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Poly(DL-lactide) nanocapsules containing diclofenac: I. Formulation and stability study

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Abstract

The aim of this work was to formulate nanocapsules prepared from poly(DL-lactide) containing a non-steroidal anti-infammatory drug, diclofenac, and to study their stability during storage at room temperature. The influence of some factors wich could affect stability, namely, the type of oily phase used or/and its concentration, the concentrations of drug and of surfactants, was investigated. The pH of the preparation, the particle size, the quantity of drug remaining (encapsulated and total) and polymer molecular weight were determined at intervals for up to 8 months after nanocapsule preparation. Although colloidal systems which were physically stable over this period could be obtained with either of the two oils tested, polymer degradation was more rapid in the presence of benzyl benzoate than with Miglyol 810®. The optimal concentration of the latter was found to be 3.33%. The highest loading of diclofenac consistent with a stable preparation was 1.00 mg/ml. Stable nanocapsules could be obtained with as little as 0.75% lipophilic surfactant together with a similar concentration of hydrophilic surfactant. These concentrations are considerably lower than those described in the literature for the formulation of this type of colloid.

Keywords: Nanocapsule; Diclofenac; Poly(DL-lactide); Stability

1. Introduction

Recently, much interest has been generated by drug delivery systems prepared from biodegradable polymers because of the possibilities for controlled release, increased drug efficacy and reduced toxicity after parenteral administration (Magenheim and Benita, 1991; Julienne et al., 1992). DL-Lactic and glycolic acids copolymers have been widely studied for this sort of application, since their degradation products are innocuous and the degradation rates vary according to the composition (Bodmeier and McGinity, 1988; Julienne et al., 1992). Poly(lactic acid) is extremely interesting for sustained drug delivery because it is completely biodegradable to non-toxic metabolites and well tolerated by tissues (Kulkarni, 1971). This polymer is degraded via non-enzymatic hydrolysis in aqueous solution in a temperature- and pH-dependent fashion (Makino

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et al., 1985). Therefore, the chemical stability of any poly(DL-lactide)-based drug delivery system must be evaluated (Magenheim and Benita, 1991).

One of the colloidal drug delivery systems currently under study is nanocapsules. In contrast to solid nanospheres, nanocapsules are characterized by a central oily core surrounded by a thin wall of polymer. They may be obtained by several methods. Al-Khouri et al. (1986) developed a technique involving the interfacial polymerization of alkylcyanoacrylate monomers around sub-microscopic droplets of a lipophilic solvent. However, the presence of residual monomers or oligomers and the possibilty of cross-reaction between the nanocapsule contents, particularly the drug molecules (Gallardo at al., 1989), might limit the potential use of these nanocapsules. The procedure patented by Fessi et al. (1988) overcomes these problems by using pre-formed, biodegradable polymers such as polylactides or poly(lactide-gycolide) co-polymers. Nanocapsules are formed by interfacial deposition following displacement of a semi-polar solvent miscible with water from a lipophilic solution.

Many factors may affect the stability of the colloidal systems. Generally, the suspension does not tend to separate because submicronic particles sediment slowly so that the effect is counteracted by the mixing tendencies of diffusion and convection (Magenheim and Benita, 1991), however, aggregation is possible. However, to date, few studies of the stability of PLA nanocapsules have been made. Stability testing involving particle size measurement, drug loss and TEM examination was conducted at given intervals over a 7 month storage period at room temperature by Fessi et al. (1989). A comprehensive long-term stability study on lipidiol/PIBCA monocapsules was performed by Al-Khouri Fallouh et al. (1986). Poly(DL-lactide) microcapsules containing phenobarbitone were prepared using a w/o emulsion method and the effect of storage on the microcapsule characteristics was studied (Jalil and Nixon, 1990).

