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Preparation and purification of polyisohexylcyanoacrylate nanocapsules

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Summary

Nanocapsules (200 nm diameter) were prepared by interfacial polymerization of isohexylcyanoacrylate in an oil-in-water emulsion. Sulphur dioxide was added to the monomer prior to its dissolution in the oil-ethanol phase in order to avoid immediate polymerization. Upon addition to the aqueous phase, interfacial polymerization occurred resulting in the formation of nanocapsules. The effect of different variables on nanocapsule size was evaluated. Miglyol concentration played a major role by controlling the size of emulsified droplets while SO_2 and pH only slightly affected nanocapsule size. Monomer concentration showed a significant effect on the density, indicating that polymeric walls of different thicknesses can be obtained. Finally, a purification method based on centrifugation and redispersion was developed.

Introduction

Increasing the specificity of drugs for diseased tissue by combination with a suitable targeting device is a topic of interest in pharmaceutical research. Polyalkylcyanoacrylate nanoparticles, consisting of a plain polymeric sphere, have been suggested as a tumour-targeting system owing to their biodegradability and their capacity to modify the body distribution of cytostatic drugs (Kante et al., 1980; Couvreur et al., 1985). Among the various polymers tested, it has been shown that isohexylcyanoacrylate has a lower toxic impact compared to shorter homologues (C1–C4) in both acute and subacute study patterns (Couvreur et al., 1989). More recently, isobutylcyanoacrylate was used for the preparation of nanocapsules consisting of an oily core surrounded by a polymeric wall (Al Khoury et al., 1986). These capsules could prove useful in the targeting of lipophilic compounds and were also suggested for peroral administration of insulin (Damgé et al., 1988). It was desirable to prepare and test polyisohexylcyanoacrylate (PIHCA) nanocapsules, since their toxicity should be even lower. However, the process of nanocapsule preparation involves mixing

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the monomer with ethanol. With IHCA this normally results in rapid polymerization and a bulk polymer is obtained. The aim of this work was to develop a formulation of PIHCA nanocapsules and a purification method. The effect of several formulation variables on nanocapsule size and density was also studied.

Materials and Methods

Materials

Isobutylcyanoacrylate was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Isohexylcyanoacrylate (purity > 99%) was received from Dr H. Vranckx (SOPAR S.A., Zelzate, Belgium) and used without further purification. Miglyol 810 (caprylic/capric triglyceride) and 829 (caprylic/capric diglyceryl succinate) were obtained from Dynamit Nobel (Montreal, Que, Canada). Absolute ethanol was purchased from Consolidated Alcohols Ltd (Toronto, Ont, Canada). Poloxamer 407 was obtained from BASF Inc. (Montreal, Que, Canada). Sulphur dioxide (SO_2) was purchased from Liquid Carbonic Ltd (Scarborough, Ont, Canada).

Nanocapsule preparation

Prior to nanocapsule preparation, part of the isohexylcyanoacrylate (IHCA) monomer was saturated with sulphur dioxide at room temperature (25°C). SO_2-free monomer was mixed with SO_2-saturated monomer in order to obtain concentrations ranging from 20 to 100% saturation. The monomer (0.5–2.0% v/v) was then dissolved in a mixture of Miglyol (0.5–8% v/v) in ethanol. This solution was slowly added in a (1 : 2) ratio to an aqueous phase at a flow rate of 0.5 ml/min using a peristaltic pump (Masterflex 7520-35, Cole-Parmer Instrument Co., Chicago, IL, U.S.A.). The aqueous phase consisted of a solution of 0.031–0.5% poloxamer 407 and 10 mM phosphate buffer (pH 3–9). After addition of the whole organic phase, stirring was interrupted and samples were drawn off for size determination.

Isobutylcyanoacrylate (IBCA) nanocapsules were prepared using the same method and the commercially available monomer without further addition of SO_2.

Size measurement

After suitable dilution in water, the size was determined by photon correlation spectroscopy using an N4SD nanosizer (Coulter Electronics, Hialeah, FL, U.S.A.). The population of nanocapsules studied can be considered to be infinite. Results, expressed as a histogram of distribution (% number of particles per size class), were transformed into a mean and standard deviation for unimodal Gaussian distributions.

