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Poly(ε-caprolactone) and Eudragit® microparticles containing fludrocortisone acetate

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Abstract

Substitutive hormonal therapies have to be administered for long periods. Thus, the development of sustained-release forms, as microparticle suspensions, is interesting in order to improve patient compliance by reducing dosing frequencies and side effects. The aim of this work was to compare different formulations of fludrocortisone microparticles for the treatment of mineralocorticoid insufficiency. The study was done with different polymers (poly(ε-caprolactone), Eudragit® RS and Eudragit® RL) and different processes (O/W solvent evaporation methods and S/O/W evaporation methods). The use of a suspension of micronized drug in dichloromethane as dispersed phase (S/O/W method) significantly improved the process. Whereas low concentrations of FLU dissolved in the dispersed phase led to smooth-surface homogeneous microparticles and poor incorporation efficiency (5.8–7.3%); suspensions of FLU led to microparticles with numerous crystals on their surfaces (S/O/W microparticles) and high incorporation efficiency (about 79%). However, the best release profiles were obtained with microparticles prepared with 7.5 mg/ml of dichloromethane, near saturation. Moreover, the use of mixtures of poly(ε-caprolactone), Eudragit® RS and RL did not improve the release profiles.

Keywords: Fludrocortisone; Microparticles; Polymer; Sustained release; Polycaprolactone

1. Introduction

Fludrocortisone acetate (9α-fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione, 21-acetate; FLU) is currently used for the treatment of several diseases from endocrine and non-endocrine origin. Owing to its strong adrenocortical action, this fluorine-containing steroid has been used in Addison's disease (Orth, 1994), congenital adrenal hyperplasia (Merke and Cutler, 1997) and orthostatic hypotension (Benarroch, 1997). Considering the chronicity of these diseases and the high potency of this drug, the long-term administration of daily doses lower than 100 μg is usually required.

After oral administration, the highest blood levels were measured after 1.7 h and the half-life of pure drug is about 30 min (Vogt et al., 1971). Thus, the development of sustained-release form of FLU could be interesting in order to improve compliance and stabilize serum concentrations.

Beside this, anti-inflammatory corticoids, used at higher doses, have been incorporated in small microparticles (0.5–10 μm) to improve biodisponibility, pass through the gastro-intestinal barrier and target inflammatory tissues (Lewis et al., 1992).

The aim of this work was to study sustained release formulations of fludrocortisone, as hormonal

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treatment. We have compared different formulations of PCL-based and Eudragit®-based microparticles of fludrocortisone: we have investigated the influence of various parameters on the characteristics of the microparticles, as the volume of the aqueous phase, the influence of the FLU concentrations in the organic phase and the influence of biodegradability of the polymer.

2. Materials and methods

2.1. Materials

Micronized fludrocortisone acetate (FLU, crystalline form A, 93% of crystals having a size <5 μm, British Pharmacopoeia grade) was a generous gift from Pharmacie Centrale des Hôpitaux de Paris (Paris, France). Poly(ε-caprolactone) (PCL) was purchased from Aldrich (Milwaukee, USA), Eudragit® RS and Eudragit® RL was supplied by Röhm GmbH (Darmstadt, Germany). The crystalline state of PCL (MW: 61,640) was about 60%, as determined by differential scanning calorimetry (DSC) according to Ali et al. (1993). Polyvinyl alcohol (PVA) 68,000 Daltons (Da), was supplied by Sigma (St. Louis, USA). All other reagents were of analytical grade from either Merck (Darmstadt, Germany) or Acros (New Jersey, USA).

2.2. Preparation of microparticles

To obtain the microparticles (MP), a modified O/W emulsion-solvent evaporation method was used (Chang et al., 1987). Each formulation is detailed in Table 1. In each case, 250 mg of polymer (PCL, Eudragit® RL, Eudragit® RS or mixtures of these polymers) was dissolved in 10 ml of dichloromethane and various amounts of FLU (Q_{OP}), ranging from 10 to 150 mg (1–15 mg/ml), were added under magnetic stirring. Since the solubility of FLU in dichloromethane

Table 1
Formulations of fludrocortisone microparticles

Parameter studied	Formulation name	Method	Concentrations in 10 ml of dichloromethane				
			Fludrocortisone (mg/ml)	PCL (mg/ml)	Eudragit® RS (mg/ml)	Eudragit® RL (mg/ml)	Aqueous phase (ml)
Assay 1: volume of the aqueous phase	Flu^{5}-PCL^{25}-800	O/W	5	25			800
	Flu^{5}-PCL^{25}-600	O/W	5	25			600
	Flu^{5}-PCL^{25}-400	O/W	5	25			400
	Flu^{5}-PCL^{25}-200	O/W	5	25			200
	Flu^{5}-PCL^{25}-100	O/W	5	25			100
Assay 2: concentration of fludrocortisone	Flu^{1}-PCL^{25}-800	O/W	1	25			800
	Flu^{2}-PCL^{25}-800	O/W	2	25			800
	Flu^{5}-PCL^{25}-800	O/W	5	25			800
	$Flu^{7.5}$-PCL^{25}-800	O/W	7.5	25			800
	Flu^{10}-PCL^{25}-800	S/O/W	10	25			800
	Flu^{15}-PCL^{25}-800	S/O/W	15	25			800
Assay 3: concentration of fludrocortisone	Flu^{1}-RS^{25}-800	O/W	1		25		800
	Flu^{2}-RS^{25}-800	O/W	2		25		800
	Flu^{5}-RS^{25}-800	O/W	5		25		800
	$Flu^{7.5}$-RS^{25}-800	O/W	7.5		25		800
	Flu^{10}-RS^{25}-800	S/O/W	10		25		800
	Flu^{15}-RS^{25}-800	S/O/W	15		25		800
Assay 4: polymer	$Flu^{7.5}$-PCL^{25}-800	O/W	7.5	25			800
	$Flu^{7.5}$-$PCL^{12.5}$/$RS^{12.5}$-800	O/W	7.5	12.5	12.5		800
	$Flu^{7.5}$-$PCL^{6.25}$/$RS^{18.5}$-800	O/W	7.5	6.25	18.75		800
	$Flu^{7.5}$-RS^{25}-800	O/W	7.5		25		800
	$Flu^{7.5}$-$RS^{18.5}$/$RL^{6.25}$-800	O/W	7.5		18.75	6.25	800
	$Flu^{7.5}$-$RS^{12.5}$/$RL^{12.5}$-800	O/W	7.5		12.5	12.5	800

was about 9 mg/ml, solutions were obtained for concentrations lower or equal to 7.5 mg/ml and the method was called O/W (oil-in-water). Stable suspensions were obtained for higher concentrations (10 and 15 mg/ml) and the method was called S/O/W (suspension-in-oil-in-water).

Under mechanical stirring (1500 rpm), the organic phase was poured into various volumes (100–800 ml) of water containing 0.1% PVA. The stirring was maintained for 2 h, leading to a total evaporation of the solvent. The microparticles were then recovered by filtration (HA filter, Millipore, 0.45 μm), washed three times with water and dried under vacuum during 24 h. Unloaded microparticles were prepared, following the same processes. For each FLU concentration, at least three independent batches were prepared. To calculate the yield of each preparation, the weight of the recovered dry particles was recorded.

2.3. Particle size analysis

The microparticle size distribution was estimated by optical microscopy. About 5 mg of particles were vortexed in 1 ml of 0.1% PVA. A drop of the suspension was then poured onto a glass slide and observed at 100× magnification using a microscope equipped with a digital-camera and an image analysis software (Kappa Image base, Kappa Opto-Electronics, Gleichen, Germany). Three fields containing about 100 particles were randomly recorded. The sizes were expressed as the mean ± S.D. of the Feret diameters ($n = 3$). The sphericity of the MP was estimated by the roundness parameter where a value of unity corresponds to a perfect circle, which was given by

$$\text{Roundess} = 4\pi \left\lfloor \frac{\text{area}}{\text{perimeter}^2} \right\rfloor \quad (1)$$

2.4. Determination of drug loading

The amount of FLU entrapped in the microparticles was determined by reversed-phase high-performance liquid chromatography (HPLC). Accurately weighed samples of MP (about 10 mg) were dissolved in 100 ml of acetonitrile. Fifty microliters were then injected onto a C_{18} column (5 μm, 4.6 cm × 25 cm, Macherey-Nagel, Eckbolsheim, France) using an autosampler (WISP 712, Waters). The mobile phase was a mixture of acetonitrile and water (50/50; v/v) at a flow rate of 1 ml/min (SP8800 pump, Spectra Physics, TSP, CA). Detection was performed by UV spectrometry at 238 nm (Waters 490E detector) using a SP-800 integrator (Spectra Physics). The method was linear up to 500 μg/ml with a detection limit <0.1 μg/ml. The amounts of FLU (μg) incorporated per mg of microparticles (Q_{MP}) and per mg of polymer (Q_{POL}) were expressed as the mean ± S.D. of three independent experiments.

