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Flurbiprofen-loaded nanospheres: analysis of the matrix structure by thermal methods

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Abstract

We report the preparation and evaluation of flurbiprofen loaded-poly-ε-caprolactone nanospheres obtained by solvent displacement method. Characterization by thermal methods, infrared spectroscopy and X-ray diffraction analysis can reveal the dispersion state of the drug inside the nanospheres. Such information predicts the stability of the particles and the drug release behaviour. The study has indicated the presence of a molecular dispersed system. In addition, construction of a phase diagram has allowed us to determine the drug/polymer ratios at which flurbiprofen has the highest entrapment efficiency. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ocular drug delivery; Nanospheres; Poly-ε-caprolactone; Thermal methods

1. Introduction

In order to improve ocular administration of drugs, new systems have been developed recently for ophthalmic use, and successful results have been obtained with polymeric colloidal suspensions. Treatment with these systems aims to increase bioavailability, reduce the frequency of administration and promote targeting of drugs to specified regions. Polymer systems for drug delivery may thus be based on principles of drug diffusion and/or polymer degradation. Poly-ε-caprolactone (PεCL) is a partially crystalline, rather hydrophobic, high molecular weight polymer (Puisieux, 1985) that may be used in diffusion-controlled delivery systems for ocular administration (Marchal-Heussler et al., 1992). Nanoparticles prepared by the solvent displacement method (Fessi et al., 1988), may present

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Table 1
Entrapment efficiency, mean size (Z), polydispersity (Q), presence of nanocrystals and Poloxamer 188 remaining

Sample	FB initial concentration (mg/ml)	Entrapment efficiency (%)	Z (nm)	Q	nanocrystals	Poloxamer 188 remaining (mg/ml)
Empty nanospheres			211.2	0.079		0.199
F1	0.25	98.00	201.2	0.073		0.166
F2	0.50	94.00	221.4	0.096		0.199
F3	0.60[a]	99 05	283.9	0.090		0.174
F4	0.65	85.10	206.4	0.082		0.183
F5	0.70	84.90	213.6	0.073		0.166
F6	0.80	80.70	284.1	0.078	+	0.191
F7	1.00	76.00	218.0	0.081	++	0 100
F8	2.00	41.50	275.3	0.058	++++	0.100

[a] Eutectic composition.

various internal structures depending on the chemical nature of the two components and the entrapment ratio of the drug (Bissery et al., 1983).

The aim of this paper is to study the interaction between a slightly water soluble drug, flurbiprofen (FB), and a polymer (PεCL), in order to determine the dispersion state of the drug in the particles. The morphometrical properties of nanospheres were determined by photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM). Interactions were studied by infrared spectroscopy (IR), X-ray diffraction, thermal methods and phase diagram construction. Moreover, this diagram provided information about the eutectic composition, invariant point whose physical characteristics, particularly the dissolution kinetics or solubility, are improved in comparison with the drug in isolation (Lacoulonche et al., 1997).

2. Materials and methods

2.1. Materials

FB was obtained from Sigma Chemical (St. Louis, USA). PεCL was purchased from Birmingham Polymers (Birmingham, USA). Poloxamer 188 (Lutrol F68®) was kindly supplied by BASF. All reagents used were analytical grade.

2.2. Methods

2.2.1. Preparation of nanospheres

Empty and FB-loaded PεCL nanospheres were prepared by precipitation using the solvent displacement method (Fessi et al., 1988). Briefly, 66 mg of PεCL and different amounts of FB ranging between 5 and 40 mg were dissolved in 20 ml of acetone (Samples F1–F8, respectively). This solution, organic phase, was emulsified in 40 ml of an aqueous Poloxamer 188 solution (8.3 mg/ml) using magnetic stirring, for 5 min. Finally, the solvent was evaporated at 45°C under vacuum to a final volume of 20 ml.

2.2.2. Nanosphere characterization

Nanosphere particle size distribution was determined by PCS in a Malvern Autosizer IIC (Malvern Instruments, Malvern, UK, (McConnell, 1981). Morphological examination of entire nanoparticles was performed using a Philips 1011, transmission electron microscope (TEM), following negative staining with phosphotungstic acid/uranil acetate (Magenheim and Benita, 1991).

FB content in the nanospheres was determined by reversed-phase high-performance liquid chromatography (HPLC), using a modification of a procedure reported elsewhere (Albert et al., 1984). The samples were first subjected to ultracentrifugation at 40 000 r.p.m. (Centrikon T-1170, Kontron) for 2 h, to separate the two phases of the