Preparation of nanocapsules for freeze-fracture study

Freeze-fracture study of the nanocapsules was performed to verify the presence of the polymeric wall. Nanocapsules in a suspension were prepared as described above. The suspension of nanocapsules contained a final concentration of 10% sucrose which served as cryoprotectant. For freeze-fracture, a small drop of the nanocapsule suspension was transferred using a drawn Pasteur pipette with a tapered end onto a standard Balzers-type specimen support plate (without central bore). Following the transfer of the nanocapsules, another specimen plate (with a central bore) was placed on the top of the drop of suspension so that the nanocapsules were sandwiched between two support discs. Excess nanocapsules in the suspension were squeezed out through the central bore and were removed with a filter paper. The sample was then frozen by plunging rapidly into Freon 22 cooled by liquid nitrogen. Freeze-fracture by the double-replica method was performed in a Balzers-type freeze-fracture unit (BAF 400T, Balzers AG, Balzers, Liechtenstein) at −130°C under a vacuum of 2×10^{-6} Torr, followed by shadowing with platinum at a fixed angle of 45° and then coating with carbon at 90°. The thickness of the replica was ~2 nm platinum and ~20 nm carbon as determined by a Balzers crystal thin-film monitor. After replication, replicas were floated off the specimen support plate on a well of dimethylformamide (DMF) and were left in the DMF solution for at least 1 h in order to remove the nanocapsule debris. The replicas were washed three times in bi-distilled water and mounted on parlodion-coated grids for examination on a Philips 300 electron microscope.

Nanocapsule density

In order to evaluate nanocapsule density as a function of monomer concentration, the size of pellets obtained by centrifugation in media of increasing density was evaluated as follows. Nanocapsules were prepared using Miglyol 810 and increasing concentrations of monomer (0.3–2% of the organic phase). The density of the aqueous phase of the final suspension was 0.965 as measured by pycnometry. After centrifugation (55 000 × *g* for 90 min, Beckman Ultracentrifuge L2-65B, rotor 75Ti) the nanocapsule pellets were redispersed in ethanol 12% (density = 0.980). The suspensions were centrifuged a second time and the pellets were redispersed in water. Finally, nanocapsules were centrifuged a third time and the pellets were redispersed in water. The turbidity of each redispersion was evaluated by nephelometry using a laser beam (wavelength 632.8 nm) after proper dilution and was used as an indication of the size of the pellet after standardization. The whole set of experiments was performed at 4°C and at least in duplicate.

Nanocapsule purification

Nanocapsules were centrifuged at 55 000 × *g* for 2 h. The supernatant was discarded and the pellet redispersed in double distilled water by mechanical stirring. Nanocapsule sizes before centrifugation and after redispersion were compared. In some instances, the procedure was applied four times consecutively to evaluate the stability of the poloxamer coating. The purity of the redispersed preparations was assessed by determining residual ethanol concentration using alcohol dehydrogenase in the presence of NAD^+ and spectrophotometric determination of NADH at 340 nm (Beutler et al., 1977).

Results and Discussion

Isohexylcyanoacrylate nanocapsules

In order to prepare nanocapsules, the monomer must first be dissolved in a mixture of Miglyol and ethanol. However, it is well-established that ethanol can initiate the polymerization by its nucleophilic hydroxyl group (Leonard et al., 1966).

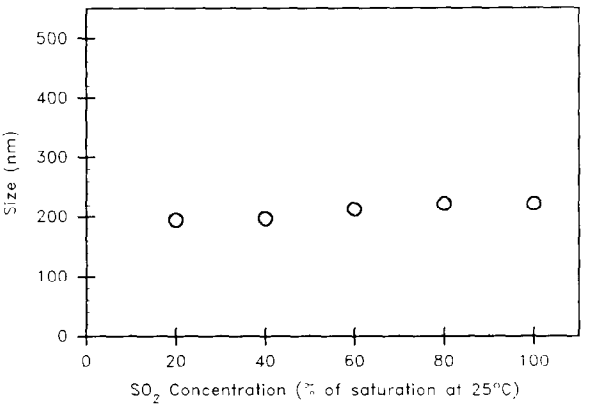

Fig. 1. Size of PIHCA nanocapsules as a function of initial SO_2 concentration in the monomer at room temperature (expressed as % of saturation).

Therefore, it is necessary to add a polymerization inhibitor to the monomer prior to mixing with the organic phase. It is equally important that the inhibitor be inactivated rapidly upon addition of the organic phase to the aqueous phase. This is indeed necessary for interfacial polymerization to occur. Sulphur dioxide was selected because its inhibitory potential has been well-established (Leonard et al., 1966). Moreover, upon addition to the aqueous phase, it rapidly diffuses into water (Lenaerts et al., 1989) and is inactivated by transformation into sulphurous acid. Any subsequent variation of the pH of the aqueous phase is easily controlled by addition of a buffer.

