Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Padova, Italy

Effect of the surfactant on the availability of piroxicam as a poorly hydrosoluble drug from suppositories

M. Dal Zotto, E. Franceschinis, A. Punchina, N. Realdon

Received June 26, 2011, accepted July 28, 2011

Prof. Dr. Nicola Realdon, Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Padova, Via F. Marzolo, 5, 35131 Padova
nicola.realdon@unipd.it

Pharmazie 67: 37–45 (2012) *doi: 10.1691/ph.2012.1092*

The use of surfactants in suppository formulations has been suggested to improve availability of poorly soluble drugs. In the present study, different kinds of surfactants have been investigated to clarify the influence on piroxicam release from suppositories formulated with both lipophilic and hydrophilic bases. Two hydrophilic glucose-derivate surfactants, and a polyoxylglyceride amphiphilic surfactant, all with high HLB values, were investigated for their use in improving drug availability. The two glucose derivate surfactants reduced drug availability from both lipophilic suppositories and hydrophilic formulations, according to longer disintegration times and drug micellization. The more complex surfactant, a lauroyl macrogolglyceride, showed an increase in piroxicam availability from lipophilic suppositories at the higher tested concentrations (15% and 20 %). Otherwise, when used in hydrophilic formulations, it was less effective in promoting drug release and even reduced drug availability.

1. Introduction

The intrarectal route can be used for advantageous drug administration to obtain a systemic effect. By this route, more than 75% of the drug can achieve systemic circulation without undergoing the first-pass effect in the liver (Abd-Elbary et al. 1992; Plaxo 1976). After release from the suppository and dissolution into the intrarectal fluid, most of the dissolved drug is absorbed by passive diffusion through the rectal *mucosa* into the plasmatic phase of the middle and inferior haemorrhoidal veins, which convey rectal blood directly to the inferior *vena cava*. Only a reduced amount of drug is absorbed through the superior haemorrhoidal vein, which drains blood into the hepatoportal system. Both drug solubility and base characteristics play a crucial role in the drug absorption rate.

Suppositories can be formulated with both lipophilic and hydrophilic bases. Mixtures of glyceride esters, named in the PhEur VI ed. as "*Adeps solidus*", which have standard chemical-physical characteristics, can be used as bases for lipophilic formulations, and mixtures of polyethylenglycols (PEG), namely macrogols, can be used as bases for hydrophilic formulations.

The mechanism of drug release depends on the kind of base. When suppositories are formulated with hydrophilic excipients, drug release takes place through the dissolution of the base into rectal fluids. When lipophilic excipients are used, drug release occurs with the melting of the lipophilic base at rectal temperature. It is therefore influenced by the melting point and viscosity of the molten mass.

In any case, the drug solubility in the intrarectal water phase plays an important role in absorption (Moolenaar and Schoonen 1980). Piroxicam, a non steroidal anti-inflammatory drug, is generally used for treatment in acute and chronic osteoarthritis and rheumatoid arthritis. According to the Biopharmaceutic Drug Classification System (BCS) (Amidon et al. 1995), it is a II-class drug with poor solubility in water, which can affect dissolution rate and drug absorption.

It has been suggested that the use of surfactants in suppository formulation improves the availability of poorly soluble drugs.

In the present study, different kinds of surfactant, two hydrophilic glucose derivates and an amphiphilic polyoxylgliceride, have been investigated as useful in improving drug availability to clarify the influence of their different chemical-physical characteristics on the release of piroxicam from suppositories.

2. Investigations and results

2.1. Melting point of suppositories

The melting point of the mass can influence drug release rate from lipophilic suppositories and different kinds of lipophilic masses can be used to produce suppositories. Witepsol H15 was chosen in the present study because of its low hydroxyl value and its melting range between 33.5 and 35.5 °C. Increasing concentrations of Tego Care 450, Montanov 68 PHA or Gelucire 44/14, were then added (Table 1).

Since the tested surfactants are solid or waxy at RT, their presence in the lipophilic base could modify the melting point of Witepsol H15 depending on their concentration in the mixture. The melting point produced by increasing the amounts of each surfactant in the mixture was therefore determined to define the highest concentration of surfactant maintaining the melting point below 37 °C.

The melting points of the bases formulated by adding Tego Care 450, Montanov 68 PHA 7.5%, or Gelucire 44/14 20%, to Witepsol H15 are shown in Table 2. Masses containing higher contents of each surfactant had melting points higher than 37 °C and were therefore unsuitable for rectal dosage forms. The effect of the drug on melting point was also investigated. As can be seen in

Table 1: Tested formulations

Base		surfactant	
	type	concentration (%w/w)	
Lipophilic bases	*Adeps solidus* (Witepsol H15)	–	–
		Polyglyceril-3 methylglucose distearate (Tego Care 450)	5 7.5
		Alkyl glucose (Montanov 68 PHA)	5 7.5
	Adeps solidus (Witepsol H15)	Lauroyl macrogolglycerides (Gelucire 44/14)	5 10 15 20
	Macrogol mixture PEG 400 40% PEG 4000 60%	–	–
		Polyglyceril-3 methylglucose distearate (Tego Care 450)	5 7.5
Hydrophilic bases	Macrogol mixture PEG 400 40% PEG 4000 60%	Alkyl glucose (Montanov 68 PHA)	5 7.5
		Lauroyl macrogolglycerides (Gelucire 44/14)	5 20

Table 2, the normal dose of piroxicam (20 mg *per* rectal dose unit) produced no important modification on the melting points of the lipophilic bases tested.

2.2. Suppository disintegration test

The disintegration of a solid dosage form is the first step towards drug availability. According to the PhEur VI ed., the disintegration process has to take place within 30 min for suppositories with lipophilic bases and within 60 min for suppositories with hydrophilic bases.

The disintegration test was performed on the lipophilic and hydrophilic formulations tested and the results, expressed as minutes required for disintegration, are shown in Table 2.

According to PhEur requirements, all the lipophilic formulations showed disintegration times within 30 min. The disintegration time increased with increasing concentrations of surfactant; however, in the presence of the highest tested concentration of Gelucire 44/14 (20%*w/w*) the disintegration time of the suppositories was faster than with Witepsol H15 alone. In the case of hydrophilic formulations, only the suppositories containing Gelucire 44/14 satisfied the EurPh requirements and disintegrated within 60 min, independent of surfactant content (Table 2).

