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ARTICLE TYPE

Two-way effects of surfactants on Pickering emulsions stabilized by the self-assembled microcrystals of α-cyclodextrin and oil

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The influence of surfactants on the stability of cyclodextrin (CD) Pickering emulsions is not well understood. In this study, we report two-way effects of Tween 80 and soybean lecithin (PL) on the long term stability of Pickering emulsions stabilized by the self-assembled microcrystals of α-CD and medium chain triglycerides (MCT). The CD emulsions in the absence and presence of Tween 80 or PL at different concentrations were prepared and characterized by the droplet size, viscosity, contact angle, interfacial tension and residual emulsion values. After adding Tween 80 and PL, similar effects on the size distribution and contact angle were observed. However, changes of viscosity and interfacial tension were significantly different and two-way effects on the stability were found: (i) synergistic enhancement by Tween 80; (ii) inhibition at low and enhancement at high concentrations by PL. The stability enhancement of Tween 80 was due to the interfacial tension decrease caused by the interaction of Tween 80 with CD at the o/w interface at lower concentrations, and significant viscosity increase caused by Tween 80-CD assembly in the continuous phase. For PL at low concentrations, the replacement of α-CD/MCT by α-CD/PL particles at the o/w interface was observed, leading to inhibitory effects. High concentrations of PL resulted in an extremely low interfacial tension and stable emulsion. In conclusion, the extensive inclusion of surfactants by CD leads to their unique effects on the stability of CD emulsions, for which the changes of viscosity and interfacial tension caused by host-guest interactions play important roles.

1 Introduction

In addition to surfactants, solid colloidal particles have been recognized recently as emulsion stabilizing agents in forming the Pickering emulsions, which have high resistance to coalescence and retain the basic properties of classical emulsions stabilized by surfactants [1]. Various inorganic and organic colloidal particles with different sizes, shapes and surface chemistry have been studied to prepare Pickering emulsions, including silica [2-4], clays [5], wax [6], layered double hydroxides particles [7], carbon nanotubes [8], magnetic particles [9] and thermosensitive particles like poly(N-isopropylacrylamide) [10].

In consideration of the practical application of emulsion in the fields of pharmaceutics, Pickering emulsions stabilized by organic particles have some unique advantages over the conventional emulsions, such as biocompatibility, biodegradability, and antioxidizability [11-13]. These emulsifier free systems can also be used as drug carriers as well as templates in the production of microspheres to achieve controlled release and/or drug stabilization. For example, Pickering emulsions stabilized by the polysaccharides starch [14-16] and cellulose nanocrystals [17] have been employed as vehicles for topical drug delivery with oil content as high as 56% (w/w). Besides, Pickering emulsions prepared with microcrystalline cellulose [18] and whey protein isolate [19] have been reported to reduce the lipid oxidation rate of o/w emulsions.

Cyclodextrins (CDs), which can form surface active complexes at oil-water interfaces, are attractive alternative emulsion stabilizers due to a capability to form host-guest inclusion complex [20-25]. It was suggested that such emulsions are a type of Pickering emulsion stabilized by the self-assembled inclusion complexes of CD-oil. Shimada et al. [26] stabilized o/w emulsions using triglycerides as the oil phase, which had been emulsified with aqueous solutions of α-CD and β-CD. The authors found that the interfacial tension of vegetable oil (soybean oil or coconut oil)/water interface decreased with the increase of CD concentration. It was reasoned that a partial inclusion complex formed at the oil-water interface by CDs interacting with one fatty acid chain of the triglycerides, with the CD preferentially orientated towards the continuous phase, while the other two fatty acid chains in the complex oriented towards the oil phase. From phase diagrams of n-alkanols/CD/water systems [21, 22], the emulsion region extended as the chain length of n-alkanols and the amount of α-CD added increased. Furthermore, the emulsion was not formed in the region where the α-CD/n-alkanols complex did not precipitate. A three-dimensional network structure in the continuous phase to prevent

aggregation among dispersed phases also contributed to the stability of CD emulsions, which was confirmed by observations using polarized light microscopy and powder X-ray diffraction measurement [20]. The prepared n-alkane/water emulsions were of the o/w type, and the stability of emulsions was in the order of n-hexadecane > n-dodecane > n-octane. Emulsifications of various common oils including squalane, soybean oil and liquid paraffins were also investigated using natural β-CD and its derivatives [25]. An o/w emulsion was formed using β-CD, whereas triacetyl-β-CD gave rise to both o/w and w/o emulsions. The authors determined the contact angle of the CD emulsions, finding that o/w emulsion at $\theta < 90°$ and w/o emulsion at $\theta > 90°$ were formed when the composition of each oil and water was mixed with natural β-CD or triacylated β-CDs [23].

Recently, Mathapa et al. investigated the self-assembly of CD molecules at the tetradecane-aqueous solution interface through formation of inclusion complexes and studied the effects of temperature, pH, chaotropic agent concentration and the length of the guest molecule on the formation of microcrystals of inclusion complexes with CDs [27]. They also reported the preparation of novel CD-polyallylamine hydrochloride co-polymer microcapsules using an interfacial cross-linking reaction with epichlorohydrin on the surface of o/w emulsion templates, which was stabilized by the CD-oil inclusion complex [28,29]. The novelty of this emulsion stabilisation mechanism was that molecularly dissolved CD from the continuous phase assembled into colloid particles directly onto the emulsion drop surface, i.e. molecular adsorption leads to effective Pickering stabilisation. They produced the cyclodextrinosomes by removing the oil from the CD-stabilized o/w emulsion by evaporation of the CD-stabilized emulsion for the first time and demonstrated that the obtained structures were stable upon redispersing in water, which had promising potential application as drug delivery vehicles and surfactant free formulations for cosmetics and personal care products.

