ELSEVIER

International Journal of Pharmaceutics 129 (1996) 113–121

international journal of pharmaceutics

Development of a new colloidal drug carrier from chemically-modified cyclodextrins: nanospheres and influence of physicochemical and technological factors on particle size

Mohamed Skiba, Dominique Duchêne, Francis Puisieux, Denis Wouessidjewe*

Laboratoire de Physico-chimie, Pharmacotechnie et Biopharmacie, URA CNRS 1218, Centre Pharmaceutique, Université de Paris XI, Rue Jean Baptiste Clément, 92290 Châtenay Malabry, France

Received 2 June 1995; accepted 21 July 1995

Abstract

A new nanosphere carrier system has been obtained from amphiphilic β-cyclodextrin (fatty acid chains varying from 2 to 14 carbons grafted at the O_2 and O_3 positions of β-cyclodextrin). The nanospheres, with a mean diameter varying between 90 and 150 nm, are prepared by progressive dispersion of an organic solution of modified β-cyclodextrin in an aqueous phase with or without surfactant. Various physicochemical parameters have been studied: the effect of the chain length of acyl groups (βCD with 6, 12 and 14 fatty acid carbons), and type of surfactant on the size and physicochemical properties and stability of the nanospheres. A preliminary investigation of water-soluble and insoluble drug entrapment by nanospheres was carried out.

Keywords: Amphiphilic β-cyclodextrins; Nanosphere; Drug carrier; Stability; Zeta potential; Poloxamer; Indomethacin; Progesterone; Metronidazole; Doxorubicin

1. Introduction

The ability to target drugs to specific tissue sites is one of the major challenges in pharmaceutical technology. To achieve this goal, a number of technical approaches have been proposed, including the use of submicronic colloidal carriers, such as liposomes and nanoparticles. Nanoparticulate supports, which are generally made with suitable biodegradable polymers, such as polyalkylcyanoacrylate or poly(lactide-co-glycolide) seem to be particularly attractive (Fessi et al., 1988; Puisieux et al., 1994). The purpose of this paper is to describe a new colloidal carrier (nanospheres) prepared from non-polymeric material such as amphiphilic β-cyclodextrin (Skiba et al., 1993). The physicochemical properties of the nanospheres were investigated; particle diameter, zeta potential and drug entrapment.

* Corresponding author. Current address: Laboratoire de Physico-chimie, Pharmacotechnie et Biopharmacie, URA CNRS 1218, Centre Pharmaceutique, Univesité de Paris XI, Rue Jean Baptiste Clément, 92290 Châtenay Malabry, France.

SSDI 0378-5173(95)04272-C

The operating factors studied were: type of amphiphilic β-cyclodextrin; effect of stirring rate; temperature of aqueous phase; type and concentration of added surfactants; type and concentration of added electrolyte on the surface charge of amphiphilic β-cyclodextrin nanospheres.

2. Materials and methods

2.1. Materials

2,3-Di-acyl-*O*-β-cyclodextrins derivatives with different chain length (pentyl, undecaonyl and tridecaonyl) were obtained by acylation of the secondary hydroxyl groups on C-2 and C-3 positions of each glucopyranose using pentyl, undecaonyl and tridecanoyl chloride. For the sake of clarity of presentation 2,3-di-pentyl-, undecaonyl- and tridecanoyl-*O*-β-cyclodextrins will be expressed as βCD-C_6, βCD-C_{12} and βCD-C_{14}, respectively. The derivatives were synthesized in Laboratoire de Chimie Organique (CNRS ER 45) according to the method described by Zhang et al. (1991) and reported by Skiba et al. (1994). Indomethacin, doxorubicin hydrochloride and metronidazole were purchased from Sigma Chemicals (USA) and progesterone was kindly provided by Laboratoires Besins Iscovesco, Montrouge, France. Poly(ethylene oxide)-poly(propylene oxide) block copolymers were chosen as nonionic surfactants: Pluronic® PEF68; PEF87; PEF88; PEF108; PEF127; PEP85; obtained from ICI Surfactants, Clamart, France. Sorbitan fatty acid esters were used as lipophilic surfactant (Span 85®); benzethonium chloride as cationic surfactant ($C_{27}H_{42}NO_2Cl$ (BZD^+)); sodium lauryl sulfate as anionic surfactant ($C_{12}H_{25}O_4SNa$ (SDS^-)); acetone; ethanol; sodium chloride (NaCl); calcium chloride ($CaCl_2$, $2H_2O$) were supplied by Sigma, St. Quentin Fallavier, France.

