Poly(Alkylcyanoacrylate) Nanocapsules: Physicochemical Characterization and Mechanism of Formation

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Nanocapsules of poly(isobutylcyanoacrylate) and poly(isohexylcyanoacrylate) were prepared by addition of the monomer to an organic phase and subsequent mixing of the organic phase to an aqueous phase containing poloxamer 188, 238 or 407. Gel permeation chromatography indicated that in contrast to literature reports, polymerization occurred in the organic phase and nanocapsules were obtained by interfacial precipitation of the polymer without any significant change of the molecular weight. Addition of SO_2 to the organic phase before the introduction of the monomer allowed preparation of nanocapsules with a lower molecular weight. Nanospheres were prepared in a similar way albeit using an organic phase that was completely miscible within the aqueous phase so that solid spheres were obtained. Density gradient centrifugation revealed that nanocapsules had a density intermediate between nanospheres and an emulsion prepared in the same way without addition of monomer to the organic phase. Further, the process used to prepare nanocapsules had a high yield since no oil droplets or nanospheres were obtained by this process. Zeta potential of the nanocapsules and spheres was found to be related to the molecular weight of the polymer: values as high as ≈ -42 mV were obtained for low molecular weight nanocapsules (MW $\approx$ 1000) compared to ≈ -10mV for the emulsion and the high molecular weight nanocapsules (MW $\approx$ 100 000). Surface charge of the nanocapsules and molecular weight of their polymeric wall conditioned the adsorption capacity of poloxamers. Moreover, the highest adsorption was measured with the most hydrophobic poloxamer. These observations agree with previous work conducted on hydrophobic surfaces.

KEY WORDS: nanocapsules; colloidal drug carrier; poly(alkylcyanoacrylate); poloxamers; characterization.

INTRODUCTION

Colloidal drug carriers made of biodegradable polymers are of interest for the controlled delivery of drugs at specific sites in the body. Poly(alkylcyanoacrylate) nanospheres have been thoroughly studied with applications ranging from ophthalmic delivery to carriers in cancer chemotherapy (1). They are generally prepared by polymerization of alkylcyanoacrylate in an acidic aqueous medium containing the drug to be adsorbed. Their high surface area and porosity allow the adsorption of significant amounts of a wide variety of drugs provided they are not too hydrophobic to be dissolved in the aqueous medium where the nanospheres are prepared. More recently, nanocapsules of poly(isobutylcyanoacrylate) (PIBCA), composed of an oily core surrounded by a polymeric film have been developed to enable better delivery of lipophilic compounds (2,3). They are obtained by mixing an organic phase containing the monomer and the drug to be encapsulated into an aqueous dispersion medium. Conventionally, nanocapsules and nanospheres are considered to be members of a broader family termed nanoparticles, which includes all colloidal particles of less than 1 μm.

The prospect of using nanocapsules by several routes and with different drugs has been supported by the successful encapsulation of phthalocyanines (4), cyclosporin (5) and by the oral administration of insulin (6). The potential toxicity for macrophages has become of lesser concern with the advent of poly(isohexylcyanoacrylate) (PIHCA) nanocapsules, a more slowly biodegradable analog. In comparison to nanospheres, nanocapsules have a reduced clearance rate from the blood compartment (7) and a lower liver uptake, which might be related to their firmly-bound hydrophilic coating (3).

While poly(alkylcyanoacrylate) nanospheres are prepared by a process of emulsion polymerization (8), interfacial polymerization is believed to be the predominant mechanism for the formation of poly(alkylcyanoacrylate) nanocapsules (2). The objective of the present study was to elucidate the formation mechanism and structure of PIHCA nanocapsules, through the analysis of bulk properties, such as nanoparticle density and polymer molecular weight, and of surface characteristics such as zeta potential and poloxamer adsorption.

