ELSEVIER

International Journal of Pharmaceutics 138 (1996) 1–12

international journal of pharmaceutics

Research papers

Steric stabilization of nanoparticles: size and surface properties

Cristina Lourenco, Maribel Teixeira, Sérgio Simões, Rogério Gaspar*

Faculty of Pharmacy and Center for Neurosciences (CNC) — University of Coimbra, Rua do Norte, 3000 Coimbra, Portugal

Received 12 December 1995; accepted 8 February 1996

Abstract

Nanoparticles of poly-(isobutylcyanoacrylate) (PIBCA), poly-lactic acid (PLA), poly-lactic-co-glycolic copolymer (PLAGA) and poly-ϵ-caprolactone were prepared according to conventional methods. In the preparation of PIBCA nanoparticles different steric stabilizers were used. Formulations included poloxamer 188, polyvinilic alcohol (PVA) or polyethyleneglycol 2000 (PEG 2000), with or without dextran, in order to characterize the influence of different steric stabilizers. All the formulations were tested for their electrophoretic mobility, zeta potential and particle size using DELSA. Particle size measurements were also performed using a PCS technique, employing two sets of apparatus, in an attempt to evaluate the influence of measuring principle and detection angle in the final results. Results indicate that PIBCA nanoparticles can be sterically stabilized by dextran, poloxamer and PVA, but PEG 2000 could need the presence of another steric stabilizer. The good correlation for the same detection angle between PCS and DELSA demonstrates the importance of DELSA in the characterization of size and surface properties associated with colloidal drug carriers like nanoparticles, as demonstrated by PLA, PLAGA and PCL nanoparticles (monodisperse particles). The differences between the two techniques could be explained by different detection angles and strongly suggests an integrated approach using PCS and DELSA when considering polydisperse particle populations.

Keywords: Drug carriers; Nanoparticles; Size analysis; Zeta potential; Polymers; Steric stabilizers

1. Introduction

Polymeric drug carriers have long been used as systems designed to achieve targeting to specific cell populations or controlled release of bioactive substances. The possibilities of modulating their biodistribution parameters and their colloidal sta-

Abbreviations: DELSA, Doppler Electrophoretic Light Scattering Analysis; PCS, Photon Correlation Spectroscopy; MPS, Mononuclear phagocyte system.

* Corresponding author. Tel.: + 351 39 4109998; fax: + 351 39 27126; e-mail: rgaspar@cygnus.ci.uc.pt

PII S0378-5173(96)04486-9

bility are dependent on physicochemical properties such as size and surface characteristics. Sizes above 100 nm will tend to restrict their biodistribution, contributing to an increase in their capture by Küpffer cells or other phagocytic cell populations within the MPS (Wisse and Leeuw, 1984).

Steric stabilizers have long been used to achieve greater stability of colloidal particles or to prevent capture by phagocytic cells (Illum et al., 1987). Colloidal systems like PLA nanoparticles can be sterically stabilized by covalently coupling PEG to PLA, achieving colloidal stability but, more importantly, also significantly reducing uptake by the MPS (Bazile et al., 1992). By avoiding opsonisation, polymeric nanoparticles can overcome removal by the mononuclear phagocyte system achieving the goal of having a slow-constant release of drug in the circulation for extended periods of time, increasing drug pharmacokinetic parameters like AUC (Gref et al., 1994; Peracchia et al., 1994; Bazile et al., 1995).

Stabilization of colloidal systems is traditionally viewed as arising from either electrostatic or steric effects. Electrostatic repulsion is sensitive to the added electrolyte, whereas steric repulsion is sensitive to changes in the solvency and molar mass of the polymer adsorbed layer. In many cases stability is impaired by a combination of mechanisms. This is the case in electrostatic stabilization, an intermediate regime in which both electrostatic and steric effects contribute to the stability of the dispersion. In this case, the electrostatic component may originate from the particle surface charge and/or charged sites on the adsorbed polymer (Napper, 1983). PACA nanoparticles can be prepared by conventional methods, but need to be stabilized by macromolecules in the polymer's outer surface to obtain stable pharmaceutical formulations, with homogeneous known size and surface properties. Dextran has long been used and constitutes a reference steric stabilizer for obtaining PACA nanoparticles (Couvreur et al., 1985). Without dextran or another stabilizer, PACA nanoparticles are not stable enough to be used in therapeutics. Steric stabilizers could also modify the polymerization mechanism of cyanoacrylates (Vauthier-Holtzscherer et al., 1991).

The results within a given group of non-ionic stabilizers may be related to the molecular structure as stated by the accepted theories of steric stabilization. As particle size, among other factors, is known to control tissue distribution and drug release of drug carrier systems, the ability to produce various sizes of nanoparticles is of importance for their potential use in drug targeting (Douglas et al., 1985).

In the present work, nanoparticles of polyisobutylcyanoacrylate (PIBCA), poly-lactic acid (PLA), poly-lactic-co-glycolic copolymer (PLAGA) and poly-ϵ-caprolactone were prepared. In the preparation of PIBCA nanoparticles, different steric stabilizers were used. Formulations included poloxamer 188, polyvinilic alcohol (PVA) or polyethyleneglycol 2000 (PEG 2000), with or without dextran. PLA, PLAGA and PCL were also used to prepare nanoparticles sterically stabilized with poloxamer 188.

All the formulations were tested for their electrophoretic mobility, zeta potential and particle size using Doppler Electrophoretic Light Scattering Analysis (DELSA). Particle size measurements were also performed using Photon Correlation Spectroscopy (PCS) techniques, with two sets of apparatus, in an attempt to evaluate the influence of the measuring principle and of the detection angle on the final results.

