

## Influence of Xanthan, Emulsification Temperature, and Environmental Stresses on the Preparation of Water-in-Corn Oil Emulsions Droplets Coated by Polyglycerol Polyricinoleate

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**Abstract** The purpose of this study was to prepare stable water-in-corn oil (W/O) emulsion droplets coated by polyglycerol polyricinoleate (PGPR). W/O emulsions (20 wt% aqueous phase, 80 wt% oil phase containing 8 wt% PGPR) were produced by high pressure homogenization (Emulsions 1), however, appreciable amount of relatively large water droplets ( $d > 10 \mu\text{m}$ ) were found. To facilitate droplet disruption, viscosity of each phase was adjusted: (i) increased the viscosity of aqueous phase by adding 0.1 wt% xanthan (Emulsions 2); (ii) decreased the viscosity of oil phase and aqueous phase by heating them separately at 50°C for 1 hr immediately before emulsification (Emulsions 3). Homogenizing at the elevated temperature clearly led to a smaller water droplet size, whereas xanthan neither improved nor adversely affected on the microstructures of the emulsions. In addition, the Emulsions 3 had good stability to droplet aggregation under shearing stress, thermal processing, and long term storage.

**Keywords:** water-in-oil emulsion, polyglycerol polyricinoleate, high pressure homogenization, emulsion stability

### Introduction

Emulsions can be conveniently classified into an oil-in-water (O/W) emulsion system that consists of oil droplets dispersed in an aqueous phase and a water-in-oil (W/O) emulsion system that consists of water droplets dispersed in an oil phase (1). W/O emulsions can be used in many different areas such as foods, pharmaceuticals, and cosmetics because they have potential as delivery systems or prolonged release systems for hydrophilic components (2-4). W/O emulsions can be also used as the inner phase in W/O/W multiple emulsions that have a number of potential benefits over conventional O/W emulsions such as controlled or triggered release, reduction of fat content, and protection of labile ingredients (5). Nevertheless, relatively limited works have been dedicated on the technology or theory related to W/O emulsions such as stabilizing mechanism and interactions at the interface, while those related to O/W emulsions have been well established (1,6-12).

In the case of W/O emulsions, it has been of interest to stabilize W/O emulsion using natural products such as proteins, hydrocolloids, or phospholipids as emulsifiers to develop emulsion systems suitable for functional food application (6-8). Among them, proteins and hydrocolloids were too hydrophilic to act as good emulsifiers at W/O interfaces. However, they somewhat improved the long-term stability of W/O emulsions when used as additives such as thickening or gelling agents in the aqueous phase of the emulsions (6,8). Johannson *et al.* (13) reported that hydrophobic lecithin with reduced phosphatidylcholine content acted as an emulsifier in W/O emulsions. However,

the droplets of the lecithin-stabilized emulsions formed aggregates thus the resulting emulsions were very viscous (7-9). On the other hand, polyglycerol polyricinoleate (PGPR), a synthetic polymer that is permitted in certain food systems, is known as one of the most efficient hydrophobic emulsifiers for W/O emulsions (6-8,14). In a direct comparison between lecithin and PGPR as emulsifying agents, PGPR-stabilized emulsions showed smaller water droplets than those stabilized by lecithin, which was attributed to polymeric steric repulsion among PGPR chains attached on the droplet surface that prevented the hydrophilic droplets from aggregating in non-polar continuous media (7,15). It is well documented that emulsifier's physical properties such as degree of polymerization and molecular weight are strongly correlated to the stability of emulsion (6). Therefore, polymeric PGPR, albeit synthetic, has been considered for utilization as a hydrophobic emulsifier. However, it should be noted that the use of this synthetic emulsifier is somewhat strictly regulated thus reducing the use and/or partially substituting PGPR with other food grade materials have recently been an interest to food emulsion scientists (13,14,16-18).

It has been emphasized that the hydrophobicity of oil phase is another critical factor that strongly influences on the stability of W/O emulsions (2,7,19,20). Several studies investigated the effect of various lipid types on the properties and stabilities of W/O emulsions (2,7,16). When PGPR was used as an emulsifier, it was found that long chain triacylglycerol formed better emulsions than medium-chain triacylglycerols (MCT), and which phenomenon was explained with the difference in the hydrophobicity of each lipid (2). In contrast to this, it is also well known that the flocculation of W/O emulsions is induced by the presence of long-chain lipids in the continuous phase of the emulsions (21). In W/O emulsions stabilized with phosphatidylcholine depleted lecithin, appreciably large

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water droplet aggregation occurred with sunflower oil as a continuous phase, slight droplet aggregation with butter oil, and no aggregation with MCT and MCT-oil/fat (7). It was attributed to the increase in the refractive index (which increases van der Waals interaction) and the increase in the size of the oil molecules (which increases depletion attraction) in the order of MCT, butter oil, and sunflower oil, i.e., MCT-oil has exclusively C8/C10 fatty acids and the lowest refractive index, butter oil has intermediate percentage of long-chain fatty acids, and sunflower oil has the highest refractive index and the highest percentage of C18 fatty acids (7,21). In light of these previous studies, water in vegetable oil emulsion appears particularly difficult to prepare and to stabilize. In addition, the vegetable oils tend to be somewhat hydrophilic and contain more surface active impurities or unsaturated components that prevent the formation of uniform film around water droplets. Therefore, no satisfactory suggestions have been established for the preparation of water in vegetable oil systems.

Therefore, the overall objective of this study was to prepare a stable food-grade W/O emulsion in which corn oil was used as an oil phase. In particular, I wanted to determine under which conditions PGPR-stabilized emulsions containing small and homogeneous droplets could be formed, and if the emulsions had good stability to environmental stresses such as shearing, thermal processing, and long-term storage. As a way of generating fine W/O emulsion droplets, here the viscosity of aqueous phase or oil phase was adjusted by using xanthan or homogenizing at an elevated temperature, and its influence on the properties of the W/O emulsions was investigated in relation to particle size, microstructures, turbidity, and sedimentation stability.

