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Preparation of amphiphilic cyclodextrin nanospheres using the emulsification solvent evaporation method. Influence of the surfactant on preparation and hydrophobic drug loading

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Abstract

In this study we verified the feasibility of preparing nanospheres from an amphiphilic 2,3-di-*O*-hexanoyl-γ-cyclodextrin (γCDC_6) using the emulsification solvent evaporation method. This preparation method consists in emulsifying an organic phase containing the cyclodextrin in an aqueous phase containing Pluronic F68 as surfactant. The influence of the process parameters, i.e. surfactant concentration and initial γCDC_6 content, on the characteristics of nanosphere preparation, as well as on the nanosphere loading of a hydrophobic drug, progesterone, was evaluated. Cyclodextrin nanospheres presenting a mean diameter varying from 50 to 200 nm were obtained, even in the presence of low surfactant concentration. The formation of colloidal particles in these conditions was associated with the amphiphilic properties of the cyclodextrin derivative. However the partitioning of the γCDC_6 molecule between the organic and aqueous phases was observed as being a function of surfactant concentration in the continuous phase. This partitioning was related to the formation of very small aggregates of the order 10 to 20 nm, probably Pluronic F68/γCDC_6 mixed micelles as evidenced by the micrographs obtained by TEM. In the case of the nanospheres loaded with progesterone, the partitioning of the drug between the dispersed phase containing the cyclodextrin and the continuous aqueous phase containing Pluronic F68/γCDC_6 aggregates was also demonstrated. The drug content found in the final nanospheres ranged from 4 to 5% (w/w) of the carrier. Finally, dilution experiments were carried out to evaluate the stability of the drug particle association.

Keywords: Amphiphilic cyclodextrins; Emulsification evaporation method; Nanospheres; Progesterone

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1. Introduction

Amphiphilic and hydrophobic esters of cyclodextrins, obtained by the introduction of fatty acid chains into the secondary face of the natural molecule, have been used to prepare colloidal suspensions of nanospheres which are considered as promising carriers for hydrophobic drugs. The method currently used to prepare these nanosphere suspensions, called nanoprecipitation (Fessi et al., 1988), is based on the spontaneous assembling of the cyclodextrin molecules previously dissolved in a water-miscible organic solvent when the latter is injected into an aqueous phase. The hydrophobic drug is usually added to the organic phase of the formulation.

Previous studies concerning the loading mechanisms of progesterone have demonstrated that, by the nanoprecipitation method, the drug is preferentially associated at the surface of the particle in its individual molecular state, but not entrapped in the matrix core (Lemos-Senna et al., 1998a). In this same study, it was shown that, in sink conditions, the hydrophobic drug is instantaneously released after dilution of the suspensions, and only a partition phenomenon of the drug between the carrier and the continuous medium governed the drug release. These results, although interesting when the increase in bioavailability of the hydrophobic drug is desired, have led to the development of new procedures of cyclodextrin nanosphere preparation capable of allowing drug matrix encapsulation.

Another kind of method, usually described to encapsulate hydrophobic drugs in polymeric matrices, involves the emulsification of water-immiscible organic solutions of preformed polymers in aqueous phases containing soluble surfactants (Puisieux et al., 1994). This method, also called emulsification solvent evaporation, has been widely employed to prepare biodegradable microspheres (Bodmeier and McGinity, 1987a,b; Jeffery et al., 1991). However, in order to obtain submicronic particles, modifications of the emulsification procedure have been applied, such as the salting-out process (Allémann et al., 1992, 1993), high-pressure emulsification (Bodmeier and Chen, 1990; Ueda et al., 1997), or emulsification solvent diffusion (Niwa et al., 1993; Leroux et al., 1995).

In this work, we first verified the feasibility of preparing nanospheres from amphiphilic cyclodextrins using the procedure of organic emulsification solvent evaporation. The influence of the surfactant on nanosphere preparation was investigated. Recent studies have demonstrated that nonionic detergents can interact with cyclodextrin nanospheres in a reversible process (Lemos-Senna et al., 1998). Therefore, this parameter can be considered as the most important factor in the pharmacotechnical development of these new carriers. The capacity of the nanospheres to associate with a hydrophobic drug and the effect dilution on this association were also evaluated using progesterone as the model.