2.2. Methods

2.2.1. Preparation of amphiphilic β-cyclodextrin nanospheres

The preparation method developed in this study was based on the spherical crystallization technique (Kawashima et al., 1982; Kawashima et al., 1989) developed by Skiba et al. (1994a). An organic solution (12.5 ml of acetone or absolute ethanol) of amphiphilic β-cyclodextrin (25 mg) was injected into an aqueous phase (25 ml) with or without dissolved surfactant (31 mg) under magnetic stirring at room temperature. Nanospheres were formed spontaneously. The organic solvent, and part of the water, were removed by evaporation under vacuum to give the desired concentrated colloidal suspension. The preparation of drug-loaded nanospheres was carried out in the same manner with soluble and insoluble drug dissolved, respectively, in the aqueous phase and in the organic solvent.

2.2.2. Size distribution evaluation

The mean nanosphere size and size distribution were determined by photon correlation spectroscopy using a Nanosizer instrument N4MD (Coultronics SA, Andilly, France) which analyses the fluctuations in scattered light intensity generated by diffusion of nanospheres in suspension. Experimental conditions were: temperature, 25°C; reference index, 90°; viscosity, 0.899×10^{-3} Pa; refractive index, 1.330.

2.2.3. Long-term stability study

Fifteen-ml aliquots of the nanosphere suspension prepared as above were transferred into ampules, which were sealed and kept at 4, 25 and 40°C. Every week, an ampule was opened and the sample was pipetted, diluted to the appropriate concentration with water and the mean size and size distribution were determined.

2.2.4. Zeta potential

The charge of nanospheres was measured with a Malvern Zetasizer 2C instrument equipped with a tubular cell of 2.6 mm. The operating principle of this instrument is based on the Doppler shift caused by the movement of nanospheres across interference fringes which are produced by the intersection of two laser beams. The nanospheres were suspended in 10^{-3} M KCl, and the measurement was made at 25°C.

Table 1
Influence of modified β-cyclodextrin and surfactant on the mean diameter size (nm) of the βCD-C_m nanospheres (m = 6, 12 and 14)

βCD-C_m	βCD-C_6	βCD-C_{12}	βCD-C_{14}
Without surfactant	107 ± 17 (0.045)[a]	103 ± 12 (0.032)	89 ± 26 (0.093)
Pluronic PEF68®	103 ± 14 (0.096)	106 ± 17 (0.069)	98 ± 16 (0.048)
Span 85®	120 ± 31 (0.072)	101 ± 19 (0.075)	100 ± 18 (0.076)

[a]Polydispersity index (PI) shown in parentheses.

2.2.5. Drug uptake studies

2.2.5.1. Progesterone content. The progesterone content of the nanospheres was analyzed using a Waters HLPC system (division Millipore, St-Quentin-en-Yvelines, France) equipped with a variable wavelength ultraviolet detector Waters 484, a Waters integrator 746 and a 25-cm, 4.6-mm i.d. reverse phase column, SFCC Nucléosil C_{18}, 10 mm. The column was eluted with acetonitrile/water (60/40 by volume), at a rate of 1 ml/min; the column eluent was monitored at 240 nm and the chromatograph was operated at a pressure of 2000 psi at room temperature. The calibration curve was constructed using freshly prepared samples of standard solutions containing 0.025–0.2% progesterone in methanol. The nanospheres were dissolved (1/10) in acetonitrile and injected into a 20-μl loop. The nanosphere suspensions were centrifuged for 30 min at 100 000 × g. The supernatants were separated, diluted and analyzed. The amount of drug associated with the nanoparticles was determined from the difference in the initial amount dosed and the amount free in the supernatant.

2.2.5.2. Metronidazole content. The HLPC operating conditions were the same as those for progesterone assays, except that the eluant was a 0.05 M methanol-sodium acetate mixture (30/70 by volume) at a rate of 0.8 ml/min, the column eluent was monitored at 320 nm.

