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Stability of poly (ε caprolacton) nanospheres in sterile aqueous media

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Abstract

Polyester nanoparticles have shown promising results in the ophthalmic area for optimization of the bioavailability of drugs. However, polyesters can undergo hydrolysis during storage in aqueous media and their degradation can be enhanced by micro-organisms. Poly (ε caprolacton) (PEC) nanospheres were formulated for an ophthalmic application, i.e. sterile, isotonic and containing a preservative. Their physical and chemical stabilities were studied during a 6-month storage at 25 and 40°C. The influence of temperature, surfactant, viscosifiant addition, and buffered or non-buffered pH was evaluated. The physical stability of nanospheres was followed by the study of visual appearance and mean particle size. The pH and tonicity of the suspensions, and the molecular weight and crystallinity of PEC were additionally analyzed to evaluate their chemical stability. The influence of surfactants was observed only on the nanosphere physical stability. Better results were obtained with Pluronic F127. The degradation of PEC was especially affected by pH and temperature. At 25°C and pH 7, the variation of polymer molecular weight was negligible. At 40°C, the hydrolysis rate increased with a loss of 50% of the initial molecular weight after 6 months in pH 7 buffer, and 60% in non-buffered pH 7 medium. Finally, the mean particle size of all nanospheres remained unchanged throughout this study.

Keywords: Poly (ε caprolacton); Nanospheres; Stability; Polymer degradation

1. Introduction:

In the past decade, several authors have studied colloidal carriers in order to enhance the ocular bioavailability of drugs. Pilocarpine (Harmia et al., 1986; Diepold et al., 1989; Vyas et al., 1992;

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Zimmer et al., 1994), amikacin sulphate (Losa et al., 1991a), indomethacin (Masson et al., 1992), betaxolol and carteolol (Marchal Heussler, 1991) associated with polymeric nanospheres or nanocapsules have shown promising results.

Only a few publications focused on the physical or chemical stability of this particulate dosage form during storage in aqueous media. Most of them refer to polyalkylcyanoacrylate nanoparticles (Al Khouri et al., 1986; Müller et al., 1990; Losa et al., 1991b; Valero, 1993), polystyrene nanoparticles (O'Mullane et al., 1990) and to polylactide, polyglycolide and copolymers as nanospheres (Gurny et al., 1981; Coffin and McGinity, 1992a,b; Wallis and Müller, 1993; Leray et al., 1994; Niwa et al., 1994; Belbella, 1995) or nanocapsules (Guterres et al., 1995).

The stability of poly (ε caprolacton) (PEC) in submicronic carriers has been studied by only a few authors. Losa et al. (1991b) have prepared some PEC nanocapsules, charged with metipranolol, and studied the evolution of the polymer's molecular weight during the in vitro liberation of the drug. More recently, Pilar Calvo et al. (1991) discussed the interaction of PEC nanospheres or nanocapsules with lysozyme, which is a major component in the lachrymal fluid. Finally the most complete study was carried out by Coffin and McGinity (1992a,b). These authors studied the chemical and physical stability of PEC pseudolatexes prepared with different surface active agents. The influence of surfactants, temperature, pH and mean particle diameter was evaluated and results compared with those obtained from poly (D,L lactide) nanospheres, stored in the same conditions.

All these studies were undertaken in non-sterile media. However, it has been reported that PEC can undergo microbial and fungal degradation (Potts and Clendinning, 1973; Fields et al., 1974; Benedict, 1983a,b; Jarrett et al., 1983; Tokiwa et al., 1990). Furthermore, any formulation instilled in the eye has to be sterile.

The purpose of our study was to evaluate the physical and chemical stability of PEC nanospheres, in conditions required for an ophthalmic administration, i.e. sterile, isotonic and containing a preservative.

The influence of several factors like pH, temperature, buffer, preservative and viscosifiant was measured in order to determine the best stability conditions.

