ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Using Resonance-Enhanced Multiphoton Ionization Time-of-Flight Mass Spectrometry to Evaluate the Movement of a Constituent in a Multiple Emulsion

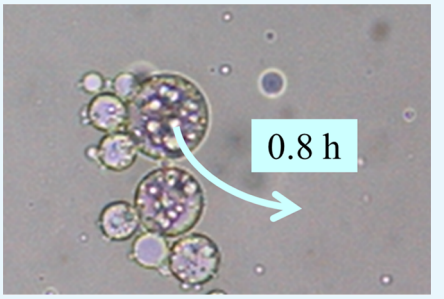
Tomonobu Sugiyama, Minori Minami, and Tomohiro Uchimura*

Cite This: *ACS Omega* 2022, 7, 2099−2104

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Herein, we propose a method for evaluating the movement of a constituent in a multiple emulsion while maintaining its original dispersed condition. In this study, an oil-in-water-in-oil ($O_1/W/O_2$) emulsion was prepared using a two-step emulsification method with styrene as an analyte species in the inner phase (O_1). The emulsion was measured using resonance-enhanced multiphoton ionization time-of-flight mass spectrometry without pretreatment such as centrifugation. From a series of obtained mass spectra, a time profile for the peak areas arising from styrene was constructed. When the emulsion was measured immediately following preparation, a time profile composed of a base, positive, and negative signals confirmed the presence of styrene in the O_2, O_1, and W phases, respectively. Moreover, while a small amount of styrene was present in the inner O_1 phase, almost all of the styrene was found in the outer O_2 phase. Furthermore, the results of the obtained time profile were converted into a box plot, and a method for the selection of the base, positive, and negative signals was tentatively determined. Then, the movement of styrene among the phases could be evaluated using the time courses of these signals; the time constant of the movement of styrene from an O_1/W droplet to the O_2 phase was calculated to be 0.8 h.



INTRODUCTION

Emulsions are composed of liquids that are immiscible. An example is water and oil where one liquid is dispersed in the other liquid as small droplets. These systems are classified as either an oil-in-water (O/W) type where small oil droplets are dispersed in water or vice versa, which is a water-in-oil (W/O) type. In addition, there are multiple emulsions, the simplest of which is sometimes called double emulsions that are composed of three phases: inner, middle, and outer. Oil-in-water-in-oil ($O_1/W/O_2$) emulsions are formed when water droplets containing an inner O_1 phase are dispersed in an outer O_2 phase, and water-in-oil-in-water ($W_1/O/W_2$) emulsions involve oil droplets containing an inner W_1 phase dispersed in an outer W_2 phase.[1] Multiple emulsions have a release property, whereby a constituent in an inner phase is intended to be released to an outer phase over time.[2] Many studies have focused on controlling the release behavior.[3−6] Due to such property, multiple emulsions have a wide range of applications[1] that include delivery systems for active ingredients and drugs in the cosmetics and pharmaceutical fields[7−9] and as carriers of vitamins in food and beverages.[10,11] Therefore, it is important to understand the time courses of the movements of constituents among phases in a multiple emulsion for the development and the quality control of these types of products. The measurement of absorbance is often used to evaluate the release behavior; in this case, at first, an outer phase and other phases are separated beforehand via centrifugation, and then the absorbance of the outer phase can be measured.[12] In this way, the movement from an inner phase to an outer phase with time can be easily evaluated. However, the dispersed condition and characteristics of a multiple emulsion are dramatically changed once it is pretreated. Therefore, a method that requires separation processes in advance is not necessarily applicable to evaluating the release behavior as an inherent dispersed condition of multiple emulsions.

Resonance-enhanced multiphoton ionization time-of-flight mass spectrometry (REMPI-TOFMS) using ultraviolet laser pulses is a highly selective analytical method for analyzing a sample with a complex mixture.[13−17] For example, real-time analysis of volatile compounds generated from roasting coffee[18] and of aromatic compounds in the environment[19] have been reported. Recently, we used REMPI-TOFMS for real-time monitoring of an emulsion without pretreatment.[20−26] In that case, the time profile of a peak area of a species of analyte was obtained from a series of mass spectra. Until now, when an O/W emulsion containing a hydrophobic compound (toluene) as a species of analyte was measured, a