## Materials and Methods

**Materials** Polyglycerol polyricinoleate (PGPR 4150; Palsgaard, Juelsminde, Denmark) prepared by the esterification of condensed castor oil fatty acids with polyglycerol was obtained from Palsgaard Industri de Mexico (Palsga, Mexico). As stated by the manufacturer, the polyglycerol moiety of the PGPR was predominantly di-, tri-, and tetraglycerols (min. 70%) and contained not more than 10% of polyglycerols equal to or higher than heptaglycerol. Xanthan, analytical grade hydrochloric acid (HCl), sodium hydroxide (NaOH), hexadecane, sodium phosphate (monobasic, anhydrous), and sodium azide ( $\text{NaN}_3$ ) were purchased from the Sigma-Aldrich (St. Louis, MO, USA). Sodium phosphate (dibasic, anhydrous) was purchased from Fisher Science (Chicago, IL, USA). Corn oil was purchased from a local supermarket and used without further purification. Distilled and deionized water was used for the preparation of all solutions.

**Preparation of solutions and emulsions** Emulsifier solution was prepared by dispersing 8 wt% PGPR into corn oil. This PGPR concentration was selected because previous studies on water-in-hydrocarbon (kerosene,  $\text{C}_{10}\text{H}_{22}$  to  $\text{C}_{16}\text{H}_{34}$ ) emulsions that used buffers as aqueous phases have shown that it is capable of forming W/O emulsions containing small water droplets with narrow droplet size distribution (17,22). Two kinds of aqueous phases were prepared: (i) aqueous phase solution 1 (5 mM phosphate

buffer) was prepared by dispersing the desired amount of monobasic and dibasic sodium phosphate into distilled water containing 0.02 wt% sodium azide (as an antimicrobial agent) and adjusting the pH to 7.0 using 1 M HCl or 1 M NaOH. The buffer system, rather than distilled water, was used as an aqueous phase to buffer the influence of pH extreme on emulsions stability; (ii) aqueous phase solution 2 was prepared by dispersing 0.1 wt% xanthan additionally into the aqueous phase 1 (5 mM phosphate buffer, pH 7.0) as a thickening agent and adjusting its pH back to 7.0. The xanthan was selected because it is capable of increasing the viscosity of a liquid exponentially with a very small quantity of the gum (23); e.g., the viscosity of a 0.5% solution decreased by over 3 orders of magnitude from low to high applied shear rates (24). The emulsifier solution and the aqueous phase solution 1 were then heated at 50°C for 1 hr or kept at room temperature (ca. 23°C) before emulsification. Separately, for the preparation of W/O emulsion droplets containing xanthan inside them, the aqueous phase 2 and the oil phase were not heated before emulsification because it was found that xanthan gum formed hydrogels when aqueous solutions were annealed at a temperature higher than characteristic temperature (ca. 40°C) and subsequently cooled (25,26).

Each W/O emulsion was prepared with an aqueous fraction of 20 wt%. The water phase with or without 0.1 wt% xanthan was dispersed gradually into the oil phase under agitation with a magnetic stirrer and blended together using a high-speed blender (M133/1281-0; Biospec Products, Inc., Bartlesville, OK, USA) for 2 min. The coarse emulsions were then passed through a 2-stage high-pressure valve homogenizer (LAB 1000; APV-Gaulin, Wilmington, MA, USA) 3 times: 2,700 psi the 1<sup>st</sup> stage, 300 psi the 2<sup>nd</sup> stage. The emulsions were then stored at ambient temperature for 24 hr before being analyzed.

In summary, 3 different PGPR-stabilized W/O emulsions were prepared:

- Emulsion 1 was prepared by homogenizing 20 wt% aqueous phase (5 mM phosphate buffer, pH 7) with 80 wt% oil phase (8 wt% PGPR in corn oil). Both phases were not heated before emulsification.
- Emulsion 2 was prepared by homogenizing 20 wt% aqueous phase (0.1 wt% xanthan, 5 mM phosphate buffer, pH 7) with 80 wt% oil phase (8 wt% PGPR in corn oil). Both phases were not heated before emulsification.
- Emulsion 3 was prepared by homogenizing 20 wt% aqueous phase (5 mM phosphate buffer, pH 7) with 80 wt% oil phase (8 wt% PGPR in corn oil). Both phases were heated at 50°C for 1 hr before emulsification.

**Influence of environmental stresses on PGPR-stabilized W/O emulsion stability** The properties and stability of the 3 different PGPR-stabilized W/O emulsions were compared by measuring their particle sizes, microstructures, turbidity, and sedimentation stability after they were subjected to certain environmental stresses. Influence of thermal processing (30 to 90°C with an interval of 10°C, each for 30 min) and subsequent storage (1 day or 1 week) at room temperature (ca. 23°C) on the stability of PGPR-stabilized W/O Emulsions 1 was examined. Thermal stability of Emulsions 2 was examined after heating them

at 80°C for 30 min. Influence of environmental stresses such as shearing, thermal processing, and storage on the properties and stability of Emulsions 3 was also examined as follows:

**Shearing:** Emulsion samples were subjected to constant shear for 0-7 min (0, 0.5, 1, 2, 3, 4, 5, and 7 min) at room temperature using a high-speed blender (M133/1281-0; Biospec Products, Inc.). The emulsions were then stored at room temperature for 24 hr before being analyzed.

**Thermal processing:** Ten g of emulsion samples were transferred into glass test tubes (i.d. 15 mm, height 125 mm), which were then incubated in a water bath for 30 min at different temperatures ranging from 30 to 80°C. After incubation, the emulsion samples were immediately cooled to ambient temperature in a water bath containing cold tap water. The emulsions were then stored at room temperature for 24 hr prior to analysis.

**Storage:** Emulsions were stored at room temperature for 1 day, 1, 2, and 3 week before being analyzed.

**Particle size determination** The mean size of the droplets in the W/O emulsions was determined by dynamic light scattering. The W/O emulsions were diluted to a droplet concentration of ca. 0.5 wt% with hexadecane (refractive index=1.434, viscosity=3.13 mPa·sec at 25°C) as a dispersant to avoid multiple scattering effects. The hexadecane was selected as an alternative to corn oil that used as a continuous phase of the W/O emulsions. Corn oil is unsuitable as a dispersant of the W/O emulsions because it has relatively high viscosity (ca. 50 mPa·sec at 25°C) thus might cause the water droplets to move too slowly through the oil continuous phase during the dynamic light scattering measurement. The particle size of the emulsions was then measured at 25°C using a commercial dynamic light scattering instrument (Zetasizer Nano-ZS; Malvern Instruments, Worcestershire, UK). This instrument measures the rate of diffusion of particles via intensity fluctuations. Particle size was reported as the scattering intensity-weighted mean diameter, Z-average (Z-avg). It should be noted that the measurements should be treated with caution because dynamic light scattering is not sensitive to the presence of slow-moving particles larger than about 3  $\mu\text{m}$  (1).

