Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 339 (2007) 121–129
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

α-Cyclodextrin/oil beads: An innovative self-assembling system

A. Bochot*, L. Trichard, G. Le Bas, H. Alphandary, J.L. Grossiord, D. Duchêne, E. Fattal

Univ Paris-Sud; CNRS UMR8612; Physico-chimie – Pharmacotechnie – Biopharmacie, Faculté de Pharmacie, 5 rue JB Clément, Châtenay-Malabry F-92296, France

Received 20 December 2006; received in revised form 22 February 2007; accepted 23 February 2007
Available online 6 March 2007

Abstract

The aim of this work was to characterise a new type of particulate system, named beads, prepared by a straightforward technique starting from a mixture of α-cyclodextrin aqueous solution and soybean oil without the use of any organic solvent or surface-active agent. Mechanisms involved in bead formation were also investigated. Optimal ratio between α-cyclodextrin (6%, w/w), soybean oil (19.6%, w/w) and water (74.4%, w/w) led to homogeneous bead size (1.6 mm) with a fabrication yield superior to 80% after a continuous external shaking during 2.5 days. After freeze-drying, oil and α-cyclodextrin contents were estimated at 80% (w/w) and 20% (w/w), respectively. X-ray diffraction studies revealed that beads presented a crystalline organisation and microscopic techniques showed that their inner structure was constituted by a matrix containing oily compartments. Beads offer interesting prospects for the microencapsulation of lipophilic and poorly stable molecules. Due to their semi-solid consistency and their ability to be freeze-dried, these beads have great potentialities for pharmaceutical (oral and topical routes) and cosmetic applications.

Keywords: Cyclodextrins; Vegetable oil; Inclusion complexes; Particulate system; Beads

1. Introduction

Cyclodextrins (CD) are cyclic oligosaccharides consisting of 6, 7 or 8 glucopyranose units. Due to the chair conformation of the glucopyranose units, CD molecules display a truncated cone shape with a hydrophilic surface and a lipophilic cavity. In an aqueous environment, CD molecules form partial or total inclusion complexes with lipophilic guests. The molecules located within the cavity are in a dynamic equilibrium with the free molecules in solution. Thanks to their inclusion capacity, CD change the properties of entrapped molecules. For this reason, they are considered as an interesting tool for molecular entrapment (Loftsson and Brewster, 1996; Loftsson et al., 2005; Challa et al., 2005) being applied for the solubilisation of poorly water-soluble drugs and for the stabilisation of poorly stable compounds (Loftsson and Brewster, 1996; Loftsson et al., 2005; Challa et al., 2005). Nowadays, several cyclodextrin-based formulations are commercialised by the pharmaceutical and the cosmetic industries (Buschmann and Schollmeyer, 2002; Challa et al., 2005).

During the last decade, the use of CD in the formulation of particulate systems (Challa et al., 2005; Trichard et al., 2006) such as microparticles (Bibby et al., 2000) (spheres and capsules), nanoparticles (spheres and capsules) (Duchêne et al., 1999a,b; Memişoğlu-Bilensoy et al., 2005) and liposomes (McCormack and Gregoriadis, 1994) has been reported. The major problems encountered during the entrapment of poorly water-soluble drugs in dispersed systems are the low loading efficiency and/or the slow or incomplete drug release rate. When introduced in dispersed systems, CD can enhance drug solubility, drug stability and drug loading (Trichard et al., 2006).

Interestingly, CD themselves (natural CD or their derivatives) can be employed as the main material for particle preparation (Trichard et al., 2006). Indeed, amphiphilic CD derivatives substituted on the primary or secondary face possess interesting interfacial characteristics which make them self-aggregate in the form of nanoparticles (nanocapsules and nanospheres) using classical preparation methods (nanoprecipitation or emulsion solvent evaporation) (Memişoğlu-Bilensoy et al., 2005; Trichard et al., 2006). Despite the chemical modifications, these new CD derivatives are still capable of including molecules (Memişoğlu-Bilensoy et al., 2005). Other amphiphilic CD can form lipidic

Abbreviations: CD, cyclodextrin; TG, triglycerides; TOG, triolein; TLG, trilinolein; TLnG, trilinolenin

* Corresponding author. Tel.: +33 146835579; fax: +33 146835308.
E-mail address: amelie.bochot@u-psud.fr (A. Bochot).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.02.034

vesicles, whose structure is very close to liposomes when associated with phospholipids, or vesicles delimited by bilayer of "amphiphilic" molecules (Memişoğlu-Bilensoy et al., 2005). However, for all the systems mentioned above, the synthesis of large amount of amphiphilic CD is an important limit for their use in drug delivery. In addition, their toxicity is still insufficiently documented. Microcapsules in which the membrane is made of β-CD are obtained by interfacial reticulation of β-CD (Pariot et al., 2000, 2002). Cyclodextrin polymer beads used for chromatographic columns or cosmetic applications can also be prepared by cross-linking the cyclodextrins at a temperature of about 50–90 °C in an aqueous sodium hydroxide solution (Kazuaki and Satoshi, 1983, 1985; Vanzo, 1991; Kazuyuki et al., 1993). However, the high temperature and the pH of the preparation are a limit for the encapsulation of fragile molecules in such systems. Finally, β-CD polymer (synthesised by reticulation of β-CD using epichloridrin) and dextran bearing hydrophobic lauryl side chains can completely associate together in water to spontaneously form supramolecular nanoassemblies (nanogels) of spherical shape (Gref et al., 2006; Daoud-Mahammed et al., 2007).

In the present work, we describe the preparation, using soft conditions (no organic solvent, no cross-linking or surface-active agents, moderate heating) and the characterisation of a novel particulate system named beads made of safe and well-known materials: α-CD and soybean oil. α-CD is employed for its ability to interact with components of vegetable oil and more especially with triglycerides (Duchêne et al., 2003). The mechanism involved in bead formation has also been investigated.