Nanospheres were studied with regard to polymer degradation and physical stability of the suspensions. As excipients may themselves alter the different parameters measured, we prepared some formulations containing all the nanosphere constituents except the polymer, and studied their stability in the same conditions.

2. Materials and methods

2.1. Materials

PEC was purchased from Aldrich Chimie SARL (Strasbourg, France). Polyoxyethylene–polyoxypropylene block copolymers: synperonic PE/F68 (PF68) and PE/F127 (PF127) were provided by ICI (Verfilco, Fontenay-sous-Bois, France), and Polyoxyethylene (40) hydrogenated castor oil (Cremophor RH40) (CRH40) by BASF Corporation (Levallois-Perret, France). Hydroxyethylcellulose (Natrosol 250XH Pharm) (HEC) was obtained from Aqualon (Rueil-Malmaison, France). Thimerosal was purchased from Sigma Chimie (St. Quentin Fallavier, France), and glucose monohydrate from Prolabo (Vaulx-en-Velin, France). All other reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of nanospheres

Nanospheres were prepared according to the method developed by Fessi et al. (1991, 1992). The final concentrations of polymer and surfactant were 1.56% (w/w) and 2.5% (w/w), respectively. Each batch, corresponding to one polymer/surfactant association, was divided into two aliquots. The first one was used for the preparation of pH 4, pH 5, pH 6, or pH 7 unbuffered samples and the second one for pH 6 or pH 7 buffered suspensions. Isotonicity was obtained after addition of glucose in adequate quantities. 0.3% of HEC was added in some for-

mulations in order to prevent the sedimentation of particles. Thimerosal (0.01%) was introduced as preservative, except in some pH 4 samples, as this product may precipitate in acidic conditions. Phosphate buffer, HCl, or NaOH were then added as concentrated solutions. Finally the volume was adjusted to obtain a polymer concentration of 1.25% (w/v).

Some control solutions were prepared according to the same formulation as the nanospheres, but omitting the polymer. These formulations were prepared using PF68, PF127, or CRH40. The preparation, sterilization, repartition and conservation processes were the same as for nanospheres.

2.2.2. Sterilization

Sterilization of nanospheres was carried out by sterile filtration according to the process previously presented elsewhere (Masson et al., submitted). In the case of formulations without HEC, a prefiltration was carried out using successively a 2-μm, 1-μm and 0.45-μm filter. Then, a filtration was carried out in a sterile area on a 0.20-μm filter (Minisart NML, Sartorius, Palaiseau, France). This process could not be used for viscosified formulations as a clogging of the filter appeared rapidly in the prefiltration step. The following method was thus used: prefiltration on 2-μm, 1-μm, 0.8-μm and 0.45-μm filters and final filtration in a sterile area on a 0.45-μm membrane (Minisart, Sartorius).

Each nanosphere or control solution formulation was sterilized and then placed in 5-ml brown glass vials in a laminar air flow workstation. Vials were sealed with a rubber stopper and an aluminium cap. One sample of each batch was removed for controls at $T = 0$. All the other vials were then stored at 25 or 40°C.

2.2.3. Physico-chemical evaluation of nanospheres and control solutions

The nanosphere integrity was evaluated by the study of visual appearance and particle sizes of the carriers. The pH and tonicity of the suspensions, and the molecular weight and crystallinity of PEC, were additionally analyzed to evaluate the polymer degradation. In the case of control solutions, only visual aspect, pH and tonicity were studied and compared to the results previously obtained for the carriers.

The suspensions were examined with respect to sedimentation, flocculation, aggregation and colour. The mean particle diameters and size distributions were determined by photon correlation spectroscopy using a nanosizer N4MD (Coultronics, France). The pH was measured using a Mettler delta 320 pHmeter. Osmolarity was evaluated by a freeze point measurement using a Fiske MS TM cryoscope (Radiometer Tacussel SA, Neuilly-Plaisance, France). No measure was performed on samples containing HEC as their viscosity was too high to allow any analysis. All these controls were carried out on every formulation at $T = 0$ and after 1, 2, 4, 5 and 6 months of storage.

