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In vitro methods for the evaluation of drug availability from suppositories: comparison between biological and artificial membranes

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Drug availability from suppositories is currently evaluated *in vitro* by means of a model consisting of a dialysis tube (porous membrane) or isolated biological membrane (animal rectum). We propose a new alternative *in vitro* method to determine drug availability from suppositories consisting of an artificial membrane soaked with *n*-octanol, coupled with a filter paper sheet soaked with phosphate buffer. This method provides for an integrated hydro-lipophilic simulation of the biological membrane, including the mucus layer adhering to the rectal mucosa. By simply using the porous membrane, the amount of drug released varied directly according to its solubility for formulations with lipophilic excipients. For formulations with hydrophilic excipients, drugs with low/intermediate solubility in water showed increased availability in comparison to lipophilic excipients. The *in vitro* rat rectum model provided overall results that were similar to those obtained with the porous membrane method, although the percentage values of AUC were lower. The new model of *in vitro* simulated absorption produced a degree of drug availability that was lower in comparison to both previous methods. However, the simulated model appeared to give a pattern of drug availability closer to that of the model of *in vitro* rat rectum. The new *in vitro* artificial model thus appears to be useful in suppositories preformulation studies, allowing for an estimate of drug availability and the choice of the most adequate excipient.

1. Introduction

Drug availability from suppository formulations is currently evaluated by means of a simulated model consisting of a dialysis tube, in which the suppository is placed, immersed in a beaker containing buffer at a temperature of 37 °C (Ayres et al. 1976; Tukker et al. 1983; Realdon et al. 2000, 2001). The amount of drug dissolved and passed through the membrane is determined and used as a measure of drug availability. This model represents an oversimplification of the overall process occurring in the living organism. The rectal compartment, simulated by the dialysis tube containing a small amount of buffer solution, and the plasmatic compartment, consisting of the surrounding buffer solution, are separated by a dialysis membrane, which is far from the real structure of mucous membranes of the rectum. The dialysis membrane consists of a plastic cellophane layer with pores, allowing the simple diffusion of molecules according to a concentration gradient. Contact between the two phases of the model, inside and outside the dialysis membrane, takes place through the membrane pores (Realdon et al. 2002).
Under physiological conditions, the drug contained in the suppository undergoes more complex interactions: first of all the drug dissolves in the rectal mucus, then it is transported through the complex structure of the rectal mucosa, and it is subsequently transferred to the plasmatic compartment.

The need of a more adequate method for the study of drug availability from the rectal compartment has led to the *in vitro* use of a biological membrane, obtained from the animal rectum (rat or rabbit) (Izgü et al. 1981; Urban et al. 1991), in which the suppository is placed. This *in vitro* biological model provides a simulation of a three-phase system, consisting of the rectal compartment, the rectal biological barrier and a simulation of the plasmatic compartment.
In order to find a substitute for the biological tissue, we propose here an alternative new method to determine drug availability from suppository formulations. On the basis of previous studies on different formulations and different theoretical models mimicking drug absorption (Striker 1971, 1973; Loth et al. 1978; Realdon et al. 1996; Jahn et al. 2001; Realdon et al. 2002; Mrestani et al. 2003; Mrestani et al. 2004; Alkrad et al. 2003), we created an artificial membrane consisting of a cellulose ester polymer membrane soaked with *n*-octanol, coupled with a filter paper sheet soaked with phosphate buffer, providing an integrated hydro-lipophilic simulation of the biological membrane, including the mucus layer adhering to the rectal mucosa.
Diffusion of a drug through the rectal membrane is related to the specific physico-chemical characteristics of the molecule, as well as by the composition of the formulation. In this study we aimed to evaluate the *in vitro* drug availability from suppositories according to drug solubility, and

in relation to the suppository base composition, using the three *in vitro* models described above.

2. Investigations and results

The release of drugs from suppositories prepared with lipophilic bases (Witepsol H15, W35 and S55) or hydrosoluble base (Macrogols, composed by a 60 : 40 mixture of Macrogol 4000 and Macrogol 400) is reported in Figs. 1 and 2 and expressed as $AUC_{0-360\ min}$. Aqueous solubility of the dugs, expressed as mg/ml, is reported in the Figs. and was determined according to experimental methods previously reported by our laboratory (Realdon et al. 2000).