Received: October 8, 2021
Accepted: December 24, 2021
Published: January 4, 2022

https://doi.org/10.1021/acsomega.1c05599
ACS Omega 2022, 7, 2099−2104

base signal and sudden positive signals based on the base signal appeared on the time profile. When a W/O emulsion was measured, however, a base signal and sudden negative signals based on the base signal were obtained.[25] Each base signal arose from the continuous detection of an analyte present in the respective outer phase, i.e., either the aqueous phase of an O/W emulsion or the oil phase of a W/O emulsion. In addition, sudden positive signals indicated the existence of local regions with a higher concentration of analytes, i.e., oil droplets of an O/W emulsion, and sudden negative signals indicated the existence of local regions with a lower concentration of analytes, i.e., water droplets in a W/O emulsion. In this manner, the possibility of the detection of each phase in an emulsion comprising two phases could be demonstrated without pretreatments such as centrifugation and dilution. Moreover, we directly measured multiple emulsions and proposed a method for the evaluation of aging.[21] However, the kinetics of the movement of a species of analyte among phases was not achieved.

In the present study, we proposed a methodology for the quantitative evaluation for the time course of the movement of a constituent among phases in a multiple emulsion. An $O_1/W/O_2$ emulsion was prepared via a two-step emulsification method, which is a typical method for creating multiple emulsions,[1,27,28] and a prepared emulsion was then measured via REMPI-TOFMS without pretreatment. The obtained time profile for an analyte species could then be converted into a box plot and could be simply classified as a base, positive, or negative signals, which were assumed to have arisen from the analyte species in one of three phases: the outer phase (O_2), the inner phase (O_1), or the middle phase (W). Moreover, the kinetics of movement for the species with time was demonstrated.

EXPERIMENTAL SECTION

Reagents and Preparation of an $O_1/W/O_2$ Emulsion. To prepare an $O_1/W/O_2$ emulsion, styrene, a sodium chloride aqueous solution, and cyclohexane were used as an inner O_1 phase, a middle W phase, and an outer O_2 phase, respectively. Two nonionic surfactants, Tween 20 and Span 80, were used as emulsifiers; the values of hydrophile−lipophile balance (HLB) were 16.7 and 4.3, respectively. It was reported that O/W emulsions prepared with Span and Tween blends are more stable than O/W emulsions prepared with either a Span or a Tween alone,[29] and therefore, we used these two surfactants as a reference, while the HLB values of mixed surfactants described below were tentatively determined by confirming the stability of the dispersion and of the droplet shape for a given experiment time (see Figure 2). Styrene, sodium chloride, cyclohexane, Tween 20, and Span 80 were all purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Purified water was acquired in-house using our laboratory.

A two-step emulsification method was applied for the production of an $O_1/W/O_2$ emulsion. In the initial step, solutions for the O_1 and W phases were prepared. The O_1 phase involved dissolving 0.06 g of Span 80 in 2 mL of styrene in a 50 mL vial container. For the W phase, 0.14 g of Tween 20 was dissolved in 20 mL of a 0.2 g/20 mL sodium chloride aqueous solution. The solution for the W phase was then dropped into the solution for the O_1 phase, the speed of which was ca. 1.7 mL/s, with mixing using a homogenizer (4000 rpm). With constant stirring for 5 min, an O_1/W emulsion was obtained through a phase-inversion process. The concentration of styrene was 100 g/kg where the denominator of the unit was the weight of water. The HLB value of mixed surfactants was set to be 13.

In the second step, the solution for an O_2 phase was prepared by dissolving 0.06 g of Tween 20 and 0.14 g of Span 80 in 25.67 mL of cyclohexane in a 50 mL vial container; the HLB value of the mixed surfactants was 8. The O_1/W emulsion prepared in the first step was then added to the solution for the O_2 phase with stirring using a magnetic stirrer (1500 rpm). The ratio of both liquids was adjusted to 1:99 (v/v). By stirring for 30 min, an $O_1/W/O_2$ emulsion was obtained. The concentration of styrene in the emulsion was 0.75 g/L where the denominator of the unit was the sum of the volumes of the water, styrene, and cyclohexane before mixing, which practically amounted to a volume of cyclohexane that was predominantly the largest of them.

Optical Microscope and Particle Size Analyzer. An inverted optical microscope (ECLIPSE TE2000-U, Nikon, Tokyo) with a 20× objective (NA = 0.45, Plan Fluor) was equipped with a digital camera (DIGITAL SIGHT DS-U1, Nikon) and was used to confirm the phase condition of a prepared $O_1/W/O_2$ emulsion. In addition, as a reference, the droplet sizes of particles in an emulsion were measured by dynamic light scattering (DLS) using a fiber-optics particle analyzer (FPAR-1000, typically ∼5 μm, Otsuka Electronics, Osaka, Japan).