In this study, we investigated the stability at room temperature of poly(DL-lactide) nanocapsules containing diclofenac prepared according to the method of Fessi et al. (1988). In order to obtain a satisfactory formula, various compositions were tested. The influence of several factors – the nature and concentration of the central oily core, the diclofenac concentration and the concentrations of surfactant – on the pH, the size, the amount of diclofenac encapsulated and the stability of the polymer were studied.

2. Materials and methods

2.1. Materials

Diclofenac (sodium salt) was obtained from Sigma (St Louis, U.S.A.); poly(DL-lactide) (PLA) was from Boehringer (Ingelheim, Germany); phospholipid mixture (Epikuron 170®) was supplied by Lucas Meyer (Hamburg, Germany) and poloxamer (Synperonic PE/F68®) by ICI (Clamart, France). Benzyl benzoate was purchased from Sigma (St Louis, U.S.A.) and caprylic/capric triglyceride (Miglyol 810®) from Hulls (Puteaux, France). All other chemicals and solvents used were of an appropriate grade and obtained from Prolabo (Paris, France).

2.2. Production of the free acid form of diclofenac

An aqueous solution of sodium diclofenac was acidified to pH 4.0 with acetic acid. This solution was then extracted three times with chloroform. After washing in water and filtering through sodium sulphate, the solvent was removed by evaporation. The diclofenac obtained in this way was characterized by infrared spectroscopy (Perkin Elmer 16 PC, St. Quentin en Yvelines, France) and ^{1}H-NMR (Bruker 80 MHz, CT, U.S.A.).

2.3. Preparation of nanocapsules

Nanocapsules of PLA containing diclofenac were prepared using the process described by Fessi et al. (1988). The lipophilic solution consisted of benzyl benzoate or caprylic/capric triglyceride, diclofenac (acid form), phospholipid mixture, poly(DL-lactide) (Mol. Wt 88000) and acetone. This organic solution (40 ml) was poured into the aqueous phase (80 ml) containing polox-

amer under moderate magnetic stirring. The acetone and some water were evaporated under reduced pressure and the final volume adjusted to 15 ml.

2.4. Nanocapsule characterization

The particle size of nanocapsules was estimated by laser light scattering using an N4 Coulter Nanosizer (Hialeah, U.S.A.). Free diclofenac was determined in the clear supernatant following separation of nanocapsules from the aqueous medium by a combined ultrafiltration-centrifugation technique (Ultrafree MC®, Millipore, Bedford, U.S.A.). Total diclofenac was determined after dissolution of nanocapsules in acetonitrile. The diclofenac content of the nanocapsules was calculated from the difference between the total and free drug concentrations measured in the nanocapsule suspension and the filtrate, respectively.

2.5. Assay procedure

Diclofenac was assayed by high-performance liquid chromatography. (El-Sayed et al., 1988; Ammoury, 1990) The system consisted of a Waters (St. Quentin en Yvelines, France) microBondapak C18 column, a Waters TM 717 Autosampler, a Waters F6000A pump, and a Waters 484 UV detector. The mobile phase consisted of acetonitrile/0.0025 M sodium acetate (65:35% v/v) adjusted to pH 4.0 with glacial acetic acid.

The total sample volume injected was 20 μl. Diclofenac was detected by absorption at 280 nm at an approximate retention time of 8.9 min. The linear response range was 0.2–20 μg/ml with a correlation coefficient of 0.9995.

2.6. Molecular weight determination

Polymer molecular weights were determined by gel permeation chromatography (GPC) using a Waters 510 pump. The eluent used was tetrahydrofuran (THF). Columns packed with Ultrastyragel (10 and 500 Å) (Waters, Milford, U.S.A.) were used simultaneously, coupled with a Waters refractive index detector. Polymers dissolved in THF were analysed at 1 ml/min at 40°C. Polystyrene molecular weight standards were used to calibrate the column. PLA and nanocapsules were dissolved in THF and filtered through a 0.45 μm filter, after which 100 μl was injected.

2.7. Stability studies

The effects of storage time (0, 1, 3, 6 and 8 months) on the pH, particle size, drug loading, drug content, and polymer molecular weight of nanocapsules were assessed. The suspensions were stored at room temperature protected from light.