The molecular weight of PEC was determined by gel permeation chromatography (GPC), using a Waters Associates chromatography system fitted with two Ultrastyragel columns of 10^4 and 500 Å. Tetrahydrofuran (THF) was used as the eluent, with a flow rate of 1 ml per min. A differential refractometer (Waters 410) was used (sensitivity = 16, temperature = 35°C) and the elution profile was acquired through interfacing with a Data Module Waters 730. The temperature of the columns was maintained at 40°C with a Waters TM Temperature Control-System. A calibration was carried out using a series of six polystyrene standards in the molecular range 1800–354 000. In the case of PF68 and PF127 nanospheres, stored at 40°C, experiments were performed after elimination of the surfactant from the carriers. This operation was necessary as these surfactants may interact with the polymer peak, in gel permeation chromatograms. Extraction of surfactant was carried out according to a washing–centrifugation process. In the case of Pluronic formulations stored at 25°C, and Cremophor RH40 preparations, GPC analyses were directly performed after freeze-drying of nanospheres, in order to remove any aqueous phase. The lyophilizates and the extract sediment were resuspended in THF (final concentration = 0.625% w/v of polymer), and filtered through a 0.45-μm filter (Minisart SRP4, Sartorius). A 100-μl aliquot was then injected for analysis. Only a part of the

samples was studied for PEC molecular weight. These are summarized in Table 1.

The enthalpy of fusion (ΔHf) of PEC was determined by differential scanning calorimetry (DSC) at a scan rate of 20°C/min, with a Perkin-Elmer DSC 4. ΔHf was estimated from the fusion peak area of the polymer. Crystallinity was calculated considering that a totally crystalline PEC has a ΔHf of 33.4 kcal/g. Experiments were carried out on polymer extracted from nanospheres according to the same washing–centrifugation process used for GPC measurements. These analyses were performed only on Cremophor RH40 formulations as Pluronic may interact with PEC in DSC thermograms. The non-buffered pH 7 formulation, the pH 7 buffer, and the pH 4-without-thimerosal formulation were analyzed at $T = 0$ and after 6 months at 40°C.

3. Results and discussion

The main evolution in the visual aspect of the suspensions was the appearance of a dark sediment in some formulations containing thimerosal, and a yellowish coloration in buffered preparation stored at 40°C. This coloration was also observed in the control solutions and resulted from an interaction between glucose and disodium phosphate (Masson et al., submitted). The dark precipitate was probably related to the degradation of thimerosal with formation of inorganic mercury salts. This preservative can undergo precipitation in acidic conditions. In any case, no correlation could be established between black sediment and an acidification of the medium.

Table 1
Formulations tested for poly (ε caprolacton) molecular weight

Formulations	Batches	Scheduled studies
PEC/PF68	pH 4+thimerosal	$T = 0$
	pH 7	$T = 6$ months/25°C
		$T = 2$ months/40°C
		$T = 4$ months/40°C
		$T = 6$ months/40°C
PEC/PF127	pH 4+thimerosal	$T = 0$
	pH 7	$T = 6$ months/25°C
	pH 7 buffered	$T = 2$ months/40°C
		$T = 4$ months/40°C
		$T = 6$ months/40°C
PEC/CRH40	pH 4+thimerosal	$T = 0$
	pH 7	$T = 6$ months/25°C
		$T = 6$ months/40°C

Aggregation appeared in some samples stored at 25°C. The addition of HEC did not prevent this phenomenon. Better physical stability was obtained with PF127. Coffin and McGinity also observed an aggregation of PEC pseudolatexes stored at 5°C. This was attributed to the greater hydrophobicity and/or the low glass transition temperature (T_g) of PEC (Coffin and McGinity, 1992a,b).

Surface active agents and HEC did not have any influence on the chemical stability of nanospheres. The results presented will thus focus especially on PF127 nanospheres without viscosifiant.

