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Development of a new process for the manufacture of polyisobutylcyanoacrylate nanocapsules

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Summary

A new process for the manufacture of nanocapsules is described. The dispersion of an alcoholic solution of isobutylcyanoacrylate and oil in water, by interfacial polymerization, allows the formation of nanocapsules with an average diameter of about 200–300 nm. Physical and technical parameters are studied: temperature of preparation, pH of aqueous phase, concentration of surfactant and ethanol. Investigated with different active molecules and particularly with a radiological tracer, nanocapsule manufacture presents some advantages: (i) preparation is easily transposable to an industrial scale; and (ii) the method allows for a high level of entrapment for lipophilic substances.

There is a need for innovation in the area of dosage-form, in particular the development of a sustained release system allowing the adjustment of drug bioavailability and kinetics, and carriers that specifically deliver active molecules to target.

Increasing the specificity and effectiveness of drugs by combining the active ingredient with a suitable delivery system is a major research objective in pharmaceutical engineering. As a rule, the active ingredients contained in a conventional dosage form are distributed without sufficient distinction between biological targets and a wide variety of sites. Effectively devoid of tropism for the intended target, they are distributed in the organism in accordance with their physicochemical properties alone.

In a drug carrier system, the drug molecule, instead of being in the free state, is associated with a carrier designed to enhance its affinity for the intended target. Drug carriers currently under investigation are molecular, vesicular or particulate (Juliano, 1980; Gregoriadis et al., 1982). The former two groups include liposomes and nanoparticules. Liposomes, which are small phospholipid based vesicles, are undeniably the most intensively investigated carriers (Gregoriadis, 1979; Gregoriadis and Allison, 1980; Knight, 1981; Gregoriadis, 1984; Puisieux and Delattre, 1984). Nanoparticules are either small solid spheres (nanospheres) or small capsules formed of a central cavity surrounded by a wall (nanocapsules). The

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former include albumin microaggregates (Marty et al., 1978; Oppenheim, 1981), polyalkylcyanoacrylate nanospheres (Couvreur et al., 1979, 1980, 1982) and polymethylmethacrylate nanospheres (Kreuter, 1978, 1983), while the latter include polybutylcyanoacrylate nanocapsules (Giamalidis, 1981; Al Khouri, 1984).

The work reported here describes a new process for the manufacture of nanocapsules, relying on the interfacial polymerization technique. The results include a morphological analysis, an examination of the influence of the main physical and technological factors on the nanocapsule characteristics, and a number of preliminary studies on certain essential applications.

Materials and Methods

Raw materials

The *monomer* used to prepare the nanocapsules is isobutylcyanoacrylate [1] which polymerizes spontaneously in contact with water to give a biodegradable polymer (Couvreur et al., 1979; Grislain et al., 1983). The *alcoholic phase* consists of absolute ethanol [2]. The *lipophilic phase* is either an oil, such as Miglyol [3], a non-miscible organic solvent, such as benzylic alcohol, or a radiological tracer like an iodized oil (Lipiodol [4]). The *surfactant* is Pluronic F68 [5], a poloxamer.

Preparation of nanocapsules

The method used to obtain the nanocapsules consisted of mixing two immiscible phases A and B. *Phase A* consists of 4 ml of an oil (oily active ingredient or oil + liposoluble active ingredient), 0.5 ml isobutylcyanoacrylate, and 50 ml absolute ethanol. After mixing, the solution obtained is perfectly clear. *Phase B* consists of 200 ml of aqueous phase containing 0.5% non-ionic surfactant (Pluronic F68). The pH of this solution is around 6, and the temperature of both phases about 20°C. Alcoholic solution A, containing the oil and monomer, is slowly injected, through a silicon tube fitted with a fine tip, into aqueous phase B subjected to magnetic agitation (Fig. 1). The nanocapsules are formed immediately. The colloidal suspension obtained is then concentrated by evaporation under vacuum to about one-fifth of the initial volume, and then filtered through sintered glass (9–15 μm). The suspension can be sterilized in the autoclave at 120°C for 20 min. In these conditions, sterilization has no effect on the size of the nanocapsules obtained.