The different influence of the tested surfactants on the disintegration time of the hydrophilic bases can be ascribed to their chemical structure and their different capacity to disperse in polyethylenglycols at 48 °C during preparation. As reported in previous studies (Realdon et al. 2001), when the molten mass undergoes solidification, a solid solution forms which produces a compact structure characterized by slow disintegration.

2.3. Rheological characteristics of suppositories

As referred in the literature, the viscosity of the molten mass is related to the structural organisation, and the type and number of

Table 2: Melting points and rheological characteristics of lipophilic suppositories; disintegration times of lipophilic and hydrophilic suppositories

Bases		Melting point (lipophilic bases) (°C)	Melting point (lipophilic suppositories) (°C)	Viscosity (lipophilic suppositories) (Pa*s)	Disintegration times (t)
lipophilic formulations					
Adeps solidus (Witepsol H15)		34.36 °C	34.90 °C	0.041	21′40″
Witepsol H15 +	5%	35.50	35.80	0.063	16′44″
Tego Care	7.5%	35.90	36.30	0.088	17′00″
Witepsol H15 +	5%	35.70	36.41	0.044	11′44″
Montanov	7.5%	36.12	36.56	0.044	13′28″
	5%	34.80	35.12	0.044	16′35″
Witepsol H15 +	10%	35.27	35.18	0.044	14′00″
Gelucire 44/14	15%	35.26	35.15	0.047	18′40″
	20%	35.52	35.17	0.048	20′45″
hydrophilic formulations					
Macrogol mixture (PEG 400 40% PEG4000 60%)		–	–	–	16′00″
Macrogol mixture +	5%	–	–	–	1h 26′00″
Tego Care	7.5%	–	–	–	57′16″
Macrogol mixture +	5%	–	–	–	1h 26′00″
Montanov	7.5%	–	–	–	2h 12′00″
Macrogol mixture) +	5%	–	–	–	15′35″
Gelucire	20%	–	–		15′50″

particles from which it is produced. As is well known, viscosity under rectal conditions plays an important role in drug release from lipophilic suppositories.
The characteristics of viscosity of the molten suppositories were therefore investigated to evaluate the effect of the surfactant. As displayed in Table 2, the addition of Gelucire 44/14 and Montanov to the base generally produced only a slight increase in viscosity values. On the other hand, when Tego Care 450 was added, the viscosity of the molten mass increased to a greater extent and was related to surfactant concentration.

2.4. Drug release from suppositories

When the suppositories are formulated with lipophilic excipients, drug availability takes place after melting at body temperature, either by diffusion of drug particles in the molecular state, when the drug is soluble in the base, or, when the drug is insoluble, by movement of solid particles to the *interfacia* between the molten mass and the rectal *aqueous* phase, in which drug particles must dissolve. Due to this complex path, a series of chemical-physical characteristics, such as the melting range and melting rate, viscosity of the base and solubility of the drug, can influence drug release (Ragazzi et al. 1984; Zuber et al. 1988; Moes 1989; Al Gohary and Foda 1993; Calis et al. 1994; Scinoda et al. 1995).
To investigate piroxicam release, a test was carried out to evaluate the direct release of piroxicam at rectal temperature ($37 \pm 0.5\,^{\circ}C$) to a suitable volume of phosphate buffer pH 7.4 1/15 M as acceptor fluid.
In Fig. 1, the courses of direct release of piroxicam are compared in relation to the composition of bases.
When lipophilic base was used, the addition of 7.5% of glucose surfactants, Tego Care 450 and Montanov 68 PHA, reduced the release of piroxicam after three hours to about 50% compared with suppositories with Witepsol H15 alone (see Fig. 1A, B). This effect was slightly more marked with Montanov 68 PHA. When the lower concentration (5%) of surfactant was added, the effect on drug release was reduced.
When the polyethylene glycol derivate Gelucire 44/14 was added to the lipophilic base, the lower tested concentration (5%) reduced to 50% the amount of piroxicam released at the test end point. Otherwise, in the presence of higher concentrations of surfactant, drug release was complete and occurred faster as the content of Gelucire increased (Fig. 1C).
When suppositories were formulated with a hydrophilic macrogol base, drug availability depended on the dissolution rate into the rectal phase (Puech et al. 1981; Marvola et al. 1981; Abd El-Bary et al. 1983; Möller 1984; Vromans et al. 1984).
The PEG excipient dissolves progressively into the rectal phase, and its dissolution rate is influenced by the percentage and molecular weight of the solid PEG in the base. It is known that polyethylene glycols can be used to enhance the solubility or dissolution characteristics of poorly soluble compounds by making solid dispersions. Under this condition, once the suppository is dissolved, the drug particles are available in the rectal phase for systemic absorption by passive diffusion through the rectal *mucosa*. The direct release curves from hydrophilic formulations are also displayed in Fig. 1.
Piroxicam content was released within 40 min from suppositories prepared with the macrogol base alone. When the glucose surfactants were added, both Tego Care 450 and Montanov 68 PHA reduced the drug release rate to a similar extent, independent of the tested concentration. Further, only about 40% of the piroxicam content was released after three hours (Fig. 1D, E). The influence exerted by the polyoxyethilene glycol derivate on drug release was different. Both the tested concentrations produced a complete drug release within 40 min (Fig. 1E).