2. Materials and methods

2.1. Materials

Isobutylcyanoacrylate (IBCA) was obtained thanks to a gift from Loctite (Loctite Co., Dublin,

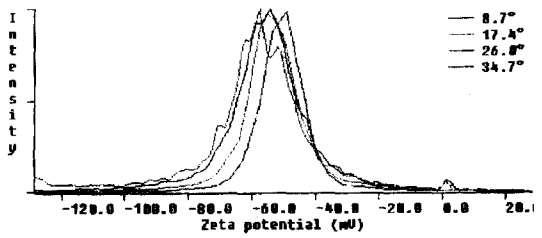

Fig. 1. Typical Coulter® DELSA 440 result in terms of zeta potential distribution at the four detection angles (8.6°, 17.1°, 25.6° and 34.2°).

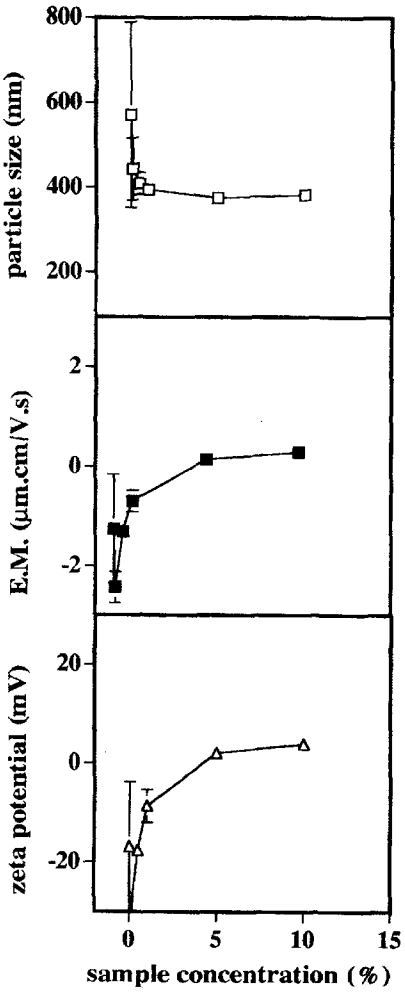

Fig. 2. Influence of sample concentration on particle size, electrophoretic mobility and ζ potential obtained with Coulter® DELSA 440. Error bars = ± S.D.

Ireland). Poly-lactic acid (PLA, MW = 90 000), poly-lactic-co-glycolic copolymer (PLAGA, MW = 50 000–75 000) and poly-ϵ-caprolactone (PCL, MW = 128 000) were all obtained from Sigma (Sigma Chemical Co., St. Louis, MO). Polyvinilic alcohol (PVA, MW = 150 000) and polyethyleneglycol (PEG, MW = 2000) were obtained from Fluka (FLUKA, Fluka Chemie, Buchs, Switzerland). Dextran (MW = 70 000) was from Sigma (Sigma Chemical Co., St. Louis, MO). Poloxamer 188 (Synperonic F68) was kindly provided by ICI (ICI, France). Glucose anhydrous, citric acid and acetone were from Merck (Merck, Darmstadt, Germany).

2.2. Preparation of PIBCA nanoparticles

PIBCA nanoparticles were obtained by emulsion polymerization of isobutylcyanoacrylate as described by Couvreur et al. (1985). The polymerization media contained citric acid (0.01 M), glucose (5%, w/v) and a steric stabilizer like dextran, poloxamer 188, polyethyleneglycol 2000 (PEG) or

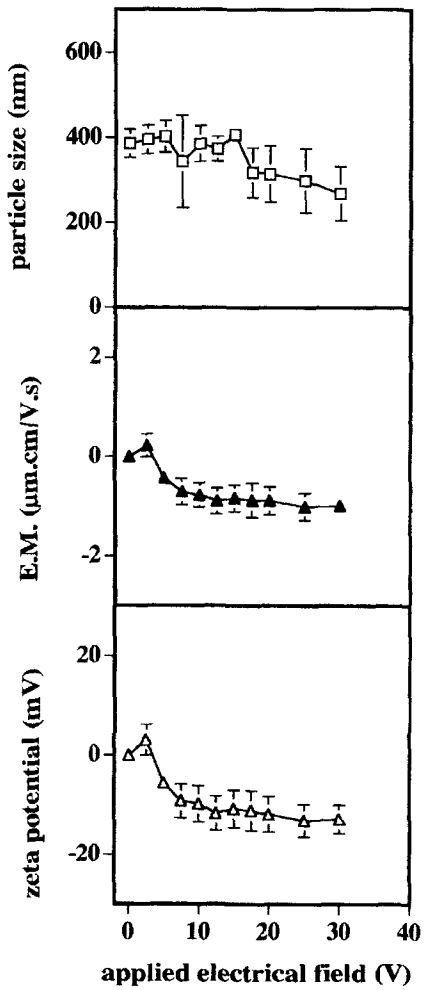

Fig. 3. Influence of applied electrical field on particle size, electrophoretic mobility and ζ potential obtained with Coulter® DELSA 440. Error bars = + S.D.

Table 1
DELSA and PCS results for PIBCA nanoparticles when polymerization medium includes dextran and/or poloxamer 188

		Medium and stabilizer				
		A. dextran 1% (pH ≈ 2.5)	B. dextran 1% poloxamer 1% (pH ≈ 2.6)	C. dextran 1% poloxamer 2% (pH ≈ 2.6)	D. poloxamer 1% (pH ≈ 2.6)	E. poloxamer 2% (pH ≈ 2.5)
DELSA	d	416 ± 29	510 ± 59	470 ± 73	552 ± 148	554 ± 128
	EM	−0.75 ± 0.43	−0.25 ± 0.15	−0.20 ± 0.14	−0.38 ± 0.25	−0.3 ± 0.25
	ζ	−9.6 ± 5.5	−3.3 ± 1.9	−2.6 ± 1.8	−3.8 ± 1.4	−3.8 ± 3.2
PCS	d	192 ± 18	81 ± 11	82 ± 22	89 ± 12	75 ± 9
	PI	0.05–0.18	0.35–0.45	0.35–0.50	0.35–0.45	0.35–0.55

d, diameter (nm); EM, electrophoretic mobility (μm·cm/V·s); ζ, zeta potential (mV); PI, polydispersity index; errors (± S.D.).

polyvinilic alcohol (PVA). The monomer, isobutylcyanoacrylate, was added dropwise (40 μl) to the polymerization media (4 ml) under magnetic stirring. After 4 h of polymerization, the colloidal suspensions obtained were filtered through a paper filter and analysed for particle size and surface characteristics (electrophoretic mobility and zeta potential).