REMPI-TOFMS. A schematic of the REMPI-TOFMS setup used for this study is shown in Figure 1. The details of the instrument were reported previously,[20,25] and are only briefly described here. The TOFMS setup was developed by Kyushu University, Japan. A technique that uses a pair of concentric capillary columns was used for the sample introduction.[20] Inner and outer capillary columns were both inactivated fused silica capillary columns (GC Sciences, Tokyo). The length and inner and outer diameters for the inner capillary were 45 cm, 25 μm, and 150 μm, and those for the outer capillary were 22 cm, 320 μm, and 450 μm, respectively. These capillaries and a stainless steel tube for a flow of ambient air were connected using a union tee (SS-100-3, Swagelok). Ambient air was introduced from an outer capillary column; a flow meter was used to adjust the flow rate to 2 mL/min. The pressure of the vacuum chamber was ca. 1×10^{-2} Pa during the measurement. The mass resolution was typically 140 at m/z 104 when measuring an $O_1/W/O_2$ emulsion. The temperature of the inlet nozzle was kept at 40 °C.

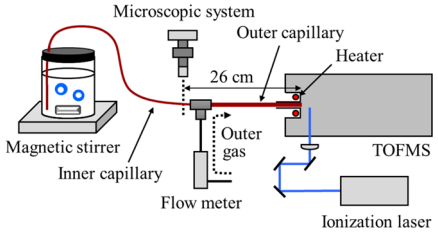


Figure 1. Schematic diagram of the REMPI-TOFMS setup used in the present study.

The prepared sample was measured at 0, 1, 2, 4, and 6 h after the preparation. First, by inserting an inner capillary column into an $O_1/W/O_2$ emulsion through a hole in the lid of a vial container, the sample just after the preparation flowed

https://doi.org/10.1021/acsomega.1c05599
ACS Omega 2022, 7, 2099−2104

through the capillary where it was subjected to TOFMS via the evacuation force of TOFMS vacuum. The position of the tip of the inner capillary was adjusted to 1 mm above the bottom of the vial container. To monitor an emulsion passing through the inner capillary column, a microscopic system was constructed;[22] this was composed of an objective (G plan Apo 20×, NA 0.28, Mitutoyo) and a digital camera as described previously. The observation position was 26 cm away from the tip of the outer capillary column at the TOFMS side. The fourth harmonic emission of a Nd:YAG laser (GAIA II, wavelength 266 nm, pulse width 4 ns, repetition rate 10 Hz; Rayture Systems) was used for the REMPI of the analyte species (styrene). The pulse energy was adjusted to ca. 10 μJ, and the laser pulses were focused using a plano-convex lens (f = 200 mm). The ion signals were recorded using a digitizer (AP240, sampling rate 1 GS/s, bandwidth 1 GHz; Acqiris/Agilent Technologies). A sample was measured for 5 min; the recording was begun with the simultaneous insertion of the inner capillary column into a sample. After the measurement, the inner capillary was pulled out, and the hole in a lid was covered using parafilm until the next measurement to suppress the vaporization of the sample. To suppress the creaming of the emulsion for measurements, the sample was gently stirred using a magnetic stirrer (300 rpm) for 6 h after the preparation was completed. The measurements were performed in triplicate.

The peak areas for styrene, which included the molecular ion peak (m/z 104) and the fragment ion peak (m/z 103), were extracted from the mass spectrum, and a time profile was constructed from the series of mass spectra. The time interval between the plots on a time profile was 0.1 s, which was determined by the repetition rate of the ionization laser (10 Hz). Each individual signal intensity of a time profile was divided by the averaged signal intensity (calculated from the data for 80−300 s) for normalization. As will be described later, the results of the time profiles were converted into a box plot (Figure 4). The box plot shows the first (Q_1), median (Q_2), and third quartiles (Q_3) of the entire dataset. The interquartile range (IQR) is $Q_3 - Q_1$, and the upper and lower whiskers with distances 1.5 times that of the IQR are illustrated. The cross mark in the box indicates the average value of the dataset.

RESULTS AND DISCUSSION

Microscopic Image and Droplet Size of the Prepared Emulsion. Figure 2 shows the microscopic images of an $O_1/W/O_2$ emulsion immediately following preparation and 6 h later. In both images, many water droplets (a middle phase) appear to contain several smaller oil droplets, i.e., O_1/W droplets can be seen. In this manner, the $O_1/W/O_2$ emulsion was prepared. As a reference, the average diameters of O_1 droplets and O_1/W droplets of the biggest three droplets in this field of view in Figure 2a were ca. 10 and 30 μm, respectively. Incidentally, when using only the naked eye, several water droplets seemed to show no oil droplets. There was almost no characteristic difference between the emulsions immediately following preparation (Figure 2a) and 6 h later (Figure 2b).

