

# Formulation and characterisation of beads prepared from natural cyclodextrins and vegetable, mineral or synthetic oils

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## Abstract

A continuous external shaking for 2.5 days of a mixture composed of  $\alpha$ -cyclodextrin (6%), soybean oil (19.6%) and water (74.4%) resulted in a calibrated lipid carrier namely bead with a high fabrication yield. The purpose of this work was to explore the possibility to substitute  $\alpha$ -cyclodextrin by other natural cyclodextrins, i.e.  $\beta$ - and  $\gamma$ -cyclodextrin and then soybean oil by mineral (Primol<sup>®</sup> 352 and Marcol<sup>®</sup> 82) or synthetic (Silicon 200<sup>®</sup> fluid 10, 50 or 100cSt) oils. Beads can be successfully prepared using Marcol<sup>®</sup> 82 with  $\alpha$ -cyclodextrin and Silicon 50 or 100cSt with  $\gamma$ -cyclodextrin. The area inside oil/cyclodextrin/water ternary diagram corresponding to bead occurrence was superior for the Marcol<sup>®</sup> 82/ $\alpha$ -cyclodextrin couple compared to that observed with soybean oil/ $\alpha$ -cyclodextrin couple. Only a few ratios of Silicon 50 and 100cSt/ $\gamma$ -cyclodextrin/water led to beads. The combinations which did not induce bead occurrence gave either emulsions, two non-miscible liquids or a solid mixture. Whatever the materials used, beads exhibited similarities: presence of a crystalline organisation and viscoelastic properties. Manufacturing process of paraffin- and silicon-based beads need further optimisation to increase fabrication yield and later on, to take advantages from the high stability of both oils for the formulation of drugs with beads.

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**Keywords:** Beads; Cyclodextrins; Emulsions; Vegetable oil; Mineral oil; Silicone oil

## 1. Introduction

A novel lipid carrier named “beads” is of great interest for pharmaceutical and cosmetic applications. Indeed, the mixture of soybean oil (19.6% w/w),  $\alpha$ -cyclodextrin (CD) (6% w/w), and water (74.4% w/w) leads to homogeneous bead size with a high fabrication yield employing a straightforward manufacture process (Bochot et al., 2007). Interestingly, soybean/ $\alpha$ -CD beads are able to efficiently encapsulate and increase oral bioavailability of a lipophilic model drug in rats (Trichard et al., 2007). Furthermore, they are well-tolerated by skin and permit the release of therapeutic component as efficiently as currently commercialised medicines (Trichard et al., 2008). Morphologically, soybean oil/ $\alpha$ -CD beads appear as minispheres consisting of a partial crystalline matrix of CD surrounding micro-domains

of oil (Bochot et al., 2007).  $\alpha$ -CD is employed to interact with triglycerides (TG) present in soybean oil and strongly participates in bead formation and organisation. The following mechanism involved in bead formation was previously proposed (Bochot et al., 2007): (i) a partial inclusion complex would be formed between TG and  $\alpha$ -CD at the oil/water interface. That would explain the presence of a film which is visualised between the oily and aqueous phases. (ii) Submitted to an external shaking the film would curve and surround oily globules to form O/W emulsions. Indeed,  $\alpha$ -CD–TG inclusion complexes displaying amphiphilic property (Shimada et al., 1991) are described capable to stabilise emulsions (Shimada et al., 1992; Yu et al., 2001; Duchêne et al., 2003). (iii) Thanks to  $\alpha$ -CD–TG interaction, a crystallisation of CD molecules would occur around the oily globules. (iv) Finally, after few days of continuous shaking, beads are obtained in suspension in an aqueous medium.

In the present work, we investigated bead formation from other ingredients using the method previously optimised for soybean oil/ $\alpha$ -CD/water-based formulation (Bochot et al., 2007). We evaluated the possibility to substitute  $\alpha$ -CD by other natural

*Abbreviations:* CD, cyclodextrin; TG, triglycerides.

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cyclodextrins, i.e.  $\beta$ - and  $\gamma$ -CD as well soybean oil by mineral (Primol<sup>®</sup> 352 and Marcol<sup>®</sup> 82) or synthetic oils (Silicon 200<sup>®</sup> fluid 10, 50 or 100cSt). These oils differ by their chemical composition and viscosity. They are of high-interest in medicine or cosmetic formulations and advantageously are odourless and more stable than soybean oil. They also exhibit attractive intrinsic properties such as emollient effect and are approved for oral or topical administrations.

