Preparation and Characterization of Functional Compounds Encapsulated Microemulsion with Nonionic Surfactants

FANG ZHONG[1]*, WENTING XU[1], TINGTING FU[1] AND YUE LI[1]

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, China

ABSTRACT

Influence of hydrophobic chain lengths of surfactant, co-surfactant, oil and different surfactant to co-surfactant ratios on phase behavior of Tween/alcohol/alkane/H2O was investigated with pseudo-ternary phase diagrams. The total areas of the microemulsion (ME) regions were determined. The largest ME region was formed when the ratio of surfactant: co-surfactant was 2 : 1 (w/w). The capacity for formation of MEs increased with an increase of hydrophobic chain length of the Tween, which was opposite to the trend for alkane (oil). N-butanol formed the largest ME region among the alcohols tested as co-surfactants. Sub-ME phases were identified by conductivity measurements. O/W MEs were observed when the water content was higher than 62 - 68%. The O/W sub-phase regions had significantly lower particle sizes and viscosities than either the W/O or bi-continuous sub-phases.

Key words: Microemulsion, Phase behavior, Surfactant, Microemulsion region, Particle size, Viscosity

INTRODUCTION

Microemulsions (ME) are defined as systems which are thermodynamically stable, low viscosity, isotropic clear dispersions. MEs have several advantages such as enhanced hydrophobic solubility, good thermodynamic stability, ease of manufacturing[1], and stability against oxidation and controlled release[2]. The microstructure, phase behavior and solubilization capacity of MEs depend on the nature of the four main components, surfactant, co-surfactant, oil and aqueous phase mixture[3].

In order to increase lipo-nutrients solubilization capacity in oil in water (O/W) MEs, numerous formula optimization attempts had been made by different researchers. Selection of surfactants that can decrease the interfacial tension to an ultra low values which solubilize otherwise immiscible components have been the most common reports. Suggestions have been made that surfactants with hydrophile–lipophile balance (HLB) values (8 - 20) maybe effective surfactants for the formation of O/W MEs, but no generalize guide for choice of surfactants for the encapsulation of various nutrients has been proposed.

The co-surfactants used in the non-ionic MEs systems are mostly medium chain length alcohols, such as ethanol, propylalcohol and butanol. The roles of co-surfactant in the formation of MEs include reducing interfacial tension, adjusting the HLB of the surfactant. Ezrahi, *et al.*[4] reported that the water solubilization capacity of ME with linear chain alcohols was higher than that of branched chain alcohols. Out of 40 compositions with 10 different oil phases, MEs with the co-surfactant n-pentanol exhibited a higher viscosity when compared to n-butanol. As to

the influence of oil phase, a study[5] suggested that the molecular volume of oil phase appeared to be more importance in determining phase behavior compared to its polarity.

The structural compatibility between components is also considered to be an important factor for ME formation. Bansal, *et al.*[6] tested the solubilization capacity of W/O MEs formed with fatty acid soaps and alcohols; their results suggested that water loading capacity reached a maximum when the oil chain length as well as that of the alcohol, was equal to that of the surfactant. Although later investigations[7] indicated that this did not applicable to all non-ionic surfactant W/O ME systems, it revealed the existence of common regulation on ME formulation. However, no such reports were found for O/W type MEs.

Based on these reports, the objectives of this study was to investigate the effect of hydrophobic chain length of surfactant, amount of surfactant, type of co-surfactant, type of oil, mixtures of surfactants on the formation and properties of W/O microemulsion to facilitate future compositional optimization procedure for microemulsion encapsulation of hydrophobic food nutrients and functional compounds. Suitable surfactants for food grade MEs are limited. Polyoxyethylene sorbitan esters (Tweens) are probably the most widely used, as they are readily available and inexpensive food grade surfactants. Therefore, a Tween/alcohol/alkane/H_2O system was employed as one of the model system.

MATERIALS AND METHODS

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* Author for correspondence. Tel: 86-510-85913625;
Fax: 86-510-85913622; E-mail: fzhong@jiangnan.edu.cn

I. *Apparatus and Conditions*

The conductivity was measured at $30 \pm 0.01°C$ with a Mettler Toledo FE-30 conductivity meter (Columbus, OH, USA). Conductivity measurements were carried out during the titration of oil and surfactant/cosurfactant (S/CS) mixtures with water.

Viscosities were measured with an AR-1000 rheometer (TA instruments, New Castle, DE, USA) at 25°C. The viscosity of the MEs with different water contents and different shear rates were measured.

The particle size measurements were made using a laser scatter analyzer (Nano-Zs90, Malvern Instruments, UK), the temperature was controlled at $25 \pm 0.01°C$.