The effect of SO_2 concentration of monomer on nanocapsule size was studied. Nanocapsules were prepared using SO_2-saturated monomer and mixtures of this latter with SO_2-free monomer. The organic phase consisted of Miglyol 829 (0.1 ml), monomer (25 μl) and ethanol (5 ml). The water phase was a 10 ml 0.25% poloxamer 407 solution. As seen in Fig. 1, SO_2 concentration in the monomer had only a slight influence on nanocapsule size which had a tendency to decrease with decreasing concentration. Below 20% saturation, SO_2 concentration in the monomer was too low and polymerization occurred instantly in the Miglyol-ethanol medium.

Nanocapsules were prepared using two different types of Miglyol. Miglyol 810 and Miglyol 829 have a density of 0.949 and 1.009 g/ml, respec-

tively. SO_2-saturated monomer was used and the aqueous phase contained 0.25% poloxamer 407. When Miglyol 810 was used, the nanocapsules were found in the pellet following centrifugation at 55 000 × *g* for 2 h. However, after centrifugation of an emulsion prepared under the same conditions without monomer, the oily phase accumulated at the surface. The absence of any residue on top of the aqueous phase following centrifugation of nanocapsules indicates that the oily phase was entirely encapsulated. This difference in density between nanocapsules and oil droplets of the same size is likely to result from the presence of a polymeric wall. The observation of freeze-fracture preparations of nanocapsules supports this hypothesis (Fig. 2). In these preparations, the nanocapsules were frequently exposed as cross-fractured profiles having a spherical oily core surrounded by a thickening envelope.

The effect of Miglyol concentration on nanocapsule size was investigated. Fig. 3 shows that

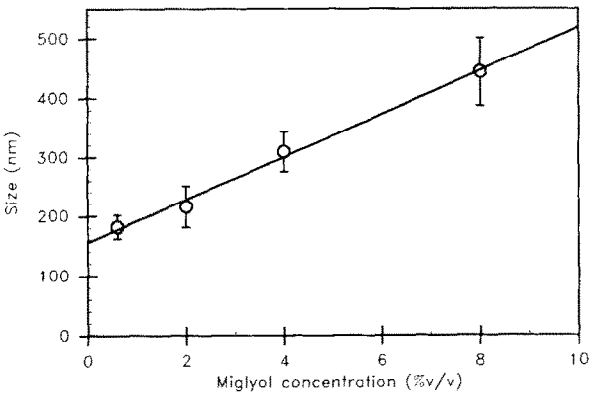

Fig. 3. Size of PIHCA nanocapsules as a function of Miglyol concentration in the organic phase (mean ± S.D., $n = 3$).

increasing Miglyol concentration significantly increased nanocapsule size. It must be pointed out that, in all cases, nanocapsule size was identical to that of emulsions in which no polymer was present. This indicates that nanocapsule size is mainly determined by the size of the initially formed

