University of Szeged, Faculty of Pharmacy
Department of Pharmaceutical Technology
Head: Prof. Dr. Habil. István Erős Ph.D., D. Sc.

PhD Thesis

Formulation of rectal suppositories containing diuretic drugs and their biopharmaceutical studies

By
Szilvia Berkó

Supervisor:
Dr. Géza Regdon jr. Ph.D.
Assistant Professor

Szeged
2002

TABLE OF CONTENTS

1. INTRODUCTION

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 [1-4]. 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 [5-10].

In view of the above the future objective of research can be to formulate a diuretic rectal suppository of proper biological effectiveness, which is missing from present pharmaceutical trade in spite of the fact that internists expressed concrete therapeutic need for the formulation of a rectal preparation containing furosemide. Regdon et al. were the first to deal with this task [11]. 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 oral and intravenous administration should be avoided (vomiting, shock, patient with bad compliance, injury of oesophagus, diseases of liver).

2. LITERATURE SURVEY

2.1. Rectal absorption

The lowest section of the intestinal tract is the 16-20 cm-long rectum, which is moistened by about 1-3 ml mucus and the pH value of which varies between 6.8-7.9 [12-13]. Anastomoses are found between the arteries and veins of the rectal area. The absorbed drug is transported by blood in two different directions. From the anal region the absorbed drugs enter the blood circulation bypassing the liver, which yields useful advantages in certain cases: on the one hand the onset of the effect is very rapid, it can equal even the speed of an intravenous injection [14-15], and on the other hand drugs enter the organism bypassing the first-pass metabolising effect of the liver, which can be a therapeutic advantage in the case of liver diseases and also in the case of drugs which are biotransformed by the liver into ineffective products [16-18]. Drugs absorbed from the upper part of the rectum enter the circulation

through the liver, thus the rate and intensity of the effect of the administered drugs can be characterized similarly to oral administration [12, 19-20].

In view of the fact that the melted (or dissolved) rectal suppository spreads in the rectum, the lower few centimetres of which are not separated sharply from the pelvic upper part with respect to blood paths either, if a drug is administered rectally into the body, the rate of a drug administered in the form of an intramuscular injection can be expected [12-13, 21].

2.2. Biopharmacy of the suppositories

Modern drug administration today requires that not only the quantity and stability of the active agent have to be ensured but the subsequent fate of the administered drug should also be known in the organism. According to Ritschel's definition [22] biopharmacy deals with the physical and chemical properties of both drugs and drug preparations, as well as with the biological effectiveness after application, that is with the availability of the drug from a given dosage form in the human or animal organism.

Similarly it was Ritschel who pointed out the importance of liberation [21]. Absorption and thereby therapeutic effect can take place only after liberation. Thus it is indispensable to be familiar with the factors influencing drug liberation, the major ones of which are the following [22-25] :

- the properties of the drug to be used (chemical structure, solubility, particle size, polymorphy, etc.)
- properties of the vehicle (melting point, lipophilic or hydrophilic nature, spreadability, HLB value, hydroxyl value, etc.)
- use of various additives (additives increasing solubility, viscosity, melting point, consistence softening additives, etc.)
- the relationship of the drug with the vehicle (solubility, distribution quotient, dissolved or suspended form, concentration used, interactions)
- properties of the medium used (pH, temperature, quantity, solubility distribution between the acceptor phase and the base).

Thus it must be emphasized that the fate of the drug in the living organism depends largely on the dosage form and on the production technology used. The primary task of pharmaceutical technology is to select the bases and additives which suit the biopharmaceutical purpose the best and also work out the best composition from among the available and ever-increasing choice [26-28].

2.3. Rectal route of drug application nowadays

Rectal drug administration has undergone major changes for the last decades, which is partly shown by the increased number of rectal dosage forms. In Ph. Eur. 3 and in Ph. Eur. 4 official as of 2002 the following rectal dosage forms are official: suppositories, rectal capsules, rectal solutions and suspensions, powders and tablets for rectal solutions and suspensions, semi-solid rectal preparations, rectal foams, rectal tampons [29-30].

In addition to the traditionally formulated rectal suppositories, rectal drug carrier systems providing *sustained effect* [14, 31-33] and *controlled drug release* [34-37] have come to the foreground. Several studies have reported *layered suppositories* [38-39], *coated suppositories* [40], *Suppo-Kap* [41-43] and rectal dosage forms such as:

- *"thermo-reversible liquid suppositories"* which are easy to administer to the anus, since they are in a liquid form at room temperature and turn into a gel instantly at physiological temperature and are also mucoadhesive to the rectal tissues without leakage after the dose [36, 44-45],
- *"solid-reversed-micellar-solutions (SRMS) suppositories"* after contact with water or any physiological aqueous media exhibit an application induced transformation into a semisolid system of liquid crystalline microstructure [34],
- *"pre-microemulsified suppositories"* are mixtures of oil, surfactants and co-surfactants, they are solid at room temperature, and they self-emulsify into water (at 37 °C) under moderate stirring [46].

Moreover, in addition to the frequently used traditional active agent groups (antipyretic, analgesic, spasmolytic, antiemetic, purgative, anti-haemorrhoidal), several new drugs have proved to be suitable for rectal administration **(Table 1)**.

Table 1 Some drugs used rectally [13,48]

Therapeutic indications, purpose of effect	Active agents	Literature sources
Narcotics	Morphine Hydrochloride, Pethidine, Methadone	35, 48-51
Cardiacs	Nitroglycerin, Lidocaine	14, 52
Hormones	Progesterone, Testosterone, Insulin, hCG	44, 53-59
Diuretics	Furosemide, Spironolactone	11, 60-61
Anticoagulants	Heparin	62
Antihypertensive	Propranolol	45, 63-64
Antibiotics	Beta-lactam antibiotics, cephalosporins	65-66
Chemotherapeutics	Amoxicillin, Ampicillin, Chloramphenicol, Erythromycin, Gentamicin, Trimethoprim, Sulfonamide derivatives, Metronidazole	67-71
Anxiolytics	Diazepam, Nitrazepam	72-75

2.4. Influencing factors of drug liberation and rectal absorption

2.4.1. Properties of suppository bases

Drug liberation can be greatly enhanced, decreased, delayed or almost entirely prevented by the vehicle. [12-13]. For this reason the choice of the proper vehicle is of primary importance in developing a new suppository composition with a given drug.

Hydrophilic (water-soluble) and lipophilic (water-insoluble) suppository bases are distinguished according to solubility.

Water necessary for dissolution is very often taken by the *hydrophilic* base from the surrounding interstitial space. This process is on the one hand opposite to absorption, and on the other hand it can induce local irritation and thereby a stimulus of defecation. Therefore hydrophilic suppositories cannot be expected to give a rapid effect. Their application is indicated in cases when this base is specifically prescribed by the doctor, is recommended by FoNo or when so-called tropics-resistant suppositories are formulated [76-77].

The melting of *lipophilic* suppository bases is a faster process. Besides the melting point, the rheological behaviour of the melt is extremely important as it determines the extent of spreading and consequently the area of the contact surface with the rectal fluid. With respect to absorption, the HLB value of the suppository base is also essential, absorption is generally worse from a purely triglyceride base than from a base which contains a certain amount of monoglyceride, too.

The active agent can be present in both types of bases in a dissolved or suspended form. Experiments have shown that in the case of both bases faster drug release can be expected from the suspension from as here passing from the base to the rectal fluid takes place depending on the distribution coefficient.

Further requirements expected from an „ideal" suppository base are listed by Rácz and Selmeczi [12, 78], such as e.g. that it should melt under 37°C or dissolve in the rectal juice, it should solidify fast, should not have a polymorphic modification, should have proper viscosity, etc. Unfortunately, the available choice of Hungarian suppository bases is very limited, there are only 5 official bases partly in the pharmacopoeia [79] and partly in the FoNo [80]. In the fourth enlarged edition of Fiedler published in 1996 [81] approximately 200 suppository bases are mentioned. Therefore it is worth obtaining information about other suppository bases widely used in European countries, such as e.g. about the French Suppocire or German Witepsol products.

2.4.2. Effect of surfactants

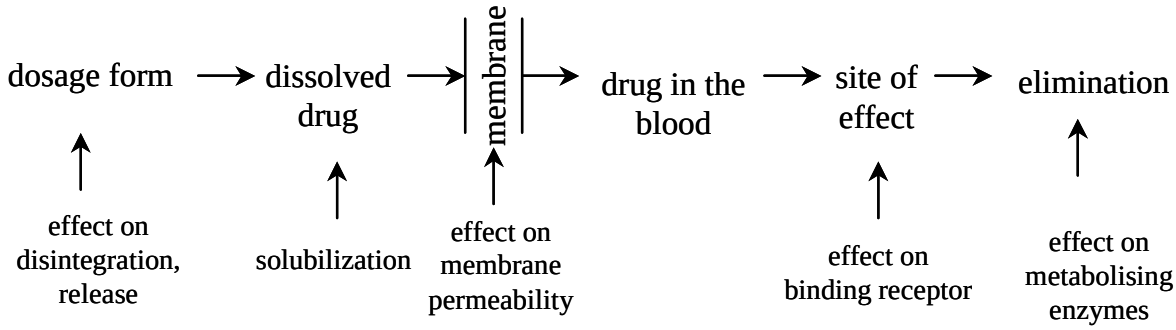
Lipophilic suppository bases can be turned into lipohydrophilic by adding a few per cent of surfactants. Their characteristic property is that they do not dissolve in water but they

moisten. The surfactant component has a favourable effect on consistency, shortens the disintegration time and frequently accelerates drug liberation, which is mainly due to the change in the moistening ability of the drug, and at the same time the spreading of the melt on the rectal mucosa is also influenced favourably [82-83].

The rate of absorption is usually enhanced, too, with the exception of cases when large-molecular surfactants form a stable complex with the drug molecule, or if the dissociation conditions change unfavourably due to the effect of the additive. Besides the moistening of the drug, there can be two reasons for the increase of the absorption rate by surfactants: one reason is that they denature the proteins on the intestinal mucosa and thus disrupt its integrity, the other is that by cleaning the surface of the mucosa they increase the number of the absorption places **(Fig. 1)** [12, 84-87].

However, some of their unfavourable properties must also be mentioned. Surfactants are not inert biologically, if their concentration is too high, they may damage epithelial cells. They may cause irritation and topical hyperaemia, which may lead to a stimulus of defecation or to effects on absorption. However, today fourth-generation non-ionic surfactants are available (BASF, SEPPIC products), which have a negligible mucosa-irritating effect [88-89].

Fig. 1 Effect of surfactants



2.4.3. Increase of drug solubility by cyclodextrin

The solubility of the drug is a decisive factor with respect to liberation and absorption, therefore it may be important to make substances insoluble or poorly soluble in water to be soluble. [2, 90-91]. One possible method for this is to form inclusion complexes. Inclusion

complexes are molecule compounds in which the molecule of the other component (guest substance) is found in the inner cavity of the structure carrier (host molecule) without any change in the structure of the latter one. The size and shape of the available cavity typically remains unchanged. The best-known host molecules are cyclodextrins (CD). CDs can form an inclusion complex with every compound which has a molecular size suitable for the size of the cavity. Complexes can be formed with larger molecules, too, but in this case only a certain group of the molecule or its side chain is built into the cavity.