2. Materials and methods

2.1. Materials

α-Cyclodextrin (α-CD) (Cavamax® W6 Pharma), β-cyclodextrin (β-CD) (Cavamax® W7 Pharma), γ-cyclodextrin (γ-CD) (Cavamax® W8 Pharma) and soybean oil (Cropure® Soybean oil) were purchased respectively from Wacker-Chemie (France) and Croda (France). Cropure® soybean oil is mainly composed of triglycerides (TG) (>98%) in which fatty acid chains possess 16 or 18 carbons. Fatty acid distribution is given by the manufacturer as 9.0% of palmitic acid (C16:0), 4.0% of stearic acid (C18:0), 24.0% of oleic acid (C18:1), 52.0% of linoleic acid (C18:2) and 8.0% of linolenic acid (C18:3). Pure TG, i.e. triolein (TOG, 1,2,3-trioleylglycerol, 99% pure), trilinolein (TLG, 1,2,3-trilinoleylglycerol, 99% pure) and trilinolenin (TLnG, 1,2,3-trilinolenylglycerol, 98% pure) were obtained from Sigma Chemical Co. (Saint-Louis, USA). Calcein and Nile red were purchased respectively from Sigma Chemical Co. (Saint-Louis, USA) and Molecular Probes (USA). All organic solvents used were analytical grade.

2.2. Ternary diagram study

Three ternary diagrams were performed using soybean oil, water and α-, β- or γ-CD to identify the domain of bead formation. Diagram exploration was limited to the domain of cyclodextrin water solubility (14.5%, 1.85% and 23.2% (w/v) at 25 °C for α-, β- or γ-CD, respectively). For each point of the diagram, the soybean oil/cyclodextrin/water ratio was determined and then, required quantities of each component were calculated by keeping constant the total volume of preparation (25 mL). Each sample was continuously shaken at 200 rpm in a gyratory shaker (Salvis, Bioblock Scientific, Illkirch, France) at 25 °C and classified into one of the following states: two non-miscible liquids, fluid milky mixture, viscous milky mixture or beads.

2.3. Bead preparation

Beads were prepared by adding 5.8 mL of soybean oil to 20 mL of a α-CD aqueous solution 8.1% (w/v), which corresponded to the most homogeneous formulation. The preparation was continuously shaken in a gyratory shaker (Salvis, Bioblock Scientific, Illkirch, France) in a range of 150–250 rpm at controlled temperature (25 °C, 28 °C, 37 °C) for few days. The conditions were eventually fixed considering the macroscopic aspect of the samples (bead occurrence and turbidity) and the time to obtain a monodisperse population of beads. In optimised conditions, beads were collected at the end of the process, washed and then freeze-dried (48 h in a Christ LDC-1 alpha1-4 freeze-dryer, Bioblock Scientific).

Bead fabrication yield was determined by the following equation:

$$\text{Bead fabrication yield (\%)} = \frac{\text{weight of freeze} - \text{dried beads}}{\text{weight } (\alpha - \text{CD} + \text{ oil})} \times 100$$

2.4. Microscopic observations of beads

2.4.1. Optical microscopy

Beads were observed with a stereomicroscope (MPO) employing a 2.0 mm calibrated glass particle. Diameter and size distribution were determined before and after freeze-drying with a Leitz Diaplan microscope (Leica Microsystèmes, France) equipped with a Coolsnap ES camera (Roper Scientific). Samples collected during bead preparation at 2, 6 and 24 h were also examined using the same microscope.

2.4.2. Scanning electron microscopy

Freeze-dried beads were deposited on a carbon conductive double-sided tape (Euromedex, France) and coated under argon with a gold layer of about 1 nm thick (Edwards Sputter coater S150). Bead surface was then observed by scanning electron microscopy (SEM) using a LEO 1530 Electron Microscope (Inc., Thornwood, NY) equipped with a Gemini column γPGT (Princeton Gammatech, USA).

2.4.3. Freeze-fracture electron microscopy

An aliquot of a suspension of fresh beads was mixed to a 30% (w/w) glycerol solution used as cryoprotectant. The preparation deposited on a copper holder was rapidly frozen in liquid

propane and all further handlings were performed in liquid nitrogen. Fracturing, shadowing using platinum (2 nm thick layer) and stabilisation with carbon (15 nm thick layer) were performed in a Balzers BAF 400T freeze-etch unit (BAL-TEC, Liechtenstein). The replicas were removed from samples in water and washed successively by an ethanol/water mixture, ethanol, chloroform and finally again by ethanol. Replicas were placed on a copper grid (Formvar/carbon) and examined by transmission electron microscopy using a JEOL 1200 EX microscope working at 80 kV.

2.4.4. Confocal microscopy

Beads were prepared as described above with two different fluorescent probes, calcein (hydrophilic) or Nile red (lipophilic) dissolved in the aqueous or oily phase respectively before bead preparation. Dye localisation within the beads was determined using a LSM 510 META Zeiss (Jena, Germany) confocal inverted microscope equipped with a Plan–Apochromat 63X/1.4NA oil immersion objective lens. Differential Interference Contrast visualisation was possible. Green fluorescence was observed with a 505–550 nm band pass emission filter under 488 nm laser illumination and red fluorescence was observed with a 560 nm long pass emission filter under 543 nm laser illumination. Images were collected with a multitrack mode. The pinhole diameter was set at 1.0 Airy unit (optical slices: 0.8 μm thick).

2.5. X-ray diffraction studies

Wide Angle X-ray Scattering experiments were performed at DCI Synchrotron Facility of LURE (Université Paris-Sud, Orsay, France) on the D43 beamline. The monochromatic X-ray beam ($\lambda = 1.45$ Å) was oriented onto a 1.7 mm diameter capillary containing the sample. Two-dimensional scattering patterns were recorded during 10–20 min on a photosensitive plate (15.72 cm × 20 cm), placed 245 mm far from the sample and oriented perpendicular to the incident beam. The imaging plate was then read using a PhosphorImager 400E scanner, (Molecular Dynamics, Sunnyvale, CA).

All samples exhibited powder diffraction Debye-Sherrer rings. Hence, the intensity (arbitrary units) as a function of the scattering vector q (Å^{-1}) $= 4\pi/\lambda \times \sin(\theta)$ was determined by circular integration using a homemade procedure (Zantl, 2001) under IGOR-PRO 3.1.1 software (Wavemetrics Inc., Lake Oswego, USA). Then, the interplanar distances d (Å) ($d = \lambda/2\sin(\theta) = 2\pi/q$) in the direct spacing were calculated according to the Bragg law.