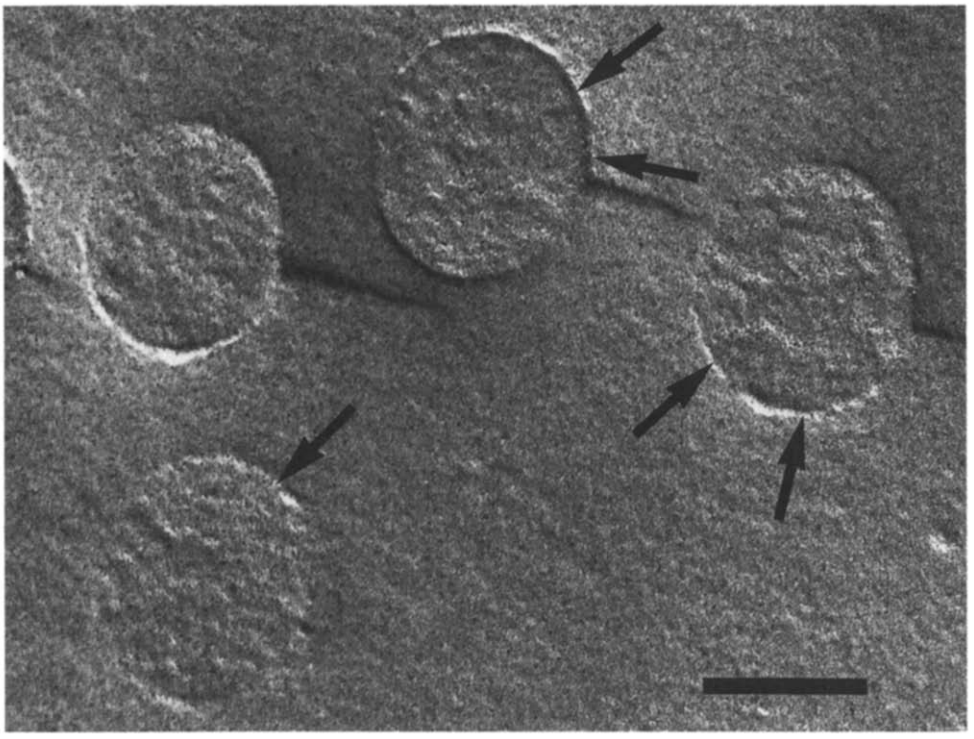

Fig. 2. Electron photomicrograph of freeze-fracture nanocapsules shown in cross-fracture. The presence of a polymeric wall is demonstrated by the thickening of a delimiting envelope (arrowheads) enclosing the solid core. × 102 600; bar = 200 nm.

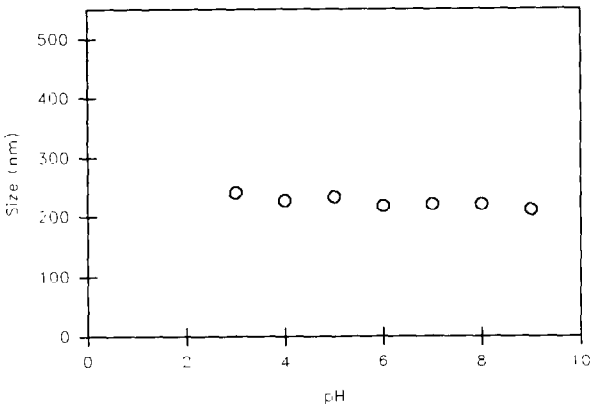

Fig. 4. Size of PIHCA nanocapsules as a function of pH of the aqueous phase.

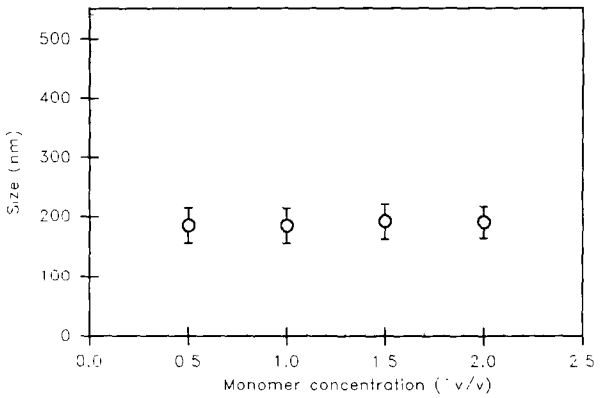

Fig. 5. Size of PIHCA nanocapsules as a function of monomer concentration in the organic phase (mean ± S.D., $n = 3$).

emulsion droplets. Consequently, one can assume that the dispersion of oil droplets following addition to the aqueous phase takes place before the formation of a uniform polymeric coating. Additional evidence that polymerization does not affect nanocapsule size significantly was provided by the preparation of nanocapsules in aqueous media ranging from pH 3.0 to 9.0 (Fig. 4). According to the anionic polymerization mechanism generally accepted for cyanoacrylates (Donnelly et al., 1977), polymerization should proceed much faster at pH 9.0 than at pH 3.0. However, even when polymerization was extremely fast, no modification of nanocapsule size was observed, indicating that the polymerization kinetics has no influence on nanocapsule size. These results suggest that, owing to the absence of a three-dimensional network, polymeric coating does not prevent nanocapsules from reaching the size dictated by emulsification conditions even after complete polymerization. In this case, the linear polymer should be viewed more as a mobile emulsion stabilizer rather than as parts of a rigid capsule wall.

Nanocapsules prepared with increasing concentrations of monomer showed no significant difference in size (Fig. 5). Since the volume of both phases was practically constant, this means that the number of nanocapsules per unit volume also remained unchanged, irrespective of monomer concentration. In view of this finding, one can assume that nanocapsules prepared with increasing concentrations of monomer are surrounded by a thicker and/or denser polymeric wall. This observation suggests that drug diffusion rate from nanocapsules could possibly be modulated by properly adjusting polymeric wall thickness. The density of nanocapsules prepared with increasing monomer concentrations was estimated by centrifugation in media of different densities. Fig. 6 shows that nanocapsule density increased with monomer concentration. Since the polymer density is higher than that of Miglyol (1.01 and 0.949 g/ml, respectively; Rollot et al., 1986), these results confirm that the amount of polymer per nanocapsule is directly related to monomer concentration in the organic phase. Furthermore, the

