Polyglycerol polyricinoleate stabilised water-in-oil emulsion: Structural characteristics and functional performance

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Abstract

In the present work, the relationship between structural characteristics and functional performance in polyglycerol polyricinoleate (PGPR) stabilised water-in-oil (W/O) emulsions was investigated. Novel W/O emulsions with a PGPR concentration of 4%, and oil volume fractions (φ_O) of 0.7 and 0.8 showed the smallest mean droplet size (~5 μm) and highest emulsification index (~97%). Both W/O emulsions showed shear-thinning and elastic-dominant properties with a weak frequency dependence. Both W/O emulsions possessed superior thixotropic recovery properties with recovery percentages of 131.26% ($\varphi_O = 0.7$) and 114.56% ($\varphi_O = 0.8$). The thermo-responsive properties could be closely related to the PGPR aggregation induced by hydrophobic interaction and interfacial rearrangement. The present work helped to design and develop functional W/O emulsions as alternatives to butter spreads, bio-lubricants, and other thermo-responsive delivery systems.

Introduction

Water-in-oil (W/O) emulsion consists of the oil phase, water phase, and an emulsifier, in which water is the dispersed phase, and oil is the continuous phase. W/O emulsion can structure complex food matrices (*e.g.*, margarine and butter) and encapsulate water-soluble bioactive (Afoakwa *et al.,* 2007). Norton and Fryer (2012) reported that the W/O emulsion could decrease the fat in chocolate by controlling emulsification and temperature. Mehrnia *et al.* (2016) found that non-ionic surfactant-stabilising W/O micro-emulsions showed superior encapsulation of crocin. However, the W/O emulsion applications are largely limited due to the high mobility of water droplets, leading to poor stability with rapid coalescence and sedimentation (Massel *et al.,* 2014). The problems of poor stability have been overcome by using a high-concentration emulsifier, and introducing other ingredients such as gelling biopolymers and hydrophilic emulsifiers (Gülseren and Corredig, 2012; Wang *et al.,* 2016).

W/O emulsion properties (stability and functionality) are a joint function of multiple factors including emulsifier type, density, viscosity, and oil type (Ushikubo and Cunha, 2014). Therefore, the correlation between structural characteristics and bulk properties of W/O emulsion is crucial for the design and development of stabilised W/O emulsion-based products.

Polyglycerol polyricinoleate (PGPR) is a powerful W/O type emulsifier used extensively by the food industries (Gómez *et al.,* 2011). PGPR has been recognised as a safe food additive with a recommended acceptable daily intake of 25 mg/kg body weight per day (Mortensen *et al.,* 2017). PGPR has also been demonstrated to maintain the stability of W/O emulsion, especially in the chocolate industry, as an effective emulsifier and viscosity modifier (Afoakwa *et al.,* 2007). Moreover, PGPR is also commonly used in the water-in-oil-in-water (W/O/W) emulsion as a novel internal hydrophobic emulsifier for controlled release and targeted delivery of resveratrol (Hemar *et al.,* 2010), sodium chloride

(Sapei *et al.,* 2012), carmine (Marefati *et al.,* 2015), and anthocyanin (Xiao *et al.,* 2017; Lin *et al.,* 2019).

Numerous investigations have focused on PGPR to stabilise W/O and W/O/W emulsion as a potential approach of viscosity modifier and delivery carrier of bioactive. However, understanding the relationship between structural characterisation (droplet state and distribution) and functional performance (rheological and thermal properties) of PGPR-stabilised W/O emulsion remains limited, which is essential for maximising potential applications of W/O emulsion in the food, cosmetic, and pharmaceutical industries.

The present work thus investigated the effects of emulsifier concentration and oil volume fraction on the droplet size and emulsification index (% EI) of PGPR-stabilised W/O emulsions. Their structural characteristics were further analysed using microscopic observation, droplet size distribution, and rheological properties. The present work is the first to evaluate the thixotropic recovery and thermo-reversible properties of PGPR-stabilised W/O emulsions.