X-ray diffraction was firstly performed on fresh or freeze-dried beads and α-CD. Then, TG:α-CD inclusion complexes were prepared by adding 20–40 mg of triolein (C18:1), trilinolein (C18:2) or trilinolenin (C18:3) to a α-CD aqueous solution (l0%, w/v). The samples were shaken during 2 weeks at 200 rpm, 28 °C, in a gyratory shaker, collected after centrifugation and then analysed by X-ray diffraction employing the conditions described above. X-ray diffraction studies were also performed on solid material extracted from beads and from samples collected 3, 6 and 30 h after starting the preparation process. Extraction was performed as follows: freeze-dried aliquots were put in a mortar and triturated with a pestle after addition of isopentane (3 washes of 10 mL) to remove oil and to concentrate solid material.

2.6. Rheological measurements

The rheological behaviour of samples collected during bead preparation process (1, 2, 4, 6, 8, 10, 13, 16, 20, 24, 30, 37, 48, 60, 72 h) was evaluated using a controlled stress rheometer (RS600, Thermo Electron, France). The preparations were analysed immediately after sampling with a plate-plate geometry (diameter, 20 mm; gap, 2.5 mm). Measurements were performed at 28 °C (±0.20) (Universal Thermal Controller coupled with a Thermo Haake F6/C35 circulating heat chilled water bath, ThermoHaake). The imposed stress was sinusoidal (frequency, 1 Hz) and increased logarithmically from 1 to 10,000 Pa (6–10 steps per decade). The imposed sinusoidal stress can be described as a function of time by the following equation $\tau = \tau_0\cos(\omega t)$. In a linear regime, stress and strain are two sinusoidal functions of time with the same frequency ω but shifting by a phase angle of δ. The strain may thus be written as $\gamma = \gamma_0\cos(\omega t - \delta)$. When $\delta = 0$, the system is purely elastic; when $\delta = \pi/2$, the system is purely viscous; and when $0 < \delta < \pi/2$, the system is viscoelastic. At every sampling time during the shaking, sample response remained linear and viscoelastic ($0 < \delta < \pi/2$) from imposed stress 1 Pa to a critical value, τ_c. This τ_c was calculated for each sample as the value of τ corresponding to $\delta = \pi/4$. Stress and strain can be written as complex numbers: $\tau^* = \tau_0 e^{i\omega t}$ and $\gamma^* = \gamma_0 e^{i(\omega t - \delta)}$. The complex shear modulus is thus complex: $G^* = \tau^*/\gamma^* = G' + iG''$. G' and G'' are, respectively, the storage (or elastic) and loss (or viscous) moduli, $G' = (\tau_0/\gamma_0)\cos\delta$ and $G'' = (\tau_0/\gamma_0)\sin\delta$, corresponding to the in-phase and out-of phase part of the signal. G' (Pa) and G'' (Pa) were calculated for each sample in linear regime (plateau value). Rheological analyses, performed on one sample at different times during bead preparation, were triplicated, mean and standard deviation of G', G'' and τ_c (Pa) were calculated.

3. Results

3.1. Bead fabrication process

A preliminary formulation study was performed in order to define the domain of bead formation. Beads were formed employing 12–24% (w/w) of soybean oil, 70–82% (w/w) of water and 3–6% (w/w) of α-CD (Fig. 1). Outside this domain, milky mixtures (fluid or viscous) or two non-miscible liquids were visualised (Fig. 1). With β- and γ-CD, the shaking of soybean oil and cyclodextrin solutions resulted in milky mixtures but never in beads.

Inside the domain of bead formation, the proportions corresponding to 19.6% of soybean oil, 74.4% of water and 6% of α-CD (Fig. 1) allowed the obtaining of beads with satisfying characteristics (very low turbidity of the dispersion medium and homogeneous bead diameter). This formulation was chosen to prepare all bead samples studied in this work but also to opti-

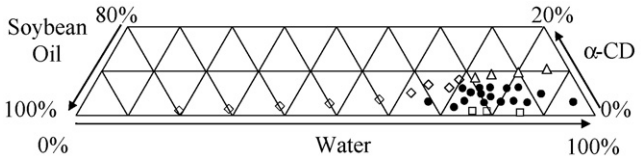

Fig. 1. Soybean oil/α-cyclodextrin/water ternary diagram at 25 °C. Each sample was continuously shaken at 200 rpm in a gyratory shaker at 25 °C and classified as: (□) two non-miscible liquids; (△) fluid milky mixture; (◊) viscous milky mixture; (●) beads.

mise the parameters of the fabrication process. The temperature and the rate of shaking influenced bead occurrence. For example, beads were not formed at 37 °C or when 150 rpm was employed, i.e. soybean oil and α-CD aqueous solution remained as two non-miscible liquids. Optimal conditions were found to be 28 °C and 200 rpm taking into account the macroscopic aspect of the samples and the shortest time (2.5 days) to obtain a monodisperse population of beads.

The different steps to obtain beads could be described as follows: (i) Addition of soybean oil to a α-CD aqueous solution resulted in two non-miscible phases separated by a self-forming film present at the oil/water interface. (ii) When submitted to a gyratory shaking, the preparation progressively turned into (after around 1 h) a stable milky mixture where the viscosity increased in time. (iii) Finally, after a continuous shaking of 2.5 days, beads were in suspension in an aqueous medium exempt of any visible trace of oil (Fig. 2a). Beads presented a semi-solid consistency and could be easily flattened by finger pressure.

Optical microscopy performed after washing showed that beads were almost spherical and had a diameter of 1.6 ± 0.2 mm (Fig. 2b). Bead fabrication yields calculated after freeze-drying reached at least 80% (Table 1).

Samples collected during the different steps of the fabrication were also examined by optical microscopy. After 2 h of shaking, oily globules mixed with solid material were observed (Fig. 3a). The size and the shape of the globules were not homogeneous. After 6 h, some beads enclosed within a viscous medium were visible (Fig. 3b). The size of the beads was ranging from 150 to 600 μm. After 24 h, the number of visible beads was considerably increased (Fig. 3c), their size being between 150 and 900 μm.