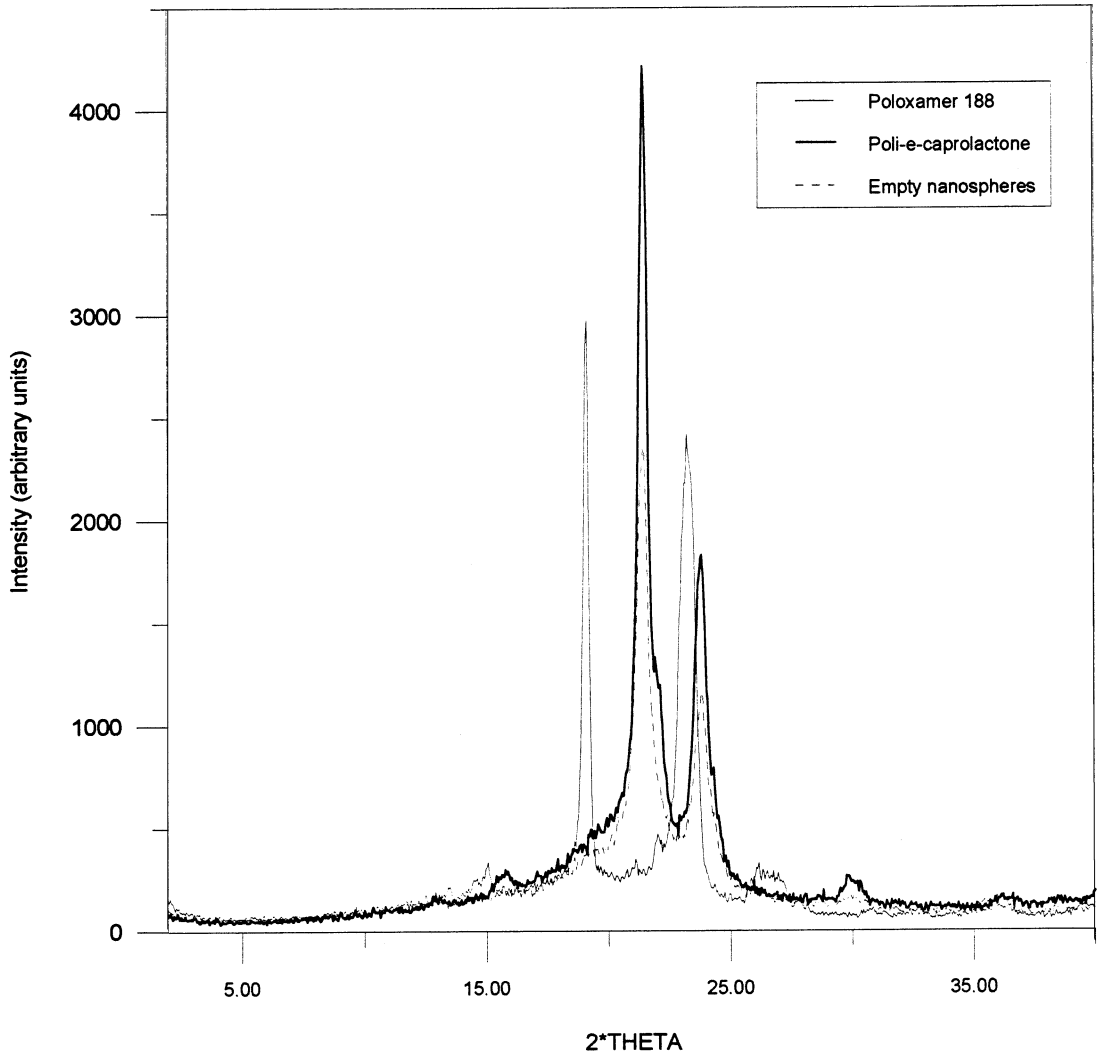

Fig. 1. Powder X-ray diffraction patterns of Poloxamer 188, poly-ε-caprolactone and empty nanospheres.

colloidal system. The samples were diluted before injection into the HPLC system (Kontron) with UV detection. Separation was achieved by using a reversed-phase column (Spherisorb) ODS-2, Tracer (BC Aplicaciones Analíticas S.A., Spain), with a flow rate of 1.5 ml/min. The mobile phase consisted of acetonitrile/water (650:350) acidified with phosphoric acid (pH 2.5). The UV detector was set at 254 nm. The content of drug in the nanospheres was calculated by determining the difference between the total amount of drug present in the colloidal suspension after dissolving samples in dimethylformamide, and the amount of free drug in the aqueous phase. Entrapment efficiency was expressed as the percentage of drug in relation to drug initially dissolved in organic phase.

The amount of Poloxamer 188 remaining in the nanospheres was determined by IR spectroscopy, using a procedure developed by the authors (Gamisans et al., in press). This method has a detection limit of 0.035 mg/ml. The samples were first ultracentrifuged at 40 000 r.p.m. for 2 h in order to separate two phases of colloidal system,

