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RESEARCH PAPER

# Stability and In Vitro Drug Release of Flurbiprofen-Loaded Poly-ε-Caprolactone Nanospheres

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## ABSTRACT

*The effects of temperature and two different initial pH (2.67 and 7.00) on poly-ε-caprolactone (PεCL) nanospheres loaded with flurbiprofen (FB) (aqueous suspensions) were studied to investigate their influence on the stability and physicochemical characteristics of these drug delivery systems. The drug release behavior was also studied. Release of the associated FB occurred very fast on high dilution in a buffered medium. The stability of the polymeric system depends on the temperature and the initial pH value; it is more degradable with the particles stored at 40°C with an initial pH value of 2.67.*

***Key Words:*** *In vitro release; Nanospheres; Ocular drug delivery; Poly-ε-caprolactone; Stability; Thermal methods.*

## INTRODUCTION

Colloidal carriers have produced promising results for the enhancement of the ocular bioavailability of drugs. According to these principles, many authors have studied several types of particles for increasing the precorneal residence time of drugs and prolonging their penetration into the intraocular structures (1–4). In this way, we have prepared poly-ε-caprolactone (PεCL) loaded with flurbiprofen (FB) by the solvent displacement method (5). PεCL is a semicrystalline polymer, rather hydrophobic, with a high molecular weight (6) that may be used in diffusion-controlled delivery systems for ocular administration (7). The main mode of degradation for caprolac-

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***Table 1***

*Entrapment Efficiency (%), Mean Size Z, Polydispersity Q, and Presence of Nanocrystals of Flurbiprofen-Loaded Poly-ε-Caprolactone Nanospheres*

| Sample | Initial FB Concentration (mg/ml) | Entrapment Efficiency (%) | *Z* (nm) | *Q* | Nanocrystals |
|---|---|---|---|---|---|
| F1 | 0.25 | 98.00 | 201.2 | 0.073 | — |
| F2 | 0.50 | 94.00 | 221.4 | 0.096 | — |
| F3 | 1.00 | 76.00 | 218.0 | 0.081 | + |

tone polymers is hydrolysis. This degradation proceeds first by diffusion of water into the material (initially into the more amorphous zones), followed by random hydrolysis fragmentation of the material, and finally more extensive hydrolysis accompanied by phagocytosis, diffusion, and metabolism. The hydrolysis is affected by the size, hydrophilicity, and crystallinity of the polymer and the pH and temperature of the environment (8). FB is a slightly water-soluble drug that could be useful for ocular applications.

The aim of this paper was to study the in vitro release profiles of FB from PεCL nanospheres (PεCLN) (aqueous suspensions) and the stability of these colloidal systems during 6-month storage at 4°C, 25°, and 40°C. The effect of pH evolution was also studied for two different initial pH values, 2.67 and 7.00.

## EXPERIMENTAL

### Materials

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

### Methods

#### Preparation of Nanospheres

FB-loaded PεCLN were prepared by precipitation using the solvent displacement method (5). Briefly, 66 mg of PεCL and different amounts of FB (ranging between 5 and 20 mg) were dissolved in 20 ml of acetone (samples F1–F3, respectively). This solution, the 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 obtain a final volume of 20 ml.

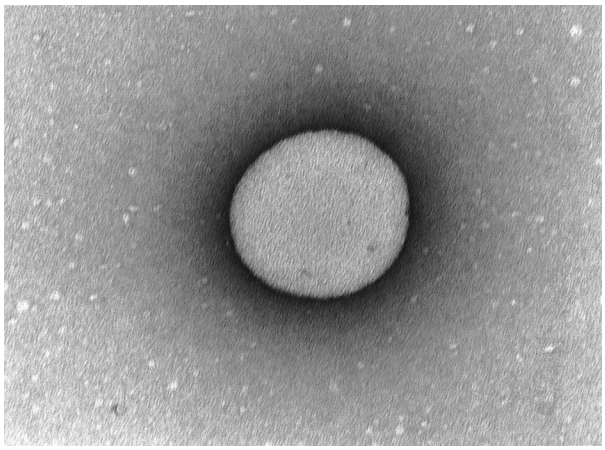
#### Nanosphere Characterization

Nanosphere particle size distribution was determined by photon correlation spectroscopy (PCS) in a Malvern Autosizer IIC (Malvern Instruments, Malvern, UK) (9). Morphological examination of entire nanoparticles was performed using a Philips 1011 transmission electron microscope (TEM) following negative staining with phosphotungstic acid solution (0.5%) (10).