2.2.5.3. Indomethacin content. The drug loaded and drug lost in the aqueous suspension medium were assayed by an HPLC method described previously (Skiba et al., 1996).

2.2.5.4. Doxorubicin hydrochloride content. The drug loaded and drug lost in the aqueous suspension medium were assayed by an HPLC method described previously (Verdun et al., 1986).

3. Results and discussion

3.1. Feasibility of nanospheres

The general preparation procedure described above allowed us to produce nanospheres with high reproducibility from batch to batch. The average mean size of the particles varied between 90 and 150 nm, depending on the materials used and on the experimental conditions.

3.2. Influence of the operating parameters on the characteristics of the nanospheres

Two main characteristics were selected to analyze the influence of manufacturing factors: mean particle diameter and long-term stability of the colloidal suspension.

3.2.1. Influence of the type of amphiphilic β-cyclodextrin and surfactant

Table 1 shows that it was possible to obtain fresh and stable colloidal suspensions of nanospheres without the use of a surfactant in the preparation medium. The mean diameter in all cases is about 100 nm, and the polydispersity index indicated a very narrow size distribution. These results suggest a highly favourable thermodynamic capability of amphiphilic β-cyclodextrin to form nanospheres. The size of nanospheres varied from 90 to 107 nm indicating no linear

relationship between size and the length of the esterified alkyl chain.

3.2.2. Influence of stirring rate

Fig. 1 shows that nanospheres could be obtained by mixing the aqueous phase and the organic phase without stirring. Moreover, the variation in stirring speed does not affect the particle mean diameter size of the nanospheres greatly. Long-term stability of nanospheres made from βCD-C_6 at 4, 25 and 40°C without and with magnetic stirring are shown in Fig. 2. Nanospheres prepared with stirring could be conserved for 27 months at 4, 25 or 40°C without any change in their diameter or polydispersity index. In contrast, when the nanospheres were prepared without stirring a small increase in size was observed at all temperatures.

3.2.3. Influence of concentration of non-ionic, anionic and cationic surfactants on the particle size and zeta potential of βCD-C_6 nanospheres

The influence of F68, $Benz^+$ and SDS^- concentration on βCD-C_6 nanosphere size, polydispersity index, mobility and zeta potential is shown in Table 2.

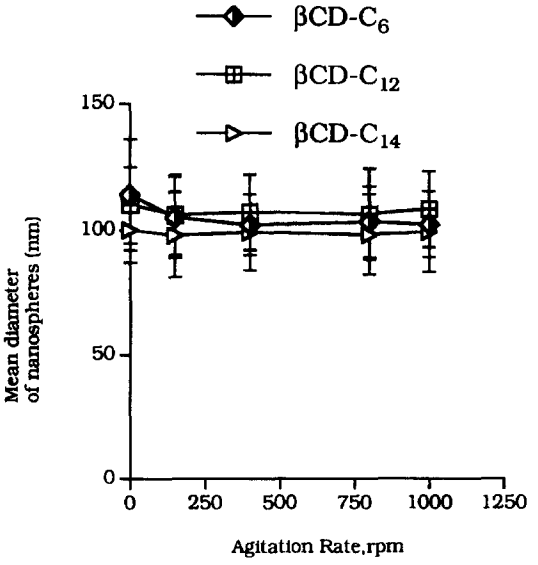

Fig. 1. Effect of stirring rate on the mean diameter size of βCD-C_m nanospheres.

Anionic and cationic surfactants can influence the size, mobility and charge of colloidal dispersions considerably. In the presence of SDS^-, the nanospheres are always negatively charged at the surface; when BZD^+ is used as surfactant, the zeta potential values indicated a positive charge on the surface, which increased with surfactant concentration but was not proportional to it.

The nonionic surfactant F68 had little effect on the size and zeta potential of the nanospheres. This is probably due to its chemical structure 'poly(ethylene oxide)-poly(propylene oxide)' with two long hydrophobic chains flanking a hydrophilic segment (Table 3). During the formation of nanospheres this surfactant might be incorporated into the matrix formed by the cyclodextrins. In contrast, the ionic surfactants with a single hydrophobic chain will be only partially included in the matrix, leaving the charged head-group on the surface, leading to a change in zeta potential.

