Combined Poly(isobutylcyanoacrylate) and Cyclodextrins Nanoparticles for Enhancing the Encapsulation of Lipophilic Drugs

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Purpose. The aim of this study was to prepare and characterize nanoparticulate systems constituted of poly(isobutylcyanoacrylate) and cyclodextrins and intended for increasing the loading of the particles with lipophilic substances. Progesterone was used as a model substance.
Methods. Nanoparticles were prepared by polymerization of isobutylcyanoacrylate in presence of cyclodextrins or progesterone/ hydroxypropyl-β-cyclodextrin complex. Particle size, zeta potential, cyclodextrin and progesterone loading of the particles were determined.
Results. Nanoparticles could be easily prepared in presence of cyclodextrins. An increase in hydroxypropyl-β-cyclodextrin concentration resulted in small nanoparticles (less than 50 nm). It was found that large amounts of cyclodextrins remained associated to the particles, resulting in a 50 fold increase in progesterone loading compared to nanoparticles prepared in absence of cyclodextrins.
Conclusions. The poly(isobutylcyanoacrylate)—cyclodextrin nanoparticles were characterized by the presence of many lipophilic sites belonging to the cyclodextrins which were firmly anchored to the structure of the particles. Therefore, this new type of nanoparticles offers probably an opportunity for increasing the loading of nanoparticles with various lipophilic drugs.

KEY WORDS: nanoparticles; poly(isobutylcyanoacrylate); cyclodextrins; hydroxypropyl-β-cyclodextrin; progesterone; drug loading.

INTRODUCTION

The design of novel drug delivery systems has as primary objective the controlled delivery of a pharmacological agent to its site of action at a therapeutically optimal rate and dosage regimen (1). The improvement of therapeutic index can be obtained by site-specific or targeted delivery, combined with controlled release which promote the efficacy of the drug and would also reduce its toxic side effects. Colloidal drug delivery systems, including liposomes, microemulsions, nanocapsules and nanospheres, are the most promising to achieve this goal. All these formulations have different advantages and disadvantages, despite their very similar characteristics of size, shape and mode of administration. However, nanospheres are very stable systems and their solid polymeric structure can be engineered for targeting or controlled-release purposes.

Despite those interesting characteristics, drug loading capacities of nanospheres, expressed as the amount of drug associated to a unit mass of polymer, is often limited, especially when drugs are very weakly soluble in water. When prepared by conventional methods, the relatively low capacity of nanospheres to carry a suitable amount of the drug from the site of administration to the target site in the body would often lead to the necessity of administering considerable amounts of polymer, consequently limiting their usefullness.

In the present study we describe the preparation and characterization of nanoparticle systems constituted of poly(isobutylcyanoacrylate) and hydroxypropyl-β-cyclodextrin (HPβCD), in presence or absence of surfactant. Cyclodextrins are cyclic oligosaccharides which form non-covalent inclusion complexes with numerous lipophilic molecules. Therefore, their association into the structure of nanoparticles is likely to favor the entrapment of such drugs. The aim of this investigation was to increase the drug loading of hydrophobic substances in the nanoparticles with progesterone as a model molecule.

MATERIALS AND METHODS

Materials

Isobutylcyanoacrylate and progesterone were purchased from Sigma Chemicals (St Louis, Mo, USA), α, β and γ-cyclodextrins, 2-hydroxypropyl-α, 2-hydroxypropyl-β and 2-hydroxypropyl-γ-cyclodextrins (average MS-value were 0.9, 0.6 and 0.6 respectively) were purchased from Wacker Chemie GmbH (Munich, Germany), and sulfobutylether-β-cyclodextrin (SBEβCD) was a gift from CyDex L. C. (Overland Park, Kansas, USA). Poloxamer 188 (Lutrol F68^R) was a gift from BASF (Ludwigshafen, Germany). Other chemicals were analytical grade.