2. Materials and methods

2.1. Materials

γCDC6 with an average degree of substitution of 5.5 (as checked by HPLC) was obtained by the synthetic route described by Zhang et al. (1991). Progesterone was purchased from Sigma (St-Quentin Fallavier, France). Poloxamer 188 (Pluronic F68) was supplied by BASF (Ludwigshafen, Germany). The other chemicals were of pharmaceutical or reagent grade and were used without further purification.

2.2. Methods

2.2.1. Nanosphere preparation

Progesterone-loaded or unloaded nanospheres were prepared by the emulsification solvent evaporation method. Briefly, the γCDC6 (30 or 60 mg) and the hydrophobic drug (3.0 mg) were dissolved in methylene chloride (2.4 ml). This solution was dispersed in an aqueous phase (117.6 ml) containing Pluronic F68 by using a high speed homogenizer (Ultra-turrax T25, IKA Labotechnik, Germany) at 20500 rpm for 2 min. Thereafter, the organic solvent was evaporated by mechanical stirring (600 rpm) for 12 h at room temperature. The aqueous suspensions were then concentrated in a low-pressure system to a final volume of 5.0 ml, and filtered in a 0.8 μm membrane (Millex AA, Millipore, France).

2.2.2. Particle size determination

The mean diameter and the size distribution of the particles were determined by quasi-elastic light scattering (QELS) using a Coulter Nanosizer (Model N4MD, Coultronic, France). The measurements were taken using SDP analysis and were made in triplicate for all the prepared batches.

2.2.3. Transmission electron microscopy

Morphological examination of the suspensions was carried out using a transmission electron microscope (Jeol 1010, accelerating voltage 100 kV) after negative staining with 2% (w/v) uranyl acetate solution.

2.2.4. Determination of the γCDC6 concentration

γCDC6 concentration was determined for all the colloidal suspensions after their 10-fold dissolution with methanol and in the supernatants obtained after ultracentrifugation (Beckman,) at $120000 \times g$ for 1 h at 20 °C. A reversed-phase liquid chromatography method recently described was used in the analysis (Lemos-Senna et al., 1998a). Liquid chromatography was performed on a Waters system (Milford, MA) equipped with a 484 turnable absorbance detector set at 240 nm, a model 510 pump, a WISP model 712 injector and a model 746 data module. Typically, the following conditions were employed: column, μBondapak C18 (300 × 3.9 mm, Waters, USA); mobile phase, methanol/THF (70/30, v/v); flow rate, 0.8 ml/min. The total cyclodextrin recovery was estimated correlating the γCDC6 concentration found in the final suspensions and in the initial γCDC6 content. The cyclodextrin recovery in the nanoparticles was estimated as being the difference between its total concentration and the cyclodextrin found in the supernatant after ultracentrifugation.

2.2.5. Determination of the progesterone loading

Progesterone was determined by using a reversed-phase HPLC method with a spectrophotometric detector set at 240 nm. The chromatography analysis was performed under the following conditions: column, Bondex C18 (300 × 4.6, SFCC, France); mobile phase, acetonitrile/water (70/30, v/v), flow rate, 0.80 ml/min. The progesterone concentration was determined for all the colloidal suspensions after dissolution of the nanospheres in acetonitrile (total drug, CT), and in the supernatants obtained after ultracentrifugation at $120000 \times g$ for 1 h at 20 °C (free drug, CS). The association of the drug (%) in the nanospheres was calculated as being the difference between total and free drug as follows.

$$(\%) = (1 - C_S/C_T) \times 100$$

Considering that a fraction of the progesterone can precipitate during the nanosphere fabrication, the drug recovery (%) was estimated correlating the total drug found in the all colloidal suspensions and that initially added to the formulations (see Section 2.2.1.). Finally, the progesterone content in the carrier (%, w/w or μg/mg γCDC6) was estimated as being the actual drug associated with the particles.