The variation of the Q_{POL} versus Q_{OP} (amount of FLU added in the organic phase, see Section 2.2) was also studied and the corresponding experimental points were fitted using a Hill model (Kurganov et al., 2001) modified to include the linear incorporation process of the dissolved FLU, following the equation:

$$Q_{POL} = K_s \times Q_{OP} + \left\lfloor \frac{Q_{POL\,max} \times Q_{OP}^{nH}}{K^{nH} + Q_{OP}^{nH}} \right\rfloor \quad (2)$$

where $Q_{POL\,max}$ is the maximum incorporated amount, K the incorporation constant for solid FLU indicating the concentration of FLU at which $0.5 \times Q_{POL\,max}$ is reached, K_s is the incorporation constant for dissolved FLU and nH is the Hill coefficient reflecting the cooperativity.

2.5. Scanning electron microscopy studies

The external morphology of microparticles was analyzed by scanning electron microscopy (SEM). The microparticles were fixed with carbon-glue and coated with gold–palladium under argon atmosphere. Samples were then observed with a Cambridge model S scanning electron microscope (Leica Cambridge Ltd., Cambridge, UK) at 20 kV.

2.6. Infrared studies of microparticles

The crystalline form of FLU incorporated in microparticles was assessed by Fourier transform infrared spectrometry (FTIR). A FT-IR 2000 spectrometer and the Spectrum 4.0 software (Perkin-Elmer, St. Quentin, France) were used. Samples of MP or pure FLU were grinded in an agate mortar with KBr to obtain a concentration of about 4% (w/w). Spectra were obtained by diffuse reflectance with 40 scans per spectrum at a J-Stop resolution of 4 cm^{-1}.

2.7. X-ray diffraction

Diffraction patterns (MP, FLU and PCL) were recorded with a D500 diffractometer. A voltage of 35 kV and a current of 20 mA for the generator were used, with Co as anticathode (1.78897 Å). The samples were exposed over a range of 2θ angles from 10 to 50° at an angular speed of 1° per min.

2.8. Determination of the residual water in microparticles

The residual water remaining in microparticles was determined by a coulometric Karl-Fischer micromethod (Bioblock, France). An accurately weighed sample (about 50 mg) of microparticles was dissolved in 10 ml of anhydrous dichloromethane and 750 μl of this solution were injected into the measuring cell using a precision Hamilton syringe. The measured levels were corrected by the dichloromethane water content (blank). Results, expressed as microgram of water per milligram of sample, were the mean ± S.D. of three determinations.

2.9. Release studies

In order to investigate the release of FLU from microparticles, various batches were suspended in 100 ml of phosphate buffer (0.1 M, pH 7.40) preheated at 37 °C. The fludrocortisone microparticles were suspended at a concentration of 10 μg of fludrocortisone per ml of medium, near "sink conditions" but taking account of the sensibility of the analytical method.

The suspensions were incubated at 37 °C in a shaking-bath at 200 strokes/min (Heito, France). One milliliter aliquot was taken at various times up to 24 h and centrifuged (7200 × g, 5 min, Denver Instruments, MA). The supernatants were then analyzed for their drug contents by the previously described HPLC method. Results are presented as the mean ± S.D. of three experiments. The release of FLU was expressed as the percentage of drug released at each sampling time T (h).

Experimental points were fitted using the release models described by Baker and Lonsdale (1974) for spherical geometries. If the drug was molecularly dissolved in the polymer, the following "dissolved drug" model (Eqs. (3) and (4)) was used:

The early time approximations were:

$$\frac{Q_t}{Q_\infty} = 6\left(\frac{Dt}{r^2 \times \pi}\right)^{1/2} - \frac{3Dt}{r^2}, \quad \text{which is valid for} \frac{Q_t}{Q_\infty} < 0.4 \qquad (3)$$

and the late time solutions were:

$$\frac{Q_t}{Q_\infty} = 1 - \frac{6}{\pi^2}\exp\left(\frac{-\pi^2 \times Dt}{r^2}\right), \quad \text{which is valid for} \frac{Q_t}{Q_\infty} > 0.6 \qquad (4)$$

where Q_∞ is the percent release at infinite time, D is the diffusion coefficient in the polymer in cm^2/s, r is the radius of the sphere in cm.

However, when the total concentration of drug into microparticles (dissolved plus dispersed) was larger than its solubility in PCL, the "dispersed drug" release model was used:

$$\frac{3}{2}\left(1 - \left[1 - \frac{Q_t}{Q_\infty}\right]^{2/3}\right) - \frac{Q_t}{Q_\infty} = \frac{3 \times DC_s}{r_0^2 \times C_O} \times t \qquad (5)$$

where C_s is the solubility in the polymer, C_O is the concentration of the drug in the polymer (dispersed plus dissolved).

Finally, Weibull equation (Langenbucher, 1972), was used for the free drug:

$$\text{Percent release} = Q_\infty\left\lfloor 1 - \exp\left[-\left[\frac{T - T_0}{T_D}\right]^\beta\right]\right\rfloor \qquad (6)$$

where T_0 is the latency time (h), T is the sampling time (h), T_D is the time for 63.2% release (h) and β is the curvature coefficient.

For each curve, the time for 75% of release ($T_{75\%}$) was determined.

3. Results and discussion

3.1. Influence of the volume of the aqueous phase (assay 1)

Our first results showed the importance of the volume of the aqueous phase during the preparation of the microparticles. Indeed, batches prepared with

100, 200 and 400 ml did not allow to obtain pure microparticles: many square crystals were contaminating the suspension. The recovery of fludrocortisone in the polymeric suspension obtained for 100, 200 and 400 ml, correspond to mixtures of crystals of fludrocortisone and microparticles (Fig. 1a). The sizes of the crystals were about 20 μm whereas the microparticles reached 47 μm (Table 2). It is obvious that the stability of the emulsion is not sufficient to obtain microparticles and the diffusion of the drug from the organic phase to the aqueous phase is followed by a precipitation. Larger volumes of PVA 0.1% (≥600 ml) allow to obtain pure spherical microparticles (Fig. 1b). It can be deduced that the lack of precipitation and detection of FLU crystals are mainly due to the solubility of FLU in the aqueous medium. It can also be attributed to the formation of stable O/W emulsion prior to microsphere formation. Consequently, all the other batches (Table 1: assays 2–4) have been prepared in 800 ml.

3.2. Influence of the concentration of FLU (assays 2 and 3)

The second step of our work was the assessment of the influence of the concentration of fludrocortisone in the organic phase. This parameter was found to be critical and has been extensively studied.

3.2.1. Effects on the incorporation of FLU

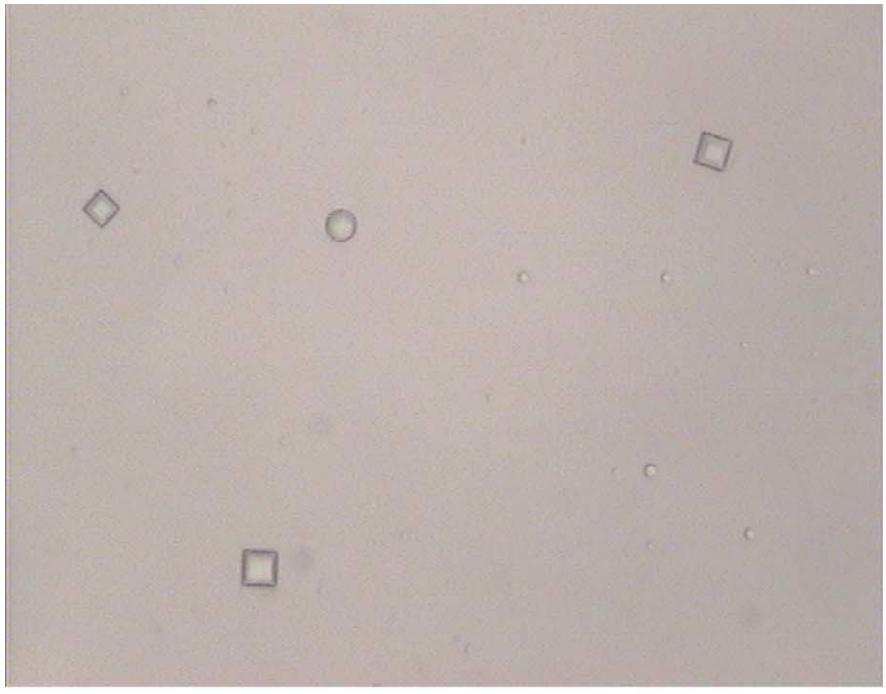
Table 3 shows the mean incorporation data, calculated. For $Q_{OP} < 200$ μg/mg, the percent IC were quite low, ranging from 5.8 to 10.0%. However, for higher amounts of FLU, this percentage increased dramatically and reached 78.6 and 68.9% for a 15 mg/ml concentration (FLU^{15}-PCL^{25}-800 and FLU^{15}-RS^{25}-800). Under our experimental conditions, the highest level of FLU incorporation achieved within microparticles was 320.4 ± 15.7 μg/mg of microparticles.