Preparation of Nanoparticles in Presence of Different Cyclodextrins

Nanoparticles were prepared by anionic polymerization (2) of 100 μl of isobutylcyanoacrylate in 10 ml of 0.01 M hydrochloric acid (pH = 2.0) containing 1% w/v poloxamer 188 and in presence of 5 mg/ml of α, β, γ, hydroxypropyl-α, hydroxypropyl-β, hydroxypropyl-γ, or sulfobutylether-β-cyclodextrins. The cyclodextrin solution was magnetically stirred (1000 rpm) at room temperature and the monomer was added dropwise. After stirring for 6 hours, the suspension was filtered with a 2.0 μm prefilter (Millex AP 500^R) and further characterized.

Preparation of Progesterone/HPβCD Complexes

Progesterone/HPβCD complexes were prepared by mixing 3.615 g of HPβCD with 3.0 g of progesterone in 150 ml water under magnetic stirring during 24 hours at room temperature. After this time the mixture was filtered (0.45 μm). The HPβCD and progesterone in the filtered solution were determined prior to being used for the preparation of progesterone loaded nanoparticles.

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Preparation of Poly(isobutylcyanoacrylate)/HPβCD Nanoparticles Loaded with Progesterone

The solution of the HPβCD/progesterone complex obtained as described above was diluted to give concentrations of 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, and 20.0 mg/ml of HPβCD in the polymerization medium. Nanoparticles were prepared as described previously, in the absence or the presence of 1% w/v poloxamer 188.

Preparation of Free-HPβCD Progesterone-Loaded Nanoparticles

For control, poly(isobutylcyanoacrylate) nanoparticles were prepared in absence of cyclodextrins in the polymerization medium. Progesterone-loaded nanoparticles were prepared by dissolving the drug in diluted hydrochloric acid (pH = 2.0) in presence of 1% w/v of poloxamer 188 (around 60 mg/ml, corresponding to the maximal solubility in this medium). The polymerization process was conducted as described above.

Determination of Progesterone and HPβCD into the Particles

The different suspensions of nanoparticles were centrifuged at 82,000 g for 30 minutes at 25°C (Beckman, L5-65 Ultracentrifuge, rotor type 70.1 Ti) and resuspended into 5 ml of distilled water. The suspensions were finally freeze dried (Christ HED 10 Freeze Dryer, Germany). For determining the progesterone payload in the particles, the lyophilized products were dissolved in HPLC grade acetonitrile and the solutions were analysed by high-performance liquid chromatography (HPLC). The HPLC system consisted in a Waters (St-Quentin-en-Yvelines, France) 510 solvent delivery unit, a WISP 712 auto sampler, a Nova-Pak C18 4 μm (250 × 4.6 mm) column a 486 absorbance detector, which was operated at 245 nm and interfaced with a 746 data module. The flow rate was 1.0 ml/min, and the mobile phase consisted of water and acetonitrile (40:60), in which the retention time was around 12 min. Results are expressed as the mean of three determinations.

For HPβCD quantification, the freeze dried nanoparticles were hydrolyzed with NaOH 0.2 M during 12 h, the pH adjusted to 7.0 (± 0.5) and the HPβCD quantified by spectrophotometric determination of the fading of phenolphthalein solutions in the presence of HPβCD. Briefly, phenolphthalein forms colorless stable inclusion complexes with CDs (3). Therefore, the color intensity of an alkaline borate buffer solution of phenolphthalein is decreased proportionally to the amount of CD in solution. Standard solutions were prepared by diluting CD stock solutions in alkaline borate buffer solution at pH 10.0 containing 2% of an ethanolic solution of phenolphthalein 0.006 M. Standard curves (λ = 550 nm) were linear for CD concentrations ranging from 1 to 100 μg/ml. Samples were added with four parts of the phenolphthalein buffer solution and assayed directly.

Characterization of the Nanoparticles

Particle size distribution, average size and polydispersity of nanoparticles were estimated by laser light scattering using a NS Coulter Nanosizer (Coultronics, Margency, France). Samples were dispersed in MilliQ water (Resistivity > 18 MΩ Millipore, St Quentin en Yvelines, France). Each analysis lasted 200 s. The temperature was 20°C and the analysis angle was 90°. The zeta potential of the particles in suspension in milliQ water was determined by laser Doppler velocimetry (Zetasizer 4, Malvern, England).