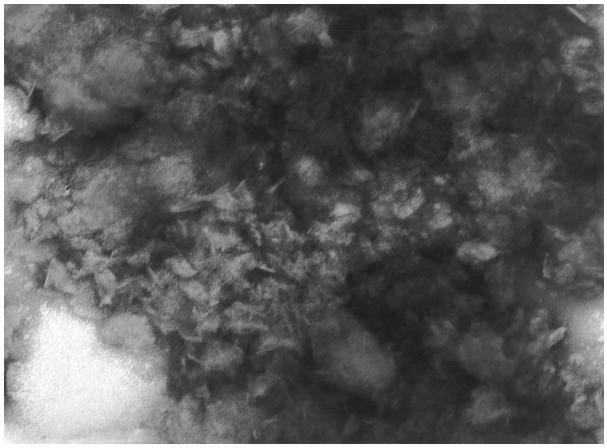
The FB content in the FB nanospheres was determined by fluorescence spectroscopy ($\lambda_{ex}$ = 248 nm, $\lambda_{em}$ = 312 nm). The samples were first subjected to ultracentrifugation at 40,000 rpm (Centrikon T-1170, Kontron) for 2 hr to separate the two phases of the colloidal system. The content of drug in the nanospheres was calculated by determining the difference between the total drug present in the colloidal suspension, after the dissolution of samples in dimethylformamide, and the free amounts of the drug in the aqueous phase. Association efficiency was expressed as the percentage of drug in relation to drug initially dissolved in the organic phase.

#### Thermal Analysis

Differential scanning calorimetry (DSC) analyses were carried out on FB nanospheres (F2). In this way, the samples (2.65–3.00 mg) were weighed (Mettler M3 Microbalance) directly in perforated aluminum pans and scanned between 0°C and 150°C at a heating rate of 10°C/min under nitrogen using a Mettler TA 4000 system fitted with a DSC 25 cell. Temperature was calibrated by the melting transition point of indium. The melting temperatures are onset temperatures (temperature at the equilibrium between phases), peak temperatures (temperature at the top of the peak), and endset temperatures. Enthalpies were determined for every sample.



**(a)**



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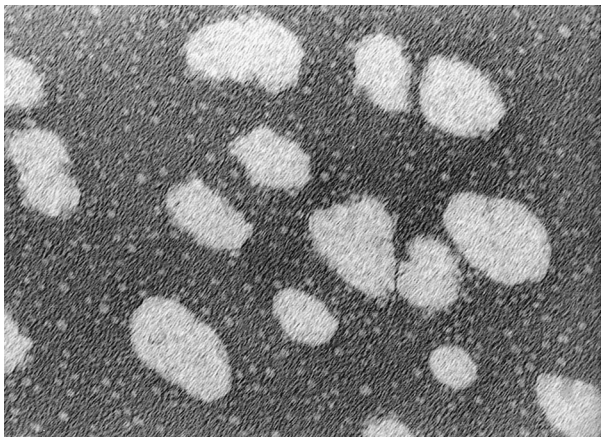

**(c)**

**Figure 1.** Transmission electron micrographs of FB nanospheres: (a) $t = 0$ days, magnification ×20,000; (b) $t = 180$ days, magnification ×20,000 (pH = 2.67); (c) $t = 180$ days, magnification ×10,000 (pH = 7.00).

To eliminate the water present in the samples, they were washed three times in deionized water, ultracentrifuged, and dried to constant weight in a desiccator.

### Stability Studies

The stability studies were performed on FB nanospheres (F2), aqueous suspensions, during 6-months storage at 4°C, 25°C, and 40°C. The initial pH of particles was 2.67, or it was adjusted to pH 7.00 because the pH of tears (7.00–7.40) is very close to that of blood. If the pH of an ophthalmic solution varies considerably from neutrality (i.e., more than 1–2 pH units), patients may complain of discomfort. Tears have relatively good buffering capacity and thus tend to neutralize the pH of any added solution. If a solution is very acidic, basic, or highly buffered, the tears then will not be able to neutralize this solution, which then will cause discomfort or, in extreme conditions, ocular damage (11).

The integrity of the FB nanospheres was evaluated by the study of morphology and particle sizes of the carriers. The pH and tonicity of the suspensions and thermal behavior were also analyzed to evaluate the polymer degradation. The suspensions were examined with respect to aggregation by phase contrast microscopy (PCM). The pH values of the colloidal suspensions were measured at room temperature with a Crison pH meter (model 2002).

### In Vitro Drug Release Studies

In vitro release experiments were performed using a bulk-equilibrium reverse dialysis technique (12). The release medium was phosphate buffer, pH 7.43, at 32°C (tear film temperature). The FB-loaded nanospheres were placed directly into 400 ml of a stirred phosphate buffered saline (PBS) solution, in which numerous dialysis sacks containing 1 ml of the same PBS solution were previously immersed, to ensure the maintenance of sink conditions during the overall release process. The amount of FB released was determined periodically by fluorescence spectroscopy ($\lambda_{ex} = 248$ nm, $\lambda_{em} = 312$ nm). Every kinetic experiment was performed in triplicate.

