Quantitative Evaluation of the Creaming of Emulsions via Resonance-Enhanced Multiphoton Ionization Time-of-Flight Mass Spectrometry

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The creaming behavior of an oil-in-water (O/W) emulsion was quantitatively evaluated via resonance-enhanced multiphoton ionization time-of-flight mass spectrometry. Styrene O/W emulsions were prepared with initial styrene concentrations of 1 and 4 g/L, and the height at the center of the sample was monitored. A peak area of the molecular ion of styrene was set as the signal intensity, for which a time profile was constructed from a series of mass spectra. As a result, the averaged time profiles showed that the signal intensities increased once and then decreased with the onset of creaming. In addition, in order to fit an experimentally obtained time profile, a modified fit function was proposed. Based on the fit results, the ratios of the increases and decreases in signal intensities were different between the two emulsions—higher in the case of an O/W emulsion with a higher initial oil concentration. On the other hand, the duration of the enhancement of the signal intensity with the onset of creaming was independent of the initial oil concentration. The present method offers the possibility to quantitatively evaluate the creaming behavior of an emulsion without pretreatment, and, therefore, would be useful for confirming the stability and quality assurance of emulsions.

Keywords REMPI-TOFMS, O/W emulsion, creaming, fit function

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Introduction

An emulsion involves immiscible liquids in a system wherein small droplets of one liquid are dispersed in another. There are two types of emulsions: an oil-in-water (O/W) emulsion involves oil droplets dispersed in water; a water-in-oil (W/O) emulsion is the opposite. One or more surfactants are generally applied to the emulsification of such immiscible liquids.

Creaming is one of the most important collapse processes of an emulsion where droplets are moved to the upper or lower portions with time, which occurs due to differences in the densities of liquids during the dispersed and continuous phases. The creaming behavior is affected by several factors such as the types and concentrations of the constituents. In addition, even if the contents are the same, the creaming behavior can either be changed by preparation conditions such as stirring or at least be affected by the size and the shape of a container. Therefore, the evaluation of creaming is very important for quality control and for improving the functionality of an emulsion.

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The creaming rate of isolated spherical particles in an infinite medium is described via Stokes’ velocity.1–3 Robins reviewed the creaming behaviors of monodispersed and polydispersed emulsions and depicted oil concentration profiles during creaming and noted the positions of the lower boundaries with time.3 Creaming behavior can be simply confirmed via the naked eye, and can be measured by analytical methods4 such as ultraviolet/visible spectrophotometry (UV-vis),5 backscattering,6,7 ultrasound velocity scanning,8 and rheology.9 Among these, turbidity evaluation via transmittance measurement using UV-vis has been the most widely applied. However, the transmittance measurement of an emulsion with high turbidity is difficult. Though dilution is a manner by which the turbidity can be decreased, emulsion properties such as creaming should completely change the original state of an emulsion. Therefore, an analytical method that could directly evaluate creaming behavior is needed, and this would be quite useful for studying the relationships between each factor concerning emulsions as well as for improving stability.

Resonance-enhanced multiphoton ionization time-of-flight mass spectrometry (REMPI-TOFMS) is a highly sensitive and selective analytical means of photo-absorbing analytes.10–12 This technique has been used in a broad range of applications such as spectroscopic study10–12 and environmental analysis.19–22 Recently, we applied REMPI-TOFMS to the analysis of an emulsion without pretreatment.23–25 In this technique, an emulsion is flowed through a capillary column and is continuously introduced into TOPMS. Then a series of mass spectra are recorded, the peak areas of a target species are extracted from the series of mass spectra, and finally a time profile of the peak areas can be constructed. In previous studies measuring an O/W emulsion containing oil droplets with diameters of a few micrometers or more, spikes for the oil component were observed on the time profile.26 Moreover, a linear relationship between the concentration of the oil component in an O/W emulsion and the average of the peak areas for the oil were demonstrated.27 Furthermore, REMPI-
TOFMS was applied to evaluate the creaming behavior of an O/W emulsion. Until now, different behaviors were confirmed between the change in turbidity, which was monitored by transmittance measurement using UV-vis, and the change in the concentration of oil, which was measured via REMPI-TOFMS. This is because turbidity depends on the number and size of small droplets, which is not necessarily directly proportional to the concentration of the oil. Very recently, using an O/W emulsion that is prone to creaming behavior, we demonstrated the differences in the time profiles of an oil component at different monitoring heights, and proposed a fit equation for the time profiles that would allow a quantitative evaluation of the creaming behavior.

In the present study, the fit function that was previously proposed was modified to allow a general-use evaluation of the creaming behavior of an emulsion. O/W emulsions with different initial oil concentrations were measured without pretreatment, and the changes in the oil concentration arising from creaming were discussed.