3.2.4. Influence of poloxamer type at 0.1% (w/v)

There are many types of poloxamer which may be characterized by their molecular blocks (x, y, z) (Table 3).

As the properties of βCD-C_6 nanospheres are affected by the type of poloxamer, it would be useful to establish the effect of molecular blocks on the size of βCD-C_6 nanospheres. In this study, various types of poloxamer were used (Table 3). The results show an increase on the particle size when $x < y$, but no effect on zeta potential.

The study on the effect of HLB on the mean size of the nanospheres, showed that this decreased disproportionately from 166 $\pm$ 75 nm at HLB 16 to 100 $\pm$ 13 nm at HLB 29, which is due to a short hydrophilic chain flanking a long hydrophobic segment (Table 3).

3.2.5. Influence of temperature of the aqueous phase on the mean diameter (nm) of the nanospheres

Five temperatures, 4, 10, 25, 50 and 100°C, were investigated (Fig. 3). The results show that this parameter does not modify the particle size for 4–50°C. However, an increase is observed at 100°C, which is probably due to the rapid evaporation of acetone at this temperature during the preparation procedure.

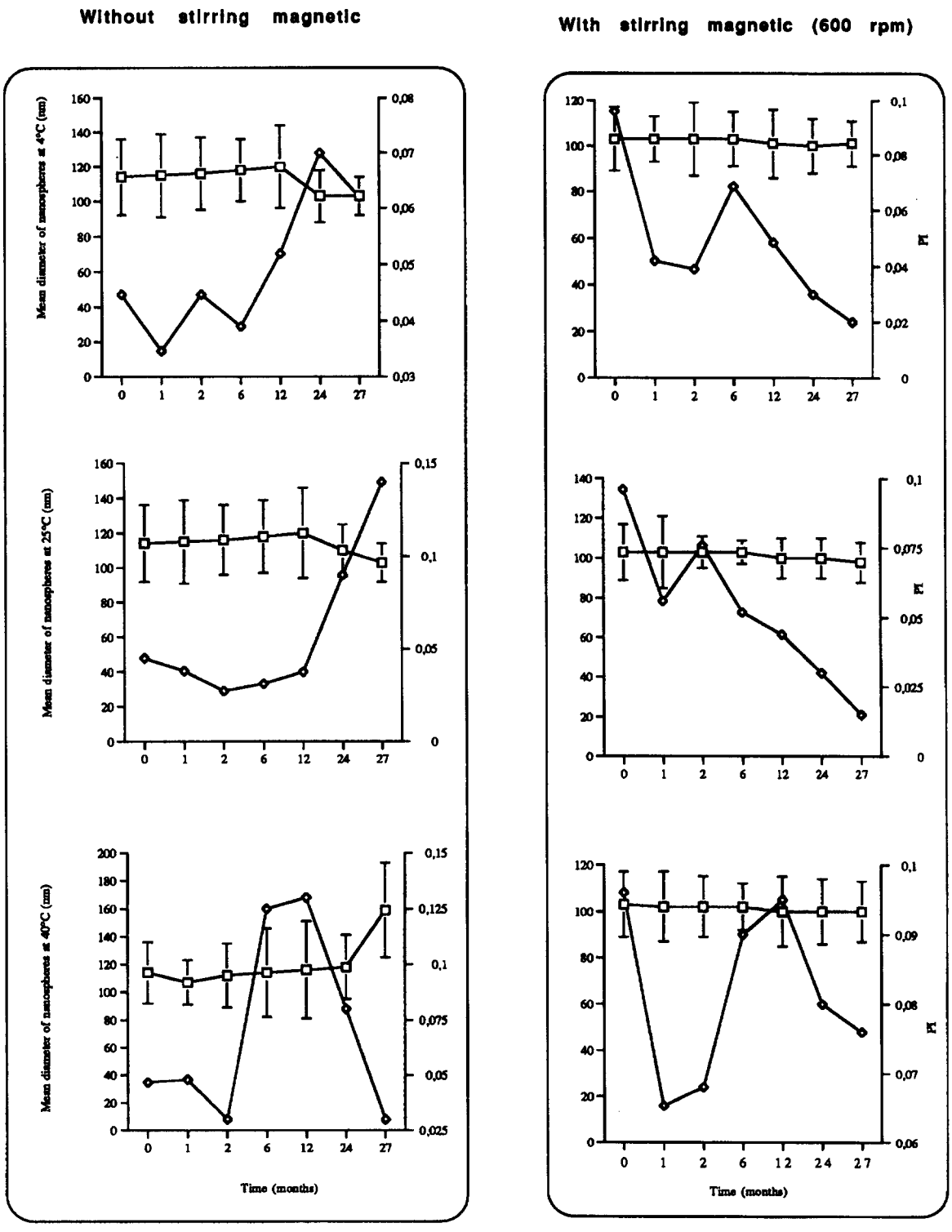

Fig. 2. Effect of magnetic stirring on mean size and polydispersity index (PI) of βCD-C_6 nanospheres and long-term stability at 4, 25 and 40°C.

Table 2
Variation of size and zeta potential of βCD-C_6 nanospheres with F68, SD^- and BZD^+ surfactant concentration

Surfactant (% w/v)	Size ± SD (nm)			Zeta potential ± SD (mV)		
	F68	SDS^-	BZD^+	F68	SDS^-	BZD^+
0	100 ± 13 (0.05)[a]	100 ± 13 (0.05)	100 ± 13 (0.05)	−26 ± 2	−26 ± 2	−26 ± 2
0.055	103 ± 17 (0.03)	136 ± 33 (0.11)	135 ± 54 (0.088)	−24 ± 3.1	−40 ± 3	+42.8 ± 4
0.156	100 ± 23 (0.04)	140 ± 24 (0.032)	159 ± 34 (0.1)	−22 ± 2	−48 ± 4	+50 ± 2
0.277	105 ± 14 (0.01)	139 ± 26 (0.018)	173 ± 29 (0.079)	−30 ± 3	−50 ± 2	+52 ± 2

[a]Polydispersity index (PI) shown in parentheses.

