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ORIGINAL ARTICLE

Flow Behavior of Oleic Acid Liposomes in Sucrose Ester Glycolipid Oil-in-Water Emulsions

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Abstract Liposomes have been used widely as carriers for active ingredients in cosmetics because of their ability to encapsulate both hydrophilic and hydrophobic compounds. In this work, fatty acid liposomes were prepared and introduced into olive oil-in-water emulsions stabilized by C_{14}–C_{18} sucrose ester mixtures at pH 8.5. Light microscopy images of the emulsions showed evidence of the coexistence of oleic acid liposomes with the emulsions. As the alkyl chain length of the sucrose ester increased, the average droplet size decreased, while the zeta potential became more negative. Further decrease in droplet size was observed when borate buffer was added to the aqueous phase. The free fatty acids in the sucrose esters and olive oil are neutralized in borate buffer; consequently, fatty acid salts were produced and served as co-surfactants. The synergistic stabilization of emulsions by the mixture of sucrose esters and fatty acid salt resulted in higher stability, smaller droplet size, and lower polydispersity. The drastic increase in negative zeta potential was possibly due to the presence of free fatty acid salts in the emulsion systems. The flow curves at steady rate displayed five distinctive regions. The polydispersity of droplets enhanced the shear thickening effect at low shear rates and shear-banding effect at middle shear rates. Formation of fatty acid salts as co-surfactants caused the viscosities of the emulsions to increase by an order of magnitude. The presence of oleic acid liposome significantly reduced the viscosities of the emulsion by half an order of magnitude; this decreased viscosity helped enhance better spreadability.

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Introduction

Liposomes are self-closed spherical structures composed of one or several bilayer membranes, encapsulating part of the aqueous medium in which they are dispersed. Fatty acid liposomes are thermodynamically stable only within a relatively narrow pH range that is close to pH 7, 8, or 9, depending on the fatty acid. A titration curve helped determine the pH region allowing formation of oleic acid liposomes (pH 8.2–10.0) [1]. For cosmetic applications, liposomes are incorporated into a lotion, gel, cream, or ointment base. Therefore, the pH of such emulsions is essential to maintaining the stability of the liposomes. On the other hand, pH and ionic strength can affect stability of the emulsions. Thus, selection of buffers is an important aspect in retaining liposomes stability as well as emulsions stability. In this work, borate buffer at pH 8.5 was chosen as the aqueous phase for the preparation of emulsions. Cosmetic creams having higher pH not only stimulate the skin to eliminate excess acids and toxins but also activate self regulation of the skin. However, excessive use of alkaline skin care products can impair the skin's outer protective layer and result in skin irritation and dryness.

In addition to long-term physical stability, rheology is another important criterion for the emulsions used in personal care applications. Flow properties determine certain important qualitative parameters of emulsions, such as consistency and spreadability. Hence, rheological measurements can be correlated to the skin feeling of cosmetic products [2]. In rheological measurements, steady state

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tests are used to examine flow properties of the emulsion [3]. The volume fraction (ϕ) of the dispersed phase and average droplet size are some of the factors that determine the flow pattern of emulsions. In the literature, more concentrated emulsions display complex flow behavior, combining characteristics of solid and liquid matter. When made to flow, the emulsions undergo a transition from a solid-like to a liquid-like state under shear. This structural evolution of the emulsion is associated with shear band development. Shear-banding phenomena involve heterogeneous flow as the formation of bands within the sample bearing different shear rates coexist. Tan et al. [4] reported that shear banding in concentrated Na-caseinate emulsions can be ascribed to the macroscopic restructuring and destruction of the emulsions.

It was demonstrated that the presence of vesicles in emulsions modifies their rheological properties [5, 6]. The emulsions were stabilized by the surfactants used to form liposomes. In other words, the vesicles were generated in situ during preparation of the emulsions. Unlike previous works, the oleic acid liposomes were mixed with the base emulsions in this study. The emulsions containing oleic acid liposomes were then compared to control emulsions without liposomes in order to investigate the effect of the presence of liposomes on the flow properties of the emulsions. Although emulsion systems containing liposomes in previous work exhibited an increase in viscosity, mixing of oleic acid liposomes with the emulsions had a negative (decreasing) effect on viscosity. Therefore, different methods of preparation and the different raw materials to form liposomes had different effects on the rheological properties of the emulsions.

Materials and Methods

Materials

Deionized water (18.2 $M\Omega cm^{-1}$) from a Barnstead Diamond Nanopure Water purification system (Dubuque, IA) was used for emulsion preparation. Cosmetic grade sucrose myristate (Surfhope® SE Cosme C-1416), sucrose palmitate (Surfhope® SE Cosme C-1616), and sucrose stearate (Surfhope® SE Cosme C-1816) were purchased from Mitsubishi-Kasei Food Corporation (Tokyo, Japan). Extra virgin olive oil (Laleli, Taylieli olive and olive oil establishment, Istanbul, Turkey) of commercial grade with minimum acidity 0.8 %, density 0.9091 g/ml, and viscosity 9.562 m Pa.s at 30 °C was used as received. Oleic acid in FCC food grade was from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide (98 %) in pellet form and concentrated hydrochloric acid (37 %) were obtained from HmbG Chemicals (Hamburg, Germany). Sodium phosphate monobasic dehydrate ($NaH_2PO_4{\cdot}2H_2O$) and sodium phosphate dibasic dehydrate ($Na_2HPO_4{\cdot}2H_2O$) for phosphate buffer preparation were supplied by Riedel-de Haën (Seelze, Germany) and Fluka (Buchs, Switzerland), respectively. Boric acid and di-sodium tetraborate decahydrate were purchased from Merck (Darmstadt, Germany).

