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Study of the mechanisms of formation of nanoparticles and nanocapsules of polyisobutyl-2-cyanoacrylate

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Summary

Biodegradable colloids suitable for use as drug delivery systems can be formed by *in situ* polymerization of isobutylcyanoacrylate monomers. In this work we have studied the mechanisms of formation of colloidal systems of PIBCA obtained in the presence of oil and ethanol. For this different analytical techniques have been used (TEM, PCS, GPC). The results have shown that the diffusing ethanol was the most important factor which leads to the structure and colloidal characteristics of the particles by convective effects, the oil playing the role of a monomer support. The existence of an ideal oil/ethanol ratio (2:100) seems to result from the combination of both mechanisms. In the absence of oil and ethanol, IBCA polymerization occurs within micelles appearing in the aqueous phase. However, in the case of nanocapsule formation, owing to the solubility of the monomer in the organic phase and the diffusibility of ethanol, polymerization can develop at the interface. In this latter case, the formation of a double population of nanocapsules and nanoparticles probably arises from the breakdown of the same interfacial polymer.

Introduction

In order to improve the efficacy of certain drugs by modifying intracellular penetration or their tropism towards a particular organ, different colloidal systems have recently been devel-

oped. Colloids may be obtained from natural macromolecules such as gelatin and albumin (Marty et al., 1978). Another kind of system uses synthetic polymers. These may be preformed, or formed at the same time as the colloidal system by *in situ* polymerization. Couvreur and others have developed polyalkylcyanoacrylate nanoparticles obtained by an emulsion polymerization process (Millich et al., 1977; Couvreur et al., 1979). Electron microscopy indicates that they consist of a dense polymeric matrix, within which an active substance may be entrapped (Rollot et al., 1986).

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More recently, a new colloidal system, nanocapsules, has been prepared using a similar monomer mixed with ethanol, Miglyol 812 and water (Al Khouri et al., 1986). On observation by transmission electron microscopy, their structure seems to be that of a nanodroplet of oil surrounded by a polymeric wall with a thickness of about 5 nm (Rollot et al., 1986). They can therefore act as reservoir system for a lipid-soluble drug. The investigative techniques used them did not provide any information about the physico-chemical mechanisms leading to the formation of nanocapsules, however, the presence of a polymeric wall and the composition of the system suggest that the colloid was formed via an interfacial polymerization process (Al Khouri et al., 1986).

This work was designed to elucidate the factors which favour the formation of either a matrix or a reservoir system and, in particular, to determine the effects of the organic components (ethanol and oil) on the morphological and structural properties of the particles. The polymerization process is one indicator of the events taking place at the moment of colloid formation. The isobutyl-2-cyanoacrylate monomer used in this study undergoes anionic polymerization (Donnelly, 1977) as shown in Fig. 1.

The degree of polymerization, and therefore the molecular weight, depend on a balance between initiation, propagation and termination (Eromosele et al., 1989). The number of growing chains depends on the concentration of initiators (OH^- , CH_3O^- , CH_3COO^- , CN^-). For the same quantity of monomer, when the number of live

chains is high, the degree of polymerization is low. Similarly, the degree of polymerization is reduced when the concentration of terminating agents (H^+) is increased (Donnelly, 1977; Pepper et al., 1978). The concentration of initiating and terminating agents available to the monomer will be determined by the physico-chemical system in which the components are dispersed at the moment of colloid formation.

In the case of a system composed of two immiscible phases, such as an emulsion, the presence and concentration of the solutes in the different phases are a function of the polarity of the solute molecules and the dielectric constant of the medium. In contrast, at an interface between an aqueous and an organic medium there can exist considerable local variations in properties which can themselves cause changes in the interfacial properties, in a dynamic system. For example, spontaneous emulsification depends on the interfacial tension. Thus, although the degree of polymerization depends on the propagation reaction, other characteristics of the colloid formed, such as particle size and morphology, depend on interfacial phenomena.

