International Journal of Pharmaceutics, 72 (1991) 1–10

ADONIS 037851739100200J

IJP 02358

Research Papers

Permeability of progesterone and a synthetic progestin through methacrylic films

Sylvene Blanchon [1], Guy Couarraze [1], Françoise Rieg-Falson [2], Gerard Cohen [3] and Francis Puisieux [1]

[1] *Laboratoire de Physico-chimie et Pharmacotechnie, U.R.A. C.N.R.S. 1218, Université Paris-XI, 92296 Châtenay-Malabry Cedex (France),* [2] *Laboratoire de Pharmacie Galénique, Université Claude Bernard, Lyon 1 (France) and* [3] *Département de Galénique, Roussel-Uclaf, Romainville (France)*

(Received 17 July 1990)
(Modified version received 21 November 1990)
(Accepted 27 November 1990)

Key words: Progestin; Permeability; Partition coefficient; Diffusion coefficient; Acrylate-methacrylate copolymer

Summary

The permeation for progesterone and a synthetic progestin was determined through films prepared from a hydrophilic acrylate-methacrylate copolymer (Eudragit^{R}). Steroid permeabilities allow us to evaluate a partition coefficient between films and water which is 5 times lower for the synthetic progestin: this difference is due to the higher aqueous solubility of this molecule despite the presence of a hydroxyl group which may interact with the polymer cation content. The values of partition coefficients show that both steroids are dissolved into the polymer films. From permeation experiments, diffusion coefficients are also calculated and different mechanisms of diffusion are presented which are 'pore' and 'solution diffusion'. The values of the diffusion coefficients show that the main mechanism is solution diffusion according to the steroid solubilities in the polymer films.

Introduction

The use of polymeric membranes in the pharmaceutical industry is becoming more and more developed in the design of controlled release devices (coating small particles, implants, transdermal matrices). For this reason, drug transit through polymeric devices must be studied. Thus, in order to have a fundamental approach to this diffusion and to compare results for different drugs and different polymers, it is first necessary to study the polymer as a film even if the final device is not this form. These studies lead to the characterization of the drug permeation through isolated films.

For this purpose, acrylic resins such as acrylate-methacrylate copolymers have been widely developed in pharmaceutical devices. But fundamental studies on these polymeric membranes seem to be rare. Some groups have pub-

Correspondence: G. Couarraze, Laboratoire de Physico-chimie et Pharmacotechnie, U.R.A. C.N.R.S. 1218, Université Paris-XI, 92296 Châtenay-Malabry Cedex, France.

lished on the permeation of drugs through this type of membrane. Different drugs were used as model molecules. Okor (1982a,b) worked on this polymer with urea because of its high permeability in the polymeric films studied. They also worked with ionic permeants (sodium chloride and sulfacetamide sodium) (Okor, 1989). In an earlier study, Gurny et al. (1976) used salicylic acid.

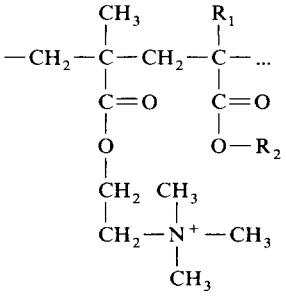
On the other hand, steroid permeability through other types of polymeric membrane has been studied; for instance, silicone (Tojo et al., 1985; Sun et al., 1987), polyether urethane, hydroxyethyl methacrylate (Zentner et al., 1978) membranes. These studies have one of two aims: either the authors try to demonstrate the influence of the polymeric membrane with a steroid as model, or they look for particular properties of different steroids in an identical membrane to determine the steroidal structure influence.

To our knowledge, no group has worked on steroids with a film made from acrylate-methacrylate copolymer. For these reasons, we wanted to investigate certain properties of steroids used in our laboratory: progesterone as a model steroid, and a new synthetic progestin: RU27987 (Roussel-Uclaf, Paris, France). In this research, we studied not only the permeation of both the steroids through an acrylate methacrylate copolymer membrane but also the behaviour of the membrane during the permeation experiment.

Materials and Methods

Materials

The polymer we used is an acrylate-methacrylate copolymer available under the trade name Eudragit (Röhm Pharma GmbH, Darmstadt, Germany). After some first experiments, the type of polymer chosen was Eudragit RL100, which is insoluble in water at 20 °C but has nevertheless hydrophilic properties because of a content of quaternary ammonium groups: 66 mol of cations per mol of polymer chain. The polymer has an average molecular weight of about 150 000. The unit of Eudragit is as follows:



R_1 = H or CH_3
R_2 = CH_3 or C_2H_5

Films were prepared by casting mixture of acetone/isopropanol 40:60 containing the polymer on a PTFE mould. The solution was made with 2.0 g of polymer in 10 ml of the solvent mixture. Glycerol triacetate (Prolabo, Paris, France) was added as a plasticizer (10% w/w of dried polymer). Solvents were permitted to evaporate for 24 h at ambient temperature before transferring the films so formed to a desiccator containing silica gel at low pressure where they were stored until use. The film thickness was measured at 10 random points on the films: it averaged 208 ± 3 μm (± SE).

