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Research Article

Formulation of a Food Grade Water-In-Oil Nanoemulsion: Factors Affecting on Stability

Mahnaz Tabibiazar[1], Hamed Hamishehkar[2]*

[1]*Biotechnology Research Center and Department of Food Science and Technology, Faculty of Nutrition, Tabriz University of Medical Sciences , Tabriz, Iran.*

[2]*Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

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ABSTRACT

Background: Water in oil (W/O) nanoemulsions can be advantageous for encapsulation of bioactive hydrophilic substance alone or as a structural unit of a double emulsion. ***Methods:*** The influence of sonication time, addition of $CaCl_2$ or bovine serum albumin (BSA) to aqueous phase, oil phase (corn or miglyol), and polyglycerol polyricinoleate (PGPR) content on droplet size and physical stability of W/O emulsions at a 30:70 ratio was investigated. Interfacial tension measurement, rheological analysis, Z-average measurement, and visual observations were used to characterize W/O emulsion stability. ***Results:*** The results showed a rapid decease in interfacial tension to 6 $mN.m^{-1}$ and mean droplet size to 347 ± 35 nm in the water/miglyol emulsion, but it was not adequate to establish physical stability. The slight solubility of miglyol in water can be attributed to increased coalescence during storage and an increase in droplet size. The addition of $CaCl_2$ and albumin appreciably reduced the mean droplet size to128 ± 37 nm and increased the physical stability of the corn oil-based W/O emulsion. ***Conclusion:*** Rheological assessment showed there is significant relation between the elastic modulus and stability of water in the corn oil emulsion. The good affinity of the formulation ingredients (PGPR-corn oil-BSA) led to formation of compact interfacial and physical stability of the emulsion.

Introduction

Water-in-oil emulsion is a structural component of many foods, drugs, and cosmetics. Water-in-oil nanoemulsion is of interest for delivery purposes and for design of stable double emulsions.[1-7] The multi-phase, compartmentalized structure of double emulsions makes them suitable for design of delivery systems and controlled release of sensitive components for production of low calorie, reduced-salt products, masking of off–flavors, prevention of oxidation, and protection of probiotics in food and drug products against gastric juices.[8-10]

Although a stable primary emulsion is critical for overall stability of double emulsions, few studies have focused on the formulation of stable water-in-oil nanoemulsions as a building unit of double emulsions.[11,12] Factors affecting the droplet size of the primary emulsion are the oil phase composition, interfacial properties and viscosity of the phases, hydrophobic emulsifier, shear rate during emulsification, and solubility of the oil phase in water.[13,14]

The steric forces that are the dominant repulsion force between droplets affect the stability of W/O emulsions. The effect of electrostatic repulsion is negligible in W/O emulsions because of the low electrical conductivity of oil. The high mobility of water droplets, which can easily coalesce, makes low stability of W/O emulsion.[15]

Stability is the most important characteristic of emulsion systems. The present study investigated the stability of W/O emulsions prepared using polyglycerol polyricinoleate (PGPR) as an emulsifier, corn, and miglyol 812 oil in the presence of $CaCl_2$ and albumin by measuring the interfacial tension, viscosity, mean droplet diameter, and by visual observation. All materials used for the preparation of the nanoemulsion were of food and pharmaceutical grade.

Materials and Methods

Materials

PGPR(E467) oil-soluble emulsifier was purchased from Oleon-Belgium. Lyophilized bovine serum albumin (BSA) was purchased from Sigma-Aldrich and

***Corresponding Author:** Hamed Hamishehkar , Tel: (+98) 41 33363234, E-mail: Hamishehkar.hamed@gmail.com

stored in the refrigerator at 4°C. Commercially-available Kasisuri corn oil (Thailand) was purchased from the local supermarket. Miglyol 812 and $CaCl_2$ were purchased from Merck. Sterile double-distilled water was used for sample preparation.

Preparation of emulsions

The PGPR was dissolved at concentrations of 4% and 6% (wt) in the corn or miglyol oil phases while being heated to 40°C and stirred with a magnetic stirrer. BSA solution was prepared by dissolving BSA powder into sterile double-distilled water that was stirred to enable complete hydration. The aqueous phases of the emulsion were prepared using the following formulas (F): (F1) Sterile double-distilled water; (F2) 0.3% (wt) $CaCl_2$ solution; (F3) 6% (wt) BSA solution; and (F4) 6% (wt) BSA solution containing 0.3% (wt) $CaCl_2$.