The droplet size distribution of an emulsion immediately following the preparation and 6 h later is shown in Figure S1. The average size is indicative of O_1/W droplets, which displayed little difference between the sample immediately following the preparation and 6 h later (19.7 and 19.0 μm, respectively).

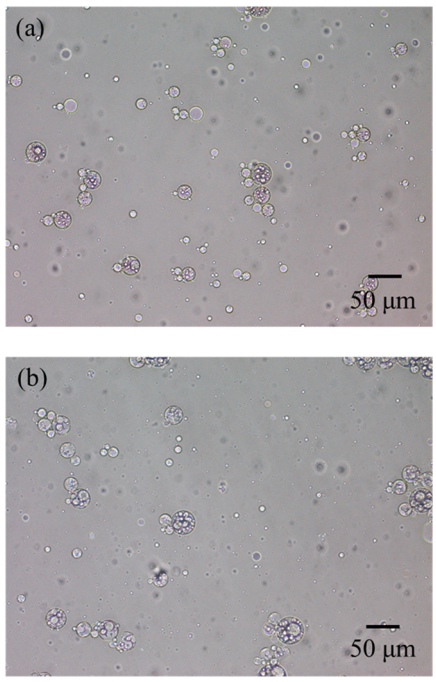


Figure 2. Microscopic images of an $O_1/W/O_2$ emulsion. Times following the sample preparation: (a) 0 h and (b) 6 h.

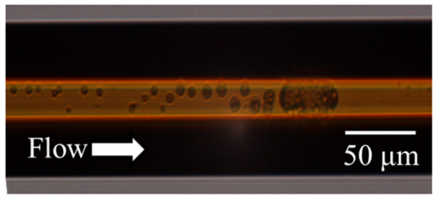


Figure 3. Microscopic image of an $O_1/W/O_2$ emulsion flowing through an inner capillary column. The time after the sample preparation was 0 h.

Emulsion Flow through the Capillary Column. Figure 3 shows a microscopic image of droplets in an $O_1/W/O_2$ emulsion flowing through an inner capillary column during the sample introduction to REMPI-TOFMS. This image confirms the passage of several O_1/W droplets, which were gathered to some extent, through the capillary column. Among them, the front O_1/W droplet was the largest—larger than the inner diameter of the column (25 μm). The diameters of the subsequent O_1/W droplets were less than ca. 10 μm. Similar flows of gathered O_1/W droplets, albeit not exactly the same, were repeatedly confirmed, though the timing between them was random.

Time Profile for Styrene Measured via REMPI-TOFMS. The time profiles for the peak areas for styrene in an $O_1/W/O_2$ emulsion (0 and 6 h after the preparation) measured via REMPI-TOFMS are shown in Figure 4. In all measurements, signals were detected several tens of seconds after starting the recording; this indicates the time needed for the passage of an $O_1/W/O_2$ emulsion through an inner capillary column. The linear velocity was calculated to be ca. 7 mm/s based on the time and the length of the inner capillary (45 cm).

The time profile shown in Figure 4a exhibits a base signal with a certain intensity and positive and negative signals based

https://doi.org/10.1021/acsomega.1c05599
ACS Omega 2022, 7, 2099−2104

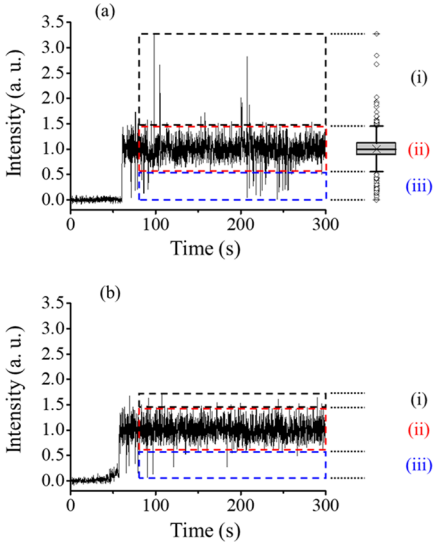


Figure 4. Time profiles of the peak areas of styrene. Times after the sample preparation: (a) 0 h and (b) 6 h. (i) The region where positive signals based on a base signal appear; (ii) the region where base signals appear; and (iii) the region where negative signals based on a base signal appear.

on the base signal. These signals can be interpreted as follows. The base signal mainly signified the detection of styrene in the outer O_2 phase. More to the point, the intensity of the base signal suggested a particular concentration of styrene in the O_2 phase, which will be described later. As shown in Figure 4a, the base signal had a certain level of intensity immediately following preparation. In other words, when the $O_1/W/O_2$ emulsion was prepared, most of the styrene was expected to be located in the inner O_1 phase but it actually existed mostly in the outer O_2 phase.