The amount of drugs released through the porous membrane, varied directly according to their water solubility (Fig. 1) in suppository formulations with lipophilic excipients (Witepsol H15 and W35). Propyphenazone and naproxen, which have a low water-solubility, gave a lower AUC value; on the contrary, aminophylline and guaifenesine (highly soluble in water) showed the highest AUC values. Release of drugs from suppositories prepared with Witepsol S55 showed a pattern which was not related to water solubility (Fig. 1). Intermediate AUC values were found for acetaminophen and aminophenazone, in accordance to their solubility. In suppository formulations with hydrosoluble excipients (Macrogols), drugs with low/intermediate solubility in water (such as naproxen and acetaminophen) showed increased availability in comparison to lipophilic excipients due to the solubilizing effect of Macrogols.

The *in vitro* rat rectum model provided overall results that were similar to those obtained with the porous membrane method (Fig. 1), although the percentage values of AUC were significantly lower ($p < 0.001$). Only in suppositories prepared with the lipophilic excipients Witepsol H15 and W35, was naproxen release not significantly different between the two compared methods. In rat rectum model, aminophylline (a drug with a high value of water-solubility) showed a value of availability lower than expected not only for suppositories with the lipophilic excipients Witepsol H15 and W35 but also with the hydrosoluble excipients Macrogols.

The model of *in vitro* simulated absorption (Fig. 2) produced a degree of drug availability that was significantly lower ($p < 0.001$) in comparison to both previous methods; in fact, under all the conditions of drug solubility and excipient composition tested, the AUC values were consistently lower. However, the simulated model appeared to give a pattern of drug availability closer to that of the *in vitro* rat rectum model. The peculiar behaviour of aminophylline was also confirmed with this model.

3. Discussion

Although the classical porous membrane model provides a simplified method for the estimation of drug release from suppositories (Ayres et al. 1976; Tukker et al. 1983; Realdon et al. 2000), it represents an oversimplification of the complex mechanisms which occur *in vivo* in the rectal compartment. The present study aimed to develop a new *in vitro* simulated absorption test for suppositories. The new method is derived from methods used in the determination of drug absorption from ointments through the skin

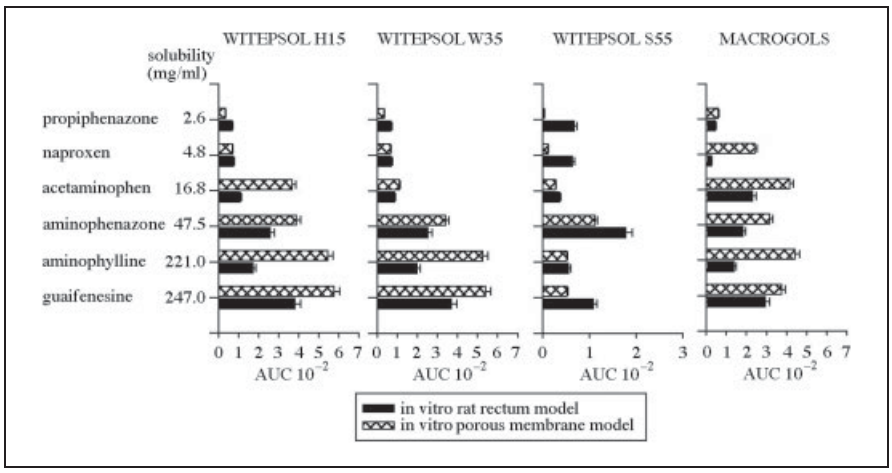


Fig. 1:
Values of AUC [percent released amount · time (0 ÷ 360 min)] calculated from the percent time-dependent release curves of the drugs in suppositories with different excipients. Data are expressed as mean ± standard deviation from 6 replicates. The water solubility of each drug is reported as mg/ml. The comparison of the *in vitro* rat rectum model and *in vitro* porous membrane model is presented. All data are significantly different ($p < 0.001$) between the two methods, except for formulations containing naproxen in Witepsol H15 and W35 excipients