Many factors could influence the type of colloid formed on polymerization of isobutyl-cyanoacrylate: the nature of the aqueous phase, pH, composition of the organic phase (monomer, oil, ethanol), ratio of monomer to the aqueous phase, and emulsification conditions. We have chosen to study two important parameters – the quantity of oil and that of ethanol – using a parametric method in which the polymerization

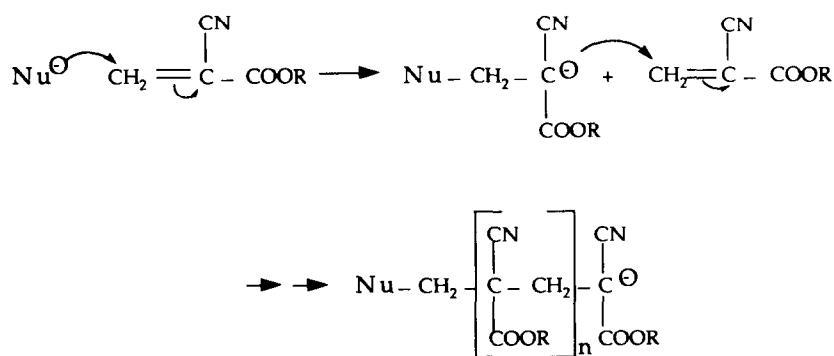


Fig. 1. Anionic polymerization of alkylcyanoacrylates.

conditions were progressively varied between two limits corresponding to the optimal conditions for the formation of nanoparticles and nanocapsules.

Materials and Methods

Materials

IBCA (Sigma, France), absolute ethanol (Pro-labo, France), Synperonic PE-F68 (ICI, France), Miglyol 812 (Dyna France) and THF (Carlo Erba, France) were obtained from the indicated sources.

Methods

Preparation of polymeric systems

Preparation of nanoparticles The aqueous phase throughout this study was 50 ml of a 0.4% solution of Synperonic PE-F68 (chosen as it allows the formation of both nanocapsules and nanoparticles) at pH 3. The organic phase was varied from monomer alone (100 μ l) to monomer in ethanol (25 ml) with 0.5 ml of Miglyol 812, according to Table 1. The colloidal systems were formed by allowing the organic phase to run slowly into the aqueous phase under magnetic stirring. Ethanol, when present, was then removed by evaporation under vacuum.

The different polymeric systems were separated and purified by ultracentrifugation at 107 000 $\times g$ for 1 h 30 min (Beckman L7-55). For analysis which required dry samples, colloids were freeze-dried.

TABLE 1

Miglyol 812 and ethanol volumes used for the preparation of different systems ($N_p - N_c$). Numbers were used as identification

Ethanol	Miglyol 812			
	0 ml	0.02 ml	0.1 ml	0.5
0 ml	1	2	3	4
1 ml	5	6	7	8
5 ml	9	10	11	12
25 ml	13	14	15	16

Morphological analysis

Transmission electron microscopy (TEM) Colloids were observed after negative staining with 2% phosphotungstic acid. Suspensions were dispersed in the staining solution for 1 h at room temperature, put on a copper grill covered with nitrocellulose, dried under vacuum for at least 24 h and observed ($\times 100\,000$) by TEM (Philips EM 301).

Determination of size by photon correlation Particle size was evaluated using a Coulter Nano-Sizer or a Coulter N4 MD (Coultronics France S.A.). The samples were dispersed in filtered distilled water. Each analysis lasted 200 s. The temperature was 20°C and the analysis angle was 90°.

Physico-chemical analysis

GPC The polymeric molecular mass of colloidal systems was evaluated by gel permeation chromatography in THF (pump: Waters® W510, differential refractometer W410, integrator and two Ultrastyragel® columns 500 and 10⁴ Å).

The polymer molecular weights were determined with reference to a calibration curve prepared using polystyrene standards.

NMR ¹H-NMR spectra were recorded on a Bruker AM 250 spectrometer (250 MHz), and ¹³C-NMR spectra on a Bruker AM 400 spectrometer (400 MHz). The dried samples were dispersed in deuterated chloroform (CDCl₃).

Results

Parametric study

Macroscopic analysis

The macroscopic appearance of the different colloidal suspensions is described in Table 2. When the quantity of ethanol was increased, there was a tendency to form a suspension of polymeric flakes.