II. *Materials and Reagents*

Surfactants (Tween 20, Tween 40, and Tween 60) and oils (n-hexane, n-heptane, n-octane, n-decane, n-dodecane, n-tetradecane and n-hexadecane) were purchased from Sigma-Aldrich (USA). Co-surfactants (ethanol, n-propylalcohol, n-butanol, n-hexylalcohol and n-octylalcohol) and other chemical reagents were purchased from Sinopharm Group Chemical Reagent Co, Ltd (Shanghai, China). All chemicals were used without further purification, and the water was double distilled.

III. *Sample Preparation*

The phase diagrams were determined by titration of water into a mixture of surfactant, co-surfactant and hydrocarbon. Various ratios of S/CS to oils (10 : 0, 9.5 : 0.5, 9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8, 1 : 9, 0.5 : 9.5, 0 : 10) (w/w) were mixed in sealed tubes, shaken vigorously in a vortex mixture, and equilibrated in a thermostatic bath at $30 \pm 0.01°C$. Water was then added with a microsyringe. The mixture was vortexed vigorously and then placed in the thermostatic bath for several minutes. Phases were visually identified as transparent, cloudy, or sharply defined separated phases. Samples that exhibited clear and transparent were considered as a monophasic areas in the phase diagram. The weight percentage of water, oil, and S/CS at the critical point of transparency change was considered as a point on the boundary. The area within the monophasic region was considered as the ME region.

RESULTS AND DISCUSSION

I. *Hydrophobic Chain Length of Surfactant and Co-surfactant*

The length of the alkyl chain of a surfactant can have an effect on the shape and extent of the ME phase area in a pseudo-ternary phase diagram. In order to find the effect of the hydrophobic chain length of surfactant and co-surfactant for the system of Tweens, the ME areas for Tweens (20, 40, 60)/alcohol/n-hexane/water with S : CS = 2 : 1 (w : w) were measured, Figure 1. Two observa-
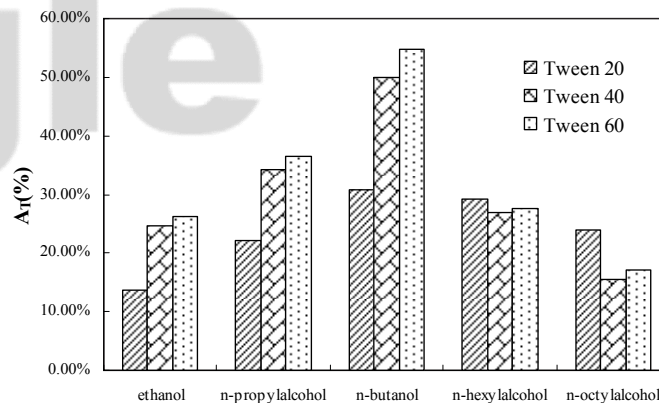
Figure 1. Effect of chain length of surfactant and alcohol on the A_T (%) for system Tweens/alcohol/n-hexane/ water systems, the ratio of Tweens : alcohol = 2 : 1 (w/w). A_T(%) represent for microemulsion area percentage.

tions could be concluded from the results: 1) of all the surfactants tested in this system, the biggest monophasic area was always occurred when n-butanol was used as co-surfactant, regardless the hydrophobic chain lengths of the surfactant, and 2) Tween 60 (C18 hydrophobic chain) were the most suitable surfactant with short chain alcohols while Tween 20 (C12 hydrophobic chain) were better with long chain alcohols with regard to producing the largest monophasic areas.

The role of the co-surfactant is related to its distribution with the surfactant in the oil-water interface layer, which is critical in reducing the interfacial tension. The presence of alcohols affects the spontaneous curvature of the interface by penetrating into the interface and destabilizing the liquid crystalline phase[8]. The fact that ME regions increased with an increasing chain length of the alcohol when the alcohol contained less than four carbons, is thought to be related to their polarities[9]. Very short chain alcohols are too soluble in the aqueous phase while long chain alcohols tend to be more soluble in oil phase, in both cases there will be a decreased concentration of cosurfactant in the interfacial layer. Besides, longer chain alcohols have large molar volumes which impede their penetration into the interfacial layer.

Larger hydrophobic chain lengths of a surfactant resulted in an increased penetration of the alkyl chain of the surfactant into the oil, which promoted the formation of a ME[10]. Our results with the C2 - C4 co-surfactant systems were in accordance with this assumption, but for the C6 - C8 co-surfactants, the contrary results were noted. Part of the reason might be the difficulty of molecular arrangement at the interface when surfactant and co-surfactant both had long hydrophobic chains, which forced co-surfactant to move from the interfacial area towards the oil phase, losing their function as a cosurfactant. Although, another possibility is that the shorter hydrophobic chain surfactant and longer chain alcohol together lead to more bi-continuous and W/O ME.