3.3. βCD-C_6 nanosphere zeta potential

Zeta potential is related to the charge on the surface of the particles, and so influences a wide range of properties of colloidal materials. In vivo, the surface charge has been found to influence the distribution of nanospheres and, in vitro, a high potential might contribute to their physical stability by reducing the rate of aggregation and fusion (Dalgleish, 1983).

Suspensions of βCD-C_6 with and without Pluronic were used to investigate the influence of: the pH; the electrolyte concentration required to obtain an inversion the zeta potential; the electrolyte concentration required to obtain the point of zero charge (PZC); the influence of the nature of the salt.

The pH-dependence of the zeta potentials of the βCD-C_6 nanospheres with and without Pluronic are shown in Fig. 4. No effect of pH was observed on the zeta potential of βCD-C_6 nanospheres with F68. The zeta potentials of the βCD-C_6 nanospheres without F68 approached zero with the exception of a narrow window around pH 7.4. Such dispersions are not electrostatically stable and tend to aggregate.

The difference in the pH-dependence of the zeta potentials of the βCD-C_6 nanospheres with and without F68 were related to the presence of the non-ionic surfactant. Nanospheres prepared without surfactant precipitated in acidic or basic medium but were stable at neutral pH in two ways: (1) masking sites at which an excess of H^+ or OH^- ions, complexing the cavity of the amphiphilic cyclodextrin, causes the nanospheres to precipitate; (2) increasing the stability by adding other interaction with surface hydroxyl groups (hydrogen bonds, hydrophobic or Van der Waals' interactions).

Two salts (NaCl and $CaCl_2$) were added in increasing concentrations to the βCD-C_6 nanospheres with and without F68 and the resulting zeta potentials were determined (Fig. 5 and Fig. 6).