However, with an increase in the volume of oil (Miglyol 812), an oily layer appeared on the surface of suspensions. For systems having a Miglyol 812/ethanol ratio of 2% (diagonal line, Table 1), the macroscopic appearance was markedly more

TABLE 2

Appearance of colloidal suspensions after polymerization in the parametric study (Table 1)

Ethanol	Miglyol 812			
	0 ml	0.02 ml	0.1 ml	0.5
0 ml	+	+	+	+
		○	○	○○
1 ml	+	+	+	+
	*	*	*	○
5 ml	+	+	◆	◆
	**	**	*	
25 ml	+	+	◆	◆
	***	***	**	

(+) Tyndall effect, (○) oil free on surface, (◆) milky aspect, (*) flakes of polymer.

homogeneous with the presence of flakes of polymer only for intermediate preparations. The limiting-case preparations, without Miglyol 812 and ethanol (true nanoparticles), i.e., preparation 1, or containing 0.5 ml of Miglyol 812 and 25 ml of ethanol (nanocapsules), namely, preparation 16, were perfectly homogeneous (Fig. 2).

Microscopic analysis

In the absence of ethanol, the sizes measured with the Coulter Nano-Sizer (Table 3) were in every case smaller than 80 nm, whereas in the presence of ethanol, the sizes were always about 300 nm.

Some preparations (on the diagonal line, Table 1) were analyzed with the Coulter N4 MD and transmission electron microscopy (Table 4). Although the general trend shown by the Coulter Nano-Sizer was confirmed, there was a considerable difference for preparation 6 which demonstrated a double population on analysis with the Coulter N4 MD and by TEM.

On the other hand, for preparation 16, while the Coulter Nano-Sizer and Coulter N4 MD both appeared to show a homogeneous preparation around 200 nm in diameter, two populations were

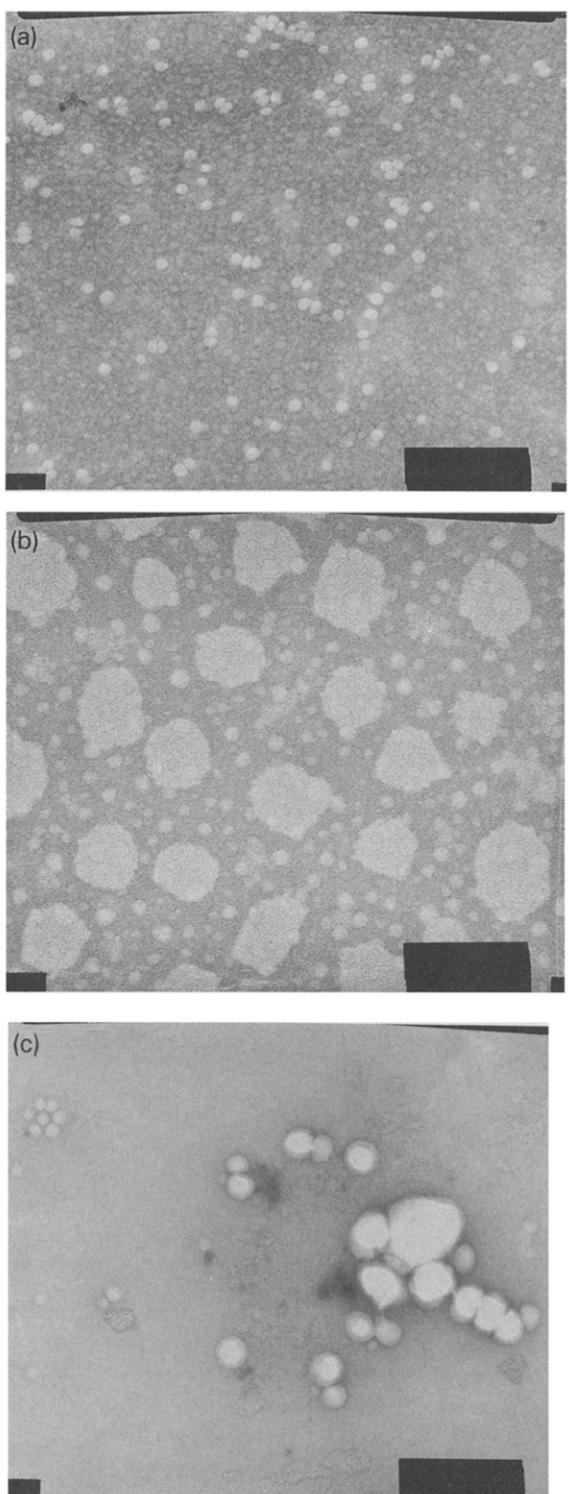


Fig. 2. TEM of: (a) preparation 1, (b) preparation 6, (c) preparation 16; (a) 1.3 cm = 500 nm, (b) 1.6 cm = 350 nm, (c) 1.3 cm = 500 nm.