Materials and methods

Materials

PGPR (E 476, > 90% of purity with the saponification value of 170 - 185 mg KOH/g) was provided from Guangzhou Yeshang Trading Co., Ltd. (Guangzhou, China). Soybean oil was food-grade oil purchased from a local supermarket. Nile red was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). All other chemicals used in the present work were of reagent grade.

Preparation of W/O emulsion

W/O emulsions with different PGPR concentrations (*c*, 1 - 5%) were prepared by mixing various volume fractions of the oil phase (φ_O, 0.5 - 0.9) with distilled water through a Silent Crusher M homogeniser (Heidolph, Schwabach, Germany) at 20,000 rpm for 2 min at room temperature (~25°C). The formulations are shown in Table 1.

Microscopic observation

The emulsions were observed using an Olympus BH-2 light microscope (Olympus Co., Japan) following the methods prescribed by Lin *et al.* (2019).

Table 1. PGPR-stabilised emulsion formulations.

Formulation	*c* (%)	φ_O
1	1	0.7
2	2	0.7
3	3	0.7
4	4	0.7
5	5	0.7
6	4	0.5
7	4	0.6
8	4	0.7
9	4	0.8
10	4	0.9

Droplet size distribution

The droplet size distributions of the W/O emulsions were measured with a Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) following the methods prescribed by Liu and Tang (2016). The results were recorded as the mean droplet size of the W/O emulsions (D [0.5]).

Emulsification index (EI)

Aliquots (10 mL) of W/O emulsions were accurately transferred into glass tubes (diameter: 2.8 cm; height: 7.5 cm) and sealed for appearance observation (Hu *et al.,* 2016; Li *et al.,* 2019b). These emulsions were monitored for 7 d, and photographed after 2 h (as initial time) and 7 d of storage. Each emulsion test was repeated three times, and *EI* was calculated using Eq. 1:

$$EI\ (\%) = \frac{H_E}{H_T} \times 100 \qquad \text{(Eq. 1)}$$

where, H_E = height of the emulsification layer, and H_T = total height of emulsions.

Rheological measurements

The rheological properties of W/O emulsions were evaluated using a HAAKE RS600 rheometer (HAAKE Co., Germany) equipped with parallel plate geometry (diameter: 35 mm; gap: 1 mm). All measurements were performed in three replicates, and the test parameters were as follows (Liu *et al.,* 2016; Li *et al.,* 2019b): amplitude sweeps, 0.1 – 1,000 Pa at the frequency of 1 Hz; frequency sweeps, 0.1 - 10 Hz at the stress of 1 Pa under the linear viscoelastic regions (LVR); flow-rate sweeps, 0.01 - 120 s^{-1} at the frequency of 1 Hz; thixotropic sweeps, 3 interval (600 s) flow-rate sweeps at 1, 10, and 1 s^{-1}, respectively;

temperature sweeps, 20 - 80°C back at a constant rate of 5°C/min.

The flow behaviour index of W/O emulsion was calculated by fitting the experimental data into Herschel-Bulkley model using Eq. 2:

$$\sigma = \sigma_\gamma + K\gamma^n \qquad \text{(Eq. 2)}$$

where, σ = shear stress (Pa), σ_γ = yield stress (Pa), K = coefficient of consistency (Pa·s^n), γ = shear rate (s^{-1}), and n = flow behaviour index.

Confocal laser scanning microscopy (CLSM)

An aliquot (1 mL) of W/O emulsion was stained with 10 µL of 1 mg/mL Nile red, and incubated on a shaker at room temperature for 30 min. The fluorescence image of the dye-stained emulsion was viewed with a TCS SP5 CLSM equipped with an argon-ion laser (Leica, Wetzlar, Germany). A 40 × 1.25 oil Leica objective lens was used, and the excitation wavelength was set at 488 nm for Nile red (Li *et al.,* 2019a).

Statistical analysis

All measurements were independently repeated three times, and results were presented as mean ± standard deviation. Analysis of variance was conducted by Duncan's multiple range test ($p < 0.05$) using statistical software (SPSS Inc., Chicago, IL, USA).