2.5. Drug availability from suppositories

Simplified models of the rectal compartment can be used to evaluate drug availability *in vitro*. In the present study, the compartment model described in the Experimental, widely validated in the literature, evaluates the amount of drug released through a dialysis membrane, which was used to simulate the rectal conditions (Tukker and De Blaey 1983; Izgü and Güngör 1981).
After rectal *in vivo* administration, the drug released from the suppository has to dissolve into the small volume (3–5 ml) of the rectal *mucous* secretion and, once dissolved, it can diffuse through the rectal barrier into the large volume of the plasmatic phase. In the model used, the two aqueous compartments, thermostated at $37\,^{\circ}C$ and separated by a dialysis membrane, can simulate rectal conditions. The small volume (5 ml) of water phase inside the dialysis membrane can simulate the intrarectal compartment, while the porous dialysis membrane simulates the rectal barrier and the larger aqueous phase (3l) simulates the plasmatic one.
The concentration of drug released into the intrarectal phase is critical for its systemic absorption, since the absorption process occurs by passive diffusion and absorption rate is, therefore, dependent on the gradient concentration between intrarectal and plasmatic compartments. Hence, the higher the concentration in the intrarectal phase, the higher the absorption rate.
The availability test was therefore performed on the studied formulations in order to evaluate the influence of various factors, such as the nature and composition of the base and the viscosity of the molten suppository, and the solubility in water of piroxicam, on the mechanisms which control availability.
The courses of piroxicam release from suppositories formulated with the two different bases alone, and with the surfactants added, are displayed in Fig. 2.
With a lipophilic Witepsol H15 base, the presence of glucose derivates as surfactants produced a similar influence on piroxicam release, as observed in the direct release test. Tego Care 450 reduced piroxicam release to a concentration-related extent: 7.5% of surfactant in the base allowed the release of about 50% of drug content (Fig. 2A). Montanov 68 PHA produced a similar decrease in piroxicam release, but in a concentration-independent manner (Fig. 2B).
The effect produced by the macrogol derivate, Gelucire 44/14, was dependent on its concentration in the lipophilic base. The lowest concentration (5%) reduced drug release to about 50%; otherwise, an increase in surfactant concentration generally increased piroxicam release, which was even higher than for suppositories with Witepsol H15 alone in the presence of the highest concentrations of Gelucire (15–20%) (Fig. 2C).
When macrogols are used as bases for hydrophilic suppositories, the melting point is higher than the rectal temperature and drug release occurs by progressive dissolution of the mass into the rectal compartment (Puech et al. 1981; Marvola et al. 1981; Abd El-Bary et al. 1983; Möller 1984; Vromans et al. 1984). As a consequence of the base dissolution, the volume of the intrarectal compartment progressively increases due to an osmotic effect exerted by the dissolved excipient which draws water from the plasmatic compartment (Ragazzi et al. 1986). Drug diffusion through the rectal barrier is, in any case, controlled by the concentration gradient between the intrarectal and plasmatic compartments, and therefore by the amount of drug available in the small volume of the intrarectal compartment.
As can be seen in Fig. 2D, after 6 h, drug availability from the suppositories formulated with the hydrophilic base alone was about 70% of the piroxicam content, and was slightly lower when compared to the lipophilic formulation with Witepsol H15.

DIRECT RELEASE

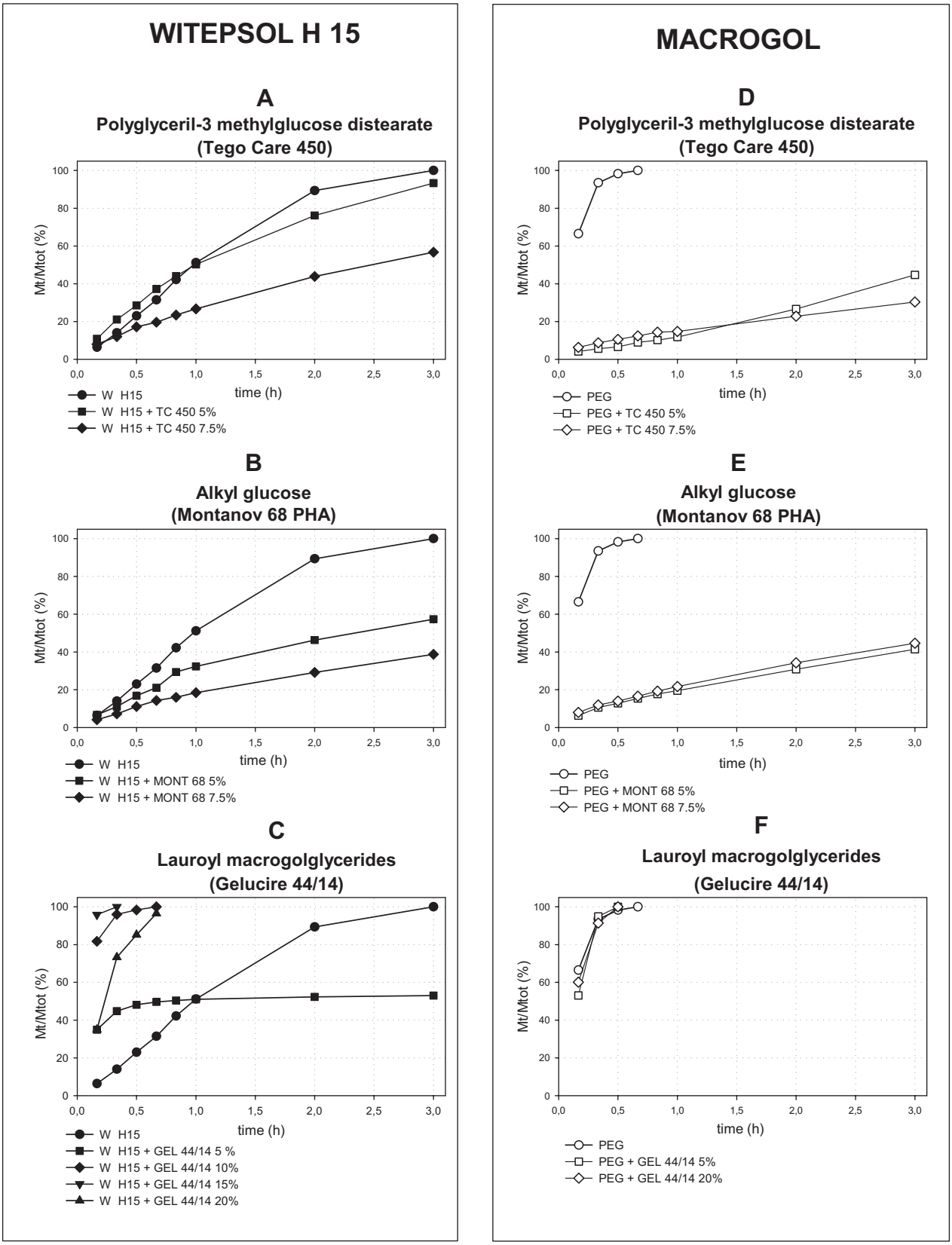

Fig. 1: Piroxicam release from lipophilic and hydrophilic suppositories with the addition of Gelucire 44/14 (A), Tego Care 450 (A, D), Montanov 68 PHA (B, E) and Gelucire 44/14 (C, F)