TABLE 3

Average size (in nm) of different systems determined by Coulter Nano-Sizer (numbers in parentheses represent the polydispersity index)

Ethanol	Miglyol 812	0 ml	0.02 ml	0.1 ml	0.5 ml
0 ml	77 (4)	57 (3)	47 (0)	63 (3)	
1 ml	262 (4)	435 (5)	305 (5)	341 (5)	
5 ml	510 (4)	243 (6)	284 (1)	638 (3)	
25 ml	238 (1)	223 (3)	232 (2)	255 (2)	

seen in TEM micrographs. The size of the particles was evaluated at about 200 and 90 nm. This result has been confirmed by Coulter N4 MD analysis and TEM observation after separation by ultracentrifugation (Table 5). Fig. 3a and b shows particles of the floating layer and sediment, respectively.

From the micrographs obtained for both systems, it would seem that the smaller particles were matrix-like systems and the larger ones vesicle-like systems.

Molecular weight analysis

Molecular weight analysis by GPC, presented in Table 6, demonstrates a large increase in molecular weight with increasing volume of ethanol (vertical axis). The increase in the volume of oil (horizontal axis) did not change the molecular weight of the polymer formed.

The two populations of particles separated by ultracentrifugation (particularly from preparation 16) did not show a difference in the molecular weight of the polymer. In fact, after separation by

TABLE 4

Comparison of results of granulometric analysis from Coulter Nano-Sizer, Coulter N4 MD and the appearance determined by transmission electron microscopy (TEM)

	Preparation 1	Preparation 6	Preparation 16
Coulter Nano-Sizer	70 nm (4)	435 (5)	255 nm (2)
Coulter N4 MD	70 ± 18 nm	90 ± 20 nm	203 ± 35 nm
TEM	Fig. 2a	Fig. 2b	Fig. 2c

TABLE 5

Granulometric analysis of particles (system 16) before and after separation by ultracentrifugation

Particles (system 16)	Size (nm)	S.D. (nm)
Before separation	215	31
After separation (floating layer)	220	26
After separation (sediment)	90	17

ultracentrifugation of system 16, the two fractions both showed a very high molecular weight ($M_n \approx 100\,000$).

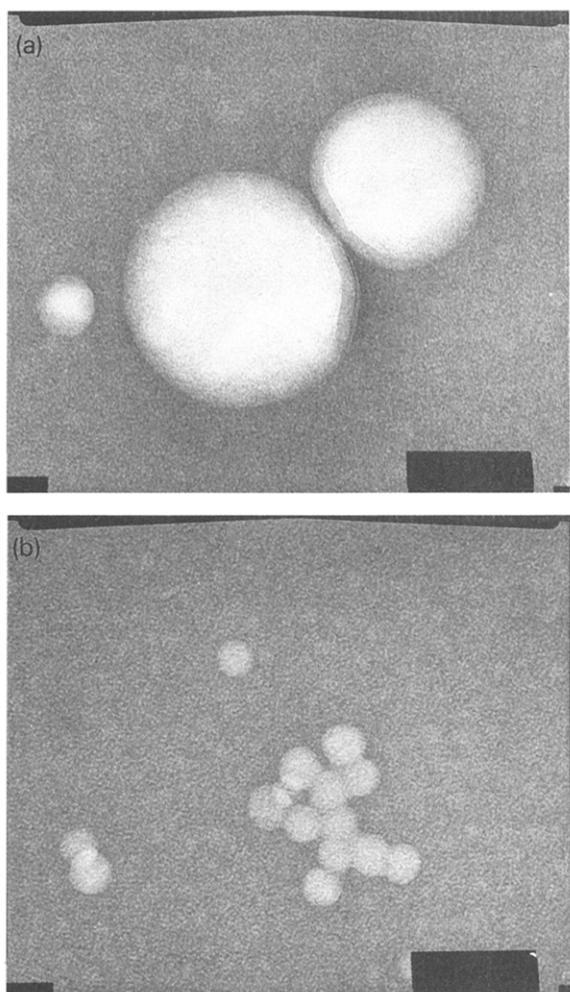


Fig. 3. TEM photographs of system 16 after separation: (a) floating layer (1.7 cm = 200 nm); (b) sediment (1.7 cm = 200 nm).