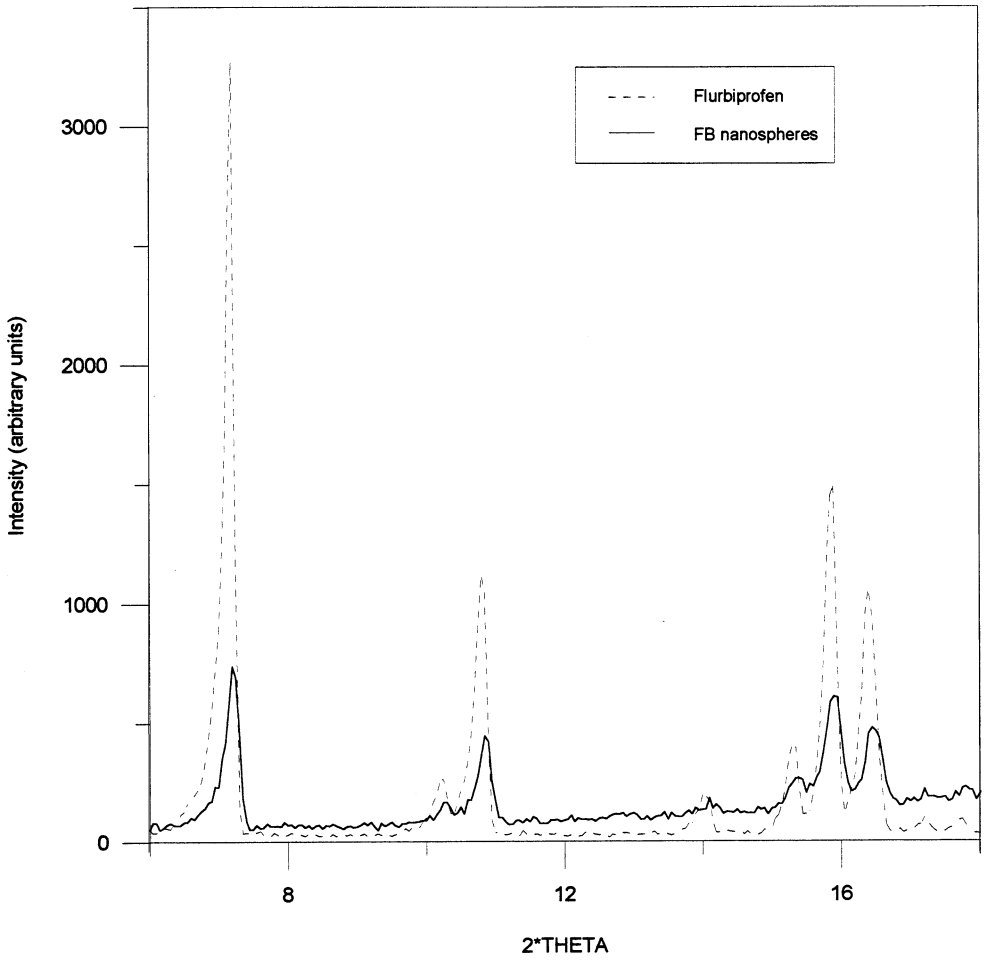

Fig. 2. Powder X-ray diffraction patterns of flurbiprofen and FB nanospheres.

pellet and supernatant. Then the surfactant was determined in the supernatant. The amount of Poloxamer 188 was calculated by determining the difference between the total surfactant present in the colloidal suspension, and the amounts present in the aqueous phase. This quantity was expressed as a concentration of Poloxamer 188 (mg/ml) in relation to surfactant originally dissolved in aqueous phase.

2.2.3. Interaction studies

2.2.3.1. Thermal analysis. In order to eliminate the water present in the nanospheres, with or without FB, they were washed three times in desionized water, ultracentrifuged and dried to constant weight in a desiccator.

Differential scanning calorimetry (DSC) analyses were carried out on samples of individual substances as well as empty nanospheres, FB nanospheres, physical mixture of FB with empty nanospheres (2:10) and FB–PεCL solid dispersions (co-precipitates and co-melts). In this way the samples were weighed (Mettler M3 Microbalance) directly in perforated aluminium pans (weight ranging from 2.65 to 3.00 mg) and scanned between 0 and 300°C at a heating rate of 10°C/min or between 0 and 140°C at a heating rate of 5°C/min (phase diagram determination), under nitrogen, using a Mettler TA 4000 system

