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

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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 hypochloraemia, 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 pharmacons 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.

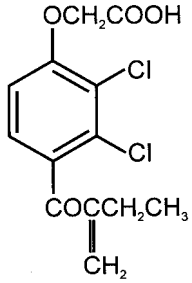

Figure 1. Chemical structure of ethacrynic acid.

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, glycocoll, 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 μg 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.

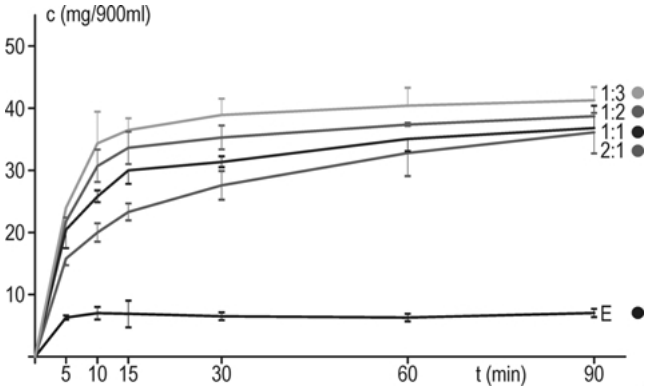

Figure 2. Dissolution of ethacrynic acid from physical mixtures (artificial gastric juice).

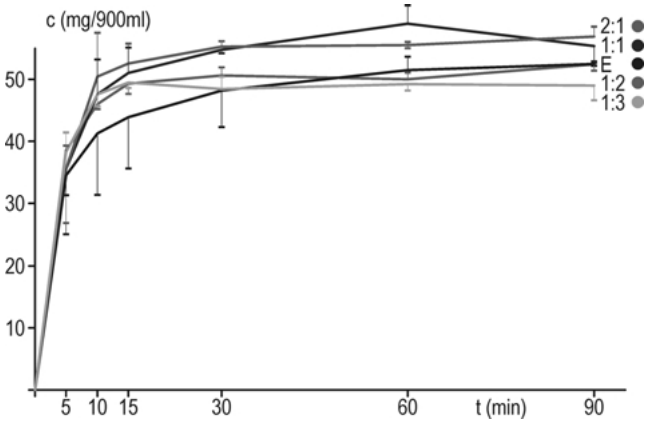

Figure 3. Dissolution of ethacrynic acid from physical mixtures (artificial intestinal juice).

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-

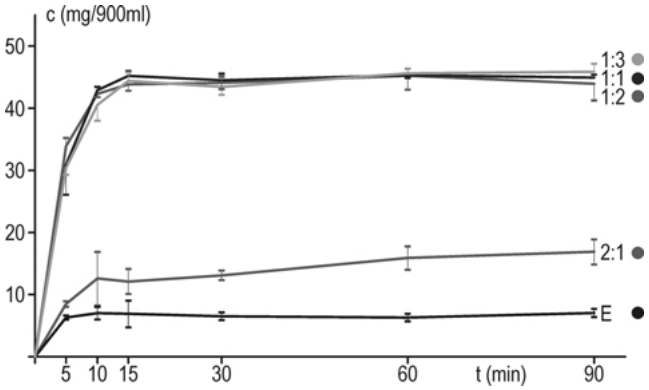

Figure 4. Dissolution of ethacrynic acid from kneaded products (artificial gastric juice).

Table 2. Composition of artificial juices

		Gastric juice	Intestinal juice	Plasma	Phosphate buffer
pH (±0.1)		1.1	7.0	7.5	7.5
1N HCl	(g)	94.0	–	–	–
NaCl	(g)	0.35	–	–	–
Glycine	(g)	0.50	–	–	–
$Na_2HPO_4 \cdot 2H_2O$	(g)	–	14.4	20.5	–
KH_2PO_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}) [cm/min]	*S*	Diff. %	K_d (10^{-4}) [cm/min]	*S*	Diff. %
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.

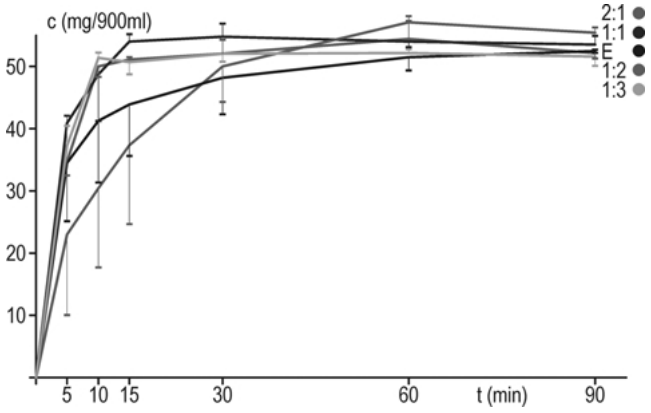

Figure 5. Dissolution of ethacrynic acid from kneaded products (artificial intestinal juice).