Preparation of Phosphate and Borate Buffer Solutions

Phosphate buffer stock solution (0.5 mol dm^{-3}) was prepared by using a mixture of sodium phosphate monobasic dihydrate ($NaH_2PO_4{\cdot}2H_2O$) and sodium phosphate dibasic dihydrate ($Na_2HPO_4{\cdot}2H_2O$) at a ratio of 2:3. The pH of the solution was adjusted using 0.1 mol dm^{-3} HCl and 0.1 mol dm^{-3} NaOH to pH 7. The solution was then made up to 100 ml with deionized water.

The ratio of di-sodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$) to boric acid (H_3BO_3) in the preparation of 0.5 mol dm^{-3} borate buffer stock solution was 1:4. The pH of the solution was adjusted to pH 8.5 using 0.1 mol dm^{-3} HCl and 0.1 mol dm^{-3} NaOH. The solution was then made up to 100 ml with deionized water.

Preparation of Oleic Acid Liposomes

Sodium oleate was first prepared by dissolving 2.824 g oleic acid with sodium hydroxide (0.22 mol dm^{-3}) using magnetic stirring. Phosphate buffer stock solution (10.0 ml) was then added to 0.2 mol dm^{-3} sodium oleate solution. Next, the pH of the solution was adjusted to pH 8.5 for the formation of liposomes. Finally, the solution was made up to 50 ml with deionized water.

Preparation of Emulsions

For preparation of the aqueous phase, 5 wt% sucrose ester was mixed thoroughly with deionized water by vortexing followed by heating in water bath at 70–80 °C until a clear solution was observed. Olive oil was added to the freshly prepared aqueous phase and homogenized at 13,000 rpm for 5 min to produce a 50 % oil-in-water emulsion.

Four different sets of emulsions (A, B, C, and D) were prepared and their composition is given in Table 1. Set A emulsions were the base emulsion systems prepared from C_{14} to C_{18} sucrose ester, respectively, and labeled as A-I, A-II, and A-III. Although the shelf life of the base emulsion stabilized by C_{18} sucrose ester was longer than that of the base emulsions stabilized by C_{16} and C_{14} sucrose esters, it eventually phase separated after 3 days due to creaming. Next, borate buffer (0.5 mol dm^{-3}) at pH 8.5 was used as aqueous phase in the preparation of the emulsions. The final concentration of borate buffer was 0.2 mol dm^{-3} as 20 wt% of deionized water was later injected and stirred

Table 1 Composition of the four different sets of emulsions. Set A emulsions were the base emulsion systems prepared from C_{14}–C_{18} sucrose esters, respectively. Borate buffer (0.5 mol dm^{-3}) at pH 8.5 was used as aqueous phase in the preparation of the Set B, C and D emulsions. Set C emulsions as control emulsions. Set D emulsions containing the oleic acid vesicle dispersion

Set	Systems	Olive oil phase (wt%)	Aqueous phase (wt%)[a]	Aqueous phase (wt%)[b]	Aqueous phase (wt%)[c]	Aqueous phase (wt%)[d]	Sucrose esters (wt%)		
							C_{18}	C_{16}	C_{14}
A	I	47.5	47.5				5		
	II	47.5	47.5					5	
	III	47.5	47.5						5
B	I	47.5		47.5			5		
	II	47.5		47.5			4.5	0.5	
	III	47.5		47.5			4.5	0.45	0.05
C	I	47.5			47.5		5		
	II	47.5			47.5		4.5	0.5	
	III	47.5			47.5		4.5	0.45	0.05
D	I	47.5				47.5	5		
	II	47.5				47.5	4.5	0.5	
	III	47.5				47.5	4.5	0.45	0.05

[a] Deionized water

[b] Borate buffer

[c] 27.5 wt% borate buffer; 20 wt% control solution prepared for formation of oleic acid liposomes containing phosphate buffer

[d] 27.5 wt% borate buffer; 20 wt% oleic acid liposome solution

with the emulsions. There was no separation of the emulsion stabilized by C_{18} sucrose ester over the storage period of 28 days, whereas shorter chain length sucrose esters such as C_{16} and C_{14} sucrose ester were not able to maintain the stability of the emulsion. A series of emulsions containing borate buffer with a variation of C_{14}–C_{18} sucrose esters was then prepared for emulsion-accelerated stability test by storing all the emulsions in an oven at 45 °C for 28 days. Thereafter, the ratios 1:0:0, 0.9:0.1:0, and 0.9:0.09:0.01 w/w of C_{18}:C_{16}:C_{14} sucrose esters were selected for further investigation. Set B emulsions containing these compositions were labeled as B-I, B-II, and B-III, respectively. Set C emulsions (C-I, C-II, and C-III) as control emulsions were made by injecting 20 wt% of solution prepared for formation of oleic acid liposomes, but without adding the oleic acid. Set D emulsions containing the oleic acid vesicle dispersion were labeled as D-I, D-II, and D-III. Oleic acid vesicle solutions were introduced into the base emulsions by injecting them slowly into the emulsions with a syringe and stirring slowly for 2 min. All emulsions were kept in an oven at 45 °C for over 7 days.