RESULTS

Results showed that it was possible to prepare and to stabilize nanoparticles in presence of cyclodextrins. The characteristics of the particles obtained with different cyclodextrins and in the presence of 1% poloxamer 188 are reported in Table I. Particle size, zeta potential, cyclodextrin content and stability (data not shown) were influenced by the nature of the cyclodextrin. Nanoparticles formulated with HPβCD and HPγCD were the most appropriated, showing interestingly a mean particle size in the range of 100 nm and a zeta potential close to zero mV. Further studies were conducted with HPβCD.

The method used for the determination of cyclodextrin contents of particles by detection of the fading of phenolphthalein solutions was efficient for the dosing of β- and γ-cyclodextrins, but despite the results published by Vikmon (1981), the correlation between α-cyclodextrin concentration and absorption intensity were not linear, possibly because of the small size cavity of this cyclodextrin type and/or the weak complex stability constant ($K_{c_{\alpha CD}}$ = 150). The amount of the different cyclodextrins bound to the particles ranged between 20 and 35% (w/w) of the total weight of the particles.

In the presence of HPβCD in the polymerization medium, the addition of the surfactant poloxamer 188 was not essential for the production of nanoparticles. On the one hand, as shown in Figure 1, the size and the zeta potential of the particles were not changed by the presence of poloxamer 188. On the other hand, the concentration of HPβCD influenced considerably the size and zeta potential. An increase of the HPβCD concentration from zero to 12.5 mg/ml resulted in a decrease in the particle size from 300 nm to less than 50 nm. Similarly, the zeta potential of the particles was progressively decreased from highly negative values (−40 mV) to a surface potential close to zero mV. Those trends were generally maintained when the nanoparticles were prepared in presence of progesterone (Figure 2). Compared to progesterone-free particles, the zeta potential was slightly negative on the range of HPβCD concentrations under study. Moreover, in the absence of poloxamer 188, there was a rapid increase in the particle size of the nanoparticles up to 450 nm, followed by a rapid decrease when the HPβ CD concentration

Table I. Characteristics of the Particles Prepared in Presence of 5 mg/ml of Different Cyclodextrins and 1% Poloxamer 188

CD (5 mg/ml)	Size (nm) ± S.D.	ζ potential (mV) ± S.D.	CD content (μg CD/mg of particles)
alpha	228 ± 69	−34.4 ± 4.0	ND[a]
beta	369 ± 7	−24.7 ± 8.2	360
gamma	286 ± 9	−22.9 ± 0.6	240
HPalpha	244 ± 25	−27.0 ± 2.2	ND
HPbeta	103 ± 6	−8.6 ± 0.9	247
HPgamma	87 ± 3	−2.6 ± 2.2	220
SBEbeta	319 ± 10	−45.4 ± 2.4	ND

Note: Mean of 3 replicated preparations ± SEM.
[a] ND = not determined.

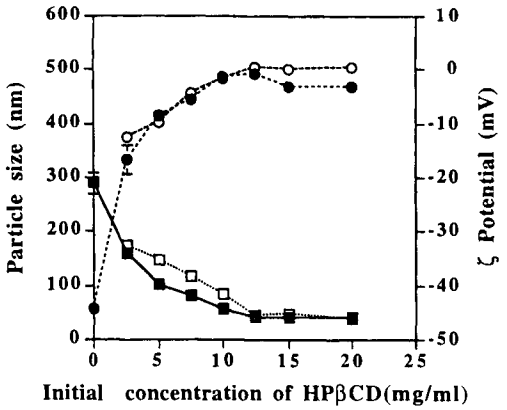

Fig. 1. Particle size and zeta potential of poly(isobutylcyanoacrylate) nanoparticles prepared in presence of hydroxypropyl-β-cyclodextrin. Particle size of nanoparticles obtained with 1% of poloxamer 188 (■) and without poloxamer (□). Zeta potential of nanoparticles obtained with 1% of poloxamer 188 (●) and without poloxamer (○) (mean of 3 replicates ± SEM).