## RESULTS AND DISCUSSION

The results of the physical analysis of FB nanospheres (F1–F3) are listed in Table 1, which shows the FB initial concentrations, the morphometrical properties (mean size and polydispersity), the entrapment efficiency, and the formation of nanocrystals. Some FB (see F3) precipitated as nanocrystals after all the acetone had evaporated from

the aqueous phase during the manufacture of the nanospheres due to the fact that the amount of FB present in the solution was greater than its solubility in water (0.03 mg/ml).

The morphology of the FB nanospheres was analyzed by TEM. The nanospheres were spherical and regular ($Q = 0.100$), which is a characteristic of a monodispersed system (Table 1). The aspect of FB nanospheres (initial pH value 2.67) at the end of the stability study was considerably modified, as can be seen in Fig. 1b. When temperature increases, the FB nanospheres lose their spherical initial form (Fig. 1a), and it is not possible to recognize the form of the particles stored 180 days at 40°C. These changes in the morphology probably can be due to a decrease of molecular weight of the polymer. This decrease was observed by other authors by gel permeation chromatography techniques (8,13). The particles, with an initial pH of 7, showed a more extend form (Fig. 1c) at the end of this study and at the higher temperature.

The particles stored at 40°C, which had an initial pH value of 2.67, showed aggregation and degradation. For this pH value, the aggregation appeared in all samples after 60 days and for all temperatures tested (Table 2). Indeed, the aggregation on heating is directly related to the precipitation and/or phase separation of the surfactant at a temperature above its cloud point, at which point this molecule is likely to dissociate from the particle. The unprotected vector can aggregate in clusters. On cooling, the surfactant redissolves in the solution and coats the aggregate particles, preventing them from dissociating into smaller ones (14). Better physical stability was obtained for FB nanospheres with an initial pH of 7.00 (Table 3). In this case, the aggregation did not appear in any sample.

The storage temperature and pH affected the stability of the colloidal suspension. In fact, according to results obtained by Coffin and McGinity (13), the PεCL was in the rubbery state between 4°C and 40°C. In this state, a polymer is at a higher free energy level than in its glassy state and thus is much more reactive; moreover, in the rubber state when temperature increases, the degradation is faster. In our case, the 25°C and 40°C storage temperatures caused instability in the samples, while the 4°C tem-

***Table 2***

*Influence of Temperature and Storage on the Polydispersity* Q*, Entrapment Efficiency, Osmolarity, and Aggregation of FB-PεCL Nanospheres (Initial pH 2.67)*

| Time (Days) | Temperature (°C) | *Q* | Osmolarity (mOsm) | Drug Content (%) | Aggregation |
|---|---|---|---|---|---|
| | 4 | 0.099 | 23.5 | 97.22 | — |
| 0 | 25 | 0.099 | 23.5 | 97.22 | — |
| | 40 | 0.099 | 23.5 | 97.22 | — |
| | 4 | 0.060 | 23.0 | 96.35 | — |
| 30 | 25 | 0.167 | 23.0 | 96.35 | — |
| | 40 | 0.904 | 24.0 | 94.29 | + |
| | 4 | 0.073 | 22.0 | 97.01 | + |
| 60 | 25 | 0.100 | 22.0 | 97.25 | + |
| | 40 | 1.000 | 22.0 | 94.25 | ++ |
| | 4 | 0.124 | 29.5 | 96.07 | + |
| 90 | 25 | 0.100 | 26.5 | 95.61 | + |
| | 40 | 1.000 | 36.0 | 94.14 | +++ |
| | 4 | 0.309 | 22.0 | 95.93 | + |
| 120 | 25 | 0.135 | 29.0 | 95.17 | + |
| | 40 | 1.000 | 46.0 | 95.94 | ++++ |
| | 4 | 0.340 | 26.0 | 95.48 | + |
| 150 | 25 | 0.171 | 24.0 | 95.84 | + |
| | 40 | 1.000 | 48.0 | 94.23 | ++++ |
| | 4 | 0.355 | 24.0 | 92.22 | + |
| 180 | 25 | 0.174 | 26.0 | 93.60 | ++ |
| | 40 | 1.000 | — | 89.26 | ++++ |

