Original Research Article

Characterization of O/W Pickering emulsion by NMR

Wiphada Mitbumrung[1], Shingo Matsukawa[2], Surangna Jain[3] and Thunnalin Winuprasith[1]*

[1]*Institute of Nutrition, Mahidol University, NakhonPathom 73170, Thailand*

[2]*Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan*

[3]*Department of Biotechnology, Mahidol University, Bangkok 10400, Thailand*

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ABSTRACT

Nuclear magnetic resonance (NMR) is defined as a non-invasive technique for measuring emulsion droplet size. The diffusion of oil molecules in oil-in-water (O/W) emulsions is restricted within oil droplet which directly affected by the oil droplet radius. Thus, restricted diffusion of oil molecules can be used to determine the oil droplet diameter. This research was aimed to investigate the influence of emulsifier type on emulsion droplet diameter by NMR comparing with conventional light scattering technique. The 10% wt oil O/W emulsions were prepared using nanofibrillated cellulose (NFC) or whey protein isolate (WPI) at concentration of 0.3, 0.5 and 0.7% wt as an emulsifier. Diffusion coefficients (D) were measured by pulsed-field-gradient stimulated spin-echo (PFG-STE) pulse sequence using High-field NMR. The results showed that the diffusion coefficients of the O/W emulsions were dependence on diffusion time (Δ). The increase in diffusion time found the decay of attenuation of a coherent magnetic signal. Since the diffusion of oil molecules was limited by adsorbing of NFC at oil-water interfaces, the restricted diffusion was found in NFC-stabilized emulsions by observing constantly of mean square displacement (Z^2) when the diffusion time was increased. On the other hand, free diffusion was found in WPI-stabilized emulsions which may occur from the diffusion of oil molecules within oil droplet and the movement of oil droplet itself. The droplet size determined by NMR technique showed the results in the same agreement with the light scattering technique. Hence, the restricted diffusion measurement by PFG-NMR is a novel and an alternative technique for monitoring emulsion droplet size. However, it is more suitable for emulsions with slow movement of oil droplets or emulsions with high viscosity of continuous phase.

* *Corresponding author*: Tel.: +6-692-435-4343; fax: +0-000-000-0000
E-mail address: thunnalin.win@mahidol.ac.th

INTRODUCTION

An emulsion is a dispersion of two immiscible liquids where one of the liquids is dispersed as spherical droplets in another. The emulsions can be classified according to the distribution of the oil and aqueous phases. The emulsions that contain oil droplets dispersed in aqueous phase are called oil-in-water (O/W) emulsions and the emulsions that contain water droplets dispersed in aqueous phase are called water-in-oil (W/O) emulsions (McClements, 2005; Song et al., 2015). Generally, the emulsions are thermodynamically unstable where the individual droplets recombine until the two phases completely separate out which is referred to as coalescence (Pawar, Caggioni, Hartel, and Spicer, 2011). To stabilize the emulsions against coalescence, it is required to add substances, called emulsifiers, that can increase stability and postpone the emulsions phase separation for a certain time period (McClements and Decker, 2000; McClements, 2005). Surfactants are commonly used to produce emulsions. They have the ability to produce small emulsion droplets by easily adsorbing at the oil/water interface and reducing surface tension. In this research, the emulsions were stabilized by solid particles, and are called Pickering emulsions (Pawar, Caggioni, Hartel, and Spicer, 2011; Wen, Yuan, Liang, and Vriesekoop, 2014). It has been reported that Pickering emulsions provides stability against coalescence because the solid particles have a high energy of attachment which allows them to irreversibly adsorb at the oil/water interface (Chevalier and Bolzinger, 2013; Pawar, Caggioni, Hartel, and Spicer, 2011; Tavernier, Wijaya, Meeren, Dewettinck and Patel, 2016).