**Experimental**

**Reagents and sample preparation**

Styrene (density 0.902 – 0.910 g/cm³, Wako Pure Chemical Industries, Osaka) and sodium dodecyl sulfate (SDS, Wako Pure Chemical Industries) were used as the oil phase and the emulsifier, respectively. Styrene was used as an analyte species for the detection by REMPI using a nanosecond UV laser, as described below. Distilled water was produced in our laboratory.

In the present study, O/W emulsions with styrene concentrations of 1 or 4 g/L were prepared. The preparation procedure was similar to that in a previous report. First, 0.12 g of SDS was dissolved in 40 mL of water in a 50-mL vial container. Next, either 44 or 176 μL of styrene, for emulsions with concentrations of either 1 or 4 g/L, respectively, was added, and processed in a homogenizer at 5000 rpm for 10 min. The final height of the sample in the vial container was ca. 5 cm.

**REMPI-TOFMS**

Figure 1 shows the experimental apparatus. The REMPI-TOFMS used in the present study was similar to that used in the previous study, and is briefly described here. The fourth harmonic emission of a Nd:YAG laser (GAIA II, 266 nm, 4 ns, 10 Hz, Rayture Systems, Tokyo) was used as an ionization light source for REMPI. The pulse energy was adjusted to 20 μJ. The laser pulses were focused using a plano-convex lens (f = 200 mm) for TOFMS.

For the sample introduction, a technique consisting of a pair of concentric fused-silica capillary columns (deactivated, GL Sciences, Tokyo) was applied. The inner diameter, the outer diameter, and the length of the inner capillary was 25 μm, 150 μm, and 60 cm, and that of the outer capillary was 320 μm, 450 μm, and 40 cm, respectively. Ambient air was introduced via the outer capillary; flow meter (Kofloc, Kyoto) was used to adjust the flow rate to 2 mL/min. Immediately after the sample preparation, an inner capillary was inserted into the sample through a hole in the lid of a vial container, and the emulsion sample was then stirred at 500 rpm using a magnetic stirrer. Simultaneously, recording of the mass spectra was started using a digitizer (AP240, bandwidth 1 GHz, sampling rate 1 GS/s, Acqiris/Agilent Technologies, Tokyo). The sampling position for the tip of the inserted inner capillary was adjusted to 2.5 cm from the bottom of the middle of the container. The laser pulses were not introduced into a TOFMS for the first 30 s after starting the recording in order to determine the ground (zero) level. The stirring was stopped 10 min after starting the recording, which was the beginning of creaming. A peak for the molecular ion of styrene (m/z 104) was set as the signal intensity, for which a time profile was constructed from a series of mass spectra. As described later, not only this raw time profile but also a time profile that represents the average of every 2 min (every 1200 plots) was plotted. The latter time profile was fitted by an equation proposed in the present study using scientific data-analysis software (Origin, LightStone, Tokyo).

**Results and Discussion**

**Visual observation of prepared emulsions**

The outstanding characteristics of the prepared O/W emulsions are shown in Fig. 2. These emulsions were homogenized for 10 min in the preparation process, and then stirred for another 10 min using a magnetic stirrer. The times displayed in the figures indicate the times after stirring was stopped. At the moment the stirring was halted (0 min), the O/W emulsion with a styrene concentration of 1 g/L was translucent, and that with a styrene concentration of 4 g/L had higher turbidity. Incidentally, the white foam observed at the top of the former emulsion was caused by bubbling rather than by an oil component of creaming. These emulsions showed creaming behavior toward the upper side because the density of styrene was lower than that of water and, as a result, the turbidity of both emulsions was decreased at the middle position in terms of height.

**REMPI-TOFMS**

The time profiles of the signal intensities of styrene in O/W emulsions with concentrations of 1 or 4 g/L are shown in Fig. 3. In the present study, an emulsion was stirred for 10 min after the insertion of the capillary column for sample introduction. In addition, a few minutes are typically required for passage through the capillary column to confirm the signal. Based on the results, when the stirring of the sample was stopped, i.e., the onset of creaming, was set as 0 min in the figure.

As shown by both of the time profiles in Fig. 3, many intense signals appeared after the creaming began, which suggests the introduction of large oil (styrene) droplets. Specifically, in both time profiles, intense signals composed of multiple plots were confirmed at the beginning of the creaming, and then intense spike signals composed of a single plot gradually began to appear with time. The appearance of intense signals composed of multiple plots can be considered as follows. When the
creaming started, large oil droplets reached the height of the monitoring position from the lower side at first, and then large numbers of such droplets were introduced into the capillary column. Some of these droplets were considered to be aggregated during passage through the capillary; such aggregation at the beginning of the creaming was reported in a previous paper. As a result, intense signals composed of multiple plots appeared at the beginning of the creaming.