3.2. Microscopic observations of beads

SEM performed on freeze-dried particles showed particles exhibiting a rough surface (Fig. 2c). Freeze–fracture replicas of fresh beads examined by TEM indicated that beads were mainly

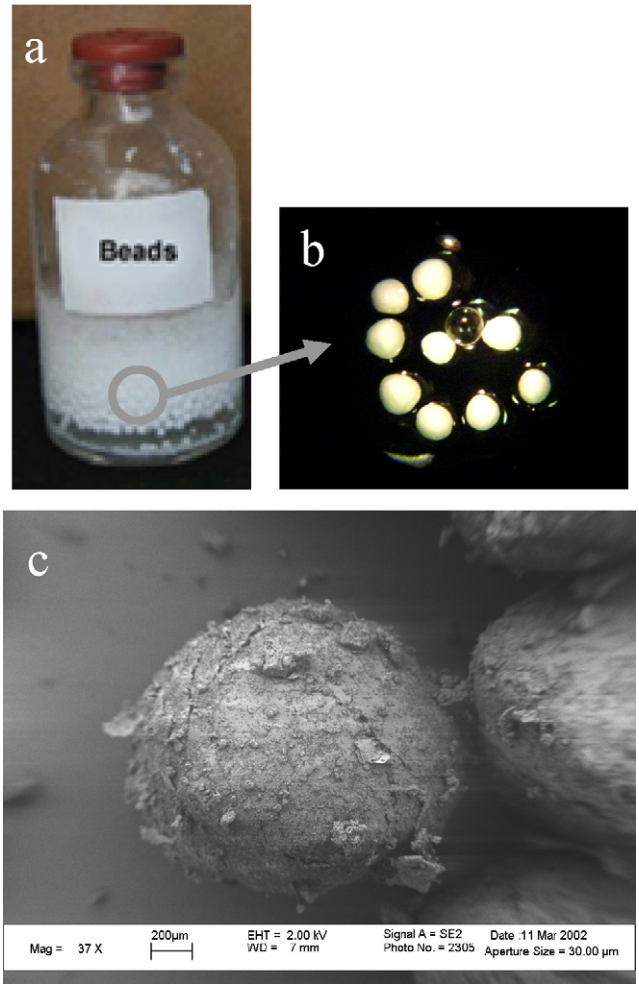

Fig. 2. Photograph of beads: (a) at the end of the fabrication process; (b) zoom of beads after washing; (c) scanning electron micrograph of freeze-dried beads.

of matrix type (Fig. 4). However, numerous ovoid shapes were observed with a size ranging from 0.01 to 1.5 μm.

Confocal microscopy examinations allowed the visualisation of the inner structure of beads. Calcein (hydrophilic probe) was preferentially concentrated on bead surface (Fig. 5a) whereas Nile red (hydrophobic probe) was localised inside the beads demonstrating the presence of oily compartments (Fig. 5b).

3.3. Crystalline structure of beads

X-ray diffraction was used to study the structure of beads. Diffraction spectra of fresh beads were reported in Fig. 6 and showed at least two peaks ($q = 0.528$ and 0.917 $Å^{-1}$) and one

Table 1
Bead size ($n = 50$) and characteristics of three different bead batches

	Fresh beads mean diameter (mm)	Freeze-dried beads mean diameter (mm)	Bead preparation yield after freeze-drying (%)	Bead preparation duration (days)
Batch 1	1.7 ± 0.3	1.6 ± 0.2	80	2.5
Batch 2	1.6 ± 0.2	1.5 ± 0.2	84	2.5
Batch 3	1.5 ± 0.2	1.4 ± 0.2	87	2.5

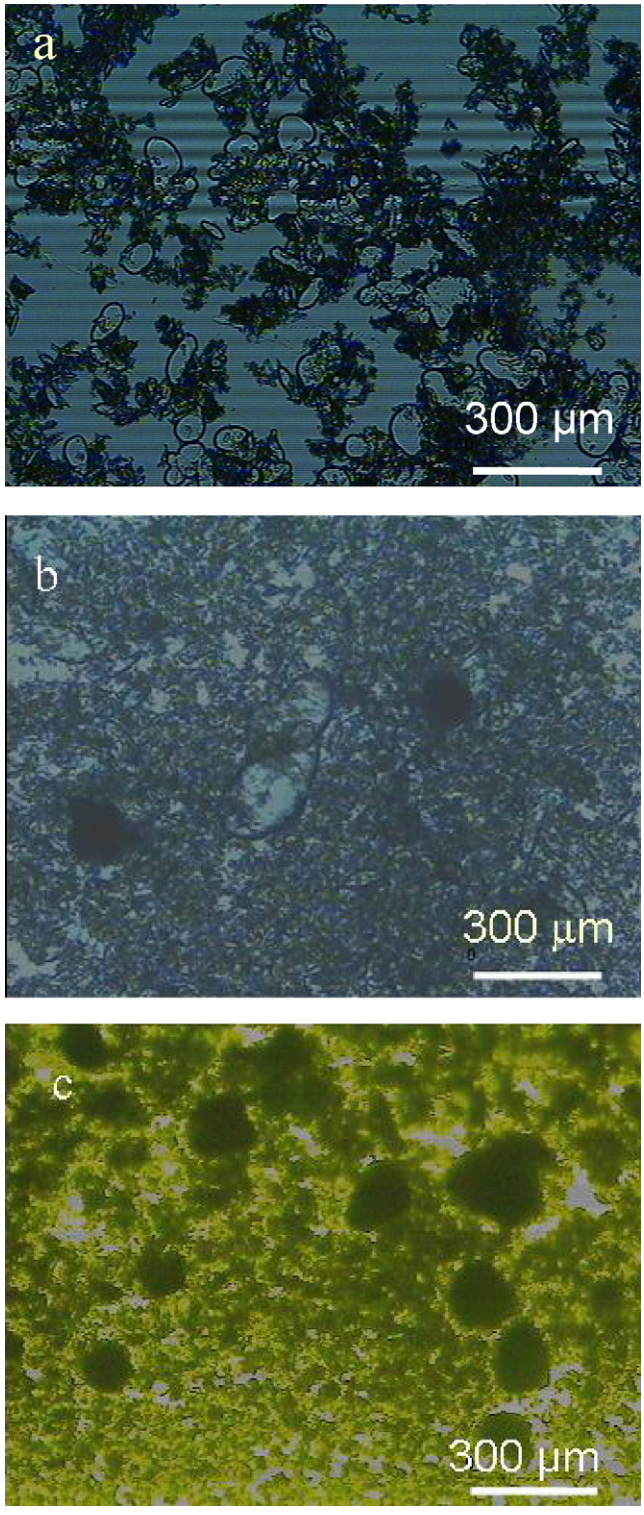

Fig. 3. Optical micrographs of samples collected during bead fabrication process after (a) 2 h, (b) 6 h, (c) 24 h of shaking.