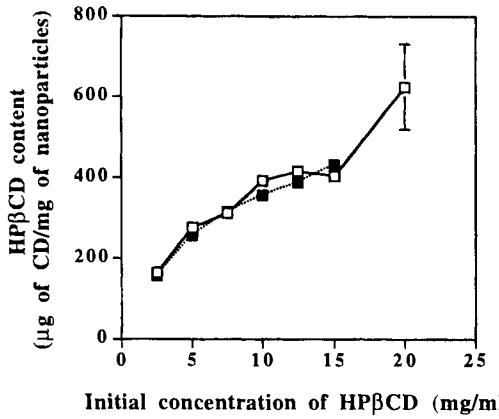

Fig. 3. Hydroxypropyl-β-cyclodextrin content of poly(isobutylcyanoacrylate) nanoparticles prepared in presence of hydroxypropyl-β-cyclodextrin, with (■) and without (□) poloxamer 188. (mean of 3 replicates ± SEM).

was higher than 10 mg/ml. This effect was suppressed in presence of poloxamer 188. Such a phenomenon reflected the probable complexity of the mechanism of aggregation of the oligomeric chains of poly(isobutylcyanoacrylate) during the formation of the nanoparticles in absence of poloxamer micelles in the polymerization medium.

The addition of HPβCD in the polymerization medium resulted in the association of large amounts of HPβCD to the nanoparticles (Figure 3). The amount of HPβCD associated to the particles was continuously increased and could be as high as 60% of the weight of the particles. When the initial mass of HPβCD and isobutylcyanoacrylate in the polymerization medium were equal, the amount of HPβCD associated to the particles was about 35% of the total mass of the particles. Moreover, the association of HPβCD to the particles was not influenced by the presence of poloxamer 188. The HPβCD content of the nanoparticles was not considerably affected by the presence of progesterone in the polymerization medium (Figure 4).

The progesterone loading of the particles was dramatically increased when the particles were prepared in the presence of HPβCD. The progesterone loading in the absence of HPβCD was 0.79 μg/mg of particles and was progressively increased up to 50 folds, which corresponded to 45 μg/mg of particles (Figure 5). There was no significant differences between the particles prepared with or without poloxamer 188 ($p = 0.05$).

DISCUSSION

Structure of the Nanoparticles

The anionic polymerization of alkylcyanoacrylate in aqueous medium produces nanospheres which must be stabilized by the addition of a steric stabilizer. The choice of the stabilizer should be based on the ability of the material to be adsorbed onto the polymer surface. Macromolecular stabilizers, such as dextrans or poloxamers, are more frequently encountered during the preparation process of poly(isoalkylcyanoacrylate) nanopar-

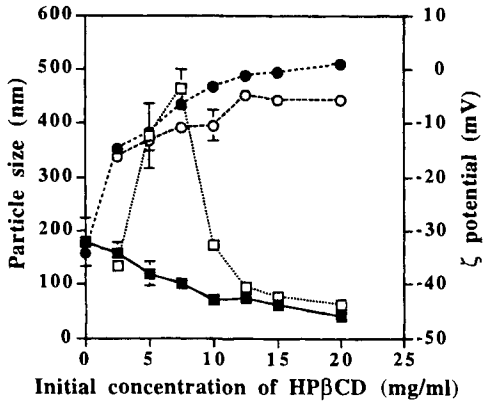

Fig. 2. Particle size and zeta potential of poly(isobutylcyanoacrylate) nanoparticles prepared in presence of progesterone hydroxypropyl-β-cyclodextrin complex. Particle size of nanoparticles obtained with 1 % of poloxamer 188 (■) and without poloxamer (□). Zeta potential of nanoparticles obtained with 1% of poloxamer 188 (●) and without poloxamer (○) (mean of 3 replicates ± SEM).