Figure 4 shows the averages of the time profiles for every 2 min, which were processed from the data in Fig. 3. The previous study used a styrene O/W emulsion, and a linear calibration curve was obtained between the concentration of styrene and the peak area extracted from a series of mass spectra. In the same way, we assumed there was a linear relationship between the averaged signal intensity and the styrene concentration in the present study. Here, an emulsion sample was first stirred for 10 min, and at that point the obtained signal intensities roughly reflected the initial concentrations of styrene—1 and 4 g/L. Average signal intensities of ca. 15 and 60 were obtained during stirring. These assumptions and results are reflected in the calculations for concentrations that are indicated in the right-hand side of each figure.

In Fig. 4(a), the signal intensity of styrene in an O/W emulsion with a styrene concentration of 1 g/L was slightly increased at first, and was then roughly maintained for 14 min. On the other hand, as shown in Fig. 4(b), in the case of the O/W emulsion with a styrene concentration of 4 g/L, the signal intensity broadly fluctuated at that time. As shown in Fig. 3(b), many intense signals were detected at that time, and as a result, the fluctuation shown in Fig. 4(b) persisted even after averaging. In both of the time profiles shown in Fig. 4, the signal intensities were then decreased, but certain signal intensities remained.

**Fitting**

In the previous report, we proposed a fit function for fitting the signal behavior obtained from an emulsion during creaming. In that study, we used this fit function to evaluate the creaming of O/W emulsions measured at different monitoring heights. In the present study, as follows, a modified fit function was proposed for fitting the obtained time profiles shown in Figs. 4(a) and 4(b), and a constant, \( I_0 \), was added to the previous fit equation.

\[
I = I_0 + \frac{A_1}{1 + e^{-a_1(t-\gamma_1)}} - \frac{A_2}{1 + e^{-a_2(t-\gamma_2)}}
\]

The fit results of both experimentally obtained time profiles are shown in Fig. 4(c), and the calculated coefficients are included in Table 1. In the present study, the plots obtained at –8 to –2 min were used for the fitting, even though the sample was stirred during the period, because only a few plots indicated the tendency of a signal rise and the fit results did not accurately reflect the experimentally obtained time profiles. The fit results obtained before creaming are indicated as dashed lines.

The newly added constant, \( I_0 \), reflects the signal intensity...
Incidentally, the slope at the inflection point can be calculated using the parameter related to the slope at the inflection point, and the sigmoid curve. A logistic curve is a common model of two logistic functions, which are the same as the fit equation mentioned previously, and the values were manually substituted for the values of $I_0$.

For fitting, a value of 240 was tentatively manually substituted for $A_1$ for the time profile of an O/W emulsion with a styrene concentration of 4 g/L, because the fit did not accurately reflect the experimentally obtained time profile when the value was automatically calculated.

According to the resultant fit curves, the signal intensities increased once the creaming started. An O/W emulsion with a concentration of 1 g/L maintained the increase in signal intensity for a while, but then it decreased. The fit corresponded well with the experimentally obtained time profile. For an O/W emulsion with a concentration of 4 g/L, the fit also indicated that the signal intensity increased and then decreased. The signal intensities fluctuated heavily, as shown in Fig. 4(b), even when reflecting an average for every 2 min, and, therefore, the fit was dependent on the degree of averaging. In all cases, the initial increase in the concentration caused an increase in the signal intensity even though no increase in turbidity could be visually confirmed, as shown in Fig. 2.

Several interesting conclusions were reached as a result of the fits. First, as mentioned previously, the signal intensity increased once, and the maximum intensities were found to be 50 (obtained at around 5 min) and 289 (obtained at 8 min) based on each of the fit results. The averaged signal intensities obtained before the creaming for styrene O/W emulsions with concentrations of 1 and 4 g/L were 15 and 60, respectively, during stirring as ca. 15 and 60, respectively, and, therefore, the signal intensities were found to have increased roughly 3- and 5-fold, respectively. Therefore, the increase in signal intensity was relatively greater in the case of the O/W emulsion with a concentration of 4 g/L, which also was the O/W emulsion with a higher initial concentration.