***Table 3***

*Influence of Temperature and Storage on the Polydispersity* Q*, Entrapment Efficiency, Osmolarity, and Aggregation of FB-PεCL Nanospheres (Initial pH 7)*

| Time (Days) | Temperature (°C) | *Q* | Osmolarity (mOsm) | Drug Content (%) | Aggregation |
|---|---|---|---|---|---|
| | 4 | 0.084 | 55.0 | 98.65 | — |
| 0 | 25 | 0.084 | 55.0 | 98.65 | — |
| | 40 | 0.084 | 55.0 | 98.65 | — |
| | 4 | 0.064 | 41.0 | 40.44 | — |
| 30 | 25 | 0.066 | 50.0 | 39.46 | — |
| | 40 | 0.109 | 65.0 | 77.33 | — |
| | 4 | 0.055 | 40.0 | 29.48 | — |
| 60 | 25 | 0.074 | 46.0 | 13.60 | — |
| | 40 | 0.117 | 60.0 | 45.00 | — |
| | 4 | 0.055 | 44.0 | 24.37 | — |
| 90 | 25 | 0.062 | 48.0 | 7.12 | — |
| | 40 | 0.133 | 62.0 | 34.13 | — |
| | 4 | 0.066 | 38.0 | 20.92 | — |
| 120 | 25 | 0.081 | 40.0 | 4.06 | — |
| | 40 | 0.122 | 64.0 | 34.84 | — |
| | 4 | 0.060 | 36.0 | 16.60 | — |
| 150 | 25 | 0.098 | 32.0 | 3.65 | — |
| | 40 | 0.212 | 60.0 | 33.03 | — |
| | 4 | 0.075 | 32.0 | 10.55 | — |
| 180 | 25 | 0.082 | 33.0 | 2.57 | — |
| | 40 | 0.290 | 66.0 | 32.90 | — |

perature produced effects less significant. Figure 2 shows the pH evolution observed during the study. When the initial pH is 2.67, there are no significant modifications in the evolution of pH values during storage. Nevertheless, when the initial pH is 7.00, a progressive decrease in pH values is observed with time. This decrease is more important for solutions stored at 25°C and 40°C.

These results are in accordance with those obtained by other authors, who explain that the degradation of polyester in aqueous media generally induces an acidification of the medium (8). Another possible explanation of the decrease in the pH is the production of free ε-hydroxycaproic acid as a result of PεCL degradation (15). An increase of tonicity appeared in the samples stored at 40°C for two initial pH values studied, which could be correlated with the acidification (Tables 2 and 3). This phenomenon could be related to the oligomers released by caprolactone hydrolysis (8).

The study of samples by DSC has shown that FB lowers the solid-liquid melting temperature of PεCL (64.5°C ± 0.1°C). A single peak characterizes the melting temperature of the nanospheres because the composition of the nanospheres is near the eutectic composition (16). In our study, this temperature decreases in every sample, for each initial pH value, and for every temperature tested (4°C, 25°C, and 40°C) (Tables 4 and 5). This decrease is more significant for the samples stored at 25°C (53.4°C ± 0.1°C) and 40°C (44.1°C ± 0.1°C) for an initial pH of 2.67. From 90 days of storage at 40°C, Tables 4 and 5 show shoulders and double peaks in DSC tracings at initial pH 2.67 and 7.00. Figures 3 and 4 show this in DSC tracings for 180 days of storage.

This can be explained by the degradation of the polymer, which begins in the amorphous region. PεCL is a semicrystalline polymer: It contains amorphous and crystalline regions. Its metastability depends on reorganization, recrystallization, and annealing, which are processes for relaxation of a crystal that is not in equilibrium (17). A special case of polymer reorganization occurs because of the presence of a metastable strained amorphous melt. In this case, the Gibbs energy is increased because of a decrease in entropy that results from a conformational change on extension of the flexible macromolecule (rotation about the backbone bond of the flexible molecule). Strains can be maintained when entanglements or chemical crossing links keep molecules from relaxing. The