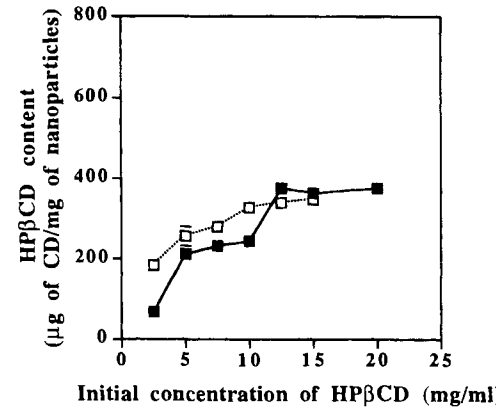

Fig. 4. Hydroxypropyl-β-cyclodextrin content of poly(isobutylcyanoacrylate) nanoparticles prepared in presence of progesterone hydroxypropyl-β-cyclodextrin complex, with (■) and without (□) poloxamer 188. (mean of 3 replicates ± SEM).

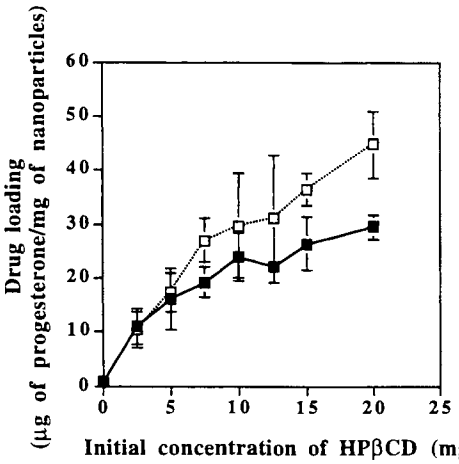

Fig. 5. Progesterone content of poly(isobutylcyanoacrylate) / hydroxypropyl-β-cyclodextrin nanoparticles,with (■) and without (□) poloxamer 188. (mean of 3 replicates ± SEM).

ticles (4). The present study shows that cyclodextrins can be used for preparing and stabilizing poly(isobutylcyanoacrylate) nanoparticles. Interestingly, the concentration of HPβCD in the range of 0–2% w/v in the polymerization medium determined the size and the zeta potential of the particles. Moreover, relatively low HPβCD concentrations allowed the preparation of particles of less than 50 nm in size. When increasing the cyclodextrin concentration in the polymerization medium, the zeta potential became close to electrical neutrality as similarly reported for dextran (5). Those results suggest that HPβCD was effective at the surface of the particles as a steric stabilizer. Alternatively, quantitative determination of the content of the nanoparticles revealed that considerable amounts of cyclodextrins (30–60% in weight) were associated to the particles. The association was rather thight, as evidenced by the fact that there was no modification of the cyclodextrin content when the tensioactive poloxamer was added during the preparation.

Cyclodextrins combined to the nanoparticles can have at least four different localizations, which are not exclusive. They can be: (i) adsorbed at the surface of the particles; (ii) bonded to the poly(isobutylcyanoacrylate) chains; (iii) entrapped into the polymeric matrix; or (iv) exist as inclusion complexes with polyisobutylcyanoacrylate chains.

A surface localization of the cyclodextrins was very probable as suggested by their strong influence on the surface properties of the particles. The surface activity of cyclodextrins is yet not very well documented. However, a detailed molecular lipophilicity pattern of cyclodextrins have been provided recently by computer modeling studies (6), suggesting a strong anisotropy of those molecules. Contrarily to a commonly widespread view that only the cavity of the cyclodextrins is hydrophobic, these authors suggested that the 2-OH/3-OH side of the macrocyclic molecule, corresponding to the wider torus rim, was highly hydrophilic and that the hydrophobic regions were not only localized into the central cavity, but extended well out of the cavity towards the primary hydroxyl face (6-CH_2OH). Therefore, such a strong anisotropy of the cyclodextrins is favorable to their adsorption at the surface of the particles.