Polarizing Light Microscope and Droplet Size Analysis

Photomicrographs of emulsions stored at 45 °C for 1 day, 3 days, and 7 days were taken using a light polarizing microscope (Leica model DM RXP, Germany, 20× magnification objective lens) equipped with JVC Color Video Camera (model KY F550) and Leica QWin image analysis software. The droplet sizes of the emulsions can be determined with the aid of the image analysis software where photomicrographs are processed as electronic documents. Diameters of 600 droplets each from five photomicrographs, i.e., a total of 3,000 droplets for each sample were measured. The mean droplet size for these 3,000 droplets was then calculated. The polydispersity index (PDI) can also be calculated from the ratio between the standard deviation of the droplet size and mean droplet size [7].

Zeta Potential Analysis

Zeta potentials were determined by electrophoretic measurements with a Malvern Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). All emulsions were previously diluted 100 times in 0.01 M potassium chloride solution. The solutions were then filled into the folded capillary cell and placed into the Zetasizer. Before measurement, the sample was equilibrated at 30 °C for 5 min. The magnitude of the electrophoretic mobility was measured with a combination of laser Doppler velocimetry and phase analysis light scattering (PALS) in a patented technique called M3-PALS. Thereafter, the potential was calculated from the electrophoretic mobility by the application of the Smoluchowski theories on the Henry equation.

Rheological Analysis

Measurements of flow property were performed using a stress/rate-controlled Bohlin CVO-R Rheometer with temperature controller. For all measurements, the temperature was maintained at 30.0 ± 0.1 °C. A 4°/40 mm cone and plate geometry with a gap of 150 μm was employed in this study. Shear rate was increased from 0.0001 s^{-1} to 200 s^{-1} to obtain the flow curves. All measurements were performed after 7 days storage at 45 °C.

Results and Discussion

Presence of Oleic Acid Liposomes in Emulsions

At intermediate pH (7.5–9.5), oleic acid/oleate molecules self-assemble to form close bilayer vesicle structures (liposomes). The presence of these liposomes can be observed under a polarized light microscope by the appearance of Maltese crosses. In accordance with previous studies [5], the typical characteristic of Maltesian crosses with a strong halo at the center under cross-polarized light suggested the presence of a vesicular type lamellar liquid crystalline phase in the olive oil-in-water emulsion system (Fig. 1).

The Maltese crosses are seen because liposomes produce a birefringent effect; therefore, vivid blue and yellow interference colors [8] can be seen in Fig. 2. This optical interference is the result of the different refractive index of the liposome due to the ordered molecular assembly of the bilayers. The oleic acid liposome structure was maintained when dispersed in prepared olive oil-in-water emulsion. The oleic acid liposome can be seen only in emulsions

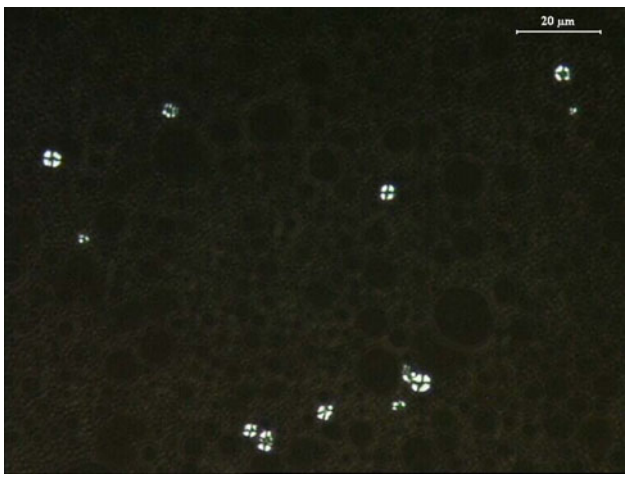

Fig. 1 Light micrograph of oleic acid liposomes (100 mM) in olive oil-in-water emulsion under dark field technique. The appearance of Maltese crosses suggests the presence of vesicular type of lamellar liquid crystalline phase in the olive oil-in-water emulsion system

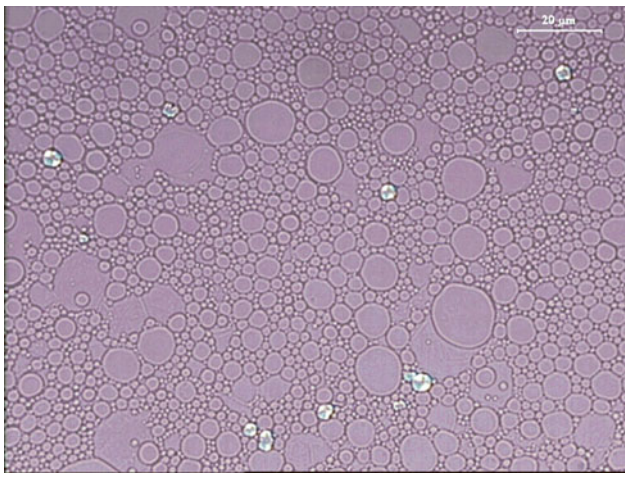

Fig. 2 Polarized light micrograph of oleic acid liposomes (100 mM) in olive oil-in-water emulsion. The birefringence of the liposomes gives rise to vivid *blue* and *yellow* interference colors (color figure online)

containing borate buffer pH 8.5 as an external aqueous phase. In other words, oleic acid liposomes are stable in the emulsion system at intermediate pH conditions.

