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Research paper

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

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**Abstract**

The aim of our experimental work was to formulate furosemide-containing rectal suppositories, to study drug release with in vitro membrane diffusion examinations and to increase drug liberation with the use of non-ionic surfactants (Solutol HS 15, Cremophor RH 60, Montanox 60 DF), which were incorporated in the suppository base in various concentrations. Suppocire $AS_2X$ proved to be the best suppository base (diffused drug: 69.78%). The use of 1% Cremophor RH 60 additive with the Witepsol H 15 base increased the quantity of the diffused drug from 62 to 75%. The membrane diffusion examinations were followed by studying the influence of suppository bases and additives exerted on the actual diuretic effect in Sprague–Dawley male rats. Once again the Suppocire $AS_2X$ suppository base gave the best results compared to the control; the quantity of the animals' urine showed a fourfold increase. Used with the Witepsol H 15 base, even 1% of all the three additives resulted in a considerable increase of diuretic effect, so their use proved to be advantageous. The comparison of the membrane diffusion examinations with the in vivo diuretic effect reveals that in vitro drug release and the pharmacological effect usually showed the same tendency, that is a greater extent of in vitro furosemide release was associated with a greater quantity of rat urine. 

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

## 1. Introduction

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

Nowadays one of the basic tasks of drug formulation is to develop an already existing dosage form in a way which makes drug release the best possible under the given circumstances, that is to enhance bioavailability in this way [2–4]. The other important aim is to widen the choice of products with respect to dosage, that is to make a given drug available in as many dosage forms as possible [5–9]. Furosemide is a weak acid (pKa = 3.9) which is absorbed incompletely from the gastrointestinal tract after oral administration. There have been reports of considerable intra- and interindividual variability in its bioavailability. This is due to the fact that absorption of furosemide depends on pH, food intake and dosage form [10,11].

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

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Table 1
Properties of suppository bases

| Name of base | Chemical description | Melting range (°C) | Hydroxyl value | Function |
|---|---|---|---|---|
| Witepsol® H 15 | Triglycerides (C10–C18) | 33.5–35.5 | 5–15 | Lipophilic base |
| Witepsol® W 35 | Higher proportion of mono- and di-glycerides (C10–C18) | 33.5–35.5 | 40–50 | Lipophilic base |
| Massa Estarinum® B | Higher proportion of mono- and di-glycerides (C12–C18) | 33.5–35.5 | 20–30 | Lipophilic base |
| Massa Estarinum® BC | Higher proportion of mono- and di-glycerides (C12–C18) | 33.5–35.5 | 30–40 | Lipophilic base |
| Suppocire® AML | Triglycerides (C8–C18) with the presence of a phospholipid | 35–36.5 | Max. 6 | Lipophilic base |
| Suppocire® AP | Saturated polyglycolyzed glycerides | 33–35 | 30–50 | Amphiphilic base |
| Suppocire® $AS_2X$ | Higher proportion of mono- and di-glycerides (C8–C18) with the presence of a non-ionic emulsifying additive | 35–36.5 | 15–25 | Lipophilic base |

## 2. Materials and methods

### 2.1. Materials

Furosemide (CHINOIN, Hungary) was used as drug. Suppocire® AML, AP, $AS_2X$ (GATTEFOSSÉ, France), Witepsol® W 35, H 15, and Massa Estarinum® B and BC (CONDEA Chemie GmbH, Germany) were used as suppository bases. Cremophor® RH 60, Solutol® HS 15 (BASF, Germany), and Montanox® 60 DF (SEPPIC, France) were used as surfactants.

### 2.2. Formulation method

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

### 2.3. In vitro release study

Experiments were performed with the method of dynamic membrane diffusion, which is a useful method for following the rate of drug release and membrane diffusion from the powder without excipient and from the different suppository compositions, too. The acceptor phase was phosphate buffer at pH 7.5 (modelling the rectal pH). The suppositories were individually packed in a kidney dialysing membrane (VISKING® Dialysis Tubing 36/32 SERVA, Germany) and placed into buffer of body temperature (37 ± 0.5 °C). The samples were exposed to slight shaking and the acceptor phase was replaced after 30, 60, 120, and 240 min. The quantity of furosemide in these samples was measured with a spectrophotometer at $\lambda = 274$ nm, using the absorbance value. The mean values were calculated from five parallel measurements each time (±SEM) [14].