The principle of complex formation with cyclodextrins is the following: the outer surface of the CD ring is polar and as such is surrounded by a hydrate envelope in an aqueous medium. However, due to the apolar cavity wall, the water molecules in the ring cavity are in an unfavourable energetic situation. During complex formation the apolar part of the guest molecule enters the cavity of the ring and thus an apolar-apolar interaction results, which is more favourable energetically. The dissolution rate largely depends on the crystalline structure of the substance and on the size of the crystal. The cyclodextrin complexes of substances which have poor water solubility and consequently are absorbed to a limited extent can be dissolved better because the complex separating the molecules of the drug has to be broken during dissolution. This represents a much weaker bond than the crystal lattice of the drug [92-94].

2.4.4. Influence of pH change on drug release

In the gastrointestinal tract the pH increases gradually from the stomach towards the rectum. The pH of the fluid in the lumen of the rectum ranges between 6.8-7.9 [12-13]. However, experimental results indicate that immediately before the epithelic membrane a mucus layer of pH 5.4 is found, which the drug has to pass through in order to be absorbed [13]. The distribution behaviour of substances with various pK values and the laws of their passing through the membrane can be explained only if the pH of the membrane is 5.4. The buffer capacity of the rectal fluid is small so substances with an acidic or alkaline character can change the pH of the rectum, which may elicit irritation and may influence absorption.

75 % of the materials available in commerce is a weak base, 20 % is a weak acid and 5 % has a non-ionic, amphoteric character, thus the bioavailability of the majority of drugs depends on the pH of the site of application to a great extent. The solubility and membrane permeability of the drug is decisive with respect to bioavailability [82, 91].

The proportion of the dissociated and non-dissociated forms of weak acids and bases is determined by the dissociation constant and by the pH of the medium according to the Henderson-Hasselbach equation [12]:

1. For weak acid

$$pH - pKa = \log \frac{\text{(dissociated form)}}{\text{(non-dissociated form)}} \qquad (1)$$

2. For weak base

$$pH - pKa = \log \frac{\text{(non-dissociated form)}}{\text{(dissociated form)}} \qquad pKa = 14 - pKb \qquad (2)$$

The solubility of a slightly acidic substance can be increased with pH increase as the dissociation equilibrium is shifted towards the dissociated form. In the stomach, however, it is present mainly in a non-dissociated form, its solubility is generally poor, but it has good permeability. In this case the decrease of the particle size can improve solubility and thereby increase the quantity of the drug available for absorption. After entering the duodenum, its solubility is considerably improved as a result of salt formation, but at the same time the absorption ratio will decrease due to the pronounced ionic character, as only a small part of the drug molecules is present in a non-dissociated lipoid-soluble form.

In view of the above the drug with a weak acidic character can be expected to have good solubility and bad permeability on the rectal pH, while weak bases can be expected to exhibit bad solubility and good absorption. However, it must be pointed out here that the pH of a given absorption area may be different from the general pH of the content of the gastrointestinal tract. Consequently, absorption from an ionic solution is not necessarily so limited as could be expected according to the principle of pH distribution [12, 84].

2.5. Examination of suppositories

Table 2 presents the examinations concerning the rectal preparations official in the Hungarian Pharmacopoeia VII [79] and in the fourth edition of the European Pharmacopoeia [30] published in 2002:

Table 2 Official pharmacopoeial examinations of rectal suppositories

Ph. Hg. VII. (1986)	Ph. Eur. 4. (2002)
Quality requirements	
♦ Dosage form examination ♦ Individual and average mass ♦ Composition examination	♦ Uniformity of content ♦ Uniformity of mass
Methods for controlling physical parameters	
♦ Melting point ♦ Drop point ♦ Solidification temperature ♦ Penetrometric examination	♦ Melting point ♦ Drop point ♦ Freezing point ♦ Measurement of consistency by penetrometry
Biopharmaceutical examinations	
♦ Dissolution, melting	♦ Disintegration test ♦ Dissolution test

The comparison of the data of the two pharmacopoeias reveals that only the biopharmaceutical examinations have been extended. The in vitro determination of drug liberation was not official in the Hungarian Pharmacopoeia VII although various models have been set up based on different principles for studying the influence of various bases and additives on drug liberation during formulation (agar diffusion method, test tube shaking method, membrane diffusion method, Cox model, Dibbern-Wirbitzki rotation cell, flow-through cell method, etc.) [13, 95-96]. All of them essentially determine the first kinetic concept of the LADMER system, that is liberation, according to Ritschel's viewpoints. From

among them the flow-through cell method is recommended by the fourth edition of the European Pharmacopoeia for the examination and classification of rectal suppositories [30].

The examination of drug liberation forms part of the biopharmaceutical assessment of suppositories but the results published in various sources of literature cannot be compared even if the drug is the same. Yet, as biopharmaceutical preliminary experiments, they can give reliable predictions about in vivo results [13, 97-100].

The release apparatus used for determining the active agent liberated from various suppository bases is presented by Bornschein et al [101] and the in vitro / in vivo correlation coefficients are determined. They found that the applicability order of suppository bases for a given drug can be given with proper in vitro examinations. The correlation of in vitro / in vivo results was reported by Regdon et al in several publications, e.g.: sodium-salicylate [102-103], sulphadimidine [104], theobromine-sodium-salicylate [61], diazepam [105-106]. They found that in vitro examinations constituted proper grounds for choosing the suppository bases which later gave good in vivo results. **(Table 3)**.

Table 3 Possibilities for determining bioavailability [22]

Phenomenon	Method	Example
1. liberation and dissolution	rate of dissolution	in vitro: water, buffer, artificial gastric juice, saliva, rectal fluid
2. free drug in the systemic circulation	blood level curve, blood level peak, time to reach this, AUC	in vivo: whole blood, plasma, serum
3. pharmacological effect	onset of effect, duration of effect, intensity of effect	in vivo: distinctive measurement of pharmacological effect
4. clinical response	controlled clinical blind or double blind test	in vivo: evaluation of clinical response
5. elimination	entire quantity of the selected drug	in vivo: urine

3. AIMS

1. In order to extend the therapeutic possibilities, the formulation of diuretic rectal suppositories from which the liberation and absorption of the two studied active agents (ethacrynic acid and furosemide) is to the greatest extent possible.

2. Formulation of the active agents in suppository bases with various physical-chemical properties, examination of several vehicles not official but obtainable in Hungary, such as e.g. Witepsol bases (CONDEA Chemie GmbH) or Suppocire products (Gattefossé).

3. Examination of in vitro drug release as the function of the pH of the acceptor phase.

4. Improvement of drug liberation by adding various surfactants, with special respect to examining how the concentration of the additives influences in vitro drug liberation.

5. Examination of in vitro drug liberation of the ethacrynic acid+cyclodextrin inclusion complex with good water solubility, comparison of the results with those of poorly water-soluble ethacrynic acid.

6. Determination of the in vitro / in vivo correlation of furosemide in the case of rectal suppositories.

4. EXPERIMENTAL WORK

4.1. Materials

4.1.1. Active agents

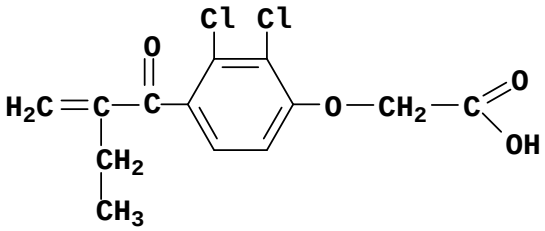
Ethacrynic acid and furosemide, which belong to the group of loop-diuretics, are very effective (high-ceiling) in draining all kinds of oedemas (of cardiac, hepatic or renal origin), in mild or moderate hypertension (in itself or combined with other antihypertensive drugs), or used in greater doses in acute and chronic renal failure, in oliguria [84]. Loop diuretics block the $Na^+/K^+/2Cl^-$ carrier at the luminal side, thus inhibiting the absorption of sodium, potassium and chloride ions in the thick ascending limb of the loop [107]. Currently they are available as oral and parenteral solutions, tablets, capsules or granules for oral administration [108].

ETHACRYNIC ACID (UREGYT®) [109]

Physical properties: white or almost white, odourless, crystalline powder, very slightly soluble in water

Chemical structure **(Fig. 2***)*: phenoxyacetic acid derivative, weak acid, pKa=3.5 [12]

Fig. 2 Structural formula of ethacrynic acid



Dosage form: tablet, injection

Dose: 50 mg - 200 mg

Pharmacokinetics: The absorption of ethacrynic acid is very rapid. When administered orally, its effect can be detected in half an hour and it lasts for 6-8 hours, with intravenous administration the onset of the effect is within 5 minutes and lasts for 2 hours. It is largely

bound to proteins, it is secreted by the proximal tubules of the kidney. Animal experiments have confirmed that it is decomposed into its active metabolite in the liver. Its 30-65 % is excreted in the urine, 35-40 % is secreted in the bile in the form of active metabolite.

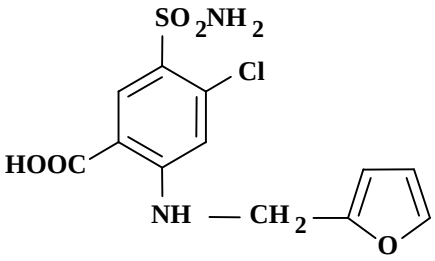
When given orally, ethacrynic acid may cause watery diarrhoea and other gastrointestinal disturbances. Gastrointestinal bleeding occurred in some patients during intravenous therapy. For example abnormal results of liver function tests have been reported rarely [107].

FUROSEMIDE *(*FURON®, FUROSEMID PHARMAVIT®, FUROSEMID®, HUMA-SEMIDE®) [109]

Physical properties: white or slightly yellow, odourless, almost tasteless crystalline powder, practically insoluble in water

Chemical structure **(Fig. 3)***:* sulphonamide, weak acid, pKa=3.9 [12]

Fig. 3 Structural formula of furosemide



Dosage form: tablet, injection, infusion

Dose: 20 mg - 600 mg

Pharmacokinetics: it is absorbed from the gastrointestinal tract extremely well, diuresis arises within 30 minutes after oral administration and its effect lasts for 6-8 hours. With intravenous administration diuresis is elicited within 5 minutes, peak diuresis is reached in 30 minutes and the diuretic effect lasts for about 2 hours. It circulates in the blood bound to proteins (mainly to albumin). The half-life of furosemide is approximately 2 hours, it is excreted rapidly with glomerular filtration and tubular secretion.

The bioavailability of oral furosemide is 60% to 69% in normal subjects but is reduced to 43% to 46% in patients with end-stage renal disease. Some generic products may show lower

bioavailability. Food slows the rate of absorption but does not alter the total amount of furosemide absorbed [107, 110-111].

4.1.2. Suppository bases

Table 4 contains the properties of Witepsol and Massa Estarinum type bases produced by the German CONDEA Chemie GmbH and those of the Suppocire suppository bases of the French Gattefossé. More than 20 types of Witepsol suppository bases are commercially available in Germany, while in Hungary only Witepsol W 35 and Massa Estarinum 299 are official from among them under the name of "Adeps solidus 50" and "Adeps solidus 3". Adeps solidus compositus is a lipohydrophilic suppository base official in FoNo VI, it contains not only Witepsol W 35 base but Polysorbatum 20 and Polysorbatum 61 as well in a concentration of 10 % each. Macrogolum 1540 is a suppository base official in Ph.Hg. VII.

4.1.3. Surfactants

Four surfactants were tested for enhancing the liberation of poorly water-soluble drugs. Solutol HS 15, Cremophor RH 40, Cremophor RH 60 (BASF, Germany) Montanox 60 DF (SEPPIC, France) non-ionic surfactants were added to 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 and emulsification. 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, cosmetics water-soluble and to improve bioavailability in solid dosage forms. The Montanox products are used to obtain oil/water emulsion, for the dispersion or solubilization of essential oils or vitamins, for some problems of gelification, in cosmetic and the pharmaceutical industries **(Table 5)**.