## 2. Materials and methods

### 2.1. Materials

Alpha-cyclodextrin ( $\alpha$ -CD) (Cavamax<sup>®</sup> W6 Pharma), beta-cyclodextrin ( $\beta$ -CD) (Cavamax<sup>®</sup> W7 Pharma), gamma-cyclodextrin ( $\gamma$ -CD) (Cavamax<sup>®</sup> W8 Pharma) were purchased from Wacker-Chemie (France). Cropure<sup>®</sup> soybean oil ( $\rho=0.92$  g/mL,  $\eta=51 \pm 1$  mPa s) purchased from Croda (Trappes, France) is mainly composed of triglycerides (TG > 98%) in which fatty acid chains possess 16 or 18 carbons (Fig. 1a). 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). Both liquid paraffins (Fig. 1b), Primol<sup>®</sup> 352 ( $\rho=0.86$  g/mL,  $\eta=124 \pm 1$  mPa s, average molecular weight = 480 g/mol, paraffinic carbons 66–67%, naphthenic carbons 33–34% and aromatic carbons 0%) and Marcol<sup>®</sup> 82 ( $\rho=0.85$  g/mL, light liquid paraffin,  $\eta=20 \pm 1$  mPa s, average molecular weight = 350 g/mol, paraffinic carbons 66–67%, naphthenic carbons 33–34% and aromatic carbons 0%) were produced by Esso (Reuil-Malmaison, France). Silicon 200<sup>®</sup> fluid 100cSt ( $\rho=0.97$  g/mL,

linear polydimethylsiloxane,  $97 \pm 1$  mPa s, average molecular weight = 5500 g/mol), Silicon 200<sup>®</sup> fluid 50cSt ( $\rho=0.95$  g/mL, linear polydimethylsiloxane,  $51 \pm 1$  mPa s, average molecular weight = 3400 g/mol) and Silicon 200<sup>®</sup> fluid 10cSt ( $\rho=0.92$  g/mL, linear polydimethylsiloxane,  $10 \pm 0.5$  mPa s, average molecular weight = 900 g/mol) were provided as a gift from Dow Corning (Sophia Antipolis, France) (Fig. 1c). The viscosity values were measured at 25 °C using a rheometer equipped with coaxial geometry (RotoVisco 1, Haake, Courtaboeuf, France).

### 2.2. Ternary diagram studies

Ternary diagrams were performed to delimit the domain of bead formation on the following oil/cyclodextrin/water mixtures: soybean oil/ $\beta$ - or  $\gamma$ -CD/water, Primol<sup>®</sup> 352 or Marcol<sup>®</sup> 82/ $\alpha$ - or  $\beta$ - or  $\gamma$ -CD/water, Silicon 200<sup>®</sup> fluid 100 or 50 or 10cSt/ $\alpha$ - or  $\beta$ - or  $\gamma$ -CD/water. Diagram exploration was limited to the domain of cyclodextrin water solubility (14.5, 1.85 and 23.2% (w/v) at 25 °C for  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD, respectively). This limit is represented as a line on each diagram (Figs. 2 and 3). For each point of the diagram, the oil/CD/water ratios were firstly determined and then, required quantities of each component were calculated by keeping constant the total volume of

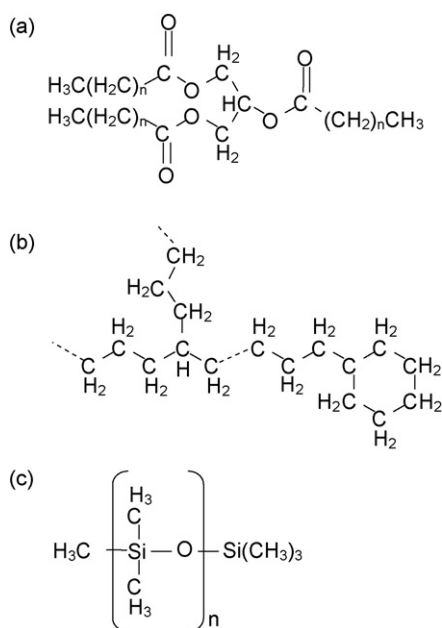


Fig. 1. Chemical formulae of oil components: (a) triglycerides in soybean oils (fatty acids of 16 or 18 carbons,  $n=14$  or 16), (b) saturated hydrocarbon chains (linear, ramified or cyclic) in paraffin oils, and (c) linear polydimethylsiloxanes in silicone oils.