Fig. 1 presents the pH variation of the unbuffered samples stored at 25 and 40°C. The pH of the suspensions stored at 25°C reached a value between 5 and 6, whatever the initial pH. Thimerosal, when present in pH 4 preparations, seemed to maintain or promote the acidification of the medium. At 40°C, the pH decreased to a final value of 4–4.5 in the case of Pluronic, and 5 in the case of CRH40. The influence of the preservative on pH reduction was especially observed in Pluronic formulations, after 1 month.

The degradation of polyester in aqueous media generally induces an acidification of the medium. This phenomenon was observed with PLA D,L nanocapsules (Guterres et al., 1995), and nanospheres (Coffin and McGinity, 1992a,b; Belbella, 1995). In the same way, Coffin and McGinity noted a pH decrease in PEC pseudolatexes stored at 37°C for 60 days, especially in formulations containing anionic surfactants. The final pH of carriers prepared with non-ionic surfactant, stored in the same conditions, was 4.8 (Coffin and McGinity, 1992a,b). In any case, as shown in Fig. 2, pH variation of the control solutions was the same as for the corresponding suspensions. Acidification should thus be ascribed to excipients rather than to polymer degradation. In fact, Klang et al. (1994) also observed an acidification of submicronic emulsions stored at 4°C, the

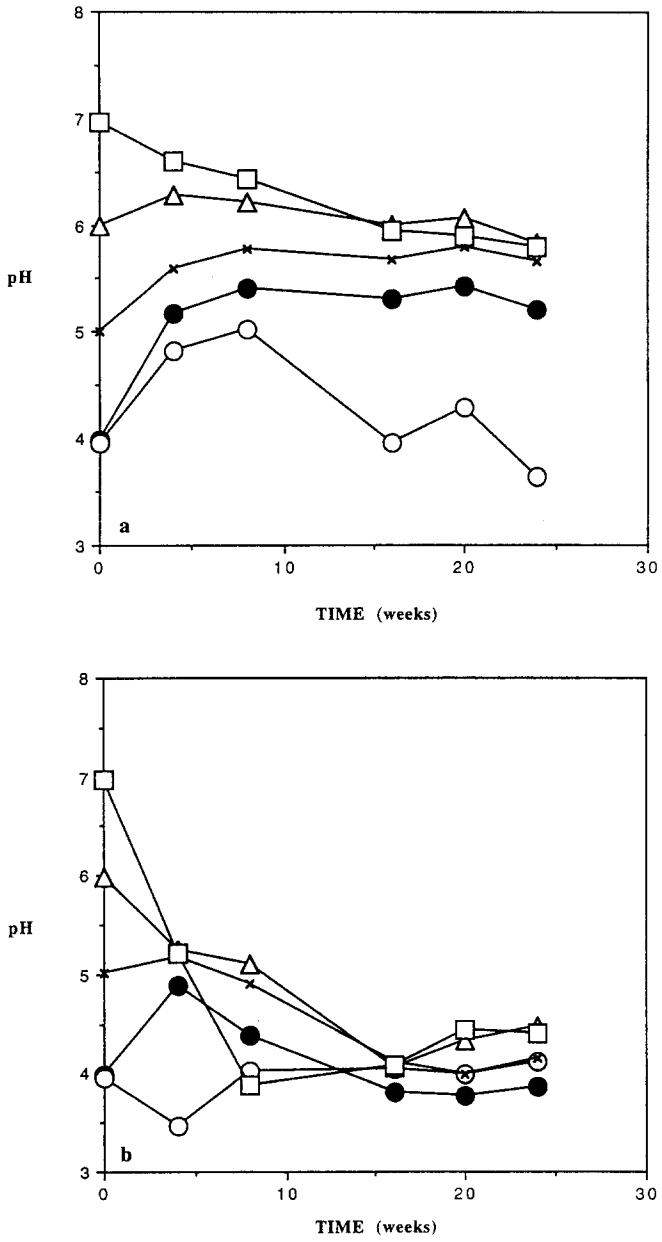

Fig. 1. Evolution of pH in unbuffered Pluronic F127 nanospheres without HEC, stored at 25°C (a) or 40°C (b). (Allowed standard variation: ±0.5). (●), pH 4 without thimerosal; (○), pH 4 + thimerosal; (x), pH 5; (△), pH 6; (□), pH 7.

acidification being related to the Pluronic concentration.