### 2.4. In vivo study

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

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

### 2.5. Mathematical evaluation

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

## 3. Results and discussion

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

Table 2
Properties of surfactants

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

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

In the course of the in vivo trials the dose–effect relationship was examined after the administration of furosemide orally and rectally (suppository with the Witepsol H 15 base) (Fig. 2). The ED50 value was calculated from the figure in both cases ($ED50_{supp} = 15.39$ mg, $ED50_{peros} = 19.03$ mg), which revealed that rectal administration is slightly more effective than oral administration. In the case of furosemide the hepatic first-pass effect is almost negligible; the major site for the first-pass metabolism of the drug in rats is probably the GI tract. Gastrointestinal and intestinal first-pass effect has been described in rats concerning furosemide,

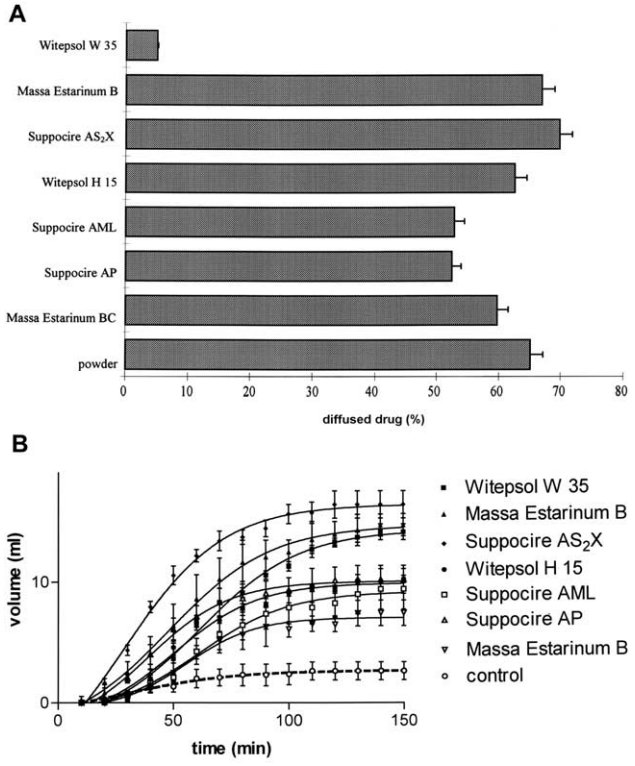


Fig. 1. (a) Furosemide release from different suppository bases after 240 min. (b) Diuretic effect of different suppository bases containing furosemide in rats. The data are the averages of the results of six experiments (±SEM).

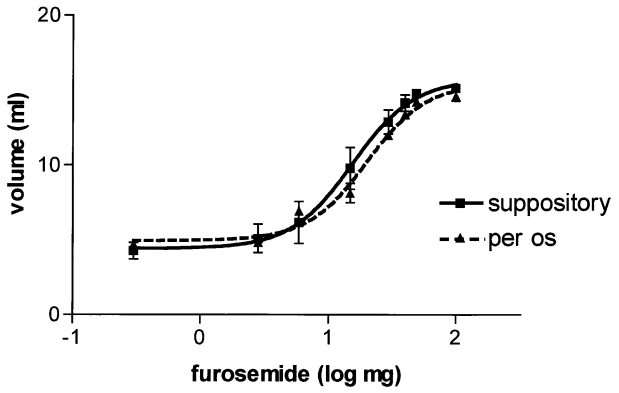


Fig. 2. Dose-dependent effect of furosemide. After suppository and per os application 20 ml/kg water was given per rat. The urine was collected for 150 min. The error bars represent the SEM.

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

Furosemide was incorporated in suppository bases, and after application in rats urine was collected for 150 min. Compared to the control, a significant increase was observed in the quantity of urine when Suppocire AP (*$P < 0.05$), Witepsol H 15 (*$P < 0.05$), Witepsol W 35 (**$P < 0.01$), Massa Esterinum B (***$P < 0.001$) and Suppocire $AS_2X$ (***$P < 0.001$) 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. 1b). The effectiveness of Suppocire $AS_2X$ and Massa Estarinum B is clearly shown by the fact that the amount of urine collected for 150 min came near to the 24 h urine quantity of rats [19].