Many commercial products based on emulsions include both surfactants and particles, hence the characterization of emulsions prepared with surfactants and CD based particles are of great importance. The influence of surfactants on stabilization of Pickering emulsion mainly depends on the types and amounts of surfactants and the preparation protocol. The effects of Tween 60, sodium caseinate and lecithin with different concentrations on the stability of o/w emulsions stabilized with hydrophilic silica particles had been investigated by Pichot et al. [30]. They found that the behaviours of o/w emulsions prepared with both particles and any of the three surfactants depended on the type of surfactants and their concentrations. When Tween 60 or sodium caseinate was selected, surfactants at low concentrations improved the stability of the emulsion, however, surfactants at high concentrations resulted in the removal of particles from the interface of the system thus impairing the stability of the emulsion. For lecithin, the behaviour of emulsions was not dependent on the concentrations of surfactant: no removal of particles was observed. For an o/w Pickering emulsion containing mixtures of particles and oppositely charged surfactants, Binks et al. [31, 32] found that the most stable emulsions were prepared under conditions where particles had negligible charge and were most flocculated with sufficient amount of adsorbed surfactant. Synergistic interaction was evidenced by adding cationic cetyltrimethyl ammonium bromidethey (CTAB) to systems stabilized by negatively charged silica particles, or anionic sodium dodecyl sulphate (SDS) to systems stabilized by positively charged alumina-coated silica particles. The most stable emulsions were found to present a gel-like consistency, with particle floccule adsorbed at the oil-water interface. Meanwhile, the initial phase in which particles were added played an important role in this interaction. The synergistic effect [33] of hydrophilic silica nanoparticles and lecithin/oleylamine in improving emulsification and stability to coalescence was evident only when silica nanoparticles were initially added to the oil phase. When nanoparticles were added from the water phase, no synergistic effect existed due to electrostatic bridging or unfavourable attachment like hydration forces and repulsive electrostatic forces. In another study [34] involving addition of SDS solutions to the formed Pickering o/w emulsions stabilized by partially hydrophobic silica particles, it was found that surfactant above the critical micelle concentration induced rapid creaming and flocculation. However, the investigations of Pickering emulsions stabilized by the mixture of surfactants and particles have been mainly focused on inorganic particles, and there are no relevant reports on the Pickering emulsion of α-CD.

Despite the recent efforts made in using CD-based assembly as promising candidates for surfactant-free emulsion stabilization, no connection between the influences of traditional surfactant on the stability of the emulsions has been established so far. In this paper, o/w Pickering emulsions stabilized by the self-assembled microcrystals of α-CD and MCT are prepared and the interfacial properties of the microcrystals are verified. Then, the effects of adding surfactants on the stability of Pickering emulsion are investigated using optical morphology characterization, drop size analysis, zeta potential, viscosity contact angle and interfacial tension measurement, X-ray diffraction analysis and long term stability.

2 Experimental

2.1 Materials

Alpha-cyclodextrin (α-CD, 100.1%) was provided from Wacker-Chemie (Germany). Medium chain triglycerides (MCT, 99.8%) were purchased from Avic Pharmaceutical Co., Ltd (Tieling, China). Soybean lecithin (PL, 96.8%) was provided by Lipoid GmbH Co., Ltd (Ludwigshafen, Germany), Tween 80, Tween 60, Tween 20 were provided by Hanhua Medicinal Excipients Co., Ltd. (Sichuan, China), sucrose fatty acid ester (SFE S270, S1670) was kindly donated by Shineroad Industrial Development Co., Ltd (Shanghai, China), hydrogenated castor oil (EL 35), caprylic/capric macrogolglycerides (Labrasol®) were provided by Yunhong Pharmaceutical Excipients Co., Ltd.(Shanghai, China), poloxamer 188 was provided by Changwei Medicinal Excipients Co., Ltd. (Shanghai, China), sodium alginate sodium dodecyl sulphate (SDS), cetyltrimethyl ammonium bromidethey (CTAB), sodium glycocholate, and alginate sodium were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). They were used as the surfactant components. Methanol, n-hexane and isopropanol (HPLC grade)

were purchased from Fisher Co., Ltd. (America). Water was purified by passing through a reverse osmosis unit and then a Milli-Q® reagent water system.

2.2 Methods

2.2.1 Preparation and characterization of the CD emulsions

2.2.1.1 Preparation of the Pickering emulsion with α-CD and MCT

The α-CD was dissolved in ultrapure water at 70 °C, and then the solution was cooled down to 25 °C as the continuous phase to prepare the emulsion. The Pickering emulsions were prepared by adding MCT to the continuous phase and the mixture was emulsified at 20,000 rpm for 5 min using a T25 high shear mixer (IKA Co., Ltd., Germany) at the temperature of 25 °C controlled by a water bath. In this study, F_{CD}-1 was chosen as an unstable emulsion to investigate any synergistic effect of Tween 80 and F_{CD}-2 as the stable emulsion to investigate the effect of PL. The detailed amounts of the formulations are listed in Table 1.

2.2.1.2 Characterization of the Pickering emulsion stabilized by α-CD/MCT microcrystals

Microstructure characterization. The morphology of the emulsions was examined using a Nikon Eclipse TS-100F inverted microscope (Nikon Co., Ltd., Japan) with phase contrast objectives and images were captured with a digital camera (Japan) using NIS Element software. The emulsions were diluted 8 times with ultrapure water and 10 μL was placed on a glass slide with cover glass for microscopic observation.

Creaming stability assessment. The creaming stability of the emulsions was considered as the most important characteristics to evaluate the stabilities of the emulsions. In this study, the emulsions were monitored immediately after their preparation by pouring the samples into glass cylinders with stoppers (1.3 cm diameter, 15 cm high). As the emulsions may separate into a "cream layer" (H_c) and a "continuous phase (serum layer)", the residual emulsion (E_r) was calculated as:

$$E_r = \frac{H_c}{H_e} \times 100\% \quad (1)$$

where H_c is the height of cream layer and H_e is the height of total emulsion.

Viscosity measurements. The viscosities of the prepared emulsions were measured by rheometer (BROOKFIELD, LVDV-III) at room temperature. The rheometer was equilibrated for 10 min and then calibrated before measurement. The rotor and the revolving speed were selected according to the viscosity of samples. The viscosity of F_{CD}-2 was measured using the rotor of $63^{\#}$ at 50 rpm. The emulsions containing Tween-80 with concentrations of 0.5%, 1.0% and 2.0% were determined using the rotor of $64^{\#}$ at 50 rpm. For other samples, the viscosities were measured using the rotor of $18^{\#}$ at 100 rpm.

Droplet size and zeta potential measurements. Droplet sizes of the emulsions were determined by the MS 3000 laser particle size analyser (Malvern Instruments Co., Ltd., Worcestershire, UK) after two days of their preparation. Meanwhile, the emulsions without any surfactants (F_{CD}-1 and F_{CD}-2) were centrifuged using a Kokusan-Superior centrifugal separator (Kokusan Co., Ltd., Japan) at 10,000 rpm for 10 min, and droplet size distributions of the supernatant creaming were then analysed. Zeta potential was measured by the Nano-ZS particle analyser (Malvern Instruments Co., Ltd., Worcestershire, UK) after diluting the prepared emulsions 25 times.

X-ray diffraction analysis. The α-CD/MCT microcrystals were prepared by adding 0.02 g of MCT to 9.38 mL of ultrapure water containing 0.60 g of α-CD. The mixture was continuously sonicated (40 KHz, Branson 3510 E-MTH, America) for 15 min. Then the mixture was emulsified at 20,000 rpm for 5 min. The α-CD/MCT microcrystals were obtained by centrifuging the emulsion at 10,000 rpm for 10 min. The obtained precipitate was washed by ultrapure water for three times and then dried at 40 °C under vacuum for 12 h. The α-CD/PL particles were prepared the same way except that MCT was replaced by PL.