As shown in Fig. 2, the amount of FLU incorporated per mg of polymer (Q_{POL}) versus the amount of FLU added in the organic phase (Q_{OP}), expressed per mg of polymer, followed a Hill equation model modified to include a linear incorporation step (Eq. (2); $r = 0.9999$). This unusual incorporation profile suggested a cooperative process for the drug loading since the Hill coefficient *nH* was >1 (Table 4). The initial portion of the curve, up to 200 μg FLU/mg of polymer (5 mg/ml), should reflect the linear incorporation of FLU in the polymer under an amorphous form, depending both on the partition coefficient in the liquid phases and on the intrinsic affinity of the drug for the solid polymer, both reflected by the incorporation constant K_s, thus leading to a molecular or colloidal dispersion of the drug in the polymer matrix. However, this incorporation process was poorly efficient since K_s was low (Table 4; Fig. 2). By linear extrapolation, the maximum incorporation of FLU in PCL at its solubility limit in dichloromethane (9 mg/ml) was estimated at 18 μg/mg of PCL and 22.6 μg/mg of Eudragit® RS. However, for a concentration of 300 μg FLU/mg PCL (7.5 mg/ml) and despite the fact that the drug was always soluble at its concentration, the incorporation was higher ($FLU^{7.5}$-PCL^{25}-800: $Q_{POL} = 82.0 \pm 25.0$ μg/mg of PCL) than expected by linear extrapolation, which could reflect a facilitated incorporation by precipitation into the drug-saturated PCL matrix before the complete solidification of the polymer. When concentrations exceeded the drug solubility (S/O/W method), a dramatic increase of the incorporation was observed, which could correspond to the direct inclusion of crystals into the hardening polymeric core.

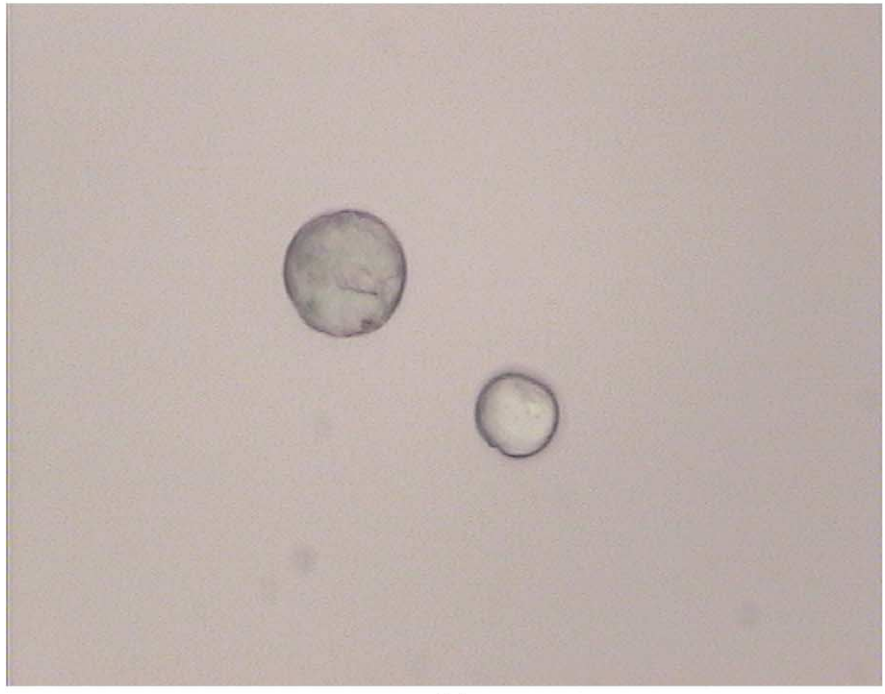
Table 2
Characteristics of each suspension (assay 1) as function of the volume of the aqueous phase

Formulation name	Volume of the aqueous phase (ml)	Presence of crystals in the suspension	Residual water (μg/mg MP)	Mean Feret diameter (μm)	Roundness
Flu^5-PCL^{25}-100	100	+	ND	ND	ND
Flu^5-PCL^{25}-200	200	+	ND	ND	ND
Flu^5-PCL^{25}-400	400	+	ND	ND	ND
Flu^5-PCL^{25}-600	600	–	1.27 ± 0.18	45.12 ± 22.80	0.74 ± 0.27
Flu^5-PCL^{25}-800	800	–	1.24 ± 0.16	47.21 ± 27.20	0.85 ± 0.21

The surface aspect, the size and the residual water were determined as described in Section 2 (mean ± S.D.; $n = 3$). ND: not determined.



(a)



(b)

Fig. 1. Suspensions obtained with (a) 200 ml of aqueous phase (Flu^{5}-PCL^{25}-200), and (b) 800 ml of aqueous phase (Flu^{5}-PCL^{25}-800).

Table 3
Incorporation data of fludrocortisone acetate (Q_{MP}, Q_{POL} and % IC) in microparticles as function of the fludrocortisone concentration in the organic phase (Q_{OP}) (assays 2 and 3)

Formulation name	Method	Q_{MP} (μg/mg MP)	Q_{POL} (μg/mg of polymer)	Q_{OP} (μg/mg of polymer)	% IC
Flu^{1}-PCL^{25}-800	O/W	2.9 ± 0.5	2.9 ± 0.5	40	7.3 ± 1.2
Flu^{2}-PCL^{25}-800	O/W	4.6 ± 0.5	4.6 ± 0.5	80	5.8 ± 0.7
Flu^{5}-PCL^{25}-800	O/W	11.8 ± 3.1	11.9 ± 3.1	200	6.0 ± 1.6
$Flu^{7.5}$-PCL^{25}-800	O/W	75.8 ± 23.1	82.0 ± 25.0	300	27.3 ± 8.3
Flu^{10}-PCL^{25}-800	S/O/W	226.9 ± 20.7	293.5 ± 26.8	400	73.4 ± 6.7
Flu^{15}-PCL^{25}-800	S/O/W	320.4 ± 15.7	471.5 ± 23.1	600	78.6 ± 3.8
Flu^{1}-RS^{25}-800	O/W	3.1 ± 1.2	3.1 ± 1.2	40	7.7 ± 3.0
Flu^{2}-RS^{25}-800	O/W	4.9 ± 4.6	4.9 ± 4.6	80	6.1 ± 5.7
Flu^{5}-RS^{25}-800	O/W	12.7 ± 2.9	13.1 ± 2.9	200	10.0 ± 1.4
$Flu^{7.5}$-RS^{25}-800	O/W	20.1 ± 1.3	20.5 ± 1.3	300	6.8 ± 0.4
Flu^{10}-RS^{25}-800	S/O/W	204.2 ± 19.7	256.6 ± 20.1	400	64.1 ± 4.9
Flu^{15}-RS^{25}-800	S/O/W	289.6 ± 78.6	407.7 ± 85.3	600	68.9 ± 11.5

Parameters were obtained as described in Section 2 (mean ± S.D.; $n = 3$). Q_{OP}: amount of FLU in dichloromethane per mg of polymer; Q_{MP}: amount of FLU incorporated per mg of MP; Q_{POL}: amount of FLU incorporated per mg of polymer; % IC: incorporation percentage.

3.2.2. *Effects on the size and the aspect of the microparticles*

Microparticles prepared with low concentrations of fludrocortisone ($Q_{OP} \leq 200$ μg/mg of polymer) show a smooth and homogeneous external aspect by SEM (Figs. 3a and 4a) without evidence of crystallization on the surface, confirming the colloidal dispersion of the drug in the polymer matrix.