3. Results and discussion

The aim of this study was to prepare a stable formulation with the highest possible payload of diclofenac. Two strategies were possible: (i) increasing the concentration of diclofenac dissolved in a given volume of central oily core; (ii) increas-

Table 1
Stability of nanocapsule formulations prepared with different amounts of diclofenac

Drug content (mg/ml of supension):		Benzyl benzoate [a]					Miglyol 810® [a]				
		0.50	1.00	1.33	2.00	3.00	0.20	0.50	1.00	1.33	2.00
pH	0 months			4.0 ± 0.2					4.6 ± 0.2		
	8 months			3.2 ± 0.2					3.8 ± 0.1		
Size (nm)	0 months			234 ± 35					189 ± 20		
	8 months			248 ± 26					195 ± 23		

[a] Formulations containing PLA 1% (w/v), poloxamer 0.75% (w/v), 0.75% of phospholipid mixture (w/v) and 3.33% of oily phase (benzyl benzoate or Miglyol 810®) containing different concentrations of diclofenac.

ing the volume of central oily core used in the preparation with a fixed concentration of diclofenac.

Two series of formulations were prepared using fixed amounts of polymer, surfactants and oil (either benzyl benzoate or Miglyol 810®), with increasing concentrations of diclofenac in the oily phase. Table 1 summarizes the composition and properties of the formulations at the time of preparation.

As far as the pH measurements were concerned, all the formulations were acidic (pH 4–5) just after preparation. The diclofenac concentration did not affect the pH of the suspension. The pH of all the formulations decreased during storage. This decline in pH could reflect the production of free lactic acid as a result of PLA degradation. This hydrolysis is reported to be non-enzymatic and to depend on the temperature and the pH of the medium, accelerated under both acidic and basic conditions (Makino et al., 1986). The formulations prepared with benzyl benzoate were more acidic to begin with, probably because of traces of free acids (0.07% according to the supplier's specifications) in the central oily core, and this could have resulted in a more extensive degradation of the polymer. This hypothesis was confirmed by determining the polymer molecular weight by GPC. As shown in Fig. 1, just after preparation, no PLA degradation could be detected in either formulation. In contrast, after 8 months of storage, considerable polymer degradation was evident in the formulations with benzyl benzoate, whereas in the preparations containing Miglyol 810 ® only minimal PLA breakdown could be seen.

Particle size is an important property of colloidal suspensions, since the tendency to sediment is determined by changes in this parameter (Magenheim and Benita, 1991). All the nanocapsule preparations retained acceptable particle size distributions after the 8 month storage period. In contrast, the diclofenac concentration had no significant effect on the particle size. As a general rule, the particles prepared from Miglyol 810® were smaller than those prepared from benzyl benzoate. According to Yu et al. (1993), the viscosity of the oily phase could explain the differences in size of injectable emulsions prepared by spontaneous emulsification. A similar phenomenon may occur during nanocapsule preparation.