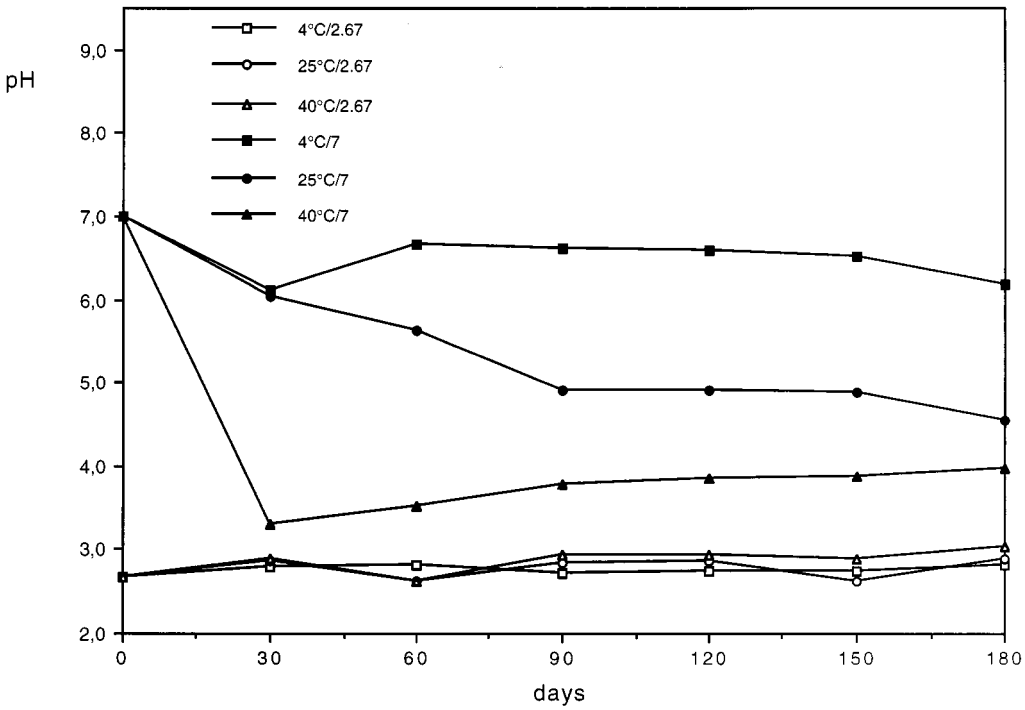


**Figure 2.** Evolution of pH of FB nanospheres during storage at different temperatures (4°C, 25°C, and 40°C).

ideal entropic elastic behavior of the polymer gives an explanation for the decrease observed in melting point when strain is relaxed by breaking of the tie molecules (macromolecules that participate in more than one crystal; the intermediate part of the molecule that acts as the tie is not crystalline). Furthermore, a recrystallization of the tie segments is possible and can explain the presence of the first peak observed in DSC (Figs. 3 and 4) at 40°C; the melting temperature of the tie segments is lower than of the nanospheres. The degradation proceeds faster in the amorphous regions, which thus results in an increase of the crystallinity level during storage.

In spite of the modifications of FB nanosphere characteristics, only significant variations of nanosphere diameter were observed for particles stored at 40°C and from 60 days of storage with an initial pH value of 2.67. In the rest of samples, the nanosphere diameter remained without significant changes throughout this experiment (Fig. 5).

The effect of storage temperature and initial pH value on FB content is shown in Tables 2 and 3. A clear decrease of FB entrapment could be noted in the particles stored at 4°C and 25°C, which had initial pH values of 7. This decrease was minor for FB nanospheres stored at 40°C for the same initial pH value. This behavior can be explained since a decrease in the pH value diminished the FB solubility. For this reason, the drug diffuses with major difficulty from the polymeric system when the pH value of the colloidal suspensions was less than the $p_K$ of the drug ($p_K$ = 4.3). Indeed, it is known that ionized molecules are distributed in the nonaqueous phases to a lesser extent than homologous neutral forms (18). In this way, the FB nanospheres with an initial pH value of 2.67 stored at 4°C, 25°C, and 40°C retained almost 90% of their FB content even after 180 days of storage in spite of the progressive hydrolytic degradation the polymer undergoes.

The release behavior of pure FB and FB from PεCL nanospheres is illustrated in Fig. 6, when the percentage release was plotted against time. As can be seen in the graph, all the formulations tested show a biphasic pattern: one initial fast-release phase (burst effect), followed by a second slow-release phase (extended release).

The burst effect or initial release, described by many authors, is often sufficiently rapid to suggest its identification as a distinct physical process (19). This effect could be attributed to drug diffusion from near the surface (20) or to the rapid leaching of the drug through pores

***Table 4***

*Influence of Temperature and Storage on the Heat of Fusion of FB-PεCL Nanospheres (pH 2.67)*

| Time (Days) | Temperature (°C) | ΔH (J/g) | | Peak (°C) | | Onset (°C) | | Endset (°C) | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| | 4 | 77.8 | — | 58.1 | — | 50.1 | — | 62.3 | — |
| 0 | 25 | 77.8 | — | 58.1 | — | 50.1 | — | 62.3 | — |
| | 40 | 77.8 | — | 58.1 | — | 50.1 | — | 62.3 | — |
| | 4 | 73.3 | — | 56.3 | — | 51.4 | — | 59.9 | — |
| 30 | 25 | 90.0 | — | 55.5 | — | 50.1 | — | 59.7 | — |
| | 40 | 103.5 | — | 54.8 | — | 47.9 | — | 60.7 | — |
| | 4 | 88.1 | — | 56.3 | — | 50.7 | — | 60.4 | — |
| 60 | 25 | 93.5 | — | 56.0 | — | 48.4 | — | 59.9 | — |
| | 40 | 98.1 | — | 52.2 | — | 44.5 | — | 56.0 | — |
| | 4 | 87.7 | — | 56.1 | — | 49.0 | — | 60.6 | — |
| 90 | 25 | 95.2 | — | 55.2 | — | 48.5 | — | 60.0 | — |
| | 40 | 80.7 | Shoulder | 49.0 | — | 44.4 | — | 52.6 | — |
| | 4 | 80.3 | — | 56.0 | — | 49.3 | — | 60.0 | — |
| 120 | 25 | 93.2 | — | 54.5 | — | 47.7 | — | 59.1 | — |
| | 40 | 73.6 | Shoulder | 47.3 | — | 43.3 | — | 51.5 | — |
| | 4 | 86.4 | — | 56.0 | — | 50.0 | — | 60.0 | — |
| 150 | 25 | 94.5 | — | 54.6 | — | 47.3 | — | 59.1 | — |
| | 40 | 42.7 | 57.1 | 44.7 | 57.1 | 38.5 | 52.5 | 48.5 | 60.6 |
| | 4 | 85.6 | — | 55.2 | — | 48.6 | — | 59.4 | — |
| 180 | 25 | 89.6 | — | 53.4 | — | 45.7 | — | 57.9 | — |
| | 40 | 36.1 | 24.4 | 44.1 | 57.2 | 35.8 | 52.5 | 47.6 | 60.6 |