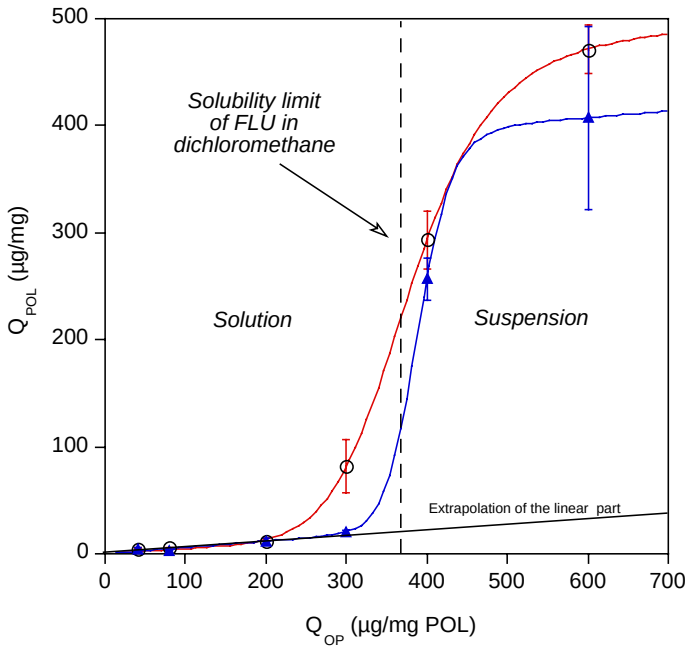


Fig. 2. Incorporation profile of fludrocortisone (FLU) into microparticles of poly(ε-caprolactone) (assay 2: ○) or Eudragit® RS (assay 3: ▲) as function of the fludrocortisone concentration in the organic phase. Q_{OP}: amount of FLU added in dichloromethane expressed in μg/mg of polymer; Q_{POL}: amount of FLU incorporated per mg of microparticular polymer. Each point represents the mean ± S.D. of three separate determinations. Solution: concentrations range corresponding to solutions of FLU. Suspension: concentrations range corresponding to FLU suspended in saturated solution.

Table 4
Parameters obtained with experimental points of the incorporation of fludrocortisone into microparticles (Q_{POL}) as function the fludrocortisone concentration in dichloromethane (Q_{OP}) and fitted using the modified Hill: $Q_{POL} = (K_s \times Q_{OP}) + \lfloor (Q_{POL\,max} \times Q_{OP}^{nH}) / (K^{nH} + Q_{OP}^{nH}) \rfloor$

	K_s	K	nH	$Q_{POL\,max}$
PCL microparticles	0.048 ± 0.014	378 ± 2	7.5 ± 0.3	456 ± 11
Eudragit® RS microparticles	0.059 ± 0.009	389 ± 3	18.9 ± 4.6	372 ± 6

$Q_{POL\,max}$ is the maximum incorporated amount, K the incorporation constant for solid FLU indicating the concentration of FLU at which $0.5 \times Q_{POL\,max}$ is reached, K_s is the incorporation constant for dissolved FLU and nH the Hill coefficient reflecting the cooperativity.

$FLU^{7.5}$-PCL^{25}-800 microparticles ($Q_{OP} = 300\,\mu g$/mg, Fig. 3b) were covered by few typical bipyramidal crystals, freshly crystallized, confirming the facilitated incorporation of the fludrocortisone by precipitation into the drug-saturated PCL matrix. On the contrary, $FLU^{7.5}$-RS^{25}-800 Eudragit® RS microparticles did not show crystals on their surface (Fig. 4b) and the concentration of the fludrocortisone incorporated was lower (20.5 ± 1.3 μg/mg of Eudragit® RS) (Table 3). This could be explained by a higher affinity of fludrocortisone for Eudragit® or by a more premature solidification of this polymer.

Finally, microparticles prepared by a S/O/W method ($Q_{OP} \geq 400\,\mu g$/mg of polymer) exhibited a heterogeneous aspect with numerous small crystals embedded in the polymer (Figs. 3c and 4c). These crystals, probably directly incorporated during the hardening process, have the same aspect as the micronized fludrocortisone.

The mean size, the size distribution profile and the shape were not different between O/W and S/O/W microparticles (Table 5). The mean particle size was about 40 μm (34–44 μm; NS) with an approximately symmetrical distribution ranging from 20 to 120 μm. Moreover, the microparticles can be considered as predominantly spherical on the basis of the shape parameters such as roundness, which was close to 1. Moreover, microscopic analysis showed neither crumbling nor clustering of particles.

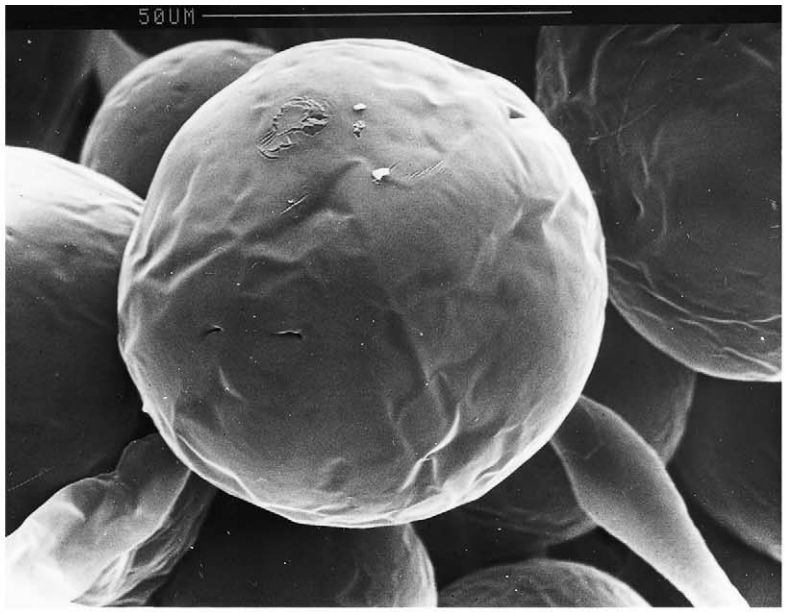
3.2.3. Effects on the crystalline state of FLU

The examination of microparticles by scanning electron microscopy have put in evidence crystals on the surface of S/O/W microparticles. Moreover, some crystals can be embedded in the core of the particles. FLU may exist under four polymorphic forms (Mesley, 1966), particularly as an amorphous form namely form C, obtained after evaporation of solutions of the crystallized form A in chloroform or acetone. This form A corresponds to the standard described by the British Pharmacopoeia. An alternative crystalline form, denoted form D, is obtained by

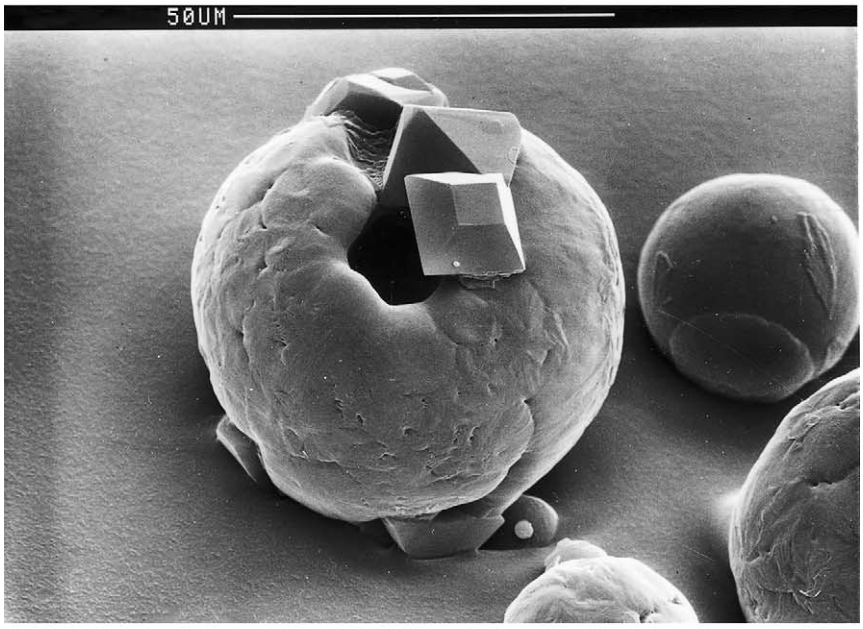
Table 5
Characteristics of microparticles as function of the fludrocortisone concentrations in the aqueous phase (Q_{OP}) (assays 2 and 3)