Table 4 Property of suppository bases [79-80, 112-113]

Name of bases	Chemical description	Melting range (°C)	Hydroxyl value	Function
Witepsol® H 15	Triglycerides (C_{10}-C_{18})	33.5-35.5	5-15	lipophilic base
Witepsol® S 58	Higher proportion of mono- and diglycerides (C_{10}-C_{18}) with the presence of cetostearyl alcohol	31.5-33	60-70	lipophilic base
Witepsol® W 35	Higher proportion of mono- and diglycerides (C_{10}-C_{18})	33.5-35.5	40-50	lipophilic base
Adeps solidus compositus	Witepsol W 35 with the presence of two non-ionic emulsifying additives	32-36	50-60	lipohydrophilic base
Massa Estarinum® 299	Triglycerides (C_{10}-C_{18})	33.5-35.5	max. 5	lipophilic base
Massa Estarinum® B	Higher proportion of mono- and diglycerides (C_{12}-C_{18})	33.5-35.5	20-30	lipophilic base
Massa Estarinum® BC	Higher proportion of mono- and diglycerides (C_{12}-C_{18})	33.5-35.5	30-40	lipophilic base
Suppocire® AML	Triglycerides (C_8-C_{18}) with the presence of a phospholipid	35-36.5	max. 6	lipophilic base
Suppocire® AP	Saturated polyglycolysed glycerides	33-35	30-50	amphiphilic base
Suppocire® AS_2X	Higher proportion of mono- and diglycerides (C_8-C_{18}) with the presence of a non-ionic emulsifying additive	35-36.5	15-25	lipophilic base
Macrogolum 1540	Polyethylen glycol (n 28-36)	**Solidification range (°C)** 40-50	70-80	hydrophilic base

Table 5 Property of surfactants [88-89]

Name of surfactants	Chemical description	Solidification range (°C)	HLB value	Hydroxyl value	Function
Solutol® HS 15	Polyethylene glycol 660 12-hydroxystearate	25-30	about 15	90-110	non-ionic solubilizer
Cremophor® RH 60	Polyoxyl 60 hydrogenated castor oil	20-28	15-17	50-70	non-ionic solubilizer and emulsifier
Cremophor® RH 40	Polyoxyl 40 hydrogenated castor oil	20-28	14-16	60-75	non-ionic solubilizer and emulsifier
Montanox® 60 DF (DF= less than 3 ppm 1-4 Dioxane and less than 1 ppm ethylene oxide)	Polysorbate 60 (Polyoxyaethylated sorbitan monostearate)	—	14.9	81-96	non-ionic emulsifier

4.1.4 Random-methyl-β-cyclodextrin (RAMEB)

β-cyclodextrin is cyclic, non-reducting oligosaccharide built up from seven glucopyranose units. Degree of substitution: DS: ~ 12-13 methyl groups / CD ring.

Formula: $C_{55}H_{95}O_{35}$
Molecular Weight: 1318.4
Appearance: White or slightly yellow powder
Melting point: 177-182 °C
Solubility: (in 100 cm^3 solvent, at 25 °C)

Water	>40 g
Methanol	>25 g
Chloroform	>25 g
Acetone	< 5 g

Cavity diameter: 0.78 nm
Diameter of outer periphery: 1.53 nm
Height of torus: 0.78 nm
Number of water molecules filling the cavity: 11
Crystal water content: 13.2-14.5 % [114].

4.2. Methods

4.2.1. Preparing of the cyclodextrin (CD) complexes

The effect of the different CD derivatives on the solubility of ethacrynic acid was determined: a mixture of 0.1 g of ethacrynic acid and 0.5 of CD derivative diluted to 20.0 g with water was stirred for 10 min with a magnetic mixer. Suspension systems were filtered through filter paper and, after suitable dilution, the UV spectra were recorded. A system without CD was used as a control. Dimethyl-β-CD, methyl-β-CD, random-methyl-β-cyclodextrin (RAMEB) had the highest influence on the solubility of the active agent.

RAMEB was chosen for further examinations on the bases of the costs and the solubility-increasing effect: the solubility was increased by a factor of 9.33 [115].

The two-component products were prepared in four different mole ratios (drug:CD mole ratio = 2:1, 1:1, 1:2 and 1:3) The ethacrynic acid content of the products was 35.91%, 21.88%, 12.29% and 8.54%.

Physical mixture: The ground components were mixed in a mortar and sieved through a 100 μm sieve.

Kneaded products: Physical mixtures of the drug and CD 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.

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

The 1:1 kneaded product was selected for further investigations on the bases of the dissolution and in vitro membrane diffusion results. This high active agent-containing composition with improved solubility and diffusibility is suitable for incorporation into lipophilic suppository bases [115].

4.2.2. Suppository formulation methods

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 50 mg drug. For the animal experiments 0.3 g suppositories were prepared, 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 %. Suppositories were stored under normal conditions at room temperature and examined after one week.

4.2.3. In vitro release study

Experiments were performed with the method of dynamic membrane diffusion [116], 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 distilled water at a pH 6.8 or phosphate buffer at a pH 7.5. The suppositories were individually packed in a kidney dialysing membrane (VISKING® Dialysis Tubing 36/32 SERVA, Germany [117]) and placed into 20 ml (lipophilic base) or 40 ml (hydrophilic base) acceptor phase of body temperature (37 ± 0.5 °C). The samples were placed into VIBROTHERM shake bath [118] and exposed to slight shaking (50/min.). The acceptor phase was replaced after 30, 60, 120, 240 min. The quantity of drug in these samples was measured with a spectrophotometer at λ= 278 nm in case of ethacrynic acid and at λ= 274 nm in case of furosemide, using the absorbance value. [119]. The results were evaluated and analysed statistically with the Prism 2.01 (GraphPad Software, USA) computer program. The data are the averages of the results of five experiments ± S.E.M. (*p<0.05; **p<0.01; ***p<0.001 versus control, analysis of variance Newman-Kleus test).

Composition of the phosphate buffer pH=7.5:

Sodium Hydroxide	2,445 g
Potassium dihydrogen phosphate	10,569 g
Distilled water	up to 1000 ml

4.2.4. 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 investigations were carried out in the Department of Pharmacodynamics and Biopharmacy of the University of Szeged. I would like to thank Prof. Dr. György Falkay, Head of Department, and Eszter Ducza, assistant lecturer, for their co-operation and assistance in evaluation.

The animal studies were performed with Sprague-Dawley male rats of 280-300 g. After 6 hours' fasting the oral administration was done with 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 minutes during 150 minutes. The control rats received only 20 ml/kg water. The results were evaluated and analysed statistically with the Prism 2.01 (GraphPad Software, USA) computer program. The data are the averages of the results of six experiments ± S.E.M. (*$p<0.05$; **$p<0.01$; ***$p<0.001$ versus control, analysis of variance Newman-Kleus test).

4.3. Experimental conditions

The conditions of the experiments carried out with the two drugs are summarized in **Table 6**. In the case of ethacrynic acid 11 various suppository bases were examined in two acceptor phases with different pH values. 3 non-ionic surfactants were tested for enhancing the membrane diffusion of the drug, and liberation was increased by making the drug water-soluble. In addition to in vitro experiments, in vivo studies were also performed, but no evaluable dose-effect relationship was found in the studied rats.

In the course of furosemide examinations 7 different suppository bases were examined in phosphate buffer of pH = 7.5. 3 non-ionic surfactants were used to facilitate drug liberation. In vitro membrane diffusion examinations were accompanied by in vivo animal investigations.

Table 6 Summary of experimental conditions

Drugs	In vitro membrane diffusion				In vivo study
	Acceptor phase	**Suppository bases**	**Surfactants**	**Cyclodextrin**	
Ethacrynic acid	distilled water pH=6.8 phosphate buffer pH=7.5	Witepsol H 15; S 58; W 35; Adeps solidus compositus; Massa Estarinum 299; B; BC; Suppocire AML; AS_2X; AP; Macrogolum 1540	Solutol HS 15 Cremophor RH 40 Cremophor RH 60	Random-methyl-β-cyclodextrin (RAMEB)	no dose-effect curve could be evaluated
Furosemide	phosphate buffer pH=7.5	Witepsol H 15; W 35; Massa Estarinum B; BC; Suppocire AML; AS_2X; AP	Solutol HS 15 Cremophor RH 60 Montanox 60 DF	no results	In vitro / in vivo correlation

5. RESULTS AND DISCUSSION

5.1 Preliminary examinations

5.1.1. Plotting of calibration lines

A stock solution of known concentration was prepared from the active agents and measurements were made from dilutions made from this stock solution at λ=278 [120] and λ=274 [120] nm in the case of ethacrynic acid and furosemide, respectively. Based on the six parallel measurements, a linear relationship was found between the concentrations of the active agent and the extinctions measured. The slope and intercept of the lines as well as the value of the correlation coefficient confirming the closeness of the correlation were determined with the help of a computer; these values are shown in **Table 7**.

Table 7 Characteristics of the calibration lines

Active agent	Slope	Intercept	Correlation Coefficient
Ethacrynic acid	0.0128	0.0085	0.9992
Furosemide	0.0616	0.0123	0.9998

5.1.2. Powder diffusion studies

50 mg of the active agents was measured on an analytical balance into the previously prepared kidney dialysing membrane and five parallel measurements were carried out in each case according to the method of dynamic membrane diffusion described above. The quantity of the diffused drug was determined in % **(Table 8)**. The powder diffusion results were taken as control in subsequent examinations. It was found that the diffusion of the drug increased considerably in the phosphate buffer of pH=7.5, a significant difference could be observed with the change of the acceptor phase at the significance level of $p<0.001$, which can be explained by the better solubility of the drug at alkaline pH.

Table 8 Power diffusion results of drugs

	Ethacrynic acid (%)		Furosemide (%)
time (min.)	distilled water pH=6.8	phosphate buffer pH=7.5	phosphate buffer pH=7.5
30	1.84	13.24	7.37
60	2.06	25.84	15.29
120	4.64	48.42	34.15
240	7.69	79.88	65.07

5.2. Influence of pH change on ethacrynic acid release from different suppository bases

The pH of the rectum varies between 6.8-7.9 [13]. The experiments were carried out in distilled water (pH=6.8) and phosphate buffer (pH=7.5). The membrane diffusion of the powder without a suppository base was regarded as control. Release values obtained with the hydrophilic Macrogolum 1540 (***$p<0.001$) base in aqueous medium were manifold higher then those determined with lipophilic bases or powder **(Fig. 4)**. This can be due to the fact that poorly water-soluble drugs are better released from hydrophilic suppositories, and the base may moisten or solubilize the drug, therefore drug solubility and membrane diffusion were increased. Results of membrane diffusion were 7-8 % from lipophilic bases, which were near the membrane diffusion of the powder **(Fig. 5)**.