2.2.6. Evaluation of the suspension dilution on the progesterone association

In order to evaluate the effect of dilution on the progesterone association, the loaded nanosphere suspensions were diluted with different volumes using two different continuous phases. These dilutions were performed in water or in a water/PEG mixture (60/40, v/v). The diluted suspensions were subjected to ultracentrifugation at $120000 \times g$ for 1 h at 20°C. The supernatants were assayed for progesterone by the HPLC method as described above and the remaining progesterone content in the nanospheres was then estimated.

3. Results and discussion

3.1. Unloaded nanospheres

In this work, we evaluated the feasibility of γCDC_6 nanosphere preparation by emulsification solvent evaporation method. According to the microsphere preparation manufacturing process, the emulsification methods involve a sequence of complex interfacial phenomena in which numerous parameters can affect the preparation pathway and the physicochemical properties of the

Table 1
Influence of both the initial γCDC_6 content in the internal phase and Pluronic F68 concentration in the aqueous phase on the QELS size and polydispersity index of the emulsion droplets and final nanospheres

γCDC_6 content	Pluronic F68 (%)		Emulsion droplets		Final nanospheres	
	Emulsions	Suspensions	Mean diameter (nm)	IP[a]	Mean diameter (nm)	IP[a]
30 mg	0.166	4.0	147 ± 57 (50%) 1000 ± 50 (50%)	0.57	145 ± 46	0.13
	0.083	2.0	159 ± 40 (22%) 975 ± 150 (73%)	0.63	165 ± 35	0.14
	0.042	2.50	148 ± 50 (60%) 932 ± 230 (40%)	0.41	136 ± 47	0.15
	0.021	1.25	292 ± 90 (74%) 1070 ± 370 (25%)	0.31	164 ± 35	0.10
	0.0104	0.625	220 ± 88 (80%) 2560 ± 820 (20%)	0.34	154 ± 43	0.12
60 mg	0.166	4.0	151 ± 55 (51%) 998 ± 150 (49%)	0.53	141 ± 47	0.013
	0.083	2.0	233 ± 60 (51%) 1040 ± 330 (49%)	0.53	152 ± 44	0.12
	0.042	2.50	231 ± 110 (86%) 1660 ± 340 (14%)	0.46	135 ± 46	0.15
	0.021	1.25	164 ± 43 (53%) 771 ± 290 (47%)	0.37	154 ± 43	0.10
	0.0104	0.625	302 ± 51 (93%) 1780 ± 260 (7%)	0.20	175 ± 26	0.086

[a] IP, polydispersity index.

final nanospheres. These parameters include the type of organic solvent or solvent mixture, the solvent/polymer/non-solvent interactions, the nature of the emulsifying agents and the rate of solvent evaporation. In our case, the amphiphilic cyclodextrin (γCDC_6) was dissolved in a volatile, water-immiscible organic solvent, methylene chloride. This solution (internal phase) was emulsified into an external aqueous phase containing Pluronic F68 as soluble surfactant. Upon the diffusion of the solvent from the surface of the droplets, the solubility of γCDC_6 is reduced and, once the solubility limit is reached, γCDC_6 aggregates are formed. Methylene chloride was selected as the organic phase and was unchanged at 2% (v/v) concentration of the total emulsion volume. Compared with other organic solvents commonly used, methylene chloride presents a higher water solubility and lower heat of evaporation, allowing diffusion of the solvent into the aqueous phase fast enough to lead to particle formation (Bodmeier and McGinity, 1988). Two different initial γCDC_6 contents were tested, 30 and 60 mg (corresponding, respectively to 1.25 and 2.5% w/v of the organic phase). The Pluronic F68 concentration added to the aqueous phase was ranged from 0.0104–0.170% (w/v), corresponding to 0.5–4.00% (w/v) in concentrated suspensions.