The accumulation of ions (Ca^{2+} and Na^+) near the surface causes the particle charges to be screened, thus reducing the zeta potential. Ions can conveniently be divided into two classes depending on how they interact with the surface of the βCD-C_6 nanospheres:

(1) Bridge ion (Ca^{2+}) (Fig. 7a) are those which are only attracted to particles by virtue of their charge in a purely electrostatic manner, a process know as non-specific adsorption. In Fig. 7 we find that the screening effect of the Ca^{2+} gradually reduces the zeta potential (not the surface potential), and this approaches zero at high Ca^{2+} concentrations. This is accompanied by particle aggregation, as previously observed by Dalgleish (1983). It is possible that these changes in zeta potential of βCD-C_6 nanospheres with and without F68 with the concentration of Ca^{2+} are responsible for their changing rates of aggregation in such suspension.

(2) Uniquely adsorbed ions (Na^+) (Fig. 7b) interact chemically with the surface or interior of one nanosphere, for example by complexation with groups on the surface. Consequently, as their concentration is increased, they also screen the zeta potential, but the additional chemical (as

Table 3
Properties of βCD-C_6 nanospheres obtained with Poloxamer type at 0.156% (w/v) concentration

Poloxamer	HLB	Molecular blocks (average value in mol)			Size ± SD (nm)	Zeta ± SD (mV)
		x	y	z		
PEF 68	29	75	30	75	100 ± 13 (0.096)[a]	−22 ± 2
PEF 88	28	97	39	97	144 ± 23 (0.064)	−28.8 ± 1.7
PEF 108	27	128	54	128	145 ± 43 (0.093)	−14.2 ± 1.7
PEF 87	24	62	39	62	136 ± 12 (0.045)	−25.3 ± 2.9
PEF 127	22	98	67	98	110 ± 19 (0.059)	−24 ± 1.6
PEP 85	16	27	39	27	166 ± 75 (0.041)	−19.7 ± 1.7

[a]polydispersity index (PI) shown in parentheses.
Poloxamer structure: $HO(-CH_2-CH_2O)_x\left(CH(CH_3)-CH_2O\right)_y(CH_2CH_2O)_zH$

distinct from electrostatic) binding on the surface causes sufficient adsorption of Na^+ for the original nanosphere charge to be neutralised and then reversed as the Na^+ concentration increases.

In such a system we see a point of zero charge (PZC) at a well-defined Na^+ concentration, prior to charge reversal (Fig. 6).

The curves obtained from nanospheres prepared with or without surfactant had a similar form except that, in the case of nanospheres without surfactant, one or more plateaux were observed. These could be due to the binding of ions within the cavity of amphiphilic cyclodextrins, which is prevented in the presence of F68.

3.4. Efficiency of drug uptake

The preliminary results of drug uptake in typical βCD-C_6 nanospheres are shown in Table 4. The nanospheres are capable of combining with water-soluble drugs (such as metronidazole and doxorubicin hydrochloride) and insoluble drugs (progesterone and indomethacin).

From these results it can be concluded that the drug loading of βCD-C_6 nanospheres is much higher than that which can be obtained by forming inclusion complexes of the same drugs with

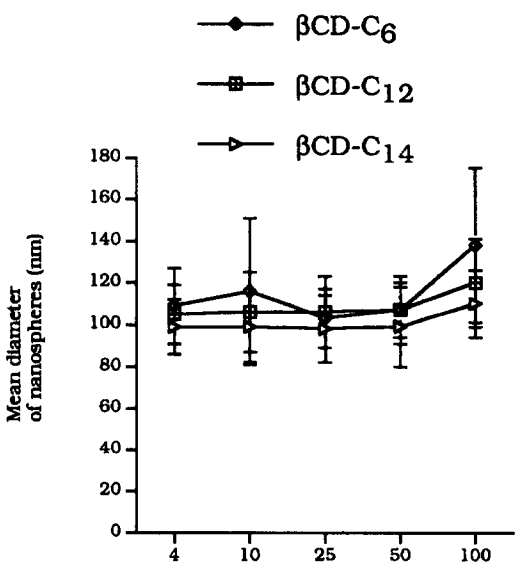

Fig. 3. Effect of aqueous phase temperature on the mean diameter (nm) of the βCD-C_m nanospheres (m = 6, 12 and 14).