MATERIALS AND METHODS

The isobutylcyanoacrylate monomer (IBCA) and dextran materials were purchased from Sigma Chemical Co. (St. Louis, MO). Isohexylcyanoacrylate (IHCA) was received from Sopar S.A. (Belgium). Miglyol 829® (caprylic/capric diglyceryl succinate), a biocompatible oil, was obtained from Dynamit Nobel (Montréal, Canada). Poloxamers 188, 238 and 407 belong to the Pluronic® family from BASF (Montréal, Canada). Stabilised tetrahydrofuran (THF) (Anachemia, Rouses Point, NY) was used for gel permeation chromatography and poloxamer adsorption studies.

Nanoparticles preparation

Low molecular weight nanocapsules (PIHCA-L and PIBCA-L) and nanospheres were prepared according to a published method (3). Isohexylcyanoacrylate or isobutylcyanoacrylate monomer (10mg) previously saturated with SO_2, was mixed with 30mg of Miglyol 829® and 2ml of 100% pure ethanol dehydrated on molecular sieves (Aldrich Chemical Co.; #20,859-0). This organic solution was then slowly added (0.5ml/min) with a peristaltic pump to 10ml of a 0.25% w/v poloxamer 188, 238 or 407 aqueous solution under stirring. The nanocapsules obtained were purified by centrifugation at 60 000g for 1 hour and redispersed in an equal volume of bidistilled water. Nanospheres were prepared us-

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ing the same procedure without addition of Miglyol®, a process which differs from the usual method (8) where the monomer is directly added to an acidic aqueous medium.

High molecular weight PIBCA nanocapsules (PIBCA-H) were obtained as above but with no SO_2 added to the monomer. High molecular weight PIHCA nanocapsules (PIHCA-H) were prepared with an organic solution consisting of a 1:1 mixture of ethanol and acetone instead of pure ethanol. In this case, the IHCA monomer was prepolymerized in a 0.01% v/v pyridine solution in ethanol, separated and dried before dissolution into the organic phase. For comparison purposes, emulsions were prepared using the same process, in which no cyanoacrylate was added to the organic phase.

Nanoparticles density

Isopycnic centrifugation in a density gradient of colloidal silica (Percoll, Pharmacia LKB, Sweden) was used to determine the density of the nanoparticles. The nanoparticles were first concentrated 20 fold by centrifugation/redispersion and 50µl of the concentrate were added to 7 ml of Percoll 45%v/v in water with a final concentration of 0.15M NaCl. Centrifugation was performed in an angle head rotor (Beckman 75Ti) at 4°C and 15 000g for 3hrs. A balanced tube containing Density Marker Beads (Pharmacia LKB) of known density was used to monitor the gradient and was treated identically to the one containing the experimental samples. During centrifugation, a gradient is generated *in situ* and calibrated by plotting the distance from the top of the meniscus to each band of density marker beads.

Gel permeation chromatography (GPC)

The nanoparticles (nanocapsules or nanospheres) were centrifuged (7ml) 1 hour at 60 000g. The supernatant was discarded and the pellet was freeze-dried. The pellet was then dissolved in THF and the solution was filtered using a 0.5µm Millex-SR filter (Millipore Corp.). The sample was injected (50µl) in a U6K injector fitted to a Shodex KF-803 and KF-804 high resolution GPC column (Millipore Corp.) with THF as eluent. The refractive index was determined by a Waters 410 Differential Refractometer (40°C) and integrated by a GPC computerized module (Waters M745B). A good agreement was found by vapour pressure osmometry where the organic phase was used as solvent. In this case, both polymers with SO_2 show a molecular weight of $\approx$1000 using indomethacin as the standard.

Zeta potential

The nanoparticles were purified and suspended in KCl 10^{-3}M and their zeta potential measured by Laser Doppler Anemometry (Malvern Zetasizer® IIc) at 25°C. Their velocity in an electric field was evaluated by the shift caused in the interference fringe produced by the intersection of two laser beams. The electrophoretic mobility was then used to calculate the zeta potential according to the following equation:

$$\zeta = \frac{U_E 4\pi\eta}{\varepsilon}$$

where ζ is the zeta potential, U_E the electrophoretic mobility, η the viscosity of the medium and ε the dielectric constant.