Droplet Size Analysis

Droplet size is known to have influence on the appearance, stability, and rheology of an emulsion and, thus, the quality of a cosmetic cream product. Table 2 illustrates the droplet size of Set A–D emulsions. Emulsion systems without borate buffer as continuous phase (Set A emulsions) are much coarser than the subsequent three sets of emulsions. It should be noted, continuing on from the Set A emulsions,

Table 2 Droplet size of the emulsion systems

Set	System	1 Day		3 Days		7 Days	
		Size (μm)	PDI	Size (μm)	PDI	Size (μm)	PDI
	I	4.99	0.59	5.42	0.64	5.71	0.65
A	II	5.24	0.70	5.97	0.68	5.81	0.69
	III	6.14	0.65	6.36	0.68	6.87	0.70
	I	3.35	0.34	4.03	0.43	3.53	0.34
B	II	3.33	0.38	3.57	0.37	3.57	0.30
	III	3.33	0.32	3.86	0.42	3.58	0.31
	I	3.34	0.31	3.29	0.35	3.78	0.35
C	II	3.42	0.36	3.28	0.34	3.76	0.35
	III	3.30	0.41	3.57	0.34	3.54	0.37
	I	3.51	0.34	3.40	0.37	3.49	0.33
D	II	3.54	0.35	3.37	0.33	3.51	0.34
	III	3.46	0.32	3.35	0.36	3.49	0.34

Emulsion systems without borate buffer as continuous phase (Set A emulsions) are much coarser than the subsequent three sets of emulsions (Set B, C and D). *PDI* Polydispersity index

that droplet size was dependent on the alkyl chain length of the surfactants. The droplet size of the emulsion decreased when the alkyl chain length of the surfactant increased. This is related to the increasing interfacial film strength at the given oil–water interface. The use of borate buffer as the continuous phase of the Set B emulsions in order to maintain the pH at 8.5 further decreased the droplet size to approximately 3.00 μm.

Set A–C emulsions exhibited pronounced creaming after being stored in an oven under 45 °C for 7 days. Overall, the droplet size of the three sets of emulsions increased significantly with time as compared to the initial size. On the other hand, no considerable separation was observed in the Set D emulsions. The presence of oleic acid vesicles enhanced the stability of the emulsions as the experimental results revealed no change in droplet size.

Droplet Size Analysis—Effect of Alkyl Chain Length of the Surfactant

The three sucrose esters that were used in the preparation of Set A emulsions have a hydrophilic-lipophilic balance (HLB) value of 16. Surfactants with high HLB values tend to have good water solubility [9]. Although all three sucrose esters have the same HLB value, these emulsifiers still exhibit slightly different water solubility. Sucrose myristate has shown good solubility in water, while sucrose palmitate and sucrose stearate need to be warmed to assist their solubilization in water (Table 3). The average droplet size of the emulsions stabilized by sucrose myristate was much larger than the emulsion droplets stabilized by the latter two surfactants (Table 2). Although the water solubilities of the latter two surfactants cannot be clearly distinguished, sucrose palmitate was found to give rise to slightly larger droplet size. This in turn indicates that a shorter alkyl chain is responsible for higher water solubility. A shorter hydrophobic chain in surfactants might significantly hinder hydrophobic interactions between the surfactant and the oil phase at the interface, and consequently weaker absorption. The poorly stabilized oil–water interface exhibits lower interfacial elasticity and, therefore, results in larger droplet size [10].

Table 3 Water solubility test for sucrose esters

Sucrose ester	Water solubility[a]
Sucrose myristate (C-1416)	Shaken for 2 min
Sucrose palmitate (C-1616)	Heated for 10 min
Sucrose stearate (C-1816)	Heated for 10 min

[a] Sucrose myristate shows good solubility in water, while sucrose palmitate and sucrose stearate need to be warmed to assist their solubilization in water

Droplet Size Analysis—Borate Buffer as Aqueous Phase

The inclusion of borate buffer to the aqueous phase resulted in a decrease in droplet size from approximately 5.00 μm to approximately 3.00 μm. Although the original purpose of incorporation of borate buffer was to control the emulsion pH, the buffer neutralized the free fatty acid originally present in the content of sucrose esters and olive oil to form co-surfactants.

In general, a single surfactant monolayer at an interface from either water-soluble surfactants or oil-soluble surfactants is not close-packed. However, the combination of these two types of surfactants normally has a good emulsifying effect. This is due to the fact that the spread of size of the lipophiles and/or hydrophiles increases surfactant packing efficiency at the oil–water interface [11]. An additional explanation for the advantageous effect of mixed surfactant film is that the supply of surfactants comes from both the oil and water phases to the interface. As a result, the surfactant interfacial film becomes more elastic and, therefore, resists rupture upon collision of emulsion droplets.

Droplet Size Analysis—Accelerated Stability Test

From a kinetic point of view, an emulsion with an activation energy 20 times greater than the thermal energy of the system is claimed to have long-term stability [12]. Nonetheless, the dynamic nature of surfactant film affects droplet stability as it changes with time. The factors that determine droplet movement and the nature of the interactions between droplets affect the kinetic stability of the emulsion.

Droplet size analysis as a function of storage time is the method most commonly used in the evaluation of possible

Table 4 Zeta potential of the emulsion systems (mV). All the emulsions show negative zeta potential

Set	Systems	1 Day	3 Days	7 Days
	I	−16.6 ± 0.115	−15.5 ± 0.208	−15.7 ± 0.306
A	II	−13.5 ± 0.866	−11.9 ± 0.173	−12.6 ± 0.208
	III	−11.2 ± 0.850	−10.9 ± 0.265	−9.93 ± 0.473
	I	−78.0 ± 0.751	−78.0 ± 1.07	−79.7 ± 0.153
B	II	−76.6 ± 0.416	−77.8 ± 0.208	−79.9 ± 0.693
	III	−75.2 ± 0.200	−76.9 ± 0.173	−79.5 ± 0.611
	I	−75.4 ± 0.361	−84.7 ± 0.557	−84.9 ± 0.404
C	II	−74.5 ± 0.643	−81.6 ± 0.208	−86.7 ± 0.0577
	III	−73.2 ± 0.153	−83.9 ± 0.819	−80.4 ± 1.31
	I	−81.7 ± 2.22	−84.0 ± 1.27	−84.5 ± 0.608
D	II	−82.8 ± 1.05	−82.1 ± 0.666	−82.3 ± 0.693
	III	−84.6 ± 1.50	−85.0 ± 2.08	−85.1 ± 0.306