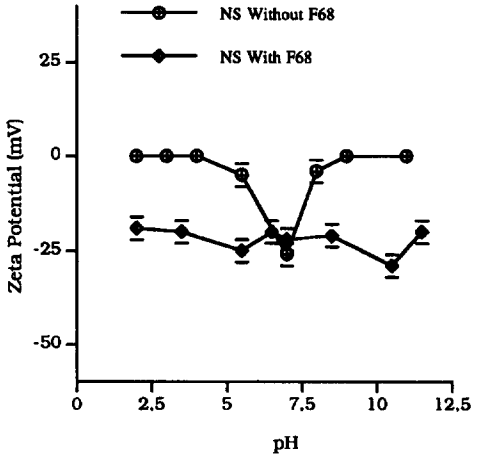

Fig. 4. Zeta potential vs. pH profiles of βCD-C_6 nanospheres with and without surfactant (F68) in 0.001 M KCl solution.

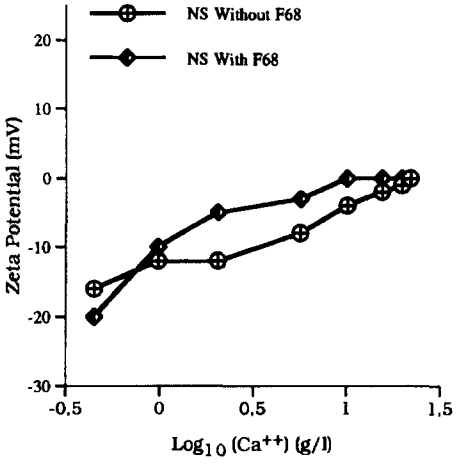

Fig. 5. Zeta potential vs. $[Ca^{2+}]$ profiles of βCD-C_6 nanospheres with and without surfactant (F68).

individual natural cyclodextrins, when the loading is at best 10%.

4. Conclusion

This article describes a process for the manufacture of the new nanospheres made from amphiphilic β-cyclodextrins and the importance of the effects of the formulation parameters on the physicochemical properties and stability of the

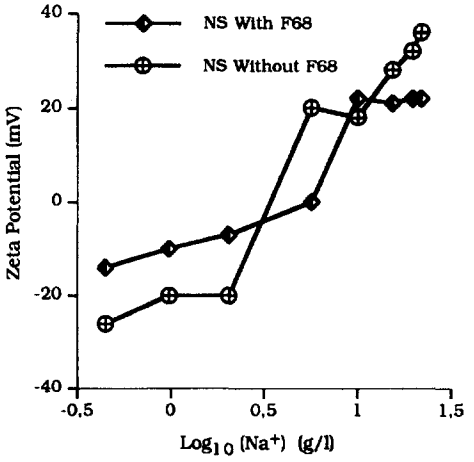

Fig. 6. Zeta potential vs. $[Na^{+}]$ profiles of βCD-C_6 nanospheres with and without surfactant (F68).

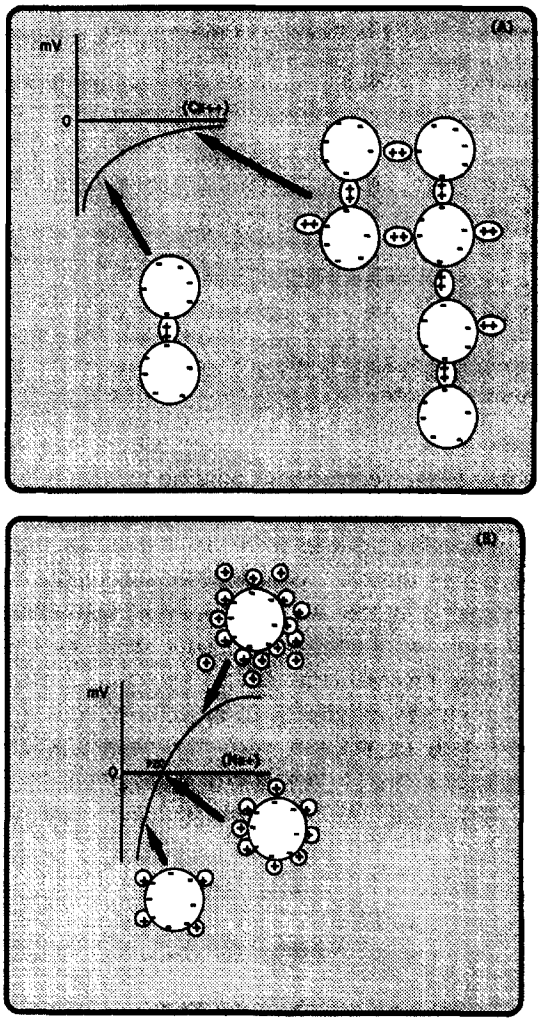

Fig. 7. Zeta potential shapes and corresponding adsorption models $[Ca^{2+}]$ and $[Na^{+}]$.