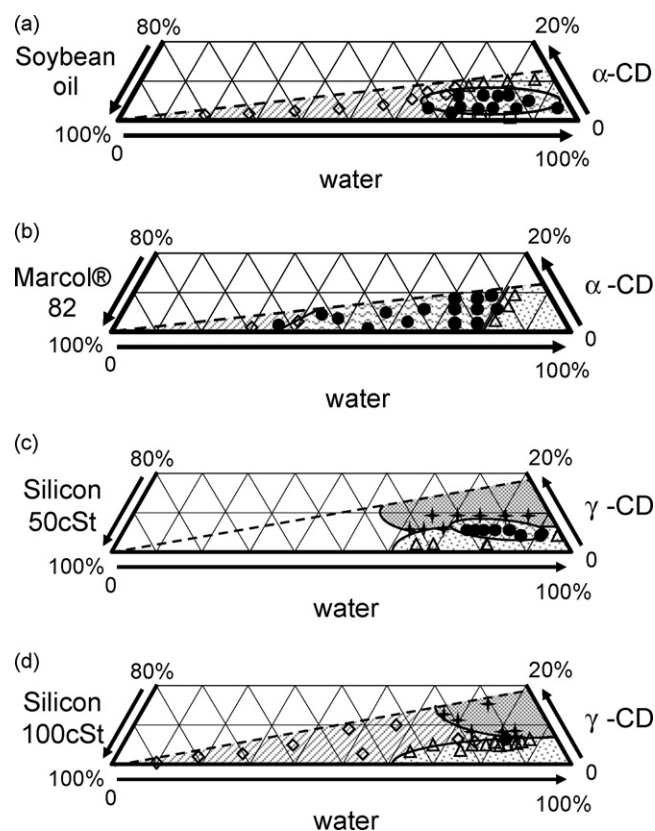


Fig. 2. Oil/CD/water ternary diagrams of (a) soybean oil/ $\alpha$ -CD/water adapted from Bochot et al. (2007), (b) Marcol<sup>®</sup> 82/ $\alpha$ -CD/water, (c) Silicon 50cSt/ $\gamma$ -CD/water and (d) Silicon 100cSt/ $\gamma$ -CD/water. Each sample was continuously shaken at 200 rpm in a gyratory shaker at 28 °C and classified as: (□) two non-miscible liquids; (△) fluid emulsion; (◇) viscous emulsion; (●) preparation containing beads; (+) solid mixture.

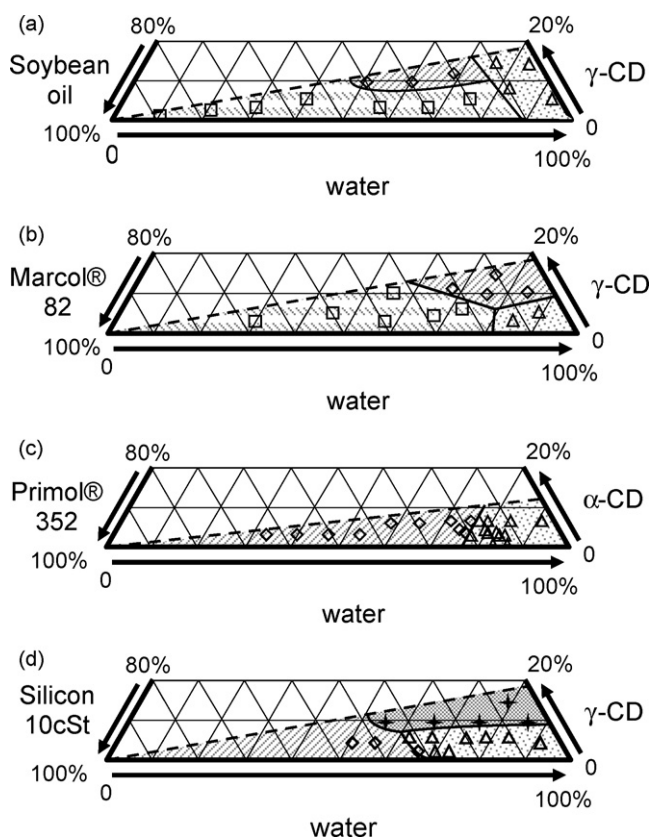


Fig. 3. Oil/CD/water ternary diagrams of (a) soybean oil/ $\gamma$ -CD/water, (b) Marcol® 82/ $\gamma$ -CD/water, (c) Primol®/ $\alpha$ -CD/water 352 and (d) Silicon 10cSt/ $\gamma$ -CD/water. Each sample was continuously shaken at 200 rpm in a gyrotory shaker at 28 °C and classified as: (□) two non-miscible liquids; ( $\Delta$ ) fluid emulsion; ( $\diamond$ ) viscous emulsion; (+) solid mixture.

preparation (25 mL). In details, the following equations were used, considering the volumic mass  $\rho_{\text{water}} = 1 \text{ g/mL}$ :

$$\text{CD quantity (g)} = \frac{(\% \text{CD}/\% \text{oil}) \rho_{\text{water}} 25 \text{ mL}}{(\% \text{water}/\% \text{oil}) + (\rho_{\text{water}}/\rho_{\text{oil}})},$$

$$\text{oil quantity (g)} = \frac{\rho_{\text{water}} 25 \text{ mL}}{(\% \text{water}/\% \text{oil}) + (\rho_{\text{water}}/\rho_{\text{oil}})},$$

$$\text{water quantity (g)} = \frac{\rho_{\text{oil}} 25 \text{ mL}}{(\% \text{water}/\% \text{oil}) + (\rho_{\text{oil}}/\rho_{\text{water}})}$$

Samples were prepared by first dissolving cyclodextrin in water, then by adding the required volume of oil. Both liquid phases were then continuously shaken at 200 rpm in a gyrotory shaker (Salvis, Bioblock Scientific, Illkirch, France) at 28 °C until preparation macroscopically reached a steady state. Samples were classified into one of the following states: two non-miscible liquids, fluid or viscous emulsions, solid mixture or preparation containing beads.