The properties of emulsion-based food products are influenced by the size of the droplets which further affects the appearance, texture, rheology, and stability of the products. Hence, an accurate droplet size determination would be desirable. There are various methods for droplet sizing including light scattering (static or dynamic), electrical pulse counting, sedimentation technique, ultrasonic spectrometry, and nuclear magnetic resonance (McClements, 2005). Our experiment focused on two specific techniques that included the static light scattering (SLS) or the laser diffraction technique and the nuclear magnetic resonance (NMR) spectroscopy. The SLS technique has been commercially available for many years and is being widely used in food industries and laboratories. However, the SLS technique is invasive and destructive to the samples because the samples need to be diluted prior to measurement. The dilution step can destroy and change the original structure of the emulsions and also affect sample characteristics by destabilizing the emulsions. Furthermore, the SLS technique has a limitation that it cannot possibly measure the size of individual droplets present as flocs (cluster) (Goudappel, Duynhoven, and Mooren, 2001; Hollingsworth, Sederman, Buckley, Gladden, and Johns, 2004; Lee, McCarthy, and Dungan, 1998). Therefore, other techniques such as ultrasonic spectrometry or high-field pulsed field gradient NMR (PFG-NMR) are used to overcome these problems. The NMR technique has been used as noninvasive method for measuring emulsion droplet size and is based on the restriction of self-diffusion of dispersed phase (hydrogen atom in oil) by the droplet interface because the distance that droplets can diffuse within an emulsion is limited by the size of the droplets (Goudappel, Duynhoven, and Mooren, 2001; Hollingsworth, Sederman, Buckley, Gladden, and Johns, 2004; KioKias, Reszka, and Bot, 2004).

In this experiment, we investigated the influence of emulsifier type and concentration on the droplet size of the Pickering emulsions by using two different techniques that included SLS and PFG-NMR. In previous studies (Winuprasith and Suphantharika, 2013 and 2015), nanofibrillated cellulose (NFC) extracted from mangosteen rind has been potentially used as a Pickering emulsifier to form O/W emulsions. Hence, we used NFC to produce O/W emulsions which represents a high viscosity emulsion. Whey protein isolate (WPI) which is a commercial emulsifier was also used to prepare at the same concentration which represents a low viscosity emulsion.

MATERIALS AND METHODS

Chemicals and reagents

Dried mangosteen rind (*Garcinia mangostana* L.); an agricultural waste from the mangosteen canning process was obtained from a local manufacturer (Chanthaburi, Thailand). All chemical reagents used in this experiment were analytical grade and deionized water used for preparation of all solutions was obtained by a Water Purifier (Autostill WA500, Yamato Scientific Co., LTD, Tokyo, Japan). D_2O (deuterium isotopic content 99.0%) and soybean oil were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Whey protein isolate (Provon 292) was obtained from Glanbia Nutritionals (NA), Inc.

Nanofibrillated cellulose preparation

Nanofibrillated cellulose (NFC) was prepared using an alkaline extraction method combined with thermal treatment following a previously described protocol (Winuprasith et al., 2018) with slight modifications. Briefly, cellulose was extracted from dried mangosteen rind powder using sodium hydroxide (NaOH) solution treated thermally (90°C) at pH12. Cellulose suspension was washed and neutralized prior to bleaching with hydrogen peroxide (H_2O_2) solution. The purified cellulose was then diluted at a concentration of 1% wt for NFC preparation and was passed through a high pressure homogenizer (APV-100, SPX flow technology, Inc., NC, USA) at a pressure of 500 bar for 20 passes at room temperature (25°C). NFC obtained was then dried in a hot air oven at 60°C, overnight and re-dispersed in D_2O for emulsion preparation.

Emulsion preparation

Oil-in-water (O/W) emulsions were prepared using a 10% oil phase with soybean oil and a 90% aqueous phase. The aqueous phase was prepared by dissolving NFC and WPI in D_2O at different concentrations of 0.3%, 0.5%, and 0.7% wt, respectively. The emulsions were prepared by mixing the oil and aqueous phases using high shear mixer (Silverson L5 series Laboratory Mixer with emulsor screen, Silverson Machines, Ltd., UK) to form coarse emulsions. Fine emulsions were then prepared by sonicating the coarse emulsions using sonicator (Qsonica Q125. Connecticut, USA) at 20 kHz, 60% amplitude, and a time interval of 5s for 5min.