DRUG AVAILABILITY

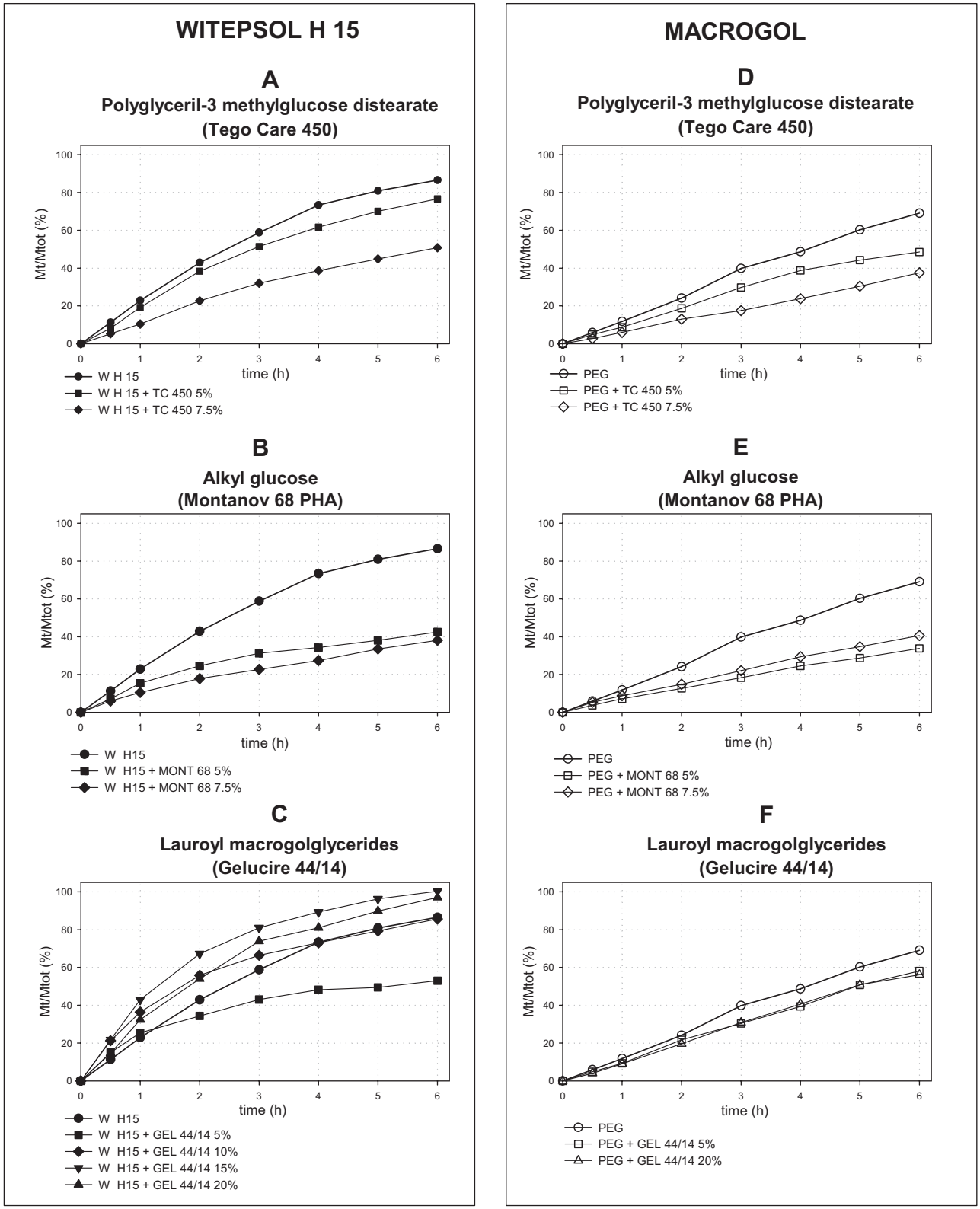

Fig. 2: Piroxicam availability through a dialysis membrane from lipophilic and hydrophilic suppositories with the addition of Tego Care 450 (A, D), Montanov 68 PHA (B, E) and Gelucire 44/14 (C, F)

When Tego Care 450 was added to the hydrophilic excipient, drug release decreased markedly in a dose-dependent manner. In the presence of 7.5% of surfactant, the availability of piroxicam in the acceptor phase simulating the plasmatic compartment was halved compared with the availability from the hydrophilic base alone (Fig. 2D). The addition of Montanov 68 PHA produced a similar decrease in drug availability, but in a dose-independent manner (Fig. 2E).

The presence of the macrogol-derivate Gelucire 44/14 in the formulation also decreased drug availability when compared to the hydrophilic base alone, but the effect was less marked and dose-independent (Fig. 2F).

2.6. *In vitro simulated absorption*

When applied *in vivo*, the drug particles released from suppositories undergo a series of interactions which are more complex than those which take place in the simplified rectal compartment model described above.

Under physiological conditions, drug particles must dissolve in the rectal *mucus*, to be then transported through the complex structure of the rectal *mucosa* and subsequently transferred into the plasmatic compartment. The need for a more adequate method to study drug availability from the rectal compartment led to the *in vitro* use of a biological membrane, obtained from animal *rectum* (rat or rabbit) (Izgü and Güngör 1981; Urban et al. 1991). On the basis of previous studies carried out on different formulations, and using different theoretical models to mimic drug absorption, to develop a substitute for biological tissue, an alternative method has been proposed to evaluate drug availability, based on the use of an artificial membrane. This model consists of a cellulose ester polymer membrane soaked with *n*-octanol, coupled with a filter paper sheet soaked with phosphate buffer. This provides an integrated hydro-lipophilic simulation of the biological membrane, including the mucus layer adhering to the rectal *mucosa*. This model was validated in previous studies on different formulations of suppositories and the results were well correlated to those obtained with *in vitro* models based on animal rectal membrane (Realdon et al. 2005).

The tested formulations were therefore tested for drug availability using this *in vitro* rectal model and the courses of drug availability are displayed in Fig. 3.

The addition of a higher concentration of Tego Care 450 in the lipophilic suppositories produced a 30% decrease in piroxicam availability compared with suppositories with the lipophilic base alone. Otherwise, relevant effect was observed in the presence of 5% of surfactant (Fig. 3A). When Montanov 68 PHA was added, the effect on drug availability was very similar, even though closely concentration-related (Fig. 3B).