and channels. In this sense, as has been reported, PεCL, because of its low glass transition temperature ($T_g$ = −60°C), is highly permeable to low molecular weight drugs (<400 D) (15) such as FB.

In our case, the drug is placed in a network of polymer matrix because the particles are porous, or in other cases, a part of the FB is placed on the surface of nanospheres as nanocrystals or free in the colloidal suspensions, such as the formulation containing an initial FB concentration of 1 mg/ml (F3). Nevertheless, in both cases the particles are easily accessible to dialysis medium. The drug release in the second slow phase could be produced by diffusion from the interior of the nanospheres. This release mechanism was also corroborated by the fact that the main factor controlling the release of FB is the volume of aqueous medium: FB releases fast and completely from the carriers on high dilution (sink conditions). The spite release of FB from the polymeric system was less than that of free drug for the same initial concentration of FB (Fig. 6). Higher drug loading showed slow release, but as the drug loading to polymer decreased, a rapid burst effect was produced. The results obtained show that the initial release rate was faster when the initial concentration of FB in the medium was minor, and the drug release was small.

The low-loaded FB nanospheres exhibited a complete release that was more rapid than high-loaded FB nanospheres (Fig. 6). The incomplete release for high-loaded FB nanospheres should be due to formation of FB crystalline domains in the nanospheres. Similar results were reported by Benita, Barkai, and Pathak for nifedipine-loaded polyacrylate microspheres (21). On the other hand, for low-loading FB nanospheres, the molecules of drug are dispersed in the nanospheres, and they are thus separated from adjacent FB molecules by the PεCL polymer molecules. The intermolecular forces of attraction between the drug and PεCL polymer molecules are probably less strong than the forces of attraction between adjacent FB molecules and can then be overcome more easily, resulting in rapid release rates, as observed in Figure 6. This behavior is in accordance with previous results obtained by infrared assays.

To consider the possible degradation of polymer in the drug release studies performed by the authors, studies carried out by Pitt have shown that no significant degra-

***Table 5***

*Influence of Temperature and Storage on the Heat of Fusion of FB-PεCL Nanospheres (pH 7.00)*

| Time (Days) | Temperature (°C) | $\Delta H$ (J/g) 1 | $\Delta H$ (J/g) 2 | Peak (°C) | Onset (°C) | Endset (°C) |
|---|---|---|---|---|---|---|
| | 4 | 82.4 | — | 58.7 | 55.8 | 62.4 |
| 0 | 25 | 82.4 | — | 58.7 | 55.8 | 62.4 |
| | 40 | 82.4 | — | 58.7 | 55.8 | 62.4 |
| | 4 | 86.8 | — | 57.1 | 52.5 | 60.8 |
| 30 | 25 | 91.0 | — | 57.6 | 52.5 | 61.9 |
| | 40 | 91.1 | — | 52.7 | 45.8 | 56.8 |
| | 4 | 85.4 | — | 58.8 | 54.2 | 63.1 |
| 60 | 25 | 89.4 | — | 56.3 | 51.3 | 60.6 |
| | 40 | 86.3 | — | 49.2 | 42.8 | 53.3 |
| | 4 | 88.8 | — | 58.5 | 53.8 | 63.1 |
| 90 | 25 | 92.5 | — | 54.5 | 48.3 | 58.5 |
| | 40 | 83.8 | — | 48.3 | 43.9 | 53.0 |
| | 4 | 106.6 | — | 56.6 | 51.6 | 60.1 |
| 120 | 25 | 129.6 | — | 58.0 | 52.6 | 62.0 |
| | 40 | 114.8 | Shoulder | 47.7 | 41.9 | 51.9 |
| | 4 | 108.1 | — | 57.8 | 53.6 | 61.6 |
| 150 | 25 | 107.8 | — | 55.2 | 49.5 | 59.7 |
| | 40 | 77.7 | Shoulder | 45.3 | 40.1 | 49.0 |
| | 4 | 102.6 | — | 57.9 | 53.5 | 61.5 |
| 180 | 25 | 94.7 | — | 56.1 | 48.8 | 59.9 |
| | 40 | 68.5 | Peak | 44.3 | 38.6 | 47.7 |