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:

$$K_d = \frac{c_{II_2} - c_{II_1}}{T_2 - T_1} \cdot \frac{1}{c_{I_0}} \cdot \frac{V_{II_0}}{F} [\text{cm min}^{-1}]$$

where c_{IIx} is the corrected drug concentration in phase II at time T_x (mg mL^{-1}); V_{II_0} 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_{I_0} 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]	*S*	Diff. %	K_d (10^{-4}) [cm/min]	*S*	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.

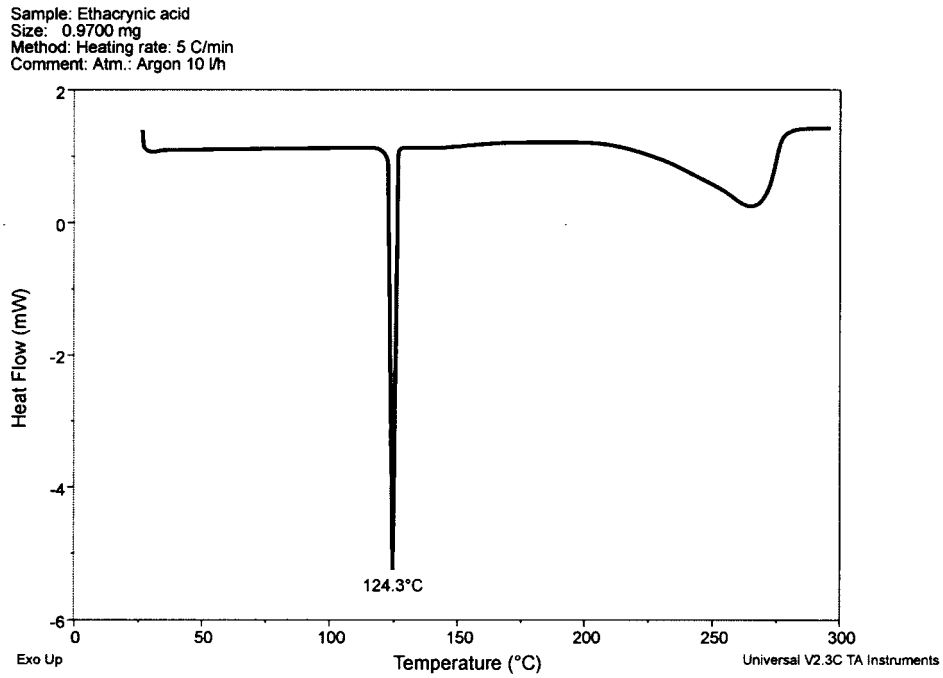

Figure 6. DSC curve of ethacrynic acid.