Poloxamer adsorption

Poloxamers are ABA block copolymers where A is poly(ethylene oxide) (PEO) and B is poly(propylene oxide) (PPO). On the basis of the molecular weight and the fraction of ethylene oxide, poloxamers 188, 238 and 407 are modelled as $(PEO)_{75}(PPO)_{30}(PEO)_{75}$ (M_W = 8350, HLB = 29), $(PEO)_{97}(PPO)_{39}(PEO)_{97}$ (M_W = 10 800, HLB = 28) and $(PEO)_{98}(PPO)_{67}(PEO)_{98}$ (M_W = 11 500, HLB = 22) respectively. These values are calculated from the stoichiometry of the polymerization where the two hydrophilic PEO blocks were assumed to be statistically equal in length. The amount of poloxamer adsorbed to the nanocapsules was determined by GPC. Nanocapsules of PIHCA-L were prepared in various concentrations of poloxamer and washed twice in 7ml of water by centrifugation (60 000g × 1h00). Nanocapsules of PIBCA-H were similarly prepared but centrifuged at 130 000g and the pellet was incubated in 2 ml of a 1:1 mixture of acetone and ethanol at 50°C for 16 hours. We had previously verified that this process reduced the molecular weight of the polymer to such an extent that chromatographic separation from poloxamer was easy to achieve. The organic solvent was allowed to evaporate during the incubation so that the final residue was completely dry and suitable for GPC analysis. The residues were then readied for GPC as explained above. Only the KF-803 column was used in this analysis. Poloxamer concentrations in the samples were determined from the peak height of the chromatogram using a calibration curve of pure poloxamer in THF. All measurements were made after two washings of nanocapsules by centrifugation and redispersion. Poloxamers adhering to the nanoparticles were found to be firmly bound and the amount released during the two consecutive washings was negligible in all cases studied. No determinations were made with poloxamer 188 since in this case, the emulsion and the PIHCA-L nanocapsules were unstable, as evidenced by erratic size distribution.

Poloxamer determination by GPC is sensitive and straightforward; but pure poloxamer 407 showed a bimodal weight distribution on the GPC chromatogram with the first peak (higher MW) averaging 80% of the total area. It appeared that only the first peak appeared in the nanoparticles chromatogram, indicating that only pure poloxamer had adhered to the nanoparticles. Consequently the adsorption data were expressed in percentage of pure poloxamer.

RESULTS AND DISCUSSION

Density of nanoparticles

It has been demonstrated that the main mechanism leading to the formation of nanocapsules is the spontaneous emulsification of the organic phase upon mixing with the aqueous phase. This emulsification was clearly shown to be mainly controlled by the composition of the organic phase (3). Since nanospheres and nanocapsules are both prepared from the mixture of two miscible solvents, the exact nature of the dispersed system must be assessed to monitor the

possible concomitant formation of nanospheres when nanocapsules are being prepared. In terms of density, the nanocapsules were found to range between emulsion droplets (no polymer) and nanospheres (solid polymer) as seen on Fig. 1. Unlike the observations reported by Gallardo et al. (9), the method used to prepare the nanocapsules in this report yielded no nanospheres since no band was detected near the nanospheres density range. In addition, a slight but significant increase in density was noticed when the amount of polymer (MW $\approx$ 1000) was doubled. These observations are in good agreement with our previous work (3) where we have reported that increasing the quantity of monomer allows the preparation of nanocapsules with a higher polymer content and, consequently, a higher density.