Progesterone (Sigma Chemical Co., St Louis, U.S.A.) is obtained commercially and RU27987 (Roussel-Uclaf, Paris, France), which is a 17β-(2-hydroxy-1-oxopropyl)-17α-methyl estra-4,9(10)-diene 3-one, is supplied by the Laboratoires Roussel-Uclaf. We had to use radiolabelled progesterone ([^{14}C]progesterone (C.E.A. Saclay, Gif/Yvette, France) to obtain sufficient sensitivity for permeation experiments.

Methods

Swelling Films were cut in pieces of 1 cm^2. After storing in a desiccator, the weight and the thickness of pieces were measured. Then the pieces were placed in water at 34 °C: the weight and thickness were determined regularly.

Microscopy Specimens of films were examined both by scanning electron microscopy before and after permeation experiments. For studies after permeability experiments, films were lyophilized (freezing at −30 °C overnight, drying at 20 °C for

4 h). Films were then stored in a desiccator containing silica gel. They were mounted using a double-sided pressure-sensitive adhesive tape and were vacuum-coated with gold.

Analytical methods Determination of unlabelled steroid concentration was performed by high-pressure liquid chromatography, followed by UV detection (Waters, St Quentin, France). A C_{18} μ-Bondapak column was used. The mobile phases were mixtures of methanol/water (80 : 20) for progesterone and of acetonitrile/water (40 : 60) for RU27987. The absorbances of progesterone and RU27987 were measured at wavelengths of 241 and 310 nm, respectively. When radiolabelled progesterone was used, samples were weighed in tared vials. Scintillation fluid (Pico Fluor ™ 40, Packard Instrument Co., Downers Grove, U.S.A.) (15 ml) was added to each vial and the concentration was determined by a scintillation counter (Model 2000 CA, Packard Instrument Co., Downers Grove, U.S.A.).

Determination of drug solubility An excess amount of drug was equilibrated with 50 ml of water at 34 °C for 48 h with stirring. The saturated drug solution was then quickly filtered and diluted in ethanol. The drug concentration was determined by HPLC.

Determination of partition coefficients The partition coefficients were determined by equilibrating a known volume of membrane in an aqueous solution of drug during 48 h. After equilibration, the concentration of the solution was assayed by HPLC. Films were wiped and dissolved in an organic solvent (chloroform) and the amount of