When the release test was performed with the porous hydrophilic membrane, it was observed that the addition of Gelucire 44/14 as surfactant led to the availability of piroxicam decreasing at the lower concentration of 5%, and increasing at the higher concentrations of 15% and 20%, compared with Witepsol H15 alone (Fig. 3C). From hydrophilic suppositories, Piroxicam availability was, in any case, lower than from lipophilic formulations (Fig. 3).

However, as far as the general effect of surfactants on drug availability is concerned, when Tego Care 450 or Montanov 68 PHA were added to a macrogol base, a similar behaviour was observed as for the lipophilic suppositories: Piroxicam availability decreased to less than half, independent of surfactant concentration (Fig. 3D, E). Otherwise, the effect exerted by Gelucire 44/14 was different from lipophilic suppositories, since the decrease in piroxicam availability was related to surfactant concentration (Fig. 3F).

2.7. *Particle size distribution*

The transfer of the surfactant from suppositories to the intrarectal phase during the availability test, and the simulated absorption through membrane, was investigated by particle size analysis. All three surfactants tested produced micelles in the small volume of the buffer solution simulating the intrarectal phase (data not reported).

3. Discussion

Polyoxyethylene derivates are reported in the literature as hydrophilic non-ionic surfactants which are currently used widely in suppository formulations. In recent years, however, an increased interest has been focused on the use of glucose derivate surfactants, such as glucose fatty esters or alkyl glycosides.

In our investigation, we tested a polyglycerylglucose ester, Tego Care 450, a polyglyceryl-3 methylglucose distearate, which consists of glucose unit with an ether bound with polyglycerol-3 chain and two ester bounds with fat acid residues, and an alkylglucoside, Montanov 68 PHA, which consists of polyglucose with an ether bound with a fat alcohol residue. In both, an oloside replaces the polyoxyethylene group typical of traditional surfactants.

More recently, a series of complex surfactants have become available, which can increase the solubility and dissolution rate of poorly soluble drugs. Gelucire 44/14 is a waxy amphiphilic material with a nominal melting point of 44 °C and an HLB value of 14. Unlike the glucose derivates previously described, Gelucire 44/14 is a complex mixture of 20% mono-di-triglycerides, 72% mono-diesters of fat acids with PEG 1500, and 8% free PEG 1500. Due to the particular balance between short, medium and long chain residues, Gelucire 44/14 forms fine dispersions of excellent stability in contact with gastrointestinal fluids at body temperature and it tends to self-emulsify in an aqueous phase.

The aim of our investigation was to evaluate the effects of these materials, which are proposed as additives with advantageous toxicological properties, on the availability of a poorly soluble drug (piroxicam) from lipophilic and hydrophilic bases.

The results obtained indicate that the availability of piroxicam from the lipophilic base is influenced by the kind of surfactant. Independently of the type and concentration of surfactant, the melting point of the mass was lower than 37 °C, a first requirement for drug release. Moreover, the lipophilic suppositories meet the disintegration requirements stated in the PhEur showing disintegration times which are, in any case, faster than 30 min (Table 2). Nevertheless, the three surfactants can influence drug release depending on their chemical structure.

When compared with suppositories formulated with the lipophilic base alone, the presence of the glucose derivates Tego Care 450 and Montanov 68 PHA in the base showed a decrease in piroxicam release. At equal concentrations of surfactant, the effect of Montanov 68 PHA was more marked than Tego Care 450. These results are based on the melting points observed. In the presence of both surfactants at 7.5%, the melting points of the suppositories were about 1.5 °C higher than those of suppositories with Witepsol H15 alone. However, no correlation could be found with the disintegration times, which were faster in the presence of both Tego Care 450 (17 min) and Montanov 68 PHA (12 min).

Since the disintegration time is a critical parameter for predicting drug release from suppositories, the disintegration results should predict a release behaviour different from what we observed.

The decrease in piroxicam release can probably be ascribed to the micelle organization of the surfactants which can entrap drug particles in the base, thus slowing its release into the diffusion compartment. Moreover, the lower release of piroxicam observed in the presence of Montanov 68 PHA, compared with Tego Care 450, can be explained by the characteristics of Montanov 68 PHA, which is reported in the literature to form liquid crystals at 5% concentration, independently of the oil phase. These liquid crystals produce solid shells consisting of double layer lamellar envelopes surrounding oil droplets, inside which drug particles are entrapped, thus explaining the reduced release.

As described above, the polyoxyethylene derivate surfactant, Gelucire 44/14, produced a different effect on piroxicam release. When added at 10%, 15% and 20%, drug release was complete within 40 min, although at different release rates. This behaviour can be ascribed to the capacity of Gelucire to increase drug aqueous solubility about twenty-fold when added at 15% to the water phase, as described by Karataş et al. (2005). The increase in drug