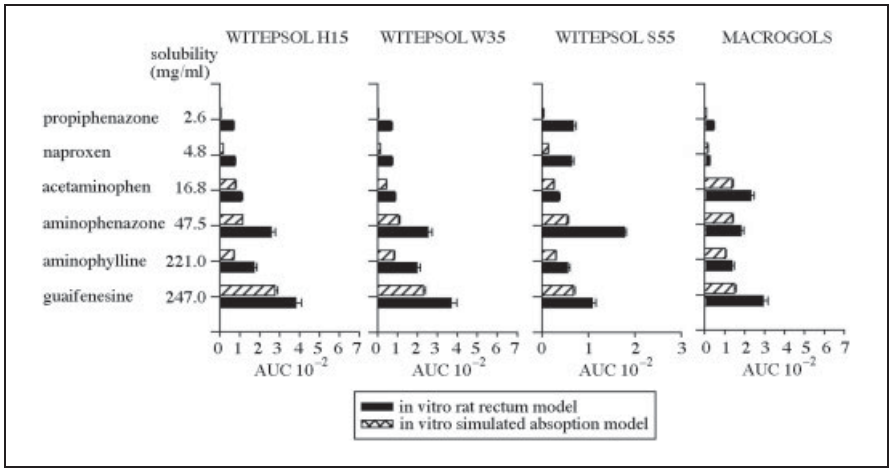


Fig. 2:
Values of AUC [percent released amount · time (0 ÷ 360 min)] calculated from the percent time-dependent release curves of the drugs in suppositories with different excipients. Data are expressed as mean ± standard deviation from 6 replicates. The water solubility of each drug is reported as mg/ml. The comparison of the *in vitro* rat rectum model and the new proposed *in vitro* simulated absorption model is presented. All data are significantly different ($p < 0.001$) between the two methods

(Striker 1971, 1973; Loth et al. 1978; Realdon et al. 1996, 2002). The use of multi-layer models for the study of drug release and penetration through tissues has become a new approach in preformulation studies, which also use complex synthetic multilayer systems that can mimic biological membranes (Realdon et al. 1996; Jahn et al. 2001; Alkrad et al. 2003; Mrestani et al. 2003; Mrestani et al. 2004). The new method consists of an artificial membrane of cellulose ester polymer soaked with *n*-octanol, coupled with filter paper soaked with phosphate buffer. The polymeric membrane simulates the rectal barrier; the *n*-octanol impregnation, which occupies the membrane pores, represents the lipoid barrier between the rectal and plasmatic phases (Realdon et al. 2002); the filter paper soaked with buffer simulates the mucus adhering to the rectal mucosa. The present data indicate that the use of an artificial structure mimicking an integrated hydro-lipophilic biological membrane, including the mucus layer, provides a good *in vitro* method for the prediction of *in vivo* drug absorption from suppositories.

Before considering the new artificial membrane method, we evaluated for comparison another model for the measure of drug absorption from suppositories; i.e., the isolated rectum model, obtained from rat, of which a report in the literature exists (Izgü et al. 1981). This *in vitro* biological model provides a simulation of a three-phase system, consisting of the rectal compartment, the rectal biological barrier and a simulation of the plasmatic compartment. In our hands the rat rectum model produced results similar to those obtained with the classical porous membrane method. The difference was only quantitative, being the total amount of drug released lower, probably as a consequence of a more selective role exerted by the rectal mucosa on drug absorption.

With formulations made of lipophilic excipients (Witepsol H15 and W35), the release of naproxen was similarly low in both two methods. Naproxen has a low water-solubility and this causes a fast onset of saturation in the rectal compartment, limiting the rate of diffusion. In this case, the slow kinetics may be the reason for the similar behaviour evidenced with the two methods.

In rat rectum model, aminophylline (highly water-soluble) showed an availability lower than expected from suppositories with the lipophilic excipients Witepsol H15 and W35, but also with the hydrosoluble excipients Macrogols. This indicates that the *in vitro* rectal membrane presents a selective influence on drug absorption, independent of excipient. This result suggests that the simple diffusion rate through a porous membrane is not the sole mechanism to be considered for a complete evaluation of rectal absorption of all types of drugs and indicates the need for a more adequate model.