The ratio of aqueous phase to oil phase was set at 30:70. A coarse emulsion was prepared by homogenization using a high-speed Ultra-Turrax blender (Hiedoloph; Germany**)** at 22000 rpm for 5 min. The coarse emulsion was further emulsified using a 20 kHz UP200S ultrasonicator (Dr. Hielscher; Germany) with a maximum power output of 200 W. Energy input was provided by a H3 sonotrode containing a piezoelectric crystal with a titanium probe 3 mm in diameter.

The amplitude of oscillation was set at 100 microns. The temperature difference between the primary coarse emulsion and the final emulsion was less than 10°C. An increase in temperature during ultrasonication was prevented by placing the sample container in a bigger beaker containing ice. The volume of the coarse emulsion was set to 5 ml for all samples and the sonotrode was located 0.5 cm below the surface of the emulsion. A preliminary study showed an increase in $CaCl_2$ concentration to 0.6% (wt) induced instability in the protein structure (salting-out) in the aqueous phase.

Particle size measurement

The mean droplet diameter and size distribution of emulsions were determined by dynamic light scattering (Zetasizer-ZS, Malvern, UK). To avoid multiple scattering effect, all samples were diluted 100-fold with oil phases containing PGPR before measurement. The refractive indices of the emulsions were measured using a refractometer.

Interfacial tension measurement

Interfacial tension between the aqueous and oil phases was measured using the du Nöuy ring method with a tensiometer (White; England) at 25°C. Tests were performed in triplicate for each measurement.

Rheological measurement

The viscosity and rheological properties of the W/O emulsion were determined using a Physica MCR 301 rheometer (Anton Parr; Austria) with double-gap concentric cylinder geometry after ramping the shear rate profile from 0.1 to 1000 S^{-1} at 25°C. Oscillatory measurements were performed at 25°C on 5 ml of emulsion to determine the storage and loss moduli at a 0.1-100 Hz frequency range.

Statistical analysis

All experiments were carried out in triplicate. Two-way ANONA was performed using GraphPad Prism 5.3 to assess for significant differences between samples. Results at $p < 0.05$ were considered statistically significant. Bonferroni multiple comparisons tests were used to compare formulation treatments on droplet size in the corn and miglyol oil-based W/O emulsions.

Results and Discussion

Effect of sonication time on droplet size

The coarse emulsion was prepared at PGPR contents of 4% and 6% (wt) in the oil phase and four combinations of the aqueous phase to determine the effect of sonication time on droplet size. Sonication was performed at 200 W at increments of 1 min for 5-15 min.

A preliminary study on optimal sonication time showed that formula-containing protein was not stable after 5 min of sonication. This could be the result of denaturation of the protein duo to the sudden and uncontrollable increase in oil temperature. The cavitation threshold decreases considerably in viscose media such as oil during sonication [16] ; Therefore, Sonication was performed in 30 s increments for 5-15 min. Both the corn and miglyol oil-based formulations with 4% (wt) PGPR in the oil phase were not stable and the oil phase gradually separated from the aqueous phase after sonication at different rates over the course of 3 days.

Figure 1 shows mean droplet diameters of 30 wt% water in oil emulsions stabilized by 6% (wt) PGPR decreased significantly as function of sonication time in miglyol and corn oil.

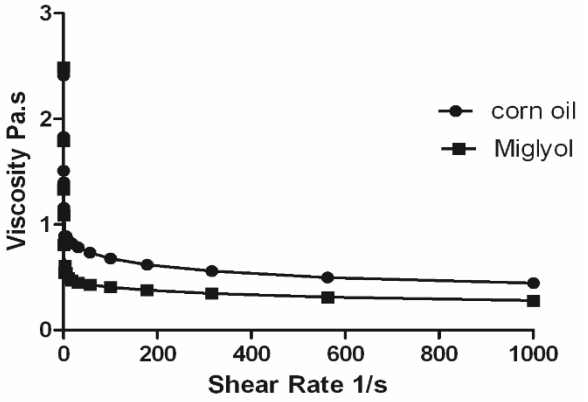

Figure 1. Mean droplet diameters of 30 wt% water in oil emulsions stabilized by 6wt%PGPR as function of sonication time immediately after preparation.

The miglyol oil-based formulation with 6% PGPR showed low physical stability. Despite the moderately low Z-average (347 ± 35 nm; Table1), phase separation

occurred after 3 days. More information is required about the physicochemical properties of miglyol and PGPR to explain these conflicting results. Studies have shown that W/O/W emulsion can be successfully formulated using miglyol 812.[6] A previous report on the molecular structure of both oil and emulsifier are important when identifying the stability of the emulsion.[15]

Table 1. Droplet size and physical stability of different formulation of 30 % wt water in oil (corn and miglyol 812) emulsion prepared with 6%wt PGPR in oil phase after 10 min sonication.