As comparison, beads made of soybean oil/ $\alpha$ -CD/water (19.6/6.0/74.4 wt%) were prepared according to Bochot et al. (2007) using the same protocol. Briefly, 5.8 mL of soybean solution was added to 20 mL of a  $\alpha$ -CD aqueous solution (8.1% w/v).

### 2.3. Bead characterisation

For each oil/CD/water combinations allowing bead formation, an optimal preparation considering high bead number and low turbidity of the dispersion medium was selected for further particle characterisation. Beads made of soybean oil/ $\alpha$ -CD/water (19.6/6.0/74.4 wt%) were also evaluated as a reference.

#### 2.3.1. Optical microscopy

Beads ( $n=50$ ) were observed using an optical microscope (Leitz Diaplan microscope, Leica Microsystems, France) equipped with a Coolsnap ES camera (Roper Scientific) in order to determine their shape and size.

#### 2.3.2. Rheological studies

Rheological measurements were performed on bead samples using a controlled stress rheometer (RS600, Thermo Electron, France) equipped with a cone-plate geometry (diameter = 20 mm; gap = 0.132 mm; angle = 4°). Measurements were performed at 28 °C ( $\pm 0.20$ ) (Universal Thermal Controller coupled with a Thermo Haake F6/C35 circulating heat chilled water bath, ThermoHaake). The imposed stress was sinusoidal (frequency, 1.000 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  $\omega$  but shifted by a phase angle of  $\delta$ . The strain may thus be written as  $\gamma = \gamma_0 \cos(\omega t - \delta)$ . Response of all samples was viscoelastic ( $0 < \delta < \pi/2$ ) from imposed stress 1 Pa to a critical value,  $\tau_c$ . This  $\tau_c$  was calculated as the value of  $\tau$  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) modulus,  $G' = (\tau_0/\gamma_0) \cos \delta$  and  $G'' = (\tau_0/\gamma_0) \sin \delta$  and they were calculated for each sample in linear regime (plateau value). Experiments were triplicated and mean as well as standard deviation of  $G'$ ,  $G''$  and  $\tau_c$  (Pa) were calculated.

#### 2.3.3. X-ray diffraction studies

Beads were submitted to X-ray diffraction analysis. Wide angle X-ray scattering experiments were performed using a Nanostar 1 (Bruker AXS, Karlsruhe, Germany) under vacuum from the X-ray source to the detector. X-ray source was a spot focus ceramic sealed tube for copper radiation (Microstar type) and a cross-coupled Göbel mirror provided a parallel monochromatic beam ( $\lambda = 1.54 \text{ \AA}$ ) oriented onto 1.6 mm diameter sealed capillary containing the samples. Two-dimensional scattering patterns were recorded 5 min on the two-dimensional detector (photon counter, Histar type) placed 125 mm far from the sample. All samples exhibited powder diffraction Debye-Scherrer rings and corresponding intensities (arbitrary units) were determined as a function of the scattering vector  $q \text{ (\AA}^{-1}\text{)} = (4\pi/\lambda) \sin(\theta)$  by circular integration (DIFFRAC<sup>plus</sup> Software). Interplanar distances  $d \text{ (\AA)} = \lambda/(2 \sin(\theta)) = 2\pi/q$

Table 1  
Characteristics of beads prepared from optimised oil/CD/water ratios

	Oil/cyclodextrin/water		
	Soybean oil/ $\alpha$ -CD/water (19.6/6.0/74.4 wt%)	Marcol <sup>®</sup> 82/ $\alpha$ -CD/water (22.7/6.2/71.1 wt%)	Silicon 50cSt/ $\gamma$ -CD/water (9.1/5.1/85.8 wt%)
Process duration (days)	2.5	8	1.5
Bead size (mm)	1.6 $\pm$ 0.2	Length from 0.2 to 0.8; width from 0.2 to 0.5	Length from 0.2 to 1.4; width from 0.1 to 0.7
Viscoelastic parameters			
$G'$ (Pa)	1.5 $\pm$ 0.2 $\times 10^6$	4.0 $\pm$ 1.0 $\times 10^4$	2.1 $\pm$ 0.8 $\times 10^3$
$G''$ (Pa)	1.8 $\pm$ 0.1 $\times 10^5$	8.3 $\pm$ 1.2 $\times 10^3$	4.4 $\pm$ 1.0 $\times 10^2$
$\tau_c$ (Pa)	3.2 $\pm$ 0.4 $\times 10^4$	3.5 $\pm$ 0.8 $\times 10^2$	1.2 $\pm$ 0.3 $\times 10^1$

in the direct spacing were calculated according to the Bragg law.