Table 1 shows the mean diameters and polydispersity indices determined by QELS for the emulsion droplets and the final particles as a function of the surfactant concentration and initial γCDC_6 content. Emulsions droplets with polydispersed distributions were obtained in all the formulations tested. Nevertheless, a slight increase in size and a decrease in the polydispersity index were observed with decreasing surfactant concentration. It is worth nothing that, contrary to data related in the literature (Jeffery et al., 1991), the formation of nanometric droplets was observed even using low surfactant concentration in the external phase. This can be explained by the amphiphilic charac-

teristics of the cyclodextrin derivative. Indeed, the formation of the nano-sized droplets during the emulsification process and subsequent nanoparticle formation have been related to the reduction in the interfacial tension between the two immiscible phases caused by the addition of polymeric surfactants or cosolvents in the organic phase of the emulsion (Bodmeier and Chen, 1990; Niwa et al., 1993). It was recently demonstrated that γCDC_6 is able to reduce the interfacial tension between hexane and water when this compound is added to the organic phase (unpublished data). Although at the concentrations employed the cyclodextrin molecules alone are too diluted to allow the emulsification of the organic phase, it is very probable that these molecules are located at the interface between the two phases participating in the stabilization of the emulsion droplets.

After 0.8 μm filtration, which removes the large aggregates such as microspheres formed during the evaporation step, γCDC_6 nanospheres were obtained. The size of the particles, determined by QELS, was not influenced by the initial γCDC_6 content. The cyclodextrin recovery in the final suspensions and in the nanospheres was evaluated as a function of both surfactant concentration and γCDC_6 initial content (Fig. 1). According to Fig. 1, the recovery of the cyclodextrin in the final suspensions increased with the increase in surfactant concentration. The recovery of the cyclodextrin molecules in the nanospheres, estimated from the difference between the γCDC_6 concentration found in the total suspensions and in the supernatants after ultracentrifugation, was surprisingly decreased by the increase in Pluronic F68. Furthermore, when surfactant concentration was plotted against the concentration of cyclodextrin in the supernatant, a linear relationship was found with a correlation coefficient of 0.999 for both γCDC_6 initial content examined (Fig. 2). These results suggest that γCDC_6 interacts with Pluronic F68 forming aggregates like mixed micelles during nanosphere preparation as the cyclodextrin derivative is very insoluble in water. The formation of small aggregates exhibiting several nanometers in diameter together with particles with sizes ranging from 50 to 200 nm was evidenced by TEM (Fig. 3a,b). Although doubts have been raised concerning the values of critical micellar concentration of polaxamer surfactants (Kataoka et al., 1993), the formation of aggregates with a hydrodynamic radius of around 6 nm (at 20°C) was demonstrated for Pluronic F68 (Al-Saden et al., 1982). This value is very close to the size of the small aggregates observed in the TEM micrographs. Besides, after ultracentrifugation at $120000 \times g$ during 1 h, these small aggregates were remained in the supernatants, as confirmed by the micrograph obtained from nanospheres recovered after ultracentrifugation (Fig. 3c). This is in agreement with the impossibility of separating micelles by centrifugation mainly