The particles were examined by X-ray diffraction analysis using a D8 Advance (Bruker, Germany) with a locked coupled scan type. The scan speed was 0.1 s/step and the increment was 0.02°. The running condition was 40 kV, 40 mA, and the scan range was 3°-40°.

Interfacial tension measurements. The interfacial tension was measured by JK99B/C automatic tension apparatus (Shanghai Zhongchen Digital Technic Apparatus Co., Ltd, China) with platinum loop at 25℃. The α-CD solutions of 5.0% and 6.0% (w/w) were used as aqueous phases for F_{CD}-1 and F_{CD}-2, respectively. MCT was used as the oil phase. The tension apparatus was calibrated before each measurement. Before measuring, the platinum loop was firstly immersed into the aqueous solution, and the oil phase was added into the aqueous phase drop by drop. The interfacial tension was recorded when the platinum loop was pulled out from the aqueous phase to the oil phase. For samples with surfactants, the interfacial tension was determined by adding Tween 80 to the aqueous phase and adding PL to the oil phase.

Contact angle measurement. The development of a reliable method for determining the contact angle, θ, of small particles attached to liquid-liquid interface has been a long-standing challenge over the past few decades. The method of Washburn-Ridea [35], relying on measuring the liquid penetration rate in a compressed powder tablet, was chosen in our study.

The α-CD/MCT microcrystals of 80 mg used for X-ray diffraction analysis were carefully compressed into a circular disk to form relatively smooth particle layers with 1.0 cm diameter and 3.0 mm thickness. The disk was then placed at the bottom of an open, transparent glass vessel with internal area of 4.0 cm2. The contact angle was measured as follows: 4 μL of pure water was carefully placed on the particle surface with a syringe. 1.5 mL of MCT was then carefully poured on the particle. The water drop was photographed and the contact angle was obtained from the equilibrated shape treated with the software of Image Pro Analyse 3D. When the influences of surfactants were investigated, Tween 80 was added in the aqueous phase and the PL was added in MCT.

2.2.2 Preparation and Characterization of surfactants related emulsions

Tween 80 related Pickering emulsions. Tween 80 (0.05%-2.0%, w/w) was added to α-CD solution and sonicated for 3 min. Then it was kept at the temperature of 25 °C to be used as the continuous phase. The Tween 80 related Pickering emulsion was prepared the same way as F_{CD}-1. The mass fraction of MCT was also kept constant as 3.0%, and α-CD was kept at 5.0%.

PL related Pickering emulsions. The PL related Pickering emulsion was prepared the same way as F_{CD}-2 except that the PL (0.1%-5.0%, w/w) was added to MCT. In this case, the mass fraction of the oil phase was kept at 20% and α-CD was at 6.0%.

The mass of all components, used in the sample preparation stages described above, are listed in Table 1. The concentrations (%) of all materials listed in the table were calculated as weight per weight percentage (%, w/w).

Table 1 The formulation of the Pickering emulsion of α-CD and surfactant concentrations used in the surfactant and mixed-emulsifier stabilized emulsions.

The mass fraction (%) of the materials used in emulsions		
Pickering emulsion stabilized by α-CD/MCT microcrystals		
Formulation	α-CD (%)	MCT (%)
F_{CD}-1	5.0	3.0
F_{CD}-2	6.0	20
CD emulsions prepared in the presence of surfactants		
Tween 80 (%)		PL (%)
0.05		0.1
0.1		0.2
0.5		0.5
1.0		1.0
2.0		3.0
/		5.0

The surfactants related emulsions and Pickering emulsions were characterized by the same methods as the CD emulsions, including microstructure characterization, creaming stability, viscosity, droplet size, zeta potential, interfacial tension and contact angle measurements.

3 Results and discussion

3.1 Self-assembly of α-CD and oil complexes at the oil-water interface

Recently, Mathapa et al.'s investigated the self-assembly of CD molecules at the o/w interface with tetradecane through formation of inclusion complexes [27-29]. They observed in real time the self-assembly and threading of CDs along a single tetradecane molecule at the oil-water interface by monitoring the intensity of light transmitted through the oil droplet in aqueous solution onto a CCD camera. These inclusion complexes were found to form microcrystals, which could decrease the o/w interfacial tension. Compared with β-CD microcrystals, the formation of α-CD microcrystals was faster and its ability to reduce the interfacial tension was stronger. The stability of these microrods at the oil-water interface was possibly enhanced by a strong hydrogen bonding network from neighbouring microrods. They discovered that the morphology and the size of the microcrystals were dependent on the type of CD and oil used. Lamella sheets and long microrods were obtained from α-CD molecules and tetradecane. In contrast, β-CD-tetradecane gave short microrods. The self-assembly at the oil-water interface around the oil droplet represented a different mechanism for stabilization of o/w emulsions, which started with molecular adsorption of CD at the oil-water interface but ended up as a Pickering emulsion due to the retention of the formed microcrystals at the interface.

Similar results were obtained in our study. A white film was evident when mixing the oil and continuous phases even without any shaking and a stable emulsion was obtained. It was shown that the α-CD/oil complexes self-assembled at the oil-water interface and α-CD/MCT microcrystals were formed. The solid microcrystals were able to be separated after centrifuging the emulsions at 10,000 rpm for 10 min and collecting the precipitates. The crystallinity of the microcrystals was characterized by powder X-ray diffraction patterns. As shown in Fig. 1, the microcrystals obtained from α-CD/MCT/water systems were different from that of the natural α-CD, which exhibited partial crystallinity of α-CD. This result strongly confirmed the formation of α-CD/MCT microcrystals of a solid state form different from α-CD and MCT. It was also shown that the microcrystals consisted of crystalline and amorphous regions, a feature which is consistent with Mathapa et al.'s findings [27]. These authors observed that α-CD/tetradecane microcrystals showed some birefringence when observed under the cross-polarized light optical microscopy and most crystals exhibited Janus characteristics with one shiny end of the rods while the other end was less shiny or not shiny at all.