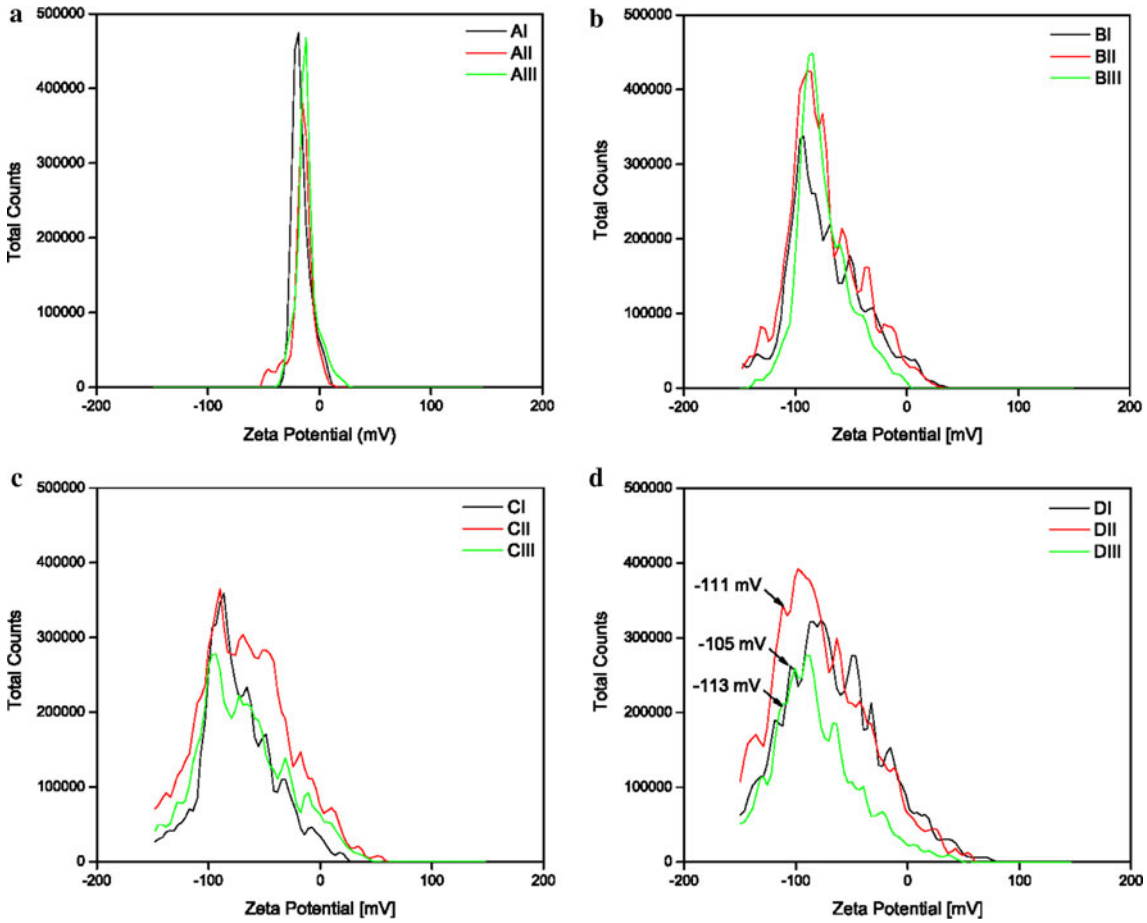

Fig. 3 Zeta potential distributions for Set A–D emulsions diluted in 0.01 mol dm^{-3} KCl. **a** A single zeta potential distribution peak was obtained for the Set A emulsions, suggesting homogeneous systems as only non-ionic sucrose ester is present on the surface of the emulsion droplets. **b**, **c** The observed peaks for Set B and C emulsions are much broader with multiple side peaks, indicating the more heterogeneous nature of the emulsion droplets due to the varying number of differently charged surfactant molecules (sucrose ester and ionized carboxyl group) on the surface of the droplets. **d** The existence of a side peak at the range of −105 to −120 mV suggests the presence of oleic acid liposomes in Set D emulsions

changes in the kinetic stability of an emulsion. The aging of the emulsions was studied over 7 days, as noted in the section on "Preparation of Emulsions". An increase in size of the Set A–C emulsions droplets was observed. All the emulsion systems in this study were categorized as moderate emulsions since the oil fractions were fixed at 50 %. The relatively low viscosity of these moderate emulsions and the loosely packed droplet structure increases the probability of the droplet collisions that consequently lead to coalescence. The relatively small changes in mean droplet size of Set B and C emulsions were due to the dense packing of mixed surfactants at the interface. Furthermore, the present of a salt form of fatty acid with negatively charged carboxylate head groups exerts electrostatic repulsion between the interfaces to prevent droplet aggregation.

The Set D emulsion droplets were significantly more stable as compared to the previous three set of emulsion droplets. This phenomenon can be attributed to the presence of negatively charge oleic acid/oleate vesicles between the emulsion droplets, which further reduces the collision rate of droplets.