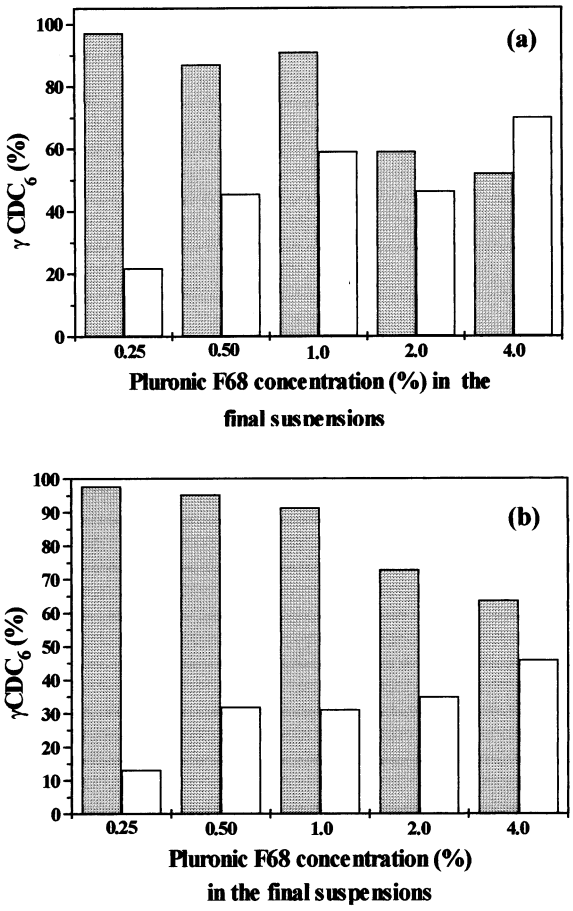

Fig. 1. Influence of Pluronic F68 concentration on the recovery of γCDC_6 in the nanospheres (black) and in the whole suspensions (white) for an initial γCDC6 content in the internal phase of (a) 30 mg and (b) 60 mg.

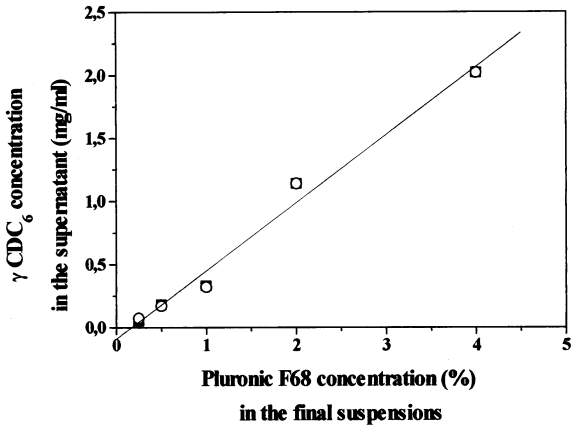

Fig. 2. Plots of γCDC_6 concentration in the supernatants as a function of Pluronic F68 concentration in the final suspensions for an initial cyclodextrin content in the internal phase of 30 (■) and 60 mg (○).

due to their monophasic behavior and low density. In Fig. 3c, only larger particles presenting a spherical to elongated shape are observed, and no more micelles are found. These results agree with the diffusion of γCDC_6 molecularly dissolved in the organic solvent towards the external phase during the evaporation step of nanosphere preparation. This partitioning probably depends on the interactions between cyclodextrin and surfactant. This is supported by the fact that the γCDC_6 concentration found in the aqueous phase does not depend on the initial cyclodextrin content in the internal phase, but only on the Pluronic F68 concentration (Fig. 2). Moreover, the formation of mixed micelles composed of γCDC_6 and a non-ionic surfactant, octyl glucoside, was recently evidenced (Lemos-Senna et al., 1998). Similarly to octyl glucoside, polyoxyethylene-polyoxypropylene block copolymers have been shown to form complexes with natural or modified cyclodextrins through interactions with both hydrophilic and hydrophobic sites of the molecule (Topchieva et al., 1994), supporting our hypothesis.

3.2. Nanospheres loaded with progesterone

In order to determine the loading capacity of a hydrophobic drug in γCDC_6 nanospheres prepared by emulsification solvent evaporation, the progesterone association and progesterone recovery were evaluated as a function of the initial drug content added to the organic phase of the formulations (Fig. 4). In these experiments, the Pluronic F68 concentration in the external phase was fixed at 0.170% (w/v), corresponding to 4.0% (w/v) in concentrated nanosphere suspensions according to the procedure described above. The initial γCDC_6 content was 30 mg corresponding to 1.25% (w/v) of the internal phase. As shown in Fig. 4, the drug association curve presents a biphasic pattern. An increase in the first portion of the curve is observed, but rapidly reaches a plateau corresponding to 50% of progesterone association. On the contrary, progesterone recovery decreased drastically with the increase in drug added to the formulations. The excess of progesterone was precipitated probably with the concentration of the aqueous suspensions and was removed by filtration.