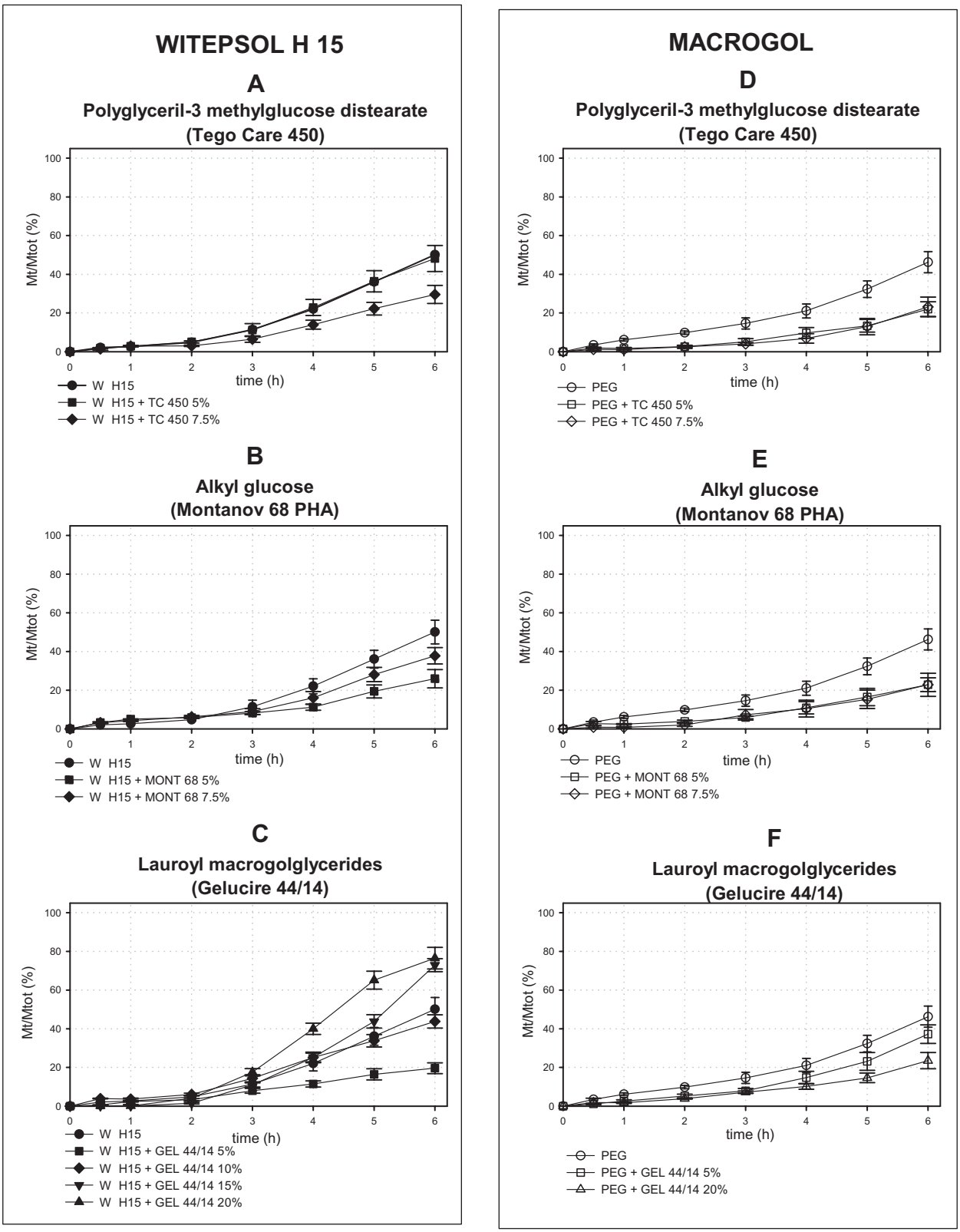

Fig. 3: Piroxicam *in vitro* simulated absorption through double layer membrane from lipophilic and hydrophilic suppositories prepared with Tego Care 450 (A, D), Montanov 68 PHA (B, E) and Gelucire 44/14 (C, F)

solubility can be explained by the association of two mechanisms, such as the increase in drug wettability and drug micellar solubilisation (Leuner and Dressman 2000; Damian et al. 2000; Hörter and Dressman 1997). Drug solubilization can be influenced by specific kinds of interaction related to both drug and solvent molecular structures (Anderson and Marra 1999). Specific interactions with mono-, di- and triglycerides, fat alcohols and polyoxyethylene fat esters, may increase the solubility of poorly soluble drugs.

The differences in release rates of piroxicam from lipophilic suppositories containing Gelucire 44/14 may be referred to the different disintegration times. In the presence of 20% Gelucire

44/14, with a disintegration time of 21 min, a slower release rate was found. Likewise, with a disintegration time of 18 min, in the presence of 15% Gelucire 44/14, a faster release rate was observed (Table 2, Fig. 1C).

The availability of piroxicam through the hydrophilic membrane was found to relate to its release rate (Fig. 2). When Tego Care 450 and Montanov 68 PHA were added to the lipophilic base, the availability of piroxicam decreased and this decrease was related to the surfactant content (Fig. 2, A and B).

The drug dissolution rate from suppositories containing Tego Care 450 and Montanov 68 PHA is influenced by the micellization of piroxicam, which is surrounded by the surfactant molecules that are oriented with the hydrophilic portion towards drug particles and the lipophilic portion towards the glyceride mass. When Tego Care 450 is added to the base at the highest concentration of 7.5%, the higher decrease in release rate is also related to the higher viscosity of the mass at 37 °C, due to the more complex structure of Tego Care 450. In this case, the viscosity values are about double the other formulations tested and must be considered as a factor affecting the migration of drug particles into the *interfacia* with the small volume of rectal phase. Moreover, the mass viscosity may also reduce the spreading process on the membrane simulating the rectal *mucosa*, thus reducing the area of dissolution surface.

When the suppositories are formulated with Montanov 68 PHA, the decrease in release rate, which is independent of surfactant concentration, may be related to the production of liquid crystals, as described above.

The drug concentration in the aqueous intrarectal fluid is a critical parameter for absorption through the rectal membrane, and the higher the quantity of drug in the rectal phase, the higher the concentration gradient with the plasmatic compartment. For an adequate concentration in the intrarectal compartment, the drug must dissolve into the small volume of the rectal fluid.

The increased availability of piroxicam from the suppositories formulated with 15% and 20% Gelucire 44/14, compared with Witepsol H15 alone, can be ascribed both to the increase in piroxicam dissolution rate at pH 6.8 (similar to rectal pH 7.4) described in the literature (Karataş et al. 2005) and to the increase in piroxicam solubility in the presence of Gelucire 44/14 described above. Both these effects produce a high concentration gradient through the membrane simulating the rectal barrier.

When the suppositories were tested for availability with the more complex rectal compartment, both the amount of drug diffused through the membrane and the differences in drug availability related to the surfactant content were reduced.

The extent of the decrease in drug availability may be congruent with the more complex structure of the membrane. In this model, the rectal barrier is simulated through a coupled membrane. To be available in the aqueous phase simulating the plasmatic compartment, piroxicam has to undergo double partitioning, first in the hydrophilic layer and then in the lipophilic layer of the membrane, until it dissolves again in the aqueous acceptor phase. In any case, the overall behaviours of the tested formulations were confirmed. Taking into account previous validation studies relating the results from this model to biological *ex vivo* models with rat or rabbit membrane, we can predict that 15% and 20% of Gelucire 44/14 added to the lipophilic base in suppository formulation could also be effective in increasing the *in vivo* availability of piroxicam.

When the suppositories are formulated with hydrophilic excipients, drug release occurs through the dissolution process of the base.

In our investigation, the availability of piroxicam from suppositories formulated with 40% PEG 400 + 60% PEG 4000 as a hydrophilic base was complete within 40 min. However, when the two glucose derivates, Tego Care 450 and Montanov 68 PHA, were added, the availability of piroxicam decreased (Fig. 2D, E). Unlike the lipophilic formulations, the reduced drug availability from the hydrophilic base could be related to the disintegration times of the suppositories, since the addition of glucose surfactants could increase disintegration times four- to eight-fold (Table 2).