### 3. Results

#### 3.1. Oil/cyclodextrin/water ternary diagrams

Analysis of oil/CD/water ternary diagrams showed that  $\alpha$ - and  $\gamma$ -CD permitted to form beads employing mineral and silicone oils, respectively (Fig. 2b–d). Soybean oil/ $\alpha$ -CD/water ternary diagram established previously by Bochot et al. (2007) is reported in Fig. 2a as a reference. Bead formation was always preceded by the following successive stages: (i) addition of oil to CD aqueous solution resulted into 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 a stable emulsion where the viscosity increased in time. (iii) Finally, after several days (1.5–8 days) (Table 1), beads were in suspension in a medium more or less turbid.

Domains of bead formation are reported in Table 2 and were found to be strongly influenced by the nature of oil/CD combinations. Indeed, the area corresponding to bead occurrence was superior for the Marcol<sup>®</sup> 82/ $\alpha$ -CD couple (Fig. 2b) compared to that observed with soybean oil/ $\alpha$ -CD one (Fig. 2a). On the opposite, only a few ratios of Silicon 50 and 100cSt/ $\gamma$ -CD/water (Fig. 2c and d) led to beads.

Oil/CD combinations which did not induce bead formation were composed of soybean oil/ $\beta$ - or  $\gamma$ -CD, Primol<sup>®</sup> 352/ $\alpha$ -,  $\beta$ - or  $\gamma$ -CD, Silicon 100 or 50cSt/ $\alpha$ - or  $\beta$ -CD and Silicon 10cSt/ $\alpha$ -,  $\beta$ - or  $\gamma$ -CD. The shaking of most of these samples (Fig. 3) as well as those prepared outside of bead occurrence domain (Fig. 2) resulted only in fluid or viscous emulsions.

Their viscosity increased when oil content was enhanced in the formulation.

Whatever the nature of the oil, most of the preparations involving  $\beta$ -CD appeared as two non-miscible liquids (data not shown). Emulsions were only observed for proportions of soybean oil, Marcol<sup>®</sup> 82 and Primol<sup>®</sup> 352 inferior to 40, 25 and 25%, respectively. Silicone oils/ $\alpha$ -CD couples also resulted into two non-miscible liquids whatever the grade employed (data not shown). Finally, for the formulations prepared from silicone oils in proportions inferior to a range from 20 to 30% and  $\gamma$ -CD superior to 10%, a precipitation occurred as soon as oil was added onto the  $\gamma$ -CD solution and resulted in preparations of solid consistency (Figs. 2c and d and 3d).

#### 3.2. Bead characteristics

Inside the domain of bead occurrence, the proportions corresponding to Marcol<sup>®</sup> 82/ $\alpha$ -CD/water (22.7/6.2/71.1%) and Silicon 50cSt/ $\gamma$ -CD/water (9.1/5.1/85.8%) allowed the formation of optimised beads regarding their number and the turbidity of the dispersion medium. These formulations were chosen to characterise bead samples. Beads formulated from soybean oil (19.6%)/ $\alpha$ -CD (6.0%)/water (74.4%) were used as reference because they presented satisfying properties such as homogeneous bead size, high fabrication yield (Table 1). Optimal ratios between soybean oil/ $\alpha$ -CD/water and Marcol<sup>®</sup> 82/ $\alpha$ -CD/water were closed together although oil compositions were very different. Otherwise, the optimal point providing the best Silicon 50cSt/ $\gamma$ -CD-based bead characteristics involved twice less amount of oil.

Bead characteristics are reported in Table 1. Beads reached a steady state in longer time (8 days) for Marcol<sup>®</sup> 82/ $\alpha$ -CD and shorter (1.5 days) for Silicon 50cSt/ $\gamma$ -CD compared to the ref-

Table 2  
Oil/CD/water domains of bead formation

	Bead feasibility			
	Soybean oil/ $\alpha$ -CD/water <sup>a</sup>	Marcol <sup>®</sup> 82/ $\alpha$ -CD/water	Silicon 50cSt/ $\gamma$ -CD/water	Silicon 100cSt/ $\gamma$ -CD/water
Oil ratio (wt%)	12–24	10–60	5–20	12.3
CD ratio (wt%)	3–6	1–10	5–6	5.9
Water ratio (wt%)	70–82	40–80	75–93	81.8

<sup>a</sup> According to Bochot et al. (2007).

erence (2.5 days). Silicon- and paraffin-based beads were more elongated-shaped (Fig. 4) and had smaller and less homogeneous size (Table 1) than soybean oil-based ones. Finally, compared to the reference, Marcol<sup>®</sup> 82/ $\alpha$ -CD and Silicon 50cSt/ $\gamma$ -CD-based preparations appeared as highly turbid mixtures in which particles were too small to permit their separation from the dispersion medium. The quantification of the fabrication yield was therefore not possible.