Results and discussion

Effect of PGPR concentration

The mean size of droplets affects the emulsion appearance, storage stability, texture, release characteristics, and biological fate (Berton-Carabin and Schroen, 2015). Figure 1A shows the mean droplet size and *EI* of PGPR-stabilised W/O emulsions as a function of *c* (1 to 5%) with φ_O of 0.7. The mean droplet size of PGPR-stabilised W/O emulsions decreased from 33.66 to 7.87 µm as *c* increased from 1 to 4%. As *c* increased, more PGPR molecules could be adsorbed at the oil-water interface, thus resulting in smaller droplet size due to the increase in interfacial area (Norton and Fryer, 2012). This result should be responsible for their increased *EI* with the increasing of *c* from 1 to 4%, wherein small droplet is beneficial for the emulsion stability (Li *et al.,* 2019b). However, there was no significant difference for the mean droplet size and *EI*

of W/O emulsions when *c* increased from 4 to 5%. This phenomenon could be attributed to the interfacial concentration at PGPR saturation, consistent with a previous study which reported that 4% of PGPR was sufficient in the coverage of the water droplets to form small and stable droplets (Massel *et al.,* 2014). Therefore, φ_O of 0.7 and PGPR concentration of 4% were used in the subsequent study.
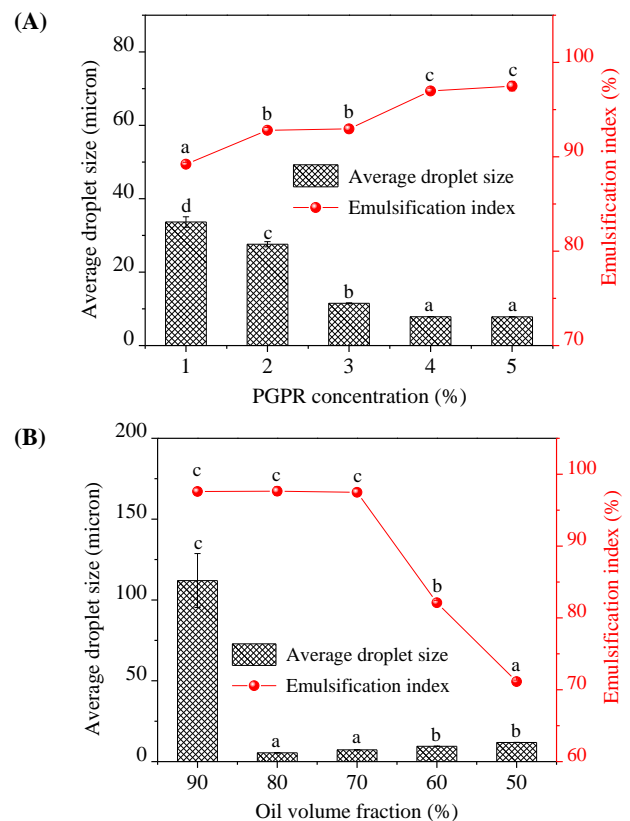

Figure 1. Mean droplet size and emulsification index of the PGPR-stabilised emulsions under different PGPR concentrations **(A)**, and oil volume fractions **(B)**.

Effect of oil volume fraction

Figure 1B presents the mean droplet size and *EI* of PGPR-stabilised W/O emulsions as the function of φ_O (0.5 - 0.9) with the fixed *c* of 4%. As φ_O decreased from 0.9 to 0.8, the mean droplet size of W/O emulsions decreased from 112 to 5.46 µm. Besides, there was no significant difference in the mean droplet size of φ_O at 0.7 and 0.8. The droplet size increased as φ_O decreased from 0.7 to 0.6, possibly due to the effect of emulsifier:oil ratio. In PGPR-stabilised emulsions with $\varphi_O > 0.7$, excessive PGPR contributed to the formation of micelles or micellar aggregates (Moulik, 1996). On the contrary, with $\varphi_O < 0.7$, there was an emulsifier deficit, possibly

promoting the coalescence of the water droplets (Li *et al.,* 2019a). Interestingly, the *EI* of W/O emulsions showed no significant difference when φ_O decreased from 0.9 to 0.7, but decreased dramatically when φ_O decreased to 0.5. This phenomenon could be explained by the high viscosity which slowed down the mobility and separation of water droplets (Ushikubo and Cunha, 2014; Lin *et al.,* 2019). Based on these results, φ_O of 0.7 and 0.8 were suitable for preparing the novel PGPR-stabilising W/O emulsion with 4% of PGPR concentration, and hence were chosen for the following structural and functional characterisation.