II. *Effect of S/CS Ratio on the of ME Phase Area*

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As has been demonstrated earlier, the molecular structure of co-surfactant had an impact on the size of ME area. In order to further understand the role of co-surfactant in the formation of a ME, S : CS ratios from 4 : 1, 2 : 1, 1 : 1, 1 : 2, to 1 : 4 (w : w), were used in Tween 60/n-butanol/n-hexane/water systems. ME areas of each system were recorded, Table 1. The results showed an increase of the ME area from 18.99 to 54.65% as the S : CS ratio increased from 4 : 1 to 1 : 2, but a further increase in the co-surfactant decreased the ME area to 46.53%. It could also be seen that high surfactant concentration lead to the emergence of liquid crystal phase, but if the concentration of surfactant was too low, which fail to critical micellar concentration will not be able to form the ME. With this system, a ratio of surfactant to co-surfactant of 2 : 1 led to an optimal ME formation.

III. *The Effect of Hydrocarbon Chain Length of the Oil on the ME Phase Area*

To investigate the effect of the chain length of oil on the size of the ME phase, Tween 60 : n-butanol (2 : 1) and Tween 40 : n-propylalcohol (2 : 1) were used as the S/CS along with various oils, n-heptane, n-octane, n-decane, n-tetradecane, and n-hexadecane, Figure 2. In both systems, there was a decrease in the ME area with an increase in the chain length of oil. The decrease was more rapid when the alkane carbon was larger than ten. N-hexane produced the largest ME phase area with either systems. The Tween 60 system was also observed to form larger ME phase areas than Tween 40; this was also consistent with the observations from the above studies.

The results were consistent with a previous report[5] which observed that within the same series of oils, the oil with shorter chain length was solubilized to a greater extent than the longer chain length oils in a ME. The solubilization capacity decreased upon increasing the chain length of the oil due to the increase in the attractive interaction among droplets. As the chain length of oil increased, the concentration of alcohol in the oil phase decreased, forcing the alcohol to penetrate into the interface by destroying the stability of the interface.

### IV. *Conductivity Measurements*

Conductivity is a structure-sensitive property and it has been used to investigate the phase structure of the ME. Electric conductivity was measured as a function of water content in various compositions of MEs (Tween/alcohol/n-hexane/water system, S : CS = 2 : 1). Five water free mixtures of hexane and S/CS were prepared with different concentrations of hexane (5, 10, 20, 30 and 40%) with respect to hexane and S/CS. Each was titrated with water, and the change in the conductivity versus % water was recorded. The shape of the conductivity/titration curves has been attributed to three sub-phases within the ME phase, W/O,

Table 1. Influence of the S/CS (w/w) ratio on the area of ME for Tween 60/n-butanol/hexane/water

S/CS Ratio	4 : 1	2 : 1	1 : 1	1 : 2	1 : 4
ME Area	46.34[a]	54.65[b]	40.40[c]	26.02[d]	18.99[d]
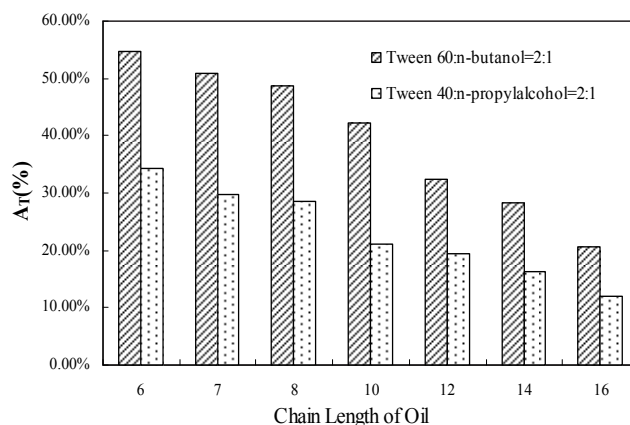
Figure 2. Effect of the chain length of oil on the A_T(%)r, the ratio of Tweens : alcohol = 2 : 1 (w/w). A_T(%) represent ME area percentage.
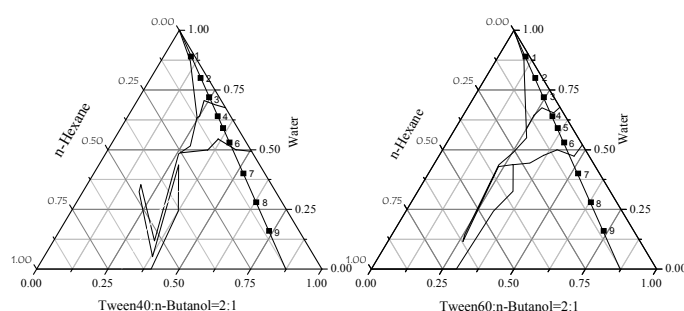
Figure 3. The pseudo-ternary phase diagram for a) water/hexane/ Tween40/n-butanol with S : CS at 2 : 1 and b) water/hexane/Tween60 /n-butanol with S : CS at 2 : 1.