Afterwards, the initial progesterone content was fixed at 3.0 mg and the influence of either the surfactant concentration in the continuous phase and initial γCDC_6 content in the internal phase on drug association was evaluated (Fig. 5). The concentration range was the same as that employed in the preparation of unloaded nanospheres. An increase in the progesterone association was observed with decreasing surfactant concentration. In addition, for a given concentration of Pluronic F68, the progesterone association appeared to decrease with lower initial γCDC_6 concentration. These results clearly evidenced the partitioning of progesterone between the internal phase containing the γCDC_6 and the continuous phase containing the surfactant. The drug partitioning between the dispersed and continuous phases has been described as the limiting factor of encapsulation of active compounds within matrix systems by using this method. The success of encapsulation was related to be highly dependent on the solubility of the drug in the medium (Bodmeier and McGinity, 1987a; Ueda et al., 1997). Similarly, in our case, progesterone diffuses into the large volume of aqueous phase initially employed. After concentration and filtration of the suspensions, a fraction of the progesterone molecules non-associated with the

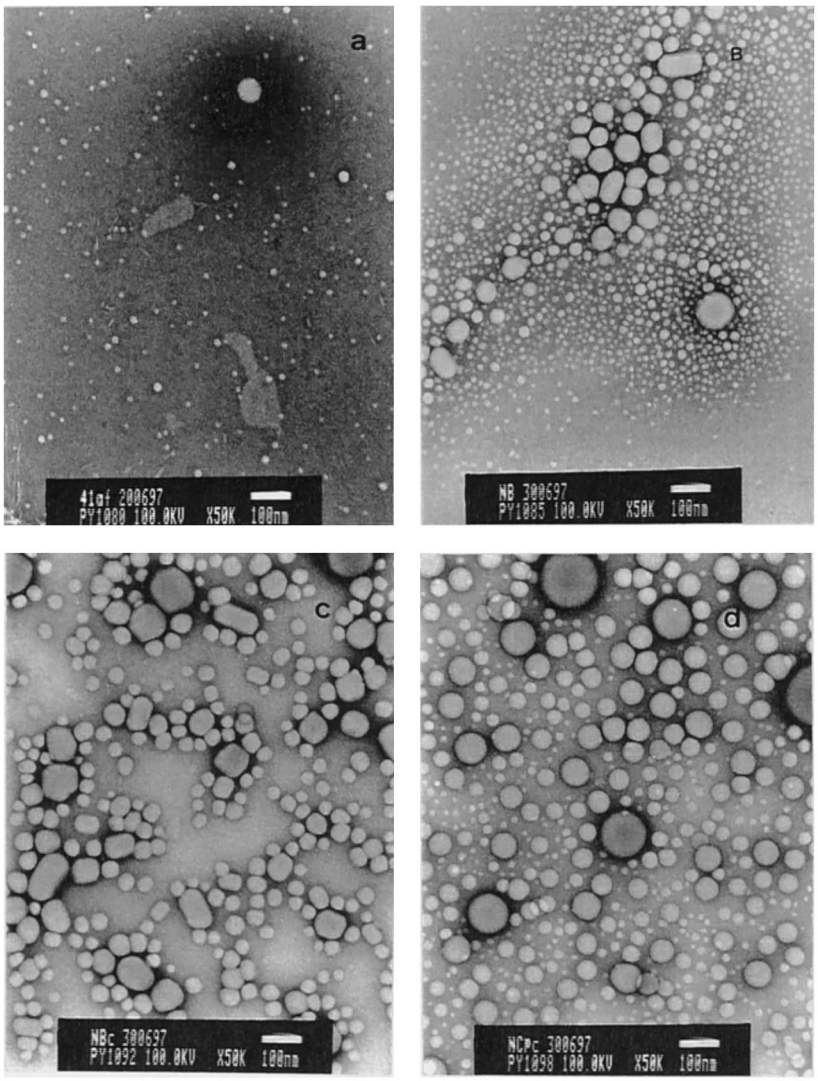

Fig. 3. Transmission electron micrographs of (a) unloaded nanospheres obtained before concentration and ultracentrifugation; (b) unloaded nanospheres before ultracentrifugation; (c) unloaded nanospheres; and (d) progesterone-loaded nanospheres obtained after ultracentrifugation of the suspensions.