It, however, the results obtained in aqueous medium and buffer medium are compared, it can be seen that the change of the acceptor phase did not have a significant influence on drug release from Macrogolum 1540, but from lipophilic bases it was increased about tenfold in the acceptor phase of pH= 7.5 **(Fig 6)**. This result can be explained by the change of the solubility of the drug, as ethacrynic acid is a weak acid so its solubility will increase with the increase of pH, which facilitates drug liberation from lipophilic bases, and the membrane diffusion of the drug will also be enhanced. As concerns lipophilic bases, bases with a small hydroxyl value

Fig. 4 Ethacrynic acid release from different suppository bases after 240 minutes
Acceptor phase: Distilled water

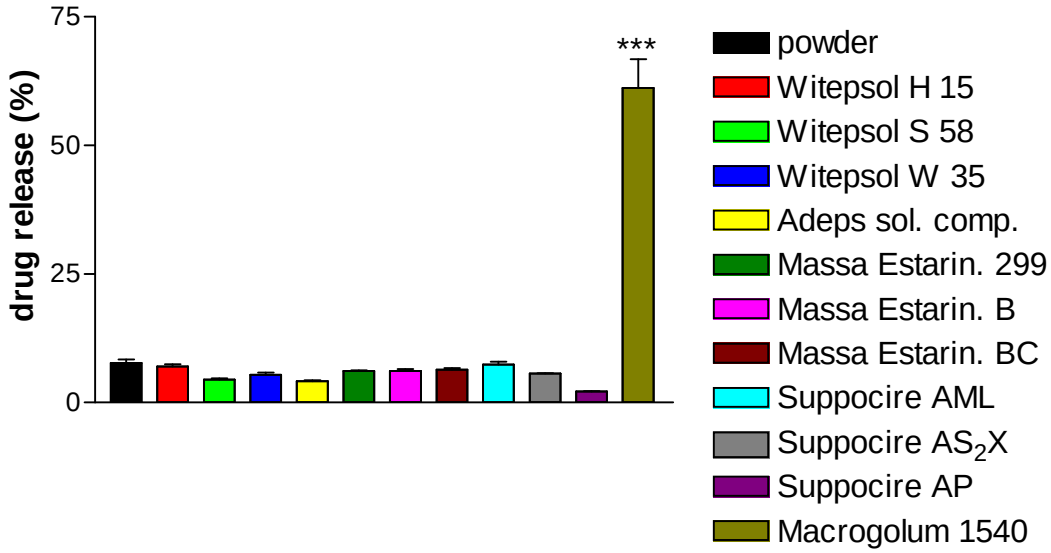


Fig. 5 Ethacrynic acid release from different lipophilic suppository bases after 240 minutes
Acceptor phase: Distilled water

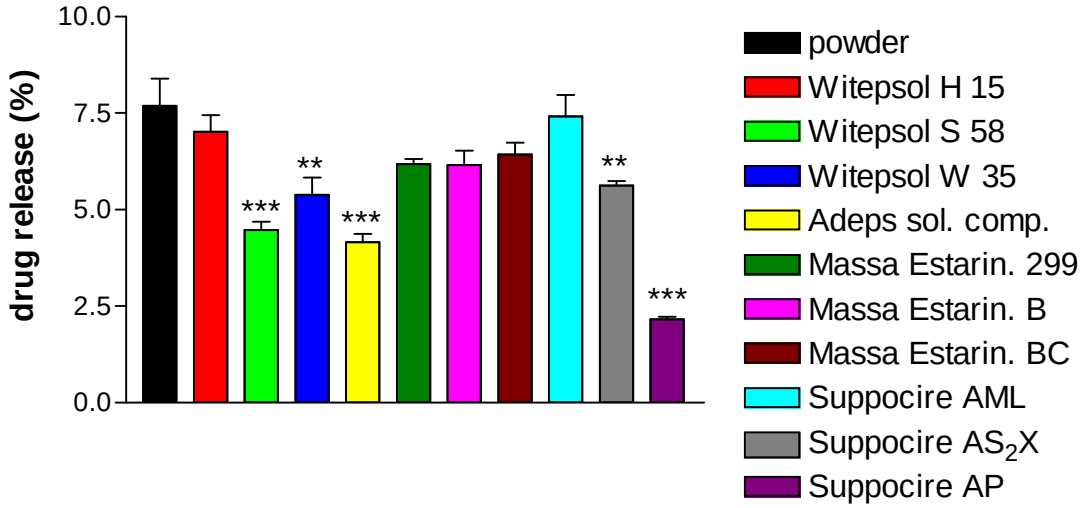
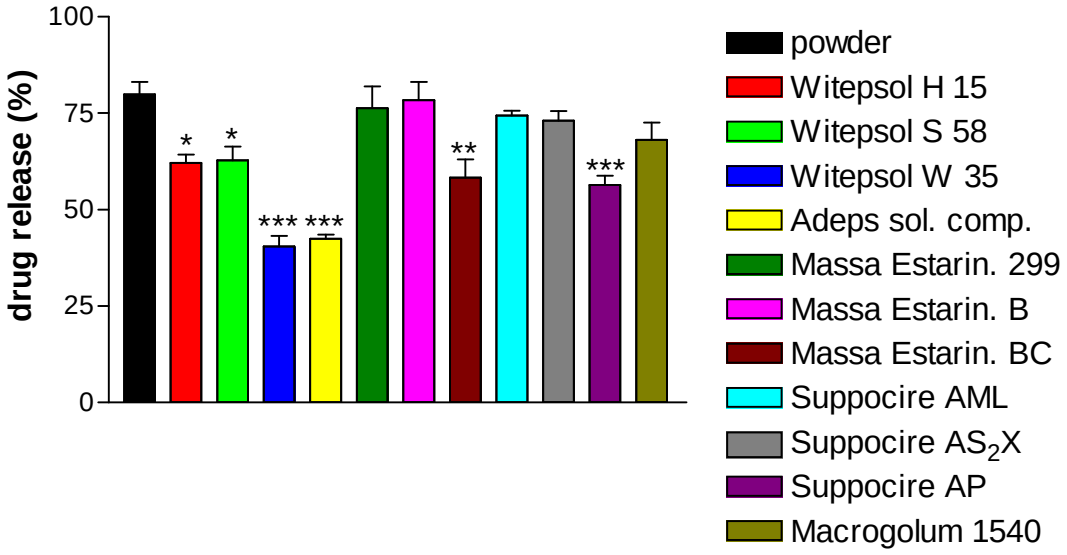


Fig. 6 Ethacrynic acid release from different suppository bases after 240 minutes
Acceptor phase: Phosphate buffer



gave better results. In the case of Massa Estarinum 299, Massa Estarinum B and Suppocire AML there was no significant decrease ($p>0.05$) compared to the membrane diffusion values of the powder either in aqueous or buffer medium, so the suppository bases did not have a retaining effect. Witepsol W 35 (***$p<0.001$), Adeps solidus compositus (***$p<0.001$) and Suppocire AP (***$p<0.001$) with a greater hydroxyl value gave the worst results in both acceptor phases. Adeps solidus compositus contains Witepsol W 35 and two non-ionic surfactants, too, so drug diffusion could be expected to increase with the moistening, solubilization of the drug and by making the base lipohydrophilic. The membrane diffusions of Witepsol W 35 and Adeps solidus compositus showed no significant difference, which can probably be explained by the fact that the joint quantity of 20 % of the two surfactants has an unfavourable influence on drug liberation (see the figures in 5.2).

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 dissolving time of hydrophilic bases. As the efficiency of the two active agents used in the study is not independent of time, in order to

achieve faster and better effect, the combinations of lipophilic bases and various additives (surfactants, cyclodextrins) were used to further improve the results.

5.3. Influence of surfactant concentration on ethacrynic acid release from Witepsol H 15 base

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 in the two acceptor phases, so the use of additives was expected to enhance drug liberation. The membrane diffusion of the drug from Witepsol H 15 base was regarded as a control. The diffusion of the drug was found to vary with their concentration. When distilled water was used as the acceptor phase, the concentration of 3 % yielded the best results in the case of all the three surfactants, this led to about a twofold increase in liberation. Except for 5% of Cremophor RH 60, their use in a concentration of 1-5-10 % did not change or decrease drug liberation **(Fig 7)**, 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, while a too small or too great amount of surfactants may lead to its decrease.

When the same examinations were performed in a buffer medium, 1, 3, 5 % of Solutol HS 15 (***$p<0.001$) and Cremophor RH 40 (**$p<0.01$) led to increase in diffusion, while the use of Cremopor RH60 ($p>0.05$) (which gave the best results in distilled water) did not bring about a change in the extent of drug release **(Fig. 8)**.

Consequently, it can be established that the increase of the pH of the acceptor phase decreased the drug liberation-increasing effect of Cremophor RH 60 surfactant, while Solutol HS 15 and Cremophor RH 40 were more effective in a buffer medium. However, in the phosphate buffer 1 % of the given additive was sufficient for eliciting the required effect.

Fig. 7 Influence of additives on drug release after 240 minutes

Acceptor phase: Distilled water

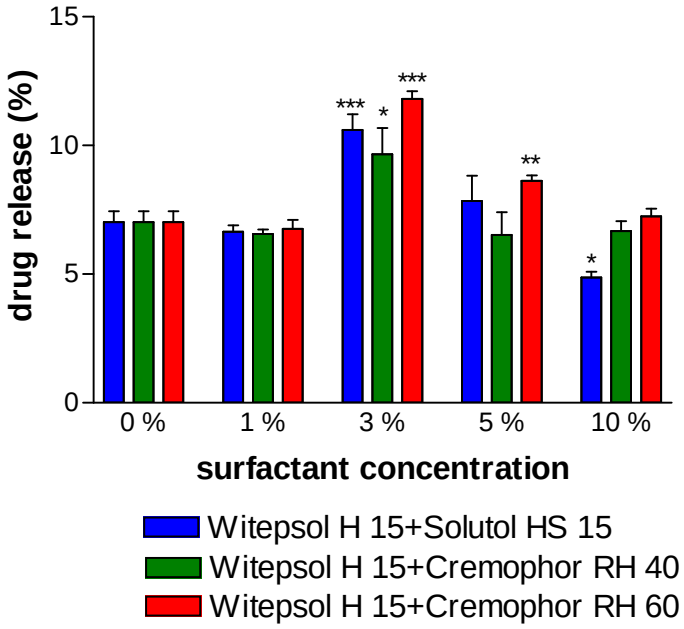
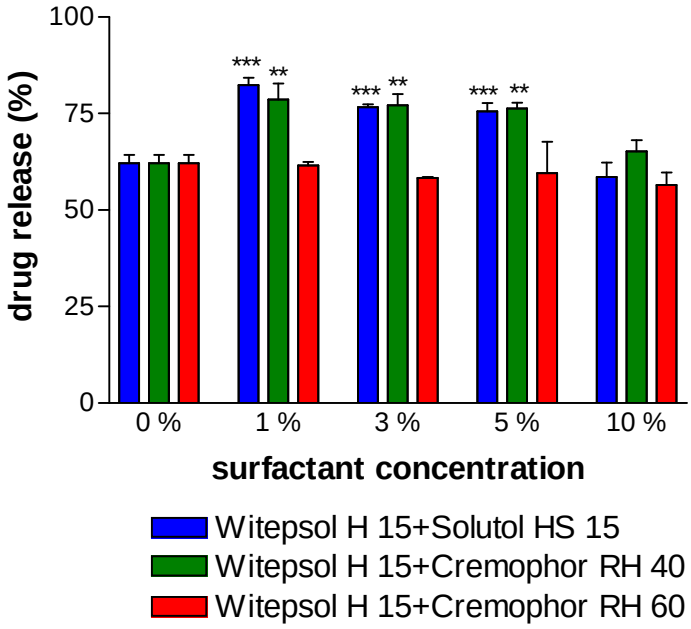