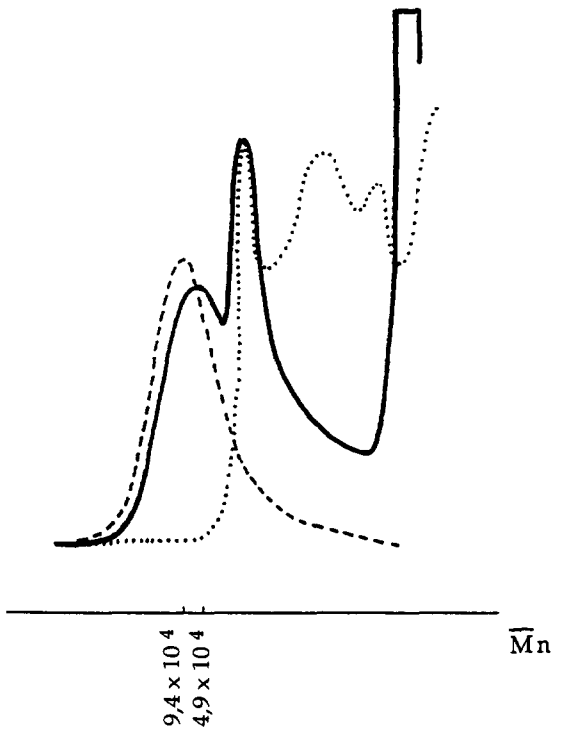

Fig. 1. GPC chromatograms for nanocapsules prepared from benzyl benzoate (dotted line) or Miglyol 810® (continuous line) after 8 months of storage. PLA alone (broken line).

The percentage of diclofenac incorporated into the nanocapsules was always close to 100, even in the formulations with the highest concentration of drug and this was maintained after 8 months of storage. The partition coefficients determined for diclofenac between the central oily core (benzyl benzoate or Miglyol 810®) and the aqueous phase at different pH showed that very little drug passed into aqueous solution (results not shown), which predicted that a high percentage encapsulation and good drug retention could be obtained. However, for the formulations with the highest drug content (concentrations higher than 2.00 mg/ml for the formulations containing benzyl benzoate and 1.33 mg/ml for those based on Miglyol 810®), crystals of diclofenac could be observed on the walls of the recipient, after 8 months of storage.

In these formulations, the total quantity of diclofenac detected declined during the stability study (Fig. 2), while the other parameters, namely, size and percentage encapsulation, remained constant. These results can be explained with reference to the solubility of diclofenac in the presence of hydrophilic surfactant. A significant increase in the aqueous solubility of the drug ($p < 0.001$) was observed under these conditions (results not shown), however, the absolute value (3.33%) remained low. In fact, surfactants can adsorb onto the surface of solid particles and thereby improve their stability in solution (Attwood and Florence, 1983). During the formation of nanocapsules, when the concentration of diclofenac exceeds its solubility in the central oily core, nanocrystals of drug, stabilized by surfactant could be formed at the same time. If these nanoparticles have the same size distribution as nanocapsules, this could explain why a satisfactory granulometric result is obtained. With time, these nanocrystals start to grow and precipitate and the quantity of drug found in the colloidal suspension is thus reduced.

When benzyl benzoate was used, formulations containing 1.33 mg/ml of diclofenac were stable for 6 months. After 8 months, only the preparation with 0.50 mg/ml remained stable. At higher drug concentrations (2.00 and 3.00 mg/ml) precipitation was observed after 1 month. With Miglyol 810®, formulations with 1.00 mg/ml diclofenac or less were stable for 8 months. The preparation with 1.33 mg/ml was only stable for 6 months, whereas that containing 2.00 mg/ml