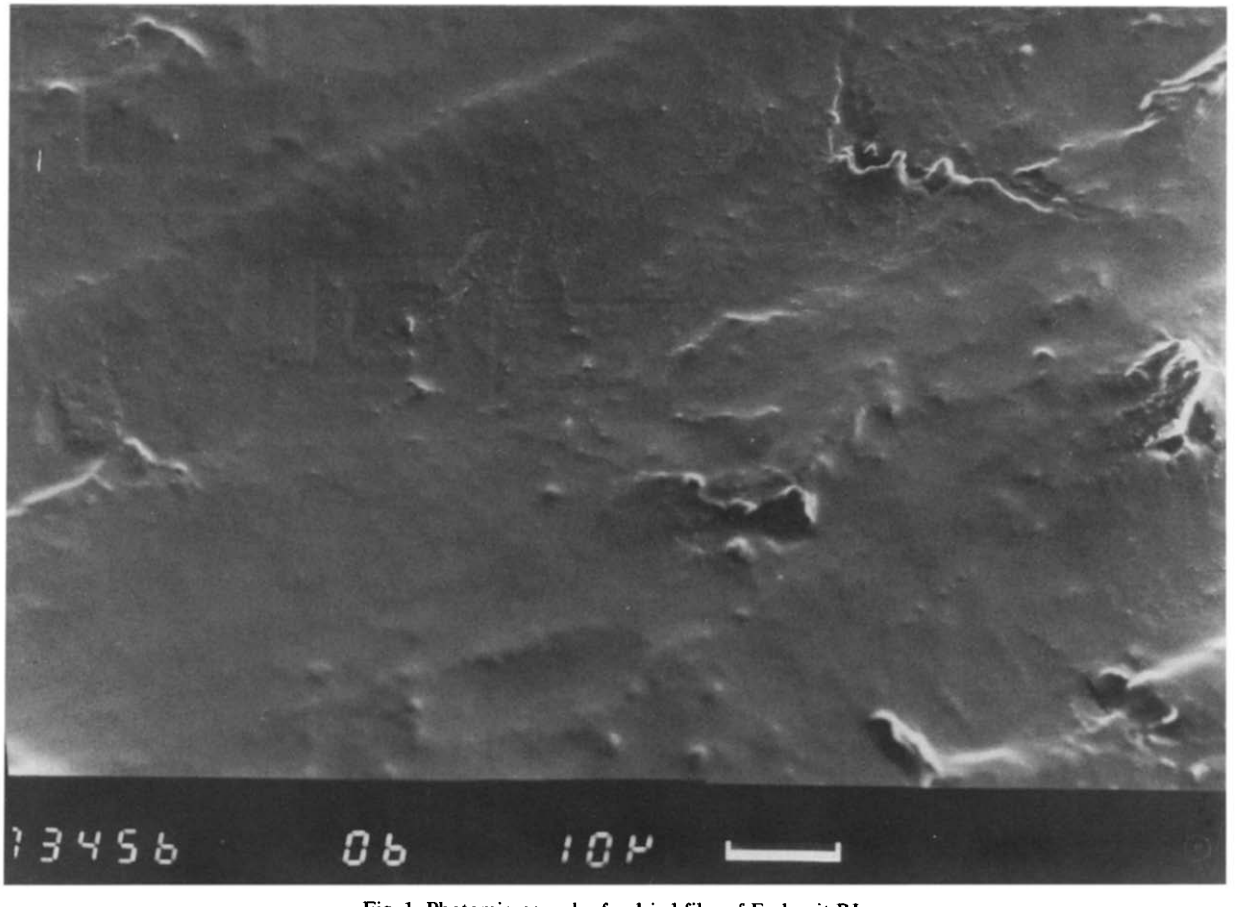


Fig. 1. Photomicrograph of a dried film of Eudragit RL.

drugs was analysed by HPLC after dilution with ethanol. The partition coefficient was then calculated as follows: $K = C_m/C_s$ where C_m is the concentration of drug in the membrane and C_s is the concentration of drug in the solution.

Permeation The permeation coefficients of the steroids in the polymer films were determined in a glass diffusion cell with two compartments of equal volume (65 ml). The membrane of 9.6 cm^2 was clamped between the two compartments with PTFE joints. Each compartment was stirred continuously. The efficacy of the stirring was verified with a colouring agent. The donor compartment was filled with water containing a known amount of drug and the permeation was followed by determining the increase of concentration in the receptor compartment: at various times, samples of 2 ml were taken. Distilled water was immediately added to the receptor compartment. Samples were assayed by liquid scintillation for progesterone and by HPLC for RU27987.

Results

Microscopy

Fig. 1 shows a dried film: there is no apparent porosity, as we have later confirmed with a mercury porosimeter analysis. After permeation experiments, photomicrographs of lyophilized films were taken: as shown in Fig. 2, pores in the