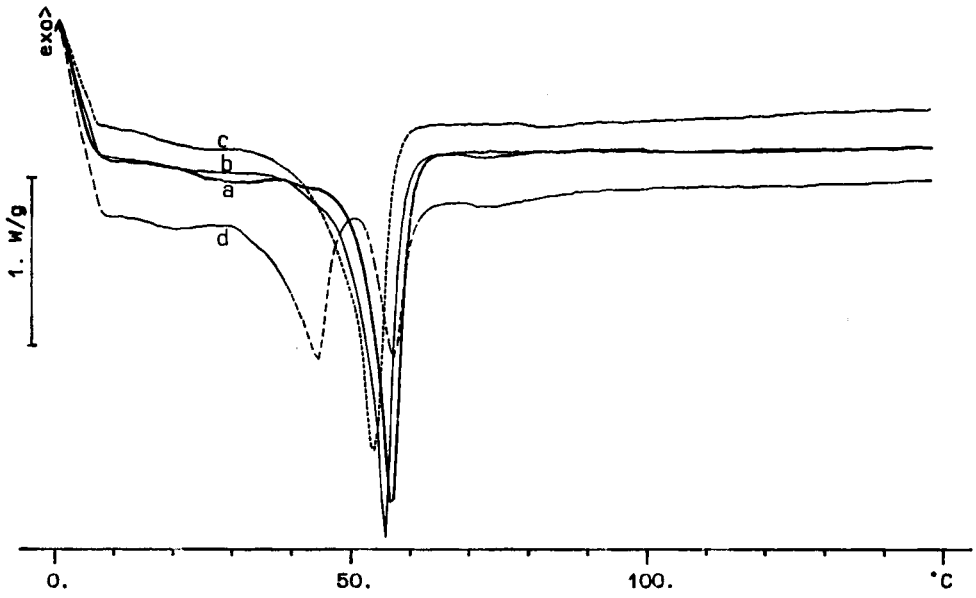


**Figure 3.** DSC tracings of FB nanospheres: (a) $t = 0$ days; (b) $t = 180$ days (4°C, pH 2.67); (c) $t = 180$ days (25°C, pH 2.67); (d) $t = 180$ days (40°C, pH 2.67).

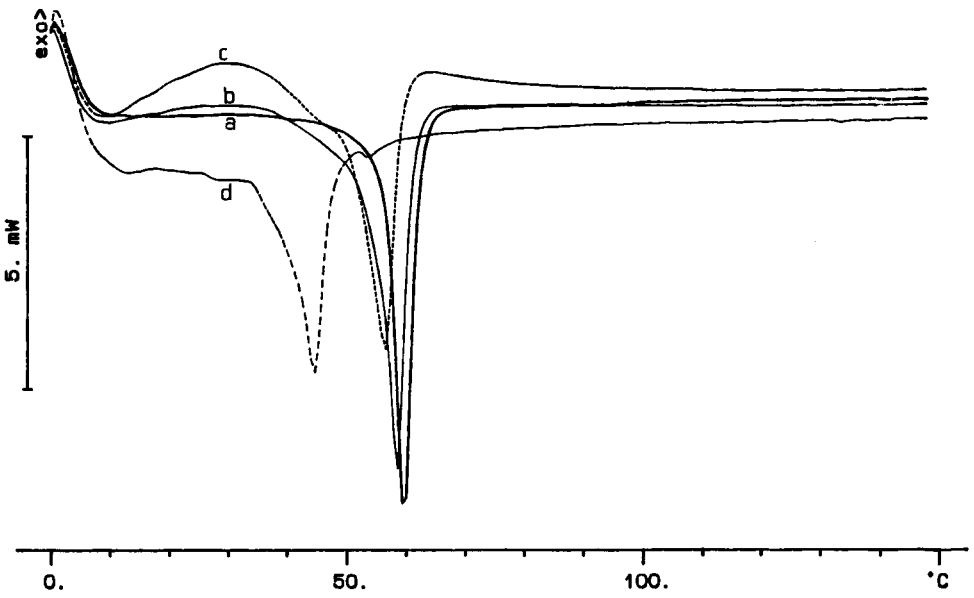


**Figure 4.** DSC tracings of FB nanospheres: (a) $t = 0$ days; (b) $t = 180$ days (4°C, pH 7.00); (c) $t = 180$ days (25°C, pH 7.00); (d) $t = 180$ days (40°C, pH 7.00).