Unlike glucose surfactants, the addition of Gelucire 44/14 did not modify the disintegration time when compared with the macrogol mixture alone. This behaviour was expected because of the presence of PEG in the composition of Gelucire 44/14. The fast disintegration times of hydrophilic formulations with and without Gelucire 44/14 could explain the high release rate of piroxicam observed (Fig. 1F).

In spite of these fast release rates, the availability of piroxicam through the porous membrane was low and similar for both macrogol, and macrogol and Gelucire, suppositories. This behaviour can be explained by considering that, despite the increase in piroxicam solubility produced by PEG, the same base exerts an osmotic effect, which can hinder drug diffusion through the membrane of the rectal compartment (Fig. 2F).

When the complex model of the rectal compartment was used to evaluate simulated absorption, the *in vitro* absorption of piroxicam was reduced by the presence of the surfactant, and the effect was related to its concentration (Fig. 3).

The decrease in drug availability with increasing concentrations of Gelucire 44/14 is due to the complex pathway of drug. Although the solubility of piroxicam in water is increased by the surfactant, the micellization process can hinder drug diffusion into the lipophilic layer of the hydro-lipidic membrane used in the complex rectal model.

4. Experimental

4.1. Materials

Micronized piroxicam of USP/PhEur grade was purchased from F.I.S. S.p.A. (Alte di Montecchio Maggiore, Vicenza, Italy). Witepsol H15 (Hüls, Werk Witten, Germany) and Macrogol 400 and 4000 of PhEur VI ed. grade (ACEF, Fiorenzuola d'Arda, PC, Italy) were used as the basis for suppository preparation. Alkylpolyglucoside Montanov™ 68 PHA (Seppic Italia, Milano, Italy), lauroyl macrogolglycerides Gelucire 44/14 (Gattefossé Italia, Milano, Italy) and poliglyceryl-3 methylglucose distearate, Tego Care 450, (Th. Goldschmidt AG, Essen, Germany) were used as surfactants.

4.2. Methods

4.2.1. Suppository preparation

Suppositories (2 ml) containing 20 mg piroxicam per unit were prepared by the melting method, according to the percentage (*%w/w*) formulations reported in Table 1.

When a lipophilic excipient was used, Witepol H15 was melted at 38 °C and the surfactant, if used, was added. Piroxicam was then homogenously dispersed with the aid of a Silverson turbomixer (Waterside, Chesham, Bucks, UK). The resulting molten mass was poured into PVC moulds at 36 °C and maintained at RT (18–20 °C) for 72 h until solidification. When hydrophilic base was used, macrogol mixtures were melted and poured at 48 °C because of their higher melting point. In any case, the obtained suppositories were stored at + 5 °C until use.

4.2.2. Melting-point

The melting point was determined by the open capillary method. Glass capillaries were used, open at both ends, with a length of 10 cm, internal diameter 1.0 mm and external diameter 1.5 mm.

The melted mass was introduced into five capillaries to obtain a 1 cm high column and maintained at 8 °C for 24 h. Each capillary was fixed to a probe thermometer and introduced in a beaker containing 1 l of water, with the end of the probe 1 cm from the bottom of the beaker. The water temperature was progressively increased by 1 °C/min. The temperature (± 0.1 °C) at which the mass leaks from the capillary was detected as the melting point and the result expressed as the average of the five values.

4.2.3. Disintegration time

Disintegration time was determined by a Sotax DT2 disintegrator, according to the PhEur VI ed. Three suppositories of each sample were tested for disintegration in water at 37 °C.

4.2.4. Drug release

Six glass cylinders, 8 cm high and 4.7 cm in diameter, were hung by side hooks to the edge of a cylindrical basket, 9.3 cm high and 22.5 cm in diameter, with the lower part of the six cylinders at least 3 cm from the bottom. The basket was filled with 3 l buffer solution (phosphate 1/15 M pH 7.4), stirred constantly at 70 rpm and thermostated at 37 ± 0.5 °C. A suppository was placed inside each cylinder and held in the buffer solution between two steel nets. Samples of the dissolution fluid (2 ml) were collected from the basket every 10 min during the first hour and every 60 min during the next two hours, and replaced with the same volume of buffer. The amount of piroxicam released was spectrophotometrically determined at 353 nm (UV-Cary 50 Scan, Varian).

4.2.5. In vitro availability through porous membrane

A 2-ml suppository was placed in a dialysis membrane, 12 cm long and 2.86 cm in diameter (Visking® Dialysis Tubing 36/32, 12–14 kDa, Medicell International ltd, London, UK), previously hydrated overnight at room temperature, and containing 5 ml of 1/15 M, pH 7.4 phosphate buffer solution. Six tubes were sealed and placed horizontally and radially in a cylindrical basket containing 3 l of the same buffer thermostated at 37 ± 0.5 °C, stirred constantly at 70 rpm using a 5-cm paddle stirrer.
From the diffusion fluid simulating the plasma compartment, 2 ml samples of the acceptor phase were collected for each tube at 15-min intervals for a total of 6 h. The volume of each sample was replaced with the same volume of buffer solution. The amount of piroxicam released was determined as previously reported.

4.2.6. In vitro simulated absorption

A 2-ml suppository was placed inside a Perspex diffusion cell with a central cavity 6 cm in diameter and 1.5 cm deep, together with 10 ml phosphate buffer. The cell was closed with a cellulose ester membrane (Millipore HAWP 09000, pore size 0.45 μm) previously soaked with n-octanol 99% and coupled with a filter paper sheet (E/2, 350 g/m^2, Cartiera di Cordenons, Italy) previously soaked with phosphate buffer (1/15 M, pH 7.4), gently pressed between two filter sheets to remove excess buffer and n-octanol. The suppository was in direct contact with the soaked filter paper simulating the mucous layer of rectal barrier. The cell was then placed horizontally in a beaker containing 1 l buffer solution thermostated at 37 ± 0.5 °C and stirred at 100 rpm with a paddle stirrer.
From the fluid simulating the plasma compartment, 2-ml samples were collected at 15-min. intervals for a total of 6 h and then replaced with the same volume of buffer solution. The amount of piroxicam released was determined as previously described. The test was simultaneously performed on three replicates for each formulation.