nanospheres. A narrow and reproducible particle size distribution and a maximum drug loading between 2 and 190% w/w can be obtained. Because of their satisfactory physical stability in pH from 1 to 11, there might be potential for such formulations to be used in oral, ocular and intravenous administration.

This new type of nanospheres could have therapeutic value in several areas: labile drug protection in aqueous medium, insoluble drug administration by intravenous route, absorption and bioavailability by oral administration, modification of the drug distribution in tissues.

Table 4
Entrapment of various drugs in βCD-C_6 nanospheres

Drug	Drug recovery in nanospheres, βCD-C_6 (%)	Drug content in nanospheres, βCD-C_6 (% w/w)
Metronidazole	85	190
Doxorubicin hydrochloride	60	6
Progesterone	86	9
Indomethacin	90	2

References

Dalgleish, D.G., coagulation of renneted bovine casein micelles: dependence on temperature calcium ion concentration and ionic strength. *J. Dairy Res.*, 50 (1983) 331–340.

Fessi, H., Puisieux, F. and Devissaguet, J.-P., Procédé de préparation de systèmes colloïdaux dispersibles d'une substance sous forme de nanosphères. US Patent No. 5118528 (1988).

Kawashima, Y., Okumura, M. and Takenaka, H., Spherical crystallization: Direct spherical agglomeration of salicylic acid crystals during crystallization. *Science*, 216 (1982) 1127–1128.

Kawashima, Y., Niwa, T., Handa, T., Takeuchi, H., Iwamoto, T. and Itoh, K., Preparation of controlled-release microspheres of ibuprofen with acrylic polymers by a novel quasi-emulsion solvent diffusion method. *J. Pharm. Sci.*, 78(1) (1989) 68–72.

Puisieux, F., Barratt, G., Couarraze, G., Couvreur, P., Devissaguet, J.-P., Dubernet, C., Fattal, E., Fessi, H. Vauthier, C., Benita, S., Polymeric micro- and nanoparticles as drug carriers. In Dumitriu (Ed.), *Polymeric Biomaterials*, Marcel Dekker Inc., New York, 1994, pp. 749–794.

Skiba, M., Wouessidjewe, D., Coleman, A., Fessi, H., Devissaguet, J.-P., Duchêne, D. and Puisieux, F., Préparation et utilisation de nouveaux systèmes colloïdaux dispersible à base de cyclodextrine, sous forme de nanosphères, PCT Application FR 93/00594 (1993).

Skiba, M., Wouessidjewe, D., Coleman, A., Fessi, H., Devissaguet, J.-P., Duchêne, D. and Puisieux, F., Development of new submicronic particles from chemically-modified cyclodextrins. Part 1. Nanospheres, *Proc. 7th Int. Symp. on Cyclodextrins*, Tokyo (25/28 April 1994).

Skiba, M., Morvan, C., Duchêne, D., Puisieux, F. and Wouessidjewe, D., Evaluation of gastro-intestinal behaviour of amphiphilic β-cyclodextrin nanocapsules, loaded with indomethacin. *Int. J. Pharm.* 126 (1996) 275–280.

Verdun, C., Couvreur, P., Vranckx, H., Lenaerts, V. and Roland, M., Development of a nanoparticle controlled-release from formulation for human use. *J. Controlled Release*, 3 (1986) 205–210.

Zhang, P., Ling, C.-C., Coleman, A., Parrot-Lopez, H. and Galons, H., Formation of amphiphilic cyclodextrins via hydrophobic esterification at the secondary hydroxyl face. *Tetrahedron Lett.*, 32 (1991) 2769–2770.