**Optical microscopy** Emulsions were gently agitated in a glass test tube before analysis to ensure that they were homogenous. A drop of emulsion was placed on a microscope slide and then covered with a cover slip. The microstructure of the emulsion was then observed using conventional optical microscopy (Nikon Microscope Eclipse E400; Nikon Corporation, Tokyo, Japan). The images were acquired using a CCD camera (CCD-300T-RC; DAGE-MTI, Michigan City, IN, USA) connected to Digital Image Processing Software (Micro Video Instruments Inc., Avon, MA, USA) installed on a computer.

**Sedimentation stability measurement** Sedimentation stability measurements were carried out on the W/O emulsions, where the water droplets tend to move downwards because they are heavier than the surrounding oil phase. Ten g of emulsion were transferred into a test tube, tightly sealed with a plastic cap, and then centrifuged at 716 g for 30 min at room temperature (Model 225A Centrifuge® Centrifuge;

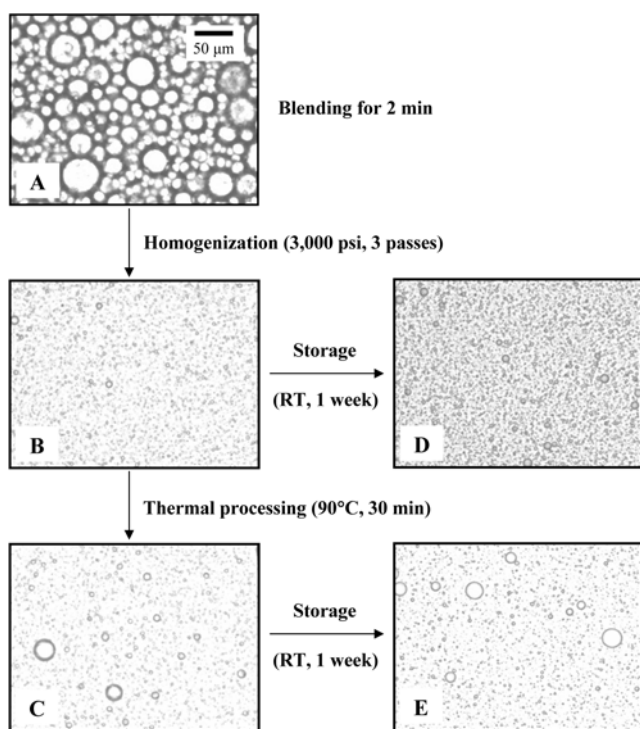
Fisher Scientific, Dubuque, IA, USA). Sedimentation was determined visually based on the phase separation after the centrifugation step. The Emulsion 3 was not centrifuged to determine its long-term stability as it was. However, water separation was not apparent in any of the W/O emulsions investigated.

**Spectro-turbidity measurements** An indication of droplet aggregation in the emulsions was also obtained from the measurements of turbidity vs. wavelength, since the turbidity spectrum of a colloidal dispersion depends on the size of the particles it contains (27). The emulsions diluted to a droplet concentration of ca. 0.15 wt% with hexadecane were transferred into 10-mm path length plastic spectro-photometer cuvettes. The emulsions were inverted a number of times prior to measurements to ensure that they were homogeneous so as to avoid any changes in turbidity due to droplet sedimentation. The change in absorbance of the emulsions was recorded when the wavelength changed from 800 to 400 nm using a ultraviolet (UV)-visible spectrophotometer (UV-2101PC; Shimadzu Corporation, Tokyo, Japan), with hexadecane as a reference.

**Statistical analysis** Experiments were performed twice using freshly prepared samples. Averages and standard deviations were calculated from these duplicate measurements.

## Results and Discussion

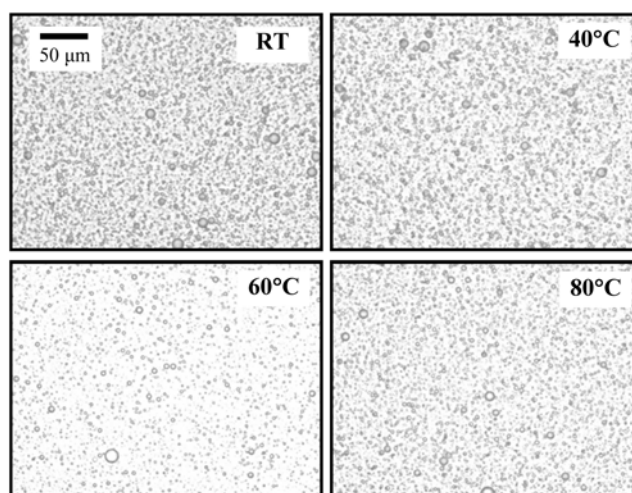
**Preliminary examination on the ability of PGPR to form water-in-corn oil emulsion droplets** Whether PGPR could produce stable water-in-vegetable oil emulsion droplets was determined by examining the microstructures of systems containing corn oil, water, and PGPR that were subjected to a series of processing steps such as blending, homogenizing, heating, and storing (Fig. 1). Microscopy pictures taken after simply blending both oil phase containing PGPR and aqueous phase showed water droplets clearly, although the droplets were appreciably large ( $d > 20 \mu\text{m}$ ) (Fig. 1A), which indicates that the PGPR could be used to form water-in-corn oil emulsion droplets. To produce fine water droplets, the preexisting coarse emulsions were then subjected to high pressure homogenization process (3 times at 3,000 psi). As the coarse emulsions pass through the homogenizer they experience a combination of intensive disruptive forces that cause the larger droplets to be broken down to smaller ones (1). Apparently much smaller water droplets were generated by the sufficient increase in energy input (Fig. 1B). Nevertheless, small populations of large droplets were still observed in the emulsions. There are a number of possible reasons to account for the observation: (i) vegetable oils have lower viscosity compared with other lipid types such as MCT-oil and vegetable fats. The higher the viscosity of the lipid used, the larger the emulsion's specific surface area and thus the smaller the emulsion's droplet size (7); (ii) vegetable oils have relatively high contents of unsaturated fatty acids and long-chain lipids. Unsaturated components in sunflower oil and olive oil were reported to prevent the formation of a uniform film at the interface of W/O emulsions and thus to lead to an increase in droplet size (19). It was reported that the presence of long-chain