4.2.7. Rheological properties

The viscosity of the melted suppositories was evaluated using a Rotovisco RV20 viscosimeter (Haake, Karlsruhe, Germany) with a RC20 Rheocontroller programmer and NV measurement equipment. The rheological determinations were carried out at 37 ± 0.2 °C with a shear rate from 0 to 700 s^{-1}.

4.2.8. Particle size distribution

Particle size distribution was determined on samples obtained from five molten lipophilic suppositories formulated with Witepsol H15, to which was added Tego Care 450 7.5%, Montanov 68 7.5%, and Gelucire 44/14 15%, in 100 ml of thermostated (37 ± 0.5 °C) phosphate buffer solution (1/15 M, pH 7.4) and stirred constantly. The dispersion obtained was strained with filter paper and then analyzed by mastersizer (Mastersizer 2000, Malvern Instruments). Samples obtained from mixtures of Witepsol H15 with the surfactant alone, were used as reference.

References

Abd El-Bary A, Ibrahim SA, Elsorady H, Abd Elmonen H (1983) Availability of paracetamol from different suppository bases. Pharm Ind 45: 307–309.

Abd-Elbary A, Tayel S, Amin ST, Osman A (1992) Bioavailability of salbutamol sulphate from different suppository formulations. Egypt J Pharm 33: 1031–1043.

Al Gohary OMN, Foda NH (1993) Availability of mebeverine hydrochloride from suppositories. Pharm Ind 55: 396–400.

Amidon GL, Lennernäs H, Shah VP, Crison JR (1995) A theoretical basis for a biopharmaceutic drug classification: The correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res 12: 413–420.

Anderson BD, Marra MT (1999) Chemical and related factors controlling lipid solubility. Bull Tech Gattefossé 92: 11–18.

Ayres JW, Lorskulsint D, Lock A, Kuhl L,. Laskar PA (1976) Absorption and distribution of radioactivity from suppositories containing 3H-benzocaine in rats. J Pharm Sci 65: 832–838.

Calis S, Summu M, Hincal AA (1994) Effect of suppository bases on the release properties of a potent antimicrobial agent (C31G). Pharmazie 49: 336–339.

Damian F, Blaton N, Naesens L, Balzarini J, Kinget R, Augustijns P, Van Den Mooter G. (2000) Physicochemical characterization of solid dispersions of the antiviral agent UC-781 with polyethylene glycol 6000 and Gelucire 44/14. Eur J Pharm Biopharm 10: 311–322.

Fokkens JG, Blaey CJ, Niederer RR, Zullinger HW (1984) Optimization of the *in vitro* release of zomepirac from suspensions and suppositories. Int J Pharm 19: 177–187.

Hörter D, Dressman JB (1997) Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv Drug Deliv Rev 25: 3–14.

Izgü E, Güngör Ű (1981) The use of natural membranes for the *in vitro* determination of absorption rates of drugs from suppository bases Int J Pharm 9: 107–120.

Karataş A, Yüksel N, Baykara T (2005) Improved solubility and dissolution rate of piroxicam using gelucire 44/14 and labrasol. Farmaco 60: 777–782.

Leuner C, Dressman J (2000) Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm 50: 47–60.

Marvola M, Jokila P, Komulainen M. (1981) Notes about absorption of diazepam from a hydrophilic and from a lipophilic suppository base in man. Acta Pharm Fenn 90: 211–215.

Moes AJ (1989) Etude de l'évolution de propriétiés physiques d' excipients gras pour suppositoires au cours de la conservation. Pharm Acta Helv 55: 307–311.

Möller H. (1984) Bioverfügbarkeit von rektalen Indometacin-Präparaten Pharm Ind 46: 285–291.

Moolenaar F., Schoonen AJM (1980) Biopharmaceutics of the rectal administration of drugs. Pharm Int 7: 144–146.

Palmieri A, Danson T, Groben W, Jukka R, Dummer C (1983) Dissolution of suppositories III: effect of insoluble polyvinylpyrrolidone on acetaminophen release. Drug Dev Ind Pharm 9: 421–442.

Parrott EL (1975) Influence of particle size on rectal absorption of aspirin. J Pharm Sci 64: 878–880.

Plaxo JM (1976) Suppositories, Dispensing of Medications (8th ed). Mack Pub. Comp, p. 163–176.

Puech A., Lasserre Y, Jakob N, Duru C (1981) Availability of phenobarbital from suppository form. J Pharm Belg 36: 332–336.

Ragazzi E, Dalla Fini G, Ragazzi E, Portioli E (1984) Effetto delle condizioni termiche di preparazione e della concentrazione del farmaco in sospensione sulla cessione da supposte con eccipiente lipofilo. Farmaco, ed. pr. 39: 3–19.

Ragazzi E, Dalla Fini G, Dal Zotto M (1986) The utilization of polyethylene glycols as vehicles in the administration of drugs rectally. Boll Chim Farm 125: 234–249.

Realdon N, Ragazzi E, Ragazzi E (2001) Effect of drug solubility on *in vitro* availability rate from suppositories with polyethylene glycol excipients. Pharmazie 56: 163–167.

Realdon N, Ragazzi E, Morpurgo M, Ragazzi E (2005) *In vitro* methods for evaluation of drug availability from suppositories: comparison between biological and artificial membranes. Pharmazie 60: 756–760.

Scinoda M, Akita M, Hasegawa M, Nadai M, Hasegawa T, Nabeshima T (1995) Pharmaceutical evaluation of carbamazepine suppositories in rats. Biol Pharm Bull 18: 1289–1291.

Tukker JJ, De Blaey CJ (1983) Optimisation of Release Testing from suppositories: introduction of a dynamic dissolution test. Acta Pharm Technol 29: 131–136.

Urban M, Arnaud P, Zuber M, Chaumeil JC (1991) Influence of enanchers on the physicochemical properties and on the release of suppositories of clomipramine hydrochloride. Drug Dev Ind Pharm 17: 1325–1342.

Vromans H, Moolenaar F, Visse J, Meijer DKF (1984) Rectal absorption of metronidazol from polyethylene glycol suppositories. Pharm Weekbl sc. ed. 6: 18–20.

Zuber M, Pellion B, Arnaud P, Chaumeil JC (1988) Kinetics of theophylline release from suppositories *in vitro*: influence of physicochemical parameters. Int J Pharm 47: 31–36.