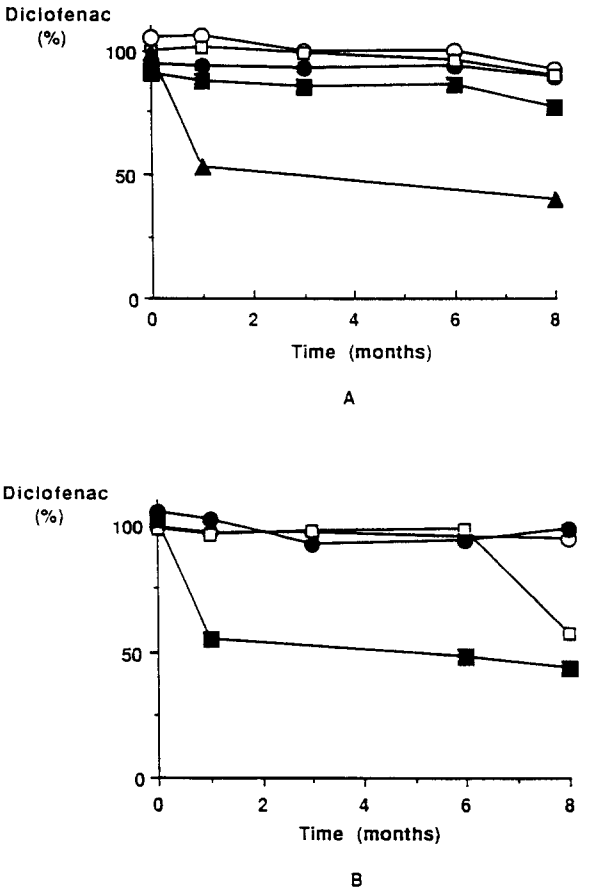

Fig. 2. Percentage of the initial load of diclofenac found as a function of storage time in nanocapsules prepared at different drug concentrations: (A) benzyl benzoate; (B) Miglyol 810®. Content of diclofenac (mg/ml): (○) 0.50, (●) 1.00, (□) 1.33, (■) 2.00, (▲) 3.00.

Table 2
Stability of nanocapsule formulations prepared with different amounts of oily phase

		Benzyl benzoate [a] (20 mg DIC/ml)				Miglyol 810® [a] (15 mg DIC/ml)			
Oily phase (% v/v):		3.33	5.00	6.67	10.00	3.33	5.00	6.67	10.00 [b]
pH	0 months			4.0 ± 0.1				4.6 ± 0.4	
	8 months			3.2 ± 0.3				3.8 ± 0.1	
Size (nm)	0 months			249 ± 22				219 ± 13	
	8 months			308 ± 48				212 ± 27	

[a] Formulations containing PLA 1% (w/v), poloxamer 0.75% (w/v) and 0.75% phospholipid mixture (w/v).
[b] The size distribution of this formulation could not be measured, due to the formation of oil droplets. It is therefore not included in the mean.

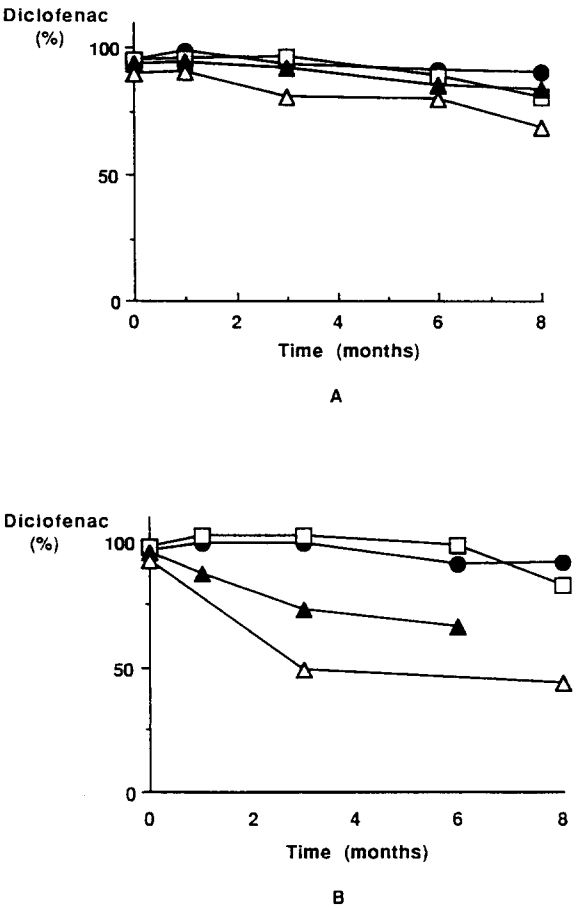

Fig. 3. Percentage of initial load of diclofenac found as a function of storage time in nanocapsules prepared with different quantities of oily phase: (A) benzyl benzoate; (B) Miglyol 810®. Content of diclofenac (%): (●) 3.33, (□) 5.00, (▲) 6.67, (△) 10.00.

showed considerable precipitation after 1 month, so that the total quantity of drug could not be retrieved.