Formulation name	Q_{OP} (μg/mg of polymer)	Presence of crystals on the MP surface	Residual water (μg/mg MP)	Mean Feret diameter (μm)	Roundness
Flu^{1}-PCL^{25}-800	40	–	1.21 ± 0.15	41.9 ± 20.0	0.81 ± 0.21
Flu^{2}-PCL^{25}-800	80	–	1.20 ± 0.12	43.2 ± 25.8	0.82 ± 0.21
Flu^{5}-PCL^{25}-800	200	–	1.24 ± 0.16	34.0 ± 17.6	0.87 ± 0.22
$Flu^{7.5}$-PCL^{25}-800	300	+	1.37 ± 0.10	38.7 ± 18.4	0.81 ± 0.21
Flu^{10}-PCL^{25}-800	400	+++	1.74 ± 0.08*	41.9 ± 22.9	0.82 ± 0.21
Flu^{15}-PCL^{25}-800	600	+++	1.71 ± 0.09*	44.4 ± 34.0	0.83 ± 0.25
Flu^{1}-RS^{25}-800	40	–	1.30 ± 0.11	40.6 ± 20.6	0.81 ± 0.20
Flu^{2}-RS^{25}-800	80	–	1.25 ± 0.06	41.7 ± 24.8	0.82 ± 0.20
Flu^{5}-RS^{25}-800	200	–	1.29 ± 0.05	32.2 ± 11.9	0.76 ± 0.24
$Flu^{7.5}$-RS^{25}-800	300	–	1.32 ± 0.09	33.7 ± 12.5	0.95 ± 0.22
Flu^{10}-RS^{25}-800	400	+++	1.77 ± 0.10*	32.6 ± 15.1	0.78 ± 0.23
Flu^{15}-RS^{25}-800	600	+++	1.83 ± 0.10*	30.0 ± 13.5	0.85 ± 0.23

The surface aspect, the size and the residual water were determined as described in Section 2 (mean ± S.D.; $n = 3$).
* $P < 0.02$ vs. Flu^{1}-PCL^{25}-800 to $Flu^{7.5}$-PCL^{25}-800.

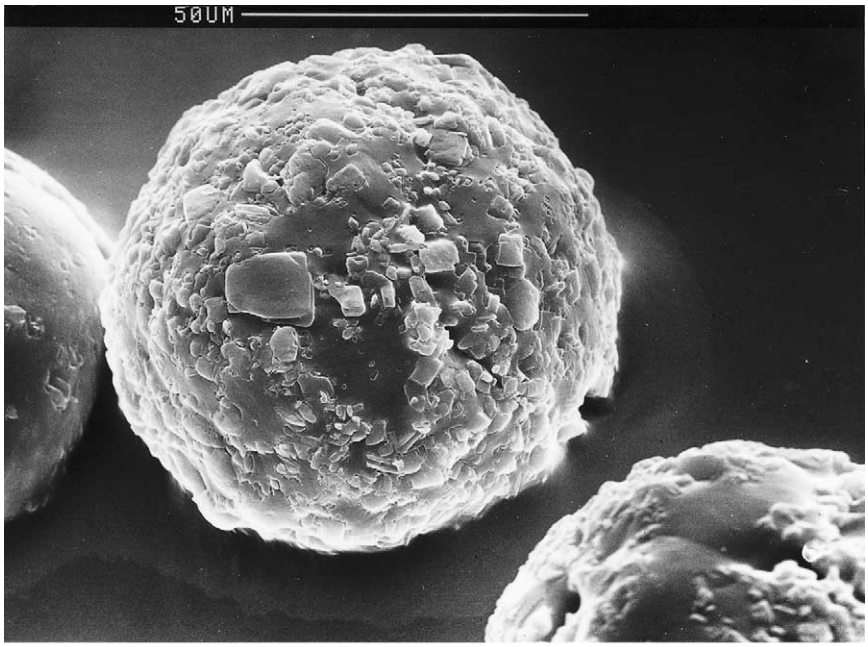


(a)



(b)

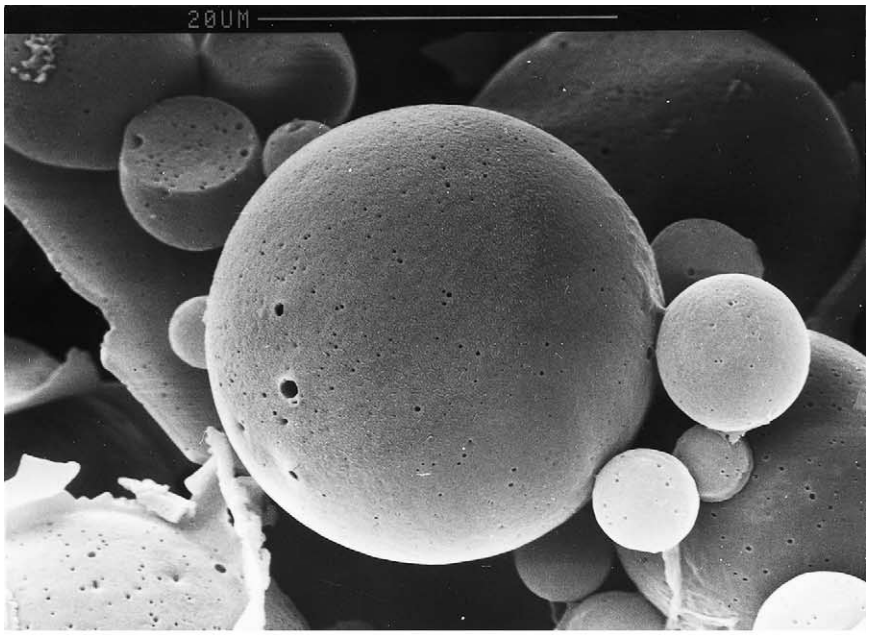
Fig. 3. Poly(ε-caprolactone) microparticles of fludrocortisone (assay 2) observed by scanning electron microscopy (SEM). (a) Flu^{5}-PCL^{25}-800 ($Q_{OP} = 200\,\mu g/mg$ PCL). (b) $Flu^{7.5}$-PCL^{25}-800 ($Q_{OP} = 300\,\mu g/mg$ PCL). (c) Flu^{15}-PCL^{25}-800 ($Q_{OP} = 600\,\mu g/mg$ PCL).



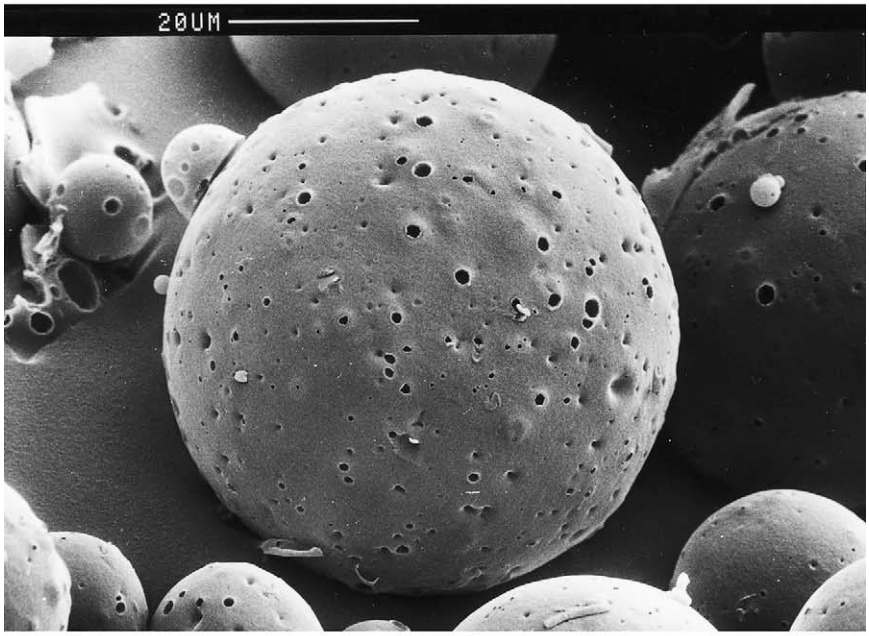
(c)

Fig. 3. (*Continued*).