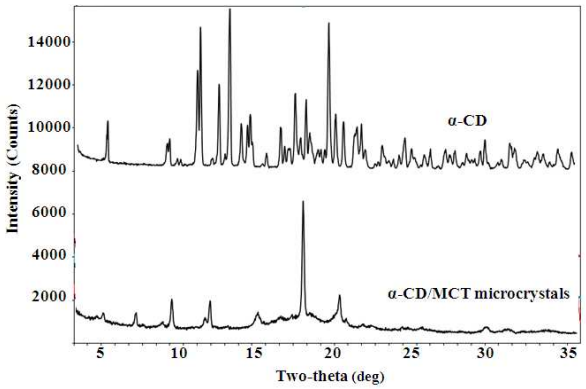

Fig. 1 Powder X-ray diffraction patterns of α-CD/MCT microcrystals and α-CD

3.2 Pickering emulsions stabilized by α-CD/MCT microcrystals

For emulsions with different oil volume fractions (F_{CD}-1 with small and F_{CD}-2 with large oil volume fractions), different stabilities were found. The emulsion stability decreased with the reduced oil volume fraction, while the formation of α-CD/MCT microcrystals in the sample bottom layer increased. The micrographs of the CD emulsion are shown in Fig. 2. It can be seen that when the emulsion was prepared with 5.0% α-CD, 3.0%

MCT and 92% water, the droplets were of highly nonspherical shapes. This phenomenon has been reported as "interfacial jamming" [36], causing a loss of interfacial mobility and making it possible to arrest interfacial tension-driven morphological coarsening. When the percentage of MCT increased to 20%, the interfacial mobility ameliorated. Thus the droplets had a good spherical shape with smooth surfaces (Fig. 2).

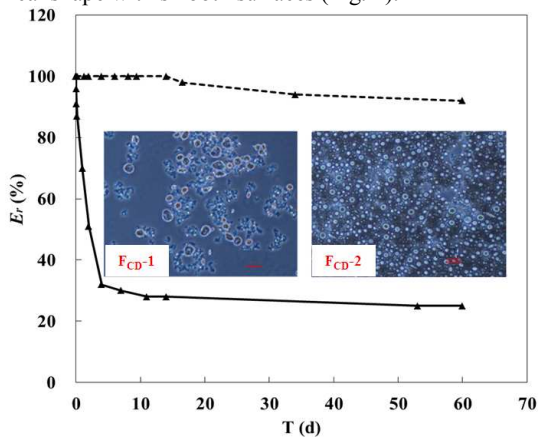

Fig. 2 The creaming stability of Pickering emulsions stabilized by α-CD/MCT microcrystals (solid line: F_{CD}-1; dotted line: F_{CD}-2) and their micrographs (the scale bar was 50 μm)

The creaming stability of CD emulsions against coalescence was evaluated by monitoring the height of the emulsion layer with E_r. It was obvious that the stability of F_{CD}-1 was poor with the emulsions separating into two layers within 1 h and the E_r decreasing quickly to 32% on the fourth day. While, F_{CD}-2 was relatively stable, E_r of which was larger than 90% after two months (Fig. 2). These results highlight the importance of the oil content on the emulsions stability. However, the separated layers of both samples formed creaming emulsions, namely, the emulsion destabilization occurred through coagulation and emulsions never fully separated into oil and water phases. All the emulsions described here did typically release a clear continuous phase as the drops creamed, indicating that the particles were either adsorbed onto the drop surfaces or had become part of a three-dimensional interconnected network of drops and particles as reported in the literature [20].

The stability of Pickering emulsions are also usually characterized by the viscosity, particle size distribution of the emulsions, the interfacial behaviour and wettability of the solid particles. According to the Stokes equation [37] (Eq. 2), the extent of phase separation of emulsions is directly proportional to the droplet size, the viscosity of the medium and the difference in density of the two immiscible phases.

$$v = \frac{d^2 \cdot (\rho_s - \rho_m) \cdot g}{18\eta_0} \qquad (2)$$

where v is the creaming rate, d is the droplet size, ρ_s and ρ_m are the density of the dispersible and continuous phases, respectively, g is the acceleration of gravity, and η_0 is the viscosity of the continuous phase. According to Eq. 2, the increases in viscosity of the medium and the decreases in droplet size would enhance the stability of emulsions. The viscosity of F_{CD}-1 was 3.6 cP. The viscosity of F_{CD}-2 was 290.3 cP, which was much higher than that of F_{CD}-1 (about 81 times). These data verified the results in Fig. 2 that the stability of F_{CD}-2 with higher viscosity was much better than that of F_{CD}-1 with relative lower viscosity, indicating the important role of viscosity that played in the stability of the emulsions.

The size distributions of droplets and particles can be studied after the centrifugation of the emulsions. To understand their composition, the emulsions were centrifuged at 10,000 rpm for 10 min, with three layers appearing: upper creamed layer, middle aqueous layer and bottom precipitated layer. The droplet size distributions of the upper creaming and full emulsions were analysed. The average size values ($D_{v,90}$) of F_{CD}-1 and F_{CD}-2 were 18.6 μm and 22.0 μm, respectively. Two size populations were observed in the size distributions of the full emulsion (Fig. 3), with the peak of the large size population approximately 12 μm and small size population around 3 μm. However, for the upper creaming, a single population peak for F_{CD}-1 ($D_{v,90}$ = 19.0 μm) and F_{CD}-2 ($D_{v,90}$ = 27.7 μm) was observed, which overlapped with the large size population of the full emulsion. According to Frelichowska et al.'s study [38], the Pickering emulsion stabilized by micro- or nano-particles consisted of o/w droplets and excess solid microparticles. When centrifuged, the upper creamed layer contained the o/w droplets and the sediment contained excess microparticles. Thus the smaller droplet size population disappeared after centrifugation. Non-adsorbed excess microparticles were available to stabilize the emulsion dispersed in the aqueous and constructed the networks surrounding the droplets. Therefore, the large size population of the full emulsion was identical to the droplet size distribution of the upper creamed layer collected after centrifugation.

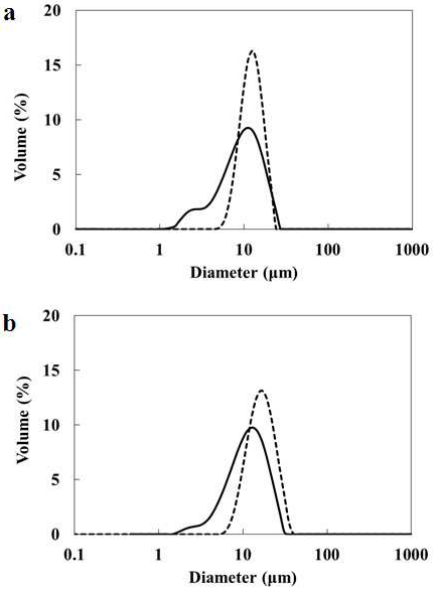

Fig. 3 The size distributions of the full Pickering emulsions (solid line) and their supernatant creaming (dotted line) after centrifuge at 10,000 rpm for 10 min (a: F_{CD}-1; b: F_{CD}-2)

To study the surface-activity of α-CD/MCT microcrystals, the interfacial tension of MCT/α-CD solution was measured. When MCT was dropped into α-CD solution, there appeared a precipitated layer of the complex at the oil-water interface. The interfacial tensions of F_{CD}-1 and F_{CD}-2 were 29.62 mN/m and 23.09 mN/m, close to the results reported in literature (about 35

mN/m for tetradecane-CD interface) [24]. They decreased with the increasing concentration of α-CD, indicating that the precipitated complex had surface activity, which was consist with the reports [21-22].