nanospheres is eliminated. However, most of the non-associated drug is probably solubilized in the continuous medium of the final suspensions. In order to evaluate the ability of Pluronic F68 to solubilize progesterone, formulations were prepared as described above, but in the absence of the amphiphilic cyclodextrin. The amount of progesterone solubilized by Pluronic F68 was then compared with the concentration of the drug non-associated with the particles. These results are demonstrated in the plots in Fig. 6. The concentrations of Pluronic F68 used did not solubilize significantly the progesterone molecules. The progesterone concentrations found in the Pluronic F68 solutions are clearly smaller than the values of the progesterone concentrations found in the supernatants obtained after the ultracentrifugation of the suspensions. This solubilizing effect is

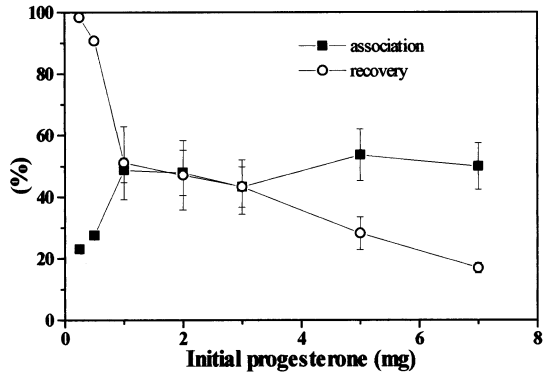

Fig. 4. Influence of the initial content of progesterone on the association and recovery of the drug in the final suspensions.

seemingly connected with the formation of the small aggregates, assumed to be Pluronic F68/γCDC$_6$ mixed micelles as described above. The increase in progesterone in these aggregates can be explained by an increase in the interaction of the drug with micelles induced by the hydrophobic characteristics of the amphiphilic cyclodextrin. On the other hand, after regression analysis, a linear correlation between Pluronic F68 concentration and solubilized progesterone in the supernatant was found for the three curves shown in Fig. 6 ($r > 0.95$). By analogy with micellar drug solubilization, the slope of the straight lines can be used to evaluate the solubilizing ability of the surfactant systems. The bulk solubility of the drug

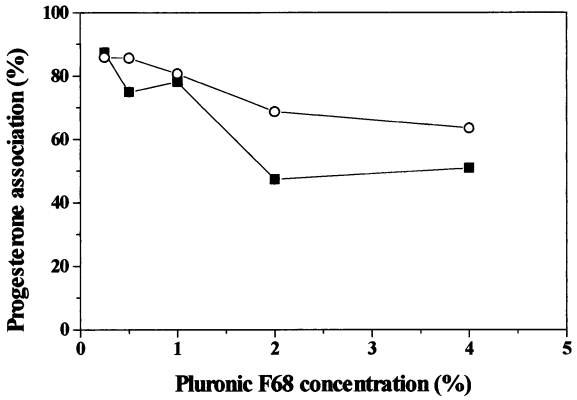

Fig. 5. Influence of the surfactant concentration on the progesterone association in the nanospheres for an initial γCDC$_6$ content of 30 (■) and 60 mg (○).

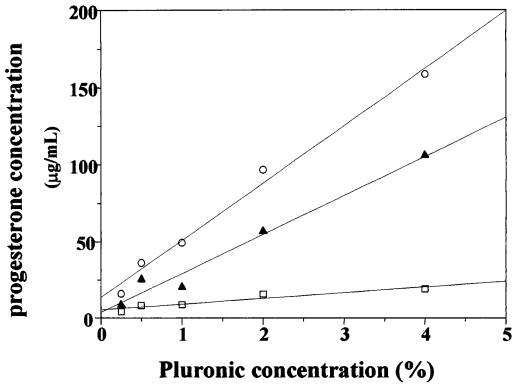

Fig. 6. Plots of Pluronic F68 versus progesterone concentration in the supernatants obtained for (□) Pluronic without γCDC$_6$, (○) nanospheres prepared with 30 mg and (▲) nanospheres prepared with 60 mg of γCDC$_6$.