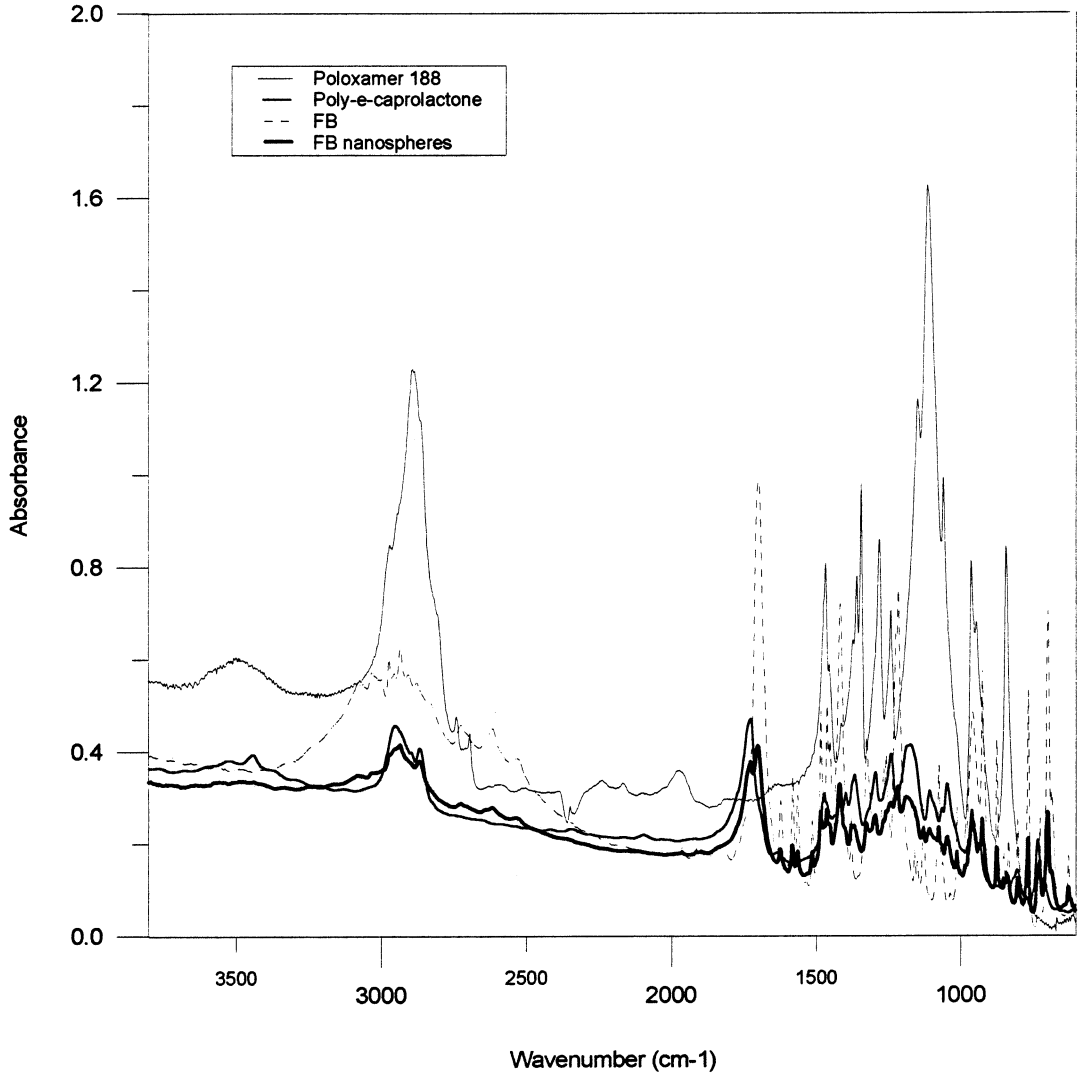

Fig. 3. IR spectra of flurbiprofen, Poloxamer 188, poly-ε-caprolactone and FB nanospheres.

equipped with a DSC 25 cell. Temperature was calibrated by the melting transition point of indium. Both the eutectic temperatures and temperatures at the end of the melting correspond to the onset temperatures (temperatures at the equilibrium between phases). Both the peak transition temperature and heat of fusion were calculated for all samples.

Thermogravimetric analyses (TG) were performed on the following samples (2.5–3 mg per sample): individual substances, empty nanospheres, FB nanospheres and physical mixture of FB and empty nanospheres (2:10) from 25 to 500°C at a heating rate of 10°C/min under an atmosphere of nitrogen.

Thermomicroscopy analyses (TMA) was performed using a polarising microscope Leitz SM POL connected to a Mettler FP52 hot stage and a Mettler FP5 temperature controller. Observations were recorded using a Sony DXC-101P colour video camera connected to the microscope.

To perform the phase diagram, solid dispersions (co-precipitate) of drug and polymer (be-

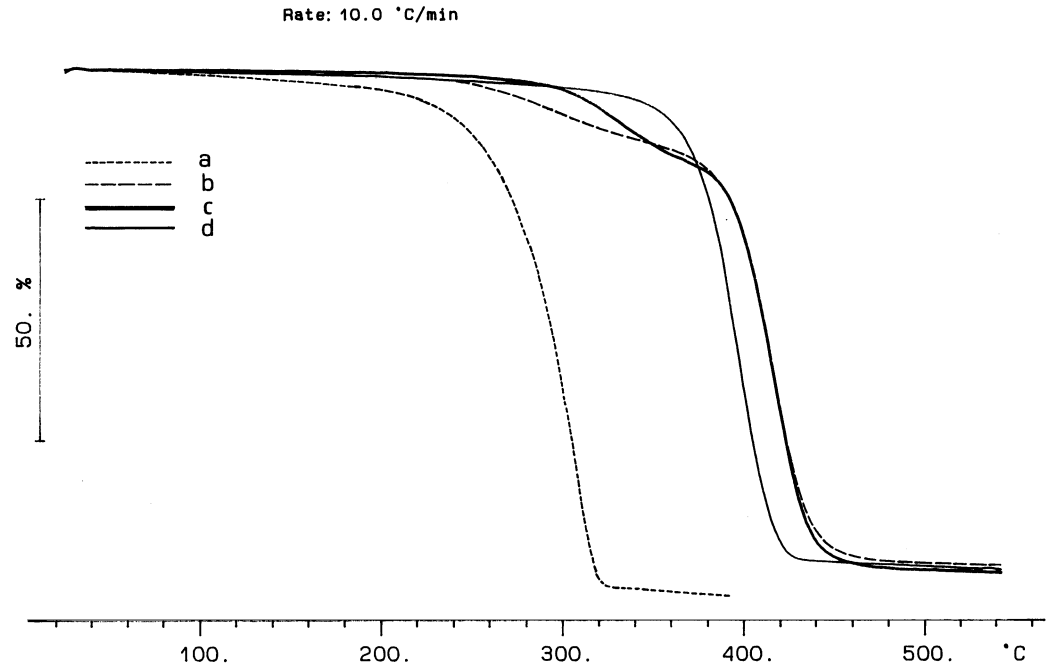

Fig. 4. TG tracings obtained with: (a) flurbiprofen; (b) FB nanospheres F2; (c)poly-ε-caprolactone; (d) Poloxamer 188.

tween 3 and 97% of PεCL, w/w) were prepared mixing the desired amounts of drug and polymer in a small volume of acetone. Co-melt were obtained from the melting of co-precipitates in the pans used in DSC.