The wettability of the solid particles by a liquid can be characterized by the three-phase contact angle θ. When θ is smaller than 90°, emulsions are favoured to be of the o/w type. Conversely, particles of θ larger than 90° favours w/o emulsions. When θ is close to 90°, the particles in the systems are well-suited for the stabilization of o/w emulsions and optimum stability of Pickering emulsions can be obtained.

The θ of α-CD/MCT microcrystals was determined by a modified powder tablet method using triplicate measurements. The average θ for the α-CD/MCT microcrystals was 46.1±3.4°, which was consistent with Inoue et al.'s results [25]. They reported that all the native CDs (α, β and γ) formed inclusion complexes with n-alkanes that showed contact angles of the β-CD complexes below 90°. While the θ of α-CD complexes was much lower than that of β-CD complexes. These results showed that the CD inclusion complex particles were hydrophilic and would stabilize o/w emulsions.

The zeta potential for the droplets was also determined. The droplets of F_{CD}-1 and F_{CD}-2 were negatively charged with the zeta potential of -24.8 mV and -22.5 mV, respectively. The electrostatic repulsive forces coming from the negative charge also contributes to the stability of the emulsions but there is no obvious difference between the two formula.

3.3 Emulsions prepared in the presence of α-CD/MCT microcrystals and Tween 80

The microstructures of emulsions prepared in the presence of α-CD/MCT microcrystals and Tween 80 at the concentrations of 0.05%-0.1% were similar to those of F_{CD}-1, with highly nonspherical droplets. Fig. 4 showed the residual volume ratio of α-CD/MCT emulsions as a function of Tween 80 with time elapsed. Adding Tween 80 to the continuous phase improves the creaming stability of the CD emulsion, although emulsions prepared with Tween 80 concentrations of 0.05% or 0.1% exhibited a degree of phase separation. The higher the concentration of Tween 80, the more stable the emulsion. For the sample with the lowest Tween 80 concentration of 0.05%, the equilibrium E_r improved from 28% to 44%. For concentrations of Tween 80 of 0.5% or above, the emulsion systems were stable against phase separation with E_r maintained at 100%. Whereas, when Tween 80 was used alone (0.05%-2.0%), a few emulsion droplets formed and the system separated into two phases within few minutes (data not shown).

In summary, the stabilities of α-CD/MCT microcrystals or Tween 80 stabilized emulsions were very poor, creaming within minutes or hours, whilst, the stability of emulsions prepared by α-CD/MCT microcrystals together with Tween 80 in appropriate concentrations was improved. These findings support the proposed synergistic effect of α-CD/MCT microcrystals and Tween 80 in the formation and stabilization of the Pickering emulsion. There is evidence of a threshold concentrations (≥0.5%) required to synergistically stabilize the emulsions. Above the synergistic threshold, the emulsions exhibited little creaming on storage.

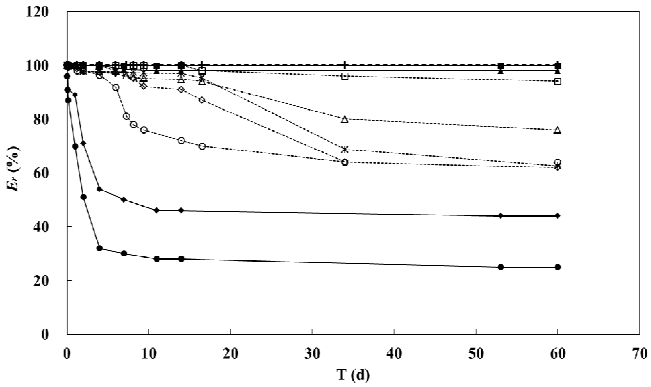

Fig. 4 The creaming stability of CD emulsions with surfactants of different concentrations (solid line: series of Tween 80 related Pickering emulsion, • : 0% (F_{CD}-1); ◆ : 0.05%; ▲ : 0.1%; ■ : 0.5%, 1.0% and 2.0%; dotted line: series of PL related Pickering emulsion, □ : 0% (F_{CD}-2); ○ : 0.1%; ◇ : 0.2%; * : 0.5%; △ : 1.0%; + : 3.0% and 5.0%)

More importantly, the additional Tween 80 increased the viscosity of the emulsions greatly, from 3.6 to 695.9 cP (data shown in Fig. 5). When the concentrations of Tween 80 between 0.0-0.1%, the viscosities of the emulsions increased from 3.6 to 10.2 cp. While for emulsions with the concentrations of Tween 80 of 0.5% or above, the viscosities of the emulsions jumped dramatically to 180.0-695.9 cp and the systems possessed a gel-like consistency. The viscosity increase was mainly caused by the interaction and assembly of Tween 80 with α-CD in the continuous phase, which was reported by Zhou et al. in the study of the self-assembly of nonionic surfactant Tween 20 and β-CD inclusion complexes in diluted solution [39]. They found that the assembly of Tween 80 and CD could be formed into nonamphiphilic vesicles and infinite two-dimensional bilayer driven by hydrogen bonding in diluted solution. the bilayer membranes bend into vesicles and the 2:1 inclusion complex was verified to be the building block in these vesicles. The increasing viscoelastic structure of the emulsion is likely to reinforce the mutually acting forces between droplets, and the increase of viscoelasticity will retard the free motion of emulsion droplets and excess particles, thereby improving the creaming stability of the emulsions. This may be the dominating contribution to the synergistic effect of Tween 80 on the stability of the CD emulsions.

The volume weighted size distributions of emulsion droplets prepared by α-CD, MCT under different Tween 80 concentrations are shown in Fig. 5. The CD emulsions with low concentrations of Tween 80 (≤0.1%) exhibited similar size distribution characterization when compared with F_{CD}-1, the drop sizes of which were relatively poly-dispersed with two size populations. When the concentrations of Tween 80 were lower than 0.1%, the volume of the lager sized population was approximately five times of that for the small population. The smaller size population is clearly distinguished from the larger size population. For example, the average diameter ($D_{v,90}$) of the emulsion with 0.05% Tween 80 was 19.2 μm, with two populations of 2.0 μm (16%) and 11.2 μm (84%). For emulsions with Tween 80 at higher concentrations (≥0.5%), the two populations described above partially merged, with the percentage of the larger size population decreasing and that of the small size population increasing dramatically. For example, the average diameter ($D_{v,90}$) of the emulsion with Tween 80 of 2.0% was 13.6 μm, with two populations of 2.8 μm (35%) and 6.7 μm (65%).