Droplet size distribution and optical microscopic examination

The droplet size distribution and morphological properties of PGPR-stabilised emulsions with φ_O of 0.7 and 0.8 were characterised, and are shown in Figure 2. The main peak of the profile at ~10 μm with φ_O of 0.7 and 0.8 could be observed. Specifically, there was a weak peak of the profile at ~1 μm and φ_O of 0.8, which could possibly be associated with the formation of micelles or micellar aggregates due to excessive PGPR (Moulik, 1996). With the increase of φ_O, the aqueous phase fraction decreased, and PGPR saturated the interface of the water droplets (Albert *et al.,* 2019), as the optical micrography showed smaller droplets of φ_O at 0.8 (Figure 2B). In addition, high-speed shear known as a primary homogenisation to form a coarse emulsion (Berton-Carabin and Schroen, 2015) possibly results in the non-homogeneous droplet size distribution for φ_O of 0.8.

Rheological properties

For more information on the functional properties of PGPR-stabilised W/O emulsions with φ_O of 0.7 and 0.8, a small amplitude oscillatory shear test was performed. Figure 3A presents the amplitude sweep (0.1 - 1000 Pa) test of PGPR-stabilised W/O emulsions with φ_O of 0.7 and 0.8 at a fixed frequency of 1 Hz. The elastic modulus (*G'*) was much higher than the viscous modulus (*G''*) in LVRs for both W/O emulsions, revealing elastic-dominant properties. The values of *G'* and *G''* in the LVR were higher in the W/O emulsion with the φ_O of 0.8, as compared to that for φ_O of 0.7. Moreover, the delayed crossover point of oscillatory yield stress (indicated by arrows) further confirmed that the strength and stiffness of W/O emulsion were enhanced at higher φ_O, which

could be attributed to the interfacial arrangement of PGPR, thus contributing to enhanced interfacial film (Gülseren and Corredig, 2014). In addition, *G'* values were higher than *G''* values in the entire frequency range, thus suggesting the formation of the elastic W/O emulsion, as shown in Figure 3B.
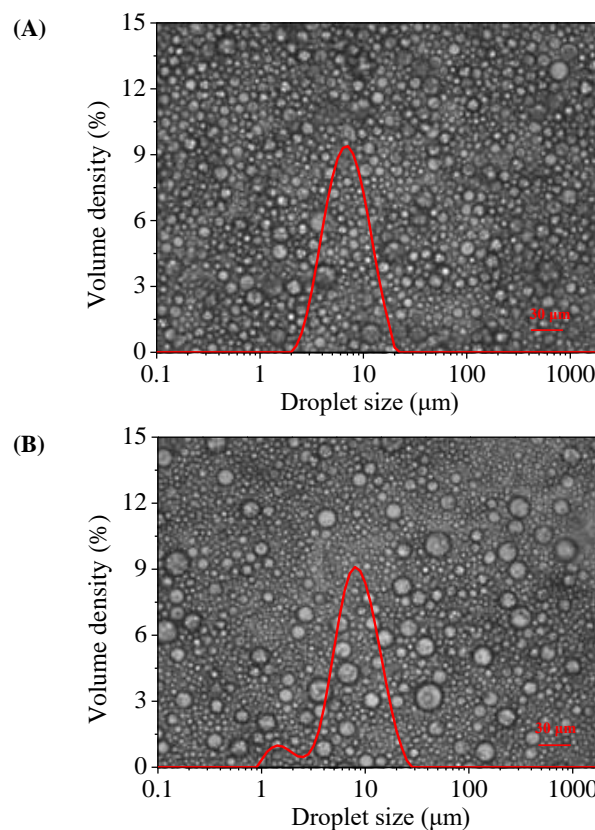

Figure 2. Volume density and droplet size distributions of the PGPR-stabilised emulsions with oil volume fractions of 0.7 **(A)**, and 0.8 **(B)**.