TABLE 6

Values of the molecular weight of parametric study preparations (Table 1)

System	\bar{M}_n	\bar{M}_w	PI	Wt
1	1400	3000	2.1	2400
2	1400	3000	2.25	2900
3	1800	3600	2.01	2900
4	1600	3400	2.09	1900
5	~	~	~	~
6	30000	57000	1.84	28000
7	37000	60000	1.62	35000
8	~	~	~	~
9	63000	93000	1.48	77000
10	80000	130000	1.57	92000
11	62000	120000	1.85	100000
12	93000	140000	1.48	140000
13	~	~	~	~
14	104000	160000	1.5	170000
15	105000	156000	1.49	139000
16	106000	158000	1.49	139000

\bar{M}_n , number average molecular weight; \bar{M}_w , weight average molecular weight; PI, polydispersity index; Wt, molecular weight at the top of the peak (for systems 5, 8 and 13, the characterization of molecular weight could not be made from the same monomer sample).

Effect of ethanol

The higher molecular weight of the polymer formed in the presence of ethanol suggested that polymerization might take place in this solvent. We therefore incubated the monomer in ethanol alone for 3 h and analysed the resulting mixture by GPC and NMR. GPC analysis showed a re-

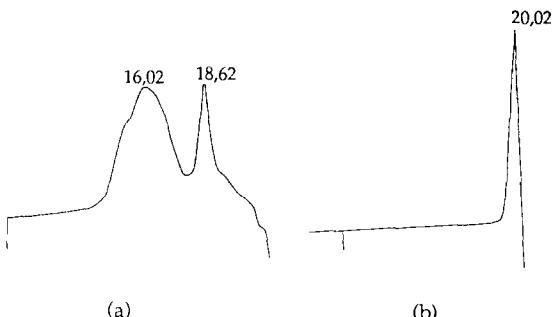


Fig. 5. GPC chromatograms and retention time at the top of the peaks: (a) IBCA in ethanol and addition of water after 3 h; (b) IBCA alone.

duction in the retention time from 20.02 to 18.62 min, indicating an increase in molecular weight.

NMR analysis demonstrated the presence of an ether link at 3.55 ppm (Fig. 4), resulting from a covalent linkage between ethanol and monomer, the ethanol acting as nucleophile and initiating the polymerization reaction. A similar phenomenon has been described by Pepper (1978) for methanol.

However, the polymerization reaction initiated by ethanol did not lead to an extensive propagation reaction. In fact, it appears that with ethanol alone, short live chains (oligomers) are formed which should be able to grow when the polymerization conditions are modified.

Fig. 5 shows GPC chromatograms demonstrating that polymerization recommences when water is added to the monomer/ethanol complex after 3 h.

Discussion

The results obtained from the analysis of the colloids formed under different conditions allow us to draw a number of conclusions about their mechanisms of formation.

Monomer /water systems (true nanoparticles)

In the case of the monomer/water system, the particles are solid, small (90 nm diameter), homogeneous (Seijo et al., 1990) and of low molecular

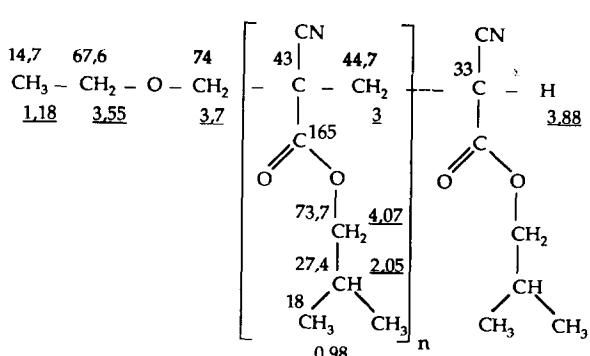


Fig. 4. Molecular structure of IBCA-ethanol complex determined by ^1H -and ^{13}C -NMR analysis.