spontaneous crystallization of the form C whereas form B require an additional heating (15 min, 100 °C). The hydrate and several solvates have been observed (Kuhnert-Brandstätter and Gasser, 1971). These polymorphic forms have distinct infrared spectra (Mesley, 1966). Hence, we used FTIR spectrometry to assess the crystalline form of FLU in S/O/W PCL microparticles (assay 2). Several characteristic peaks of the form A were observed for FLU powder used to prepare the microparticles, ascertaining thus its polymorphic form: 847, 988, 1274 cm^{-1} (21-acetoxy-20-ketone); 879 cm^{-1} (4-ene-3-ketone); double peak at 3340 and 3350 cm^{-1}. As presented in Fig. 5, the spectrum of FLU incorporated in S/O/W microparticles was almost identical at ± 0.1 cm^{-1} for numerous characteristic peaks observed in the spectra of the form A, in wavelength ranges where PCL do not absorb as from 600 to 700 and 800 to 925 cm^{-1}, especially the triplet at 879, 892 and 902 cm^{-1}. These results demonstrate that FLU was incorporated under its initial crystalline form. Moreover, these results indicate the absence of chemical interactions between FLU and PCL under solid state. However, due to the low quantities of FLU incorporated in O/W microparticles, no drug-related peak was observed. Therefore, we were unable to confirm by this method that FLU was incorporated in O/W microparticles under its amorphous form, as expected, or under the form D, which could result from the crystallization of the dissolved molecules during the diffusion and the evaporation of dichloromethane. However, an amorphous form for FLU should be assumed for $Q_{OP} \leq 200$ μg/mg since the slow extraction of the solvent from the organic droplets by the external aqueous phase during the polymer hardening step, could be assimilated to an evaporation process. Therefore, FLU could solidify under its amorphous form C inside the forming particles, as observed during the evaporation from its chloroform solution, solvent similar to dichloromethane. The formation of the crystalline form D by a slow crystallization of the amorphous FLU cannot be however excluded, albeit unlikely. Nevertheless, our results strongly suggest that FLU was mainly incorporated into S/O/W microparticles under its initial form without notable polymorphism as expected by the preparation process. Moreover, these hypotheses were also supported by X-ray analysis (Fig. 6). It was obvious that the pure drug exhibited crystalline characteristics. Unloaded



(a)



(b)

Fig. 4. Eudragit® RS microparticles of fludrocortisone (assay 3) observed by scanning electron microscopy (SEM). (a) Flu^{5}-RS^{25}-800 (Q_{OP} = 200 μg/mg Eudragit® RS). (b) $Flu^{7.5}$-RS^{25}-800 (Q_{OP} = 300 μg/mg Eudragit® RS). (c) Flu^{15}-RS^{25}-800 (Q_{OP} = 600 μg/mg Eudragit® RS).

(c)

Fig. 4. (*Continued*).

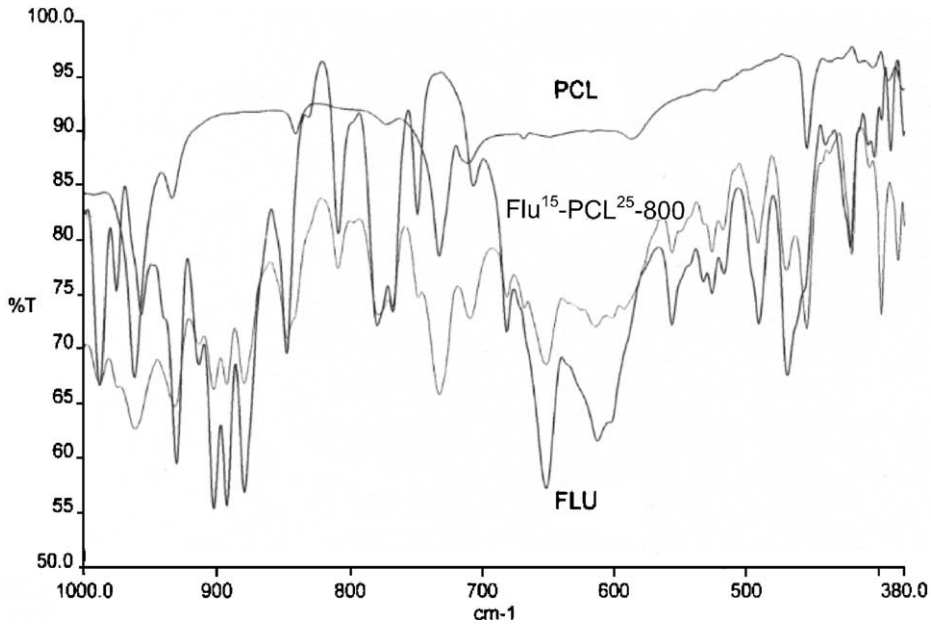


Fig. 5. Infrared study of fludrocortisone (FLU), poly(ε-caprolactone) (PCL), and "S/O/W" fludrocortisone-loaded microparticles (Flu^{15}-PCL^{25}-800).

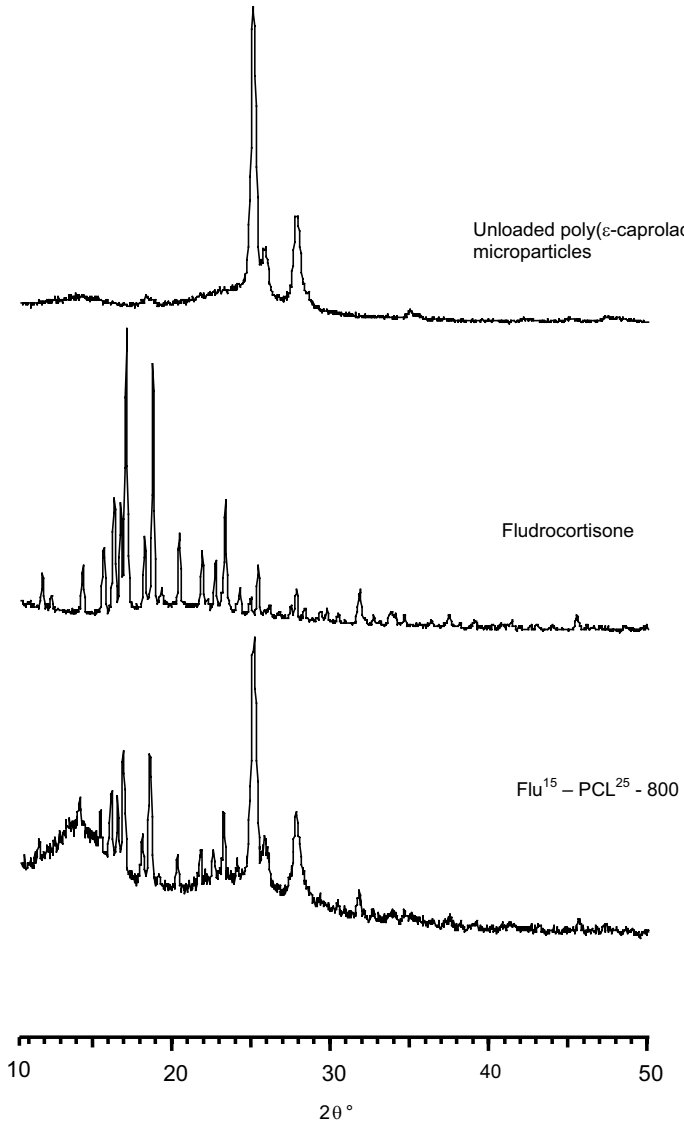


Fig. 6. X-ray diffraction spectra of poly(ε-caprolactone), fludrocortisone, and fludrocortisone microparticles (Flu^{15}-PCL^{25}-800).

PCL microparticles showed the typical X-ray profile of crystalline materials and the FLU^{15}-PCL^{25}-800 S/O/W microparticles show an interference due to the pure drug peaks (Fig. 6).

3.2.4. Effects on the hydration of microparticles

The determination of water in microparticles showed low residual levels (Table 5), which were not different for the O/W microparticles of various drug loads, whereas the S/O/W microparticles were slightly more hydrated ($P < 0.03$). However, these levels were similar to those found in drug free-PCL microparticles and can be reduced to about 0.2% by extensive drying under vacuum.

3.2.5. Effects on the in vitro release of FLU

The in vitro release experiments performed with PCL-based and Eudragit®-based microparticles

showed that FLU could be released from the polymeric matrix. The release studies were not prolonged up to 12-h since a significant FLU decomposition in the incubation medium was observed for longer periods, corresponding to the hydrolysis of acetate ester, as shown by HPLC. Experimental results, up to 12 h, were well described by the " dissolved drug" model and the "dispersed drug" according to Baker and Lonsdale (1974). For the O/W PCL microparticles (FLU^{1}-PCL^{25}-800, FLU^{2}-PCL^{25}-800, FLU^{5}-PCL^{25}-800, $FLU^{7.5}$-PCL^{25}-800) the maximum release, estimated by Q_{∞}, was higher ($P < 0.02$) than for the correspondent S/O/W microparticles (FLU^{10}-PCL^{25}-800, FLU^{15}-PCL^{25}-800) and was not significantly different from this obtained with the free drug (Table 6; Fig. 7a). As regards Eudragit® RS microparticles (Table 6; Fig. 7b), the release was very low but, as for PCL microparticles, the release from S/O/W microparticles was the lowest.

Using the $T_{75\%}$ parameter as estimation of the release rate (Eqs. (4)–(6)), the dissolution of FLU was very rapid (about 0.22–0.55 h) and not different of the free drug (about 0.19 h) for FLU^{1}-PCL^{25}-800 FLU^{2}-PCL^{25}-800 and FLU^{5}-PCL^{25}-800 O/W microparticles, whereas the release rates were strongly reduced for the S/O/W microparticles (about 3.5 h; $P < 0.001$).