The drug availability evidenced with the model of *in vitro* simulated absorption was significantly lower in comparison to both previous methods at all the conditions of drug solubility and excipient composition. The AUC values confirmed once again that the amounts of drug absorbed were conditioned by drug aqueous solubility, accordingly to the principle demonstrated above, common to all the three methods: the higher was drug aqueous solubility, the higher was the amount of drug solubilized into the intra-rectal phase and consequently the higher was its availability for the absorption. In each case, the barrier modulated the intensity of the absorption in spite of a rapid and high intra-rectal availability as a consequence of a rapid release from the suppository. The new model produced an amount of drug availability closer to that found with the *in vitro* model of the rat rectum. The peculiar behaviour of aminophylline, while possessing high water-solubility has a low absorption, was confirmed also with this model, indicating the similarity with rat mucosa. The behaviour of aminophylline is particularly highlighted by an alternative way of presenting our data, as in the example of Fig. 3, which compared the AUC values *vs* drug solubility (log scale) obtained with the three *in vitro* methods with Witepsol H15 excipient.

In suppositories prepared with Witepsol S55, which contains surfactant substances, unpredictable effects were found. This suggests that complex interactions may occur among excipients, drugs and membranes, requiring a complete evaluation of the absorption phenomena, e.g. by using very different *in vitro* methods proposed here.

By using different types of *in vitro* models it is possible to elucidate the influence of general parameters, including the effects of the excipient type, viscosity of the molten mass of the suppository and water solubility of the drug. Since rectal absorption occurs fundamentally by diffusion,

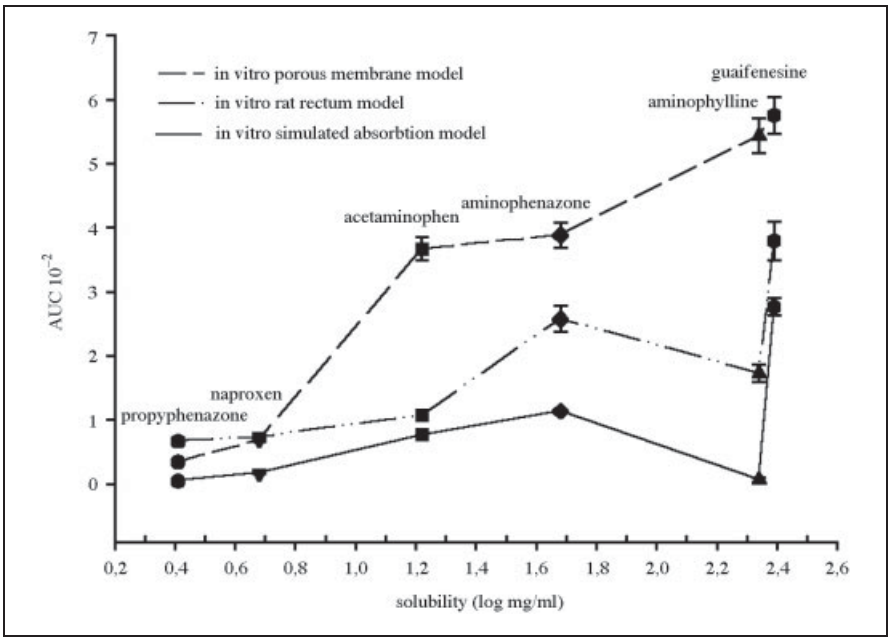
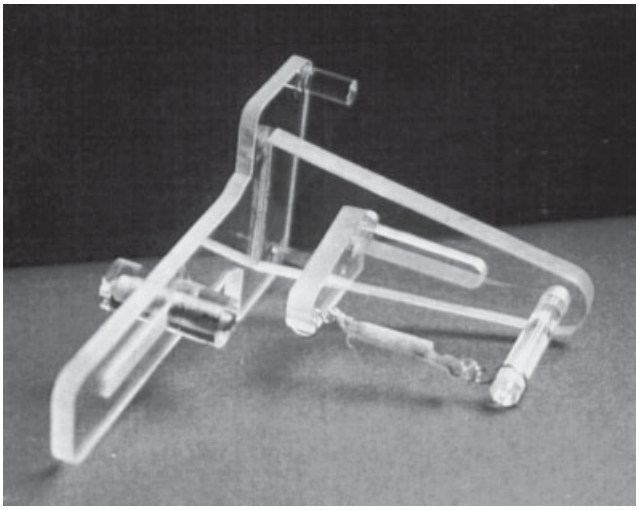
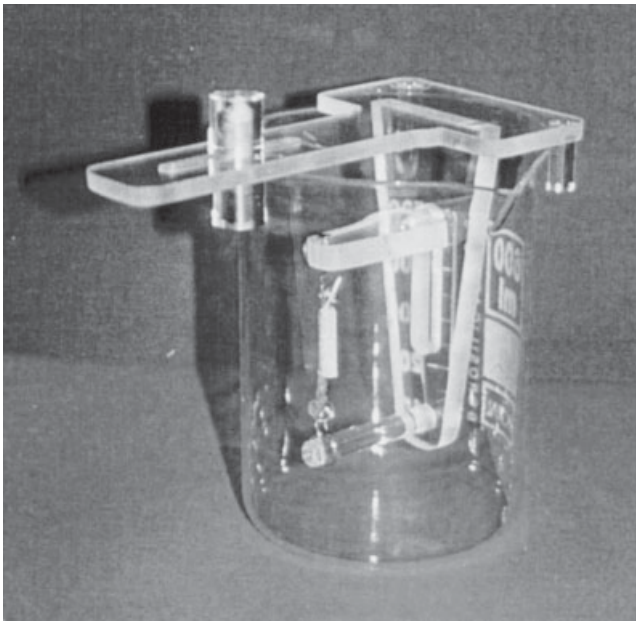