weight. The mechanism of formation proposed in the literature is an emulsion polymerization process (Couvreur et al., 1979). Vansnick et al. (1985) have studied the molecular weight of PIBCA as a function of the pH of the medium (pH 2–4). Their results showed a reduction in molecular weight with increase in the acidity. An interpretation of this phenomenon was made on the basis of the balance between initiating and terminating agents. In an acidic medium (low pH), the concentration of OH^- is very low, whereas that of H_3O^+ is very high. Under these conditions, the reaction is very rapidly stopped and the molecular weight is low.

Monomer / oil / water system

In the presence of oil, a population of polymeric chains with a slightly greater molecular weight was found. The size of the colloidal system was almost the same and the oily phase was sometimes found free on the surface.

In the presence of oil alone, the oily phase only solubilizes the monomer in reservoir drops, but does not induce by itself monomer diffusion towards the aqueous phase.

In this situation, the monomer was dispersed in oily droplets. Thus, the surface/volume ratio of the monomer was changed slightly and the monomer became less available to the aqueous phase at each instant. Diffusion of the monomer to the aqueous phase was delayed and the probability of a reaction between the monomer and initiating agents at the drop surface was greater (Millich and Carraher, 1977). The oligomeric 'live chains' can diffuse from the oily to the aqueous phase in order to integrate into the growing particles of latex. This process could explain the formation of chains of slightly higher molecular weight.

Monomer / ethanol / water system

In the case of systems which were obtained with a mixture of monomer and ethanol injected into the aqueous phase, a very large increase in molecular weight of the polymer was found. The formation of flakes of polymer was also observed (Fig. 6).

The partition coefficient of ethanol favours



Fig. 6. TEM of preparation 5 (in the presence of ethanol alone in the organic phase) (1.3 cm = 500 nm).

distribution into the aqueous phase. Thus, a strong diffusion flux during preparation would be expected (Van Oss et al., 1989). In order to ascertain whether the presence of ethanol itself or its diffusion explains the increase in molecular weight with the quantity of ethanol, an experiment was performed in which the total volume of ethanol was kept constant but the diffusing volume was varied between 0 and 100% of the total. For this purpose, the rest of the ethanol was added to the aqueous phase. In the absence of diffusing ethanol (monomer injected alone in the ethanol/water mixture), the molecular weight of the polymer was similar to that obtained in the aqueous phase without ethanol. Furthermore, the molecular weight increased with increase in the volume of diffusing ethanol, reaching a value comparable to that obtained with nanocapsules.

Thus, it appears that the molecular weight of the polymer formed does not depend on the total amount of ethanol, but only on the portion of ethanol diffusing from the organic to the aqueous phase.

In order to establish whether polymerization of the monomer could occur in the presence of ethanol alone, we characterized the species created under these conditions: after 3 h of incubation in ethanol, the results obtained from NMR

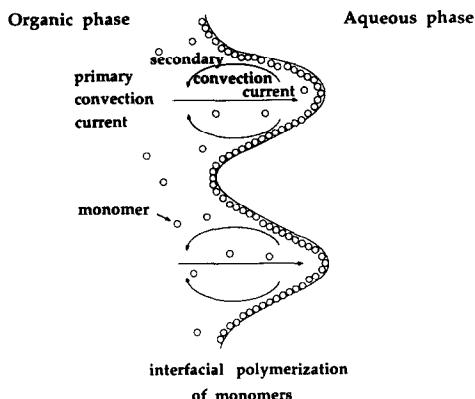


Fig. 7. Scheme of primary and secondary convection currents during diffusion of the organic phase.

and GPC analysis showed only oligomer formation. Although a reaction took place between the ethoxy anion and the monomer (Pepper, 1978; Eromosele et al., 1989), this proceeded very slowly.

In fact, during colloid preparation, the period of time before injection in which the monomer and ethanol were together in solution was very short (only a few seconds). Under these conditions, the ethanol/monomer reaction had an insignificant effect on the molecular weight and only the diffusion process appeared to yield a high degree of polymerization.