Molecular weights of poly(alkylcyanoacrylates)

Because of interferences with Miglyol®, it was not possible to determine accurately the molecular weight of poly(alkylcyanoacrylates) in nanocapsules. Therefore, molecular weights were analyzed only in preparations of nanospheres and in the organic phases used to prepare them. Results, summarized in Table I, show that polymerization occurs already in the organic solution. This is not surprising since ethanol has a relatively strong nucleophilic character and is therefore likely to initiate the polymerization reaction, as previously shown by Leonard et al. (10). In addition, when the molecular weights of the cyanoacrylates in the organic solution and in the final nanospheres preparations are compared, no significant differences are noted, indicating that the reaction in the organic solution goes to completion. In view of these findings, one can assume that the mechanism of formation of nanospheres corresponds in fact to the precipitation of a pre-formed polymer in a large excess of non-solvent. Following addition to the water phase, the polymer precipitates in the form of nanospheres when no Miglyol® is present. In the presence of Miglyol®, the polymer is deposited at the oil/water interface, resulting in the formation of nanocapsules. These findings are in contradiction with a previous hypothesis (2,9) where cyanoacrylates were assumed to polymerize at the oil/water interface. Gallardo et al. (9) found that the molecular weight of poly(alkylcyanoacrylates) in the organic solution was around 1000 without addition of SO_2 and increased to around 100 000 after addition of the organic solution to the aqueous phase. The molecular weight of 1000 in the organic solution is significantly lower than what we found for SO_2-free polymer. We attribute this difference to the effect of the process used to remove ethanol on the stability of the polymer. Indeed, when applying the process used by Gallardo et al. to remove ethanol (evaporation at 40°C) we have observed a progressive reduction of molecular weight with time, indicating that the polymer was progressively being degraded. In contrast, our process (SO_2 or N_2 flow at room temperature) did not affect the molecular weight of the polymer.

Sulphur dioxide has been used as a polymerization inhibitor. When sulphur dioxide was dissolved in the monomer prior to addition to the organic solution, polymerization did occur but was limited and a molecular weight of around 1000 was obtained (see Table I). When the organic solution was mixed to the aqueous phase, no increase of the molecular weight was detected, indicating that the polymerization which had occurred in the organic solution had reached completion.

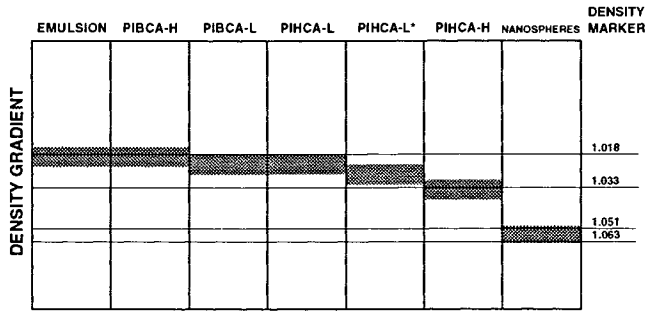

Figure 1: Banding of nanocapsules preparations in gradients of Percoll with the position of the Density Marker Beads at their buoyant density (g/ml). *PIHCA-L nanocapsules with twice the amount of polymer.

Zeta potential

Nanospheres, nanocapsules and emulsion were all prepared in the presence of poloxamer 407 for stabilization. In addition, three other poloxamers were tested with nanocapsules. It must be emphasized that, for comparative purposes, all measurements were made in 1mM KCl, therefore making any attempt to correlate the data with the possible in vivo fate of the nanoparticles tentative. As shown in Table II, nanocapsules prepared with poloxamer 407 and with a SO_2 saturated monomer (PIBCA-L, PIHCA-L) possess a stronger negative zeta potential as compared to an emulsion. Therefore, one can conclude that the increase in net surface charge results from the presence of a polymeric wall at the oil/water interface. The negative charge might be associated with the carbanion which has been assumed to be present at the termination end of the polymeric chain (11). This carbanion is indeed relatively stable and can only be neutralized by strong acidity (11). With an equivalent mass of polymer, a small molecular weight yields higher amounts of small chains per nanocapsule, thus resulting in a higher number of carbanions and a higher charge density. In this respect PIBCA-L and PIHCA-L were found to be equivalent since the same zeta potential was calculated for both preparations, having similar molecular weight. In contrast, the type of poloxamer used to coat the nanoparticles appears to have an influence on the zeta potential. Since the zeta potential of coated particles is a complex function of several variables, i.e. particle size, surface roughness and coating thickness among others (12), which can all be variously affected by the coating, it is tentative to present an explanation to the effect of the different poloxamers on zeta potential. It is interesting to note that positively-charged nanocapsules could be obtained by simply using a 500 000 MW dextran coating, an approach which might be used to modify the body distribution of colloidal drug carriers and should prove toxicologically safer than the DEAE-dextran used to date.