Table 2. The properties of the sub-phases of MEs formed with Tween40 and Tween60

S	ME Type	Sample	Water (%)	Particle Size (nm)	Zeta potential (mV)	Viscosity[a] (mPa s)
T w e e n 4 0	O/W	1	89.0	11.9	-5.59	1.62
		2	80.0	19.3	-4.67	4.50
		3	72.0	25.5	-0.754	6.72
		4	64.0	37.1	-0.166	21.8
	BC	5	59.0	38.5	-0.345	25.6
		6	53.0	37.1	-0.162	21.6
		7	40.0	150	-0.347	17.2
	W/O	8	28.0	137	-0.245	16.8
		9	16.0	540	0.123	16.6
T w e e n 6 0	O/W	1	89.0	10.9	-3.23	1.52
		2	80.0	13.8	-3.20	3.76
		3	72.0	19.8	-0.519	7.69
		4	64.0	23.3	0.132	22.8
	BC	5	59.0	34.1	-0.919	25.1
		6	53.0	31.9	-0.968	25.6
		7	40.0	79.8	-0.246	23.3
	W/O	8	28.0	267	-0.324	22.1
		9	16.0	5015	-0.100	21.7

[a] Viscosity measured at 40 sec^{-1}.

bi-continuous and O/W, Figure 3. The results suggested that the W/O ME had water content of up to a range of 42 - 50% and the O/W ME had water contents higher than 62 - 68%. With the data from conductivity titrations of the other initial hexane concentrations, a reformed pseudo-ternary phase diagram was constructed which subdivided the ME region which was 54.6% of the total phase area into three regions, W/O (45.0%), bi-continuous (5.6%), and O/W (4.0%). Conductivity measurements with a different S : CS (1 : 1) ratio obtained 40.4% total ME phase area, and the three sub-regions, W/O (36.5%), bi-continuous (2.8%), and O/W (1.1%). The results showed decreases in each area of the three sub phase areas with respect to the 2 : 1 S : CS ME.

V. *Viscosity and Particle Size Measurements*

The two systems are Tween 40 or Tween 60/n-butanol/n-hexane/water with a fixed ratio of S/CS to oil of 2 : 1. The viscosity was measured at a shear rate of $40s^{-1}$. As was shown in Table 1, the viscosity changed with increasing water content follow the same pattern between the two systems. The initial addition of water to the oil and S + CS mixture produced W/O MEs with increasing viscosities. An even steeper increase in viscosity was found after the water content reached 50 - 60% and the systems transformed into a bi-continuous phase. Continued additions of water decreased the viscosity as the phase changed into a W/O. The distinct parts in the viscosity changes appeared to correlate with the conductivity measurement. The big viscosity drop with the transition from bi-continuous to O/W ME was thought to be due to the low viscosity of the aqueous continuous phase. The increase of viscosity with more water in W/O ME and the decrease of viscosity with added water in O/W ME as shown in Table 1 resulted from the higher dispersed phase fraction added to the viscosity of continuous phase, but the high viscosity in bi-continuous phase might due to the contribution of high viscosity of continuous phase, high disperse phase fraction and small particle size altogether. The Particle size distribution measurements of the samples, Table 2, were made with dynamic light scattering. The results suggested that even high HLB Tween surfactants can form W/O MEs but the sizes of those particles are out of the nanorange. The particle size of the O/W MEs decreased with higher concentrations of water at fixed surfactant to oil ratio. The possible mechanism could be that with the higher water content, the viscosity is low and the absolute value of zeta potential is high (Table 2), which would facilitate the formation of small particles.

CONCLUSIONS

The phase behavior of Tween/alcohol/alkane/H₂O for different chain lengths of surfactant, co-surfactants, oils and different S/CS ratios were investigated. The study reported how to optimize the process to obtain a maximum ME area. Tween 60/n-butanol (2 : 1, w/w) were found to be the most effective S : CS for the formation of the largest ME phase areas with Tweens/alcohols/alkanes/H₂O. At larger ratios liquid crystals were formed. If the ratio was too low and the concentration of surfactant was too low, it was different to form ME phases. With longer chain co-surfactnats than butanol, the ME phase area decreased.

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