The rheological behaviour of these preparations was more elastic than viscous (Table 1). More precisely, elastic modulus values were constantly about one decade higher than viscous modulus one. The critical values of stress  $\tau_c$  which reflects the sample structuring was ordered as follows: soybean oil/ $\alpha$ -CD/water-based preparation  $\gg$  Marcol<sup>®</sup> 82/ $\alpha$ -CD/water-based preparation  $>$  Silicon 50cSt/ $\gamma$ -CD/water-based preparation (Table 1).

All beads showed a crystalline organisation but the packing type was different for each formulation since X-ray diffraction peak positions were not identical (Fig. 5, spectra number (1), (2) and (5)). Bead crystalline organisations were all different from natural CD hydrate crystals (spectra (4) and (7)), which unsurprisingly adopted a cage structure (Saenger and Steiner, 1998). From Marcol<sup>®</sup> 82/ $\alpha$ -CD/water-based bead spectrum (spectrum (2)), following interplanar distances were calculated:  $d = 15.8$ ; 9.7; 7.9; 6.9; 4.9; 4.6 and 4.4 Å. The first interplanar distance 15.8 Å corresponded to the height of  $\alpha$ -CD dimers and oriented toward  $\alpha$ -CD organisation as a channel structure (Noltemeyer and Saenger, 1980). Silicon 50cSt oil/ $\gamma$ -CD/water-based bead spectrum (spectrum (5)) presented the following interplanar distances:  $d = 11.8$ ; 6.0; 5.6 and 5.3 Å. Interestingly, the first interplanar distance was described as the fingerprint for the channel type structure of  $\gamma$ -CD inclusion complexes (Porbeni et al., 2001; Kawasaki et al., 2007). Marcol<sup>®</sup> 82 (spectrum (3)) and Silicon 50cSt (spectrum (6)) presented broad band of diffusion ( $q_{\max} = 1.28$  and  $0.86 \text{ \AA}^{-1}$ , respectively) which was visible also in the corresponding bead spectra (spectra (2) and (5), respectively).

#### 4. Discussion

The aim of this work was to investigate bead feasibility from different raw materials using the method previously optimised for soybean oil/ $\alpha$ -CD/water formulation. This study demonstrated firstly that  $\gamma$ -CD can also be used to prepare beads and secondly that soybean oil can be replaced by mineral or synthetic oils. Interestingly, beads presented similarities whatever the materials used. They all exhibited a crystalline organisation related to CD packing as channel structure which supports the formation of inclusion complexes between natural CD and oil component(s) at the oil/water interface (Bochot et al., 2007). All bead samples also presented viscoelastic properties. However, bead formulations prepared from Marcol<sup>®</sup> 82/ $\alpha$ -CD and Silicon 50cSt/ $\gamma$ -CD exhibited lower viscoelastic parameters than soybean oil/ $\alpha$ -CD-based ones. A dramatic increase of  $G'$ ,  $G''$  and  $\tau_c$  was identified at 6 h for sample prepared with soybean bead (data not shown). At this time, bead diameters (150–600  $\mu\text{m}$ ) measured by optical microscopy (Bochot et al., 2007) overcame