The apparent viscosity and shear stress of PGPR-stabilised W/O emulsions with φ_O of 0.7 and 0.8 as the function of shear rate are shown in Figures 3C and 3D, respectively. The apparent viscosity of both W/O emulsions decreased with the increasing shear rate, thus indicating shear-thinning properties. This trend might be due to the breakdown of water droplet clusters in the W/O emulsions under shear force (Liu *et al.,* 2016). Based on the apparent viscosity curves, the emulsion with φ_O of 0.8 showed higher apparent viscosity, possibly due to the higher φ_O contributing to larger contact surface areas between water droplets (Liu *et al.,* 2018). Furthermore, the flow parameters of the emulsions derived from the apparent viscosity curve over shear rate (Figure 3D) were analysed using the Herschel-Bulkley model. The results derived from the Herschel-Bulkley model are in good agreement with

the experimental data, as shown in Figure 3D. The yield stress σ_γ of the PGPR-stabilised emulsion with φ_O of 0.8 was higher, thus indicating that φ_O could modulate the structure of the W/O emulsion. This result is in agreement with the previously described rheological properties (Figures 3A and 3B). The flow behaviour index n was used to measure the degree of shear-thinning ($n < 1$) and shear-thickening ($n > 1$). Both the PGPR-stabilising emulsions showed good shear-thinning properties (n = 0.57 and 0.41), thus implying that these emulsions could be suitable for applications in completely or partly substituted butter spreads (Giroux *et al.,* 2013; Liu *et al.,* 2018).
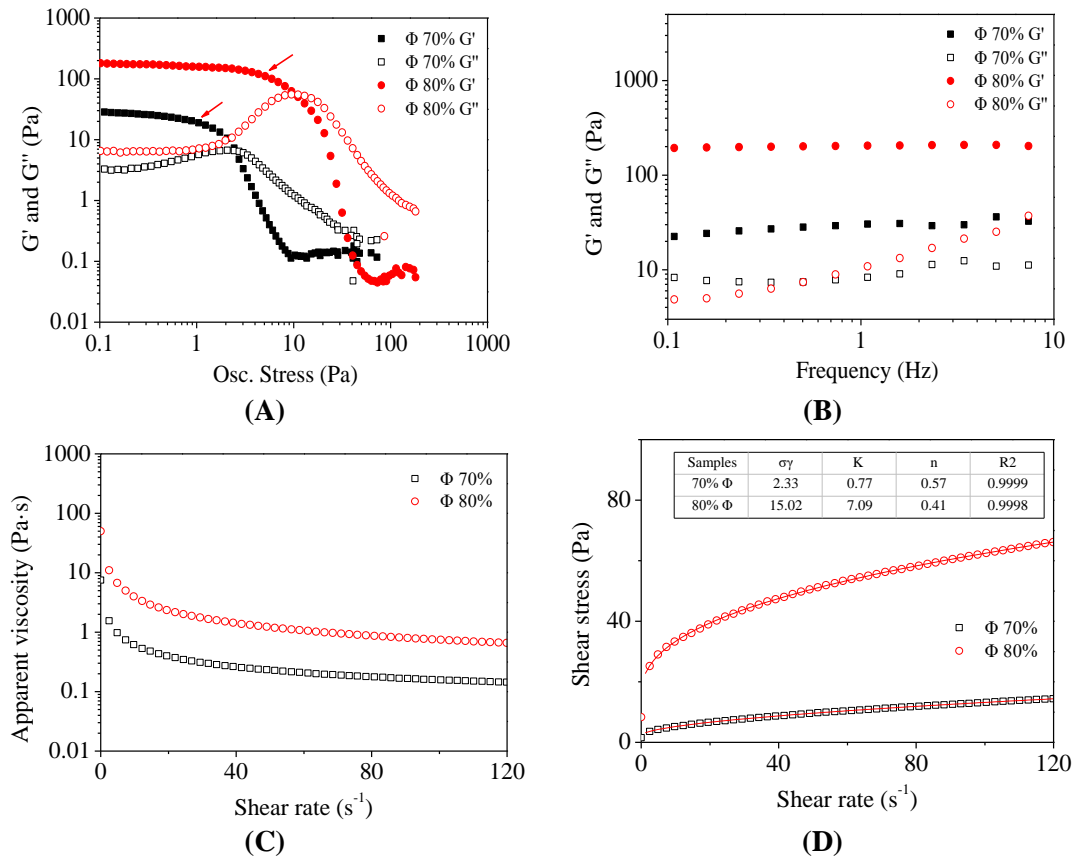