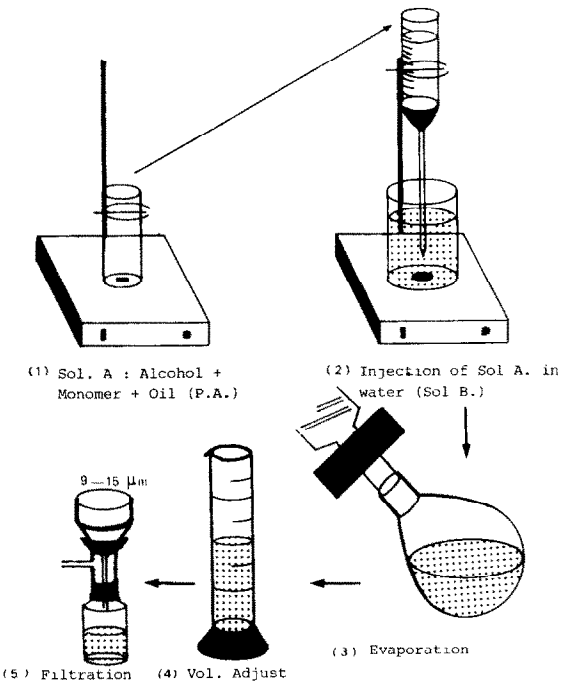

Fig. 1. Preparation of nanocapsules.

Morphological analysis

The mean diameter of the nanocapsules was estimated using a monochromatic laser ray diffusion counter (Nanosizer [6]).

Depending on the conditions and the products encapsulated, the process serves to obtain nanocapsules with a mean diameter between 200 and 300 nm.

[1] Ethnor SA, 8 rue Bellini, 75782 Paris Cédex 16, France.
[2] Prolabo, 12 rue Pelée, 75011 Paris, France.
[3] Dyna France SA, 6 rue Talleyrand, 75007 Paris, France.
[4] Laboratoires Guerbet, 16-24 rue Chaptal, BP 15, 93609 Aulnay Sous Bois, France.
[5] ICI France, 1 avenue Newton, 92142 Clamart, France.
[6] Coultronics France SA, 14 rue Eugène Legendre, Margency, 95580 Andilly, France.

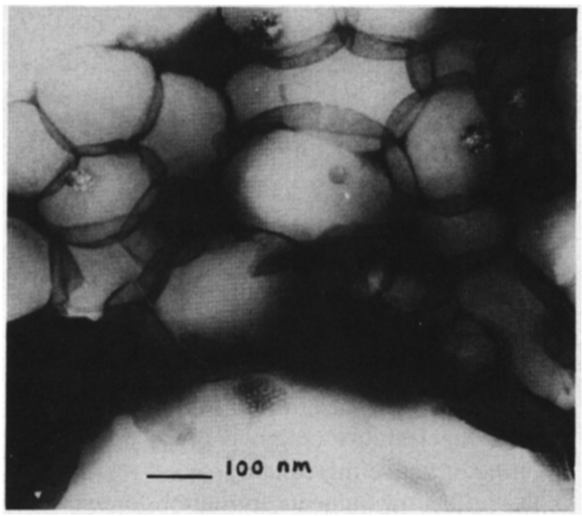

Fig. 2. Nanocapsules examined under transmission electron microscope.

The nanocapsules were examined under the transmission electron microscope after negative staining of the preparation with sodium phosphotungstate. Fig. 2 clearly shows the existence of a wall whose thickness can be estimated at 3 nm. Analysis under the scanning electron microscope was carried out in different conditions. As an example, Fig. 3 represents a photomicrograph of Lipiodol nanocapsules: the nanocapsules were concentrated by centrifugation, one drop of the suspension was placed on a suitable support, dried, and then examined without fixing. The nanocapsules envelope was perfectly smooth and uniform.