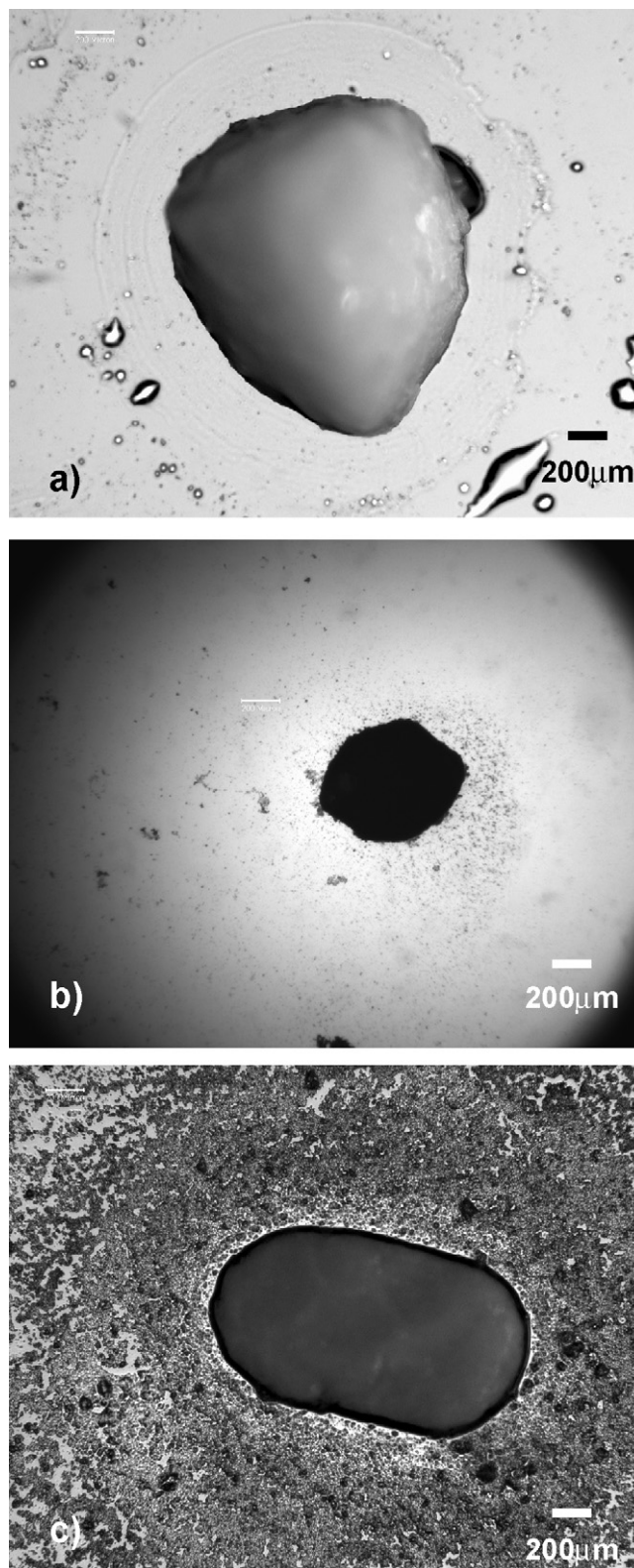


Fig. 4. Optical micrographs of (a) soybean oil/ $\alpha$ -CD/water (19.6/6.0/74.4 wt%)-based beads, (b) Marcol<sup>®</sup> 82/ $\alpha$ -CD/water (22.7/6.2/71.1 wt%)-based beads and (c) Silicon 50cSt/ $\gamma$ -CD/water (9.1/5.1/85.8 wt%)-based beads.

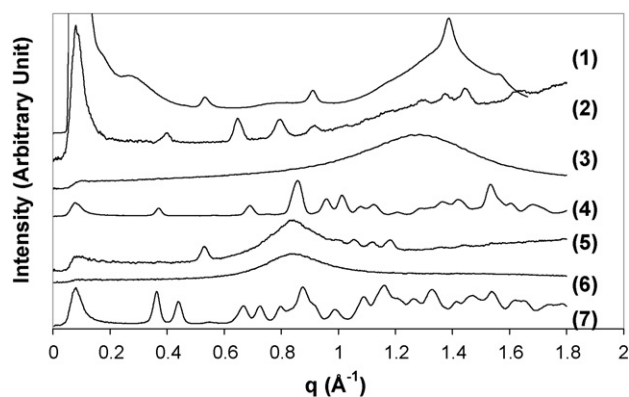


Fig. 5. X-ray diffraction spectra of (1) soybean oil/ $\alpha$ -CD/water (19.6/6.0/74.4 wt%)-based beads; (2) Marcol<sup>®</sup> 82/ $\alpha$ -CD/water (22.7/6.2/71.1 wt%)-based sample containing beads; (3) Marcol<sup>®</sup> 82; (4)  $\alpha$ -CD; (5) Silicon 50cSt/ $\gamma$ -CD/water (9.1/5.1/85.8 wt%)-based sample containing beads; (6) Silicon 50cSt; (7)  $\gamma$ -CD.

the gap of the cone-plate geometry (132  $\mu\text{m}$ ). It can be proposed that as soon as beads would be large enough to be contacted to the gap of the cone-plate geometry, rheological parameters would start increasing.  $G'$ ,  $G''$  and  $\tau_c$  values would thus be related to bead occurrence but also to their size and number. Diameters of beads prepared with Marcol<sup>®</sup> 82 and Silicon 50cSt are smaller than those prepared with soybean oil and bead fabrication yield was too low to be precisely quantified. For these reasons, rheological behaviours of these samples would be found lower than soybean oil-based ones.

Previous works showed that soybean oil/ $\alpha$ -CD beads can be considered as a new lipid carrier suitable for the microencapsulation of lipophilic drugs such as retinoids (Trichard et al., 2007,2008). Indeed, their structure, i.e. a crystalline matrix of cyclodextrins surrounding microdomains of oil, and their high oil content (80 wt%) (Bochot et al., 2007) favour high encapsulation efficiency (>90%) (Trichard et al., 2007,2008). Considering silicone oils, their interest in bead formulation seems more limited than that of soybean oil and Marcol<sup>®</sup> 82 due to restricted domains of bead formation associated with low amount of oil. Although more combinations involving Marcol<sup>®</sup> 82 than soybean oil resulted in beads, optimal oil/CD/water proportions were found very similar. However, Marcol<sup>®</sup> 82-based beads were always dispersed in a highly turbid medium. As a compromise has to be found between oil content and fabrication yield to achieve high drug loading and efficiency, soybean oil still offers the greatest interest in bead formulation.