Two groups of nanoparticles were prepared. In the first group, a model polymerization medium containing glucose, citric acid and dextran was used and other steric stabilizers (PVA, PEG or poloxamer 188) were added to this medium. In the second, only one steric stabilizer was used for each polymerization procedure.

2.3. Preparation of PLA, PLAGA, PCL nanoparticles

Nanoparticles of these three different polymers were prepared by a nanodispersion methodology (Fessi et al., 1989). Briefly the polymer was dissolved in an organic phase (acetone) and poured into 15 ml of aqueous medium with or without poloxamer 188, under magnetic stirring. The aqueous phase rapidly turned opalescent because of nanoparticle formation. The acetone was removed in a rotary evaporator, under vacuum, and the final suspension concentrated to the desired final volume.

2.4. Surface properties

Electrophoretic light scattering (ELS) was used to directly measure the velocity of particles moving under the influence of an electric field. With this technique, electrophoretic mobility is detected by the Doppler shift in frequency, $\Delta\upsilon$, using an heterodyne method (Woodle et al., 1992), and calculated by the equation:

$$\Delta\upsilon = U(Ek) \quad (1)$$

where U is the electrophoretic mobility, E the applied electric field and k the scattering angle, determined by:

$$k = (4\pi n/\lambda)\sin(\theta/2) \quad (2)$$

where λ is the wavelength of the helium-neon laser light in vacuum, n the refractive index of the medium and θ is the angle between the incident laser beam and the detector. From both equations, electrophoretic mobility can be calculated by the equation:

$$U = \frac{(\Delta\upsilon\lambda)}{2nE\sin(\theta/2)} \quad (3)$$

Electrophoretic mobility is related to zeta potential by the assumption derived from the Smoluchowski approximation, which assumes (Woodle et al., 1992) that the double layer thickness is small compared to the colloidal particle diameter:

$$U = \frac{\epsilon\zeta}{\eta} \quad (4)$$

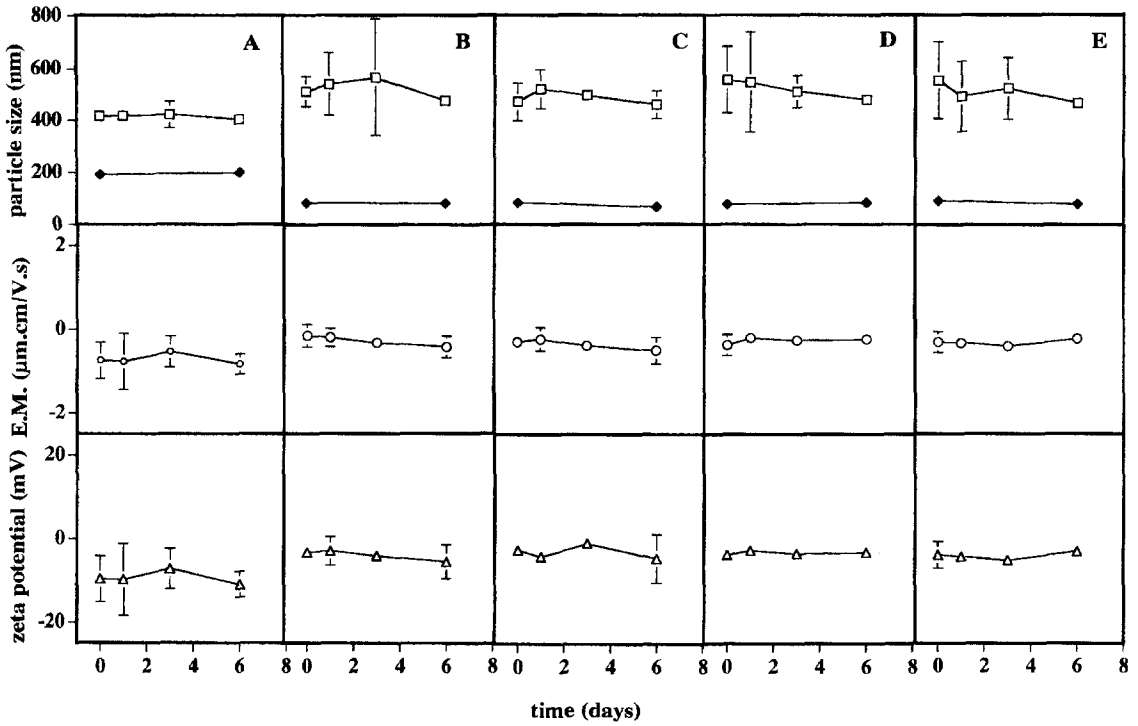

Fig. 4. Physical stability of PIBCA nanoparticles prepared in the presence of poloxamer 188, compared to reference medium (A), when particle size is determined by Coulter® DELSA 440 and PCS; electrophoretic mobility and ζ potential are determined by Coulter® DELSA 440. Open symbols (□ ○ △) are results from DELSA analysis; closed symbols are for results from PCS analysis (◆). For composition of polymerization medium, refer to Table 1. Error bars = ± S.D.

where ζ is the zeta potential and ϵ is defined by: $\epsilon = \epsilon_0 D$, ϵ_0 being the permittivity of the free space and D the dielectric constant of water, η is the viscosity, this being a valid assumption for aqueous systems of usual electrical conductivity.

For this purpose a Coulter® DELSA 440 (Coulter Electronics, Hialeh, FL) was used. It is a laser-based multiple angle particle electrophoresis analyser that measures the electrophoretic mobility and zeta potential distribution simultaneously with the hydrodynamic size of particles in suspension. The DELSA 440 optics and detection system are designed to make measurements at four different angles simultaneously (8.6°, 17.1°, 25.6° and 34.2° — after correction for the refractive index of water), with four separate detectors and four independent 256-channel analyzers. Fig. 1 shows typical results expressed in terms of zeta potential distribution at the four detection angles.