**Fig. 1. Preliminary examination on the ability of PGPR to form water-in-corn oil emulsion droplets.** 20 wt% aqueous phase and 80 wt% oil phase (8 wt% PGPR in corn oil) were blended for 2 min and homogenized 3 times at 3,000 psi. The W/O emulsions were heated or non-heated, and then stored for 1 week at room temperature. Microstructures of the emulsions were examined after each step by optical microscopy.

lipids in the continuous phase of lecithin-stabilized W/O emulsions induced the aggregation of the W/O emulsions (7,21), and which was attributed to depletion attraction originated by the presence of relatively large long-chain molecules. In addition, the high proportions of unsaturated components in the vegetable oils might contribute to lowering the viscosity of the oils; (iii) vegetable oils have high refractive indices. Aggregation tendency of droplets in W/O emulsions was magnified by increasing the refractive index mismatch between dispersed and continuous phase, i.e., by increasing the van der Waals interaction (21). It is well documented that the attractive forces between droplets are minimal when the difference between the refractive indices of the 2 phases is minimal (2). All of these 3 factors depend directly on the structural and physicochemical properties of the vegetable oils used. (iv) ionic environment created by the phosphate buffer has an adverse effect on the emulsifying ability of PGPR by interacting the ionic charge on the functional groups of the PGPR at the water-oil interface (18). However, this seems unlikely because the above emulsion systems were prepared using sufficiently high concentration of PGPR to compensate the reduced emulsifying ability of PGPR in the presence of electrolytes.

To examine the applicability of PGPR to vegetable oil-based W/O emulsions, the influence of thermal processing on the PGPR emulsions was also examined because it was widely known that the temperatures could change the viscosity of liquid phases, solubility, and partitioning of



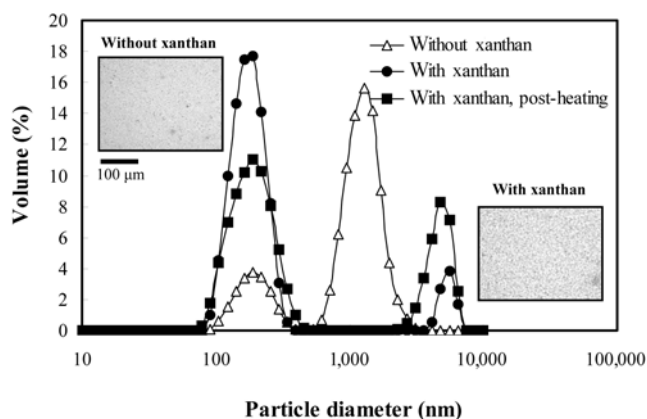
**Fig. 2. Influence of thermal processing and subsequent storage at room temperature (RT, 1 week) on the microstructures of PGPR-stabilized W/O emulsions (20 wt% aqueous phase, 80 wt% corn oil containing 8 wt% PGPR).**

molecules between the oil and aqueous phases, thus might change the functional properties of surfactants (1). Indeed, when the homogenized W/O emulsions were heated at 90°C for 30 min, relatively large droplets ( $d > 10 \mu\text{m}$ ) were observed in their microscopy pictures (Fig. 1C). However, there were no noticeable changes in microstructures with time when the emulsions that had or had not undergone heat treatment were stored at room temperature for 1 week (Fig. 1D and 1E). These observations are consistent with a previous study reporting that relatively high temperature (storage at 90°C) had a pronounced effect on the rate of increase in the droplet size of water in medium chain triglyceride oil emulsions coated by 1.0% sorbitan monooleate plus 3.5% PGPR, but not that noticeable when stored at  $\leq 70^\circ\text{C}$  (4). They attributed the thermal stability of PGPR to its low phase inversion temperature ( $\leq 0^\circ\text{C}$ ) (4). Figure 2 indicates that the PGPR did not change its own character fundamentally upon heating (30–80°C) and subsequent storage (at room temperature for 1 week), unlike many emulsifiers for O/W emulsions. Therefore, in the case of PGPR-stabilized water-in-corn oil emulsions, it is sufficiently informative to characterize the emulsions after homogenization step (here, Fig. 1B step) rather than any further processing steps.

In light of the above results, the next experiment focused, for the preparation of PGPR-stabilized W/O emulsions, specifically on facilitating droplet disruption within the homogenizer by adjusting viscosity of each phase (see below).

#### **Influence of xanthan on the properties of PGPR-stabilized W/O emulsions**

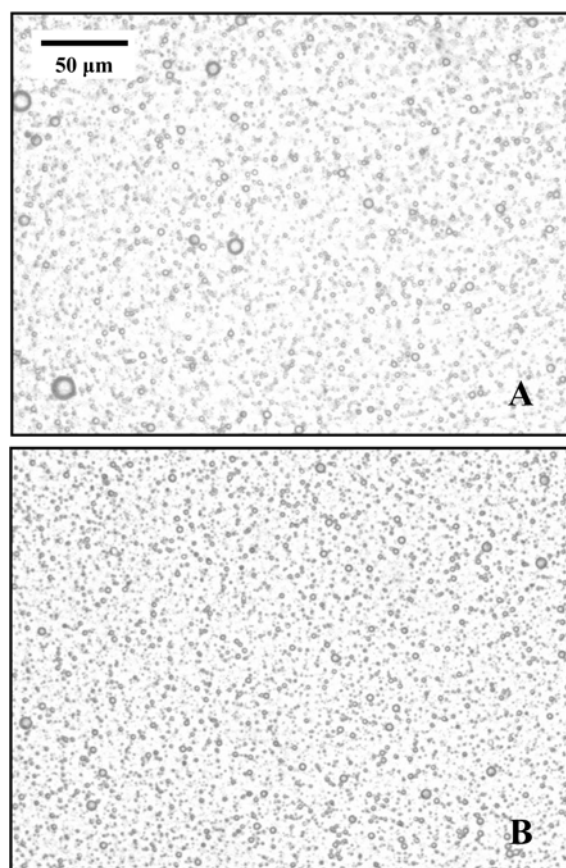
The manner in which droplets break up in a homogenizer depends on the ratio of the viscosities of the droplet and continuous phase ( $\eta_D/\eta_C$ ) (1,28–30). Thus,  $\eta_D/\eta_C$  was determined in the PGPR-stabilized W/O emulsion system consisting of 20 wt% aqueous phase and 80 wt% corn oil phase (Emulsion 1). The  $\eta_D/\eta_C$  was calculated approximately as 0.01 from the apparent viscosities of the oil phase (+PGPR) and water phase ( $\eta_C \approx 68 \text{ mPa}\cdot\text{sec}$ ,  $\eta_D \approx 0.9 \text{ mPa}\cdot\text{sec}$  at 25°C). It has