Two further series of formulations were prepared, this time containing a fixed quantity of drug dissolved in increasing volumes of benzyl benzoate or Miglyol 810®. Table 2 lists the compositions and properties of this series. Neither the particle size distribution nor the pH measured just after preparation was affected by the oily phase, except for the formulation containing 10% (v/v) of Miglyol 810® as the oily phase. Fig. 3 shows the total amount of diclofenac measured in the preparations as a function of time. When benzyl benzoate was used at more than 5%, the formulation became unstable after 3 months, as demonstrated by a precipitate which could not be resuspended and the appearance of small drops of oily phase. This limiting central oily core concentration is the same as that found by Ammoury (1990) with nanocapsules containing indomethacin. When Miglyol 810® was used at 5%, the formulations were only stable for 6 months. Generally, for the same volume of oily phase, benzyl benzoate conferred a greater stability than Miglyol 810®.

Finally, the effect of the surfactant concentra-

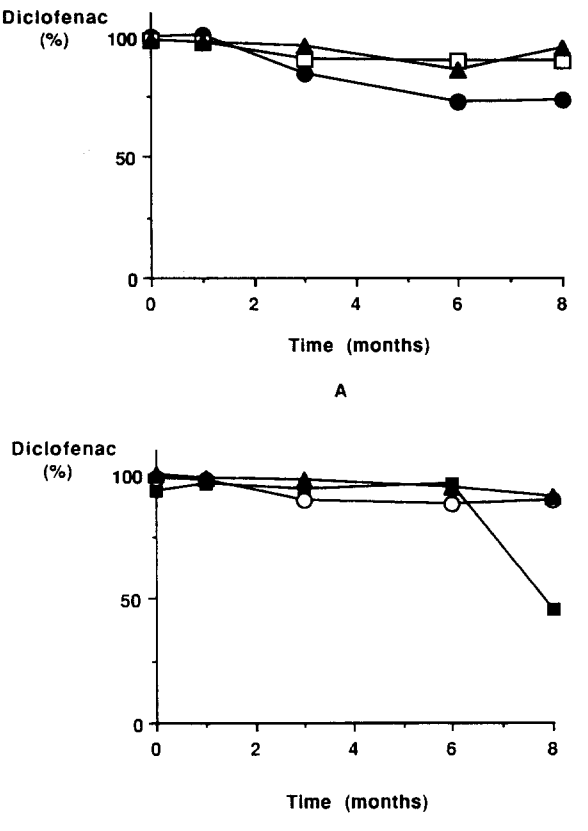

Fig. 4. Percentage of the initial load of diclofenac found as a function of storage time in nanocapsules prepared with different concentrations of surfactants: (A) benzyl benzoate; (B) Miglyol 810®. Content of surfactants (%): (●) 0.30, (□) 0.75, (▲) 1.50.

tion was studied, by preparing two series of nanocapsule formulations with a fixed concentration of central oily phase (benzyl benzoate or Miglyol 810®; 5%), of diclofenac (1.25 mg/ml) and of polymer (1%) and with increasing equal concentrations of hydrophilic and lipophilic surfactants. Fig. 4 shows that the formulation with Miglyol 810® and 0.3% surfactant was stable for 6 months after preparation. With 0.75 or 1.5% surfactants, the stability reached 8 months. Examination by electron microscopy confirmed that in the presence of 0.75% surfactant no liposome-like structures, which could have originated from the Epikuron 170®, were found. The stability was reduced when benzyl benzoate was used as the oily core: after 3 months the formulation with 0.3% surfactant was found to be unstable; whereas those with 0.75 and 1.5% were stable for 8 months.

This formulation study allowed us to define compositions containing either of the two oily phases which were stable for 8 months. As far as Miglyol 810® was concerned, the optimal content was 3.33%. The highest diclofenac concentration compatible with 8 month stability was 1 mg/ml. We sought the lowest surfactant concentration able to ensure stability and found that 0.75% of lipophilic surfactant combined with the same proportion of a hydrophilic one was sufficient. It is noteworthy that these values are lower than those which have been reported for this type of formulation containing other drugs (Fessi et al., 1989; Ammoury, 1990; Yu et al., 1993).