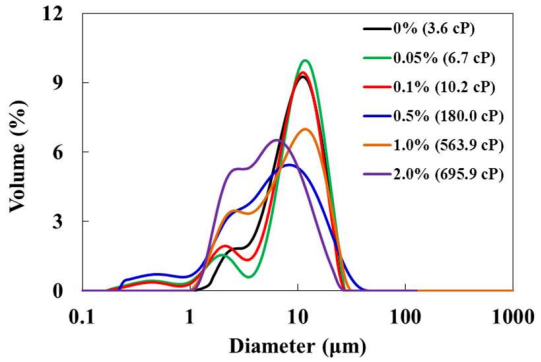

Fig. 5 The size distributions and viscosities of Tween 80 related Pickering emulsions (the $D_{v,90}$ values of these emulsions were 18.6 μm (0%), 19.2 μm (0.05%), 18.0 μm (0.1%), 18.0 μm (0.5%), 18.8 μm (1.0%), and 13.6 μm (2.0%); data in the parentheses represent the corresponding viscosities)

As demonstrated by Fig. 3 in section 3.2, the large size population relates to the emulsion drop and the small size population to the excess α-CD/MCT microcrystals. From Fig. 5, it can be seen that above the threshold concentration of Tween 80, the final size of the emulsion drops decreased a little and that of the excess particles remained almost unchanged. Interestingly, the relative percentages changed, with the percentage of emulsion drops decreasing and that of the excess particles increasing. Results also show that the addition of Tween 80 enhanced the percentages of α-CD/MCT microcrystals and the flocculation of the continuous phase, which is consistent with the improved stability illustrated in Fig. 4. This effect is attributed either to the particles absorbed on the emulsion drops being replaced by the surfactant molecules and moved into the continuous phase, or the surfactant molecules covering the oil-water interface faster, thus preventing the particles entering the oil-water interface in the first place [4].

In addition, the stability of emulsions is greatly influenced by the interfacial tension and wettability of the solid particles. Thus, the effects of Tween 80 on the interfacial tension and the contact angle of the adsorbed α-CD/MCT microcrystals have also been investigated. As shown in table 2, with the concentrations of Tween 80 increased, the interfacial tension decreased from 29.62 to 16.47 mN/m and average θ values decreased from 46.1±3.4° to 21.7±2.5°. When the concentration of Tween 80 increased to 2.0%, the continuous phase became viscous and the value of the interfacial tension could not be given. Similarly, the interfacial tension and average θ decreased with the increasing concentration of Tween 80. Meanwhile, the average θ and the interfacial tension decreased more above the threshold concentration of Tween 80 (0.5%). According to the attachment energy equation [30], the attachment energy reduces with the θ and the interfacial tension decreases, as the size of the emulsion droplets remained unchanged in our study. Therefore, the α-CD/MCT microcrystals are likely to move into the continuous phase thereby causing the increased flocculation of the continuous phase. Furthermore, adding Tween 80 also brought about a lower zeta potential on the surface of the emulsion droplets, decreasing from -32.2 mV to -40.7 mV, compared with -24.8 mV for F_{CD}-1, a factor which may also contribute to the observed improved stability of the emulsions.

Table 2 The average θ and interfacial tension measured in different concentrations of Tween 80

Tween 80 (%)	0.05	0.1	0.5	1.0	2.0
Interfacial tension (mN/m)	31.17	28.62	19.83	16.47	--
Contact angle θ (°)	39.0±3.5	36.5± 2.6	37.0± 4.9	23.9± 2.7	21.7± 2.5

It was indicated from the results described above that the ability of Tween 80 to enhance the stability of CD emulsions was mainly attributed to: i) to decrease the interfacial tension through the interaction of Tween 80 with CD at the o/w interface at lower concentrations, to increase the viscosity of the continuous phase through the assembly with CD in the continuous phase at higher concentrations; ii) For the concentrations above the threshold, the flocculation enhancement caused by the particles adsorbed on the emulsion drops being replaced by the surfactant molecules and moving into the continuous phase also facilitated the stability. These may be the important synergistic mechanism in forming of the emulsions prepared with α-CD/MCT microcrystals and Tween 80. In fact, the benefit is obvious especially since surfactants such as Tween 80 are unable to provide stable emulsions alone.

3.4 Emulsions prepared in the presence of α-CD/MCT microcrystals and PL

In contrast to Tween 80, there were antagonistic interactions between PL and α-CD/MCT microcrystals at certain concentrations ranges. Due to the antagonistic effect of PL on the emulsion, CD emulsions (F_{CD}-2) different from F_{CD}-1 were prepared and chosen to analyse the antagonistic mechanism. It is well documented that long-term stable o/w emulsions can be prepared with lecithin (>0.5%) and oil phase (10%-30%), an approach which has been widely used as drug/cosmetic delivery vehicles. The emulsions with 20% as the oil phase, and a range of 0.1%-5.0% PL as surfactant were selected.

As shown in Fig. 4, the addition of PL (0.1%-1.0%) impaired the stability of the CD emulsions in comparison with F_{CD}-2. Surprisingly, their stability exhibited an improving tendency. The PL related emulsions suffered from phase separation in about 5 h after their preparation. Their equilibrium E_r values (about 60% for PL (0.1%-0.5%) related Pickering emulsions and 75% for PL (1.0%) related Pickering emulsion) were all below F_{CD}-2 (94%). In the process of their phase separation, the higher the concentration of PL (0.1%-1.0%), the slower the sedimentation rate of PL related Pickering emulsions. At high concentrations (3.0%-5.0%), the emulsions were stable against any coalescence and phase separation. When PL (0.1%-0.5%) was used alone, the emulsions were of poor stability. At PL concentrations lower than 0.5%, the phase separation occurred quickly wherein the E_r value of the emulsion with 0.1% PL was down to 30% within 2 h. Subsequently, an oil layer appeared in the systems on the seventh day. The emulsions eventually separated almost totally into continuous phase and oil phase within 2 months, which was completely different from the emulsions adding Tween 80. At the PL concentrations of 0.5%-5.0%, the emulsions will not suffer from coalescence within 2

months but there remains gravitational separation with the droplets floating upwards and the turbid lower liquid forming (data not shown).