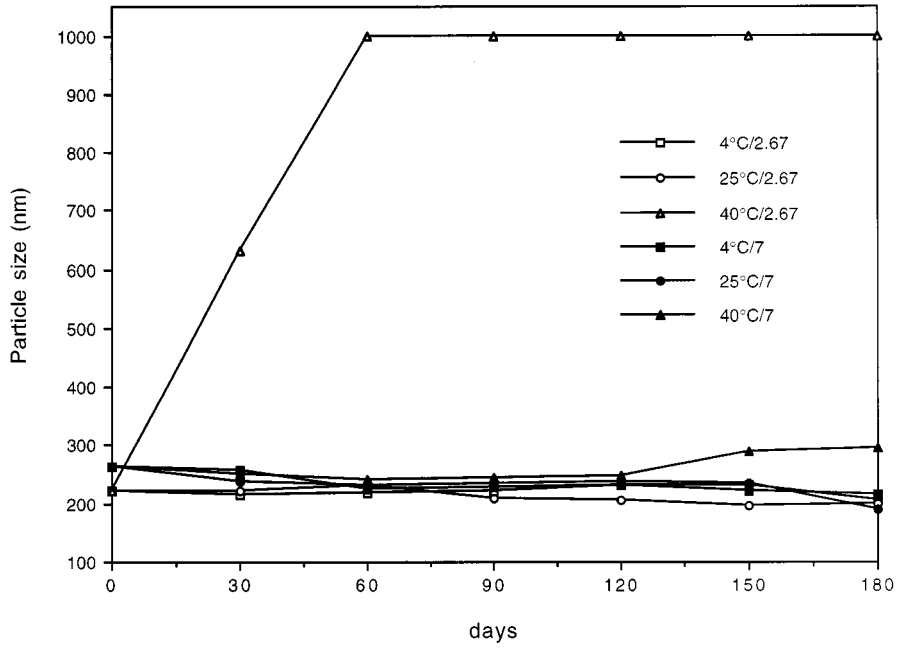


**Figure 5.** Evolution of size average of FB nanospheres at two different initial pH values during storage at different temperatures (4°C, 25°C, and 40°C).

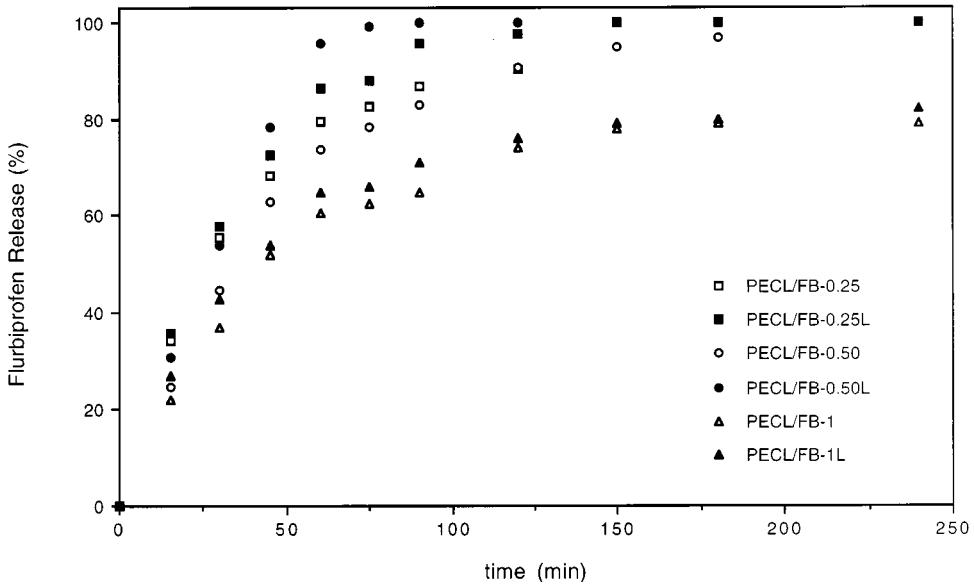


**Figure 6.** Drug release profiles of free FB (0.25, 0.50, and 1.00 mg/ml) and FB nanospheres (0.25, 0.50, and 1.00 mg/ml).

dation of PεCL in PBS, as measured by weight loss at 37°C, occurred within 12 months (15). For this reason, the observed drug release could not be explained by the erosion of the polymer matrix.

## CONCLUSIONS

Results obtained in this study show that these colloidal systems easily released their drug content completely on dilution when the initial concentration of FB was 0.25 and 0.5 mg/ml. However, for an initial concentration of 1 mg/ml, the release was incomplete (80%) in the same period of time. The particles were stable during storage for several months at 4°C and 25°C for two initial pH values studied, but results of our study showed significant degradation of particles at 40°C dependent on the initial pH of the solution. The DSC results confirm the degradation of nanospheres during the storage at 25°C and 40°C. The ternary phase diagram should be constructed (PεCL oligomers, PεCL polymers, and FB) to specify the shoulders and double peaks observed in DSC tracings. In conclusion, the results of this study emphasize the possible potential of PεCL nanospheres as a new ocular drug delivery system.

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