Fig. 8 Influence of additives on drug release after 240 minutes

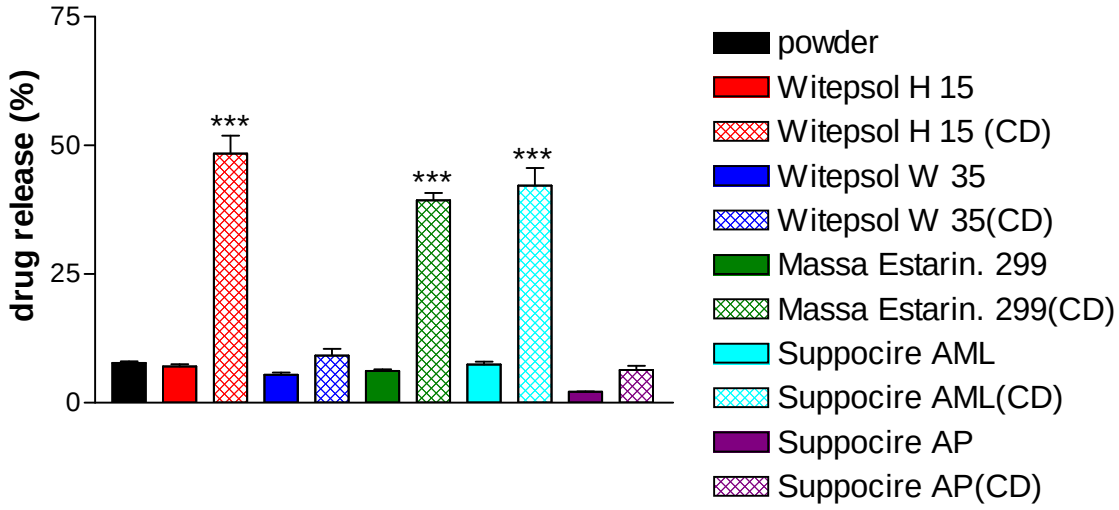
Acceptor phase: Phosphate buffer



5.4. Results of ethacrynic acid and random-methyl-β-cyclodextrin complex release from different suppository bases

Ethacrynic acid and the previously selected ethacrynic acid + RAMEB 1:1 kneaded product were incorporated into 5 different, previously examined lipophilic suppository bases (Witepsol H 15, Witepsol W 35, Massa Estarinum 299, Suppocire AML, Suppocire AP). The membrane diffusion of ethacrynic acid without a suppository base was regarded as control. The amount of ethacrynic acid released in distilled water was under 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, Suppocire AML and Massa Estarinum 299 afforded the best results as concerns the investigated suppository bases. The diffusion of the drug 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 (***p<0,001) **(Fig 9)**.

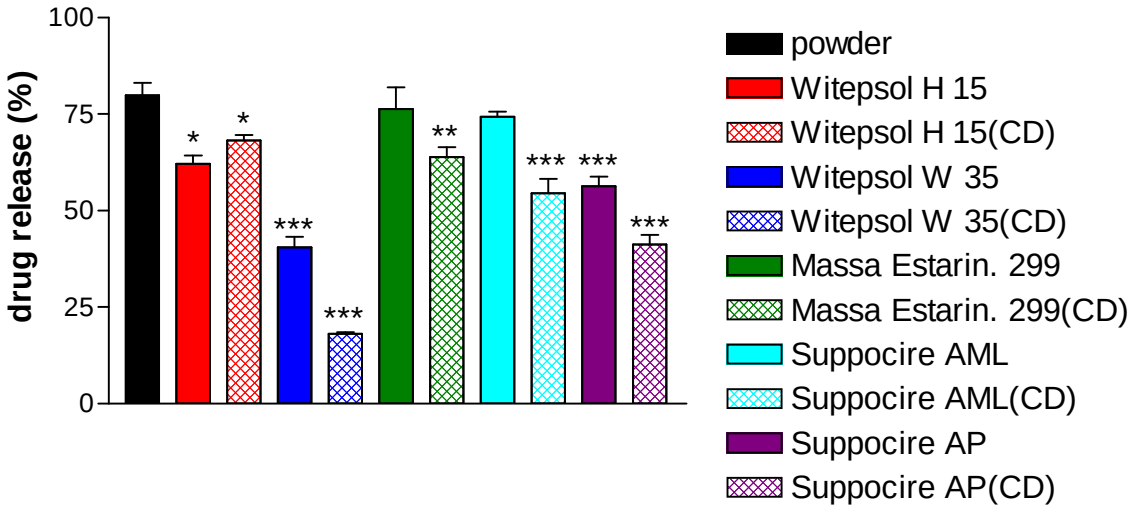
Fig. 9 Solubility and diffusibility increasing effect of RAMEB in distilled water



The solubility of ethacrynic acid increased with the pH increase of the acceptor phase, and so did the diffusibility through the membrane **(Fig 10)**. The best suppository bases in the

distilled water experiments (Witepsol H 15, Suppocire AML and Massa Estarinum 299) were also the best in the phosphate buffer medium. The diffusion results for the suppositories containing CD complexes 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 6.8-7.9. 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 that are effective independently of the pH of the surrounding media.

Fig. 10 Solubility and diffusibility increasing effect of RAMEB in phosphate buffer

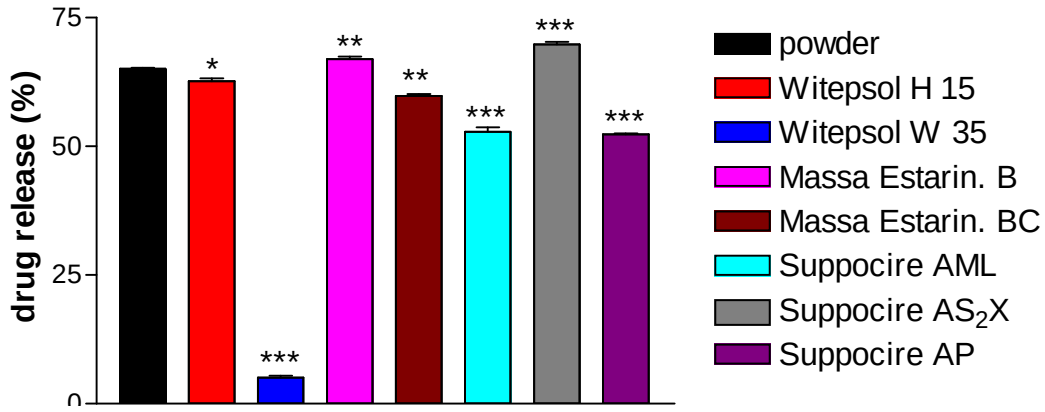


5.5. In vitro membrane diffusion of furosemide from different suppository bases

The membrane diffusion of the powder without a suppository base was regarded as control during the in vitro experiments. It can be stated that drug diffusion from Suppocire AS_2X (***$p<0.001$), 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 (**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. 11)**. 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.

Fig. 11 Furosemide in vitro release study from different suppository bases

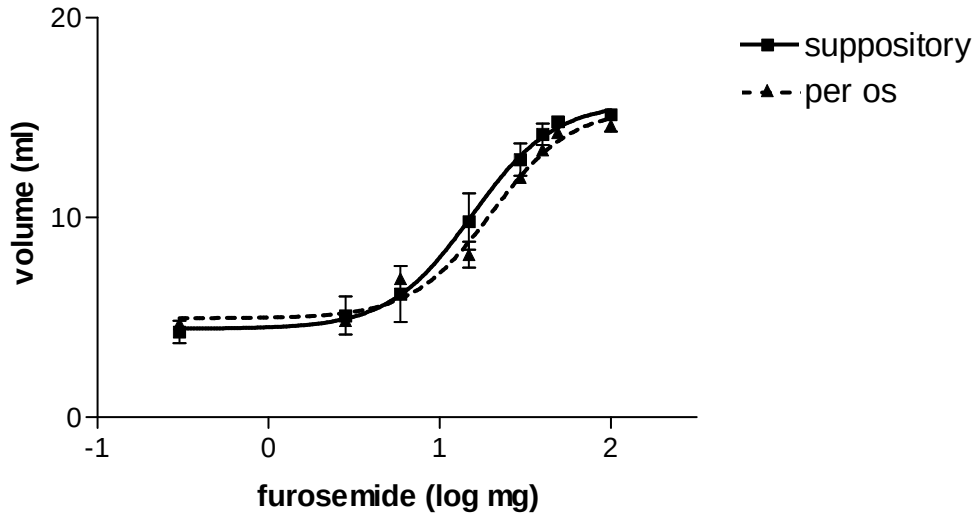


5.6. Diuretic effect of furosemide from different suppository compositions

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. 12)**. The ED50 value was calculated from the figure in both cases ($ED50_{supp}$=15.39 mg, $ED50_{per\ os}$=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, where 20-40 % of the administered drug is metabolised [46]. Further examinations were carried out with the ED50 value calculated from the dose-effect examinations.

Fig. 12 Dose-dependent effect of furosemide



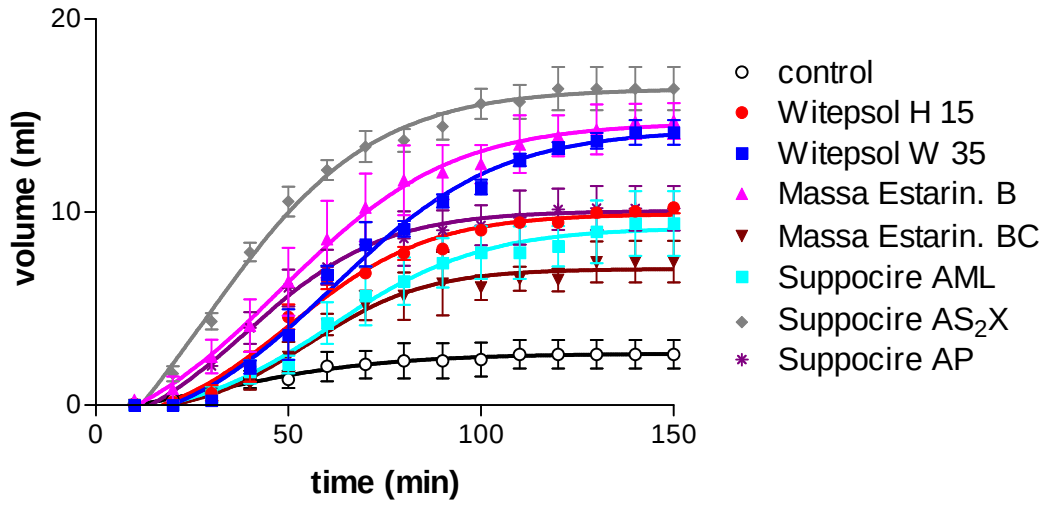
Furosemide was incorporated in suppository bases, and after application in rats urine was collected for 150 minutes. Compared to the control, a significant increase was observed in the quantity of urine when Suppocire AP (* $p<0.05$), Witepsol H 15 (* $p<0.05$), Witepsol W 35 (** $p<0.01$), Massa Estarinum B (*** $p<0.001$) and Suppocire AS_2X (*** $p<0.001$) suppository bases were used. The use of Suppocire AML and Massa Estarinum BC did not bring about a significant difference in urine quantity compared to the control **(Fig. 13)**. The effectiveness of Suppocire AS_2X and Massa Estarinum B is clearly shown by the fact that the amount of urine collected for 150 minutes came near to the 24-hour urine quantity of rats [121].