Samples from the prepared suspensions were diluted in water and placed in the measurement cell, with position adjusted to cover the stationary layer previously determined. The electric field, when applied, was between 5 and 15 V. Data were automatically converted into zeta potential using the dielectric constant for water and data processed with the software included with the system. Measurements at three electric field strength values were recorded and both electrophoretic mobility (E.M.) and zeta potential (ζ) calculations were made for each scattering angle (8.6°, 17.1°, 25.6°, 34.2°) and electric field value (5, 10 or 15 V).

2.5. Particle size measurements

In an attempt to evaluate the influence of the measuring principle and of the detection angle on the final results, particle size was evaluated by two techniques: electrophoretic light scattering (ELS) using the Coulter® DELSA 440 described, and

photon correlation spectroscopy (PCS) using a Malvern Autosizer IIc and a Coulter® N4MD.

The PCS technique uses autocorrelation spectroscopy of scattered laser light to determine its time-dependent fluctuations resulting from the Brownian motion of particles in suspension. The light intensity scattered at a given angle is detected by a photo-multiplier whose output current is passed to an autocorrelator, which analyses time dependency, determining the rate of diffusion, or Brownian motion, of the particles and hence their size. The main advantage of using two sets of equipment based on this principle is the possibility of particle size evaluation at fixed (90°) or variable detection angles, respectively with the Malvern Autosizer IIc and the Coulter® N4MD.

With the DELSA method, determination of particle size was performed without applying current and considering only the Doppler shift in light scattering at a detection angle of 34.2°.

To validate the techniques, polystyrene beads with different diameters (106, 300, 806 and 1530 nm) and a narrow distribution were analyzed. Samples and dilutions of the suspending medium (particle free distilled water) were prepared for all the techniques. The results for each sample were expressed as the average and standard deviation of at least two successive measurements for each sample (and at least three different experiments).

3. Results and discussion

The aim of this work was to characterize size and surface properties of nanoparticles sterically stabilized by different macromolecules (dextran, poloxamer, PVA and PEG).

Previously characterized PACA nanoparticles (Müller et al., 1992) possessed a relatively low surface charge of about 2 $\mu C/cm^2$ which was consistent with the ζ potential measurements. The ζ potentials in distilled water (-6 to -26 mV) were observed to fall to zero at physiological salt concentrations. After intravenous injection, the particle charge in the blood is therefore supposed to be determined solely by adsorbed serum components. Since their surfaces are similar in degree of hydrophobicity, the adsorption behavior should be identical. This assumption is in agreement with the identical adsorption of charged serum components leading to ζ potentials in serum of about -3 mV. From these data, no differences were found in the in vitro interactions with cells in culture or the in vivo distribution of nanoparticles with different alkyl chain length (Müller et al., 1992).

The preparation of polymeric colloidal carriers can be performed by polymerization of the monomer or polymer's nanodispersion in a non-solvent medium. For cyanoacrylates, emulsion polymerization has been the selected procedure to obtain nanoparticles (Couvreur et al., 1979) with interfacial polymerization to achieve the formation of nanocapsules (Al Khouri et al., 1986). In emulsion polymerization, micelles and monomer droplets are dispersed and stabilized by surfactant molecules and the nucleation site determines the particle growth mechanism (Vauthier-Holtzscherer et al., 1991). In the process for obtaining PLA, PLAGA and PCL nanoparticles by a nanodispersion methodology, the polymer is solubilized in an organic solvent and then dispersed in an aqueous solution of a steric stabilizer (Fessi et al., 1988).

For particle populations of small size, separation is diminished by increasing concentration, if the particles are of identical size or if the particles of higher ζ potential are smaller in size (Marmur, 1985). A problem often encountered in characterizing their behavior is that real colloidal dispersions are generally polydisperse in size, shape and surface properties. Therefore to fully characterize a polydisperse system, the distribution of all surface and bulk parameters is required. If the distribution of all properties is known, a polydisperse system can be considered to be a set of monodisperse systems with known properties (Dukhin and Van de Ven, 1994). When polydisperse systems are present there is an obvious need to correctly define the detection angle dependency factor, if the objective is to correlate results from PCS and DELSA methods. This is one of the more important aspects to be accounted for in the present work. The operational conditions for the DELSA method had to be optimized since it is a relatively new method with insufficient analytical

Table 2
DELSA and PCS results for PIBCA nanoparticles when polymerization medium includes dextran and/or polyvinilic alcohol (PVA)

		Medium and stabilizer				
		A. dextran 1% (pH ≈ 2.5)	G. dextran 1% PVA 1% (pH ≈ 2.8)	H. dextran 1% PVA 2% (pH ≈ 3.2)	I. PVA 1% (pH ≈ 2.8)	J. PVA 2% (pH ≈ 3.3)
DELSA	d	416 ± 29	501 ± 70	468 ± 75	519 ± 113	448 ± 13
	EM	−0.75 ± 0.43	−0.21 ± 0.17	−0.27 ± 0.35	−0.18 ± 0.09	0.12 ± 0.007
	ζ	−9.6 ± 5.5	−2.5 ± 1.6	−3.5 ± 4.6	−2.3 ± 1.14	−1.5 ± 0.03
PCS	d	192 ± 18	116 ± 14	231 ± 35	132 ± 46	202 ± 53
	PI	0.05–0.18	0.30–0.40	0.40–0.55	0.25–0.40	0.40–0.55

d, diameter (nm); EM, electrophoretic mobility (μm·cm/V·s); ζ, zeta potential (mV); PI, polydispersity index; errors (± S.D.).

experience with the particles considered. The DELSA results obtained were also compared with PCS results obtained at different standard angles. The DELSA method seems to be quite reproducible when standard operational conditions, like sample concentration and applied electrical field are maintained.