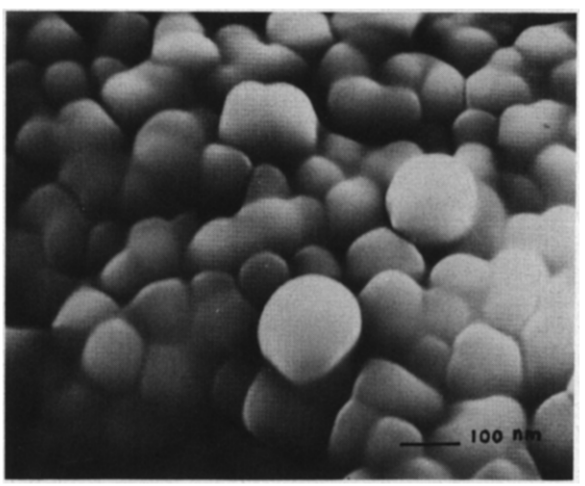

Fig. 3. Nanocapsules examined under scanning electron microscope.

Results

Influence of the main physicochemical and technological parameters on nanocapsule characteristics

The interaction of the carriers with the biological constituents and with the cell elements is undeniably influenced by their physicochemical properties, and especially by their dimensional characteristics. For more effective control of the proposed preparation method, a study was conducted on the effect of several physicochemical and technological factors on the characteristics of nanocapsules of Lipiodol, and of a number of other lipophilic substances (lomustine, progesterone). The parameters investigated were the preparation temperature, aqueous phase pH, surfactant concentration and oil type and concentration.

Table 1 shows the effect of preparation temperature (4–90°C) for Lipiodol nanocapsules. Up to 20°C, the nanocapsules display closely comparable size of about 250 nm. Higher temperatures (50–90°C) cause an increase in size and in polydispersity index.

The influence of aqueous phase pH was analyzed with the same nanocapsules. Preparation

TABLE 1

INFLUENCE OF THE AQUEOUS PHASE ON THE SIZE OF NANOCAPSULES

Temperature (°C)	Size (nm)	Polydispersity index *
0–4	249	< 1
10	249	< 1
20	270	< 1
30	281	2
40	289	< 3
50	302	2
60	294	3
70	299	3
80	312	4
90	334	4

* Nanosizer.

was carried out using pH values ranging from 1 to 10. Only pHs between 4 and 10 allowed the formation of nanocapsules of uniform size. Greater polydispersity occurs below pH 4.

Also investigated with Lipiodol nanocapsules was the surfactant concentration (0.5% w/v) which did not seem to have any significant effect on the initial characteristics of the nanocapsules. However, the presence of a surfactant is necessary to guarantee the physical stability of the preparation. Without surfactant, the nanocapsules formed tend to cluster together during storage.

The absolute ethanol concentration used to dissolve the oily phase and the monomer appears to have a significant effect on nanocapsule size, whatever the type of oil used. Up to 10% alcohol, the monomer polymerizes in flakes without formation of nanocapsules. It is only above 15% absolute alcohol that the preparation leads to the formation of isolated nanocapsules of satisfactory size. Above this concentration, nanocapsules size decreases with the alcohol content of the mixture. To provide an example, Table 2 reproduces the results observed for Lipiodol nanocapsules. Similar results were obtained with nanocapsules containing a solution of lomustine in Miglyol.

The oily phase is an important factor. Oil type and concentration in the medium must be such that the dissolution of the monomer and that of the active ingredient are suitably guaranteed. If the oily phase content is too low in comparison with the monomer, flake polymerization occurs. If it is too low in comparison with the active ingredient, the latter crystallized. With lomustine and Miglyol, for example, the concentration of active ingredient must be less than 0.2% or crystals appear at the surface of the nanocapsules after 24 h. With progesterone, crystallization can be prevented by replacing Miglyol by benzylbenzoate.

Applications

A number of preliminary experiments were conducted to evaluate the effect of the nanoencapsulation on the stability of labile drugs and on the targeting of active ingredients.