The appearance of positive signals signified a rapid increase in the concentration of styrene at an ionization point compared with when styrene is normally detected in an O_2 phase. Therefore, a positive signal was regarded as the proof of styrene concentration in one or more O_1/W droplets, or perhaps that styrene was substantially concentrated in one or more O_1 droplets. Needless to say, a positive signal could also mean the presence of styrene in the O_2 phase to some extent if an O_1/W droplet is smaller than the diameter of the inner capillary column used for the sample introduction (25 μm in the present study). In any case, a positive signal should appear when O_1 droplets containing styrene in concentrations higher than that of the peripheral region are introduced into TOFMS. Though the positive signals clearly appeared on the time profile of an $O_1/W/O_2$ emulsion immediately following preparation (Figure 4a), those signals were hardly detected from an $O_1/W/O_2$ emulsion 6 h later (Figure 4b). This result suggests that the styrene concentration was eliminated in the O_1 phase to some extent due to the movement of styrene from the O_1 phase to the O_2 phase over time.

A negative signal based on a base signal indicates that the concentration of styrene at an ionization point is, at the moment, lower than the concentration of styrene in the O_2 phase. In our previous paper, when a W/O emulsion was continuously measured, negative signals based on a base signal were obtained.[25] The emulsion contained toluene as an analyte species, and therefore, negative signals indicated the existence of less-concentrated regions of toluene, i.e., water droplets. In the same manner, the present study showed negative signals appearing due to small amounts of styrene in the O_1 phase of an O_1/W droplet. In addition, as shown in Figure 2, when water droplets have no O_1 droplets detectable by the naked eye, negative signals should be obvious. This result shows that negative signals arise mainly from a middle W phase that contains the presence of less-concentrated styrene. As shown in Figure 4, several negative signals with an intensity of almost zero were detected. In the present study, the inner diameter of the inner capillary column used for the sample introduction was 25 μm. Therefore, such negative signals could have arisen from water droplets that contained only a trace of styrene and had diameters of $\geq$25 μm. Incidentally, 6 h after preparation such negative signals were still confirmed from an $O_1/W/O_2$ emulsion, which suggests that a W phase with almost no styrene was retained even after 6 h.

Time Course of Styrene in Each Phase. As shown in Figure 4, when an $O_1/W/O_2$ emulsion was measured via REMPI-TOFMS, the base, positive, and negative signals appeared on the time profile of styrene, and are believed to have arisen from styrene in the O_2, O_1, and W phases, respectively. Next, using the results of the time profiles, we proposed a new analytical method for the evaluation of the movement of styrene among phases with time.

The time profile was studied to determine a method that could unambiguously derive the base, positive, and negative signals. First, the data from each of the time profiles of the signal intensities obtained at 80−300 s were converted into the box plot that appears on the right-hand side in Figure 4. These signals represent the points at which the emulsions were stably introduced. In the present study, a positive signal was tentatively assigned to the signal with a signal intensity that was higher than that of the upper edge of the upper whisker (i), and a negative signal was tentatively assigned to the signal with a signal intensity that was lower than that of the lower edge of the lower whisker (iii). A base signal was, of course, one that ranged between the upper edge of the upper whisker and the lower edge of the lower whisker (ii).

An example of the box plots obtained from each time profile following the sample preparation is shown in Figure 5a. From this result, the average intensities of the positive, base, and negative signals (I_{O1}, I_{O2}, and I_W, respectively) were calculated for each time. In the present study, all of the experiments were performed in triplicate, and each of the average intensities was also obtained as the mean ± standard deviation. The time course of the average intensities of the positive, base, and negative signals are shown in Figure 5b. The average intensities of the base signal (I_{O2}) were nearly constant during the measurement; in Figure 5b, a line of I_{O2} = 1 is depicted for guidance. The average intensity of 1 following normalization meant the concentration of styrene in the O_2 phase equaled that of the initial concentration of styrene (0.75 g/L). These results suggest that styrene appeared immediately following the preparation in both the inner O_1 and outer O_2 phases. In addition, the prepared $O_1/W/O_2$ emulsion was composed of mainly the O_2 phase. Therefore, the signal intensity of styrene in the O_2 phase was nearly constant because of the large capacity of the O_2 phase, while styrene was transferred from the O_1 phase to the O_2 phase by a concentration gradient.