Figure 3. Rheological properties of the PGPR-stabilised emulsions with oil volume fractions of 0.7 and 0.8: amplitude sweeps **(A)**, frequency sweeps **(B)**, apparent viscosity over shear rate **(C)**, and shear stress over shear rate **(D)**.

Thixotropic recovery properties

Thixotropic recovery is a key parameter of spreadable formulations (*e.g.*, mayonnaise) where a reversible structure breakdown is desired. The thixotropic recovery properties of PGPR-stabilised W/O emulsions with φ_O of 0.7 and 0.8 were monitored by observing the changes in apparent viscosity over alternating cycles of low and high shear rates (0.1 and 10 s^{-1}). As shown in Figure 4, recovery percentages (*i.e.*, the percentage of the peak apparent viscosity during interval 3 to the apparent viscosity at the end of interval 1) of the W/O emulsions were 131.26% ($\varphi_O = 0.7$) and 114.56% (φ_O = 0.8), which may be correlated with droplet flocculation during which the broken-down emulsion structure aggregated quickly due to the interfacial arrangement saturated PGPR (Dickinson and Casanova, 1999; Garcia and Whitby, 2012). These results correspond well with the enhanced droplet flocculation of PGPR-stabilised emulsion with φ_O of 0.7 shown in Figure 2A. Patel and Dewettinck (2015) also reported that a recovery percentage of no less than 70% is considered sufficiently good thixotropic recovery. The higher recovery percentages of the PGPR-stabilised W/O emulsions than that of wheat gluten-stabilised high internal phase emulsions (~100%) indicated their superior thixotropic recovery properties (Liu *et al.,* 2018). The applicability of the two thixotropic W/O emulsions is highly desirable in substitutes of petroleum jelly and bio-lubricants.
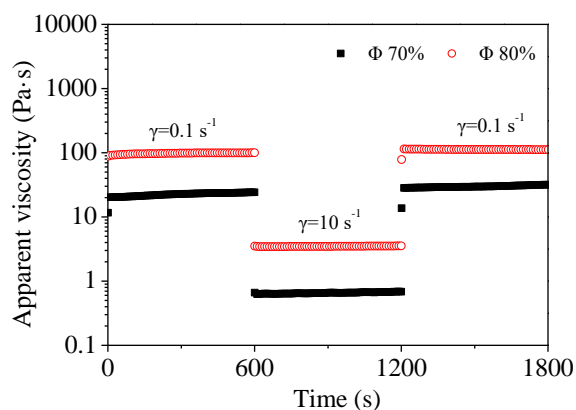
Figure 4. Thixotropic recovery property of PGPR-stabilised emulsions with oil volume fractions of 0.7 and 0.8.

Thermo-responsive properties *and emulsion microstructure*

The thermal reversibility properties of PGPR-stabilised W/O emulsions with φ_O of 0.7 and 0.8 were evaluated via heating and cooling cycles. As shown in Figure 5, the storage modulus *G'* and apparent viscosity of the two W/O emulsions increased when heating from 20 to 80°C, and decreased when cooling from 80 to 20°C. These thermal properties were the opposite of a standard polysaccharide gel system, in which *G'* and apparent viscosity decreased with the increasing temperature due to the coil-helix transition (Eha *et al.,* 2021). The dramatic increase in *G'* and viscosity over the temperature range of 20 - 80°C indicated a substantial increase in the strength of PGPR-stabilised emulsion, which could be attributed to PGPR aggregation induced by hydrophobic interaction and interfacial rearrangement (Chen *et al.,* 2000; Wan *et al.,* 2017). Similar results were found in the sodium caseinate-based emulsion gel, which exhibited thermo-reversible rheological properties when subjected to heating and cooling cycles (Dickinson and Casanova, 1999).