2.2.3.2. Fourier transform infrared spectroscopy. The IR spectra of the products and the formulations were recorded on a BOMEN DA.3 spectrophotometer with MTC detector. Potassium bromide disc containing the samples (at a 1% concentration) were prepared prior to IR analysis.

2.2.3.3. X-ray Diffraction. X-ray powder diffractograms were recorded using a Siemens D500 system and a Cu-Kα radiation (40 kV, 30 mA). The scan speed was 1/2° (2θ)/min for a step size of 0.05° (2θ) and a measuring time of 6 s.

3. Results and discussion

The results of the analysis of empty nanospheres and FB nanospheres (F1–F8) are listed in Table 1, which shows the FB initial concentrations, the mean size, the polydispersity, the entrapment efficiency, the formation of nanocrystals and the amount of Poloxamer 188 remaining in the samples. Some FB (see F6–F8) precipitated as nanocrystals after all the acetone had evaporated from the aqueous phase, due to the fact that the amount of FB present in the solution was higher than its solubility in water, which is 0.03 mg/ml.

We first analyzed the morphology of the empty nanospheres and FB nanospheres by TEM. Both types of nanospheres were spherical and regular ($Q < 0.100$), which is a characteristic of a monodispersed system.

In order to further study the structure of the nanospheres and to establish the physical state of both, the polymer and drug in the nanospheres, X-ray analyses, IR spectra determination and thermal analyses were performed.

Fig. 1 shows the results of the X-ray analysis of empty nanospheres and the different components (Poloxamer 188 and PεCL). The peaks corresponding to PεCL but not those of Poloxamer

Table 2
Thermogravimetry and kinetics decomposition results

Sample	Weight (mg)	Decomposition			Weight losses (mg)	Kinetics (mg/min)
		Phase	Begin (°C)	End (°C)		
Empty nanospheres	2.730	1	60	337	0.500	0.018
		2	337	505	2.160	0.128
		Residue[a]			0 070	
FB nanospheres (F2)	3.714	1	128	343	0.530	0.025
		2	343	501	3.160	0.200
		Residue[a]			0.020	
Flurbiprofen	2.644	1	155	360	2.064	0.100
		Residue[a]			0.580	
Poloxamer 188	3.000	1	260	460	2.950	0.150
		Residue[a]			0.050	
Poli-ε-caprolactone	3.706	1	134	366	0.600	0.026
		2	366	501	3.020	0.224
		Residue[a]			0.090	

[a] Residue evaluated at 500°C.

188, were detected when empty nanospheres were analyzed. This suggests that the surfactant only acts as an adjuvant in the nanospheres formation process, stabilizing the colloidal suspension.

The amounts of Poloxamer 188 present in the supernatants obtained after ultracentrifugation of FB-nanospheres samples prepared with different amounts of drug and empty nanospheres were determined by IR. Table 1 shows that the amount of remaining surfactant varied between 0.1 and 0.199 mg/ml. This suggests that during the ultracentrifugation of the samples some water with Poloxamer 188 is retained in the pellet. After pellet desiccation to obtain samples, the surfactant remains, but not is entrapped by the polymer, it is placed over FB nanospheres. These results were confirmed by those obtained with X-ray diffraction.

The analysis of the pattern of FB nanospheres (F2, Fig. 2) showed that they consist of a combination of two crystalline structures (FB and part of the polymer), although the original structure of the polymer is semicrystalline. The association of FB to PεCL causes a slight displacement towards the high angle zone (2θ), which may be explained by the reduction in the size of FB crystalline cell when it is entrapped.

The possibility of chemical interactions between drug and polymer was analyzed by IR spectroscopy. The results showed that in FB nanospheres (F2) the main polymer was PεCL, whereas Poloxamer 188 was not detected either as a physical mixture or as a product of a chemical reaction (Fig. 3). This was in accordance with the results obtained by X-ray and IR analysis of surfactant. The IR spectra obtained of FB nanospheres are very similar, despite the fact that small differences do appear at low wavenumbers. Therefore, it is not possible to ascertain either the presence or the lack of interactions between the drug and the polymer matrix in the FB nanospheres.

The results of the thermogravimetric analysis of the samples are shown in Fig. 4 and Table 2. The curves obtained for PεCL and FB nanospheres (F2) show that the temperature of degradation/decomposition for both polymeric systems present a difference of about 2.2°C (417.2°C for PεCL, 415.0°C for FB nanospheres), possibly caused by the FB inside the nanospheres.