In Figure 5b, the average intensity of the positive signals (I_{O1}) decreased to a certain value asymptotically with time, and that of the negative signals (I_W) seemed to slightly increase to a

https://doi.org/10.1021/acsomega.1c05599
ACS Omega 2022, 7, 2099−2104

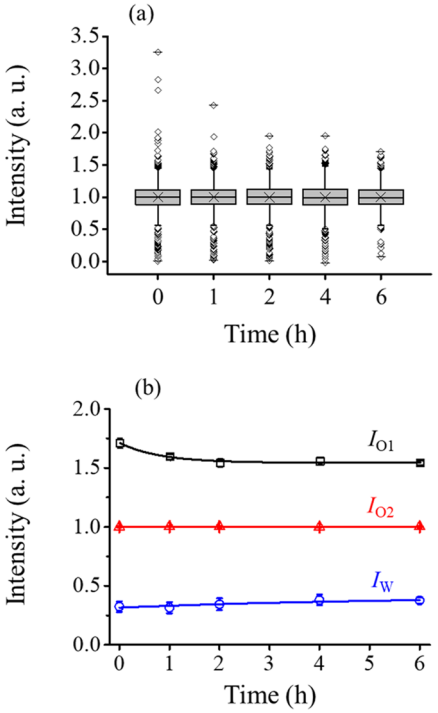


Figure 5. Evaluation of the movement of styrene in an $O_1/W/O_2$ emulsion. (a) Box plots obtained for each time course following the sample preparation (0, 1, 2, 4, 6 h). (b) Time course of the average intensities of the positive, base, and negative signals (I_{O1}, I_{O2}, and I_W, respectively) (n = 3).

certain value. In the present study, these changes were fitted with the following equations, respectively

$$I_{O1}(t) = I_{O1}(\infty) + J_{O1}\exp(-t/\tau_{O1}) \quad (1)$$

$$I_W(t) = I_W(\infty) - J_W\exp(-t/\tau_W) \quad (2)$$

where $I_{O1}(t)$ is the average intensity of the positive signal at time t, $I_{O1}(\infty)$ is the average intensity at a certain value with sufficient time, J_{O1} is the difference between $I_{O1}(0)$ and $I_{O1}(\infty)$, and τ_{O1} is the time constant of the exponential decay curve. Also, $I_W(t)$ is the average intensity of the negative signal at time t, $I_W(\infty)$ is the average intensity when it reached a certain value with sufficient time, J_W is the difference between $I_W(0)$ and $I_W(\infty)$, and τ_W is the time constant. Based on the fit of these results, τ_{O1} and τ_W were calculated to be 0.8 and 5.2 h, respectively. This result suggests that the rate of inflow from the outer O_2 phase into the middle W phase is smaller than the rate of the release from the O_1/W droplet to the outer O_2 phase. Actually, the calculated τ_W was a reference value because the increase in the average intensity of the negative signals was quite small. In future studies, the movement of constituents among phases should be discussed in detail via the use of two different compounds, e.g., isotopomers, for the analyte species arranged for the O_1 and O_2 phases, respectively. In a two-step emulsification method, efficient encapsulation in the inner O_1 phase has recently been studied.[30] To reveal the movement without pretreatment is important for the development and quality control of emulsion products, and the present method could become a novel analytical method for such requirements.

CONCLUSIONS

In the present study, a method for evaluating the movement of a constituent among phases in a multiple emulsion was proposed. An $O_1/W/O_2$ emulsion prepared by a two-step emulsification method was measured via REMPI-TOFMS without pretreatment. As a result, the base, positive, and negative signals appeared on the time profile of styrene, which arose mainly from styrene in the outer O_2, inner O_1, and middle W phases, respectively. Moreover, a time profile was converted into a box plot, and an evaluation of the movement of styrene among phases was attempted. The time constant of the time course's decay curve for the average intensity of positive signals indicated that the movement of styrene from the O_1/W droplet to the O_2 phase occurred in 0.8 h. The increase in the average intensity of negative signals suggested that the inflow of styrene from the O_2 to the W phase was very small with a calculated time constant of 5.2 h. With absorbance measurement, change in the concentration of an analyte in the outer phase could be measured following the centrifugation of a multiple emulsion. However, with pretreatment, there is a risk of droplets in an emulsion collapsing, such as that seen in the phenomenon of coalescence/destruction. By the present method, however, the movement behavior of a constituent among phases in a multiple emulsion is feasible without pretreatment.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c05599.