5.7. Influence of surfactants on furosemide release and diuretic effect

Three non-ionic surfactants were also tested for increasing furosemide liberation. 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 result 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

Fig. 13 Diuretic effect of different suppository bases containing furosemide in rats



the 1 % concentration of Cremophor RH 60 led to a significant increase, in the other cases no significant differences were observed, or furosemide diffusion even decreased with the increase of the surfactant concentration **(Fig. 14)**. The decrease in drug diffusion through the membrane is due to two causes: 1. The additive, 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 dialysing membrane which had a pore size of 25Å. 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 the significant increase in the amount of the collected urine **(Fig. 15)**. Their effect is composed of several factors: they moisten the drug, they denaturate 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. [122] suggest that

Fig. 14 Influence of additives on drug release after 240 minutes

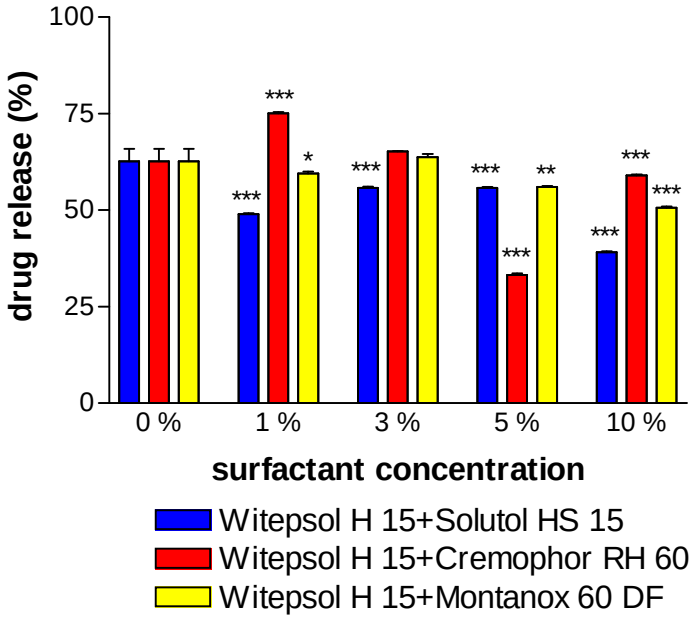
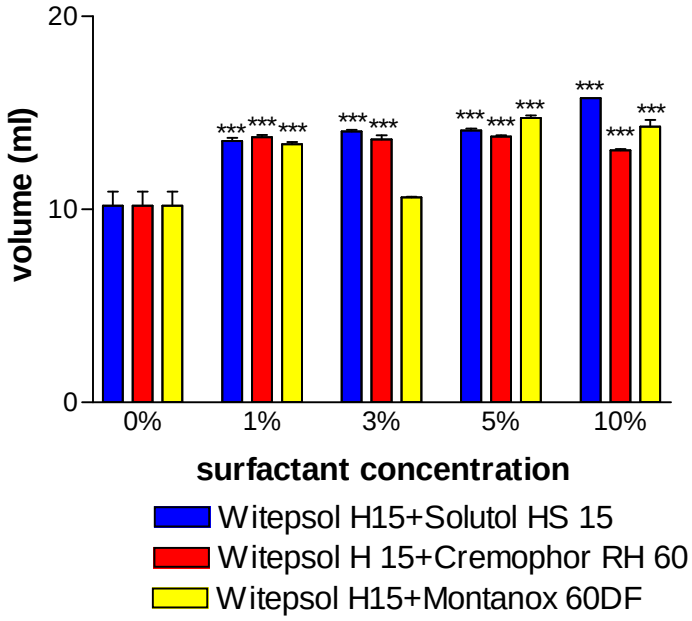


Fig. 15 Influence of additives on diuretic effect in rats



surfactants, which are commonly added to pharmaceutical formulations, may enhance the intestinal absorption of some drugs by inhibiting an apically polarized efflux system. In the animal experiments performed with rats all the three additives increased the quantity of the excreted urine approximately to the same extent, which indicates increased drug liberation. Figure 15 also shows that the increase of the surfactant concentration was not accompanied with significant changes, so a concentration of 1 % is enough to achieve the desired effect.

5.8. Mathematical evaluation of experimental data

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 the time. If the parameters of the functions are known, the extent of drug liberation or its diffusion through the membrane can be calculated at any intermediate time.

$$\log C = K * \log t + B \qquad (3)$$

where C is the amount of material released after time t, K is the slope and B is the intercept of the straight line.

The following tables **(Table 9-16)** show the slope (K) and intercept (B) of the lines, the values of the correlation coefficients indicating the closeness of the correlation (R), the time needed for the liberation of 50 % of the drug (t_{50}) and in vitro availability, the values over 90 % are presented in red colour. The slope is the rate constant of the process, the value of the intercept (liberation belonging to 0 time) should be 0 in principle. The negative values are due to the fact that first the membrane has to be impregnated with the drug, and diffusion starts after impregnation.

Table 9 Ethacrynic acid in distilled water

	slope (K)	intercept (B)	R	t_{50} (min.)	in vitro availability
powder	0.7357	-1.1578	1.0000	2977	100.00
Witepsol H 15	**0.7009**	**-1.1042**	**0.9994**	**3714**	**91.27**
Witepsol S 58	0.7596	-1.4462	0.9990	5549	58.10
Witepsol W 35	0.7772	-1.3843	0.9986	3800	70.15
Adeps solidus comp.	0.7865	-1.5221	0.9996	5160	53.99
Massa Est. 299	0.8566	-1.5246	0.9998	2581	80.36
Massa Est. B	0.6991	-1.1566	0.9997	4508	80.03
Massa Est. BC	0.7865	-1.3312	0.9997	2951	83.61
Suppocire AML	**0.8075**	**-1.3366**	**0.9995**	**2434**	**96.53**
Suppocire AS_2X	0.7126	-1.2410	0.9993	5049	73.34
Suppocire AP	0.5916	-1.3561	0.9990	45210	28.06
Macrogolum 1540	**1.2891**	**-1.3200**	**0.9778**	**128**	**795.31**

Table 10 Ethacrynic acid in phosphate buffer

	slope (K)	intercept (B)	R	t_{50} (min.)	in vitro availability
powder	0.7724	-0.2184	0.9981	123	100.00
Witepsol H 15	0.8165	-0.3866	0.9843	153	77.81
Witepsol S 58	0.8200	-0.4005	0.9920	156	78.56
Witepsol W 35	0.5091	0.1263	0.9958	314	50.61
Adeps solidus comp.	0.9811	-0.9323	0.9989	237	53.15
Massa Est. 299	**0.7149**	**-0.0763**	**0.9948**	**115**	**95.50**
Massa Est. B	**0.7975**	**-0.2485**	**0.9916**	**116**	**98.29**
Massa Est. BC	0.7743	-0.3427	0.9995	177	73.41
Suppocire AML	**0.8740**	**-0.4548**	**0.9935**	**131**	**93.06**
Suppocire AS_2X	**0.7492**	**-0.1524**	**0.9864**	**117**	**91.46**
Suppocire AP	0.8006	-0.4079	0.9963	180	70.62
Macrogolum 1540	1.7240	-2.2859	0.9740	137	85.24

Table 11 Influence of additives in distilled water

	slope (K)	intercept (B)	R	t_{50} (min.)	in vitro availability
powder	0.7357	-1.1578	1.0000	2977	100.00
Witepsol H 15+Solutol HS 15 1%	0.8208	-1.4113	0.9998	2645	86.35
Witepsol H 15+Solutol HS 15 3%	**0.7927**	**-1.1752**	**0.9991**	**1762**	**137.84**
Witepsol H 15+Solutol HS 15 5%	**0.8857**	**-1.4947**	**0.9991**	**1844**	**102.08**
Witepsol H 15+Solutol HS 15 10%	0.9526	-1.8391	1.0000	2500	63.41
Witepsol H 15+Cremophor RH 40 1%	0.9361	-1.6892	0.9979	1985	85.33
Witepsol H 15+Cremophor RH 40 3%	**0.8532**	**-1.3351**	**0.9997**	**1596**	**125.61**
Witepsol H 15+Cremophor RH 40 5%	0.9413	-1.6885	0.9971	1900	84.86
Witepsol H 15+Cremophor RH 40 10%	0.8754	-1.5535	0.9987	2352	86.82
Witepsol H 15+Cremophor RH 60 1%	0.7951	-1.3355	0.9972	2740	87.99
Witepsol H 15+Cremophor RH 60 3%	**0.9208**	**-1.3924**	**0.9990**	**1072**	**153.61**
Witepsol H 15+Cremophor RH 60 5%	**1.0336**	**-1.7984**	**0.9976**	**1237**	**112.24**
Witepsol H 15+Cremophor RH 60 10%	0.8657	-1.5947	0.9991	2863	89.14

Table 12 Influence of additives in phosphate buffer

	slope (K)	intercept (B)	R	t_{50} (min.)	in vitro availability
powder	0.7724	-0.2184	0.9981	123	100.00
Witepsol H 15+Solutol HS 15 1%	**0.7329**	**-0.0780**	**0.9936**	**103**	**103.16**
Witepsol H 15+Solutol HS 15 3%	**0.7639**	**-0.2044**	**0.9978**	**125**	**95.88**
Witepsol H 15+Solutol HS 15 5%	**0.7358**	**-0.1302**	**0.9954**	**119**	**94.54**
Witepsol H 15+Solutol HS 15 10%	0.8778	-0.5965	0.9993	187	73.27
Witepsol H 15+Cremophor RH 40 1%	**0.7470**	**-0.1563**	**0.9976**	**120**	**98.46**
Witepsol H 15+Cremophor RH 40 3%	**0.7764**	**-0.2226**	**0.9958**	**122**	**96.58**
Witepsol H 15+Cremophor RH 40 5%	**0.8297**	**-0.3568**	**0.9973**	**130**	**95.52**
Witepsol H 15+Cremophor RH 40 10%	0.8735	-0.5412	0.9992	165	81.65
Witepsol H 15+Cremophor RH 60 1%	0.7580	-0.2853	0.9979	166	77.07
Witepsol H 15+Cremophor RH 60 3%	0.7783	-0.3711	0.9999	187	72.96
Witepsol H 15+Cremophor RH 60 5%	0.9304	-0.7113	0.9990	184	74.59
Witepsol H 15+Cremophor RH 60 10%	0.8819	-0.6192	0.9994	193	70.78

Table 13 Ethacrynic acid with RAMEB in distilled water

	slope (K)	intercept (B)	R	t_{50} (min.)	in vitro availability
powder	0.7357	-1.1578	1.0000	2977	100.00
Witepsol H 15	**0.6334**	**-0.0729**	**0.9792**	**209**	**629.59**
Witepsol W 35	**0.6287**	**-0.8194**	**0.9998**	**3364**	**119.41**
Massa Est. 299	**0.9056**	**-0.8332**	**0.9987**	**290**	**511.70**
Suppocire AML	**0.7349**	**-0.3751**	**0.9861**	**258**	**548.76**
Suppocire AP	0.5401	-0.7437	0.9907	9231	82.89

Table 14 Ethacrynic acid with RAMEB in phosphate buffer

	slope (K)	intercept (B)	R	t_{50} (min.)	in vitro availability
powder	0.7724	-0.2184	0.9981	123	100.00
Witepsol H 15	0.7619	-0.2315	0.9890	137	85.42
Witepsol W 35	0.5516	-0.3051	0.9922	1223	22.73
Massa Est. 299	0.9114	-0.6119	0.9897	160	79.94
Suppocire AML	0.8965	-0.6637	0.9955	199	68.29
Suppocire AP	0.9120	-0.8208	0.9977	270	51.56

Table 15 Furosemide in phosphate buffer

	slope (K)	intercept (B)	R	t_{50} (min.)	in vitro availability
powder	1.0782	-1.0414	0.9994	182	100.00
Witepsol H 15	**0.7829**	**-0.3301**	**0.9965**	**161**	**96.24**
Witepsol W 35	0.5417	-0.8751	0.9997	15708	7.78
Massa Est. B	**0.7797**	**-0.2971**	**0.9972**	**149**	**102.92**
Massa Est. BC	**0.7287**	**-0.2350**	**0.9988**	**174**	**91.86**
Suppocire AML	0.6669	-0.1323	0.9963	197	81.19
Suppocire AS_2X	**1.0197**	**-0.8398**	**0.9966**	**156**	**107.24**
Suppocire AP	0.7444	-0.3142	0.9972	199	80.46