The effect of nanoencapsulation on stability was evaluated by using the lomustine molecule, an extremely unstable drug in the presence of water (Benoit, 1983). Table 3 gives the results obtained using Miglyol as an oily phase (in 95% alcohol solution, over 50% of the lomustine is destroyed after 10 days of storage).

Preliminary targeting tests were conducted in the rabbit, by intravenous administration, using Lipiodol, an iodized contrast product. Radiological examinations were conducted after injections of the same dose of free Lipiodol and encapsulated Lipiodol. A comparison of the radiographs (Fig. 4A and B) clearly reveals a densitometric highlighting on the liver and spleen after nanoencapsu-

TABLE 2

INFLUENCE OF THE CONCENTRATION OF ABSOLUTE ETHANOL ON THE SIZE OF NANOCAPSULES

Ethanol (%)	Size (nm)	Polydispersity index *
5	382	< 4
10	363	< 4
15	286	< 2
20	259	< 2
25	236	1
30	221	1
35	205	1
40	198	1
45	195	1
50	194	1

* Nanosizer.

TABLE 3

STABILITY OF LOMUSTINE (C.C.N.U.) AND SIZE OF NANOCAPSULES DURING CONSERVATION

Time (days)	Lomustine (No. destroyed)	Size (nm)	Polydispersity index *
0	100	201	<1
10	100	202	<1
20	100	202	<1
30	100	209	<1
40	85	196	<1
50	85	203	1
60	85	206	<1
70	75	208	1
240	24	230	1
440	12	232	<1

* Nanosizer.