The influence of concentration was determined using samples of PIBCA nanoparticles prepared in the reference medium and diluted at concentrations from 0.1 to 10% in water. Results (Fig. 2) show that the more reproducible values in terms of particle size, E.M. and ζ potential were obtained for concentrations between 0.5–10%. Values under that limit were not used because laser light diffraction was not clearly obtained, as a result of fewer colloidal particles in suspension.

The applied electric field can also affect the results of E.M. and ζ potential obtained by DELSA. Meanwhile for values greater than 5 V, variation was minimal (Fig. 3).

As standard procedure, we routinely used a range of concentrations between 1–10% (v/v). Values of applied field were considered and expressed as an average for the results obtained at 5, 10 and 15 V. Peaks for which there is an anomalous relation between angle and frequency shift were eliminated.

Dextrans of various molecular weights and concentrations can be used as stabilizing agents for the formation of poly(isobutyl-cyanoacrylate) nanoparticles over a wide size range. Even if experimental data gave limited evidence for it, the particles were previously suggested to be sterically stabilized by a surface layer of dextran covalently linked to the matrix of the cyanoacrylate nanoparticles (Douglas et al., 1985). Our results reaffirm the importance of dextran as a steric stabilizer for cyanoacrylate polymers allowing a comparison with other previously unreported and potential steric stabilizers either used alone and in association with dextran (e.g. PVA) or only in association with dextran (e.g. PEG 2000).

Stabilization of the system can also be achieved using poloxamer or polysorbate (non-ionic surfactants) and by varying the concentration and nature of the surfactant. Some authors claimed the possibility of obtaining small-sized nanoparticles down to as low as 80 nm in diameter (Douglas et al., 1985; Seijo et al., 1990).

Experiments with polymerization medium containing poloxamer 188 show the possibility of obtaining colloidal particles of PIBCA (Table 1). Results indicate a significant difference between nanoparticles prepared with reference polymerization medium containing only dextran as steric stabilizer (A) and those nanoparticles prepared in media containing poloxamer 188 (B, C, D and E). These differences are expressed either in terms of particle size (d), electrophoretic mobility (E.M.) or zeta potential (ζ). Surface characteristics such as E.M. or ζ potential show low absolute values in both situations (always negative values). Moreover there is a marked difference between size results obtained by DELSA or by PCS. This is more obvious when poloxamer 188 concentration is increased, in the absence of dextran, but is true

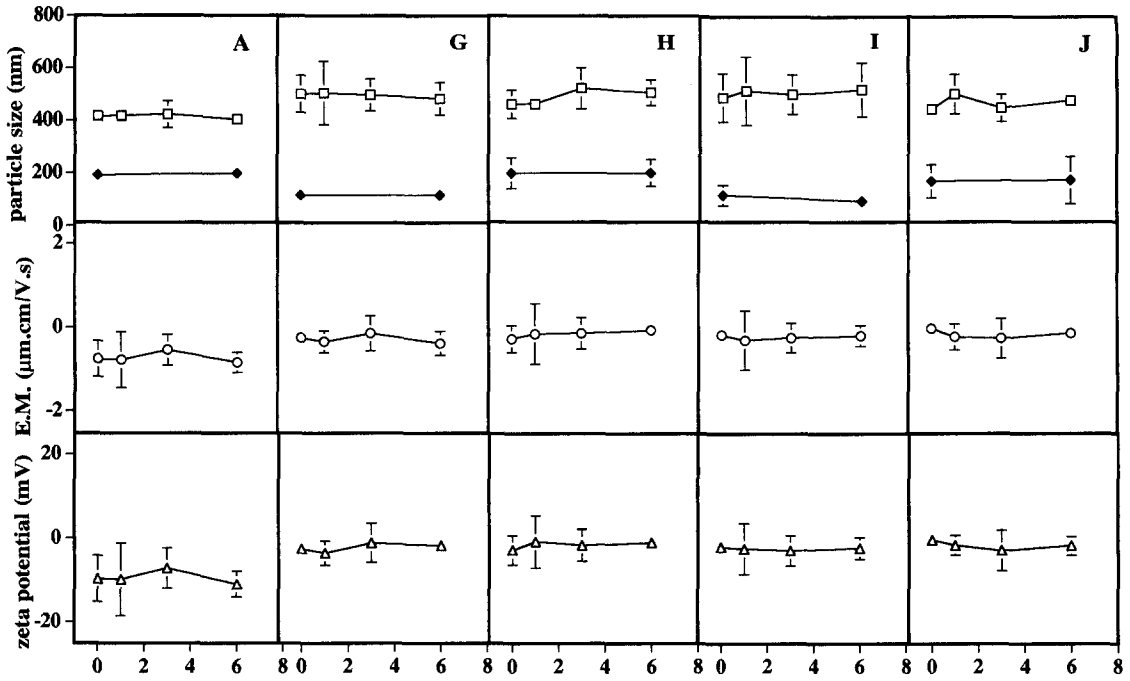

Fig. 5. Physical stability of PIBCA nanoparticles prepared in the presence of PVA, compared to reference medium (A), when particle size is determined by Coulter® DELSA 440 and PCS; electrophoretic mobility and ζ potential are determined by Coulter® DELSA 440. Open symbols (□ ○ △) are results from DELSA analysis; closed symbols are for results from PCS analysis (◆). For composition of polymerization medium, refer to Table 2. Error bars = ± S.D.

for all situations including standard polymerization media (A) as seen for their increase in polydispersity index (P.I.). We can therefore postulate that DELSA size results are not directly correlated with PCS when we are considering populations with high P.I. values.

Results obtained during the first week after polymerization show no significant difference in terms of diameter, E.M. or ζ potential for all samples examined (stored in suspension at 4°C) (Fig. 4).