Table 16 Influence of additives in phosphate buffer

	slope (K)	intercept (B)	R	t_{50} (min.)	in vitro availability
powder	1.0782	-1.0414	0.9994	182	100.00
Witepsol H 15+Solutol HS 15 1%	0.8646	-0.6586	0.9999	239	75.26
Witepsol H 15+Solutol HS 15 3%	0.9678	-0.8282	0.9985	199	85.79
Witepsol H 15+Solutol HS 15 5%	1.0667	-1.0641	0.9993	203	86.07
Witepsol H 15+Solutol HS 15 10%	1.1462	-1.3408	0.9995	245	60.17
Witepsol H 15+Cremophor RH 60 1%	**0.7005**	**-0.1155**	**0.9987**	**144**	**115.42**
Witepsol H 15+Cremophor RH 60 3%	**0.7554**	**-0.2519**	**0.9975**	**152**	**100.15**
Witepsol H 15+Cremophor RH 60 5%	0.9276	-0.8776	0.9997	283	51.02
Witepsol H 15+Cremophor RH 60 10%	0.9772	-0.8228	0.9992	187	89.69
Witepsol H 15+Montanox 60 DF 1%	**0.8075**	**-0.4895**	**0.9989**	**217**	**91.33**
Witepsol H 15+Montanox 60 DF 3%	**0.9797**	**-0.7562**	**0.9957**	**158**	**97.84**
Witepsol H 15+Montanox 60 DF 5%	1.0667	-1.0641	0.9993	203	86.06
Witepsol H 15+Montanox 60 DF 10%	1.1421	-1.3358	0.9955	247	77.78

6. SUMMARY

Having considered the characteristics of rectal drug administration, the physiological state of the rectum, the properties of drugs, bases and additives, I have drawn the following conclusions and I am proposing the following compositions for the formulation of diuretic rectal suppositories:

Considerations in the technological formulation of rectal suppositories containing ethacrynic acid:

1. The solubility of the drug was increased manifold by changing the pH of the acceptor phase. As a result drug liberation from various suppository bases changed. Liberation from lipophilic bases was increased about ten times by increasing the pH. The best results were given by bases with a small hydroxyl value and by lipophilic bases containing an additive. Hydrophilic Macrogolum 1540 gave good results both in an aqueous and buffer medium, but because of its long disintegration time it can be proposed for the formulation of diuretic suppositories only under certain conditions (e.g. tropics-resistant suppositories).

2. When non-ionic surfactants are used with lipophilic bases, drug liberation increases independently of the pH due to the base becoming lipohydrophilic. The extent of the increase was greater in distilled water (pH=6.8) as the surfactant contributed not only to making the base lipohydrophilic but it also solubilized the poorly soluble drug. The quantity of the surfactant is one of the most important factors in the formulation of rectal suppositories. Drug liberation changed according to a maximum function. In aqueous medium a surfactant concentration of 3-5 % proved to be optimal, while in a buffer medium 1 % was enough to give the best results. The physical-chemical parameters of the surfactant were also decisive, which modified the results with pH change.

3. The formulation of the cyclodextrin complex of the drug resulted in about a tenfold increase in the solubility of ethacrynic acid in distilled water, and as a consequence the membrane diffusion of the drug also improved considerably. The solubility of ethacrynic acid increases with the pH increase, so the results of cyclodextrin complexes were worse than those of the membrane diffusion of the pure drug. In this case the retaining effect of the complex may have to be reckoned with.

In view of the above summary, with the consideration of the pH of the rectum, the following is proposed for the formulation of rectal suppositories containing ethacrynic acid:

- **Witepsol H 15 base containing 3% Solutol HS 15 additive, or**
- **ethacrynic acid + random-methyl-β-cyclodextrin complex incorporated in Witepsol H 15 suppository base.**

Considerations in the technological formulation of rectal suppositories containing furosemide:

1. When the membrane diffusion examinations are compared with the actual diuretic effect, it can be stated that drug liberation and pharmacological effect showed the same tendency in 70 %, that is a greater extent of furosemide liberation was accompanied with a greater amount of animal urine. The best results were given by the Suppocire AS_2X base in both cases, which means that the liberation of the drug was about 70 % and the animal produced approximately 15 ml of urine in 150 minutes, which equals the daily urine quantity of a rat according to literature data.

2. The Witepsol H 15 base yielded better results under in vitro conditions than in the animal investigations, and in the case of the Witepsol W 35 base the pharmacological effect proved to be better than the results of the membrane diffusion examinations. This also confirms that if the best composition is to be chosen, it is essential to supplement in

vitro results with in vivo experiments in order to form a clear picture about the interactions between the active agent-base-living organism.

3. When non-ionic surfactants were used, in vitro examinations revealed a significant increase only with the use of 1 % Cremophor RH 60 surfactant concentration, in the other cases there was no significant difference or the diffusion of furosemide decreased with the increase of the surfactant concentration. In the in vivo experiments diuretic effect was definitely increased by surfactants, but 1 % of them was sufficient for eliciting maximum effect.

Based on the results, I have found two compositions suitable for formulating furosemide-containing suppositories:

- **Suppocire AS_2X suppository base in itself, which proved to be the best both in the membrane diffusion and during the animal experiments, or**
- **Witepsol H 15 suppository base with 1% Cremophor RH 60 additive, which also gave optimal results with both examination methods.**

7. REFERENCES

1. ifj. Regdon G., Regdon G., Selmeczi B.: Gyógyszerészet 42, 451-461 (1998)
2. Daharjanu, M., D., Kumaran, K. S., Baskaran, T., Moorthy, M. S.: Drug. Dev. Ind. Pharm. 24, 583-587 (1998)
3. Hermann, T. W.: Int. J. Pharm. 123, 1-11 (1995)
4. Yahagi, R., Machida, Y., Onishi, H., Machida, Y.: Int. J. Pharm. 193, 205-212 (2000)
5. Nagatomi, A., Mishima, M., Tsuzuki, O., Ohdo, S., Higuchi, S.: Biol. Pharm. Bull. 20, 892-896 (1997)
6. VanDenBerg, C. M., Kazmi, Y., Stewart, J., Weidler, D. J., Tenjarla, S. N., Ward, E. S., Jann, M. W.: Am. J. Health Syst. Pharm. 57, 1046-1050 (2000)
7. Samy, E. M., Hassan, M. A., Tous, S. S., Rhodes, C. T.: Eur. J. Pharm. Biopharm. 49, 119-127 (2000)
8. Janicki, S., Sznitowska, M., Zebrowska, W., Gabiga, H., Kupiec, M.: Eur. J. Pharm. Biopharm. 52, 249-254 (2001)
9. Makó S., Stampf G.: Acta Pharm. Hung. 71, 293-299 (2001)
10. Stampf G.: Acta Pharm. Hung. 68, 119-122 (1998)
11. Regdon, G., Fazekas, T., Regdon, G. jr., Selmeczi, B.: Pharmazie 51, 116-119 (1996)
12. Rácz I., Selmeczi B.: Gyógyszertechnológia, Egyetemi tankönyv, 4. kiadás, Medicina Könyvkiadó Rt. Budapest, 2001
13. Müller, B. W.: Suppositorien. Pharmakologie, Biopharmazie und Galenik rektal und vaginal anzuwendender Arzneiformen. Wissenschaftlich. Verlag. mbH Stuttgart, 1986
14. Kim, N. S., Umejima, H., Ito, T., Uchida, T., Goto, S.: Chem. Pharm. Bull. 40, 2800-2804 (1992)
15. Umejima, H., Kim, N. S., Ito, T., Uchida, T., Goto, S.: J. Pharm. Sci. 82, 195-199 (1993)
16. Yahagi, R., Onishi, H., Machida, Y.: J. Control. Release 61, 1-8 (1999)
17. Kato, Y., Matsushita, T., Uchida, H., Egi, S., Yokoyama, T., Mohri, K.: Eur. J. Clin. Pharmacol. 42, 619-622 (1992)
18. Lievertz, R. W.: Am. J. Obstet. Gynecol. 156, 1289-1293 (1987)
19. Knoll J.: Gyógyszertan, 8. kiadás, Medicina Könyvkiadó, Budapest, 1993

20. Pfaff, G., Zimmermann, T., Lach, P., Yeates, R., Simon, G., Wildfeuer, A.: Arzneim. - Forsch./Drug Res. 43, 391-395 (1993)
21. Ritschel, W. A.: Angewandte Biopharmazie, Wiss. Verlag mbH Stuttgart, 1973
22. Ritschel, W. A.: Handbook of Basic Pharmacokinetics Including Clinical Applications, Ed. 3. Hamilton USA Drug Intelligence, 1986
23. Hakata, T., Ijima, M., Kimura, S., Sato, H., Watanabe, Y., Matsumoto, M.: Chem. Pharm. Bull. 41, 351-356 (1993)
24. Bornschein, M., Grohmann, A., Voigt, R.: Pharmazie 35, 772-776 (1980)
25. Schmitt, M., Guentert, T. W.: J. Pharm. Sci. 79, 359-363 (1990)
26. Nishihata, T., Rytting, J.: Adv. Drug Deliv. Rev. 28, 205-228 (1997)
27. Regdon G., Selmeczi B.: A rektális gyógyszerbevitel jelentősége napjainkban. A gyógyszerészeti tudomány aktuális kérdései 10. szám, MGYT Kiadvány, Budapest, 1992
28. Regdon G.: A kúpkészítés és a kúpok gyógyszerleadását befolyásoló tényezők tanulmányozása gyógyszertechnológiai és biofarmáciai szempontok figyelembevételével. Kandidátusi értekezés, Szeged, 1975
29. European Pharmacopoeia Ed. 3. Council of Europe, Strasbourg Cedex 1997
30. European Pharmacopoeia Ed. 4. Council of Europe, Strasbourg Cedex 2002
31. Ermis, D., Tarimci, N.: Int. J. Pharm. 113, 65-71 (1995)
32. Azechi, Y., Ishikawa, K., Mizuno, N., Takahashi, K.: Drug. Dev. Ind. Pharm. 26, 1177-1183 (2000)
33. Nakajima, T., Takashima, Y., Furuya, A., Ozawa, Y., Kawashima, J.: Chem. Pharm. Bull. 37, 3145-3147 (1989)
34. Schneeweis, A., Müller-Goymann, C.: Int. J. Pharm. 196, 193-196 (2000)
35. Moolenaar, F., Meyler, P., Frijlink, E., Jauw, T., Visser, J., Proost, H.: Int. J. Pharm. 114, 117-120 (1995)
36. Mijazaki, S., Suisha, F., Kawasaki, N., Shirakawa, M., Yamatoya, K., Attwood, D.: J. Control. Release 56, 75-83 (1998)
37. Schneeweis, A., Müller-Goymann, C. C.: Pharm. Res. 14, 1726-1729 (1997)
38. Realdon, N., Ragazzi, E., Dal Zotto, M., Dalla Fini, G.: Int. J. Pharm. 148, 155-163 (1997)