Fig. 3 depicts pH variation of the different buffered PF127 suspensions stored at 25 and 40°C. The pH was stable at 25°C, whereas acidification appeared at 40°C. The acidification observed in buffered and unbuffered suspensions stored at 40°C was also observed in the corresponding con-

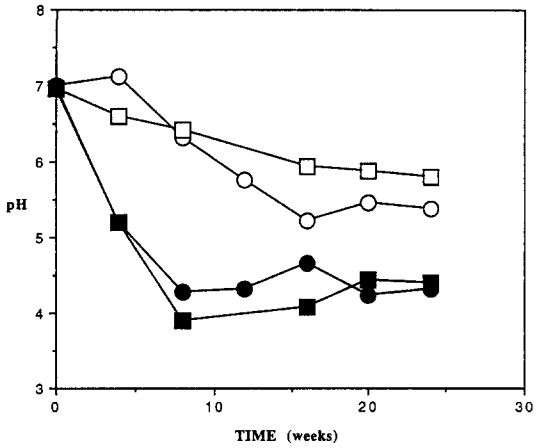

Fig. 2. Comparison of the pH evolution in pH 7 Pluronic F127 nanospheres and control solutions, stored at 25 or 40°C. (Allowed standard variation: ±0.5). (○), Control solution/25°C; (●), control solution/40°C; (□), nanospheres/25°C; (■), nanospheres/40°C.

trol solutions. Thereby, the pH decrease is not only the result of acidic functions released by polymer hydrolysis.

An increase of tonicity appeared in some samples stored at 40°C, which could be correlated with the acidification. In any case, values always remained within the allowed standards, except in some formulations adjusted to acidic pH (Fig. 4). This phenomenon, which was not observed with control solutions, could be related to the

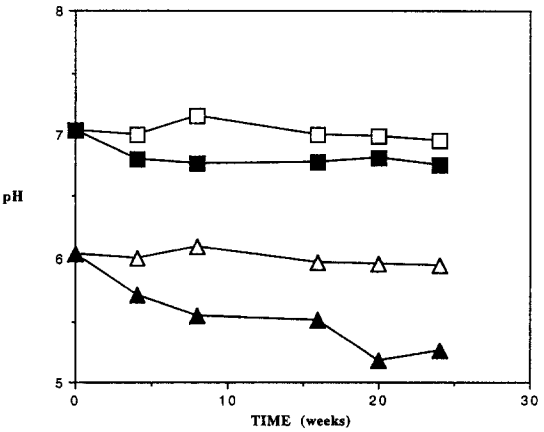

Fig. 3. Evolution of pH in pH 6 or pH 7 buffered nanospheres prepared with Pluronic F127, stored at 25 or 40°C. (Allowed standard variation: ±0.5). (△), pH 6 buffer/25°C; (▲), pH 6 buffer/40°C; (□), pH 7 buffer/25°C; (■), pH 7 buffer/40°C.

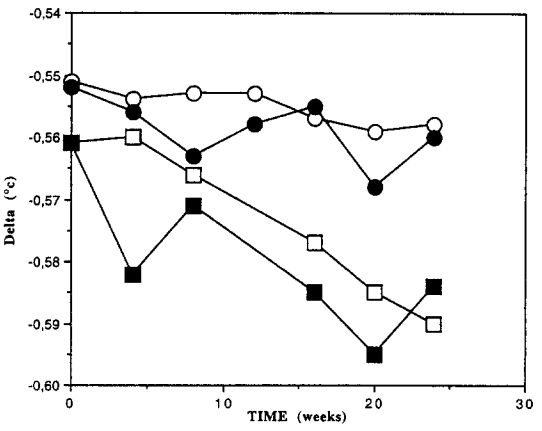

Fig. 4. Evolution of tonicity in pH 4 nanospheres and control solutions stored at 40°C. (Allowed standard variation: ± 0.02°C). (○), Control solution without thimerosal; (●), control solution + thimerosal; (□), nanospheres without thimerosal; (■), nanospheres + thimerosal.