Fig. 3:
Comparison among the AUC values *vs* drug solubility (log scale) obtained with the three *in vitro* methods by adopting Witepsol H15 as excipient.
● propyphenazone, ▼ naproxen, ■ acetaminophen, ◆ aminophenazone, ▲ aminophylline, ● guaifenesine

drug release rates from suppositories affects its own concentration in the aqueous phase and hence the absorption rate. Therefore, solubility of a drug in water should be considered as a fundamental factor influencing absorption through the rectal mucous membrane (Realdon et al. 2000). The new *in vitro* artificial membrane method appears to be less influenced by the solubility of the drug, in comparison to the other two used models. Moreover, the new method, providing a more complex layer structure with differential characteristics mimicking the rectum biological membranes, could help in considering the many aspects of drug diffusion without the need of animal tissue.
Drug dissolution rate in the intrarectal aqueous phase is influenced by parameters related to the molten suppository mass (Ayres et al. 1976; Bornschein et al. 1980; Realdon et al. 1997) and excipient composition. The viscosity of the molten suppository, depending on both the composition of the excipient and the amount of drug in suspension, is fundamental for the regulation of release rate. Suppository excipient composition may surely affect drug diffusion across the barrier within the three models considered here, suggesting similar behaviour on different membrane constituents as indicated already in our previous work (Chicco et al. 1999). The influence of the excipient composition is apparent in each model and is responsible for the overall behaviour.

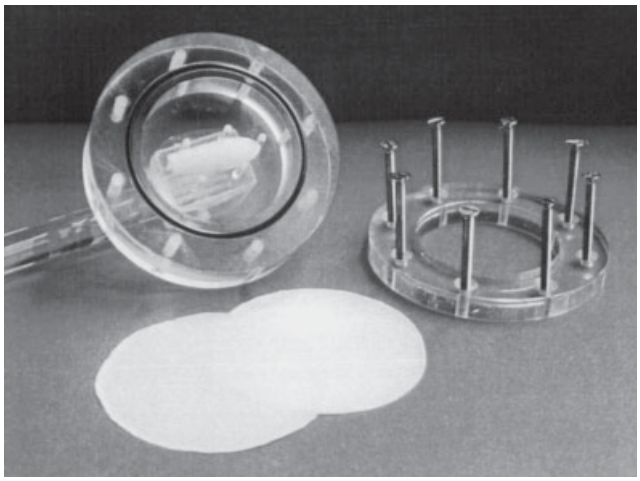


A)