**Fig. 3. Influence of xanthan as a thickening agent on droplet distribution and microstructure of PGPR-stabilized W/O emulsions.** 0.1 wt% xanthan was added into the aqueous phase (5 mM phosphate buffer at pH 7) of the emulsions before emulsification, and then heated at 80°C for 30 min after emulsification.

been well understood that droplets are resistant to breakup at low viscosity ratios ( $<0.05$ ) and high viscosity ratios ( $>5$ ) because they are able to become extremely elongated before any disruption occurs at the low viscosity ratios and they do not have sufficient time to become deformed during the application of the disruptive forces at the very high viscosity ratios. At intermediate viscosity ratios (0.05–5), droplets are susceptible to break up because they tend to form a dumbbell shape just prior to breaking up (1, 28–30). To fall  $\eta_D/\eta_C$  of the W/O emulsions into the specific range,  $\eta_D$  was increased by adding 0.1 wt% xanthan as a thickening agent into the aqueous phase, thus  $\eta_D/\eta_C$  was adjusted to ca. 4 (Emulsion 2). Xanthan has been reported to increase appreciably the consistency factor of water because of its ability to immobilize water (8). In the absence of xanthan (Emulsion 1), the particle size distribution was bimodal with a major peak consisting of micron sizes of small droplets and a minor peak consisting of submicron sizes of very small droplets (Fig. 3). In the presence of xanthan (Emulsion 2), while the volume of submicron sizes of droplets appreciably increased, the peak consisting of micron sizes droplets was right-shifted (Fig. 3). Previous studies reported that when the viscosity of dispersed phase of emulsions was increased, the rate of droplet coalescence was reduced and the resulting droplets tended to be smaller, which was attributed to the increase in activation energy for coalescence with the increase in the viscosity of internal phase (8,31). However, optical microscopy indicated that the xanthan neither improved nor adversely affected on the microstructure of the W/O emulsion (Fig. 3). The difference between the laser diffraction and optical microscopy measurements could be attributed to the instrumental limitation of dynamic light scattering measurement that is not sensitive to the presence of slow-moving particles larger than about 3  $\mu\text{m}$  (1). Clearly, there were droplets larger than this in the microscopy pictures of the W/O emulsions without xanthan (Fig. 1–3).

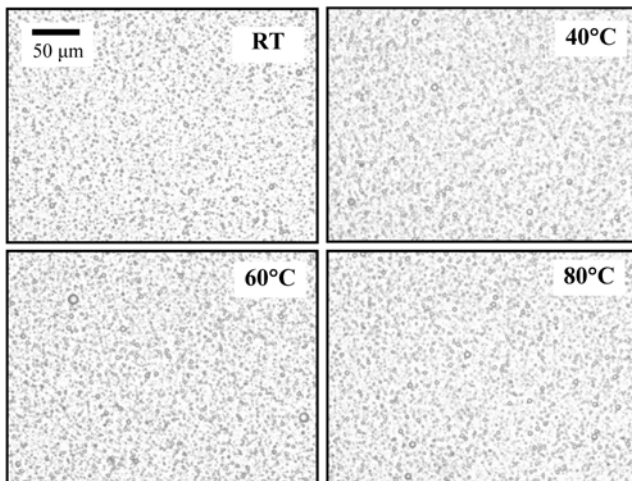
The W/O emulsion droplets containing xanthan (Emulsion 2) were unaffected by heating (80°C for 30 min) (Fig. 3), which confirmed once again the previous findings of that



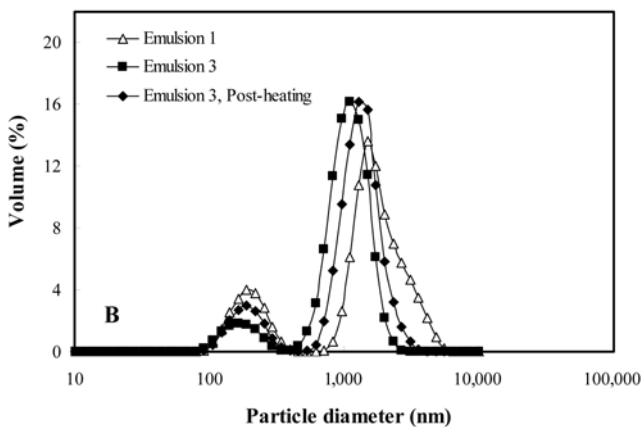
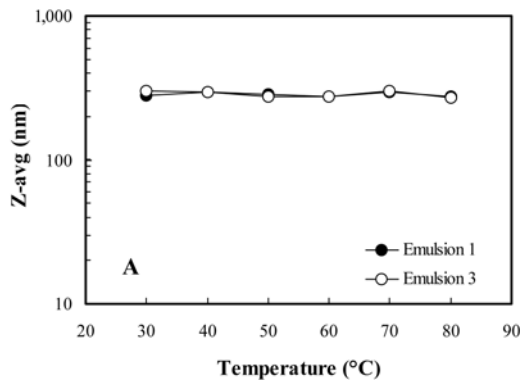
**Fig. 4. Influence of heating of both oil phase (PGPR in corn oil) and aqueous phase before emulsification on the microstructure of PGPR-stabilized W/O emulsions.** Each phase was heated at 50°C for 1 hr (B: Emulsion 3). Emulsion 1 (A) was prepared without heating both phases before emulsification.

lipophilic surfactants did not change their own characters upon heating (Fig. 2).