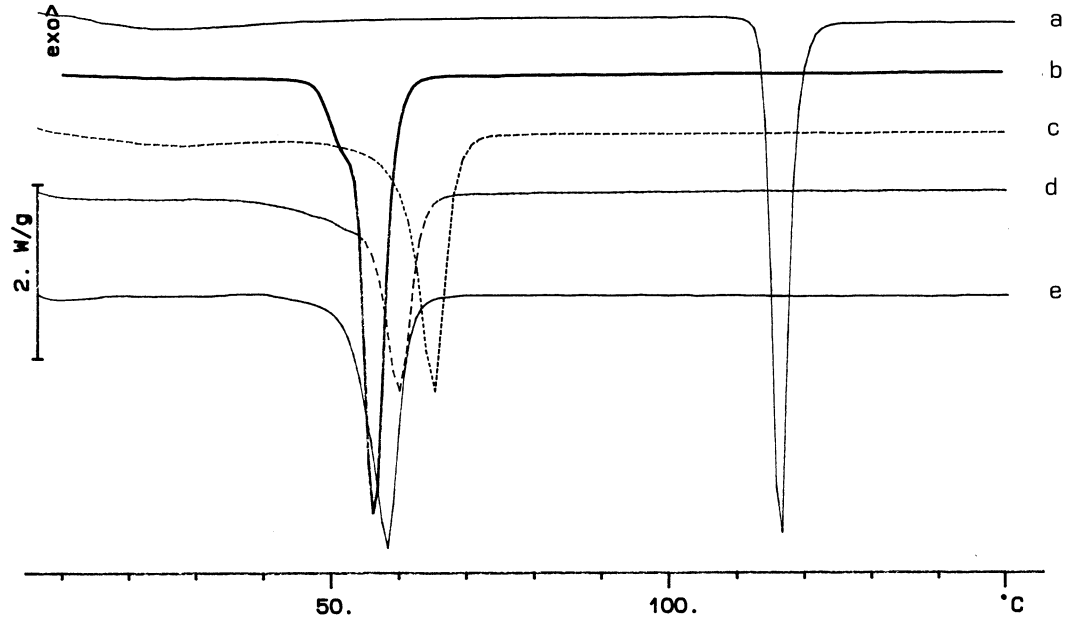

Fig. 5. DSC scans obtained with: (a) flurbiprofen; (b) Poloxamer 188; (c) poly-ε-caprolactone; (d) empty nanospheres; (e) FB nanospheres F2.

The addition of surfactant to the medium of formation of the nanospheres did not cause any significant effects when the particles are formed. This is in accordance with our results obtained with X-ray diffraction and IR spectroscopy. The loss of mass corresponding to the surfactant took place at 397°C, which is lower than that measured for PεCL (417.2°C) and for FB nanospheres (415.0°C), also the TG tracings of FB nanospheres is very close to PεCL and far from Poloxamer 188 as shows the Fig. 4. Besides, the TG tracing of empty nanospheres (not shown) displaies the second loss of mass at the same temperature (417.2°C) that PεCL and FB nanospheres (F2). The Table 2 shows differences between temperature of the first phase of decomposition for empty nanospheres (60°C) and FB nanospheres (128°C) caused by interactions between FB and PεCL (Chauvet et al., 1993). These results are according to X-ray and IR analyses, which suggest no interaction between the surfactant and the nanospheres. No weight loss was detected for any samples at the range of temperatures used in the experiment (Table 2), thus suggesting that the samples were stable between 0 and 150°C.

The DSC curves for empty nanospheres, FB, Poloxamer 188, PεCL and FB nanospheres (F2) are shown in Fig. 5. The DSC tracings of Poloxamer 188 and PεCL show a single, sharp endothermic peak that corresponds to the solid–liquid melting transition (54.1 ± 0.1°C for Poloxamer 188 and 64.5 ± 0.1°C for PεCL). The endothermic peak measured for empty nanospheres appeared at 60.8 ± 0.1°C. The enthalpy for empty nanospheres was lower than that measured both constituents (90.1 ± 0.1 J/g compared to 103.4 ± 0.1 J/g for PεCL and 124.6 ± 0.1 J/g for Poloxamer 188). The DSC curve obtained for FB also presents an endothermic event during the heat treatment ($T = 114.6 \pm 0.1$°C, $\Delta H = 106.1 \pm 0.1$ J/g) corresponding to the solid–liquid melting transition, which was also detected by thermomicroscopy. The FB nanospheres (F2) show an endothermic peak ($T = 57.2 \pm 0.1$°C, $\Delta H = 76.8 \pm 0.1$ J/g) (Table 1).

The DSC results obtained for the samples are listed in Table 3. FB nanospheres (F2) do not show any sharp thermal event corresponding to melting of FB crystals. The absence of an endotherm corresponding to the drug fusion indi-

Table 3
Enthalpy values and melting transition of FB, poly-ε-caprolactone, empty nanospheres, FB-loaded nanospheres and FB mixed with empty nanospheres, by DSC and thermomicroscopy

Sample	ΔH (J/g)		Melting transition (°C)		Thermomicroscopy
	Peak 1	Peak 2	Peak 1	Peak 2	Melting (°C)
FB		106.1 ± 0.1		114.6	114
Poly-ε-caprolactone	103.4 ± 0.1		64.5		64
Empty nanospheres	90.1 ± 0.1		60.8		61
F1	89.6 ± 0.1		60.2		63–90
F2	76.8 ± 0.1		57.2		68–95
F7	73.8 ± 0.1		58.2		75–100
F8	59.6 ± 0.1		55.2		86–105
FB mixed with empty Nanospheres (2:10)	51.8 ± 0.1	1.5 ± 0.1	58.0	114.3	98–114