39. Chicco, D., Grabnar, I., Skerjanec, A., Vojnovic, D., Manrich, V., Realdon, N., Rajavni, E., Belic, A., Karba, R., Mrhar, A.: Int. J. Pharm. 189, 147-160 (1999)
40. Mawatari, Hu Z., Shimokawa, S., Kimura, T., Yoshikawa, G., Shibata, Y., Takada K, N.: J. Pharm. Pharmacol. 52, 1187-1193 (2000)
41. Eckert, Th., van Husen, N.: Dtsch. Apoth. Ztg. 120, 55-56 (1980)
42. Sákovits J., Regdon G., Pintyéné Hódi K., Elek B-né, Benkő A., Kraszkó P., Cseke B.: Gyógyszerészet 34, 529-532 (1990)
43. Sákovics J., Benkő A., Jónás J., Elek B-né, Regdon G., Pintyéné Hódi K., Mézes É.: Gyógyszerészet 34, 581-585 (1990)
44. Yun, M-O., Choi, H. G., Jung, J. H., Kim, C. K.: Int. J. Pharm. 189, 137-145 (1999)
45. Ryu, J. M., Chung, S. J., Lee, M. H., Kim, C. K., Shim, C. K.: J. Control. Release 59, 163-172 (1999)
46. Kim, E. J., Han, K. S., Lee, M. G.: J. Pharm. Pharmacol. 52, 1337-1343 (2000)
48. Regdon G., ifj. Regdon G., Tariné Gombkötő Zs., Berényi M.: Gyógyszerészet 39, 259-265 (1995)
48. Watanabe, S., Belzile, M., Kuehn, N., Hanson, J., Bruera, E.: Cancer Treat. Rev. 22, 131-136 (1996)
49. Bruera, E., Watanabe, S., Fainsinger, R. L., Spachynski, K., Suarez-Almazor, M., Inturrisi, C.: Pain. 62, 141-146 (1995)
50. Kanamoto, I., Xin Zheng, N., Ueno, M., Koizumi, T., Adachi, I., Horikoshi, I.: Chem. Pharm. Bull. 40, 1883-1886 (1992)
51. Bredow, V.: Zentralbl. Gynakol. 114, 551-554 (1992)
52. Kamiya, A., Ogata, H., Fung, HL.: J. Pharm. Sci. 71, 621-624 (1982)
53. Watanabe, Y., Matsumoto, Y., Hori, N., Funato, H., Matsumoto, H.: Chem. Pharm. Bull. 39, 3007-3012 (1991)
54. Hosny, E., El-Ahmady, O., El-Shattawy, H., El-M. Nabih, A., El-Damacy, H., Gamal-El-Deen, S., El-Kabbany, N.: Arzneim. - Forsch./Drug Res. 44, 611-613 (1994)
55. Hacker, E., Kossowicz, J., Milde, K.: Pharmazie 46, 125-127 (1991)
56. Hosny, E. A.: Drug Dev. Ind. Pharm. 25, 745-752 (1999)

57. Iwata, M., Komiya, S., Nakamura, K., Kiuchi, M., Andoh, N., Hirahara, F., Maitani, Y., Takayama, K.: Drug Dev. Ind. Pharm. 27, 1039-1045 (2001)
58. Babar, A., Fares, H. M., Plakogiannis, F. M.: J. Pharm. Sci. 82, 389-391 (1993)
59. Kowari, K., Hirosawa, I., Kurai, H., Utoguchi, N., Fujii, M., Watanabe, Y.: Biol. Pharm. Bull. 25, 678-681 (2002)
60. Regdon, G. jr., Deák, D., Regdon, G., Muskó, Zs., Erős, I.: Pharmazie 56, 70-73 (2001)
61. Regdon G., Sztanovics K., Zádeczky S., Selmeczi B.: Gyógyszerészet 33, 297-300 (1989)
62. Nissan, A., Ziv, E., Kidron, M., Bar-On, H., Friedman, G., Hyam, E., Eldor, A.: Haemostasis 30, 225-232 (2000)
63. Sastri, M. S. P., Satyanarayana, N. V., Krishna, D. R., Diwan, P. V.: Arzneim. - Forsch./Drug Res. 43, 320-323 (1993)
64. Ogiso, T., Iwaki, M., Kashitani, Y., Yamashita, K.: Chem. Pharm. Bull. 39, 2657-2661 (1991)
65. Ofoefule, S. I., Nwankwo, C., Chukwu, A., Orisakwe, O. E.: Boll. Chim. Farm. 137, 341-344 (1998)
66. Bergogne-Berezin, E., Bryskier, A.: J. Antimicrob. Chemother. 43, 177-185 (1999)
67. Sallai, J., Vernyik, Á., Regdon, G., Gombkötő, S., Németh, J., Regdon, G. jr.: J. Pharm. Pharmacol. 49, 496-499 (1997)
68. Regdon, G., Dorogi-Jakab, I., Bándi, D., Várföldi, T., Regdon, G. jr., Selmeczi, B.: Eur. J. Pharm. Biopharm. 38, 150-153 (1992)
69. Hacker, E., Krause, D., Kossowicz, J.: Pharmazie 47, 275-276 (1992)
70. Webster, J. A., Dowse, R., Walker, R. B.: Drug Dev. Ind. Pharm. 24, 395-399 (1998)
71. Lindmark, T., Soderholm, J. D., Olaison, G., Alvan, G., Ocklind, G., Artursson, P.: Pharm. Res. 14, 930-935 (1997)
72. Sznitowska, M., Gajewska, M., Janicki, S., Radwanska, A., Lukowski G.: Eur. J. Pharm. Biopharm. 52, 159-163 (2001)
73. Kelemen-Fazekas H., ifj. Regdon G.: Orvostudományi Értesítő 71, 243-249 (1998)
74. ifj. Regdon G., Bácskay I., Kata M., Regdon G.: Gyógyszerészet 35, 219-222 (1991)

75. Bácskay I., Keki S., Deák G., Zsuga M., Mezey G.: Acta Pharm. Hung. 71,153-156 (2001)
76. Czetsch-Lindenwald, V. H.: Suppositorien, Ed. Cantor, Aulendorf i. Württ. 1959
77. ifj. Regdon G., Schirm I., Pittmann Á., Regdon G.: Acta Pharm. Hung. 65, 45-50 (1995)
78. Kovács B., Selmeczi B., Szepessy A.: A gyógyszerkészítés segédanyagai, OGYI Kiadvány, Budapest, 1983
79. Pharmacopoea Hungarica Editio VII., Medicina Könyvkiadó, Budapest, 1986
80. Formulae Normales Editio. VI., Medicina Könyvkiadó, Budapest, 1987
81. Fiedler, H. P.: Lexikon der Hilfsstoffe für Pharmazie Kosmetik und angrenzende Gebiete, vierte, völlig überarbeitete und erweiterte Auflage, Ed. Cantor, Aulendorf, 1996
82. Jinno, J., Oh, D-M., Crison, J. R., Amidon, G. L.: J. Pharm. Sci. 89, 268-274 (2000)
83. Mezey G., Regdon G.: Acta Pharm. Hung. 41, 193-202 (1971)
84. Goodman and Gilman's The pharmacological basis of therapeutics 9th Ed.., McGraw-Hill, New York 1995
85. Buszello, K., Schnier, C., Müller B. W.: Eur. J. Pharm. Biopharm. 47, 247-253 (1999)
86. Scheller, Dworniczak, S., Pogorzelska, T., Rajca, M., Shani, J.: Arzneimittelforschung 50, 72-76 (2000)
87. Buszello, K.,Harnisch, S., Müller, R. H., Müller, B. W.:Eur. J. Pharm. Biopharm. 49, 143-149 (2000)
88. Pharmazeutische Spezialprodukte. Prospekt der BASF AG. Ludwigshafen, Germany 1993
89. Pharmaceutical and Health food. Seppic, Paris, France 1990, 1991
90. Usayapant, A., Iyer, B. R.: Drug Dev. Ind. Pharm. 25, 387-390 (1999)
91. Jarho, P., VanderVelde, D., Stella, V. J.: J. Pharm. Sci. 89, 241-249 (2000)
92. Aigner, Z.: Iomeglamsav- és β-ciklodextrin-tartalmú termékek, tabletták előállítása és vizsgálata, Egyetemi Doktori Értekezés, Szeged, 1992
93. Aigner, Z., Kata, M.: STP Pharm. Sci. 9, 279-282 (1999)
94. Szejtli, J.: Cyclodextrins and their Inclusion Complexes. Akadémiai kiadó, Budapest, 1982
95. Bozsik E., Kissné Csikós E.: Acta Pharm. Hung. 63, 290-295 (1993)

96. USP 23/NF 18. United States Pharmacopeial Convention, Inc. Rockville, 1995
97. Thoma, K.: Arzneiformen zur rektalen und vaginalen Applikation. Werbe- und Vertriebsgesellschaft Deutscher Apotheker mbH, Frankfurt am Main, 1980
98. Kingsford, M., Eggers, N. J., Soteros, G., Maling, T. J., Shirkey, R. J.: J. Pharm. Pharmacol. 36, 536-538 (1984)
99. Yen, S. Y., Sung, K. C., Wang, J. J., Yoa-Pu Hu, O.: Int. J. Pharm. 220, 91-99 (2001)
100. Regdon, G., Szikszay, M., Gebri, G., Regdon, G. jr., Erős, I.: Pharmazie 54, 521-523 (1999)
101. Bornschein, M., Hoffmann, K., Voigt, R.: Pharmazie 40, 449-455 (1985)
102. Kapás M., Kedvessy Gy., Regdon G., Minker E., Magyarlaki A.: Acta Pharm. Hung. 51, 161-167 (1981)
103. Magyarlaki A., Minker E., Kapás M., Kedvessy Gy., Regdon G.: Acta Pharm. Hung. 51, 258-263 (1981)
104. Regdon, G., Magyarlaki, A., Kedvessy, Gy., Minker, E., Regdon, E.: Pharmazie 33, 67-69 (1978)
105. Regdon, G., Bácskay, I., Kata, M., Selmeczi, B., Szikszay, M., Sánta, A., Bálint, G. S.: Pharmazie 49, 346-349 (1994)
106. Bácskay I., Regdon G., Kata M., Selmeczi B., Szikszay M., Sánta A., Bálint G. S.: Acta Pharm. Hung. 63, 277-283 (1993)
107. Drug Evaluations Annual 1992, American Medical Association, Chicago, 1992
108. Martindale, in: K. Parfitt (Ed.), The complete drug reference, 32th edn. Pharmaceutical Press, London, 1999
109. Gyógyszer Kompendium 2001, MediMedia Információs Kft, Budapest, 2001
110. Chungi, V. S., Ditter, L. W., Smith, R. B.: Int. J. Pharm. 4, 27-38 (1979)
111. Hamarlund, M. M., Paalzow, L. K., Odlind, B.: Eur. J. Clin. Pharmacol. 26, 197-207 (1984)
112. Fatty bases for suppositories. Hüls AG Witten, Germany 1994
113. Suppocire brossure, Gattefossé, Saint-Priest, France, 1994
114. Sample Specification, CYCLOLAB R&D Laboratory Ltd., Budapest, 1999
115. Szepesi E.: Etakrinsav-komlexek készítése és vizsgálata. Szakdolgozat, Szeged, 2000

116. Regdon G., Kovács B., Pécsiné Szebellédi I., Regdon É.: Acta Pharm. Hung. 59, 23-31 (1989)
117. Brossure of kidney dialysing membranes, Union Carbide Co. Chicago/USA, 1986
118. Vibrotherm shake-bath. Type 609/A, MTA KUTESZ, Budapest, 1990
119. Regdon G., Kovács B., Pécsiné Szebellédi I., Regdon G.: Acta Pharm. Hung. 59, 32-41 (1989)
120. Dibbern, H. W.: UV- und IR - Spektren wichtiger pharmazeutischer Wirkstoffe. Ed. Cantor Aulendorf, 1978
121. Krinke, G. J. (Ed.): The Laboratory Rat, 1st Ed.., Academic Press London, 2000
122. Nerurkar, M. M., Burton, P. S., Borchardt, R. T.: Pharm. Res. 13, 528-534 (1996)