Meanwhile, polymer adsorption and conformation is most likely affected by the electric double layer while the charge and potential distribution

Table 3
DELSA and PCS results for PIBCA nanoparticles when polymerization medium includes dextran and PEG 2000

		Medium and stabilizer		
		A. dextran 1% (pH ≈ 2.5)	L. dextran 1% PEG 2000 1% (pH ≈ 2.5)	M. dextran 1% PEG 2000 2% (pH ≈ 2.5)
DELSA	d	416 ± 29	377 ± 21	371 ± 37
	EM	−0.75 ± 0.43	−0.65 ± 0.34	−0.40 ± 0.1
	ζ	−9.6 ± 5.5	−8.4 ± 4.5	−5.0 ± 2.1
PCS	d	192 ± 18	212 ± 27	217 ± 26
	PI	0.05–0.18	0.05–0.15	0.01–0.15

d, diameter (nm); EM, electrophoretic mobility (μm·cm/V·s); ζ, zeta potential (mV); PI, polydispersity index; errors (± S.D.).

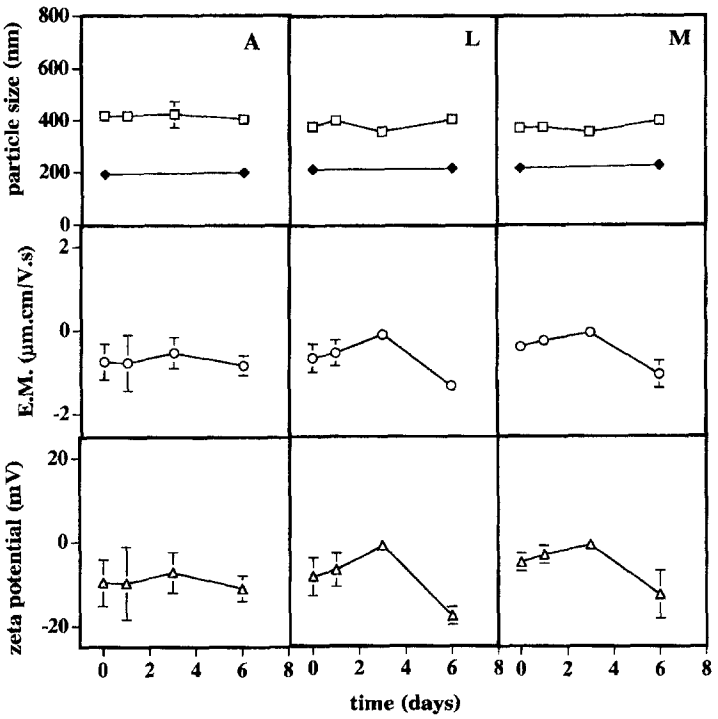

Fig. 6. Physical stability of PIBCA nanoparticles prepared in the presence of PEG 2000, compared to reference medium (A), when particle size is determined by Coulter® DELSA 440 and PCS; electrophoretic mobility and ζ potential are determined by Coulter® DELSA 440. Open symbols (□ ○ △) are results from DELSA analysis; closed symbols are for results from PCS analysis (◆). For composition of polymerization medium, refer to Table 3. Error bars = ± S.D.

of the electric double layer may be affected by the presence of the polymer adsorbed layer. For monodisperse aqueous lattices, stabilized by low molecular weight poly(ethylene oxide) poly(propylene oxide) block copolymer, both electrostatic and steric repulsions were previously found to be important in maintaining dispersion stability (Einarson and Berg, 1993).

Nanoparticles of PIBCA obtained using polymerization media with PVA also show a marked reduction in E.M. and ζ potential (Table 2) when compared with reference conditions (A). Differences between DELSA and PCS results are also clear, due to the net heterogeneity of particle populations as in the case of PIBCA nanoparticles prepared in the presence of poloxamer 188.

In systems consisting of PLA, PLAGA or PCL nanoparticles, PVA could reduce particle diameter increasing polydispersity (Jullienne et al., 1992; Scholes et al., 1993; Niwa et al., 1993; Conti et al., 1995). However, these data cannot be directly correlated since the preparation methods are different.

Colloidal stability was again preserved for at least 1 week after polymerization (samples stored in suspension at 4°C) (Fig. 5).

At pH = 3, nanoparticles could only be prepared in the presence of PEG 2000, when dextran was also included in the polymerization media. PEG 2000 by itself was not sufficient to achieve colloidal stability: nanoparticles sedimented immediately after polymerization. The presence of PEG 2000 did not significantly affect either particle size, E.M. or ζ potential for the nanoparticles obtained compared to reference conditions (A) (Table 3).

Stability studies for nanoparticles obtained with PEG and dextran as stabilizers, show no significant differences in particle size for at least 1 week. However, E.M. and ζ potential changed slightly

Table 4
DELSA and PCS results for PLA nanoparticles

		PLA: PLA 94 mg, acetone 15 ml + water 15 ml	**PLA-P:** PLA 94 mg, acetone 15 ml + poloxamer 38 mg, water 15 ml	**PCL-P:** PCL 94 mg, acetone 15 ml + poloxamer 38 mg, water 15 ml	**PLAGA-P:** PLAGA 94 mg, acetone 15 ml + poloxamer 38 mg, water 15 ml
DELSA	d	276 ± 104	186 ± 8	270 ± 11	146 ± 11
	EM	−2.7 ± 1.2	−2.2 ± 0.6	−1.9 ± 0.5	−1.7 ± 0.26
	ζ	−35.2 ± 15	−29.0 ± 8.0	−23.6 ± 6.7	−21.2 ± 3.2
PCS	d	174 ± 8	144 ± 1	219 ± 13	118 ± 3
	PI	0.02–0.12	0.009–0.12	0.002–0.10	0.007–0.12

P refers to the presence of poloxamer 188 in the external aqueous phase; d, diameter (nm); EM, electrophoretic mobility (μm·cm/V·s); ζ, zeta potential (mV); PI, polydispersity index; errors (± S.D.).

at the 6th day after polymerization (samples stored in suspension at 4°C) increasing their negative values (Fig. 6).

Ongoing studies (data not shown) have shown the dependence of these results on pH when considering the presence of PEG in polymerization medium.