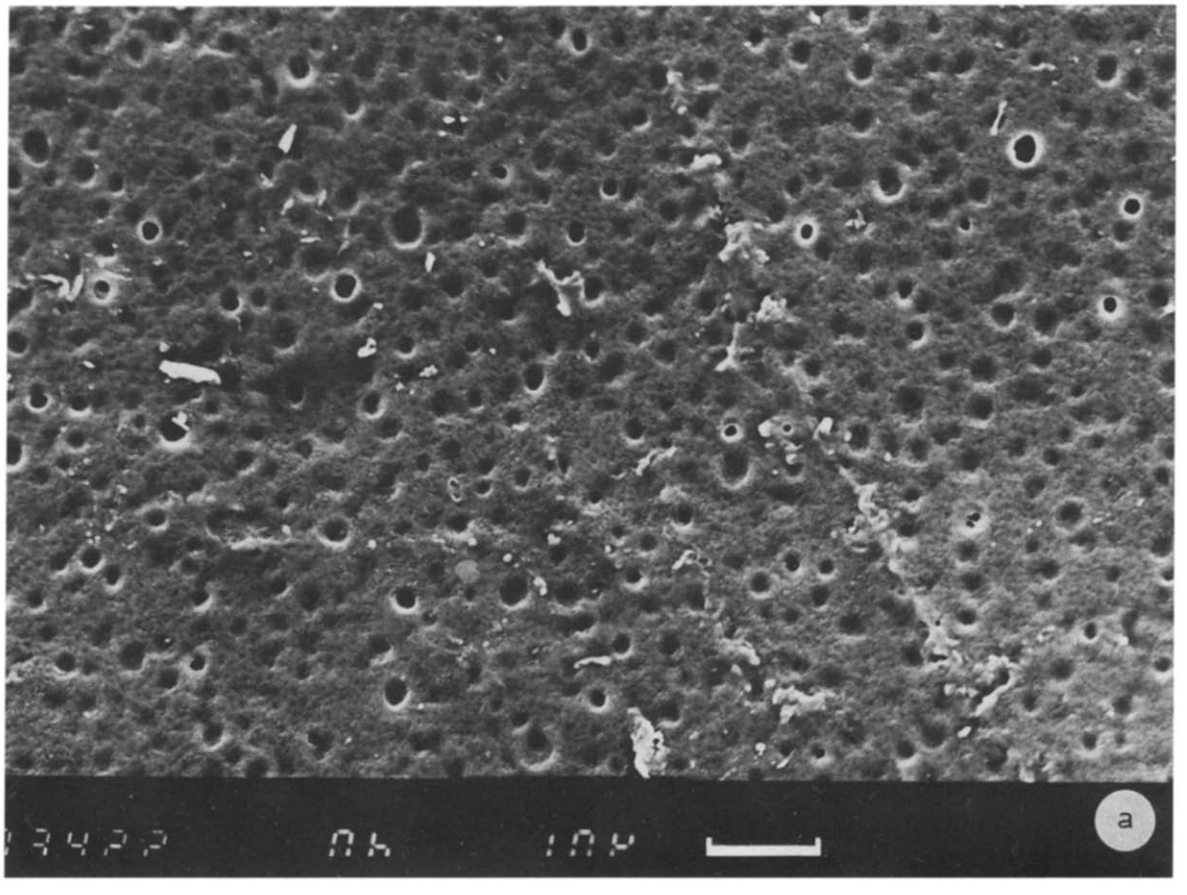


Fig. 2. Photomicrographs of Eudragit RL films after the permeation experiments without drug (a) or with different solutes: progesterone (b), RU27987 (c).

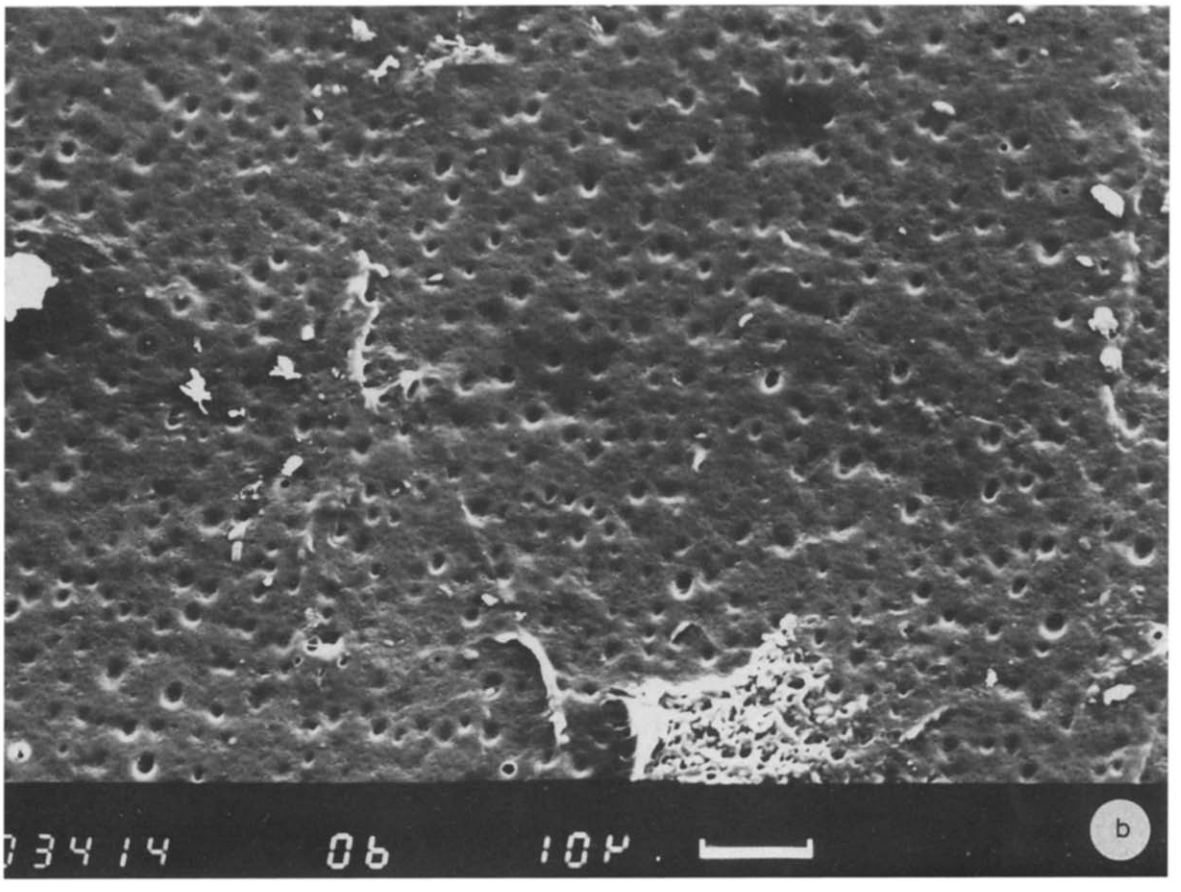


Fig. 2 (continued).

film's structure can be observed. There was no difference between films after permeation either with or without drug in the donor compartment. The pore density within the film seems to be homogeneous as does the pore size. With a greater magnification, we have estimated the average diameter of a pore: about 1.5 μm. This value is smaller than that found by Abdel-Aziz et al. (1975): this difference may be explained by a ratio of plasticizer two-fold smaller in our formulation: 10% w/w dried polymer. This existence of pores is probably due to a leak of plasticizer which is hydrophilic and not to the passage of drug which might induce channels in films (Okor, 1982b).

