A finely monodispersed oil-in-water (O/W) emulsion was obtained under high temperature and pressure conditions (240°C and 10 MPa). A coarse emulsion stabilized by Tween 20 was prepared in advance from the decane/water system to be treated under the subcritical conditions. The treated coarse emulsion was thereafter mixed with the aqueous solution including surfactants to improve the monodispersity of the oil droplets. The subcritical condition could supply sufficient energy to produce finely dispersed oil droplets (~233 nm in diameter).

1. Introduction

Emulsions are of importance not only in foods, but also in cosmetics, pharmaceuticals and petrochemicals. Their characteristics including rheology, appearance, chemical reactivity, and physical stability are all affected by the size of the droplets and droplet size distribution [1,2]. The size of oil droplets and their dispersity in general depend on the emulsification technique. Conventionally, oil droplets in emulsions have been prepared by homogenizers, ultrasound homogenizer, rotor stator systems, and microchannel [3]. These emulsification techniques are advantageous for a preparation of oil droplets of several tens of microns in diameter. Recently, alternative emulsification techniques such as membrane emulsification [4] have been reported to produce better control over fine droplet size (300 nm in diameter) and to consume less energy. However, the preparation of fine droplets with less than 300 nm in diameter would require much energy.

Subcritical water is water that maintains its liquid state at elevated temperatures under pressurized conditions; i.e. a fluid with much energy. If subcritical water is utilized in the emulsification process, much energy can be supplied for fine oil droplet formation. In recent studies, a novel preparation method using subcritical water has been reported [5,6]. Subcritical water has two distinct properties: (1) a low relative dielectric constant and (2) high ionic product. This property of subcritical water suggested that organic solvents could be solubilized in water even at high concentrations [7]. Finely dispersed oil droplets could be thereby formed in an octanoic acid (oil phase)/water system with nonionic surfactants under subcritical conditions [6], whereas, when sodium dodecyl sulfate (SDS) was used as an anionic surfactant, was finely dispersed oil droplets were not formed [5]. This is probably because of the repulsive electrostatic interaction between the octanoic acid and SDS. In the dodecane (oil phase) / water system, finely dispersed oil droplets could be formed even in the presence of SDS [8]. It is, therefore, considered that the emulsification using the non-fatty acid (oil phase) / water system under subcritical conditions might give a
better insight on the formation mechanism of finely dispersed oil droplets.

In this study, we investigated the preparation of emulsions in the decane (oil phase) / water system to avoid the influence of the electrostatic repulsive interaction between the oil phase and the surfactants. Three types of surfactants, Tween 20 (nonionic), hexadecyl-trimethyl-ammonium bromide (cationic), and SDS (anionic) were used in this study. First, the contribution of surfactants to the emulsification process was clarified. Second, the influence of surfactant type on the size of oil droplets and their dispersity / stability was examined. Finally, we discussed the emulsification efficiency of the present method.

2. Experimental

2.1 Materials
Polyoxyethylene sorbitan monolaurate (Tween20), hexadecyl-trimethyl-ammonium bromide (CTAB), sodium dodecyl sulfate (SDS), and decane were purchased from Wako Pure Chemical Industry, Ltd. (Osaka, Japan). Other chemical reagents were of analytical grade. SUS316 tubes were obtained from GL Science, Co. Ltd. (Tokyo, Japan).

2.2 Set up of the reaction system
The flow system used in this study is shown in Figure 1. Firstly, a mixture of 0.3 g of decane and 99.7 ml of water (solution I) was prepared. The solution I with and without Tween 20 was homogenized with the homogenizer PT-MR3100 (KINEMATICA, Switzerland) of 10,000 rpm for 2 min to prepare the coarse emulsion. The coarse emulsion obtained was thereafter injected into the oil bath by the HPLC pomp and heated up to 240 °C under 10 MPa. The flow rate of solution I and II were 0.3 and 0.6 ml/min, respectively. The residence time was 2 min. The solution containing surfactant (solution II) was also injected to mix with solution I under subcritical water conditions. The mixture was thereafter cooled in a quenching bath (25 °C) for 10 sec. The pressure was elevated to 10 MPa by controlling the back pressure valve.