Fig. 4. Freeze–fracture electron micrograph of fresh beads.

broad band (q_{max} = 1.392 Å^{-1}) of diffraction. Crystalline organisation was not affected by freeze-drying (Fig. 6).

TOG:α-CD, TLG:α-CD and TLnG:α-CD inclusion complexes were prepared and their X-ray diffraction spectra were compared to those of beads. As shown in Fig. 6 and Table 2, all peak positions observed in bead diffraction pattern were found in TG:α-CD inclusion complex spectra (q = 0.528, 0.917 and 1.392Å^{-1}). On the opposite, all peaks of inclusion complex samples were not visible in bead spectrum (q = 0.345, 0.648, 0.654, 0.690, 1.142, 1.443, 1.532 and 1.591 Å^{-1}) and particularly the first intense peak (q = 0.345 Å^{-1}). There was no major difference between TG:α-CD inclusion complex spectra (Fig. 6).

Crystalline structure of the samples collected during bead fabrication was also investigated. The results after 3, 6 and 30 h of shaking are presented in Fig. 7. Samples analysed after 3 and 6 h already exhibited diffraction properties. Moreover, diffraction peaks previously identified in beads (mainly q = 1.392Å^{-1}) tended to be narrower and more intense as a function of time (Fig. 7).

3.4. Rheological measurements

A rheological study was carried out to characterise the behaviour of samples collected during bead fabrication. The Fig. 8 reported the evolution of mean G', G'' and τ_c values as a function of time. During the whole fabrication process, samples displayed viscoelastic properties. However, G' values were about 10-fold higher than G'' ones demonstrating a high elastic behaviour. G', G'' and τ_c values remained constant from 1 to

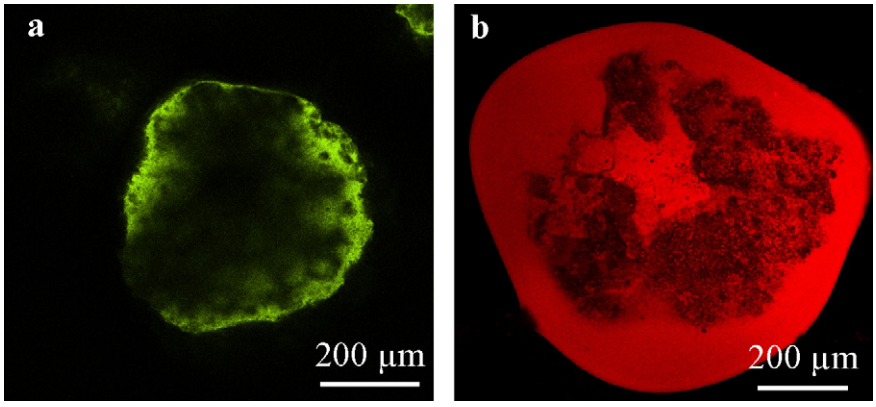

Fig. 5. Confocal micrographs: (a) fresh beads with calcein and (b) fresh beads with Nile red.

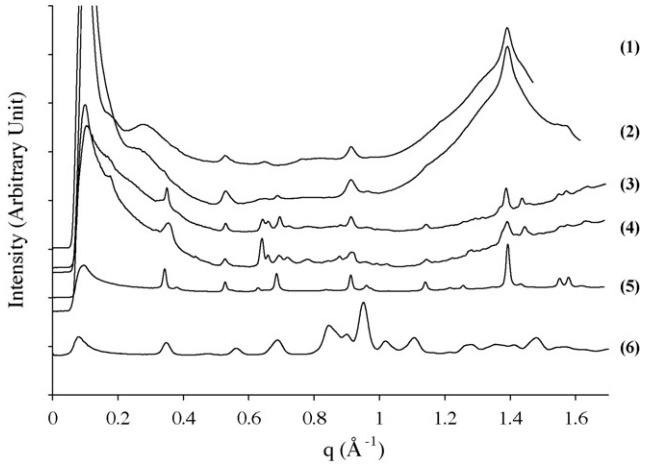

Fig. 6. X-ray diffraction spectra of fresh beads (1), freeze-dried beads (2) and inclusion complex samples of TG:α-CD: TOG:α-CD (3), TLG:α-CD (4), TLnG:α-CD (5), α-cyclodextrin (6). (TOG: triolein, TLG: trilinolein, TLnG: trilinolenin, CD: cyclodextrin).

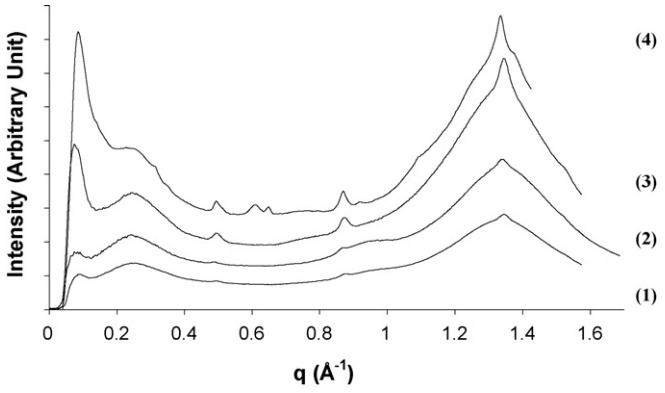

Fig. 7. X-ray diffraction spectra of samples collected during bead fabrication process at 3 h (1); 6 h (2); 30 h (3) and solid material extracted from beads (4).

about 4 h then rapidly increased until reaching a maximum 22 h after starting the preparation process. From 22 to 52 h, the three parameters slowly decreased to reach a plateau assumed as the end of the fabrication process.