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

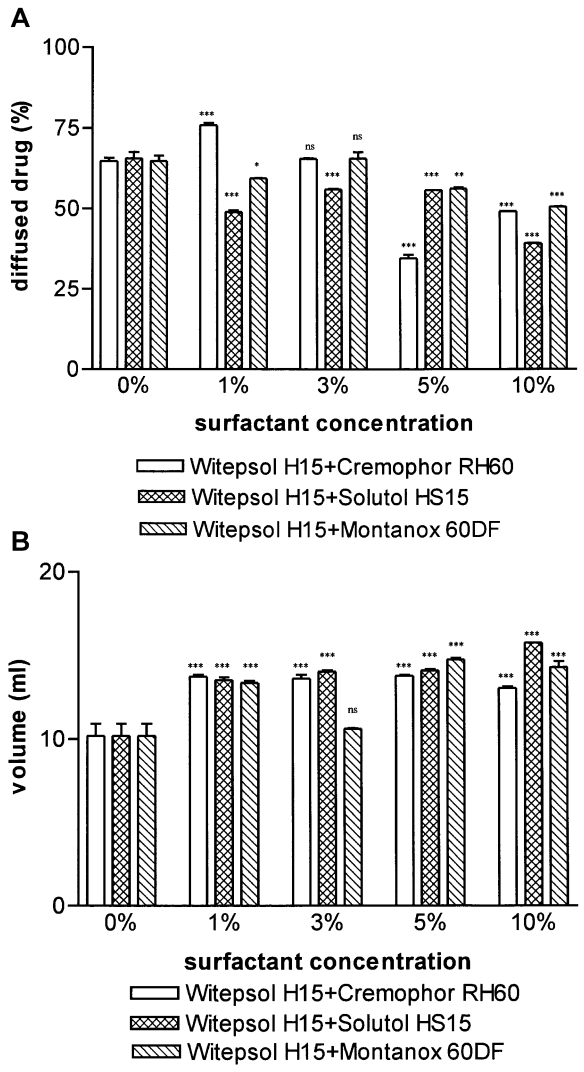


Fig. 3. (a) Influence of additives on drug release after 240 min (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus control, analysis of variance Newman–Keuls test). (b) Influence of additives on diuretic effect in rats. The data are the averages of the results of six experiments (±SEM) (*$P < 0.05$,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

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

In the in vivo examinations the use of surfactants led to a significant increase in the amount of urine collected (Fig. 3b). Their effect is composed of several factors: they moisten the drug, they 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. suggest that surfactants, which are commonly added to pharmaceutical formulation, may enhance the intestinal absorption of some drugs by inhibiting an apically polarized efflux system [23]. In the animal experiments performed with rats all three additives increased the quantity of the excreted urine approximately to the same extent, which indicates increased drug liberation. Fig. 3b also shows that the increase of the surfactant concentration was not accompanied by significant changes, so a concentration of 1% is enough to achieve the desired effect.

## 4. Conclusion

The comparison of the membrane diffusion examinations with the actual diuretic effect shows that drug release and the pharmacological effect had the same tendency in 70% of cases, so a greater extent of furosemide release was associated with a greater quantity of rat urine. The best results were achieved in both cases with the Suppocire $AS_2X$ base, which means that drug release was about 70% and the animal produced about 15 ml of urine in 150 min, and according to the literature this corresponds approximately to the daily urine quantity of a rat. The Witepsol H 15 base had better in vitro than in vivo results, while in the case of the Witepsol W 35 base the pharmacological results proved to be better than the results of the membrane diffusion examinations. This also confirms that in vivo trials are essential to get a clear picture of the drug–base–living organism interactions and thus to choose the best composition.

Based on the results obtained, two compositions were found to be suitable for formulating furosemide-containing suppositories: one is the Suppocire $AS_2X$ suppository base in itself, which proved to be the best both in the membrane diffusion and the animal experiments, and the other is the Witepsol H 15 suppository base with 1% of Cremophor RH 60 additive, which also gave optimal results with both examination methods.

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