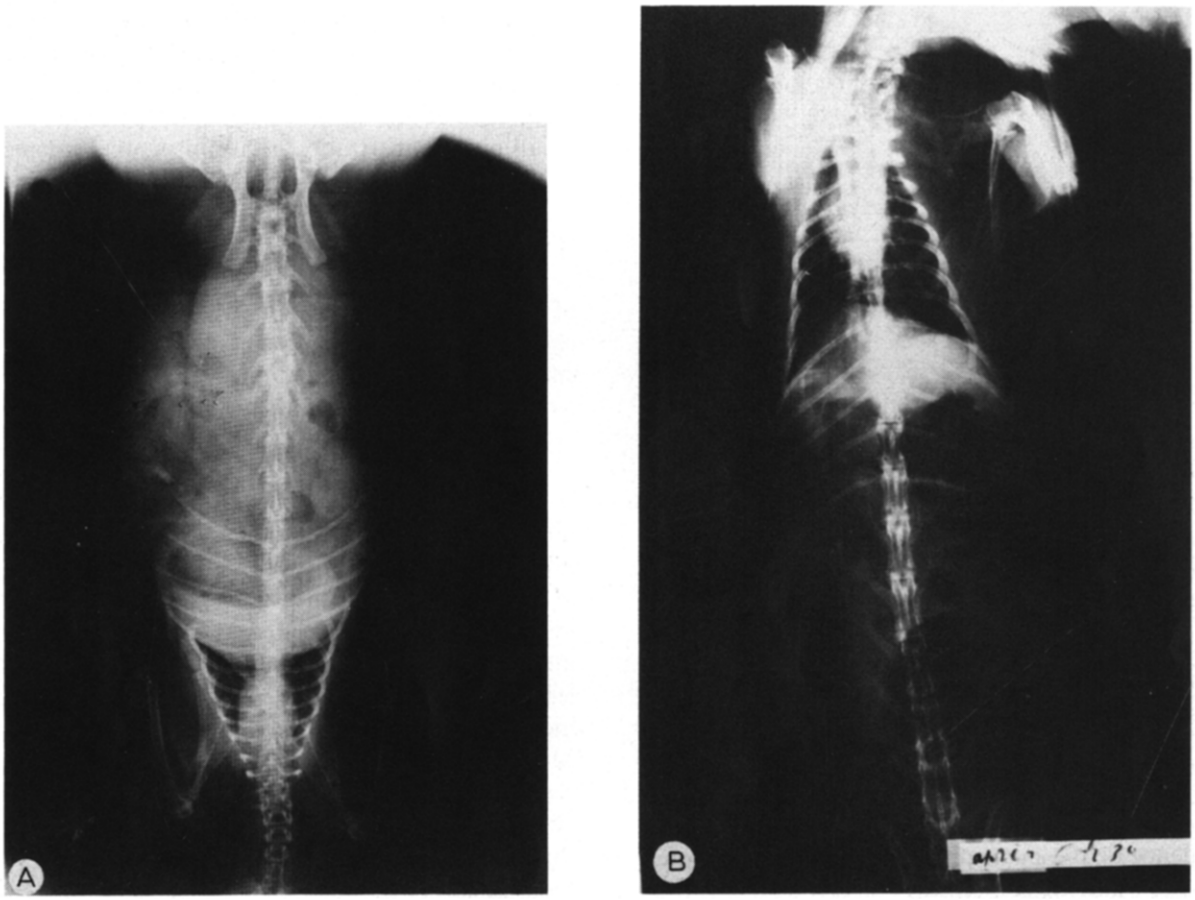

Fig. 4. A: radiograph of rabbit liver after i.v. administration of free Lipiodol. B: radiograph of rabbit liver after i.v. administration of Lipiodol in nanocapsules.

lation. As anticipated, the nanocapsules thus display a specific tropism for the reticuloendothelial system.

Discussion

This paper describes a new method to obtain polyalkylcyanoacrylate nanocapsules with a mean diameter of about 200–300 nm. The particles obtained, unlike those described by Couvreur et al. (1979), are not small solid spheres, but are formed of an envelope surrounding a liquid central cavity. Further proof has been obtained and is to be published. According to the nomenclature proposed in this article, the process therefore leads to nanocapsules and not nanospheres.

The nanocapsule formation mechanism is probably that of interfacial polymerization, described by Florence et al. (1976, 1979), (Wood et al., 1981), for the manufacture of polyalkylcyanoacrylate microcapsules. In the process proposed by these authors, an aqueous solution of the drug to be encapsulated is emulsified in an organic solvent. More organic solvent containing the cyanoacrylate monomer is added, and the interfacial polymerization allowed to occur. The reaction is carried out at 4°C. The microcapsules produced are transferred to an aqueous phase containing a surfactant. The capsules (microcapsules) range from 25 to 250 μm in size, depending on the degree of agitation used. They are formed of a polymeric

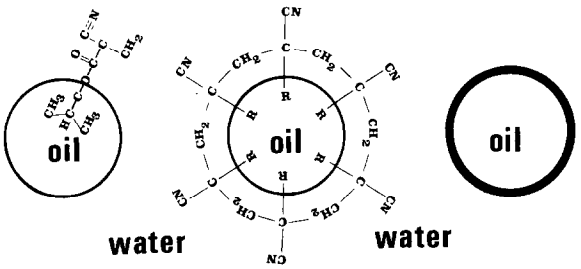

Fig. 5. Mechanism of formation of nanocapsules.

envelope surrounding an aqueous central cavity.

The originality of the process proposed in this work lies in the use of a third alcoholic solvent which serves to obtain a molecular solution containing both the monomer and the oily phase. If this solution is injected into water (Fig. 5), the oil ceases to be soluble in the medium, and rapidly dispersed by agitation, with the formation of small nanometric droplets. The amphilic monomer is then placed at the oil/water interface, where it polymerizes spontaneously to form the polyalkylcyanoacrylate polymer.

The mechanism proposed above agrees with the observations made during the study of the influence of the main physicochemical and technological parameters on the characteristics of the nanocapsules obtained. The surfactant (poloxamer) is only useful for nanocapsule physical stability. However, it is not directly involved in the capsule formation mechanism. The effects of temperature and pH are also comparable to those reported by Florence et al. (1976). In the process they describe, as in our own, a relatively low temperature and slightly basic pH are favourable to capsule formation. In the Couvreur process (Couvreur et al., 1979), which corresponds to an emulsion polymerization mechanism, the anionic polymerization, which is too rapid in alkaline medium, must be carried out in acidic medium (pH around 2–3).

