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Notes

Spontaneous formation of drug-containing amphiphilic β-cyclodextrin nanocapsules

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Abstract

A new colloidal carrier system, nanocapsules, using 2,3-diacyl-*O*-β-cyclodextrins is described. The nanocapsules were prepared by adding a mixture of an organic solution of 2,3-diacyl-*O*-β-cyclodextrins (with or without a lipophilic surfactant) and an oily phase to an aqueous solution (with or without a hydrophilic surfactant) whilst stirring. Nanocapsules with a mean size of 200 nm are formed progressively. Direct microscopic observation of the particles was carried out using transmission electron microscopy (TEM) combined with the freeze-fracture method. The influence of different constituents on the preparation process was studied: type of 2,3-diacyl-*O*-β-cyclodextrins (β-CD-C_6, β-CD-C_{12} and β-CD-C_{14}), nature of the oily phase (benzyl benzoate, Miglyol 812®), and the surfactants used (Pluronic PEF68®, Span 85®). An investigation of lipophilic drug encapsulation was also carried out. The results of this study suggest that nanocapsules of 2,3-diacyl-*O*-β-cyclodextrins could be considered as a potential colloidal drug carrier system. Copyright © 1996 Elsevier Science B.V.

Keywords: Amphiphilic β-cyclodextrins; Drug carrier; Nanocapsules

1. Introduction

Nanocapsules are described in the literature as an oily cavity surrounded by a thin polymer wall. A wide variety of polymer materials, such as poly(lactide-co-glycolide), poly-ε-caprolactone

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and polyalkylcyanoacrylate, can be used for this purpose. Nanocapsules are interesting because the system is ideal for the encapsulation of a high level of lipophilic active ingredients (Vauthier-Holtzscherer et al., 1991).

In this paper, we report the preparation of a new type of nanocapsule using amphiphilic β-cyclodextrins (Skiba et al., 1993). Two main physicochemical manufacturing parameters were studied. Firstly, the influence of the amphiphilic β-cyclodextrin used, and secondly, the nature of the oily phase. The effect of surfactants on the preparation of the nanocapsules and on their capacity to entrap lipophilic drug was also investigated.

2. Materials and methods

2.1. Materials

The amphiphilic cyclodextrins were synthesized by grafting fatty acids of 6, 12 and 14 carbons to the secondary hydroxyl groups of glucose units of natural β-cyclodextrin as reported in the previous paper (Zhang et al., 1991; Skiba et al., 1995a). The amphiphilic derivatives obtained are called respectively β-CD-C_6, β-CD-C_{12} and β-CD-C_{14}. The lipophilic phase was either an oil, such as Miglyol 812® (Lambert-Rivière, Fontenay sous Bois, France) or benzyl benzoate (Prolabo, Paris, France). Poloxamer 188 (Pluronic PEF68®) and sorbitan fatty acid esters (Span 85®) were obtained from ICI France (Clamart, France), and used as surfactants. Indomethacin and amphotericin B were purchased from Sigma Chemicals (St. Louis, MO, USA) and progesterone was kindly provided by Laboratoires Besins Iscovesco (Montrouge, France). The other ingredients used were of analytical grade.

2.2. Methods

2.2.1. Preparation of nanocapsules

Nanocapsules were obtained by the method of Skiba et al. (1993). A lipophilic phase, composed of 250 ml of Miglyol 812® or benzyl benzoate and 25 mg of amphiphilic β-cyclodextrin, was dissolved in 12.5 ml of acetone and added under mechanical stirring to 25 ml of an aqueous phase consisting of distilled water dissolved (or not) with Pluronic PEF68® (31 mg). The nanocapsules formed immediately. Acetone was totally removed by evaporation under vacuum, together with part of the water, to obtain a desired concentrated colloidal suspension. For loaded nanocapsules, the drug was added in organic phase.

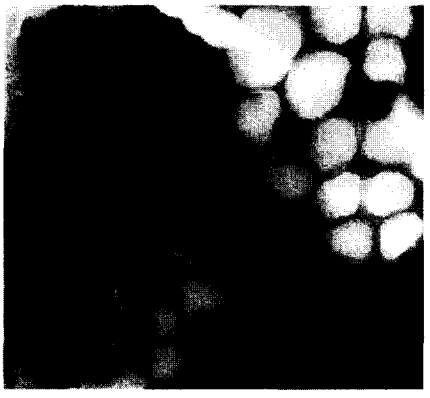

Fig. 1. Transmission electron micrographs of β-CD-C_6 nanocapsules containing benzyl benzoate.

2.2.2. Size of nanocapsules

The size of nanocapsules was estimated by quasi-elastic laser light scattering using a monochromatic laser ray diffusion counter (Nanosizer®, N4MD Coultronics France, Andilly, France).

2.2.3. Ultrastructural aspect of nanocapsules

The morphology of the nanocapsules was examined, following negative staining of the nanocapsules preparation with sodium phosphotungstate solution using a transmission electron

Fig. 2. Transmission electron micrograph of β-CD-C_6 nanocapsules containing benzyl benzoate after freeze-fracture.