Chemical bonding of cyclodextrins to poly(isobutylcyanoacrylate) should also be considered. Alkyl-2-cyanoacrylates polymerize rapidly by an anionic mechanism in the presence of weak bases at ambient temperatures. The weakly-basic hydroxyl groups of water or ethanol initiate the polymerization reaction (7). It has also, been observed that amino groups from molecules, such as: doxorubicin (8); netilmicin sulphate (9); ampicilin (10); and peptides (11) could participate to the polymerization. The covalent binding of dextrans to polyalkylcyanoacrylate chains is controversial, but it has been suggested that dextran hydroxyl groups should be bonded covalently to polyalkylcyanoacrylate polymeric chains, where each dextran molecule could contain several cyanoacrylate polymer moieties covalently linked via any of the available dextran hydroxyl groups (4). Similarly, covalent bonding of cyclodextrins molecules containing several hydroxyl groups in their structure, to poly(isobutylcyanoacrylate) should be possible. Such a chemical structure would be likely to favor the anchorage of chemically-modified cyclodextrins at the surface or within the particle itself.

Interactions of cyclodextrins with the poly(isobutylcyanoacrylate) chains were also likely to intervene during the process of formation of the nanoparticle, possibly inducing the size changes of the particles observed in the presence of HPβCD. It was reported that in the presence of surfactants such as poloxamer 188, micelles are formed in the polymerization medium which serve as a locus for the aggregation of poly(isobutylcyanoacrylate) oligomeric chains (8). However, in the absence of poloxamer micelles but in presence of cyclodextrins, the mechanism of aggregation of the oligomeric chains of poly (isobutylcyanoacrylate) became probably more complex. The introduction of hydrophilic cyclodextrin moieties in the molecular structure of the cyclodextrins poly(isobutylcyanoacrylate) conjugates formed during the polymerization process as discussed above could lead to modifications in the aggregative properties of the chains. Such conjugates could have a natural tendency to aggregate due to the interactions of their hydrophobic sites together. However, the structure of the conjugates was certainly not constant on the whole range of cyclodextrin concentrations, leading to variations in their aggregation pattern and, in turn, to variations in the final size of the nanoparticles. Due to its inherent tensioactive properties, the addition of poloxamer 188 in the polymerization medium should mask those effects.

Loading Capacity of the Nanoparticles

Progesterone was chosen as a model of a very poorly water-soluble molecule (aqueous solubility: 0.01 mg/ml) which forms easily water-soluble complexes with cyclodextrins. Generally, low water solubility of the drug in classical water emulsion polymerization procedures results in very low drug loading. Other techniques such as emulsion polymerization lead to higher drug loadings (14). However, this technique necessitates the use of intermediary solvents during the preparation process which have to be removed further. So far, poly(isobutylcyanoacrylate) nanoparticles have been prepared by anionic polymerization in the presence of the drug (3,8) and it was the aim of this study to optimize the loading of poly(isobutylcyanoacrylate) nanoparticles by lipophilic drugs by using progesterone as a model.

The loading obtained in the present study was around 0.08% (0.79 mg of progesterone/g of nanoparticle) for conven-

tional nanospheres of poly(isobutylcyanoacrylate). Similar loadings were reported when an adsorption technique was used for the loading of preformed particles (12). Very interestingly, the progesterone loading of the particles was 4.5% (45 mg of progesterone/g of nanoparticles) when progesterone-HPβCD complex was added to the polymerization medium, which represents an increase of 50 folds, compared to conventionally prepared nanoparticles. Different mechanisms can be proposed for explaining the enhancement of drug loading. Firstly, the amount of progesterone directly available in the polymerization medium for entrapment into the particles during their polymerization was considerably increased when using the progesterone/HPβCD complex instead of the progesterone solution. The phase solubility diagram of progesterone in aqueous HPβCD solutions is of AL-type, i.e., the solubility of progesterone has shown a linear dependency on the HPβCD concentration (13). Under experimental conditions, progesterone solubility in aqueous 20% (w/v) HPβCD was around 1,4 mg/ml, corresponding to a 140-fold increase. Secondly, the presence of HPβCD immobilized into the structure of the nanoparticles created numerous lipophilic sites available for the complexation of progesterone. In this respect, the nanoparticles prepared in presence of cyclodextrins offers probably a very interesting opportunity for increasing the loading of nanoparticles with various lipophilic drugs.

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