Poloxamer adsorption

The results shown on Fig. 2 represent the amount of poloxamer adsorbed per unit area of nanoparticle surface.

Table I. Molecular weights parameters of the polymers in the organic solution and the aqueous phase of nanocapsules

	Organic solution				Aqueous phase			
Polymer	Mw[a]	Mn[b]	Mp[c]	P.I.[d]	Mw	Mn	Mp	P.I.
PIHCA-L	960	682	500	1.41	1446	1142	1338	1.27
PIBCA-L	867	577	450	1.5	1957	1387	1433	1.41
PIHCA-H	162 141	114 882	189 273	1.41	196 230	121 159	266966	1.62
PIBCA-H	100 609	56 462	148 031	1.78	123 908	70 509	189 273	1.76

[a] Weight average molecular weight.
[b] Number average molecular weight.
[c] Molecular weight derived from the elution time at the peak of the chromatogram.
[d] Polydispersity index. A measurement of scatter in molecular weight distribution defined as Mw/Mn.

The surface area was determined from the mean volume-surface diameter obtained from at least 250 measurements on transmission electron photomicrographs. Figure 3 shows the spherical shape of the nanocapsules and their true diameter after staining. The following equation was used to calculate the mean volume-surface diameter:

$$d_{vs} = \frac{\Sigma nd^3}{\Sigma nd^2}$$

where, d_{vs} is the volume to surface mean diameter, n the number of particles and d the projected diameter of the particles. Table III shows the mean values of diameters and specific surface area for the three systems studied assuming that particles are spherical in shape.

With increasing concentrations of poloxamer in the aqueous phase, the quantity found at the nanoparticles/water interface increases gradually until a plateau is attained. One can observe that the adsorption of poloxamer 407 is higher than poloxamer 238 for the three colloidal systems studied. This study of a homologous series of poloxamers with practically the same molecular weight and PEO content revealed that the attachment of the poloxamer to the emulsion droplets is highly dependent on the PPO chain length. Poloxamer 407 has a longer PPO segment than poloxamer 238. Thus, the PPO chains of poloxamer 407 can be easily dissolved into the oily phase and be arranged in a conformation allowing extension of the PEO segments into the water phase. Homo-

Table II. Zeta potential of nanoparticles prepared with different cyanoacrylates and stabilizers

	Stabilizers*	Zeta potential (mV ± SD)
PIHCA-L nanocapsules	Poloxamer 407	−29.31 ± 1.39
	Poloxamer 407 dextran@	33.91 ± 2.28
	Poloxamer 407 DEAE-dextran#	46.01 ± 2.15
	Poloxamer 403	−23.18 ± 4.76
	Poloxamer 238	−42.37 ± 2.52
	Poloxamer 234	−37.09 ± 3.55
PIHCA-H nanocapsules	Poloxamer 407	−7.54 ± 0.36
PIBCA-L nanocapsules	Poloxamer 407	−30.58 ± 1.11
PIBCA-H nanocapsules	Poloxamer 407	−8.07 ± 1.29
PIHCA-L nanoparticles	Poloxamer 407	−22.04 ± 1.34
	Poloxamer 407 DEAE-Dextran	45.43 ± 0.97
EMULSION	Poloxamer 407	−9.68 ± 0.67

* All poloxamers at 0.25% w/v, all Dextrans at 1% w/v.
@ Dextran sulfate 500 000.
# Diethylaminoethyl-dextran 500 000.