Aqueous phase composition	Z average		PDI		Physical stability	
	Corn	Miglyol	Corn	Miglyol	Corn	Miglyol
F1) water	217±23[a]	347±35[a]	0.3	0.35	>11	<3
F2) 0.3 wt%$CaCl_2$	136±31[b]	238±26[b]	0.21	0.41	>23	<8
F3) 6wt% BSA	136±42[c]	275±9[b]	.036	0.35	>27	<14
F4) 6wt% BSA + 0.3wt%$CaCl_2$	128±37[c]	278±14[b]	0.45	1	>32	<19

Value with different letters are significantly different ($p<0.05$)

Effect of CaCl2 and BSA concentration on droplet size

Figure 2 shows that addition of up to 0.3% (wt) $CaCl_2$ significantly decreased droplet size ($p < 0.05$) in all formulated emulsions. It has been proposed that $CaCl_2$ influences droplet size of emulsions by reducing interfacial tension by mechanisms such as reducing the electrical double layer or the interfacial activity of fatty acid in oil and higher packing of PGPR.[17]

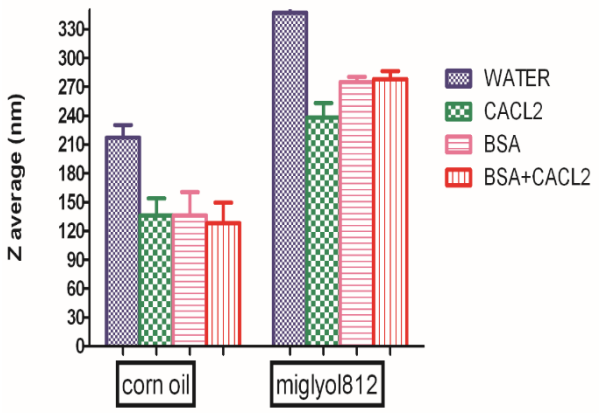

Figure 2. Effect of addition of $CaCl_2$ and BSA on Z-average in corn and miglyol oil after 10 min sonication (power: 200W).

The presence of BSA significantly decreased droplet size in corn oil-based emulsion ($p < 0.05$) and increased the physical stability of the emulsion through the steric repulsion effect (Table1). One explanation for the positive influence of protein on decreasing droplet size and stability may be related to the interaction of PGPR with the hydrophobic moieties of the protein, which causes changes in the viscoelastic properties of the interface. It has been suggested that sodium caseinate and PGPR have synergistic effects on stabilizing the W/O interface. The use of PGPR extensively decreased in combination with amphiphilic emulsifier.[18,19]

Effect of interfacial tension on droplet size

Table 2 shows that the interfacial tension of the water-corn oil was 36.8 $mN.m^{-1}$ and of the water-miglyol interface was 15 $mN.m^{-1}$. When PGPR was added to the oil phase, a considerable decrease in interfacial tension occurred to 4.7 and 6 $mN.m^{-1}$in the corn and miglyol systems, respectively. The addition of $CaCl_2$ to the aqueous phase strongly decreased the interfacial tension to 3.5 and 5.2 $mN.m^{-1}$in the corn and miglyol systems, respectively.

Table 2. The amount of interfacial tension and viscosity of different emulsion prepared with 6%wt PGPR in oil phase.

Aqueous phase composition	Interfacial tension($mN.m^{-1}$)		Viscosity(ratio)	
	Corn	Miglyol	Corn	Miglyol
water	36→4.7	15→6	0.017	0.03
0.3 wt%$CaCl_2$	3.5	5.2	-	-
6wt% BSA	7.4	13.9	0.021	0.038
6wt% BSA + 0.3wt%$CaCl_2$	8	9.6	0.021	0.038

The interfacial tension of corn and miglyol oil containing albumin were about 7.4 and 13.9 $mN.m^{-1}$, respectively, but the mean droplet size of the corn oil emulsion in all formulations was significantly lower than for the miglyol emulsion. These results demonstrated that, based on Taylor's equation, the variation in mean droplet diameter of the emulsions could not only originate from the amount of interfacial tension such as role of albumin and polyethylene glycol in the albumin-stabilized oil-in-water nanoemulsion.[20] It should be noted that miglyol showed little solubility in water, which may be the result an increase in coalescence during storage and in droplet size. Corn oil acts as a kinetic barrier to Ostwald ripening because of

its very low solubility in water.[21] PGPR is a hydrophobic and non-ionic emulsifier having one unsaturated and one hydroxyl group with a hydrophilic nature. Miglyol oil consists of two saturated oils (caprylic/capric triglyceride) with a completely hydrophilic nature. It has been reported that the chemical affinity of the oil phase and emulsifier led to a more compact molecular arrangement in the interfacial layer.[15] The interaction of PGPR at the interface was more efficient with corn oil, which has a more hydrophobic character.