Table 1
Effect of modified β-CD and surfactants on the mean diameter (nm) of the nanocapsules containing Miglyol 812® or benzyl benzoate

	Modified β-CD nanocapsules: mean diameter (nm)		
	β-CD-C_6	β-CD-C_{12}	β-CD-C_{14}
Miglyol 812®			
Without surfactant	107 ± 17 (0.045)*	103 ± 12 (0.032)	89 ± 26 (0.093)
Pluronic PEF68®	103 ± 14 (0.096)	106 ± 17 (0.069)	98 ± 16 (0.048)
Benzyl benzoate			
Pluronic PEF68®	204 ± 49 (0.083)	183 ± 48 (0.057)	178 ± 15 (0.130)
Span 85®	256 ± 66 (0.040)	223 ± 49 (0.001)	213 ± 43 (0.029)

* Polydispersity index (PI) shown in parentheses.

microscope (TEM), and also the freeze-fracture procedure (Skiba et al., 1994).

2.2.4. Stability tests

The physical stability of the nanocapsules was measured by the variation of the particle size and size distribution during storage in different conditions. A known amount of nanocapsules suspension was placed into several vials and the vials were stored for 27 months at ambient temperature, at 4, 25 and 40°C; thereafter, the samples were examined at 1 week intervals.

2.2.5. Entrapment efficiency

Indomethacin, progesterone and amphotericin B were estimated by an high-performance liquid chromatography (HPLC) technique. The determination of the percentages of drug recovery or drug contained in the nanocapsules was carried out as described previously by Skiba et al. (1995b).

3. Results and discussion

3.1. Morphological analysis

The TEM microphotograph in Fig. 1 shows spherical particles with a diameter of around 200 nm. Freeze-fracture micrographs (Fig. 2) clearly show a spherical oily core surrounded by a thickening envelope. The process of the assembly of amphiphilic cyclodextrins leading to the formation of such vesicles needs further physical analysis before being completely elucidated.

3.2. Influence of the amphiphilic cyclodextrins, the oily phase and the surfactant

It was possible to produce nanocapsules without surfactant when Miglyol® was used as the oily phase, whereas benzyl benzoate did not allow nanocapsules to be obtained. This situation is probably due to the fatty acids of Miglyol® (mixture of triglycerides of saturated fatty acid), which act as a stabilizer of the nanocapsule wall.

The use of Span 85® (lipophilic surfactant, HLB 18) with Miglyol oily phase was not suitable for stabilizing the nanocapsule wall to the interface with the aqueous phase, in contrast to the hydrophilic surfactant, Pluronic PEF68®, HLB 29), which is convenient for this purpose.

In all cases, when the nanocapsules are produced, it seems that the mean size of the nanocapsules obtained using benzyl benzoate (density 111) is slightly inferior to that of nanocapsules prepared with Miglyol® (density 095). This could be due to the higher density of benzyl benzoate, which led to smaller oil droplets during the preparation procedure (Table 1).

Table 2
Periods of storage (months) of nanocapsules containing Miglyol 812® or benzyl benzoate

	Temperature (°C)	β-Cyclodextrin nanocapsules: period of storage (months)		
		β-CD-C_6	β-CD-C_{12}	β-CD-C_{14}
Oily phase: Miglyol 812®				
Without surfactant	4	27	1	27
	25	6	12	6
	40	2	1	2
PEF68®	4	27	27	6
	25	27	27	27
	40	2	6	6
Oily phase: benzyl benzoate				
PEF68®	4	1	27	27
	25	1	27	27
	40	1	27	27
Span 85®	4	1	27	27
	25	27	27	27
	40	27	27	27

3.3. Effect of formulation variables on formulation stability

Long-term stability of nanocapsules made from β-CD-C_m ($m = 6$, 12 and 14) at ambient temperature, at 4, 25 and 40°C, with and without surfactant, are shown in Table 2. Nanocapsules containing miglyol 812 presented a variable stability with different conditions of conservation and with the type of amphiphilic cyclodextrins used. When a hydrophilic surfactant was added (PEF 68®), the conservation was better, especially at 25°C.

Nanocapsules containing benzyl benzoate and prepared with a hydrophilic (PEF 68®) or lipophilic (Span 85®) surfactant were more stable at the different temperatures of conservation, except for the nanocapsules made from β-CD-C_6 prepared with hydrophilic surfactant (PEF68®), probably because they contains only a short acyl chain.

3.4. Lipophilic drug entrapment and encapsulation efficiency

The results of encapsulation efficiency of three drugs in the nanocapsules made from β-CD-C_6 with benzyl benzoate as the oily phase are summarized in Table 3. As expected, these nanocapsules showed a higher capability to encapsulate lipophilic ingredients than the nanospheres made from the same cyclodextrins, as described previously (Skiba et al., 1996). It is probable that these drugs are largely solubilized in the oily core of the system.

Table 3
Entrapment of various drugs in β-CD-C_6 nanocapsules containing benzyl benzoate

Drug	Drug recovery in β-CD-C_6 nanocapsules (%)	Drug content in β-CD-C_6 nanocapsules (% [w/w])
Progesterone	90	21.6
Indomethacin	>98	7.5
Amphotericin B	90	11

4. Conclusions

This paper proposed a new type of nanocapsule made from amphiphilic β-cyclodextrins and prepared by nanocrystallization technique. Evaluation of the encapsulation capacity was done with lipophilic drugs, and showed that nanocapsules made from β-CD-C_6 with benzyl benzoate as the oily phase have important advantages over nanospheres made from the same cyclodextrins.

This technique offers a new type of oily vesicular carrier about 100–250 nm in diameter displaying some valuable advantages: (a) easy preparation requiring conditions favourable to the stability of active ingredients and easily transposable to an industrial scale; (b) a particularly high encapsulation level for lipophilic substances.

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