Monomers and oligomers of IBCA are very soluble in ethanol. When monomer and oligomer diffuse with ethanol from the organic into the aqueous phase, primary convective currents of diffusion arise (Davies and Haydon, 1957; Sternling and Scriven, 1959; Miller, 1978) and lead to a high concentration of IBCA at the interface (Fig. 7).

On contact with the aqueous phase, the first live chains, which are poorly soluble in water, create an initial interfacial film by stranding of polymer (Ruschak and Miller, 1972; Egbogah and Dawe, 1985). This film is a diffusion barrier for monomers, and polymer chains can grow on the organic side of the interface, protected from termination agents which are mainly found in the aqueous phase. This could explain the increase in molecular weight for preparations using diffusing ethanol.

On the other hand, monomer and oligomers of IBCA have tensio-active properties (Florence et al., 1976; Baszkin et al., 1987, 1989). The high concentration of IBCA at the interface leads to a gradient of interfacial tension giving rise to interfacial turbulences (Wielenbinski and Findenegg, 1988). These turbulences and secondary convective currents developed at the interface by the Marangoni effect (Davies and Haydon, 1957; Sternling and Scriven, 1959; Miller, 1978) can explain the fragmentation of the polymeric film and the microscopic appearance of the polymeric species created (particles and flakes).

Monomer / oil / ethanol / water system

In the presence of oil and ethanol, as in the formation of nanocapsules, the degree of polymerization is similar to that observed with the monomer/ethanol/water system. Hence, it is reasonable to believe that the conditions of polymerization and colloid formation might be similar.

Furthermore, the formation of a double colloidal population, consisting of 230 nm nanocapsules and 100 nm nanoparticles with a similar polymer molecular weight, leads us to assume the existence of a single polymerization mechanism based on the formation of polymer at the interface between the organic and aqueous phases.

In the absence of oil, the polymer generated does form spherical particles, probably due to the force of ethanol diffusion, which leads to the appearance of polymer flakes.

With the monomer/Miglyol 812/ethanol mixture, the viscosity of the organic phase is increased, and consequently the convective fluxes are slowed down (Miller, 1978).

However, the oil could play a second role, acting as a support for the monomer in the organic phase: for the monomer/ethanol mixture, the organic phase disappears as a result of the diffusion of ethanol. In the presence of oil, convective currents and interfacial turbulences, induced by ethanol diffusion and Marangoni effects, can give rise to spontaneous emulsification of the organic phase.

Through this mechanism, the polymeric film created by interfacial polymerization between the

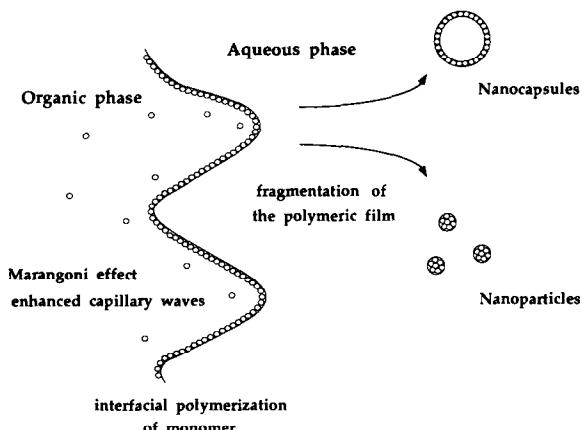


Fig. 8. Scheme of mechanism of formation of system 16 (nanoparticles and nanocapsules).

organic and aqueous phases is broken in order to surround the oil drops (Castillo and Velarde, 1985). The occurrence of this phenomenon was confirmed by the formation of polymeric films at the oil/water interface, with a smooth oily face and an undulating water face, with presence of a polymeric colloid in the water surface of a similar degree of polymerization (Gallardo, 1991). Such a scheme of formation of nanocapsules is illustrated in Fig. 8.

According to this scheme, it is possible to consider that the nanoparticles observed in this system result from fragmentation residues of the polymeric interfacial film which do not contain any oily droplets.

Thus, we can understand why the smaller-sized population in this system (sediment of system 16) has a matrix structure, whereas that of larger size has a vesicular structure.

These results therefore support the hypothesis of interfacial polymerization for the formation of polyalkylcyanoacrylate nanocapsules.

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