Particle size determination

The particle size distribution and mean particle size of the emulsions were measured by using laser diffraction nanoparticle size analyzer (SALD-7500nano, Shimadzu Co., Kyoto, Japan). The emulsions were dispersed in deionized water to a certain concentration in order to avoid multiple scattering effects. Refractive index of soybean oil was specified at 1.46 and absorption was assumed to be 0.

NMR measurement

NMR spectra were recorded on a Bruker Avance II 400WB spectrometer (Bruker Co., USA) equipped with a 5 mm diffusion probe (Bruker Diff 60). The measurements were performed at 25°C and the temperature was controlled by using a Bruker BVT-3200 temperature unit. The emulsions were filled in 8 mm diameter glass NMR tubes at 8 mm height. Diffusion coefficients (*D*) were measured

by pulsed-field-gradient stimulated spin-echo (PFG-STE) pulse sequence. The gradient field strength (g) was varied in 16 steps in the range 100-1500 G/cm. The gradient pulsed duration (δ) was applied at 1 ms and diffusion time ($\varDelta$) was varied from 10 to 300 ms. The diffusion coefficient was determined using the following equation

$$I(g) = I(0)\exp[-\gamma^2\delta^2 g^2(\varDelta-\delta/3)D] \tag{1}$$

where $I(g)$ is the measured signal intensity, $I(0)$ is the initial signal without the field gradient pulse, γ is gyromagnetic ratio of ^{1}H. According to Eq. (1) the diffusion coefficients (D) from signal attenuation in the NMR experiment exhibited molecular property which depends on the size of molecule and viscosity of solution. The mean square displacement (Z^2) of the diffusing molecule along the axis of the magnetic field gradient is dependent on D and $\varDelta$

$$<Z^2> = 2D\varDelta \tag{2}$$

As the diffusion molecules were restricted by an emulsifier wall around the oil droplets, the apparent mean square displacement values along the gradient axis is dependent on the oil droplet size and the shape of the confinement.

$$<Z^2> = 2/5R^2 \tag{3}$$

with R is the radius of the confinement of the liquid or radius of oil droplet.

RESULTS AND DISCUSSION

Light scattering measurement

The particle size distribution of the emulsions stabilized by NFC and WPI, respectively are shown in Fig.1. The particle size
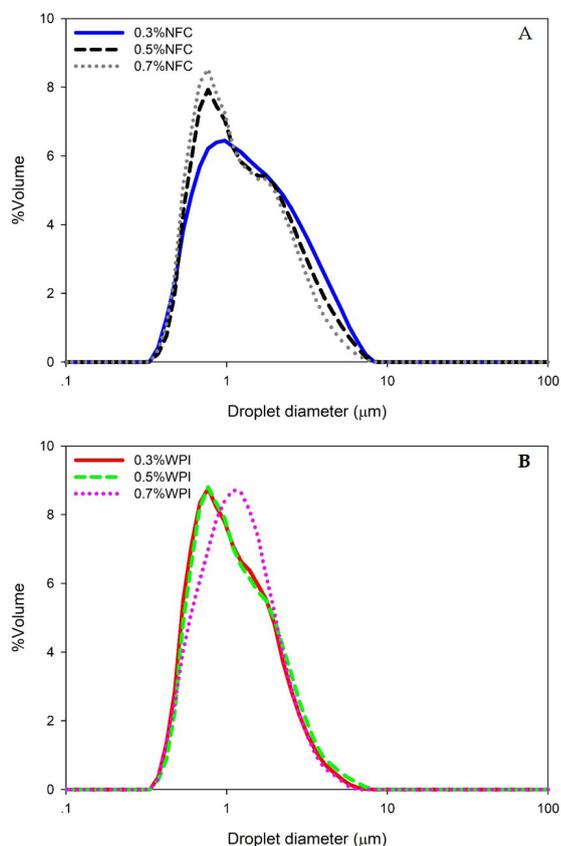
Figure 1. The particle size distribution of O/W Pickering emulsion stabilized by NFC (A) and WPI (B) at concentration 0.3%, 0.5%, and 0.7% wt.