In order to compare PIBCA nanoparticle results with other biodegradable polymeric drug carriers, nanoparticles of PLA, PLAGA and PCL

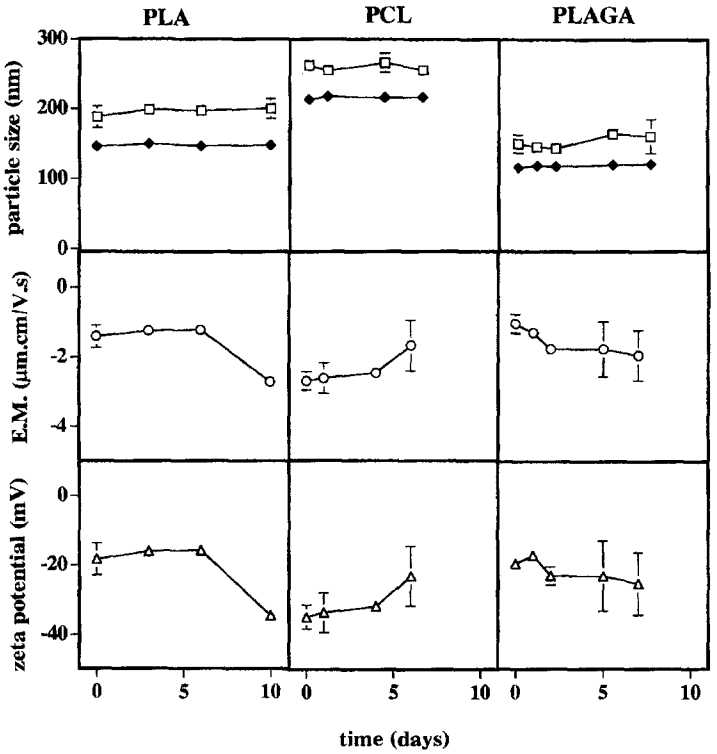

Fig. 7. Physical stability of PLA nanoparticles prepared in the presence of poloxamer 188 when particle size is determined by Coulter® DELSA 440 and PCS; electrophoretic mobility and ζ potential are determined by Coulter® DELSA 440. Open symbols (□ ○ △) are results from DELSA analysis; closed symbols are for results from PCS analysis (◆). Error bars = ± S.D.

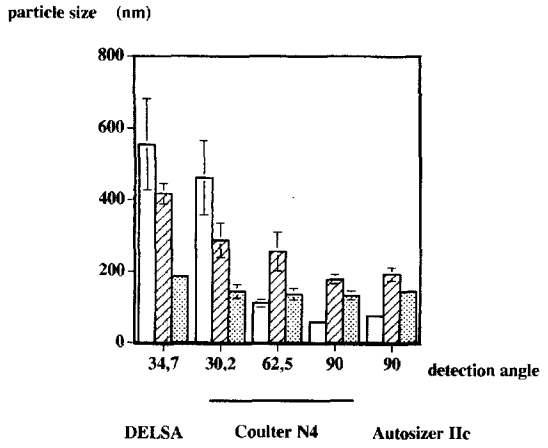

Fig. 8. Influence of detection angle on particle size results obtained for PIBCA nanoparticles prepared either in the presence of dextran (medium A) (■) or poloxamer 188 at a concentration of 2% (medium E) [□], and when considering a monodisperse population (taking into account the polydispersity index values obtained by PCS) of PLA nanoparticles (XXX). Nanoparticles were analyzed by PCS (Malvern Autosizer IIc with detection at 90° and Coulter® N4MD at three different detection angles) and DELSA (Coulter® DELSA 440 with a detection angle of 34.2°). Error bars = ± S.D.

were also prepared. Results show that nanoparticles of PLA, PLAGA and PCL can be obtained easily and particle size results are in correspondence with what should be expected for a unimodal population (Table 4). The presence of poloxamer 188 is necessary to produce stable formulations, otherwise PLA nanoparticles will sediment forming aggregates. Moreover, these nanoparticles are quite stable for at least 1 week in suspension (at 4°C) as shown by particle size analysis, electrophoretic mobility and ζ potential evaluation (Fig. 7).

In order to evaluate the angle dependency of particle size results, PIBCA nanoparticles prepared with reference medium or including only poloxamer 188 as steric stabilizer, were analyzed for particle size measurements at different detection angles using a Coulter® N4MD. Results were also evaluated by DELSA 440 and Malvern Autosizer IIc, and were compared to PLA nanoparticles in the same conditions.Results (Fig. 8) show a marked influence of detection angle in particle size measurements when PIBCA nanoparticles are considered. These differences are related to particle size heterogeneity determined by the PCS polydispersity index of the populations analyzed and are more important when poloxamer 188 is used as the only steric stabilizer. Meanwhile, monodisperse populations such as PLA nanoparticles show results largely independent of detection angle when the three pieces of apparatus are considered.

Comparing with our results from Table 1, when PCS is used, and relating to the variability shown in Fig. 8, we could assume that results are poor when using only a 90° detection angle in PCS.

It is important to understand the role of surfactants like poloxamers and other steric stabilizers (e.g. dextran, PVA, PEG) in the polymerization mechanism, mainly the nucleation and particle growth steps. The evaluation of size and surface properties is only one of the aspects to be considered. In fact, complementary studies are being performed in order to fully characterize the physicochemical interactions between steric stabilizers and cyanoacrylic or poly-lactide polymers, by vibrational spectroscopy, differential scanning calorimetry and molecular weight distribution analysis. Our results indicate the importance of establishing adequate methods for particle size analysis and surface characterization, based upon the intrinsic nature of the colloidal systems, since a simple 90° detection in a PCS analysis will be insufficient and needs to be coupled with full angle length and distribution studies. Moreover, we have established different size distributions and surface characteristics resulting from different steric stabilizers, closely correlating with previously designed theoretical analysis of the polymerization mechanism for cyanoacrylic polymeric drug carriers (Vauthier-Holtzscherer et al., 1991). Ongoing complementary studies strengthen these conclusions, showing the close interaction between the chemical nature of steric stabilizers and the polymerization mechanism for PIBCA nanoparticles.