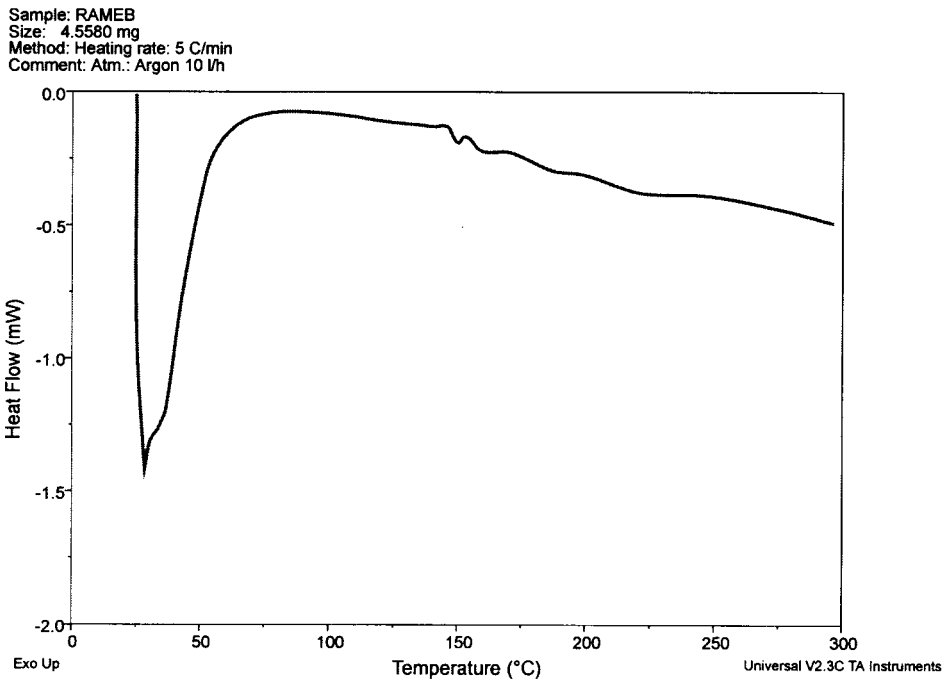

Figure 7. DSC curve of RAMEB.

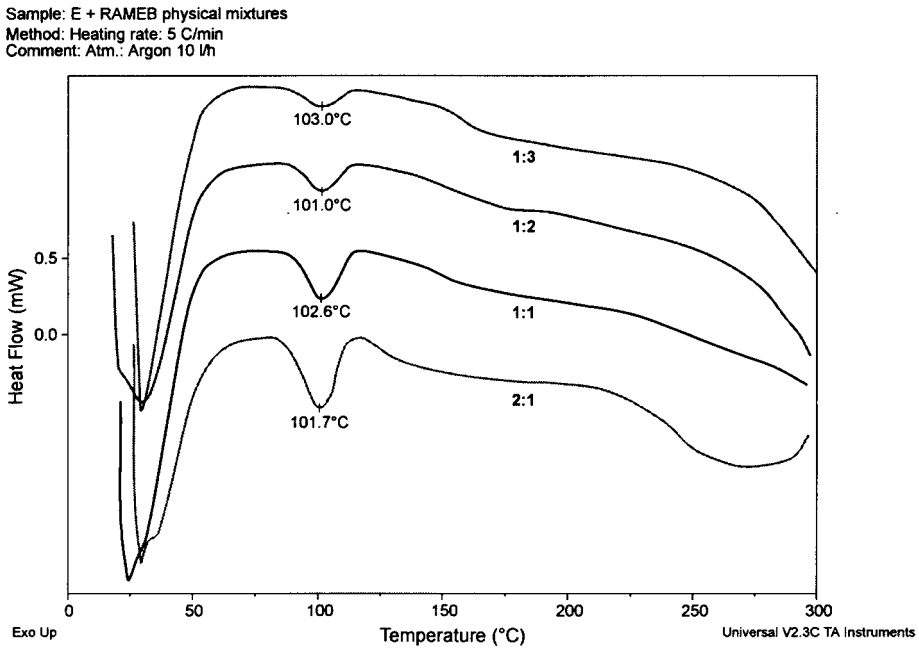

Figure 8. DSC curves of physical mixtures.

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 pharmacon. 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 pharmacon. 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

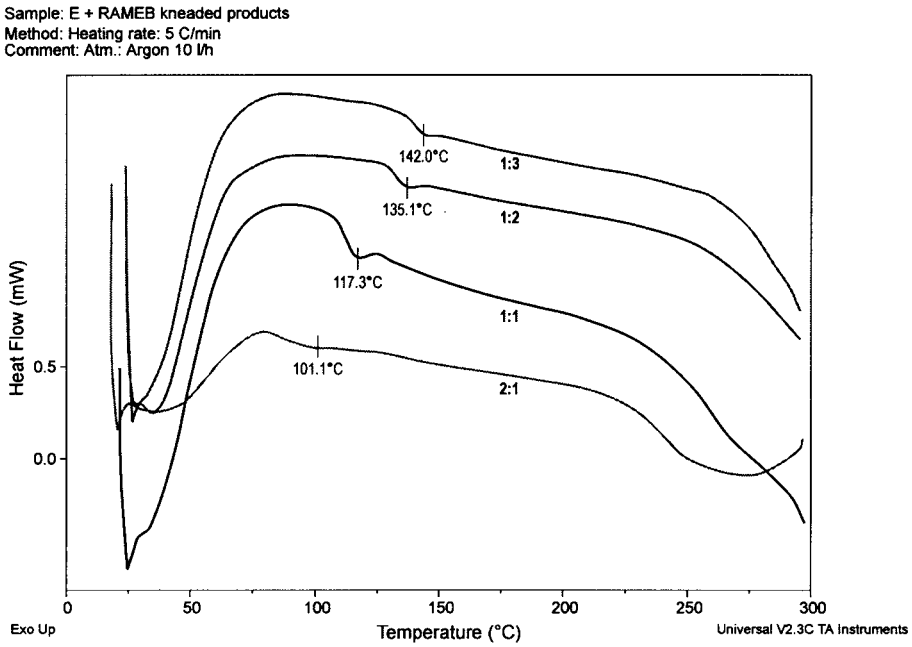

Figure 9. DSC curves of kneaded products.