**Influence of emulsification at an elevated temperature on the properties of PGPR-stabilized emulsions** The viscosity of both the oil and aqueous phase is temperature dependent and therefore the minimum droplet size that can be produced may be altered because of a variation in the viscosity ratio,  $\eta_D/\eta_C$ . Usually the viscosity of oils decreases more rapidly with increasing temperature than the viscosity of water, hence  $\eta_D/\eta_C$  for a W/O emulsion would tend to increase, thereby facilitating droplet disruption at higher temperature (1). However, the oil phase (+PGPR) and water phase were heated only to 50°C for 1 hr before emulsification (Emulsion 3) because temperature dependency of lipid oxidation (32) did not enable to select higher temperatures. The temperatures of the emulsion were ca. 45 and 44°C when it was fed into and came out of the homogenizer, respectively, thus viscosities of both oil phase (+PGPR) and water phase were calculated at 45°C ( $\eta_C \approx 34$  mPa·sec,  $\eta_D \approx 0.6$  mPa·sec).  $\eta_D/\eta_C$  of the Emulsion 3 was approximately 0.02, which is 2-fold higher than the value for Emulsion 1 but still less than 0.05. Nevertheless, homogenizing the W/O emulsions at an elevated temperature clearly led to a smaller water droplet size (Fig. 4). This was

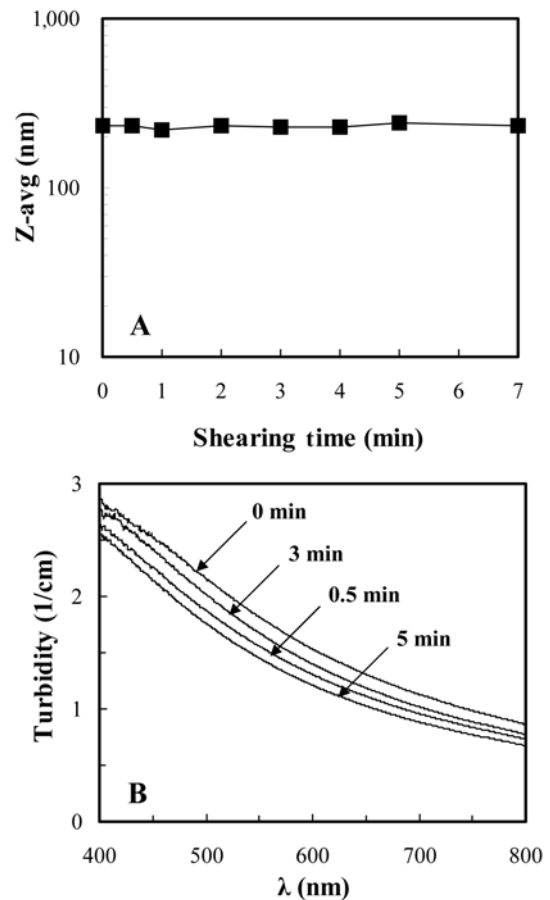


**Fig. 5.** Thermal stability of PGPR-stabilized W/O emulsion that was prepared by heating both phases before emulsification (Emulsion 3). Emulsion 3 was subjected to thermal processing at 30–80°C for 30 min.



**Fig. 6.** Influence of heating of both oil phase and aqueous phase before emulsification on droplet size (A) and particle size distribution (B) of PGPR-stabilized W/O emulsions. Each phase was heated at 50°C for 1 hr. Emulsion 3 was subjected to thermal processing at 30–80°C for 30 min.

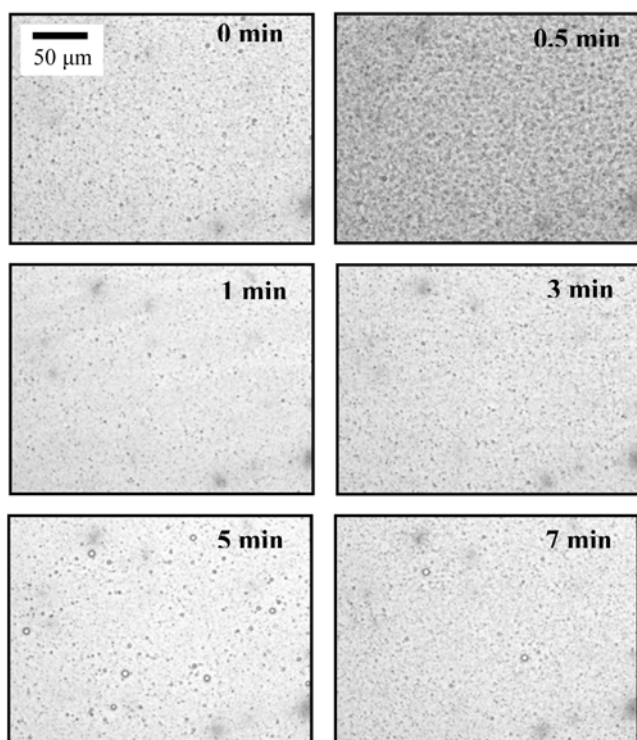
probably because the viscosity of the oil phase decreased appreciably on heating, which made it easier for droplet disruption to occur within the homogenizer. In subsequent experiment, thermal stability of the Emulsion 3 was



**Fig. 7.** Influence of shearing time on droplet size (A) and turbidity spectra (B) of PGPR-stabilized W/O emulsions (Emulsion 3).

examined. Consistently, there was no significant difference in the microstructures of the W/O emulsions that had undergone heat treatment (30–80°C for 30 min) after emulsification (Fig. 5). In dynamic light scattering measurement, the mean droplet diameter (Z-average) of both Emulsion 1 and Emulsion 3 was ca. 300 nm and which value was not changed with temperatures after emulsification (Fig. 6A). In addition, there was no noticeable shift between particle size distributions of Emulsion 1 and Emulsion 3 although the major peak reflecting the volume of micron sizes of droplets was slightly left-shifted in the Emulsion 3 compared with that of the Emulsion 1 (Fig. 6B). The different results depending on analytical technique (dynamic light scattering vs. optical microscopy) suggest that optical microscopy should be always used to complement particle size measurement and to determine the general microstructure of emulsions because the microscopy observation provides information about the tendency for droplets to aggregate in non-diluted emulsions and about whether the aggregation is due to coalescence or flocculation (1).