oligomers released by caprolacton hydrolysis. The same evolution was described by Li et al. (1990) on PLA D,L parallelepipedic samples stored in isotonic saline solution at 37°C. A marked decrease in pH value was noted between 8 and 12 weeks which was correlated with an increase in osmolarity and lactic acid release.

Table 2 presents the molecular weight (M_w) variation of PEC, and the corresponding polydispersity index, in pH 4 or pH 7 unbuffered suspensions, stored at 25°C.

After 6 months, the M_w decrease of PEC was about 10–20% and was especially obvious in pH 4 formulations, relative to pH 7. In fact, the change of M_w in pH 7 nanospheres was within the relative standard deviation of measurements. The polydispersity index variation was quite negligible. A very large decrease of PEC M_w was observed by Coffin and McGinity (1992a,b) in pseudolatexes prepared with non-ionic surfactants (initial pH = 5.1), stored at 25°C. In fact, after 22 weeks, the molecular weight dropped to 58% of its initial value. The greater molecular weight of polymer used in this study (M_w = 113 330) may have accounted for this faster hydrolysis. In fact, several authors have shown that the rate of M_w decrease is faster if the initial molecular weight is high (O'Hagan et al., 1994; Rafler and Jobman, 1994). Coffin and McGinity (1992a,b) did not observe any effect of

Table 2
Evolution of poly (ε caprolacton) molecular weight and polydispersity index (Id) in unbuffered nanospheres stored at 25°C

	pH 4			pH 7		
	PF68	PF127	CRH40	PF68	PF127	CRH40
M_w evolution ($T = 24$ weeks)	−13%	−15%	−19%	−11%	−10%	−11%
M_n evolution ($T = 24$ weeks)	−9%	−10%	−20%	−8%	−7%	−9%
Id ($T = 0$)	1.31	1.32	1.57	1.32	1.30	1.63
Id ($T = 24$ weeks)	1.25	1.24	1.58	1.27	1.26	1.59

the initial pH on the polymer degradation rate except for a value of 1.65 where an acid catalysis appeared.

Storage at 40°C induced an extensive degradation of PEC as depicted in Fig. 5. The M_w drop reached a value of 50–60% after 6 months, whatever the surfactant used. An increase of the polydispersity index was noted for the Pluronic nanospheres especially after 6 months at 40°C. GPC chromatograms showed that in the early stages of storage, the PEC peak conserved its initial monomodal morphology, while it moved towards the low molecular weight region. After 4 months, the GPC peak became larger with a small shoulder in the low molecular weight region, indicating the formation of oligomers. After 6 months, the PEC peak completely changed and became flatter and larger. This evolution in the peak morphology after 6 months could be due to the acid catalysis induced by the oligomers detected after 4 months.

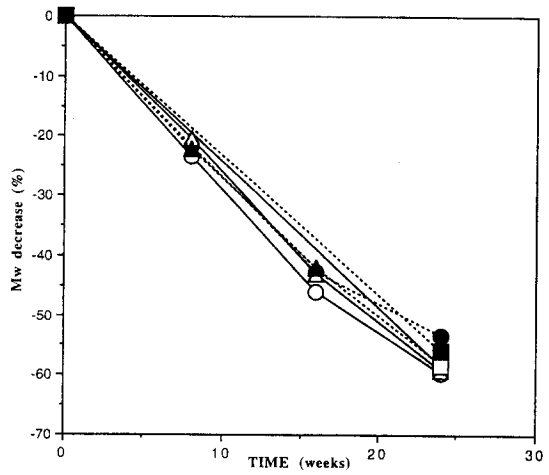

Fig. 5. Evolution of poly (ε caprolacton) M_w in unbuffered nanospheres stored at 40°C. (M_w SEM within 6% of the mean). (○), PF68/pH 4; (●), PF68/pH 7; (△) PF127/pH 4; (▲), PF127/pH 7; (□), CRH40/pH 4; (■), CRH40/pH 7).