Swelling

Although films are insoluble in water, they may swell in an aqueous medium (Abdel-Aziz and Anderson, 1976) or in biological medium (Gurny et al., 1976). For the authors who worked with this polymer, the equilibration time of swelling was about 2 or 3 h and the temperature has an influence. So we were interested to know the kinetics of the swelling of our membranes in water at

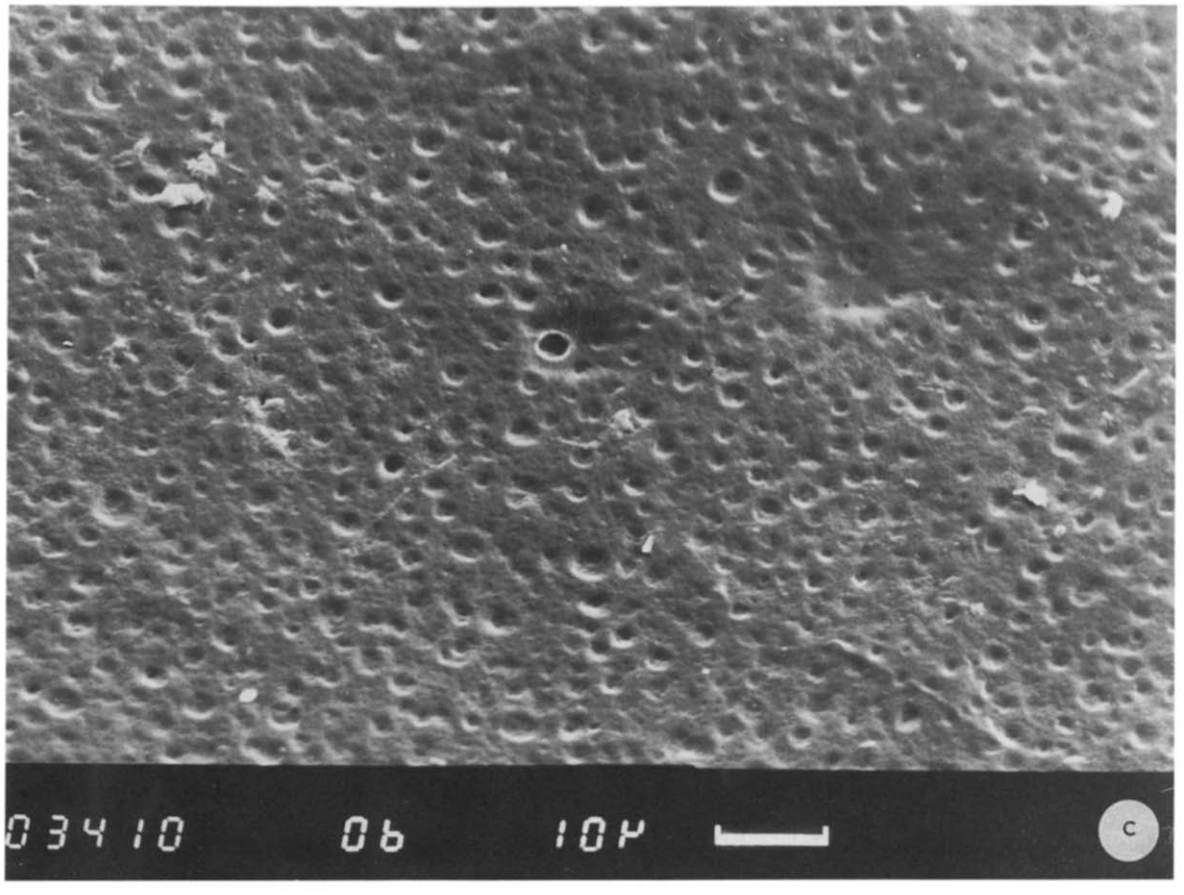


Fig. 2 (continued).

34° C. A swelling index was calculated using the formula (Eqn 1):

$$I_s = (W_s - W_d)/W_d \quad (1)$$

where W_d is the weight of the dried polymer and W_s the weight after swelling. The plot of this index vs time is shown in Fig. 3. The polymer equilibrates itself in 15 min with an increase in weight of 55%. The increase in the thickness is in the same order of magnitude, but a little smaller: 40%. So, the thickness of water-swollen membrane we take for subsequent calculations is 280 μm.

Partition coefficient and solubility

The partition coefficient is quite dependent on the experiment conditions; notably on the concentration of the initial solution. For this reason,

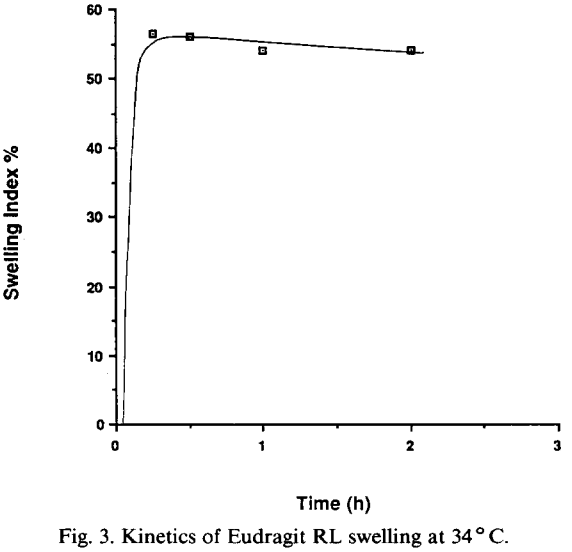


Fig. 3. Kinetics of Eudragit RL swelling at 34° C.