The many investigations conducted in the area of drug vectorization have shown that the ideal carrier must possess many qualities, some of them contradictory: submicroscopic size, to be able to circulate in the vascular system and to allow endocytosis, harmlessness and biodegradability, ability to fix a sufficient quantity of active ingredient, stability and possible sterilization, tropism for the intended signal, etc. Several systems have been proposed, but none possesses all these qualities.

Liposomes, which are small vesicles with lipoidic walls structurally related to those of biological membranes, offer the advantage of relative harmlessness, but they are not easy to obtain on the industrial scale, and their stability is limited. Moreover, the entrapment levels obtained are generally low. Proposed by Speiser (1974), and investigated by Kreuter (1978, 1983) and Sjoholm (Sjoholm and Edman, 1979), acrylic polymer based nanospheres require the use of radiation to initiate polymerization. Their biodegradability is also low, and repeated administrations incurr the risk of tissue accumulation. The polyalkylcyanoacrylate based nanospheres proposed by Couvreur (Couvreur et al., 1979) offer the major advantage of being biodegradable (Lenaerts et al., 1984). The

TABLE 4

COMPARATIVE TABLE OF DIFFERENT KIND OF NANOPARTICLES

	Nanoparticles		
	Speiser (1974)	Couvreur (1979)	Fallouh et al. (this work)
Polymer	Méthylmethacrylate	Polyalkylcyanoacrylate	Polyalkylcyanoacrylate
Surfactant	15%	< 0.8%	0.5– > 0%
Ease of prep.	–	+	+ +
Sterilization	+	+	+
Biodegradability	–	+	+
Nature of drug	hydrophilic	hydrophilic	lipophilic
Type of nanoparticle	nanosphere (nanocapsule)	nanosphere	nanocapsule

active ingredients are adsorbed or enclosed in a dense polymer network, and the entrapment levels range from 15 to 90%. In vivo, the rate of degradation of the nanospheres depends on the length of the polymer's alkyl chain. This makes it possible to prepare nanospheres that release the active substance at different rates.

In Table 4 are compared some of the advantages and drawbacks of the different types of nanoparticles reported above: those of Speiser, Couvreur and those described here.

The comparison shows that our system offers several positive advantages.

(1) Carried out at ambient temperature, without major external energy input, preparation is simple, rapid and easily transposable to the industrial scale.

(2) The manufacture pH, around neutral, enhances the stability of many active ingredients. The possibility of preparation without surfactant eliminates one potential cause of toxicity.

(3) Related to the presence of a central cavity, the entrapment level can be especially high, at least for lipophilic substances.

(4) The suspensions obtained are stable at ambient temperature and easy to sterilize by autoclaving.

(5) The biodegradability and harmlessness of the polymers used have been demonstrated (Lenaerts, 1984). As biodegradation of nanospheres is already rapid and complete, then nanocapsules, with a higher ratio of active ingredient per unit mass polymer, should be rapidly cleared.

Conclusion

This article describes a new process for the manufacture of polyalkylcyanoacrylate nanocapsules. Based on the principle of interfacial polymerization, this technique offers a new type of oily vesicular carrier about 200–300 nm in diameter, displaying some valuable advantages: (1) easy preparation requiring conditions favourable to the stability of active ingredients and easily transposable to the industrial scale; (2) particularly high encapsulation level for lipophilic substances; (3) sterilization possible by autoclaving and stability of nanocapsule suspensions; and (4) potential biodegradability and harmlessness should be at least comparable to those of the corresponding nanospheres.

Following a number of preliminary experiments, this new type of nanoparticles is now subject to intensive study in our laboratory, designed to assess its value in several areas: labile drug protection in water medium, insoluble drug administration by i.v. route, absorption and bioavailability by oral administration, modification of the drug distribution in tissues.

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