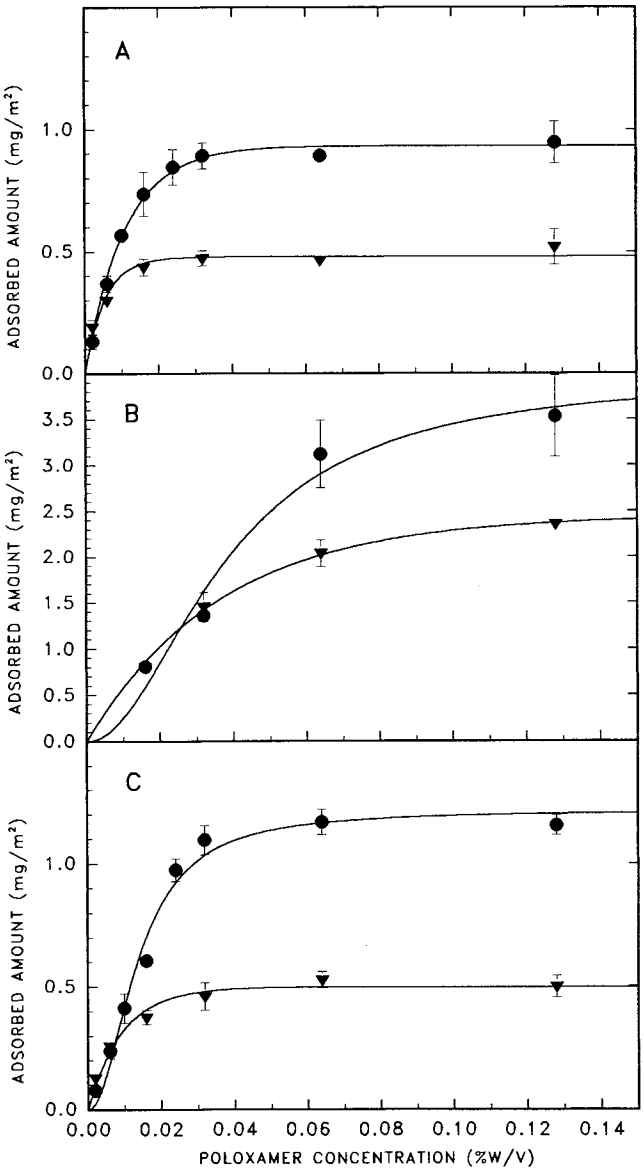

Figure 2: Specific adsorption of poloxamer 238 (filled triangle) and poloxamer 407 (filled circle) on PIHCA-L nanocapsules (A), PIBCA-H nanocapsules (B) and emulsion (C). (n = 4)