TABLE 1

Physicochemical parameters of both steroids

	Progesterone	RU 27987
S (μg/ml)	7	126
K_p	1136	179
V_m (cm^3/mol)	270	291
$R_h = (3V_m/4\pi)^{1/3}$ (cm)	4.7×10^{-8}	4.9×10^{-8}
D_w (cm^2/s)	6.9×10^{-6}	6.6×10^{-6}

S, aqueous solubility, K_p partition coefficients between water and Eudragit RL film, V_m molar volume calculated from the theory of the contribution of groups, R_h hydrodynamic radius, D_w diffusion coefficient in water at 34°C.

it must be measured under conditions as near as possible to those of permeation experiments (Zentner et al., 1979). So we chose to determine the partition coefficient with a solution in which the concentration of drug is 4 times smaller than the solubility in water. Results are given in Table 1 where the aqueous solubilities of the two steroids are also reported. The solubility of the RU27987 is higher than that of progesterone and the partition coefficient is smaller. The addition of an hydroxyl group to the steroidal structure significantly increases the aqueous solubility and leads to a reduction in the partition coefficient.

Permeation

Figs 4 and 5 are permeation curves with usual shapes: after a lag time, the drug flux can be calculated by the amount of drug which passes through the membrane per unit area and per unit of time. After an initial flux J has been established, this one decreases because of the depletion of the donor. At the end, an equilibrium is obtained, which can be proved by analysing drug concentration in each compartment of the cell. Some permeation parameters are given in Table 2. We can notice that the initial concentration C_0 in the donor is higher than solubility for progesterone and lower than solubility for RU27987. The flux of RU27987 is higher than that of progesterone: it is highly dependent on the concentration so the result is not surprising. A more interesting parameter is the permeation coefficient P,

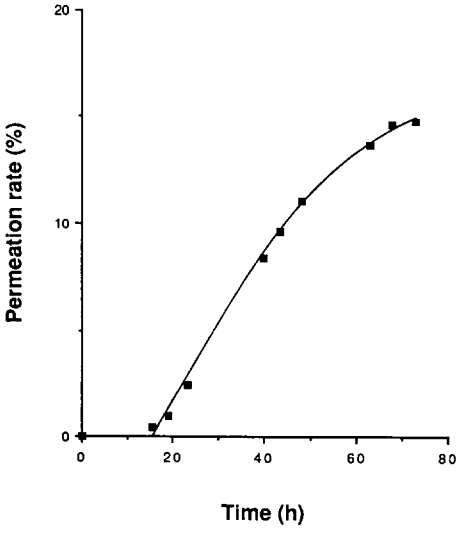


Fig. 4. Kinetics of progesterone permeation through Eudragit RL films at 34°C. % is the receptor concentration divided by the initial donor concentration.

which is calculated by the following formulae (Eqns 2):

$$P = K \cdot D/h = J/C_0 \text{ (for RU27987)}$$
$$P = K \cdot D/h = J/S \text{ (for progesterone)} \quad (2)$$

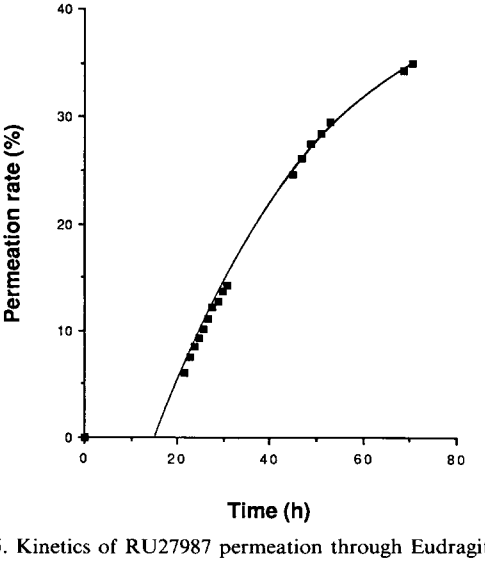


Fig. 5. Kinetics of RU27987 permeation through Eudragit RL films at 34°C. % is the receptor concentration divided by the initial donor concentration.