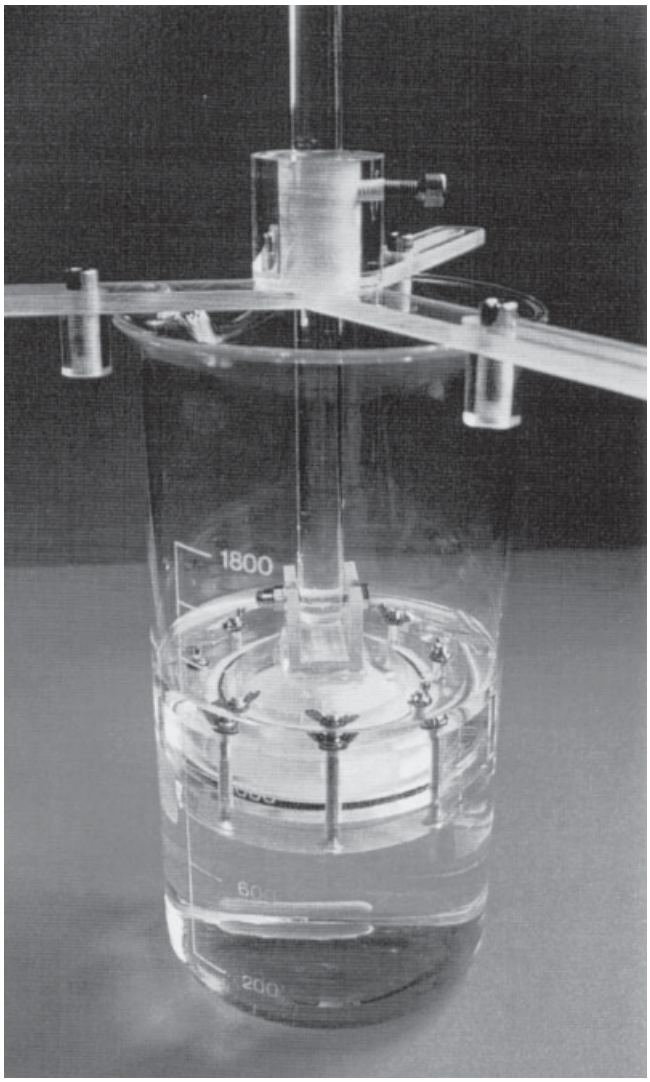


B)

Fig. 4: Experimental set-up for the *in vitro* rat rectum model. A) The Perspex support for holding the isolated rectum; B) final aspect of the assembled set-up



A)



B)

Fig. 5: Experimental set-up for the *in vitro* simulated absorption assay. A) Unassembled components of the cell and artificial membranes (a cellulose ester polymer soaked with *n*-octanol, coupled with a filter paper); B) assembled components

In conclusion, the new *in vitro* method, consisting of coupled artificial membranes, permits a good correlation with other *in vitro* models, and appears to be closer to the *in vitro* rat rectum model. Thus, the new *in vitro* artificial model appears to be useful in suppositories preformulation studies, indicating, importantly the availability of a drug to be absorbed. Moreover, in preformulation studies it represents a useful method for the selection of an adequate excipient. The model could play an important role in the quality control of batches of suppositories because constant operative conditions can be assumed.

4. Experimental

4.1. Materials

Drugs tested-Propyphenazone, naproxen, acetaminophen, aminophenazone, aminophylline, guaifenesine. All drugs were purchased from ACEF (Fiorenzuola d'Arda, Piacenza, Italy).
Excipients-Witepsol H15, W35 and S55 were obtained from Hüls AG, Werk Witten, Germany. Macrogol 4000 and Macrogol 400 were from ACEF (Fiorenzuola d'Arda, Piacenza, Italy).

4.2. Preparation of suppositories

Suppositories of 3 ml were prepared with each drug tested at the same unitary dose of 500 mg. The excipient was melted to 40 °C and the drug in fine powder form was uniformly dispersed by a Silverson turbomixer (Waterside, Chesham, U.K.). The melted mass was then poured into disposable PVC moulds and cooled to solidification at room temperature (18–20 °C). After 24 h the suppositories were refrigerated (5–10 °C) until use in the different tests.
Suppositories for the *in vitro* rat rectum test were prepared by pouring the same melted mass into moulds with a diameter of 5 mm and a height of 20 mm. Suppositories of 400 mg, containing 60 mg of drug, were obtained and preserved in the above mentioned conditions.