Taking above results together, PGPR-stabilized W/O emulsions were prepared by heating both oil phase and aqueous phase to 50°C prior to homogenization because there was an apparent decrease in the droplet size with the pre-heating process and because the W/O emulsions

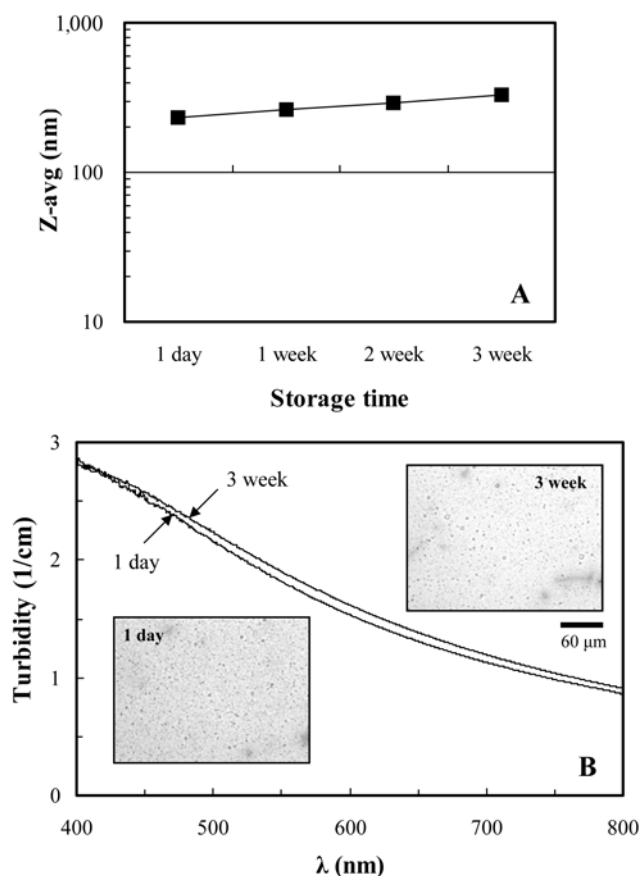


**Fig. 8.** Photomicrographs of 20 wt% W/O emulsions stabilized with PGPR (Emulsion 3) depending on shearing time. The emulsions were subjected to constant shear for 0-7 min.

contained relatively small water droplets that were evenly dispersed throughout the oil phase.

**Influence of shearing stress on the properties of PGPR-stabilized W/O emulsions** If there is a mechanical stress applied to an interface that is covered with emulsifier, some of the emulsifier molecules may be dragged along the interfaces and then coalescence could occur if 2 emulsifier-depleted regions on different droplets come into close proximity during a droplet-droplet encounter (33). Here, PGPR-stabilized W/O emulsion droplets' resistance to mechanically induced stress was assessed by measuring particle size, turbidity, microstructure, and sedimentation stability of the emulsions that had been subjected to the constant mechanical agitation by a high speed blender.

The data obtained from the dynamic light scattering indicated there was no increase in the particle diameter with shearing (Fig. 7A). Turbidity vs. wavelength measurement was also used to ascertain the extent of droplet coalescence in the emulsions. The wavelength of light varied from 400 to 800 nm and therefore the droplets of similar size to each wavelength would contribute to the measured turbidity. In all W/O emulsions regardless of shearing time, the turbidity of the emulsions was relatively high at short wavelength and decreased steeply with increasing wavelength (Fig. 7B), which is indicative of a system containing small particles. In a microscopic analysis, there was no observable evidence of droplet coalescence depending on the shearing stress (Fig. 8). In addition, all emulsions were stable to gravitational separation in accelerated sedimentation test using centrifugal field, there being no evidence of the



**Fig. 9.** Influence of storage time on droplet size (A), turbidity spectra (B), and microstructure (B) of PGPR-stabilized W/O emulsions (Emulsion 3). The emulsions were stored at room temperature up to 3 weeks.

formation of an oil-rich layer at the top of the emulsion due to downward movement of the water droplets. The stability of these emulsions may have been because the relatively high viscosity of the oil continuous phase at room temperature (ca. 68 mPa·sec) retarded movement of the water droplets. It could be also attributed to the fact of that the polymeric emulsifier PGPR provides sterically strong repulsive force between water droplets thus prevents the droplets from aggregating (15).

**Influence of long-term storage on the properties of PGPR-stabilized W/O emulsions** Sedimentation test accelerated by centrifuging an emulsion at a fixed speed for a certain length of time could be used as a means of predicting the long-term stability of emulsions. However, it should be treated with caution because the factors that determine droplet movement in a gravitational field may be different from those that are important in a centrifugal field, e.g., the continuous phase may have a yield stress that is exceeded in a centrifuge, but which would never be exceeded under normal storage conditions (1). Therefore, the PGPR-stabilized W/O emulsions were stored at room temperature up to 3 weeks and their stabilities were assessed by measuring particle size, turbidity, microstructure, and sedimentation stability of the emulsions at the time of 1 day, 1, 2, and 3 week.

There was no significant effect of long-term storage on the droplet aggregation of the emulsions. Dynamic light scattering measurement and turbidity measurement indicated that there was no evidence of droplet aggregation in the emulsions during the experimental period (Fig. 9). Regardless of storage time, the intensity-weighted mean diameters of the emulsions were around 300 nm (Fig. 9A), and there was a fairly steep decrease in the turbidity when the wavelength was increased from 400 to 800 nm (Fig. 9B). No significant difference was observed in the overall microstructure (Fig. 9B) and the sedimentation stability of the emulsions after storage, which was presumably due to the suppression of coalescence-rate through the relatively high viscosity of the continuous oil phase at room temperature.

Overall, the PGPR-stabilized W/O emulsions that were homogenized at an elevated temperature clearly contained smaller water droplets and had good stability to droplet aggregation under the environmental stresses such as mechanical agitation, thermal processing, and long-term storage. This study clearly showed that stable food grade W/O emulsions coated by PGPR could be prepared in a system containing a vegetable oil that was known to have a relatively high water solubility and viscosity. The finding of this study provides valuable information on the requirements and the properties of emulsifiers for preparing high stable W/O emulsions.