is given by the y-intercept of the straight lines. From these curves, progesterone solubilization appears more significant when a lower γCDC$_6$ content is added to the formulations. These results agree with a partitioning of progesterone between the mixed micelles and the emulsion droplets during the evaporation step of the nanosphere preparation. Considering that, for a given surfactant concentration, the cyclodextrin concentration in the external phase is not affected by the initial γCDC$_6$ content, a high concentration of cyclodextrin in the internal phase favors the retention of the drug in droplets and consequently in the nanospheres. The progesterone content in percent of the carrier for all formulations ranged from 4 to 5% (w/w). The TEM micrograph obtained from progesterone-loaded suspensions recovered after ultracentrifugation is shown in Fig. 6d. The presence of spherical particles, with diameter similar to those obtained for unloaded nanospheres, was observed.

3.3. Effect of the suspension dilution on the progesterone association

The stability of the progesterone association in the cyclodextrin nanospheres was evaluated by dilution of the suspensions using two different continuous media, water and a PEG/water mixture (40/60; v/v). The influence of formulation

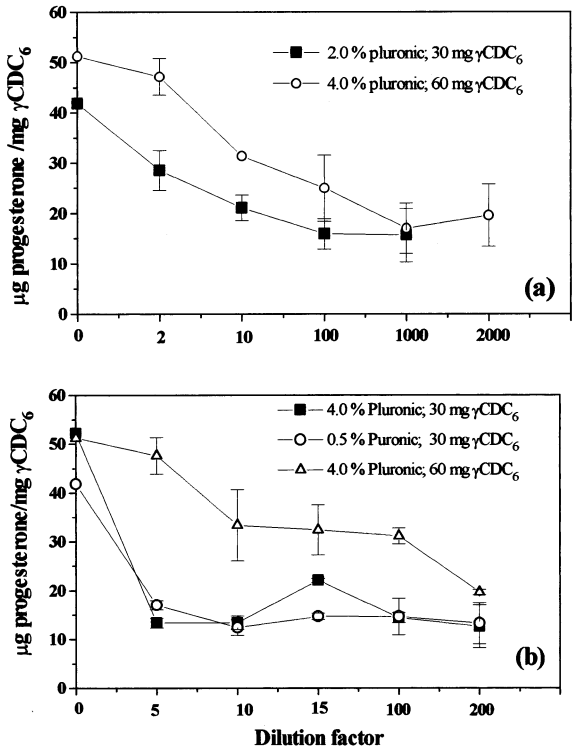

Fig. 7. Influence of the suspension dilution on the association of progesterone in cyclodextrin nanospheres in; (a) water; and (b) water/PEG mixture.

parameters, i.e. surfactant concentration and initial γCDC_6, on drug release was evaluated. These results are illustrated in Fig. 7. A fast decrease in drug association was observed at low dilutions of the suspensions. When the PEG/water mixture was employed, the higher solubility of the drug in this continuous medium led to a faster release of the drug. However, in both cases, a plateau corresponding to 1.5–2% (w/w) of drug association was reached. These results suggest that most of the progesterone is associated at the surface of the particles. However, the encapsulation of a fraction of the drug in the nanosphere matrix also occurred, opposing the results obtained by using nanoprecipitation (Lemos-Senna et al., 1998a). Concerning the formulation parameters studied, its appears that the surfactant concentration did not influence the progesterone release. The differences obtained at high γCDC_6 content can be related to drug partition phenomenon where the presence of more concentrated suspensions leads to higher retention of the drug in the carrier.

4. Conclusions

In this work we have demonstrated that it is possible to prepare cyclodextrin nanospheres by using the emulsification evaporation method, although that recovery of the cyclodextrins in the final suspensions is considerably lower when compared with that obtained with nanoprecipitation. However, several parameters of nanosphere formulation, i.e. the cyclodextrin concentration in the organic phase, the ratio between organic and aqueous phase volumes, should be tested to optimize the fabrication of nanospheres by this method.

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