A faster degradation rate was observed by Coffin and McGinity (1992b) on their PEC pseudolatexes stored at 37°C. In fact, a 41% decrease of M_w was noted after 8 weeks against 20% in our experiment. This should again be related to the greater molecular weight of PEC used to prepare pseudolatexes. Furthermore, their study was not carried out in a sterile medium. In other studies, the degradation rate of PEC in cylinders or discs stored in water at 37°c was slower than in our study (Ali, 1993; Taylor et al., 1994). The PEC M_w lost 20.6% of its initial value after 16 weeks, whereas a 40% decrease was observed with our nanospheres (Ali, 1993). This suggests that the low carrier diameter and/or the surfactant influence on polymer moistening may enhance the polymer degradation rate or change its mechanism. In any case, Ali (1993) also observed an increase of PEC polydispersity index.

The influence of buffer on PEC molecular weight decrease is presented in Table 3. The polymer degradation in unbuffered nanospheres, stored at 25°C, was quite negligible. Thus, the addition of buffer did not induce any amelioration. The influence of buffer on PEC degradation appeared after 4 months at 40°C. The buffer reduced the polymer hydolysis by about 10%. In fact, the M_w drop observed after 6 months in unbuffered particles was 58% against 50% in buffered ones. The buffer seems to influence the number average molecular weight (M_n) evolution especially. M_n lost 64% of its initial value in pH 7 formulations compared to 48% in buffered suspensions. The extended M_n loss in unbuffered

Table 3
Evolution of poly (ε caprolacton) molecular weight and polydispersity index (Id) in pH 7 buffered and non-buffered nanospheres prepared with Pluronic F127, stored at 25 and 40°C

		25°C		40°C			
		$T=0$	$T=24$ weeks	$T=0$	$T=8$ weeks	$T=16$ weeks	$T=24$ weeks
pH 7	M_w evolution	—	−10%	—	−22%	−42%	−58%
	M_n evolution	—	−7%	—	−29%	−45%	−64%
	Id	1.30	1.26	2.08	2.28	2.18	2.38
pH 7 buffer	M_w evolution	—	−7%	—	−22%	−34%	−50%
	M_n evolution	—	−5%	—	−24%	−32%	−48%
	Id	1.30	1.27	2.12	2.19	2.06	2.05

suspensions could be the result of a catalysis induced by low molecular weight products as evidenced by the increase of PEC polydispersity index observed only in this preparation. This phenomenon was further confirmed by GPC chromatograms. In the case of unbuffered nanospheres, the PEC peak showed a shoulder after 4 months at 40°C and a significant enlargement after 6 months. This enlargement did not appear in the case of buffered nanospheres, which reveals that few oligomers were produced. However, a small shoulder was visible after 6 months, which reflects that the buffer has only delayed the degradation process.

Makino et al. (1985) also observed different degradation rates of PLA in microcapsules stored at 37°C in different buffers. A M_w decrease of 41–51%, after 102 days was observed by Belbella (1995) on PLA D,L nanospheres stored in pH 7.4 buffer at 37°C. These findings are consistent with our results. A similar behaviour was also observed by Leray et al. (1994) on PLA D,L nanospheres stored in the same conditions: in this study, a M_w drop of 14.2% with a consequent increase of the polydispersity index was noted after 4 weeks.

An important acidification appeared in some pH 4 formulations containing thimerosal. In order to determine if this pH decrease resulted from a greater degradation of polymer, we measured the PEC molecular weight in those samples. Fig. 6 presents the pH and M_w changes observed. The acidification in formulations containing thimerosal was associated with a M_w reduction of 15% compared with 9% in the case of preservative-free nanospheres. In any case, this difference is not significant with regard to GPC measurement standard deviation.