4. Discussion

The aim of this work was firstly to characterise a novel particulate system consisting of beads prepared from natural components (vegetable oil, α-cyclodextrin and water) and secondly to elucidate mechanisms involved in their formation. Among natural cyclodextrins, only α-CD is able to form beads. Beads are easy to produce since a continuous external orbital shaking of the formulation only is required. Interestingly, the

Table 2
Interplanar distances deduced from X-ray diffraction patterns

Interplanar distances (Å)	Fresh beads	Freeze-dried beads	TOG:α-CD	TLG:α-CD	TLnG:α-CD
d_{001}			18.1	17.9	18.4
d_{100}	11.9	11.9	11.9	11.9	11.9
$d1\bar{1}1$			9.8	9.7	10.0
d_{101}			9.6	9.6	
d_{002}			9.1	9.0	9.2
d_{110}	6.9	6.9	6.9	6.9	6.9
$d11\bar{2}$ and $d1\bar{2}2$			5.5	5.5	5.5
d_{120}	4.5	4.5	4.5	4.5	4.5
d_{121} and $d2\bar{3}\bar{1}$ and $d1\bar{3}\bar{1}$			4.4	4.4	
$d3\bar{2}2$ and $d2\bar{3}2$ and $d1\bar{3}2$					4.1
d_{300}					3.9

Examinations were made on fresh beads, freeze-dried beads and inclusion complex samples (TOG:α-CD, TLG:α-CD, TLnG:α-CD) assuming a pseudohexagonal cell $a \approx b \approx 13.8$ Å, $c \approx 18$ Å; $\alpha \approx \beta \approx 90^\circ$ and $\gamma \approx 120^\circ$. TOG : triolein, TLG: trilinolein, TLnG:trilinolenin.

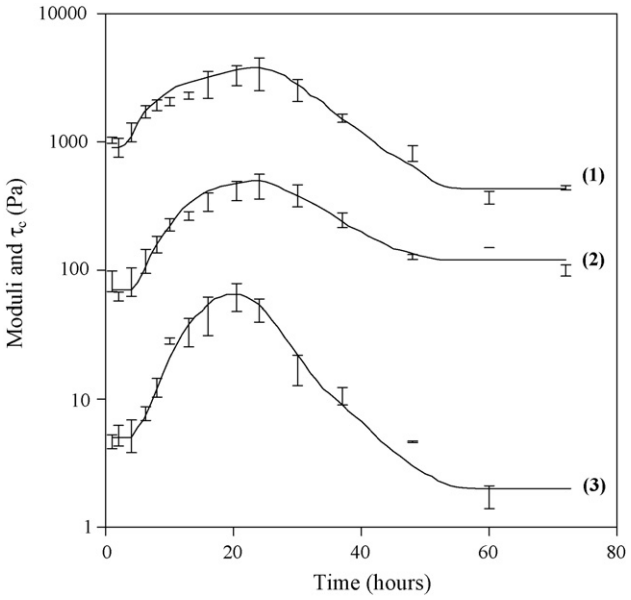

Fig. 8. Evolution of rheological characteristics of samples collected during bead fabrication process as a function of shaking time using a plate-plate geometry (gap 2.5 mm); G' (1): elastic plateau modulus (Pa); G'' (2): viscous plateau modulus (Pa), τ_c (3): critical τ (Pa).

process avoids the use of organic solvents, cross-linking agent, surface-active agents and the fabrication conditions are very soft: heating inferior to 30 °C and external stirring. The use of an optimal ratio between α-CD, soybean oil and water leads to homogeneous bead size with a high fabrication yield. Beads can be freeze-dried facilitating their handling.

The composition and the structure of beads were investigated. Calculated water content of fresh beads is 70% by assuming that the loss of bead weight before and after freeze-drying is only due to the removal of water. Considering freeze-dried beads, the oily content is estimated at 80% (w/w) by taking into account that no trace of oil remains visible in bead suspension at the end of the fabrication. Consequently, α-CD content represents around 20% of the weight of freeze-dried beads.

Confocal microscopy employing Nile red as a marker of the lipid phase and freeze-fracture electron microscopy showed that the inner structure of beads is constituted by a matrix containing micro-compartments of free oil. The large amount of oil could be interesting for the encapsulation of lipophilic drugs as previously described for nanocapsules (Couvreur et al., 2002), solid lipid nanoparticles (Müller et al., 2002a; Uner, 2006), self-emulsifying drug delivery systems and microemulsions (O'Driscoll, 2002). Moreover, as liquid lipids solubilise molecules in a higher extend than solid lipids (Müller et al., 2002b), they should favour high drug loading in beads. Indeed, preliminary study performed on retinoids showed encapsulation efficiencies of 80 to 90%. Moreover, bead structure may also permit a controlled release of entrapped molecules.