2.3 Determination of the size of the O/W emulsion
The size distribution of oil droplets in an emulsion was measured using the dynamic light scattering mode of FPAR (Ohtsuka Electronics Co. Ltd., Japan). The polydispersity (PDI) of oil droplets was also estimated from the size

Figure 1 Experimental equipment for the emulsification process in this study. Tube: inner diameter: 0.8 mm; length in oil bath and water bath were 1.41 and 0.35 m, respectively. Flow rate of solution I and II were 0.3 and 0.6 ml/min, respectively. The residence time in the oil bath was 2 min.
distribution, by using cumulant analysis. The auto-correlation function for measured scattering light intensity (G₂(τ)) is G₂(τ) = <I(t)I(t+τ)/<I(t)>², where I(t) is the scattering light intensity at time t and τ is the delay time. In the case of polydisperse materials, the auto-correlation function can be displayed as follows:

G₂(τ) = A[1 + Bg₁²(τ)]

where G(Γ) is the distribution function of the decay constant Γ. The mean value of Γ, <Γ>, and the variance μ² are defined as follows.

〈Γ〉 = ∫₀∞ ΓG(Γ)dΓ, μ² = ∫₀∞ (Γ - 〈Γ〉)²G(Γ)dΓ

Then, the polydispersity index PDI can be defined as follows and a PDI value < 0.1 signifies the monodispersity.

PDI = μ²/<Γ²

2.4 Measurement of interfacial tension

The interfacial tension of the sample was measured by using a Processor-Tensiometer K100 (KRÜSS, Germany), according to the Wilhelmy method.

3. Results and Discussion
3.1 Preparation of a fine emulsion

In the first series of experiment, we preliminarily prepared the oil droplets in the emulsion by using the continuous flow system (Figure 1). The mean diameter of the droplets in the emulsion and its polydispersity index (PDI) were determined using a dynamic light scattering method.

In the case of no surfactant in solution I and II, no emulsification was observed (entry 1), as shown in Table 1. The same was true for the case when Tween 20 was added to solution II (entry 2). This is, in both cases, because the coarse emulsion as solution I rapidly underwent phase separation before mixing with solution II. These results are in agreement with the report that the maintenance of the coarse emulsion (solution I) required heat treatment of solution I [5]. Tween 20 was then added to solution I to stabilize the coarse emulsion and the resulting size distribution is shown in Figure 2. Size distribution of emulsions obtained by using surfactants. Tween 20 (0.0017 mM) was used as the surfactant solubilized in solution I. Surfactants in solution II are Tween20, CTAB, and SDS. Pretreated by T = 240°C and 10 MPa.
Figure 2(a). The mean diameter of the oil droplets in the coarse emulsion and its PDI were 1260 nm and 0.38. Emulsification was observed in the case where the coarse emulsion (solution I) was stabilized by Tween 20 (entry 3). The size distribution curve was shifted to lower diameters (Figure 2(b), the mean diameter: 280 nm and PDI: 0.25). This indicated that the oil droplets obtained here were polydisperse. Furthermore, when Tween 20, CTAB, or SDS was added to solution II, emulsifications were observed and the size distribution curves were shifted to a lower and narrow diameter range (Figure 2(c) – (e)). Also, their PDI values were about 0.04 – 0.175 (entries 4 – 6), in contrast to entry 3. Thus, the surfactants included in solution II could improve the polydispersity of the oil droplets.

From the results, it is considered that surfactants in solution I plays a stabilizing role in the coarse emulsion (solution I) and surfactant in solution II improves the polydispersity of the emulsion spontaneously formed by mixing with solution I.