distribution results obtained by both SLS and NMR of the emulsions stabilized by NFC and WPI exhibited a unimodal distribution (1peak) and demonstrated mean particle diameter values to be around 1 µm (Table 1). From Fig. 1A, it can be observed that at low concentrations of NFC, the emulsions exhibited a broad particle size distribution and there was a formation of larger oil droplets. An increase in the NFC concentration in the emulsion systems led to a decrease in the oil droplet size and a narrower particle size distribution which indicated the NFC to be as an effective and potential emulsifier. From Fig. 1B, it was observed that the emulsions stabilized by WPI exhibited a similar behavior where an increase in the WPI concentration resulted in a more uniform particle size distribution but did not significantly affect the particle size.

Table1. The mean particle size of emulsions stabilized by NFC and WPI measuring with static light scattering and pulsed-field gradient stimulated spin-echo NMR techniques.

Sample	Mean droplet diameter (µm)	
	Light scattering	PFG-NMR
0.3%NFC emulsion	1.31 ± 0.01	1.26 ± 0.03
0.5%NFC emulsion	1.21 ± 0.02	1.18 ± 0.03
0.7%NFC emulsion	1.11 ± 0.09	ND
0.3%WPI emulsion	1.04 ± 1.04	0.96 ± 0.20
0.5%WPI emulsion	1.09 ± 1.09	1.01 ± 0.29
0.7%WPI emulsion	1.10 ± 1.10	1.14 ± 0.29

*ND represent not detect

The emulsions stabilized by NFC provided larger emulsion droplets in comparison to the emulsion stabilized by WPI. Because NFC is a polysaccharide which has larger molecules than WPI which is a globular protein (Bouyer et al., 2011) so, WPI has better ability to adsorb at the oil/water interface (Table 1). Hence, during emulsification, WPI can easily adsorb at the interface and cover the oil surface which prevents the reassembly of the oil droplets following their breakdown due to high shear whereas, NFC takes a longer time to reach at the interface as they strongly affect the viscosity of the continuous phase (Lu, Zhang, Li, and Huang, 2018). However, it was seen that the presence of NFC in the continuous phase resulted in a higher emulsion stability beyond that of WPI. The NFC-emulsions did not cream throughout the period of 20 days whereas WPI-emulsions began to cream readily from day 6 (data not shown). It has been reported in a previous study of Winuprasith and Suphantharika (2015) that NFC located in the continuous phase forms a three dimensional network and traps the oil droplets in their structure which further retards droplet movement and collision leading to droplet coalescence.

NMR diffusion measurement

Pulsed-field-gradient stimulated spin-echo (PFG-STE) NMR was used for measuring the diffusion coefficients (D) of emulsions stabilized by NFC and WPI, respectively and the results obtained are shown in Fig. 2A. From the result, it was observed that the D values decreased with an increase in the diffusion time ($\varDelta$) which can be attributed to the movement of ^{1}H of fatty acids that was previously restricted by an interfacial layer of emulsifier (NFC and WPI) located around the oil droplet surface. The emulsions stabilized by NFC and WPI also showed an echo intensity in the PFG-STE experiment which was fitted linearly in the plot of intensity decay against $\gamma^2\delta^2 g^2(\varDelta-\delta/3)$ in Eq. (1) indicating the presence of one diffusion components (data not shown) that denotes the self-diffusion of oil molecules. Fig. 2B depicted the mean square displacement (Z^2) values against diffusion time ($\varDelta$) of the emulsions stabilized by NFC and WPI, respectively.