Particle size distributions of an $O_1/W/O_2$ emulsion measured by DLS (Figure S1) (PDF)

AUTHOR INFORMATION

Corresponding Author

Tomohiro Uchimura − *Department of Materials Science and Engineering, Graduate School of Engineering, University of Fukui, Fukui 910-8507, Japan;* orcid.org/0000-0003-4816-6328; Phone: +81-776-27-8610; Email: uchimura@u-fukui.ac.jp

Authors

Tomonobu Sugiyama − *Department of Materials Science and Engineering, Graduate School of Engineering, University of Fukui, Fukui 910-8507, Japan*

Minori Minami − *Department of Materials Science and Biotechnology, School of Engineering, University of Fukui, Fukui 910-8507, Japan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c05599

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by Research Grants from the University of Fukui (FY 2021).

REFERENCES

(1) Aserin, A. *Multiple Emulsions: Technology and Applications*, 1st ed.; John Wiley & Sons: Hoboken, NJ, 2008.

(2) Pays, K.; Giermanska-Kahn, J.; Pouligny, B.; Bibette, J.; Leal-Calderon, F. Double emulsions: how does release occur? *J. Controlled Release* **2002**, *79*, 193−205.
(3) Yoshida, K.; Sekine, T.; Matsuzaki, F.; Yanaki, T.; Yamaguchi, M. Stability of vitamin A in oil-in-water-in-oil-type multiple emulsions. *J. Am. Oil Chem. Soc.* **1999**, *76*, 1−6.
(4) Stambouli, M.; Avendano-Gomez, J. R.; Pezron, I.; Pareau, D.; Clausse, D.; Grossiord, J. L. Modelization of the release from a tetradecane/water/hexadecane multiple emulsion: evidence of significant micellar diffusion. *Langmuir* **2007**, *23*, 1052−1056.
(5) Akram, S.; Wang, X.; Vandamme, T. F.; Collot, M.; Rehman, A. U.; Messaddeq, N.; Mély, Y.; Anton, N. Toward the formulation of stable micro and nano double emulsions through a silica coating on internal water droplets. *Langmuir* **2019**, *35*, 2313−2325.
(6) Attia, M. F.; Anton, N.; Bouchaala, R.; Didier, P.; Arntz, Y.; Messaddeq, N.; Klymchenko, A. S.; Mély, Y.; Vandamme, T. F. Functionalization of nano-emulsions with an amino-silica shell at the oil−water interface. *RSC Adv.* **2015**, *5*, 74353−74361.
(7) Kovács, A.; Erős, I.; Csóka, I. Optimization and development of stable w/o/w cosmetic multiple emulsions by means of the quality by design approach. *Int. J. Cosmet. Sci.* **2016**, *38*, 128−138.
(8) Okochi, H.; Nakano, M. Preparation and evaluation of w/o/w type emulsions containing vancomycin. *Adv. Drug Delivery Rev.* **2000**, *45*, 5−26.
(9) McClements, D. J.; Decker, E. A.; Weiss, J. Emulsion-based delivery systems for lipophilic bioactive components. *J. Food Sci.* **2007**, *72*, R109−R124.
(10) Saffarionpour, S.; Diosady, L. L. Multiple emulsions for enhanced delivery of vitamins and iron micronutrients and their application for food fortification. *Food Bioprocess Technol.* **2021**, *14*, 587−625.
(11) Assadpour, E.; Maghsoudlou, Y.; Jafari, S.-M.; Ghorbani, M.; Aalami, M. Evaluation of folic acid nano-encapsulation by double emulsions. *Food Bioprocess Technol.* **2016**, *9*, 2024−2032.
(12) Nollet, M.; Laurichesse, E.; Besse, S.; Soubabère, O.; Schmitt, V. Determination of formulation conditions allowing double emulsions stabilized by pgpr and sodium caseinate to be used as capsules. *Langmuir* **2018**, *34*, 2823−2833.
(13) Lubman, D. M. Optically selective molecular mass spectrometry. *Anal. Chem.* **1987**, *59*, 31A−40A.
(14) Lin, C.-H.; Murata, Y.; Imasaka, T. Analysis of photoablation products resulting from polymer materials by supersonic beam/multiphoton ionization/time-of-flight mass spectrometry. *Anal. Chem.* **1996**, *68*, 1153−1157.