cates that the drug is in the form of either a molecular dispersion or a solid solution (Ford and Timmins, 1989). These results are in accordance with those obtained by other authors (Dubernet et al., 1991) using ibuprofen-loaded ethylcellulose microspheres. Nevertheless, the single endothermic peak appeared between 40 and 65°C (Fig. 5e) which was similar to that observed for empty nanospheres (Fig. 5d). The mixture of FB and empty nanospheres (2:10) showed two peaks, one at 58.0 and another at 114.3°C (Table 3), the latter may caused by the FB in the sample. The PεCL enthalpy values decreased when FB was associated with the polymer, furthermore, this decrease correlated with the amount of FB present in the sample. In order to explain this event, we suggest that a small molecule like FB (solid-liquid melting transition = 114.6°C) when dissolved in a polymer-like PεCL (solid–liquid melting transition = 64.5 ± 0.1°C), acts as an impurity in the crystalline part of the polymer, decreasing its melting transition temperature. When the polymer was in nanospheres, the melting temperature was slightly lower (60.8°C), but after the addition of different amounts of FB (F1, F2, F7 and F8) to the nanospheres, the melting transition of PεCL falls around 55.2°C, thus confirming that the drug may act as an impurity for the polymer. In order to further study this event, we constructed the phase diagram from the results obtained by DSC and thermomicroscopy (Chiou and Riegelman, 1971) performed on the FB–PεCL solid dispersions (Fig. 6). The diagram revealed the existence of a stable invariant called 'eutectic', which is a characteristic composition of solid dispersions, for all compositions of PεCL between 3 and 97% (w/w) (excluding the formation of solid solution) with the following characteristics:

Eutectic E: FB + PεCL → eutectic liquid

Co-precipitate:	Co-melt:
$X_E = 85\%$ (w/w) of PεCL	$X_E = 85\%$ (w/w) of PεCL
$T_{fE} = 52.5 \pm 0.2$°C $= 325.0 \pm 0.2$ K	$T_{fE} = 52.5 \pm 0.2$°C $= 325.0 \pm 0.2$ K
$\Delta H_{fE} = 115.5 \pm 3.4$ J/g	$\Delta H_{fE} = 108.7 \pm 3.3$ J/g

X_E, T_{fE} and ΔH_{fE} are the experimental eutectic composition, the experimental temperature and the enthalpy of the eutectic melting respectively. The ΔH_{fE} values measured for the co-precipitates were used to construct a Tammann diagram (Tammann, 1924). Tammann diagram is constructed from the value of the melting enthalpy of the eutectic for each composition (corresponding to the first peak of the DSC tracing, the second corresponds to the melting end of the mixing). For 95%, the DSC tracing shows only a peak that corresponds to these endothermic events that are very close. For this reason the value of enthalpy of the eutectic melting can not be determined separately. The Tammann diagram for PεCL/FB

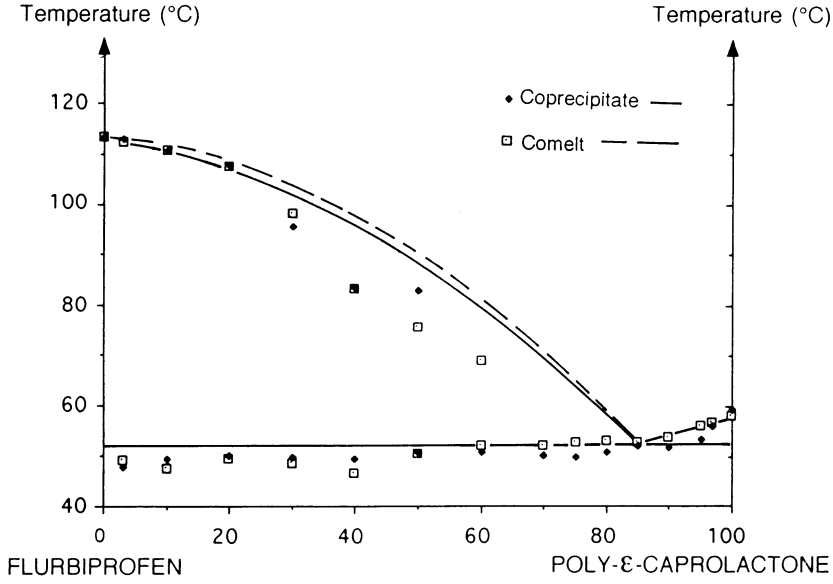

Fig. 6. Tracings of phase diagram FB–PεCL solid dispersions, co-precipitates, co-melts.

mixings gave the position of the eutectic at $X_E =$ 85% (w/w) of PεCL (Fig. 7).

Thermomicroscopy contact process revealed that when the drug and the polymer were placed side by side, a visible continuous black streak appeared at the eutectic melting temperature (Chauvet and Masse, 1983). Therefore, when the drug and mixtures of different composition were placed side by side, this technique specified the eutectic composition by the absence of this streak.