The results clearly indicated that the emulsions stabilized by NFC demonstrated a restricted diffusion which can be clearly explained by the fact that the diffusion of oil molecules in emulsions stabilized by NFC are restricted by the NFC network that embeds the oil molecules. On the other hand, the emulsions stabilized by WPI exhibited free diffusion as indicated by their Z^2 values which was found to increase with an increasing diffusion time (Δ). This may have occurred due to two diffusion mechanisms; (1) intra-droplet restricted diffusion and (2) droplet self-diffusion (Voda and Duyhoven, 2009). Also, in comparison to the emulsions stabilized by NFC, the WPI emulsions were found to exhibit some diffusion within the oil molecules. However, since an emulsion is a colloidal dispersion system, the oil droplets have some mobility which causes the self-diffusion of the oil droplets.
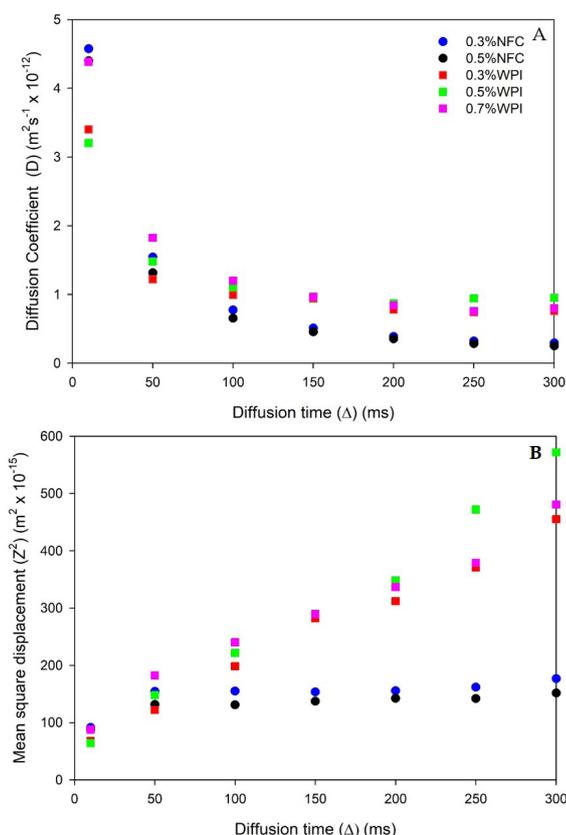
Figure 2. Influence of emulsifier type and concentration on the diffusion coefficient (D) (A) and mean square displacement (Z^2) (B) of emulsions stabilized by NFC and WPI.

The mean particle size of the emulsions stabilized by NFC and WPI, respectively were determined by static light scattering and NMR techniques and are shown in Table 1. The results obtained from the static light scattering technique which is a conventional invasive method showed a decrease in the mean particle size of the emulsions stabilized by NFC with an increasing NFC concentration and a slight increase in the mean particle size of emulsions stabilized by WPI with an increasing WPI concentration. The results obtained by the NMR technique were found to be in the same agreement with those obtained by the light scattering technique although the mean particle size measured by NMR were of smaller size. Despite the light scattering technique resulting in errors due to sample dilution they have an advantage that the sample viscosity does not affect the measurement process. On the other hand, the NMR technique is more suitable for emulsions which are highly viscous otherwise the results may be misled by droplet self-diffusion.

CONCLUSIONS

We investigated the influence of emulsifier type and concentration on the particle size of O/W Pickering emulsion measured by the static light scattering (SLS) technique and pulsed-field gradient stimulated spin-echo nuclear magnetic resonance (PFG-NMR). The emulsions stabilized by both NFC and WPI exhibited particle size values around 1µm however, the NFC-emulsions exhibited larger particles as NFC affects the viscosity of the continuous phase whereas WPI does not. However, the mean particle size of the emulsions stabilized by NFC was found to reduce with an increasing of NFC concentration. The result obtained by light scattering and NMR were found to be in same agreement. Although, the light scattering technique has sample dilution error, but the viscosity of sample would be not affect to these technique. The NMR technique is a good noninvasive method that does not alter the sample structure but it has a limitation that it measures the diffusion of emulsions with high mobility. Both the light scattering and NMR are potential methods used for measuring the particle size of Pickering emulsions where the NMR technique may be applied for measuring the droplet size of other molecules in a complex matrix such as food and encapsulation compounds by the difference in diffusion coefficients from the chemical shift of each compound.

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