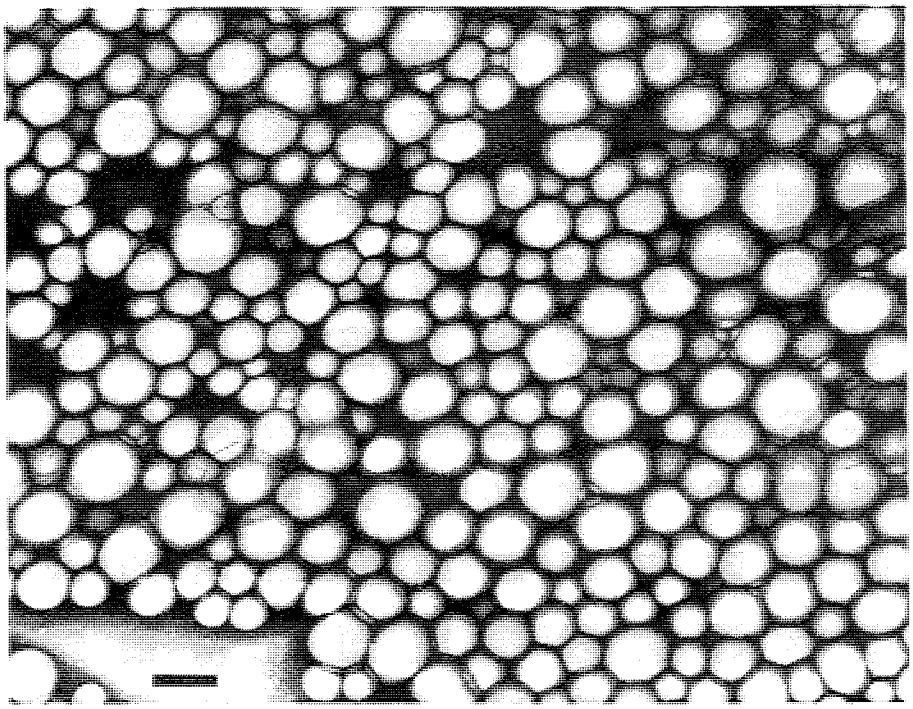

Figure 3: Electronic photomicrograph of PIHCA-L nanocapsules from negative staining with sodium phosphotungstate. The sample was prepared by an adhesion method on glow discharge treated grids (bar = 200nm).

logue 238 has shorter PPO chains and thus a lower affinity for oil droplets giving lower adsorbed amounts. Finally poloxamer 188, with an even shorter PPO moiety, did not allow satisfactory stabilization of the dispersed oil droplets during two washing cycles.

In the case of higher molecular weight PIBCA-H nanocapsules (see Fig. 2B), a higher affinity of the poloxamer for the nanocapsules is observed, suggesting that the mechanism of association may differ. The polymer is certainly less hydrophilic than short PIHCA chains and the nanocapsules have a lower net charge. Therefore, it is reasonable to assume that, in this case, poloxamer might adsorb on the polymeric surface rather than partition between phases of different hydrophilicity. Adsorption on hydrophobic surfaces has been characterized by a denser segment density distribution where the PPO moieties are enriched at the proximity of the surface and the PEO moieties are protruding into the outer layer (13,14,15). This situation, characterized by high adsorption values, is likely to prevail with the PIBCA-H nanocapsules. Van de Steeg et al. reported values on hydrophobicity gradient surfaces which were in the same range, with a plateau at 2.7mg/m^2 (16).

The high zeta potential values obtained for PIHCA-L and PIBCA-L nanocapsules was expected from the above conclusions (see Table II). It is known that latex nanospheres have their zeta potential significantly reduced by coating with non-ionic surfactants (12). The reduction is related to the extent of the diffuse layer and the poloxamer coating layer thickness. The high values observed with the PIHCA-L nanocapsules could be attributed to a thin coating layer with reduced tail formation.

Rapid uptake by the mononuclear phagocytic system (MPS) is the major obstacle to the development of a colloidal drug carrier for systemic administration (17). It has been described that hydrophilic coating with poloxamers can protect colloids such as latex particles against MPS recognition and elimination. Several authors have claimed that good stability, tightly bound PPO segment and long PEO chains extending away from the interface are required for optimum protection (12,18). In this respect, poloxamer 407 should provide the most hydrophilic coating among the three homologues studied and should be most effective in preventing MPS recognition and uptake of nanocapsules.

Table III. Volume-surface mean diameter (d_{vs}) and specific surface (Sw) of nanocapsules

	d_{vs} (nm)	Sw (m^2/g)
PIHCA-L nanocapsules	155	26.1
PIBCA-H nanocapsules	178	29.9
EMULSION	186	31.3

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