(15) Haefliger, O. P.; Zenobi, R. Laser mass spectrometric analysis of polycyclic aromatic hydrocarbons with wide wavelength range laser multiphoton ionization spectroscopy. *Anal. Chem.* **1998**, *70*, 2660−2665.
(16) Boesl, U. Laser mass spectrometry for environmental and industrial chemical trace analysis. *J. Mass Spectrom.* **2000**, *35*, 289−304.
(17) Ju, T.; Yoshinaga, K.; Imasaka, T.; Nakamura, H.; Imasaka, T. Time-correlated single ion counting mass spectrometer with long and short time-of-flight tubes and an evaluation of its performance for use in trace analysis of allergenic substances. *Anal. Sci.* **2020**, *36*, 539−543.
(18) Gehm, C.; Streibel, T.; Ehlert, S.; Schulz-Bull, D.; Zimmermann, R. Development and optimization of an external-membrane introduction photoionization mass spectrometer for the fast analysis of (polycyclic)aromatic compounds in environmental and process waters. *Anal. Chem.* **2019**, *91*, 15547−15554.
(19) Hertz-Schünemann, R.; Dorfner, R.; Yeretzian, C.; Streibel, T.; Zimmermann, R. On-line process monitoring of coffee roasting by resonant laser ionisation time-of-flight mass spectrometry: bridging the gap from industrial batch roasting to flavour formation inside an individual coffee bean. *J. Mass Spectrom.* **2013**, *48*, 1253−1265.
(20) Ishigami, H.; Tsuda, Y.; Uchimura, T. Laser ionization/time-of-flight mass spectrometry for the direct analysis of emulsions. *Anal. Methods* **2014**, *6*, 5615−5619.
(21) Tsuda, Y.; Uchimura, T. Evaluating the aging of multiple emulsions using resonance-enhanced multiphoton ionization time-of-flight mass spectrometry. *Anal. Sci.* **2016**, *32*, 789−795.
(22) Shimo, Y.; Uchimura, T. Time-profile measurement of an emulsion using multiphoton ionization time-of-flight mass spectrometry in combination with a microscope. *Anal. Sci.* **2016**, *32*, 1059−1063.
(23) Fukaya, H.; Uchimura, T. A quantitative analysis of an oil component in an emulsion by multiphoton ionization mass spectrometry. *Anal. Sci.* **2017**, *33*, 1067−1070.
(24) Iwata, M.; Uchimura, T. Resonance-enhanced multiphoton ionization time-of-flight mass spectrometry for evaluating emulsion inversion *via* temperature change. *Anal. Sci.* **2019**, *35*, 1361−1365.
(25) Sugiyama, T.; Iwata, M.; Ueyama, T.; Uchimura, T. Characteristic signal behaviors for water-in-oil and oil-in-water emulsions measured by resonance-enhanced multiphoton ionization time-of-flight mass spectrometry. *ACS Omega* **2020**, *5*, 31289−31294.
(26) Takezawa, H.; Itadani, K.; Obata, R.; Sugiyama, T.; Uchimura, T. Quantitative evaluation of the creaming of emulsions *via* resonance-enhanced multiphoton ionization time-of-flight mass spectrometry. *Anal. Sci.* **2021**, *37*, 1453−1457.
(27) Matsumoto, S.; Kita, Y.; Yonezawa, D. An attempt at preparing water-in-oil-in-water multiple-phase emulsion. *J. Colloid Interface Sci.* **1976**, *57*, 353−361.
(28) Sousa, F. L.; Santos, M.; Rocha, S. M.; Trindade, T. Encapsulation of essential oils in SiO_2 microcapsules and release behaviour of volatile compounds. *J. Microencapsulation* **2014**, *31*, 627−635.
(29) Boyd, J.; Parkinson, C.; Sherman, P. Factors affecting emulsion stability, and the HLB concept. *J. Colloid Interface Sci.* **1972**, *41*, 359−370.
(30) Guo, J.; Jiang, J.; Gu, X.; Li, X.; Liu, T. Encapsulation of β-carotene in calcium alginate hydrogels templated by oil-in-water-in-oil (o/w/o) double emulsions. *Colloids Surf., A* **2021**, *608*, No. 125548.

https://doi.org/10.1021/acsomega.1c05599
ACS Omega 2022, 7, 2099−2104