Zeta Potential

Zeta potential is commonly used along with particle size measurement to control the stability of a system. Droplet flocculation has a large influence on the stability of many cosmetic emulsions. In order to keep each droplet discrete and prevent flocculation, the electrostatic repulsion between droplets must be maximized. Zeta potential measurement provides an insight into the nature of the electrostatic interaction of an emulsion. An absolute zeta potential value of ±30 mV or higher generally implies a greater static electricity repulsion between the droplets, so the emulsion system may have better stability. The zeta

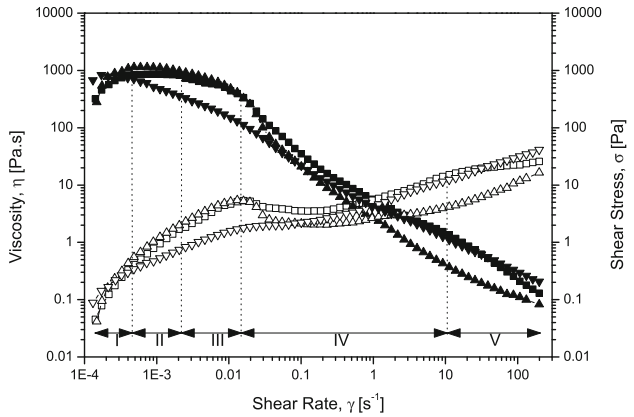

Fig. 4 Illustrative flow curves of Set A emulsions (*squares* A-I; *up-pointing triangles* A-II; *down-pointing triangles* A-III): *I* shear thickening region where viscosity increases with increasing shear rate; *II* Newtonian plateau region in the intermediate shear rate range where viscosity is constant; *III* shear thinning region where viscosity decreases with increasing shear rate; *IV* shear-banding region showing coexistence of flowing and non-flowing regions in the system; *V* high shear rate shear thinning region. *Filled symbols* η; *open symbols* σ

potential is proportional to the electrophoretic mobility, which in turn depends on the nature of the surface, size, shape, and electrical charge of a substance [13].

Negative zeta potential was observed for all the emulsions in this study, as displayed in Table 4. First of all, emulsion systems without borate buffer (pH 8.5) as dispersing medium (Set A emulsions) have a negative zeta potential, most probably caused by the adsorption of the OH^- ion at the sucrose head group of the surfactant. The presence of the $-OH$ groups at the surfactant head enabled the creation of hydrogen bonds with the aqueous OH^- groups. Similar findings by others showed that oil droplets stabilized by different nonionic surfactants are likely to have a negative charge at pH > 4 [14]. The magnitude of the zeta potential for Set A emulsions after 1 day of storage varied from -16.6 ± 0.115 to -9.93 ± 0.473 mV. Figure 3a displays the single zeta potential distribution peaks for Set A emulsions, suggesting homogeneous systems containing only non-ionic sucrose ester on the surface of the emulsion droplets. The low negative zeta potential implies that the droplets do not carry enough charge to repel each other and are more likely to aggregate. Thereby, a significant decrease in negative zeta potential was observed during storage.

Set B emulsions showed a significant increase in negative zeta potential. Generally, different conditions and ingredients have a significant effect on the zeta potential. In situ generation of fatty acid sodium salt, as discussed previously in the section on "droplet size analysis—borate buffer as aqueous phase" (see above), may contribute to the dramatic increase in negative zeta potential. This unusually high value of negative zeta potential suggests the

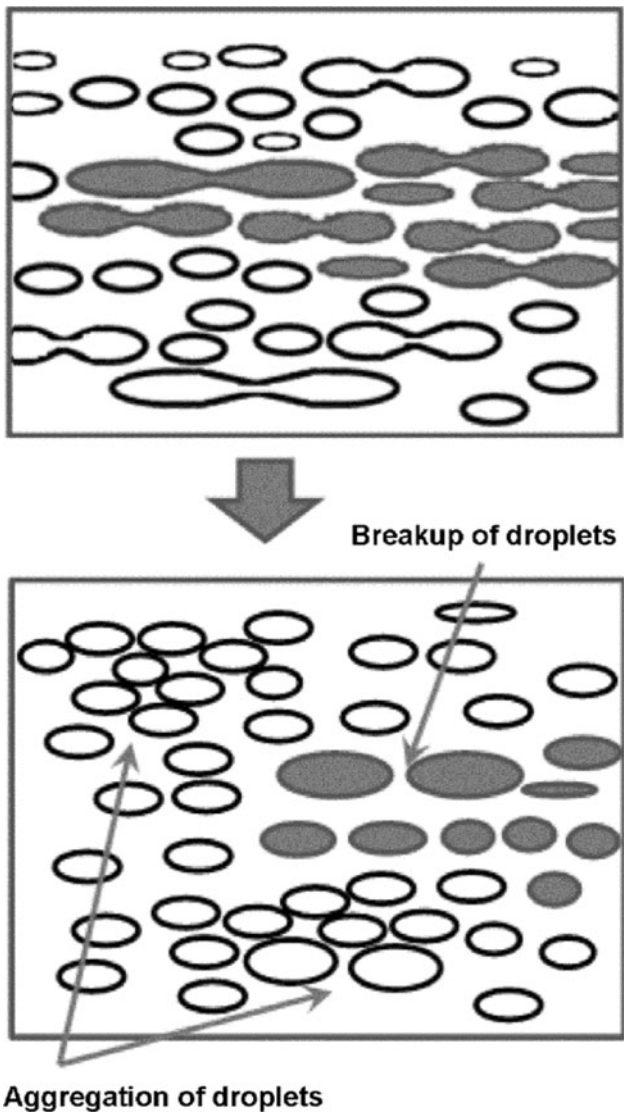

Fig. 5 Illustration of structural evolution of microstructure of emulsion droplets in a shear-banding region. The breakdown of droplet structures decreases the viscosity, while the aggregation of droplets increases viscosity

existence of ionized carboxyl groups at the droplet surface. Furthermore, the observed zeta potential distribution peaks for Set B and C emulsions are much broader with multiple side peaks (Fig. 3b, c), indicating the more heterogeneous nature of the emulsion droplets. This is due to the varying number of differently charged [15] surfactant molecules (sucrose ester and ionized carboxyl group) on the surface of the droplets.