The PL affected the creaming stability of Pickering emulsion depending upon its concentrations, with inhibition at low concentrations and enhancement at high concentrations. Regardless of the host-guest interaction between α-CD and PL, the potential influences of PL on the stability of CD emulsions might have been shown to change particle contact angle, influence the interfacial tension, and effect the flocculation of the solid particles in the continuous phase. However, the host-guest interactions between PL and α-CD have been reported previously [40, 41]. It was reported that PL could partially enter into the α-CD cavity [40], forming the partial inclusion complex, an event which was also confirmed by the powder X-ray diffraction patterns in Fig. 6 in our study. Thus, the additional PL is likely to competitively combine with α-CD, thereby impairing the inclusion complex formation between MCT and α-CD and the stability of the CD emulsions. In order to give experimental evidence of this inhibition effect, the quantities of the microcrystals formed at different concentrations of α-CD in the presence of excess amount of PL was determined and compared with that of α-CD/MCT (data not shown). As a result, there is potential for PL to combine with more α-CD compared with the same mass of MCT. The adding of 0.2% PL into 6.0% α-CD aqueous solution produced 20.3 mg/mL of precipitation, leaving the concentration of α-CD as 40.1 mg/mL. For MCT, the amount of precipitation was just 3.3 mg/mL and the concentration of α-CD was 57.0 mg/mL. When excessive PL and MCT were added to a certain concentration of α-CD aqueous solution, there was also more precipitation in the PL systems.

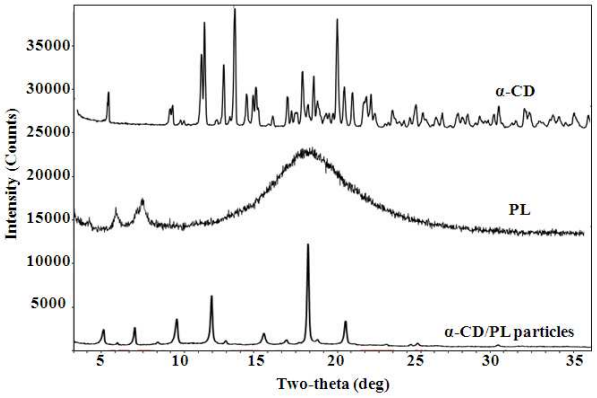

Fig. 6 Powder X-ray diffraction patterns of α-CD/PL microcrystals

When viscosities of PL related emulsions were measured, the surprising results exhibited a sharp decrease from 290.3 to 5.7 cP (data shown in Fig. 7). With the concentration of PL increased, the viscosities of the emulsions fluctuated around 10.0 cp. This kind of decrease might be the main factor to the instability of emulsions, though the viscosity of emulsions was not strictly dependent on the concentrations of PL. The decreasing viscosity suggested that the formed α-CD/MCT microcrystals might be replaced by α-CD/PL microcrystals, which could hardly stabilize the emulsions indicated by its contact angle of 0° (data not shown).

The volume weighted size distributions of emulsion droplets prepared by α-CD, MCT under different PL concentrations are shown in Fig. 7. The CD emulsions with low PL concentrations (≤0.5%) exhibited similar size distribution characterization with F_{CD}-2, the drop sizes of which are relatively poly-dispersed with two size populations (the 3 μm population and the 12 μm population). Similarly with that of Tween 80, the latter population had the larger percentage, while the small size population separated more clearly with the large size population. For emulsions with high PL concentrations (>0.5%), the two populations described above merged partially, with the percentage of the larger size population decreasing and that of the small size population increasing dramatically. Simultaneously, the smaller size population overlapped with the large size population.

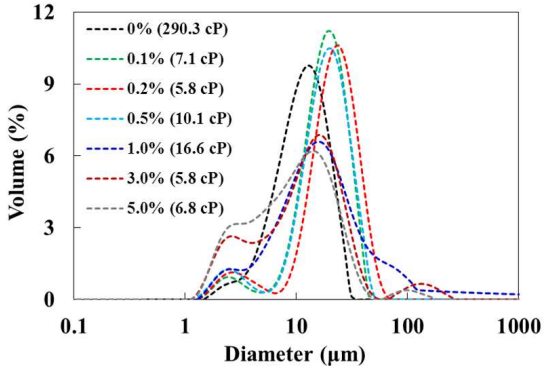

Fig. 7 The size distributions and viscosities of PL related Pickering emulsions (the $D_{v,90}$ values of these emulsions were 22.0 μm (0%), 32.5 μm (0.1%), 40.2 μm (0.2%), 34.0 μm (0.5%), 34.2 μm (1.0%), 31.4 μm (3.0%), and 26.1 μm (5.0%); data in the parentheses represent the corresponding viscosities)

With additional PL, the interfacial tension dropped greatly from 23.09 to less than 2.00 mN/m (1.99 mN/m for 0.1% PL and 1.88 mN/m for 1.0% PL) or even lower. When the oil phase containing PL dropped into the α-CD solution, the white precipitate formed immediately at the o/w interface and its amount suffered from a sharp increase along with the increasing concentration of PL. For the samples of high PL concentrations, precipitate would fall from the o/w interface down to the aqueous phase, leaving the interfacial tension too low to be determined. This was confirmed by the phenomenon that the PL related emulsions could be formed spontaneously before emulsifying by the mixer. This might be the main reason for the better stability of the PL related emulsions with high PL concentrations (3.0% and 5.0%).

Similar with the emulsions composed of Tween 80, the contact angle was smaller than that of F_{CD}-2 when PL was added. At PL concentration of 5.0%, the pressed α-CD/MCT microcrystals tablet was damaged and the contact angle could not be measured. The values of zeta potential increased from -16.5 mV to 1.42 mV, compared with -22.5 mV of F_{CD}-2.

Table 3 The average θ measured in different concentrations of PL

PL (%)	0.1	0.2	0.5	1.0	3.0
Contact angle θ (°)	14.6±4.5	17.4±4.9	41.7±1.7	37.2±1.7	35.3±2.2

In summary, the α-CD/MCT microcrystals stabilized

Pickering emulsions with 20% oil (F_{CD}-2) were stable (E_r > 90% after two months), while the low concentrations of PL stabilized emulsions (0.1%-0.5%) were unstable, creaming within minutes or hours. After adding PL at the concentration ≤ 1.0%, although the interfacial tension decreased, the stability of CD emulsions decreased due to the competitive host-guest interactions between α-CD/PL, which resulted in the dramatically decrease of the viscosity of the continuous phase. The stronger interactions of PL with α-CD resulted in the replacement of the formation of α-CD/MCT microcrystals by α-CD/PL particles. The decreased stability for the CD emulsions at PL concentration ≤ 1.0% indicated that the ability to stabilize the emulsions of α-CD/PL particles might be weaker than that of α-CD/MCT microcrystals. Finally, an inhibitory effect of PL on the stabilization of the CD emulsion was observed. However, when the concentration of PL ≥3.0%, the stabilities of emulsions prepared with PL alone and PL related Pickering emulsions were both good, which might be due to the sufficient surfactant ability to stabilize the oil droplets provided by PL. And for the CD emulsions in the presence of high concentrations of PL, the good stability was also attributed to the particles absorbed on the emulsion drops being replaced by PL molecules and moved into the continuous phase. Namely, the flocculation of the continuous phase by particles also contributed to the final increase of the emulsion stability.