Table 6
Release parameters of FLU and FLU-loaded microparticles (assays 2 and 3)

Batch name	Q_{∞} (%)	$T_{75\%}$ (h)
Flu	86.8	0.19
Flu^{1}-PCL^{25}-800	70.1	0.41
Flu^{2}-PCL^{25}-800	75.1	0.28
Flu^{5}-PCL^{25}-800	76.9	0.30
$Flu^{7.5}$-PCL^{25}-800	82.5	4.00
Flu^{10}-PCL^{25}-800	59.8	3.94
Flu^{15}-PCL^{25}-800	54.6	3.88
Flu	86.8	0.19
Flu^{1}-RS^{25}-800	22.3	0.22
Flu^{2}-RS^{25}-800	20.6	0.23
Flu^{5}-RS^{25}-800	20.4	0.55
$Flu^{7.5}$-RS^{25}-800	12.6	3.50
Flu^{10}-RS^{25}-800	14.3	3.42
Flu^{15}-RS^{25}-800	14.9	3.30

Results are calculated according to Eqs. (4)–(6). Q_{∞} denotes the release percentage at infinite time and $T_{75\%}$ indicates the time to obtain 75%.

It is generally admitted that the release of dispersed drugs from polymers, needs an initial diffusion of the solvent, a dissolution step and a retrodiffusion of the solution. It is also often assumed that the rate-limiting step is the diffusion of the drug from the matrix. Several studies have demonstrated that the release of low molecular drugs such as progesterone or phenothiazines from PCL-based microparticles was rapid, as the dissolution rate of pure drug crystals or faster; this phenomenon being attributed to the molecular dispersion of the drugs in the polymer (Pitt et al., 1979; Chang et al., 1987).

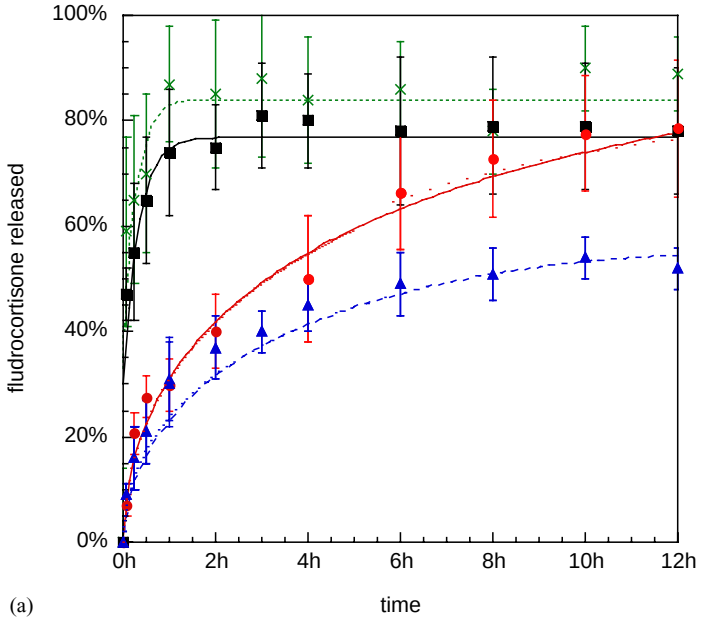
The release of solid drugs randomly dispersed in homogeneous matrices, described by Roseman and Higuchi (1970) is a very gradual process: the solid drug dissolves from the surface layer and when it becomes exhausted of drug the next layer begins to be depleted (Baker and Lonsdale, 1974).

Finally, the release patterns cannot be related to the degradation of the polymer since we ascertained its integrity by steric exclusion chromatography. Moreover, It has been shown that PCL degradation in water was a very slow process (Ali et al., 1993).

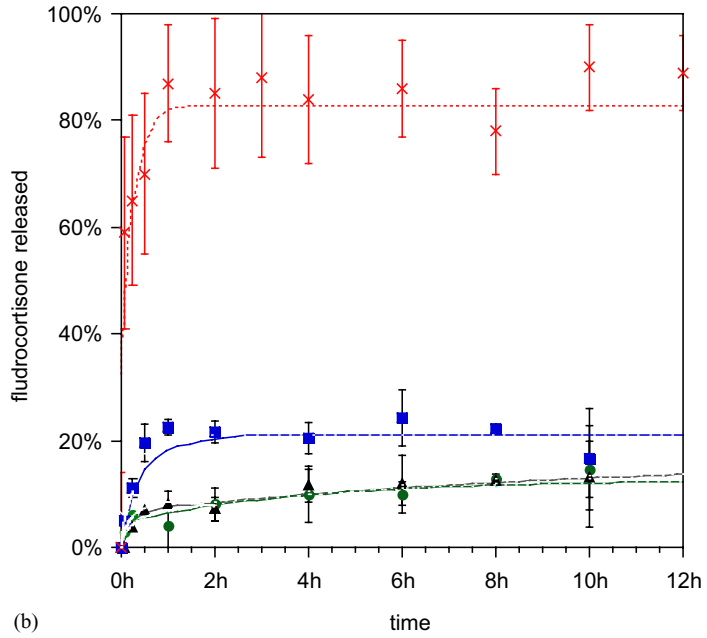
The Eudragit® RS microparticles show partial releases of fludrocortisone (Fig. 7b). Nevertheless, as for PCL-based microparticles, S/O/W Eudragit® RS microparticles (FLU^{10}-RS^{25}-800 and FLU^{15}-RS^{25}-800) release less fludrocortisone than O/W microparticles.

3.3. Assay 4: influence of the polymer

In our fourth assay (Table 1), various mixtures of poly(ε-caprolactone) and of Eudragit® were used. Mixtures of Eudragit® RS and RL are not biodegradable and are used in pharmaceutical preparations (filmcoats, microparticles) to obtain various permeabilities (Donbrow et al., 1995). The distinguishing letter RL and RS relate to the initial letters of the German words "leichtdurchlässig" (freely permeable) and "schwerdurchlässig" (slightly permeable), respectively and refer to the permeability characteristics of these coating. On the contrary, PCL is biodegradable and the mixtures of polymer allowed to modulate the characteristics of permeability and biodegradability. Moreover, this polymer had a mean



(a)



(b)

Fig. 7. Release of fludrocortisone from microparticles in phosphate buffer (0.1 M, pH 7.4, 37 °C). Influence of the fludrocortisone concentrations (assays 2 and 3). (a) Poly(ε-caprolactone) microparticles: Flu^{5}-PCL^{25}-800 (■) and $Flu^{7.5}$-PCL^{25}-800 (●), Flu^{15}-PCL^{25}-800 (▲). (b) Eudragit® RS microparticles: Flu^{5}-RS^{25}-800 (■) and $Flu^{7.5}$-RS^{25}-800 (●), Flu^{15}-RS^{25}-800 (▲). Each curve has been compared to free fludrocortisone (FLU: ×).

Table 7
Incorporation data of fludrocortisone acetate in microparticles as function of the polymer (assays 4)

Formulation name	Q_{MP} (μg/mg MP)	Q_{POL} (μg/mg of polymer)	Q_{OP} (μg/mg of polymer)	% IC
$FLU^{7.5}$-PCL^{25}-800	75.8 ± 23.1	82.0 ± 25.0	300	27.3 ± 8.3
$FLU^{7.5}$-$PCL^{12.5}$/$RS^{12.5}$-800	47.5 ± 9.3	49.9 ± 9.4	300	15.8 ± 3.1
$FLU^{7.5}$-$PCL^{6.25}$/$RS^{18.5}$-800	34.1 ± 2.5	35.3 ± 2.5	300	11.8 ± 0.8
$FLU^{7.5}$-RS^{25}-800	28.2 ± 0.2	29.0 ± 0.2	300	9.7 ± 0.1
$FLU^{7.5}$-$RS^{18.5}$/$RL^{6.25}$-800	27.3 ± 1.2	28.0 ± 1.2	300	9.3 ± 0.4
$FLU^{7.5}$-$RS^{12.5}$/$RL^{12.5}$-800	13.4 ± 0.5	13.5 ± 0.5	300	4.5 ± 0.2

Parameters were obtained as described in Section 2 (mean ± S.D.; $n = 3$). Q_{POL}: amount of FLU incorporated per mg of polymer; Q_{POL}: amount of FLU in dichloromethane per mg of polymer; Q_{MP}: amount of FLU incorporated per mg of MP; % IC: incorporation percentage.