The phase diagrams for co-precipitates and co-melts were very similar (Fig. 6). Thermomicroscopy contact process and Tammann diagram are complementary, they revealed the position of the eutectic (85% w/w of PεCL), despite having a melting temperature close to that of the polymer. Nanospheres loaded with FB (F1, F2, F7 and F8) had PεCL concentrations of 93.0, 86.8, 76.8 and 62.3%, respectively that are near FB–PεCL solid dispersions containing 95, 85 and 75% of PεCL. These dispersions showed only a single endothermic peak when analyzed by DSC, such as the FB nanospheres (F1, F2, F7 and F8, Table 3), probably because their composition is near that of the eutectic composition, whereas coprecipitate containing 50% of PεCL showed two peaks (one corresponding to the melting of the eutectic and the other corresponding to the final melting of the dispersion, Lacoulonche et al., 1998). Our results suggest that the melting of PεCL in nanospheres is greatly influenced by the presence of FB, which acts as an impurity, in terms of melting temperatures and heats of melting (Llovet et al., 1995). The DSC results for solid dispersions and FB nanospheres (F1, F2, F7 and F8) are similar. The co-precipitate diagram showed that the FB–PεCL system is a molecular dispersed system with an eutectic, not a solid solution. The X-ray diffraction experiments revealed that in FB nanospheres (F2) FB and PεCL are in crystalline form, due to lack of miscibility of FB in PεCL, as shows the phase diagram (Fig. 6). The phase diagram also allowed us to determine the eutectic composition. In spite of FB is in the crystalline state in the FB nanospheres (F2) as shows the Fig. 2, the DSC tracing of these particles show only an endotherm (Fig. 5) because the composition of these nanospheres (86.8:13.2) is near to the eutectic composition (85:15). The eutectic composition is in accordance to drug/polymer proportion at which the highest entrapment efficiency was attained and no FB nanocrystals were present in the aqueous phase of colloidal suspension (Table 1).

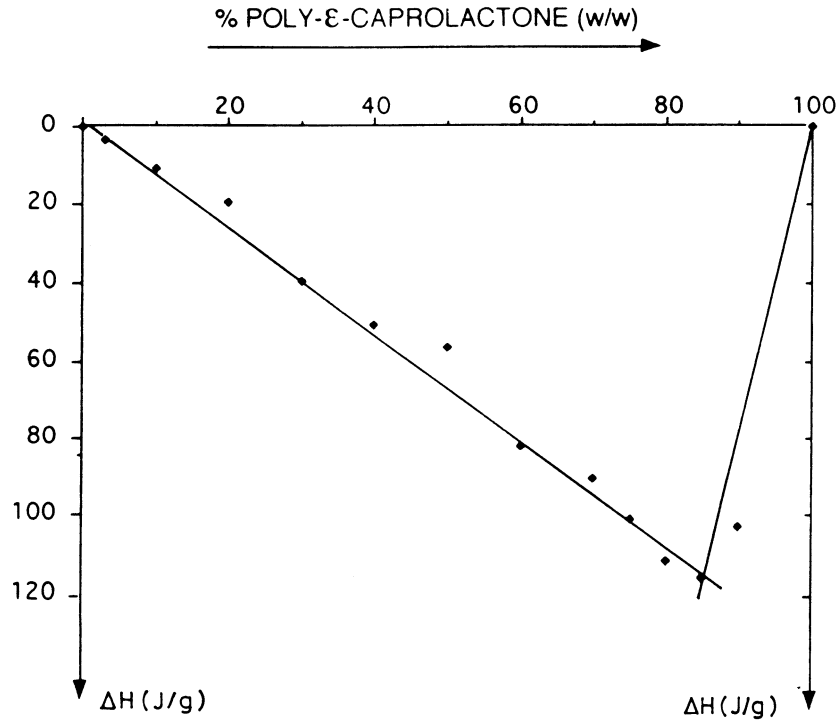

Fig. 7. Tammann diagram of FB–PεCL co-precipitates.

We also studied the effect of the initial FB concentration on the nanospheres. Our results showed that when the amount of FB is increased, a part of drug crystallizes inside the nanospheres, on their surface and even in the aqueous phase. The tendency showed by FB to spontaneously form free crystals in a wide selection of samples suggests that it has a low miscibility (0.03 mg/ml), (Benoit et al., 1986). For this reason, crystallization of drugs that dissolve easily in acetone, as is the case of FB, over PεCL nanospheres is repressed at low drug loadings due to the nature of solvent displacement method. These results are in accordance with those obtained by other authors (Benoit et al., 1986) for progesterone-loaded poly (D,L-lactide) microspheres. Therefore, when the amount of drug in the solution exceeds its solubility, some of it precipitates as nanocrystals.

Thermomicroscopy analysis confirmed the different events that take place during heat treatment of FB entrapped in the polymeric system. By using this technique, we have established that the melting temperature of the drug depends on the amount of the drug entrapped in the nanospheres (Table 3).

4. Conclusions

Results of thermoanalytical and spectral methods used in this work show that the flurbiprofen is molecularly dispersed in the polymeric matrix of nanospheres.

Construction of the FB–PεCL phase diagram revealed the existence of a stable invariant called 'eutectic' characterized by $(X_E)_{exp} = 85\%$ PεCL and by $(T_{fE})_{exp} = 52.5 \pm 0.2°C$, in which the solubility increases. This is an interesting fact for the pharmaceutical industry when choosing a carrier to drug manufacture.

In addition, the determination of eutectic composition is especially valuable, as it corresponds to the PεCL/FB proportion that presented the highest entrapment efficiency. This study can be

the first step for the design of FB ocular controlled delivery systems that prolong ocular levels and maintain appropriate clinical effects.

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