Effect of viscosity of oil phases on droplet size

All W/O emulsions formulated with corn oil had a smaller droplet size than miglyol oil emulsion (Table 2). It has been reported that droplet breakup in the emulsion system depends on dispersal to a continuous-phase viscosity ratio (ηD/ηC); this circumstance has been frequently examined in O/W systems.[21,22] The results of the current study show that, if the viscosity of the continuous phase was high enough, energy transfer occurred adequately from the continuous phase to disperse and break up the droplets to form nanosized droplets(Table 2). In agreement with previous reports, the higher viscosity of corn oil in the continuous phase exerts a strong influence on the cavitation thresholds of droplets at the same sonication time.[23]

Rheological assessment

Rheological assessment can provide valuable information on the stability and microstructure of emulsions.[24] Figure 3 shows the viscosity of the corn oil-based emulsion was greater than for the miglyol based-system. It has been reported that a small droplet size increases viscosity in a W/O emulsion.[25]

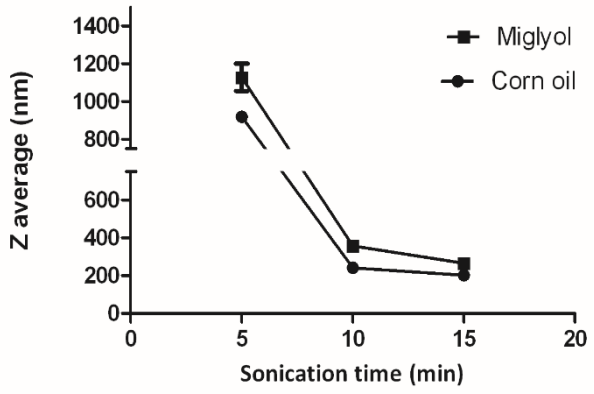

Figure 3. Fluid behaviors of 6 %wt PGPR stabilized W/O emulsion based on (F4) formula as aqueous phase in corn and miglyol oil in the ratio (30:70).

The coalescence of droplets was avoided by the viscoelastic characteristics of the interface during storage as measured by the relatively high complex modulus.[26] As shown in Figure 4, corn oil-based emulsion subjected to frequency sweep experiments behaved mostly like a gel. In the gel structure, the elastic modulus (G´) is significantly higher than the loss modulus (G″). The behavior of miglyol-based emulsion is similar to a dilute solution. These results indicate that there were no compact interfacial layers in the miglyol-based formulation. Droplet deformability depends on interfacial tension and on droplet elasticity. Droplet elasticity been infrequently considered for emulsion systems.[27]

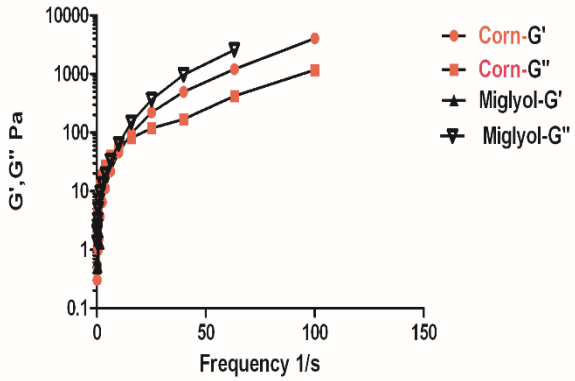

Figure 4. Elastic modulus (G´) and loss modulus (G") variations as function of frequency.

Conclusion

A stable W/O nanoemulsion with a droplet size of approximately 128 nm was prepared by ultrasonication. The presence of 6% PGPR in the corn oil phase was necessary to form a stable emulsion. The addition of $CaCl_2$ and protein to the aqueous phase resulted in a decrease the need for PGPR and increased the stability of W/O emulsions. The results showed that the interaction between oil and PGPR and protein had a great influence on the long-term stability of the emulsion. The reduction of interfacial tension and application of adequate shear rate in both the corn and miglyol systems resulted in the formation of small droplets, but a rapid increase in droplet size of the miglyol oil emulsion occurred through coalescence by different mechanisms.

Conflict of interests

The author claims that there is no conflict of interest.

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