Table 1. Effect of surfactants on emulsification

<table>
<thead>
<tr>
<th>Entry</th>
<th>Surfactant A in solution I</th>
<th>Surfactant B in solution II</th>
<th>Emulsification</th>
<th>Diameter of oil droplet [nm]</th>
<th>Polydispersity index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>none</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>none</td>
<td>Tween 20</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Tween 20</td>
<td>none</td>
<td>Yes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>280</td>
<td>0.25</td>
</tr>
<tr>
<td>4</td>
<td>Tween 20</td>
<td>Tween 20</td>
<td>Yes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>238</td>
<td>0.069</td>
</tr>
<tr>
<td>5</td>
<td>Tween 20</td>
<td>CTAB</td>
<td>Yes&lt;sup&gt;c&lt;/sup&gt;</td>
<td>233</td>
<td>0.175</td>
</tr>
<tr>
<td>6</td>
<td>Tween 20</td>
<td>SDS</td>
<td>Yes&lt;sup&gt;d&lt;/sup&gt;</td>
<td>255</td>
<td>0.04</td>
</tr>
</tbody>
</table>

a: 0.0017 mM for Tween 20, b: 0.0017 and 0.0041 mM for Tween 20 in solution I and II, respectively, c: 0.0137 mM for CTAB, d: 0.0173 mM for SDS

3.2 Effect of surfactants on the preparation of the emulsion

In the case where a nonionic surfactant Tween 20 solution as solution II was mixed with solution I (water/decane + Tween 20), the mean diameter of the droplets in the emulsion definitely decreased above 0.001 mM and was 220 nm (Figure 3(a)). The obtained droplets of 220 nm in diameter were quite monodisperse. When using a cationic surfactant CTAB as solution II, the diameter of the oil droplets was 230 nm for a CTAB concentration of 0.01 mM. Besides, the threshold in Figures 3(a) and (b) was
observed also as in our previous publication [6]. When the anionic surfactant SDS was used as solution II, the mean diameter of oil droplets was constant and independent of the SDS concentrations (Figure 3(c)). It was likely that the threshold concentration for SDS was lower than the concentration range tested here. The threshold concentration is corresponded to the amount of the surfactant for monolayer coverage of the fine oil droplet [6]. It is therefore considered that SDS could effectively form the monolayer coverage layer on an oil droplet of decane, in contrast to Tween 20 and CTAB. Previously, SDS yielded finely dispersed oil droplets in the dodecane / water system [8] but not in the octanoic acid / water system [5]. Therefore, it appeared that the electrostatic interaction between solvent molecules of the oil phase and surfactants affected the diameter of the oil droplets and their polydispersity.

In general, spontaneous oil droplet formation is driven by Laplace pressure differences [3]. Therefore, the reduction in size of an oil droplet requires a decrease in interfacial tension. The interfacial tension of the emulsions obtained here was studied as a function of the surfactant concentration (Figure 4). The critical micellar concentration (cmc) for each surfactant is shown by arrows in the figure. The values of the interfacial tension at the cmc decrease in the order SDS > Tween 20 > CTAB.
A positive correlation between the interfacial tension and the oil droplet size (15–60 μm in diameter) was seen at a concentration range below the cmc and depended on the type of the surfactants [8]. It was found that a rough correlation between both was observed in this study, from a comparison of Figures 3 and 4 (data not shown). Likewise, the mean diameter of oil droplets was also plotted against the interfacial tension at the concentration range above the cmc of Tween 20. No correlation appeared between the interfacial tension and the type of surfactants (Figure 5). This result is consistent with the previous result as the the interfacial tension above the cmc depended on the type of the surfactants [8]. Besides, this result implies the possibility that oil droplets obtained here were not at steady state. In the following, we examined the stability of the oil droplet in the emulsion.