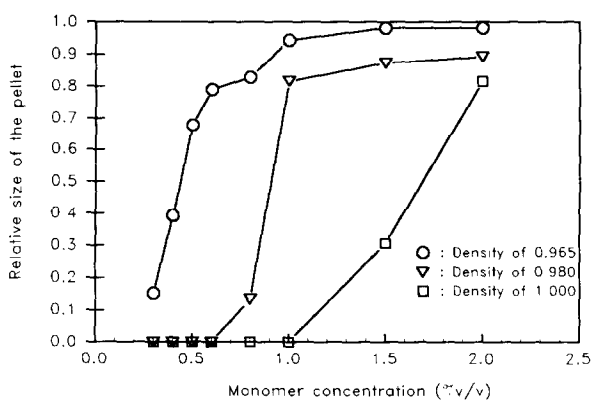

Fig. 6. Size of nanocapsule pellets following three consecutive centrifugations in media of increasing density as a function of monomer concentration expressed in percentage of the organic phase.

absence of any sediment at 1% v/v in the 1.000 density medium indicates that only nanocapsules with an oily core were formed to the exclusion of plain polymeric nanoparticles (density of nanoparticles = 1.01; Rollot et al., 1986).

Nanocapsule purification

Nanocapsules were prepared using different concentrations of poloxamer 407. As summarized in Table 1, the purification process did not alter nanocapsule size. Using isobutylcyanoacrylate, it was not possible to prepare nanocapsules below a critical poloxamer 407 concentration of 0.125%. Below this concentration, no nanocapsules were formed as demonstrated by the accumulation of Miglyol 810 at the surface after centrifugation. In contrast, with isohexylcyanoacrylate, nanocapsules were successfully prepared at a poloxamer 407 concentration as low as 0.031%. The efficiency of this process for the purification of nanocapsules was evaluated by the determination of residual ethanol in the redispersed preparation. Whereas the initial concentration was around 26% (w/v) this value dropped to less than 1% after purification, irrespective of the type of monomer used. Finally, four consecutive purifications were performed for both types of polymers using an initial concentration of 0.25% poloxamer 407. As seen in Table 2, the size was not significantly modified during any of the four purification steps. This result indicates that poloxamer attachment to the polymer was relatively stable. This represents an improvement over the method described previously (Al Khoury et al., 1986) which consisted of evaporation under reduced pressure. Indeed, whereas the latter method allowed a reduction of the ethanol content, it was ineffective in removing other impurities such as unbound poloxamer or residual cyanoacrylate monomers.

TABLE 1

Nanocapsule size (mean ± S.D.) before and after purification as a function of initial poloxamer 407 concentration

Poloxamer concentration (% w/v)	Size (nm)			
	IHCA		IBCA	
	Before	After	Before	After
0.5	218 ± 82	220 ± 65	146 ± 76	154 ± 34
0.25	222 ± 56	228 ± 44	143 ± 22	137 ± 26
0.125	223 ± 36	227 ± 59	134 ± 58	131 ± 50
0.063	217 ± 48	225 ± 61	[a]	[a]
0.031	220 ± 61	268 ± 76	[a]	[a]

[a] No nanocapsules were formed at these concentrations.

TABLE 2

Nanocapsule size (mean ± S.D.) during four consecutive purifications with an initial poloxamer 407 concentration of 0.25%

Purification number	Size (nm)	
	IHCA	IBCA
0	222 ± 56	143 ± 22
1	228 ± 44	137 ± 26
2	230 ± 68	145 ± 31
3	236 ± 49	133 ± 30
4	222 ± 83	109 ± 30

Conclusion

A novel technique was developed for the preparation of isohexylcyanoacrylate nanocapsules. Its original feature consists of dissolving sulphur dioxide in monomer before the addition to an organic phase. Sulphur dioxide acts as an inhibitor, preventing the polymerization of cyanoacrylates by ethanol. Upon addition to a water phase, sulphur dioxide and ethanol migrate in the external phase and a very fine emulsion is instantly formed. Polymerization takes place at the interface, resulting in capsules of approx. 200 nm diameter. Nanocapsule size is mainly influenced by factors acting on the droplet size, such as Miglyol concentration, and to a lesser extent by factors such as sulphur dioxide or monomer concentration. The evidence indicates that the polymeric wall can easily be prepared in different thicknesses and/or densities. Controlling polymeric wall thickness could prove a simple means of modifying the diffusion of drugs from nanocapsules into biological fluids. This possibility could be of interest for the design of nanocapsules with tailored drug release kinetics.

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