With respect to the signal intensity, the value of $A_1$ is also worthy of consideration. The values of $A_1$ were calculated as 15 and 5 from the fit results for O/W emulsions with concentrations of 1 and 4 g/L, respectively. Both values were positive, and therefore, after a sufficient amount of had time elapsed ($I_\infty$, which means the signal intensity at $t = \infty$) the signal intensity was higher than that at $t = 0$, i.e., $I_0$, in each experiment. Here, the signal intensities, $I_0$ and $I_\infty$, were discussed. In the case of an O/W emulsion with a styrene concentration of 1 g/L, $I_0 = 15$ and $I_\infty = I_0 + A_1 - A_2 = 30$. As a result, the value of $I_\infty$ was twice that of $I_0$. These results indicate that, when the creaming of the present O/W emulsion fully proceeded, the concentration of styrene at the monitoring position became 2 g/L, which was twice the initial concentration (1 g/L). On the other hand, in the case of an O/W emulsion with a styrene concentration of 4 g/L, the initial signal intensity ($I_0 = 60$) and the final signal intensity ($I_\infty = 60 + 5 = 65$) was almost the same, which means the initial and final concentrations were also almost the same. Furthermore, based on the above results, the ratios of the increase and the decrease in the styrene concentration were small in the case of an O/W emulsion with an initial styrene concentration of 1 g/L, but those changes were large in the case of an O/W emulsion with a styrene concentration of 4 g/L.

The accuracy of the parameter, $a$, remained ambiguous in the

### Table 1: Calculated coefficients in the fit equation for the time profiles shown in Fig. 4(c)

| Concentration/g L\(^{-1}\) | $I_0$ | $A_1$ | $a_1$ | $\gamma_1$ | $A_2$ | $a_2$ | $\gamma_2$ |
|-----------------------------|------|------|------|-----------|------|------|-----------|
| 1                           | 15\(^a\) | 35    | 6.1  | 2.0       | 20   | 1.5  | 15.9      |
| 4                           | 60\(^a\) | 240   | 1.0  | 3.5       | 235  | 0.4  | 16.2      |

\(^a\) $I_0$ for both concentrations and $A_1$ for 4 g/L were manually substituted.

![Fig. 4](image-url) Averaged time profiles of the signal intensities of styrene in an O/W emulsion. Initial oil concentrations: (a) 1 g/L, (b) 4 g/L. (c) Fit results of the averaged time profiles (a) (red) and (b) (black). The fit results from –8 to –2 min are indicated as dashed lines because these results were obtained before the onset of creaming (see text).
present study because only a few plots were available for fitting. Based on that fact, it was interesting that in both fits the values of $a_2$ were smaller than those of $a_1$. Also, the slope of the inflection point when the signal intensity fell was gentler compared with that when the signal intensity rose. When a monodispersed styrene O/W emulsion is measured, the concentration should sharply decrease around the boundary between the upper (oil) layer and lower (water) layer. On the other hand, the emulsions prepared in the present study should probably have been considered polydispersed. When the signal intensity falls, the slope should be gentler for an emulsion that is polydispersed. Based on the fit results, we assumed that large oil droplets passed rapidly through the monitoring point at first, and then the smaller oil droplets began to continuously reach the monitoring point.

The time of inflection point $\gamma$ suggests that the value of $\gamma_2 - \gamma_1$ can be regarded as indicating the duration of an increase in the signal intensity at the early stage of creaming. In the present study, the values of $\gamma_2 - \gamma_1$ were $13.9$ and $12.7$ min for O/W emulsions with concentrations of 1 and 4 g/L, respectively. Therefore, there was little difference between them, while the initial concentration was 4-fold different between the two emulsions. In the previous paper, the corresponding duration times were different between the heights of the monitoring positions—the duration was longer for a higher monitoring position. By contrast, the duration at the monitoring position showed almost no dependence on the initial concentration of the oil in the present study.

Conclusions

In the present study, O/W emulsions with different initial styrene concentrations were directly measured via REMPI-TOFMS, and the time profiles for the signal intensities of styrene were fitted using a modified fit function. In both emulsions, the signal intensity increased once with a similar duration, and then decreased with the onset of creaming. On the other hand, the ratios for the respective increases and decreases in the signal intensities were different—small in the case of an O/W emulsion with a styrene concentration of 1 g/L; large in the case of an O/W emulsion with a styrene concentration of 4 g/L. The signal behavior should differ according to differences in the height of the monitoring point, or be different if a preparation procedure such as the stirring condition is changed. In this manner, the creaming behavior of an emulsion is easily changed, and, therefore, the evaluation is quite important. However, the measurement of an emulsion with high turbidity is difficult via transmittance measurements using UV-vis. Moreover, it is quite difficult to pre-treat an emulsion that undergoes creaming. On the other hand, the present method using REMPI-TOFMS can be applied to the measurement of an emulsion even without pretreatment, and therefore would be useful for a quantitative evaluation of the creaming behavior.

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