X-ray diffraction studies reveal that beads have a crystalline organisation, which can be attributed to α-CD molecules. α-CD crystals can exhibit a rod shape and a herringbone packing (spectrum [6] of α-CD Fig. 6) (Manor and Saenger, 1974; Le Bas and Ryzanek, 1987). This type of crystal was not observed in beads by optical microscopy and their corresponding cell parameters were not identified by X-ray diffraction. α-CD molecules can also be organised in other crystalline lattice types resulting from interaction with exogenous entities (Le Bas and Ryzanek, 1987). Triglycerides represent around 98% of Cropure® soybean oil components. The formation of partial inclusion complexes between TG and natural CD have been already reported (Shimada et al., 1992; Szente et al., 1993; Laurent et al., 1994). Acid value of soybean oil extracted from beads remains inferior to the detection limit of the method used (< 0.2 mg KOH/g of soybean oil). Consequently, we assume that TG hydrolysis does not occur during bead fabrication. C18 fatty acids represent 88% of fatty acid composing TG of Cropure® soybean oil (data given by the manufacturer). For this reason, homogeneous C18:1 (TOG), C18:2 (TLG) and C18:3 (TLnG) triglycerides were employed to prepare inclusion complexes with an excess of α-CD. X-ray diffraction spectra of these samples were clear enough to propose a crystalline structure. Noltemeyer and Saenger (1980) described four lattice types for α-CD involved in inclusion complexes: triclinic, tetragonal, pseudohexagonal and hexagonal. The positions of experimental diffraction peaks were similar to those calculated for the pseudohexagonal lattice using the following parameters: $a \approx b \approx 13.8$ Å, $c \approx 16$ Å; $\alpha \approx \beta \approx 90°$ and $\gamma \approx 120°$ (Noltemeyer and Saenger, 1980). Moreover, relative peak intensities show that α-CD molecules are ordered as dimers in TG:α-CD inclusion complex samples. Lattice parameters were then refined from their diffraction spectra ($a \approx b \approx 13.8$ Å, $c \approx 18$ Å; $\alpha \approx \beta \approx 90°$ and $\gamma \approx 120°$) showing that c value is slightly different from 16 Å. This value corresponds to dimer repetition and could be influenced by the nature of exogenous compound.

Attribution of h, k and l indices in the reciprocal space was then performed using the new cell parameters (Table 2). Since the diffraction peak positions of beads are similar to those of TG:α-CD inclusion complex samples, we propose that bead crystalline structure corresponds to the refined pseudohexagonal lattice. Interestingly, $1 \neq 0$ peaks are all missing in X-ray diffraction bead pattern (Table 2). These results suggest that the crystals could grow in beads only in two dimensions or that no privileged order in the third dimension could emerge due to the wide range of different TG present in soybean oil.

The mechanism involved in their formation was also studied. The milky aspect and the presence of oily globules exhibited by samples resulting from external shaking orientate towards the formation of an O/W emulsion. This is in agreement with previous works describing α-cyclodextrin able to decrease interfacial tension at vegetable oil/water interface (Shimada et al., 1992) and to stabilise O/W (Shimada et al., 1991; Yu et al., 2001; Duchêne et al., 2003) and O/W/O emulsions (Yu et al., 1999; Duchêne et al., 2003). Indeed, a partial inclusion complex is formed at the oil/water interface, CD molecules interacting with only one fatty acid chain of the TG. This super-molecule constitutes a surface-active agent due to its amphiphilic property (a hydrophilic head and a hydrophobic tail) (Shimada et al., 1992).

The emulsifying properties of natural CD decrease when the temperature increases (Shimada et al., 1991). It could explain why no dispersion of soybean oil in cyclodextrin aqueous solu-

tion occurred at 37 °C during the whole process. Moreover, a sufficient rate of shaking is also necessary to form the emulsion. These results clearly demonstrate the major role played by the emulsifying step in bead formation.

The partial inclusion complex may also be responsible for the apparition of the self-forming film macroscopically visualised at the interface since soybean oil is added to α-CD solution. An interface is clearly a two-dimensional space so it could also explain that crystals expend favourably in two dimensions ($l \neq 0$) rather than in three ones. Moreover, properties of the interfacial film could play a role on emulsion formation and stability. When submitted to an external shaking the film could curve and surround oil to form and stabilise oily globules in the aqueous medium.

Rheological parameter values and more especially τ_c value (corresponding to the maximum stress tolerated by sample without destruction) increases from 4 to 24 h. It expresses a notable structuring in the samples that could result from crystal apparition and then from crystal growth. This hypothesis is supported by X-ray diffraction peaks, which become more intense and narrower as a function of time. Moreover, the structuring could also be due to the presence of beads of which size and number increase in time as observed by optical microscopy. After one day of shaking, rheological parameter values decrease and then remain stable from about 50 h assumed as the end of the process. Indeed, no more material would be available to be associated with beads and their formation would be stopped.

We describe here a new particulate system produced with soft conditions by a simple and original process. In such a system, the α-cyclodextrin is employed to interact with triglycerides and participates in bead formation and organisation whereas the high oil content offers interesting prospects for the microencapsulation of lipophilic drugs. The use of freeze-dried beads facilitates their handling. Studies are now in progress to evaluate their interests for drug delivery by the oral and topical routes.

Acknowledgments

We are grateful to Alain Marraud (Laboratoire de Mécanique, Sols, Structures et Matériaux, Ecole Centrale de Paris, Châtenay-Malabry) for the access to JEOL electron microscope, Françoise Garnier (Centre de Ressources "Microscopies", Ecole Centrale de Paris, Châtenay-Malabry) for SEM examinations, Valérie Nicolas (Plateau technique d'imagerie cellulaire, IFR141-ITFM, Faculté de Pharmacie 92296 Châtenay-Malabry) for confocal microscopy analyses, Ghislaine Fribourg (UPR 3101, CIME, Université Pierre et Marie Curie, Paris VI) for freeze-fracture. We thank Danielle Jaillard (Centre commun de microscopie électronique, développement et évolution UMR-CNRS 8080, Université Paris Sud, Orsay) for her excellent technical assistance.

References

Bibby, D.C., Davies, N.M., Tucker, I.G., 2000. Mechanisms by which cyclodextrins modify drug release from polymeric drug delivery systems. Int. J. Pharm. 197, 1–11.

Buschmann, H.J., Schollmeyer, E., 2002. Applications of cyclodextrins in cosmetic products: a review. J. Cosmet. Sci. 53, 185–191.

Challa, R., Ahuja, A., Ali, J., Khar, R.K., 2005. Cyclodextrins in drug delivery: an updated review. AAPS. Pharm. Sci. Tech. 6, 329–357.

Couvreur, P., Barratt, G., Fattal, E., Legrand, P., Vauthier, C., 2002. Nanocapsule technology: a review. Crit. Rev. Ther. Drug Carrier Syst. 19, 99–134.

Daoud-Mahammed, S., Couvreur, P., Gref, R., 2007. Novel self-assembling nanogels: Stability and lyophilisation studies. Int. J. Pharm. 332, 185–191.