It is clear that negative zeta potential of Set B emulsions goes up after 7 days of storage at 45 °C. Supposedly, higher magnitude of negative zeta potential indicates better resistance to coalescence. Nevertheless, the increasing negative zeta potential is in contrast to the droplet size measurements. The significant increase in droplet size

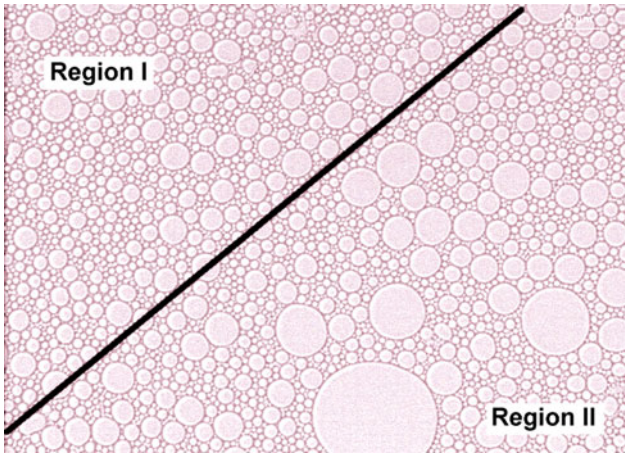

Fig. 6 Polarized light micrograph of A-II emulsion system showing two regions of different sized droplets that tend to have different shear rates when shearing at a steady flow

shows some degree of coalescence with storage. Therefore, the increment in negative zeta potential did not correlate with emulsion stability, but instead to the hydrolysis of the sucrose ester. Formation of more fatty acid sodium salt seems to have an adverse effect on emulsion stability. This effect is more severe in Set C emulsions, where the magnitude of negative zeta potential increased suddenly after 3 days of storage.

Set D emulsions have relatively higher negative zeta potential than Set A–C emulsions. This is due to the presence of oleic acid liposomes in the emulsion systems, which can be supported by the existence of a side peak at the range of −105 to −120 mV, as shown in Fig. 3d. No significant changes were observed for the negative zeta potential throughout the measured storage time. This in turn suggests that the emulsions were considerably more stable.

Rheological Flow Behavior

Flow curves of oil-in-water emulsions stabilized by sucrose esters (Set A emulsions) are shown in Fig. 4. Five distinct regions were identified in both the A-I and A-II emulsions. The shear thickening behavior (region I) can be ascribed to shear-induced clustering of droplets at low shear rate. On applying shear rate, shear forces pushing the droplets in the well-stabilized polydispersed emulsions together override the steric repulsive forces between the droplets, thereby displacing droplets from the initial equilibrium position. This leads to a disordered structure, causing an increase of the emulsion viscosity [16]. Physically, the effect of polydispersity is to allow droplets to pack more densely, thus leading to the formation of a disordered structure. This can explain why the A-II emulsion exhibited more pronounced shear thickening than the A-I emulsion.

As the rate of shear increases, a Newtonian plateau develops (region II), implying that the droplets reach a disordered solid-like state. The A-I emulsion droplets with higher interfacial elasticity are stiffer and stronger as measured by the shear modulus, G. The droplets tend to resist deformation, thus the emulsion has an extended Newtonian plateau regime. Beyond the Newtonian plateau, the emulsions displayed shear thinning behavior when the droplets started to deform (region III). High deformability of a relatively large size of A-III emulsion droplets due to less elastic surfactant film tend to elongate and align under

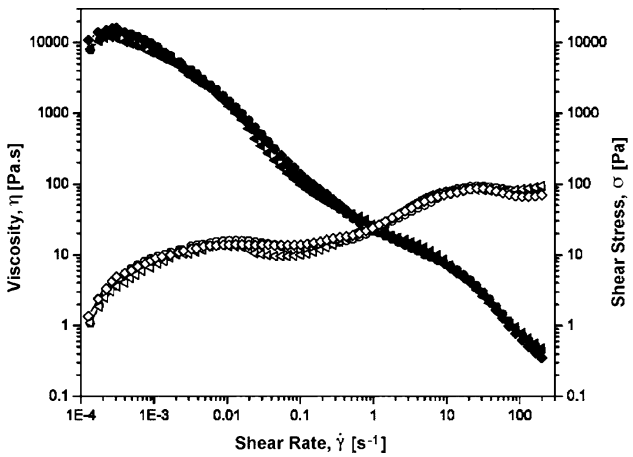

Fig. 7 Viscosity versus shear rate and shear stress versus shear rate profiles of Set B emulsions (*circles* B–I; *left-pointing triangles* B-II; *diamonds* B-III; *filled symbols* η;*open symbols* σ). The number of droplet–droplet interactions in Set B emulsions increases when the droplet concentration increases, leading to an overall increase in viscosity