Whatever the formulations and the storage conditions, the M_n of PEC always remained above 8300. It has been reported that PEC mass loss occurred when its M_n had decreased to about 5000 (Holland et al., 1986; Pitt, 1990, 1992). No mass loss was noted here as the mean particle diameter remained unchanged throughout the study. The oligomers produced by PEC degradation seem thus to be too large to dissolve in aqueous media.

The last parameter studied in order to emphasize polymer stability was its crystallinity. In the case of a semi-crystalline polymer, the degradation proceeds faster in the amorphous region which thus results in an increase of the crystallinity level (Pitt and Schindler, 1984; Pitt, 1990, 1992; Bruck, 1991; Ameche et al., 1992; Williams, 1992). Furthermore, a recrystallization of tie segments, made possible by the chain cleavage in the amorphous phase, has been described (Ali, 1993). This phenomenon seems to be facilitated by the low glass transition temperature ($T_g = -60$°C) of PEC. The PEC crystallinity should thus increase as degradation proceeds. In fact, an increase of PEC crystallinity of 17.4% and 16.3% was observed in pH 4 and pH 7 unbuffered nanospheres, respectively, after 6 months at 40°C. This increase correlates with the similar M_w drop observed in this nanospheres. A surprising 22.2% crystallinity increase was noted for pH 7 buffered nanospheres. This result is greater than in the case of

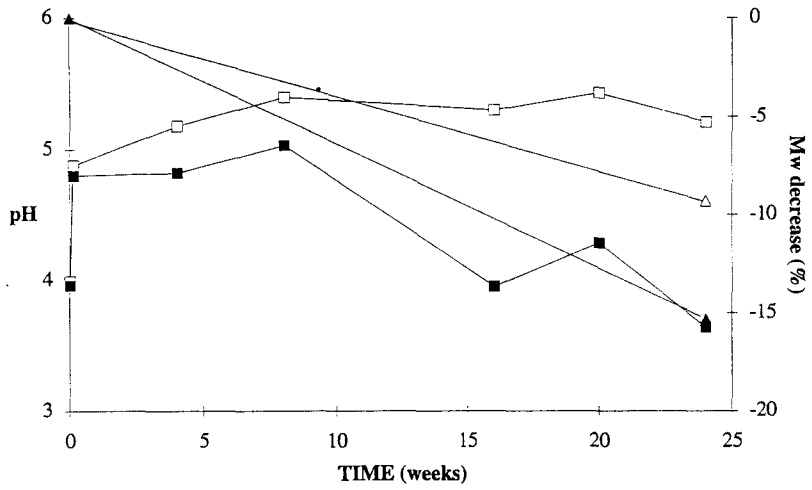

Fig. 6. Influence of thimerosal on pH and PEC M_w evolutions in pH 4 nanospheres prepared with Pluronic F127, stored at 25°C. (M_w SEM within 6% of the mean). (□), pH without thimerosal; (△), M_w without thimerosal; (■), pH + thimerosal; (▲), M_w + thimerosal.

the unbuffered preparation, although the degradation was less important in the first case.

In conclusion, this study showed that some parameters have to be controlled to enhance the stability of PEC nanospheres suspensions in aqueous media. The surfactants play a role in maintaining the physical stability of the suspension. Nanospheres coated with Pluronic F68 or Cremophor RH40 showed some aggregation when stored at 25°C. Pluronic F127 seems thus to be the better candidate. HEC, which was used to prevent nanosphere sedimentation, did not enhance their stability. The tonicity agent and preservative should be chosen carefully to prevent any interaction with other components. The pH must be controlled by a concentrated buffer to conserve the neutrality of the medium and promote the stability of the polymer. Even with a molecular weight decrease of 60%, the mean particle size remained constant. In any case, it is likely that the polymer degradation has to be reduced to a minimum, as polymer hydrolysis may influence the release rate of the drug associated with the nanospheres.

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