### 3.3 Stability of the O/W emulsion

The mean diameter of oil droplets just after their formation was about 200 ~ 300 nm, irrespective of the interfacial tension. However, the diameter of the oil droplets gradually increased within a day and reached 800 nm via flocculation or coalescence (data not shown). This implies that the oil droplets obtained by this method were only kinetically or temporarily stabilized by the balance between the break-up of droplets and their coalescence. To clarify the contribution of the subcritical condition to the formation of fine oil droplets, we investigated the temperature effect on the size of the oil droplets. Their size and polydispersity index were reduced with an increase in temperature (Figure 6), suggesting that subcritical water was advantageous for the break-up of the oil droplets. This result was consistent with the fact that the interfacial tension decreased with an increase in temperature [9]. From the temperature dependency of droplet size, it was considered that the oil droplet obtained under subcritical conditions was forced to be temporarily stabilized.

### 3.4 Emulsification efficiency

It is because the elevated temperature and pressure conditions was advantageous for the break-up of oil droplets that the fine droplets obtained were temporarily stabilized. The droplet size depends on the emulsification method [11]. According to the report by Mann et al. [3], the emulsification efficiency based on the energy density necessary for the emulsification can give a quantitative relationship between the
droplet size and the emulsification method. Here, the energy density is the energy input per unit volume of emulsion, \( E_V \) [kJ/m\(^3\)], as defined in the literature \([11]\). Table 2 shows a comparison of spontaneous emulsification techniques with shear based microsystems, and traditional emulsification systems (homogenizer and microfluidizers). Overall, the \( E_V \) values correspond to the mean diameter of the oil droplets from the comparison of the mean diameter of the oil droplet with the \( E_V \) value. It is considered that more energy is required to produce small droplets. We then estimated this to be \( 8 \times 10^8 \) kJ/m\(^3\) for the production of oil droplets (233 nm in diameter) under the representative subcritical condition (240 °C 10 MPa). This estimate is consistent with the relationship between the mean diameter and the \( E_V \) value. It is, hence, considered that the apparatus shown in Figure 1 can produce the required high energy density.

Table 2. Emulsification efficiency for the proposed and conventional methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean diameter</th>
<th>( E_V ) [J/m(^3)]</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edge-based droplet generation (EDGE)</td>
<td>(~ 7 \mu \text{m})</td>
<td>(3 \times 10^4)</td>
<td>[10]</td>
</tr>
<tr>
<td>Membrane emulsification</td>
<td>300 nm</td>
<td>(4 \times 10^5)</td>
<td>[4]</td>
</tr>
<tr>
<td>Y-junction*</td>
<td>(~ 4 \mu \text{m})</td>
<td>(1 \times 10^7)</td>
<td>[3]</td>
</tr>
<tr>
<td>Flat valve homogenizer</td>
<td>(~ 3 \mu \text{m})</td>
<td>(2 \times 10^7)</td>
<td>[3]</td>
</tr>
<tr>
<td>Microfluidizer</td>
<td>300 nm</td>
<td>(1 \times 10^8)</td>
<td>[3]</td>
</tr>
<tr>
<td>Subcritical water-assisted emulsification</td>
<td>233 nm</td>
<td>(8 \times 10^8)</td>
<td>This study</td>
</tr>
</tbody>
</table>

\* Original data were referred to Ph.D thesis (Wageningen University, 2009) by M.L.J. Steegmans, entitled “Emulsification in microfluidic Y- and T-junctions.” The \( E_V \) values were taken from the literature \([3]\), except for our study.

4. Conclusion

The emulsification of the decane / water system was examined under subcritical conditions. The present study clarified the relationship between the type of surfactant, the oil droplet size, and the emulsification efficiency, which is the first attempt to do so in the field of subcritical water-assisted emulsification to our knowledge. Surfactants could stabilize oil droplets and improve their dispersity, independent of the type of surfactants. In the decane / water system, the subcritical water-assisted emulsification is considered to be based on the effective supply of enough high energy density for the break-up of the oil droplets and the formation of temporarily-stabilized oil droplets of 200~300 nm diameter. The present result would give the better understanding on the formation mechanism of fine oil droplets although further investigations are required.

References