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.

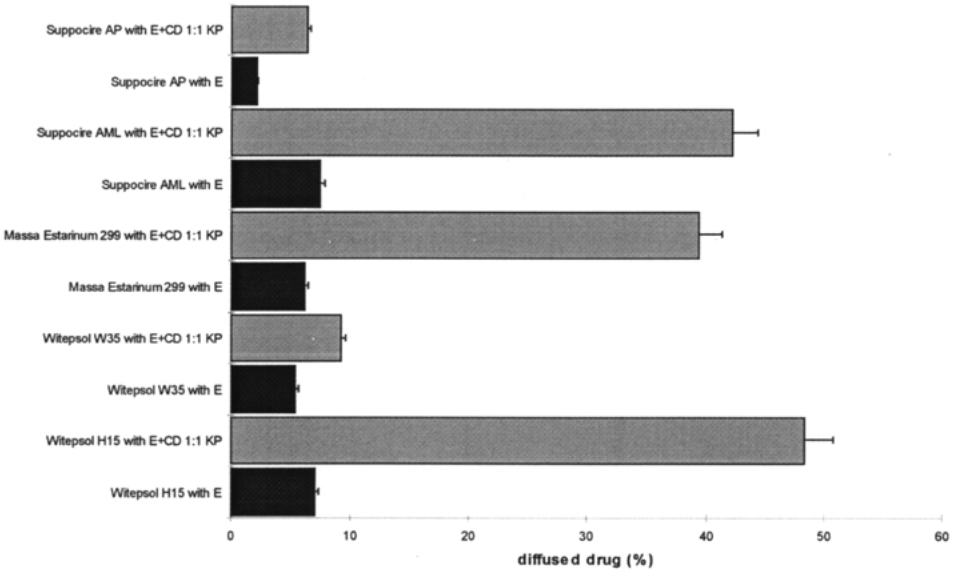

Figure 10. Ethacrynic acid release from different suppository compositions after 240 minutes. Acceptor phase: distilled water.

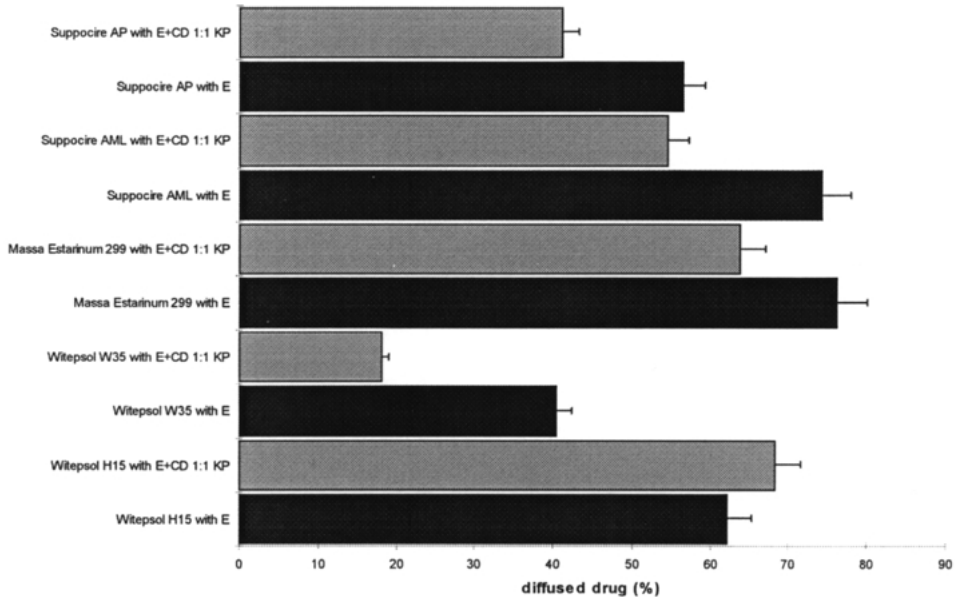

Figure 11. Ethacrynic acid release from different suppository compositions after 240 minutes. Acceptor phase: phosphate buffer (pH = 7.5).

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 pharmacon 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.

Acknowledgements

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