4.3. In vitro drug availability with the porous membrane model

A 3-ml suppository formulation was placed in a dialysis tube (12.5×2.5 cm), previously soaked in water overnight at room temperature, containing 5 ml of phosphate buffer solution 1/15 M, pH 7.4. The tube was sealed and placed horizontally in a beaker containing 2 L of the same buffer solution, maintained at 37 ± 0.5 °C and stirred at 100 rpm by a 5-cm blade stirrer. From the diffusion fluid simulating the plasma compartment, samples of 3 ml were taken at 15 min intervals for a total of 6 h. The volume of each sample was replaced with the same amount of buffer solution. Drug concentration was determined spectrophotometrically as previously reported (Realdon et al. 2000, 2001). The assay was performed simultaneously on 6 replicates for each drug and excipient.

4.4. In vitro rat rectum model

The isolated rectum, obtained from male Wistar rats (mean weigh 200 g) sacrificed after anaesthesia, was closed with suture at one end. A suppository (weighing 400 mg) was introduced and the rectum was closed at the other end, then tied vertically on a Perspex support (Fig. 4a) and placed in isotonic phosphate buffer, pH 7.4, maintained at 37 ± 0.5 °C (Fig. 4b), stirred at 100 rpm by a magnetic stirrer. From the fluid simulating the plasma compartment, samples of 1 ml were taken at 15 min intervals for a total of 6 h. The volume of each sample was replaced with the same amount of buffer solution. Drug concentration was determined spectrophotometrically as previously reported (Realdon et al. 2000, 2001). A total of 6 replicates was carried out for each drug and excipient.

4.5. In vitro simulated absorption

A 3-ml suppository formulation was inserted in a Perspex diffusion cell (Fig. 5a) with a central cavity diameter of 6 cm, 15 mm deep, provided with a membrane consisting of a cellulose ester polymer (Millipore HAWP 09000, pore size 0.45 μm) soaked for 1 h with *n*-octanol, coupled with a filter paper sheet (E/2, 350 g/m^2, Cartiera di Cordenons, Italy) soaked with phosphate buffer (1/15 M, pH 7.4). The suppository was placed in direct contact with the soaked filter paper, mimicking the mucus layer of the rectum. After placing the suppository in the diffusion cell, 10 ml of the buffer were added. The cell was then sealed and placed horizontally in a beaker (Fig. 5b) containing 1 L of the buffer solution, maintained at 37 ± 0.5 °C and stirred at 100 rpm by a magnetic stirrer. From the fluid simulating the plasma compartment, samples of 2 ml were taken at 15 min intervals for a total of 6 h. The volume of each sample was replaced with the same amount of buffer solution. Drug concentration was determined spectrophotometrically as previously reported (Realdon et al. 2000, 2001). The essay was performed simultaneously on 6 replicates for each drug and excipient.

4.6. Mathematical analysis and statistics

To provide an integrated measure to evaluate experimental data, the area under the curve (AUC) was calculated for the time-course of percent amount (*a*) of drug released from suppositories, from time 0 to 360 min. The AUC was obtained by the trapezoidal rule, i.e.:

$$\mathrm{AUC} = 1/2\,\Sigma\,(t_{i+1} - t_i) \cdot (\alpha_i + \alpha_{i+1}) \qquad (1)$$

where t_i ($i = 0, 15, 30, \ldots, 360$) is the time (min) of measurement and *a* is the percent of drug released at those times. The AUC data presented are normalized to drug amount contained in the different suppositories and the volume of the buffer solution simulating the plasma compartment. The 360 min-period has been considered in order to mimic a reasonable time of permanence in the rectum; in all the cases, the same 360 min period was considered.
Data obtained from different experimental conditions were compared using Student's t test for unpaired data. A statistically significant difference was assumed for $p < 0.05$ (two-tailed).

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