TABLE 2

Parameters of permeation kinetics through Eudragit RL films for both steroids at 34 °C

	Progesterone	RU 27987
C_0 (μg/ml)	16	100
T_l (h)	15	15
J (μg/cm^2 per s)	1.03×10^{-4}	20.08×10^{-4}
P (cm/s)	1.5×10^{-5}	2.0×10^{-5}
C_{eq} (μg/ml)	2.7	35
Q_f (μg)	689	1950
C_f (μg/ml)	2.5×10^{3}	7.0×10^{3}
$K_{p(perm)}$	911	199

C_0 is the initial concentration in the donor compartment, the lag time (T_l), the flux (J) and the permeation coefficient (P) are calculated from the data in Figs 3 and 4, C_{eq} is the final concentration in the donor and receptor compartments, Q_f and C_f are the amount and the concentration of solute in the film at equilibrium, $K_{p(perm)}$ is the partition coefficient calculated from the permeation experiments.

where D is the diffusion coefficient, h the film thickness and S the solubility in water. We can observe that the two permeation coefficients are of the same order of magnitude. Different publications give permeation coefficients of progesterone through different polymers: the order of magnitude is the same: 10^{-5} cm/s. For instance, Sun et al. (1987) found a permeation coefficient of progesterone through a silicone polymer of between 10^{-5} and 10^{-4} cm/s depending on the experimental conditions. Zentner et al. (1978) also calculated permeation coefficients of progesterone through hydrogel membranes: permeation coefficients (10^{-7} cm^2/s) are not in their calculations dependent on the thickness of the membrane. When we consider this one, we find again the same order of magnitude as our coefficients.

The equilibrium concentration is only 35% of the initial solution concentration for RU27987 and 17% for progesterone although we could expect an equilibrium concentration of 50% of the initial one. This is due to a considerable adsorption on the film as the partition coefficients show. More explanations are given in the discussion.

Discussion

The permeation of a drug through a membrane results from two different properties. The first is the partition of the solute between the solution and the film; this property is evaluated by the partition coefficient. The second one is the mobility of the drug within the membrane, this property is evaluated by the diffusion coefficient. We will now consider these two types of property for the two steroids studied.

Partition of progesterone and RU27987 between water and polymeric membrane

Considering the partition coefficients (Table 1), it appears that both progesterone and RU27987 have high values of K_p, this result is consistent with the hydrophobic characteristic of these molecules. However, we remark that, for the same partition conditions, progesterone has a relative affinity for the polymer approximately 6 times greater than RU27987. This difference was again found at the end of the permeation experiments. The assay of the remaining amount of drug within the film (after dissolution in chloroform) allowed us to also evaluate the partition of the two steroids between water and Eudragit films: the results of these determinations are given in Table 2. The greater affinity of progesterone for the film was again found. However the partition coefficient of progesterone is only 4.6 greater than that of RU27987 in this experiment. The differences between the determinations of Tables 1 and 2 can be explained easily by considering the well-known dependence of the partition coefficient on the concentration: in the experiment reported in Table 1, the remaining progesterone concentration in aqueous phase is only 5% of the solubility after equilibrium, while it is 38% of the solubility at the end of the permeation experiment (Table 2).

An interesting deduction can be made about the location of the steroids within the film: the amounts of steroids at the end of the permeation experiment cannot result from the soluble fractions in the pores of the polymer. This conclusion follows from the value of 50% of the swelling index of the film: the pore volume is therefore about the third of total volume of the swelling

film. If the steroids were principally in the water of the pores, the local concentration would be three times the average concentration within the film, which would mean 2.1×10^4 μg/ml for RU27987 and 7.5×10^3 μg/ml for progesterone. The comparison of these values with the aqueous solubility of these solutes does not allow us to retain this hypothesis.

Consequently, we can conclude that the amounts of steroids contained within the Eudragit RL films are essentially adsorbed on the polymer. The greater adsorption of progesterone would be the result of specific interactions with Eudragit RL.

Diffusion of progesterone and RU27987 through the Eudragit films

In agreement with most studies of film permeation, two types of mechanism can be considered for the drug diffusion through a polymeric membrane: either a solution-diffusion mechanism within the polymeric chains, or a pore mechanism within water-filled pores present in the film (Zentner et al., 1979).

According to the hypothesis of diffusion within the polymer, the diffusion coefficients of the two solutes can be obtained from the permeation coefficient by the relationship (Eqn 3):

$$D = Ph/K \tag{3}$$

This equation gives diffusion coefficient values of: $D = 0.37 \times 10^{-9}$ cm^2/s and 3.2×10^{-9} cm^2/s for progesterone and RU27987 respectively. It seems that these values are too different for two solutes physically and chemically rather comparable. This is proved by the lag times, which are about 15 h for both the steroids.

In contrast the hypothesis of a pore-diffusion mechanism seems to be more realistic because the permeation coefficients are of the same order of magnitude, and because the density of pores is high as it appears in Fig. 2. According to this diffusion-type mechanism, the relationship between D and P is no longer given by Eqn 3. A modified relation can be taken as Eqn 4:

$$P = D_w/ht \tag{4}$$

where D_w is the diffusion coefficient in the water of the pores, and t the tortuosity factor of the pores. So the product ht is the value of the effective diffusional path length. No partition coefficient appears in Eqn 4 since water is both the external medium and the diffusion medium within the film. According to Eqn 4, the ratio of steroid permeation coefficients is the same as the ratio of steroid diffusion coefficients in water.