## References

- McClements DJ. Food Emulsions: Principles, Practice and Techniques. CRC Press, Boca Raton, FL, USA. pp. 53-339 (2004)
- Benichou A, Aserin A, Garti N. Polyols, high pressure, and refractive indices equalization for improved stability of W/O emulsions for food applications. *J. Disper. Sci. Technol.* 22: 269-280 (2001)
- Bjerregaard S, Soderberg I, Vermehren C, Frokjaer S. Formulation and evaluation of release and swelling mechanism of a water-in-oil emulsion using factorial design. *Int. J. Pharm.* 193: 1-11 (1999)
- Bjerregaard S, Vermehren C, Soderberg I, Frokjaer S. Accelerated stability testing of a water-in-oil emulsion. *J. Disper. Sci. Technol.* 22: 23-31 (2001)
- Auweter H. Multiple-core encapsulation: Double emulsion. pp. 145-151. In: *Microencapsulation of Food Ingredients*. Vilstrup P (ed). Leatherhead Food International, Leatherhead, UK (2001)
- Goubran R, Garti N. Stability of water in oil emulsions using high molecular weight emulsifiers. *J. Disper. Sci. Technol.* 9: 131-148 (1988)
- Knöth A, Scherze I, Muschiolik G. Effect of lipid type on water-in-oil emulsions stabilized by phosphatidylcholine-depleted lecithin and polyglycerol polyricinoleate. *Eur. J. Lipid Sci. Tech.* 107: 857-863 (2005)
- Knöth A, Scherze I, Muschiolik G. Stability of water-in-oil emulsions containing phosphatidylcholine-depleted lecithin. *Food Hydrocolloid* 19: 635-640 (2005)
- Scherze I, Knöth A, Muschiolik G. Effect of emulsification method on the properties of lecithin- and PGPR-stabilized water-in-oil emulsions. *J. Disper. Sci. Technol.* 27: 427-434 (2006)
- Surh J. Influence of pH, emulsifier concentration, and homogenization condition on the production of stable oil-in-water emulsion droplets coated with fish gelatin. *Food Sci. Biotechnol.* 16: 999-1005 (2007)
- Surh J, McClements DJ. Influence of salt concentrations on the stabilities and properties of sodium caseinate stabilized oil-in-water emulsions. *Food Sci. Biotechnol.* 17: 8-14 (2008)
- Oh JK, Kim SJ, Imm JY. Antioxidative effect of crude anthocyanins in water-in-oil microemulsion system. *Food Sci. Biotechnol.* 15: 283-288 (2006)
- Johansson D, Bergenstahl B, Lundgren E. Wetting of fat crystals by triglyceride oil in water. 1. The effect of additives. *J. Am. Oil Chem. Soc.* 72: 921-931 (1995)
- Wilson R, Van Schie B, Howes D. Overview of the preparation, use and biological studies on polyglycerol polyricinoleate (PGPR). *Food Chem. Toxicol.* 36: 711-718 (1998)
- Dedinaite A, Campbell B. Interaction between mica surfaces across triglyceride solution containing phospholipid and polyglycerol polyricinoleate. *Langmuir* 16: 2248-2253 (2000)
- Weiss J, Scherze I, Muschiolik G. Polysaccharide gel with multiple emulsion. *Food Hydrocolloid* 19: 605-615 (2005)
- Su J, Flanagan J, Hemar Y, Singh H. Synergistic effects of polyglycerol ester of polyricinoleic acid and sodium caseinate on the stabilisation of water-oil-water emulsions. *Food Hydrocolloid* 20: 261-268 (2006)
- Su J, Flanagan J, Singh H. Improving encapsulation efficiency and stability of water-in-oil-in-water emulsions using a modified gum arabic (Acacia (sen) SUPER GUM™). *Food Hydrocolloid* 22: 112-120 (2008)
- Lindenstruth K, Muller BW. Parameters with influence on the droplet size of w/o emulsions. *Pharmazie* 59: 187-190 (2004)
- Roberts DD, Pollien P, Watzke B. Experimental and modeling studies showing the effect of lipid type and level on flavor release from milk-based liquid emulsions. *J. Agr. Food Chem.* 51: 189-195 (2003)
- Leal-Calderon F, Mondain-Monval O, Pays K, Royer N, Bibette J. Water-in-oil emulsions: Role of the solvent molecular size on droplet interactions. *Langmuir* 13: 7008-7011 (1997)
- Dickinson E, McClements DJ. Water-in-oil-in-water multiple emulsions. pp. 280-300. In: *Advances in Food Colloids*. Dickinson E, McClements DJ (eds). Blackie Academic & Professional, London, UK (1996)
- Lopez MJ, Vargas-Garcia MC, Suarez-Estrella F, Moreno J. Properties of xanthan obtained from agricultural wastes acid hydrolysates. *J. Food Eng.* 63: 111-115 (2004)
- Morris VJ. Bacterial polysaccharides. pp. 413-454. In: *Food Polysaccharides and Their Applications*. Stephen AM, Phillips GO, Williams PA (eds). Marcel Dekker, NY, USA (2006)
- Takahashi M, Hatakeyama T, Hatakeyama H. Change of gelation characteristics by annealing the solution of polysaccharide/water systems. *Kobunshi Ronbunshu* 55: 760-767 (1998)
- Yoshida T, Takahashi M, Hatakeyama T, Hatakeyama H. Annealing induced gelation of xanthan/water systems. *Polymer* 39: 1119-1122 (1998)
- McClements DJ. Theoretical prediction of emulsion color. *Adv. Colloid Interfac.* 97: 63-89 (2002)
- Bentley BJ, Leal LG. An experimental investigation of drop deformation and breakup in steady, two-dimensional linear flows. *J. Fluid Mech.* 167: 241-283 (1986)
- Janssen JJM, Boon A, Agterof WGM. Influence of dynamic interfacial properties on droplet breakup in simple shear flow. *AIChE J.* 40: 1929-1939 (1994)
- Williams A, Janssen JJM, Prins A. Behaviour of droplets in simple shear flow in the presence of a protein emulsifier. *Colloid Surface A* 125: 189-200 (1997)
- Kamogawa K, Kuwayama N, Katagiri T, Akatsuka H, Sakai T, Sakai H, Abe M. Dispersion and stabilization in water droplets of hydrophobic organic liquids with the addition of hydrophobic polymers. *Langmuir* 19: 4063-4069 (2003)
- Tan CP, Che Man YB, Selamat J, Yusoff MSA. Application of Arrhenius kinetics to evaluate oxidative stability in vegetable oils by isothermal differential scanning calorimetry. *J. Am. Oil Chem. Soc.* 78: 1133-1138 (2001)
- McClements DJ. Protein-stabilized emulsions. *Curr. Opin. Colloid In.* 9: 305-313 (2004)