slightly in artificial intestinal juice.
6. The TG, DTG and DSC plots of the products were analysed. The DSC curves proved most informative. The drug peak is clearly seen in the curves of the different physical mixture compositions. The area under the peak is proportional to the active agent content of the product. The peak relating to the drug is lower by more than 20 °C as compared to that for the pure drug, indicating possible eutectic formation. The temperature of the second peak for the kneaded products is shifted towards higher temperatures, depending on the active agent content. These results can stem from an interaction between the two components; clarification is planned with further compositions and preparation methods.

7. The 1 : 1 kneaded product was selected for further investigations on the basis of the dissolution and in vitro membrane diffusion results. This high active agent-containing composition with improved solubility and diffusivity is suitable for incorporation into lipophilic suppository bases.
8. The diffusion of the active agent from lipophilic suppository bases into distilled water was improved significantly after complexation with CD. The diffusion of the CD complex-containing product decreased on increase of the pH of the acceptor phase, resulting in poorer data than those for the pure drug. A correlation can be found between these results and the experiments detailed in point 5 between the solubility of the active agent, the pH of the acceptor phase, the dissolution from the suppository base and the membrane diffusion. On the basis of the pH-independent diffusion studies, Witepsol H15 is recommended for the incorporation of the 1 : 1 kneaded product of ethacrynic acid + RAMEB.

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