Duchêne, D., Ponchel, G., Wouessidjewe, D., 1999a. Cyclodextrins in targeting. Application to nanoparticles. Adv. Drug Deliv. Rev. 36, 29–40.

Duchêne, D., Wouessidjewe, D., Ponchel, G., 1999b. Cyclodextrins and carrier systems. J. Control Release 62, 263–268.

Duchêne, D., Bochot, A., Yu, S.C., Pépin, C., Seiller, M., 2003. Cyclodextrins and emulsions. Int. J. Pharm. 266, 85–90.

Gref, R., Amiel, C., Molinard, K., Daoud-Mahammed, S., Sebille, B., Gillet, B., Beloeil, J.C., Ringard-Lefebvre, C., Rosilio, V., Poupaert, J., Couvreur, P., 2006. New self-assembled nanogels based on host-guest interactions: characterization and drug loading. J. Control Release 111, 316–324.

Kazuaki, H., Satoshi, M., 1983. Production of polycyclodextrin beads. JP Patent JP 58171404, 8 Oct.

Kazuaki, H., Satoshi, M., 1985. Production of bead-foam insoluble cyclodextrin polymer. JP Patent JP 60020924, 2 Feb.

Kazuyuki, I., Yukio, T., Hiroshi, T., Kazuhiko, O., Rokuro, O., Kazuaki, H., 1993. Cosmetics containing water-insoluble cyclodextrin polymers as scrubbing particles. JP Patent JP 5105619, 27 Apr.

Laurent, S., Ivanova, M.G., Pioch, D., Graille, J., Verger, R., 1994. Interaction between α-cyclodextrin and insoluble glyceride monomolecular film at the argon/water interface: application to lipase kinetics. Chem. Phys. Lipids 70, 35–42.

Le Bas, G., Ryzanek, N., 1987. Structural aspects of cyclodextrins. In: Duchêne, D. (Ed.), Cyclodextrins and Their Industrial Uses, Editions de santé, Paris, pp. 105–130.

Loftsson, T., Brewster, M., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.

Loftsson, T., Jarho, P., Masson, M., Jarvinen, T., 2005. Cyclodextrins in drug delivery. Expert Opin. Drug Deliv. 2, 335–351.

Manor, P.C., Saenger, W., 1974. Crystal and molecular structure of cyclohexaamylose hexahydrate. J. Am Chem. Soc. 96, 3630–3639.

McCormack, B., Gregoriadis, G., 1994. Entrapment of cyclodextrin-drug complexes into liposomes: potential advantages in drug delivery. J. Drug Target 2, 449–454.

Memişoğlu-Bilensoy, E., Bochot, A., Trichard, L., Duchêne, D., Hincal, A.A., 2005. Amphiphilic cyclodextrins and microencapsulation. In: Benita, S. (Ed.), Microencapsulation Methods and Industrial Applications, pp. 269–295.

Müller, R.H., Radtke, M., Wissing, S.A., 2002a. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv. Drug Deliv. Rev. 54, 131–155.

Müller, R.H., Radtke, M., Wissing, S.A., 2002b. Nanostructured lipid matrices for improved microencapsulation of drugs. Int. J. Pharm. 242, 121–128.

O'Driscoll, C.M., 2002. Lipid-based formulations for intestinal lymphatic delivery. Eur. J. Pharm. Sci. 15, 405–415.

Noltemeyer, M., Saenger, W., 1980. Structural chemistry of linear α-cyclodextrin-polyiodide complexes. X-ray crystal structures of (α-cyclodextrin)$_2$. LiI_3. I_2. $8H_2O$ and (α-cyclodextrin)$_2$. $Cd_{0.5}$. I_5. $27H_2O$. Models for the blue amylose–iodine complex. J. Am. Chem. Soc. 102, 2710–2722.

Pariot, N., Edwards-Levy, F., Andry, M.C., Levy, M.C., 2000. Cross-linked beta-cyclodextrin microcapsules: preparation and properties. Int. J. Pharm. 211, 19–27.

Pariot, N., Edwards-Levy, F., Andry, M.C., Levy, M.C., 2002. Cross-linked beta-cyclodextrin microcapsules. II. Retarding effect on drug release through semi-permeable membranes. Int. J. Pharm. 232, 175–181.

Shimada, K., Kawano, K., Ihsii, J., Nakamura, T., 1992. Structure of inclusion complexes of cyclodextrins with triglyceride at vegetable oil/water interface. J. Food Sci. 57, 655–656.

Shimada, K., Ohe, Y., Ohguni, T., Kawano, K., Ishii, J., Nakamura, T., 1991. Emulsifying properties of alpha-, beta- and gamma-cyclodextrins. Nippon Shokuhin Kogyo Gakkaishi 38, 16–20.

Szente, L., Szejtli, J., Szeman, J., 1993. Fatty acid-cyclodextrin complexes: properties and applications. J. Incl. Phenom. Mol. Recognit. Chem. 16, 339–354.

Trichard, L., Bochot, A., Duchêne, D., 2006. Cyclodextrins in dispersed systems. In: Dodziuk, H. (Ed.), Cyclodextrins and Their Complexes: Chemistry, Analytical Methods, Applications. Wiley-VCH, Weinheim (DE), pp. 423–449.

Uner, M., 2006. Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): their benefits as colloidal drug carrier systems. Pharmazie 61, 375–386.

Vanzo, E., 1991. Spherical cyclodextrin polymer beads. US Patent US 5,075,432, 24 Dec.

Yu, S.C., Bochot, A., Le Bas, G., Chéron, M., Grossiord, J.L., Seiller, M., Duchêne, D., 1999. Design and evaluation of an original O/W/O multiple emulsion containing natural cyclodextrins as the emulsifier. S. T. P. Pharma Sci. 9, 273–277.

Yu, S.C., Bochot, A., Le Bas, G., Chéron, M., Grossiord, J.L., Seiller, M., Duchêne, D., 2001. Characteristics of O/W emulsions containing lipophilic molecules with cyclodextrins as emulsifiers. S. T. P. Pharma Sci. 11, 385–391.

Zantl, R., 2001. Flüssigkristalle aus DNA und Kationischen Lipidmembranen. Ph.D. thesis, TU München, Germany.