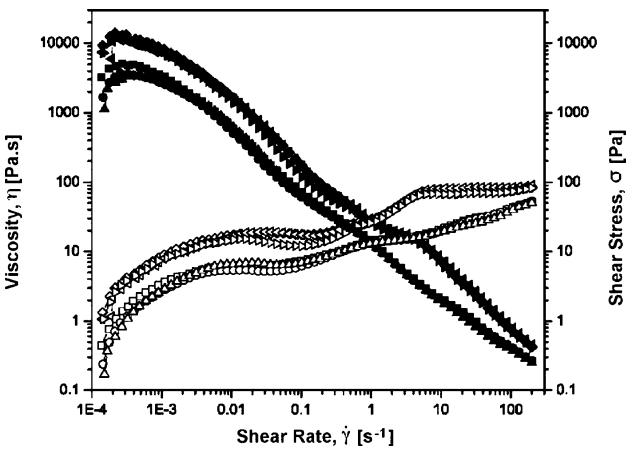

Fig. 8 Flow curves of Set D emulsions (*squares* D-I; *circles* D-II; *triangles* D-III) and control Set C emulsions (*diamonds* = C-I; *left-pointing triangles* C-II; *right-pointing triangles* C-III).*Filled symbols* η ;*empty symbols* σ . The lower viscosities of Set D emulsions were due to the possibility of packing of surfactant at the interface loosened by the presence of oleic acid molecules

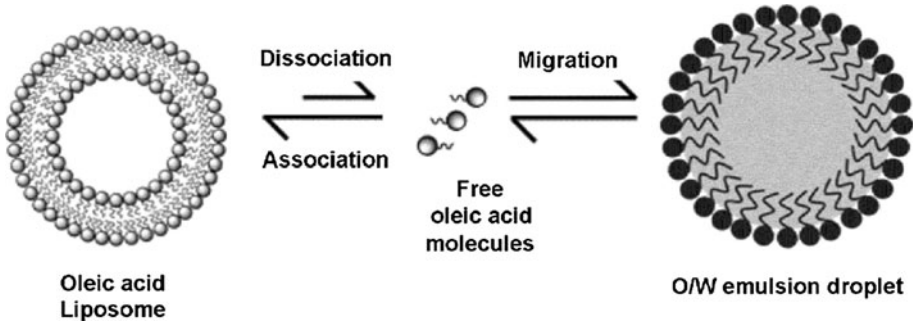

Fig. 9 Transportation of oleic acid molecules to neighboring oleic acid liposomes or oil-in-water emulsion droplets

shear, resulting in more pronounced shear thinning behavior.

The viscosities of the emulsions decreased drastically at a critical stress, representing the onset of shear banding (region IV). This region is clearly reflected in the plot of shear stress against shear rate, either a stress plateau or a notably negative slope over some range of shear rates. The shear-banding phenomenon involves heterogeneous flow as the formation of bands within the sample with the coexistence of different shear rates. The flow behavior of shear banding has been reported in several systems, including polymers, lamellar surfactant phases [17], wormlike micelles [18, 19], emulsions [4, 20], foams, and colloidal gels [21].

It was suggested that the occurrence of shear-banding states can be attributed to destructuring and restructuring of the emulsions [4, 22]. Upon the application of shear, the elongated droplets eventually break up into smaller droplets. The breakdown of droplet structures decreases the viscosity as droplets start to flow. At the same time, shear-induced aggregation of droplets increases the viscosity [21, 23, 24]. This structural evolution has been illustrated in Fig. 5. Nonetheless, this cannot fully explain the more pronounced shear-banding phenomenon in A-II as the emulsion is more polydisperse. The different size of droplets in polydisperse emulsion tend to cream at various rates, with the larger droplets creaming faster than smaller droplets [25]. Microscopy image, such as shown in Fig. 6, uniquely provide significant evidence to support the existence of two regions with different droplet size distribution. Region II is likely to have much larger droplets than region I. When shearing the emulsion at a steady flow, the two regions tend to have very different shear rates, suggesting shear banding. After structural evolution of the emulsions (A-II and A-III) under shear in the shear-banding region, droplets gradually rearrange themselves in the flow direction at higher shear rates (region V). This rearrangement of droplets produced less resistance to flow, and the viscosity decreases.

As shown in Table 2, the emulsion systems with the use of borate buffer in the continuous phase (Set B emulsions) have smaller droplet size as compared to Set A emulsions. When the droplet size decreased, the droplet concentration increased. As a result, the number of droplet–droplet interactions increases, leading to an overall increase in viscosity, as seen in Fig. 7. The increase in viscosity is more pronounced at low shear rates, not only because of the increasing number of weak droplet–droplet interactions but also because of the increasingly high negative zeta potential, which forced the droplets to strongly repel each other. Fundamentally, this prevents the droplets from flowing freely, subsequently causing the viscosity to increase.

The elasticity and consistency of Set D emulsions decreased compared to those of the control Set C (Fig. 8). During the injection and mixing of oleic acid liposome solutions to the base emulsions, there is a possibility that some of the liposomes may break down. Thereafter, some of the free oleic acid molecules may migrate to the oil–water interface of the emulsion droplets or to the surface of neighboring liposomes. The transportation of free oleic acid molecules depends on its affinity to the two interfaces as shown in Fig. 9. Unsaturation of the oleic acid tail has an influence on the packing efficiency at the interface. The packing of surfactant at the interface may be loosened by the presence of the oleic acid molecules [26].

The results presented here seemed to contradict an earlier study that showed enhanced viscosity of emulsions when vesicles were present in system [5]. This is due to the different preparation methods in producing emulsions containing vesicles. The vesicles in the previous study were formed in situ, following emulsification. The capturing of aqueous phase inside the vesicles changed the emulsion system's oil:water ratio. This in turn increased the effective volume fraction of the emulsions. In other words, the emulsions become more concentrated.

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