A theoretical estimate of the diffusion coefficient in water can be calculated by consideration of the Stokes-Einstein equation (Eqn 5):

$$D_w = kT/6\pi\mu_w R_h \tag{5}$$

where k is Boltzmann's constant, μ_w the viscosity of water at temperature T, and R_h the hydrodynamic radius of the drug. Table 1 gives the results of these calculations where the molar volumes have been estimated by the theory of contribution of groups (Flynn et al., 1974). The ratio of the diffusion coefficients does not correspond exactly with the experimental ratio of permeation coefficients: $P_{RU27987}/P_{progesterone} = 1.33$. Moreover, this assumption of a pure pore-diffusion mechanism, would lead to values of tortuosity factor a little too high (from 10 to 15), and this factor would depend on the nature of steroid; these results are not likely.

So it seems that the diffusion mechanism of these two solutes within the Eudragit RL films is complex: the experimental permeation must be the result of a dominant mechanism: the diffusion into the aqueous phase of pores, and a secondary diffusion mechanism within the polymer or within other aqueous domains, where water is not in a bulk state, similar to water in solution, but where water is associated with polymer: bound water (Jhon and Andrade, 1973); Zentner et al. (1979) have previously shown that the existence of such domains could modify the value of the diffusion coefficients of steroids through a film.

Conclusion

Our study has shown interesting differences of properties between the two steroids within a con-

trolled release device like a Eudragit RL film. The permeability of RU27987 through this membrane is increased by a factor 1.33 with respect to progesterone. This property might be explained by the existence of a hydroxyl group on the RU27987 molecule. This interpretation is in agreement with the studies of Zentner and al. (1979) on the permeation contributions of steroids function groups: for these authors, the relative factor of diffusibility within hydroxyethyl methacrylate films between 17α hydroxyprogesterone and progesterone is 1.37. In our film the same type of chemical modification on progesterone leads to a similar increase of diffusibility.

Moreover, the chemical structure of the synthetic steroid RU27987 allows a much greater aqueous solubility and an affinity for the polymer smaller than those of progesterone. Both these properties could render RU27987 suitable for use in a progestin release device.

Acknowledgements

The authors wish to thank Laboratoires Roussel-Uclaf for donation of RU27987 and their assistance, and F. Durivaux for his help.

References

Abdel-Aziz S.A.M. and Anderson W., The influence of casting solvent composition on structure and permeability of acrylic-methacrylic ester copolymer films. *J. Pharm. Pharmacol.*, 28 (1976) 801–805.

Abdel-Aziz, S.A.M., Anderson, W. and Armstrong, P.A.M., Surface differences and solute permeability in films of certain acrylic-methacrylic ester polymers. *J. Appl. Polym. Sci.*, 19 (1975) 1181–1192.

Flynn G.L., Yalkowsky S.H. and Roseman T.J., Mass transport phenomena and models: Theoretical concepts. *J. Pharm. Sci.*, 63 (1974) 479–510.

Gurny R., Mordier D. and Buri P., Réalisation et développement théorique de formes médicamenteuses à libération contrôles par des films méthacryliques. I: Fabrication et évaluation des films. *Pharm. Acta Helv.*, 51 (1976) 384–390.

Jhon M.S. and Andrade J.D., Water and hydrogels. *J. Biomed. Mater. Res.*, 7 (1973) 509–521.

Okor R.S., Effect of polymer cation content on certain film properties. *J. Pharm. Pharmacol.*, 34 (1982a) 83–86.

Okor R.S., Influence of hydrophilic character of plasticizer and polymer on certain film properties. *Int. J. Pharm.*, 11 (1982b) 1–9.

Okor R.S., Solute adsorption and concentration-dependent permeability in certain polymer films. *J. Pharm. Pharmacol.*, 41 (1989) 483–484.

Sun Y., Ghannam M., Tojo K., Chien Y.W., Lee C.L., Ulman K.L. and Larson K.R., Effect of polymer composition on steroid permeation: membrane permeation kinetics of androgens and progestins. *J. Control. Rel.*, 5 (1987) 69–78.

Tojo K., Ghannam M., Sun Y. and Chien Y.W., In vitro apparatus for controlled release studies and intrinsic rate of permeation. *J. Control. Rel.*, 1 (1985) 197–203.

Zentner G.M., Cardinal J.R. and Kim S.W., Progestin permeation through polymer membranes II: Diffusion studies on hydrogel membranes. *J. Pharm. Sci.*, 67 (1978) 1352–1355.

Zentner G.M., Cardinal J.R., Feijen J. and Song S.S., Progestin permeation through polymer membranes IV: Mechanism of steroid permeation and functional group contributions to diffusion through hydrogel films. *J. Pharm. Sci.*, 68 (1979) 970–975.