In this work, we also tried to substitute  $\alpha$ -CD by  $\beta$ - or  $\gamma$ -CD to formulate beads with soybean oil. Although the formation of inclusion complexes having emulsifying property was mentioned between  $\beta$ -CD and triglycerides (Shimada et al., 1991, 1992), beads were not obtained using this CD. Indeed,  $\beta$ -CD is much less soluble in water than  $\alpha$ -CD and the amount of partial inclusion complexes might therefore not be sufficient to allow either emulsification step or bead occurrence. Actually, for high soybean oil/ $\beta$ -CD ratios, samples appear as two non-miscible liquids. On the other hand, low oil/ $\beta$ -CD ratios led only to fluid emulsions because a too small amount of  $\beta$ -CD

would not permit the crystallisation of CD molecules required to generate bead matrix. Beads were not also obtained with the soybean oil/ $\gamma$ -CD couple. In this case, CD water-solubility is high but O/W emulsions necessitate higher CD amount to be stable (Shimada et al., 1991; Yu et al., 2001). Indeed, the cavity of  $\gamma$ -CD is too wide to lead to an optimal interaction with triglycerides (Yu et al., 2001). Consequently, inclusion complex formation between CD and TG appears essential to form beads but two other conditions seem also to be required: the formation of a sufficient amount of complexes and an adequate fitting between oil components and CD.

Interestingly, soybean oil was successfully substituted by Marcol<sup>®</sup> 82 and Silicon 50cSt to form beads with  $\alpha$ - and  $\gamma$ -CD, respectively showing the influence of the oil/CD couple on bead occurrence.  $\alpha$ -CD forms inclusion complexes with linear hydrocarbon chains (Wishnia and Lappi, 1974). As liquid paraffins contain linear but also branched hydrocarbon chain(es), we can suggest that  $\alpha$ -CD molecules could entrap linear part of hydrocarbons chains whereas ramified hydrocarbon branch(es) could not enter within the cavity. In the case of silicone oils (Fig. 1c), polydimethylsiloxane chains interact strongly with  $\gamma$ -CD to form inclusion complexes (Okumura et al., 2000; Porbeni et al., 2001; Saalwächter, 2002). As previously depicted for TG and  $\alpha$ -CD, both types of inclusion complexes would be able to stabilise emulsions due to their amphiphilic property. These complexes would be partial, CD molecules interacting with one part of oil components in the aqueous phase whereas the nonincluded one would be oriented toward the oily phase.

Crystalline organisations in bead samples differ from those of natural CD hydrate crystals and tend to confirm the presence of inclusion complexes (Le Bas and Ryzanek, 1987). When no complexation occurs as reported between polydimethylsiloxane chains and  $\alpha$ -CD (Okumura et al., 2000) no emulsion are formed and samples remain as two non-miscible liquids.

Contrary to Marcol<sup>®</sup> 82 and Silicon 50 or 100cSt, Primol<sup>®</sup> 352 and Silicon 10cSt gave only emulsions. Within the same chemical family, the silicone and mineral oils studied differ by their viscosity, their average molecular weight and thus by the length of hydrocarbon or polydimethylsiloxane chains. The chain length would influence the amphiphilic character of the partial inclusion complexes (more or less lipophilic or hydrophilic) formed at oil/water interface. It could therefore explain why bead occurrence was high with Silicon 50cSt and Marcol<sup>®</sup> 82, poor with Silicon 100cSt and absent with Silicon fluid 10cSt and Primol<sup>®</sup> 352.

For the combinations previously mentioned as resulting in beads, some areas were also identified as solid mixture or fluid/viscous emulsions demonstrating that the ratio between oil, CD and water is another key parameter in bead formation. Indeed, a sufficient amount of CD would not only be necessary to stabilise oily globules but also to structure bead sample and generate the crystalline bead matrix.

Finally, mineral and silicone oils present higher variability in their chemical composition (mixtures of components with various molecular weights) than soybean oil which is mainly composed of triglycerides (>98%) in which fatty acid chains possess 16 or 18 carbons. Thus, the composition and the vari-

ability of oil components could affect the complexation with CD and consequently the fabrication yield, the time required to form beads and finally their size.

This study demonstrates that bead can be prepared from different natural cyclodextrins and various oils, i.e. vegetable, mineral and silicone oils. Manufacturing process of paraffin- and silicon-based beads needs further optimisation to increase fabrication yield and later on, to take advantages from the high stability of both oils for the formulation of drugs with beads.

Several critical points were identified to achieve bead formation: (i) the influence of oil chemical composition; (ii) a good fitting between CD molecules and oil components to provide strong inclusion complexes; (iii) formation of partial inclusion complexes presenting emulsifying property; (iv) optimum ratios between oil components and CD.

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