Table 8
Characteristics of microparticles as function of the polymer (assays 4)

Batch name	Presence of crystals on the MP surface	Residual water (μg/mg MP)	Mean Feret diameter (μm)	Roundness
$FLU^{7.5}$-PCL^{25}-800	+	1.37 ± 0.10	38.7 ± 18.4	0.81 ± 0.21
$FLU^{7.5}$-$PCL^{12.5}$/$RS^{12.5}$-800	+/−	1.32 ± 0.07	38.0 ± 13.2	0.95 ± 0.19
$FLU^{7.5}$-$PCL^{6.25}$/$RS^{18.5}$-800	+/−	1.35 ± 0.10	32.5 ± 12.0	0.82 ± 0.22
$FLU^{7.5}$-RS^{25}-800	−	1.32 ± 0.09	33.7 ± 12.5	0.95 ± 0.22
$FLU^{7.5}$-$RS^{18.5}$/$RL^{6.25}$-800	−	1.35 ± 0.11	26.7 ± 9.4	0.93 ± 0.20
$FLU^{7.5}$-$RS^{12.5}$/$RL^{12.5}$-800	−	1.36 ± 0.12	35.4 ± 12.1	0.90 ± 0.21

Parameters were obtained as described in Section 2 (mean ± S.D.; $n = 3$). Q_{OP}: amount of FLU in dichloromethane per mg of polymer; Q_{MP}: amount of FLU incorporated per mg of MP; Q_{POL}: amount of FLU incorporated per mg of microparticular polymer; % IC: incorporation percentage.

molecular weight (MW) of 61,640 Da with a polydispersity index (MW/Mn) of 1.61, as assessed by steric exclusion chromatography; this was a polymer with a very low glass transition temperature (−60 °C) and a low melting point (about 62 °C), responsible for its high permeability to low molecular drug.

The preparations (Table 1, assay 4) have been made near the solubility limit of fludrocortisone, which had allowed to obtain the best slow-release profile (high Q_∞, and late $T_{75\%}$) in previous assays.

It was obvious that the amount of PCL is correlated with the incorporation of fludrocortisone (Table 7). Some bipyramidal crystals of fludrocortisone have been observed by SEM in each PCL-containing preparation confirming the crystallization process in the hardening polymer.

The mean size, the size distribution profile and the shape were not significantly different as function of the polymer (Table 8).

On the contrary, the release profiles (Table 9; Fig. 8) were very different: the Q_∞ was also correlated with the amount of PCL (Fig. 8b), and probably due to the biodegradation of the microparticles, to the lower

Table 9
Release parameters of FLU and FLU-loaded microparticles (assay 4)

Batch name	Q_∞ (%)	$T_{75\%}$ (h)
Flu	86.8	0.19
$Flu^{7.5}$-PCL^{25}-800	82.5	4.00
$Flu^{7.5}$-$PCL^{12.5}$/$RS^{12.5}$-800	62.0	3.50
$Flu^{7.5}$-$PCL^{6.25}$/$RS^{18.5}$-800	48.5	2.27
$Flu^{7.5}$-RS^{25}-800	12.6	3.50
$Flu^{7.5}$-$RS^{18.5}$/$RL^{6.25}$-800	68.0	2.05
$Flu^{7.5}$-$RS^{12.5}$/$RL^{12.5}$-800	70.1	1.15

Results are calculated according to Eqs. (4)–(6). Q_∞ denotes the release percentage at infinite time and $T_{75\%}$ indicates the time to obtain 75%.

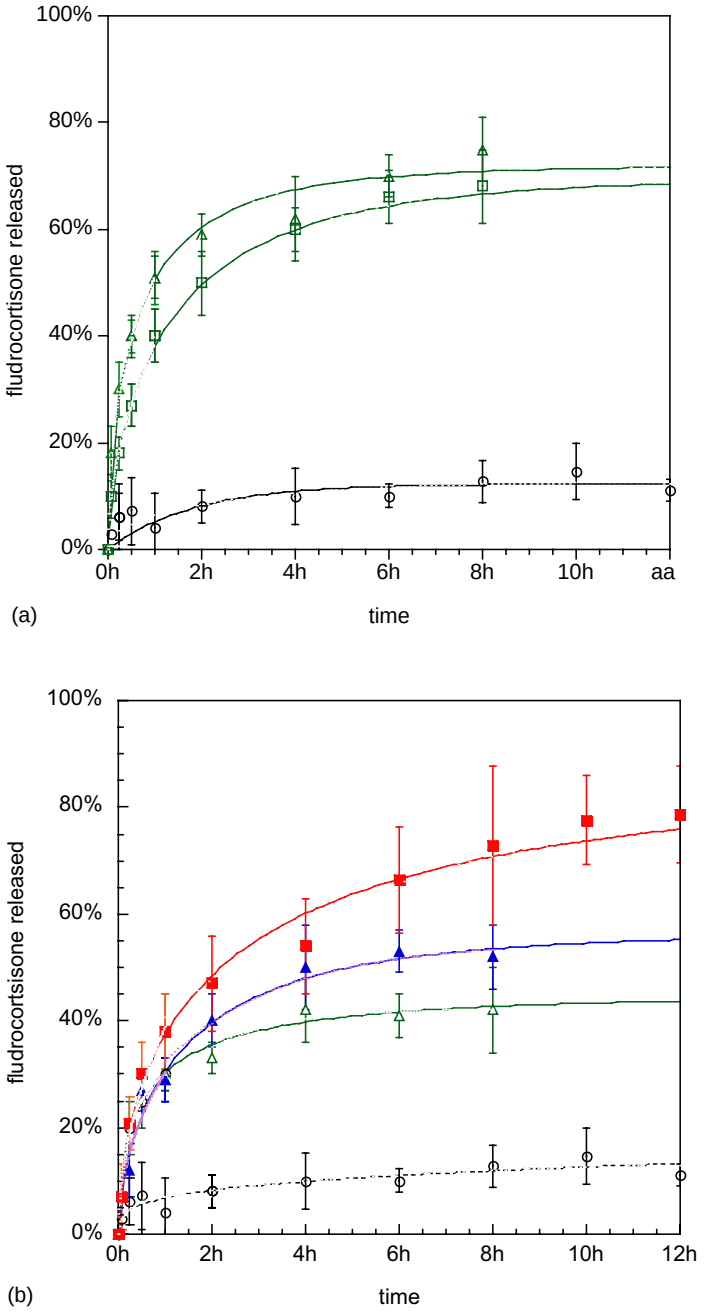


Fig. 8. Release of fludrocortisone from fludrocortisone microparticles in phosphate buffer (0.1 M, pH 7.4, 37 °C). Influence of the polymer (assay 4). (a) Mixtures of Eudragit® RS and Eudragit® RL: $Flu^{7.5}$-RS^{25}-800 (○), $Flu^{7.5}$-$RS^{18.5}/RL^{6.25}$-800 (□), and $Flu^{7.5}$-$RS^{12.5}/RL^{12.5}$-800 (△). (b) Mixtures of poly(ε-caprolactone) and Eudragit® RS: $Flu^{7.5}$-PCL^{25}-800 (■), $Flu^{7.5}$-$PCL^{12.5}/RS^{12.5}$-800 (▲), $Flu^{7.5}$-$PCL^{6.25}/RS^{6.25}$-800 (△), and $Flu^{7.5}$-RS^{25}-800 (○).

affinity of the drug for the PCL and/or to the higher permeability of this polymer.

4. Conclusion

Since the aim of this study was to obtain slow release FLU-loaded microparticles, a small size and good incorporation efficiency were the prerequisites. In the present study, we have successfully obtained small PCL-based microparticles leading to a good incorporation efficiency of FLU when the O/W emulsion-solvent evaporation method was used with 7.5 mg/ml of FLU.

Our results obtained with lower concentrations of FLU showed low levels of drug incorporation into microparticles. This poor incorporation was obviously unfavorable both for clinical purposes, requiring the administration of high amounts of microcarriers to obtain sufficient quantities of FLU, and for manufacturing aspects, considering the loss of an expensive active substance. Hence, the use of a saturated solution of FLU, leading to heterogeneous microparticles with several crystals embedded on the polymeric surface, should be a good alternative. Indeed, and regardless of their release properties, the microparticles obtained with an acceptable waste of the active substance, could necessitate less administered amounts of polymer to achieve the clinical goals. As an example, considering a mean daily dose of 50 μg, 1 mg of microparticles could be sufficient for about 1 week of treatment.

The development of an injectable form of fludrocortisone would also be suitable for this hormonal therapy. Nevertheless, poly(ε-caprolactone) (PCL) is not yet approved by the health authorities for injectable controlled release, but it is well tolerated by tissues without release of acidic metabolites unlike PLA/PLG (Benoit et al., 1999). This polymer has been recently used for the sustained release of levonorgestrel (Pitt, 1990).

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