

University of Szeged, Faculty of Pharmacy  
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## **Summary of PhD Thesis**

# **Formulation of rectal suppositories containing diuretic drugs and their biopharmaceutical studies**

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## **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. 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.

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. 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).

## **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.

## **EXPERIMENTAL WORK**

### **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. Loop diuretics block the  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  carrier at the luminal side, thus inhibiting the absorption of sodium, potassium and chloride ions in the thick ascending limb of the loop. Currently they are available as oral and parenteral solutions, tablets, capsules or granules for oral administration.

### **Suppository bases**

Witepsol (H 15, S 58, W 35) and Massa Estarinum (299, B, BC) type bases produced by the German CONDEA Chemie GmbH, Suppocire (AML, AP, AS<sub>2</sub>X) suppository bases of the French Gattefossé and Macrogolum 1540 were used. 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.

### **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.

## Methods

### Preparing of the cyclodextrin (CD) complexes

Different CD derivatives e.g. dimethyl- $\beta$ -CD, methyl- $\beta$ -CD, random-methyl- $\beta$ -CD (RAMEB) were tested for increasing solubility of ethacrynic acid. RAMEB was chosen for further examinations on the bases of the costs and the solubility-increasing effect. 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  $\mu$ m sieve.

*Kneaded products:* Physical mixtures of the drug and CD were mixed 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  $\mu$ m sieve.

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.

### Suppository formulation method

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.

### In vitro release study

Experiments were performed with the method of dynamic membrane diffusion, which is a useful method for following the rate of drug release and membrane diffusion from the powder without excipient and from the different suppository compositions, too. The acceptor phase was 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®) and placed into 20 ml (lipophilic base) or 40 ml (hydrophilic base) acceptor phase of body temperature ( $37 \pm 0.5$  °C). The samples were placed into VIBROTHERM shake

bath 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  $\lambda = 278$  nm in case of ethacrynic acid and at  $\lambda = 274$  nm in case of furosemide, using the absorbance value. The data are the averages of the results of five experiments.

### **In vivo study**

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 results were evaluated and analysed statistically with the Prism 2.01 computer program. The data are the averages of the results of six experiments.

### **Experimental conditions**

In the case of ethacrynic acid 11 various suppository bases were examined in two acceptor phases with different pH values. 3 non-ionic surfactants (Solutol HS 15, Cremophor RH 40, Cremophor RH 60) 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 (Solutol HS 15, Cremophor RH 60, Montanox 60 DF) were used to facilitate drug liberation. In vitro membrane diffusion examinations were accompanied by in vivo animal investigations.

## **RESULTS**

### **Influence of pH change on ethacrynic acid release from different suppository bases**

The pH of the rectum varies between 6.8-7.9. 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 than those determined with lipophilic bases or powder. 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. 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. 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 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.

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.

### **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.

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 Cremophor RH60 ( $p > 0.05$ ) did not bring about a change in the extent of drug release.

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.

### **Results of ethacrynic acid and random-methyl- $\beta$ -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 ( $p < 0.001$ ).

The solubility of ethacrynic acid increased with the pH increase of the acceptor phase, and so did the diffusibility through the membrane. The best suppository bases in the distilled water experiments 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.

### **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<sub>2</sub>X (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. 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.

### **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)). The ED50 value was calculated from the figure in both cases (ED50<sub>supp</sub>=15.39 mg, ED50<sub>per os</sub>=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. Further examinations were carried out with the ED50 value calculated from the dose-effect examinations.

Furosemide was incorporated in suppository bases, and after application in rats urine was collected for 150 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<sub>2</sub>X (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. The effectiveness of Suppocire AS<sub>2</sub>X 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.

### **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 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.

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. Their effect is composed of several factors: they moisten the drug, they denature the proteins found on the intestinal mucosa thereby disrupting the integrity of the membrane, and furthermore they increase the number of adsorption places by cleaning the membrane surface. 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, and the increase of the surfactant concentration was not accompanied with significant changes, so a concentration of 1 % is enough to achieve the desired effect.

## **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- $\beta$ -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<sub>2</sub>X 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<sub>2</sub>X 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.**

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## Publications related to the thesis

- I. **S. Berkó**, G. Regdon jr., I. Erős: Influence of pH change on drug release from rectal suppositories *Pharmazie* 55, (4) 324 (2000) **IF.: 0.498**
- II. **S. Berkó**, G. Regdon jr., I. Erős: Solutol and Cremophor products as new additives in suppository formulation *Drug Development and Industrial Pharmacy* 28, (2) 203-206 (2002) **IF.: 0.557**
- III. **S. Berkó**, G. Regdon jr., E. Ducza., G. Falkay, I. Erős: In vitro and in vivo study in rats of rectal suppositories containing furosemide *European Journal of Pharmaceutics and Biopharmaceutics* 53, (3) 311-315 (2002) **IF.: 1.503**
- IV. Z. Aigner, E. Szepesi, **S. Berkó**, Cs. Novák, G. Regdon jr., M. Kata: Investigation of ethacrynic acid and random-methyl- $\beta$ -cyclodextrin binary complexes *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 42, 219-226 (2002) **IF.: 1.15**

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