References

Al-Khouri, N., Fessi, H., Roblot-Treupel, L., Devissaguet, J.-P. and Puisieux, F., Etude et mise au point d'un procédé original de préparation de polyalkylcyanoacrylates par polymérisation interfaciale. *Pharm. Acta Helv.*, 61 (1986) 274–281.

Al-Khouri Fallouh, N., Roblot-Treupel, L., Fessi, H., Devissaguet, J.P. and Puisieux, F., Development of a new process for the manafucture of polyisobutylcyanoacrylate nanocapsules. *Int. J. Pharm.*, 28 (1986) 125–132.

Ammoury, N., Etude physico-chimique et biologique de vecteurs colloidaux vésiculaires d'indométacine-acide polylactique, Thesis, Université de Paris-Sud (1990).

Attwood, D. and Florence, A.T. *Surfactants Systems: Their Chemistry, Pharmacy and Biology*, Chapman and Hall, London, 1983.

Bodmeier, R. and McGinity, J.W., Solvent selection in the preparation of poly(DL)-lactide microspheres prepared by the solvent evaporation method. *Int. J. Pharm.*, 43 (1988) 179–186.

El-Sayed, Y.M., Abdel-Hameed, M.E., Suleiman, M.S. and Najib, N.M., A rapid and sensitive high-performance liquid chromatographic method for the determination of diclofenac sodium in serum and its use in pharmacokinetics studies. *J. Pharm. Pharmacol.*, 40 (1988) 727–729.

Fessi, H., Puisieux, F. and Devissaguet, J.P., Procédé de préparation de systèmes colloïdaux dispersibles d'une substance sous forme de nanocapsules. *Eur. Patent 0274961 A1*, 1988.

Fessi, H., Puiseux, F., Devissaguet, J.-P, Ammoury, N. and Benita, S., Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int. J. Pharm.*, 55 (1989) R1–R4.

Gallardo, M.M., Roblot-Treupel, L., Mahuteau, J., Genin, I., Couvreur, P., Plat, M. and Puisieux, F., Nanocapsules and nanospheres d'alkylcyanoacrylate, interactions principe actif/polymer. *APGI Ve Congrès International de Technologie Pharmaceutique*, Paris, 1989.

Jalil, R. and Nixon, J.R., Microencapsulation using poly(DL-lactic acid): IV. Effect of storage on the microcapsule characteristics. *J. Microencapsul.*, 7 (1990) 375–383.

Julienne, M.C., Alonso, M.J., Gomez Amoza, J.L. and Benoit, J.P., Preparation of poly(DL-lactide-glycolide) nanoparticles of controlled particle size distribution: application of experimental designs. *Drug Dev. Ind. Pharm.*, 18 (1992) 1063–1077.

Kulkarni, R.K., Moore, E.G., Hegyeli, A.F. and Leonarde, F., Biodegradable poly(lactic acide)polymers. *J. Biomed. Mater. Res.*, 5 (1971) 169–181.

Magenheim, B. and Benita, S., Nanoparticle characterization: a comprehensive physicochemical approach. *STP Pharm. Sci.*, 1 (1991) 221–241.

Makino, K., Arakawa, M. and Kondo, T., Preparation and in vitro degradation properties of polylactide microcapsules. *Chem. Pharm. Bull.*, 33 (1985) 1195–1201.

Makino, K., Ohshima, H. and Kondo, T., Mechanism of hydrolytic degradation of poly(L-lactide) microcapsules: effects of pH, ionic strength and buffer concentration. *J. Microencapsul.*, 3 (1986) 203–212.

Yu, W., Tabosa do Egito, E.S., Barratt, G., Fessi, H., Devissaguet, J.P. and Puisieux, F., A novel approach to the preparation of injectable emulsions by a spontaneous emulsification process. *Int. J. Pharm.*, 89 (1993) 139–146.