3.5 Two-way effects of Tween 80 and PL on the stability of CD emulsions

In general, the stability of Pickering emulsions is mainly influenced by the particle size distributions of the droplets and their dispersion in the continuous phase. The adsorbed layer of solid particles forms a rigid coating around the liquid droplets that could be compared to an egg shell. Coalescence of droplets can be prevented by this kind of mechanical barrier. The mechanical strength of the layer of particles results from aggregation of solid particles at the droplet surface. Solid particles are held together at the oil-water interface by means of attractive interactions including capillary forces, dispersion, and electrostatic forces. Besides, solid particles might also control coagulation by electrostatic repulsions between the oil droplets, steric repulsion of particles bridges and the network of flocculated particles in the continuous phase.

Therefore, the potential functions of surfactants in particle stabilized emulsion are often considered as: i) to increase the viscosity of continuous phase and promote flocculation of the solid particles in the continuous phase; ii) to reduce the interfacial tension; and iii) to change particle contact angle. And in our study, the former two were the main factors.

As described above, the effects of PL on the droplet size distribution and the contact angle were similar with that of Tween 80. Above the threshold concentration of the surfactants, the two size populations overlapped and their relative percentages changed, with the smaller one increased and the larger one decreased. The θ values decreased after adding the surfactants. However, the effects of PL and Tween 80 on the interfacial tension and the viscosity of the CD emulsions were significantly different, which lead to their two way influences on the stability of the CD emulsions. The stability was enhanced when adding Tween 80, while the stability was firstly inhibited and then enhanced when adding PL. The stability enhancement of Tween 80 was believed to be due to the interfacial tension decrease caused by the interaction of Tween 80 with cyclodextrin at the o/w interface at lower concentrations, the viscosity increase of the continuous phase caused by the assembly of Tween 80 with CD in the continuous phase and the flocculation enhancement at higher concentrations. Whereas, PL of high concentrations resulted in an extremely low interfacial tension, which contributed to the formation of the stable emulsion. At low concentrations of PL, the inhibitory effects were attributed to the replacement of α-CD/MCT microcrystals at the o/w interface by α-CD/PL particles and the insufficient viscosity to stabilize the emulsion.

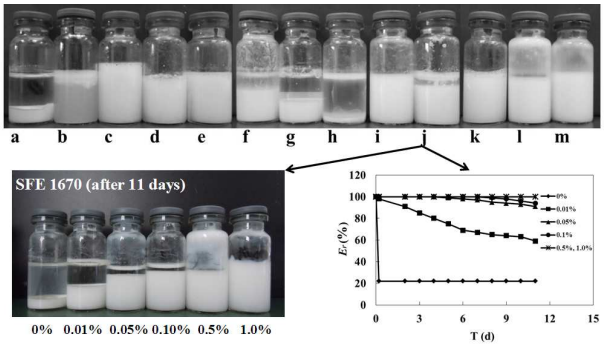

Fig. 8 The effects of different surfactants (0.5%) on the stability of CD emulsions after three days of their preparation (A: a: CD emulsions without any surfactants; b: SDS; c: CTAB; d: SFE 270; e: hydrogenated castor oil; f: caprylic/capric macrogolglycerides; g: poloxamer 188; h: sodium glycocholate; j: SFE 1670; k: alginate sodium; l: Tween 20; m: Tween 60)

The above results indicated that the changes of viscosity and interfacial tension caused by host-guest interactions played important roles on the stability of CD emulsions. In order to generalize and verify this conclusion, Tween 80 and PL were replaced with other surfactants, such as SDS, CTAB, sucrose fatty acid ester 270 (SFE 270), hydrogenated castor oil, caprylic/capric macrogolglycerides (Labrasol®), poloxamer 188, sodium glycocholate, SFE 1670, alginate sodium, Tween 20 and Tween 60, as shown in Fig. 8. As a result, two-way effects of surfactants on CD emulsions were found. For most of the surfactant, such as Tween 20, Tween 60, sucrose fatty acid ester (SFE), the synergetic effects of the surfactants on the stability of the CD emulsions were observed, similar to Tween 80. The inhibition effects similar to PL were just observed for SDS and sodium glycocholate. Therefore, two-way effects of surfactants on CD emulsions existed. However, it's better to have further studies to offer more sufficient data to elucidate the effects of these surfactants on the viscosity, the interfacial tension, contact angle and the final stability and the dependences on the surfactant concentrations.

4 Conclusions

There is no report in the literature concerning the Pickering emulsions stabilized by mixtures of CD-oil microcrystals and surfactants, and the contribution of each species to the stabilization of the oil-water interface is poorly understood. The effects of Tween 80 and PL on the long term stability of

Pickering emulsions stabilized by the self-assembled microcrystals of α-CD and MCT have been investigated in this study.

The X-ray diffraction data, consisting of crystalline and amorphous regions, confirmed the formation of α-CD/MCT microcrystals which were different from α-CD and MCT, were consistent with the reported birefringence observed under the cross-polarized light optical microscopy. Two size populations were observed in the size distributions of the full emulsion. The centrifugation separation experiments revealed that the former belonged to the emulsion droplets and the later belonged to the excess α-CD/MCT microcrystals. When adding Tween 80 and PL, similar effects on the size distribution and the θ were obtained. However, two way-effects on creaming stability were found, synergistic enhancement for Tween 80, while inhibition at low concentrations and enhancement at high concentrations for PL. The stability enhancement of Tween 80 was due to the interfacial tension decrease caused by the interaction of Tween 80 with CD at the o/w interface at lower concentrations and the viscosity increase of the continuous phase caused by the Tween 80-CD assembly in the continuous phase. Whereas, for PL at low concentrations, the replacement of α-CD/MCT by α-CD/PL particles at the o/w interface caused by the strong interactions of α-CD and PL was observed, which resulted in the inhibitory effects. Whereas, PL of high concentrations resulted in an extremely low interfacial tension, which contributed to the formation of the stable emulsion.

The results have confirmed the unique effects of surfactants to the CD emulsion formulations due to the extensive inclusion of surfactants by CD leading to the formation of the CD/surfactant particles, for which the changes of viscosity and interfacial tension caused by host-guest interactions played important roles on the stability of CD emulsions.

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Notes and references

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