Acknowledgements

The authors are grateful to Dr Patrick McDonnell (Loctite Co., Ireland) for providing the

cyanoacrylate monomer. This work was financed by project PBIC/C/BIO/1256/92 (JNICT, PORTUGAL). C. Lourenco and M. Teixeira hold grants from JNICT (Portugal). The collaboration and helpful discussions of Professor Margarida Figueiredo (Chemical Engineering Department, University of Coimbra, Portugal) also allowing the authors to use the photon correlation spectroscopy equipment is welcomed and greatly appreciated. The technical assistance of Mrs Joaquina Cristovão and Luísa Lopes are to be mentioned.

References

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

Bazile, D., Prud'homme, C., Bassoullet, M.T., Marlard, M., Spenlehauer, G. and Veillard, M., Stealth Me.PEG-PLA nanoparticles avoid uptake by the mononuclear phagocytes system. *J. Pharm. Sci.*, 84 (1995) 493–498.

Bazile, D., Veillard, M., Prud'homme, C. and Michalon, M., Nanoparticules à base d'un copolymère à blocs de polyoxyde d'éthylène et acide polylactique. European Patent no. 0520888 A1, 1992.

Conti, B., Genta, I., Modena, T. and Pavanetto, F., Investigation on process parameters involved in polylactide-co-glycolide microspheres preparation. *Drug Dev. Ind. Pharm.*, 21 (1995) 615–622.

Couvreur, P., Kante, B., Roland, M., Guiot, P., Baudini, P. and Speiser, P., Polycyanoacrylate nanocapsules as potential lysosomotropic carriers: preparation, morphological and sorptive properties. *J. Pharm. Pharmacol.*, 31 (1979) 331–332.

Couvreur, P., Roland, M. and Speiser, P., Procedé de préparation de particules submicroscopiques, particules ainsi obtenues et compositions pharmaceutiques ques les contenant. European Patent no. 0064967, 1985.

Douglas, S.J., Illum, L. and Davis, S.S., Particle size and size distribution of poly(butyl 2-cyanoacrylate) nanoparticles: II. Influence of stabilizers. *J. Colloid. Interf. Sci.*, 103 (1985) 154–163.

Dukhin, A.S. and Van de Ven, T.G.M., Electrokinetic characterization of polydisperse colloidal particles. *J. Colloid. Interf. Sci.*, 165 (1994) 9–18.

Einarson, M.B. and Berg, J.C., Electrosteric stabilization of colloidal latex dispersion. *J. Colloid. Interf. Sci.*, 155 (1993) 165–172.

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

Fessi, H., Puisieux, F. and Devissaguet, J.P., Procedé de preparation de systèmes colloidaux dispersibles d'une substance, sous forme de nanocapsules. European Patent no. 0 274 961, 1988.

Gref, R., Minamitake, Y., Peracchia, M.T., Trubetskoy, V., Torchilin, V. and Langer, R., Biodegradable long-circulating polymeric nanospheres. *Science*, 263 (1994) 1600–1603.

Illum, L., Davis, S.S., Müller, R.H., Mak, E. and West, P., The organ distribution and circulation time of intravenously administered colloidal carriers sterically stabilized with a blockcopolymer-poloxamine 908. *Life Sci.*, 40 (1987) 367–374.

Jullienne, M.C., Alonso, M.J., Amoza, J.L.G. and Benoît, J.P., Preparation of poly-(D,L-lactide/glycolyde) nanoparticles of controlled particle size distribution: application of experimental designs. *Drug Dev. Ind. Pharm.*, 18 (1992) 1063–1077.

Marmur, A., The effect of particle concentration on difficult electrophoretic separations. *J. Colloid. Interf. Sci.*, 103 (1985) 337–342.

Müller, R.H., Lherm, C., Herbort, J., Blunk, T. and Couvreur, P., Alkylcyanoacrylate drug carriers: I. Physicochemical characterization of nanoparticles with different alkyl chain length. *Int. J. Pharm.*, 84 (1992) 1–11.

Napper, D.H., *Polymeric Stabilization of Colloidal Dispersions*, Academic Press, London, 1983.

Niwa, T., Takeuchi, H., Hino, T., Kunou, N. and Kawashima, Y., Preparations of biodegradable nanospheres of water-soluble and insoluble drugs with D,L-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. *J. Control. Release*, 25 (1993) 89–98.

Peracchia, M.T., Gref, R., Minamitake, Y., Domb, A. and Langer, R., PEG-coated nanospheres for intravenous drug delivery and targeting. *Proc. Int. Symp. Control. Release Bioact. Mater.*, 21 (1994) 513–514.

Scholes, P.D., Coombes, A.G.A., Illum, L., Davis, S.S., Vert, M. and Davies, M.C., The preparation of sub-200 nm poly(lactide-co-glycolide) microspheres for site-specific drug delivery. *J. Control. Release*, 25 (1993) 145–153.

Seijo, B., Fattal, E., Roblot-Treupel, L. and Couvreur, P., Design of nanoparticles of less than 50 nm diameter: preparation, characterization and drug loading. *Int. J. Pharm.*, 62 (1990) 1–7.

Vauthier-Holtzscherer, C., Benabbou, S., Spenlehauer, G., Veillard, M. and Couvreur, P., Methodology of the preparation of ultra-dispersed polymer systems. *Stp Pharma Sci.*, 1 (1991) 109–116.

Wisse, E. and Leeuw, A.M., Structural elements determining transport and exchange process in the liver. In Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E. (Eds), *Microspheres And Drug Therapy, Pharmaceutical, Immunological and Medical Aspects*, Elsevier, Amsterdam, 1984, pp. 1–23.

Woodle, M.C., Collins, L.R., Sponsler, E., Kossovsky, N., Papahadjopoulos, D. and Martin, F.J., Sterically stabilized liposomes: reduction in electrophoretic mobility but not electrostatic surface potential. *Biophys. J.*, 61 (1992) 902–910.