The microstructure of the PGPR-stabilised W/O emulsions with φ_O of 0.7 under heating and cooling cycles was observed using CLSM, during which the oil phase was stained with Nile red. As shown in Figure 5B, the water droplets (shown in black) were surrounded with oil phase (shown in red), thus confirming the formation of a W/O emulsion. As the temperature increased from 20 to 80°C, the droplet size decreased, and the density of the interfacial structures packed around the water droplets intensified. In the meantime, the W/O emulsion fluid shifted to "emulsion gel" with arrested

dynamics as it remained immobile after inverting the glass tube. After cooling to 20°C, the "emulsion gel" returned to its original fluid state. These results indicated good thermal reversibility of the emulsions, which was closely related to the formation of gel-like networks due to the interaction between PGPR and the oil phase during the heating and cooling cycles. Researchers have speculated that heat treatment could promote unfolding and the exposure of reactive units of amphipathic molecules (hydrophobic groups and hydrophilic sites), thus leading to interfacial rearrangement and droplet flocculation that increase gel strength (Zhu *et al.,* 2018). These thermal reversibility properties should be considered in applications where oil leakage at high temperatures must be avoided.
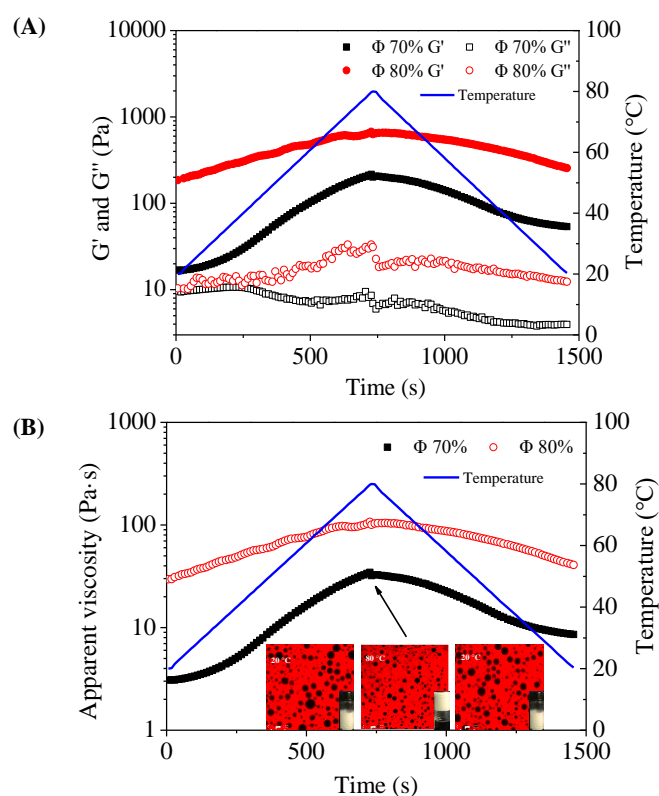

Figure 5. Thermo-reversible property of PGPR-stabilised emulsions with oil volume fractions of 0.7 and 0.8: *G'* and *G''* **(A)**, and apparent viscosity with CLSM images **(B)**.

Conclusions

Novel PGPR-stabilised W/O emulsions with PGPR concentration of 4% and φ_O of 0.7 and 0.8 prepared in the present work showed smaller droplet size and better emulsion stability. The relative quantity of PGPR amount and droplet flocculation

affected the emulsion's functional performances including the elastic-dominance, shear-thinning properties, thixotropic recovery properties, and thermo-responsiveness. The emulsion's thermo-reversible rheological properties under heating and cooling cycles could be closely related to the formation of gel-like networks due to interfacial rearrangement of PGPR that induced droplet flocculation and increased gel strength. The present work is the first to use PGPR in the design and construction of functional W/O emulsions that could be adopted in the food, pharmaceutical, or cosmetic industries.

Acknowledgement

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