International Food Research Journal 23(4): 1361-1366 (2016)
Journal homepage: http://www.ifrj.upm.edu.my

Role of xanthan gum on physicochemical and rheological properties of rice bran oil emulsion

[1]Saharudin, S. H., [1, 2*]Ahmad, Z. and [3]Basri, M.

[1]*Laboratory of Vaccine and Immunotherapeutic, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Department of Pathology, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[3]*Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

Article history

Received: 11 August 2015
Received in revised form: 23 November 2015
Accepted: 6 December 2015

Keywords

Xanthan gum
Rice bran oil
Flow behaviour
Viscosity
Topical emulsion

Abstract

Xanthan gum is a high molecular weight substance with potential in improving emulsions properties. The formulation composition was selected on Tween 80 ternary phase diagram and was modified by the addition of xanthan gum at different concentrations (0.0% – 1.0%). The main objective of this study was to determine the effect of xanthan gum on physicochemical and rheological properties of rice bran oil emulsion. Xanthan gum concentrations have affected the formulations droplet size. Yet, they did not show any effect on pH of the formulations. However, an increase in xanthan gum concentration had increased the zeta potential value, stability, and viscosity of the formulations. Xanthan gum also enhanced the flow behaviour of the formulations.

Introduction

Rice bran oil (RBO) is produced from the extraction of rice bran. It is widely used for cooking and baking in several countries due to its high smoke point and mild flavour. Generally, it contains saturated and unsaturated fatty acids. The saturated fatty acid consists of palmitic acid (21.5%) and stearic acid (2.9%). Meanwhile, the unsaturated fatty acid consists of linoleic acid (34.4%), α-linoleic acid (2.2%) and, oleic acid (38.4%) (Cicero and Derosa, 2005). On the other hand, RBO is rich in bioactive components such as tocopherols, tocotrienols, phytosterols, polyphenols, squalene, triterpene alcohol, and γ-oryzanol (Cicero and Derosa, 2005; Juliano *et al*., 2005; Hoed *et al*., 2006). It also composed of vitamins such as vitamin E as well as vitamin B (Juliano *et al*., 2005; Oluremi *et al*., 2013). Additionally, RBO contains minerals such as aluminium, calcium, chlorine, iron, magnesium, manganese, potassium, phosphorus, silicon, sodium, and zinc (Oluremi *et al*., 2013).

Owing to its beneficial compounds, the application of RBO could be expanded. Scientists and entrepreneurs have been treating this oil into useful products instead of its usage for cooking purposes. For instance, cosmetic industry has incorporated RBO in sun protection products due to its ability to shield skin from UV light along with a remedy for wrinkles and hyperpigmentation (Burlando and Cornara, 2014). Besides, RBO has been formulated for topical products which aim to slow skin ageing process (Patel and Naik, 2004) and treating skin diseases such as atopic dermatitis and psoriasis (Bernardi *et al*., 2011). Furthermore, this oil is frequently used in hair conditioner, lipstick and nail enamel (Burlando and Cornara, 2014).

Emulsion is a system containing two immiscible liquids such as water and oil. Emulsion system needs help from surfactant to mix the immiscible liquids together. There are several types of emulsion that can be developed which are water in oil (W/O), oil in water (O/W), and multiple emulsions, either water in oil in water (W/O/W) or oil in water in oil (O/W/O) (Rezaee *et al*., 2014; Oppermann *et al*., 2015). The utilization of emulsion has been increased widely in food, cosmetic and medical industries. In topical system, the emulsion works advantageously by encapsulating, protecting, and delivering poorly water soluble compounds to the target destination (Ariviani *et al*., 2015). Normally, emulsion formulations required rheology modifier to obtain the desired characteristics.

Xanthan gum is rheology modifier that is usually

*Corresponding author.
Email: *zalinah@upm.edu.my, zalinahmedic@gmail.com*
Tel: +60128712558, +60389472454; Fax: +6089412787

introduced into emulsion formulation. It is an anionic polysaccharide secreted by bacterium *Xanthomonas campestris* through aerobic fermentation. It consists of a β-(1–4)-linked glucose backbone with orderly distributed trisaccharide side chains and completely soluble in cold and hot water. This gum exhibited high viscosity in solution due to its high molecular weight. Xanthan gum improves the flow and viscosity of formulation thus facilitates the handling, mixing, swallowing, and pouring of the formulation. While having low shear rate, the viscosity is comparatively high and provides a superior stability for the system (Sun *et al*., 2007). The concentration of xanthan gum could be altered to achieve the desired characteristic of emulsion. Thus, this study was carried out to investigate the influence of xanthan gum concentration on physicochemical and rheological characterization of rice bran oil emulsion.

Materials and Methods

Materials

Rice bran oil was obtained from Alfa One (UK). The non-ionic surfactants, Tween 20 was purchased from R&M Chemicals (UK), Tween 80 was obtained from Sigma-Aldrich (USA), and Tween 85 was purchased from Acros Organic (USA). Glycerine was procured from Systerm (Malaysia) and phenoxyethanol was obtained from Fluka (USA). Other than that, disodium EDTA was purchased from Bio Basic Inc (USA). The rheology modifier, xanthan gum was acquired from Sigma-Aldrich (USA). Distilled water was used for ternary phase diagram study and deionised water was used in the rest of the study.

Construction of ternary phase diagram

Construction of the ternary phase diagram was started by mixing the RBO and Tween 85 at various mass ratios ranging from 0:100 to 100:0 and all the mixtures were vortexed (Vortex mixer VTX-3000L, LMS, Japan). After that, water was poured into every tube in various concentrations from 0% (w/w) to 90% (w/w) of the total weight with 5% (w/w) intervals. Next, each and every tube was vortexed for 5 minutes after distilled water was added. Afterwards, they were centrifuged at 4000 rpm for 15 minutes (Centrifuge Universal 320R, Hettich, Germany) and ready to be observed under polarized light. The whole study was performed at room temperature. Finally, the ternary phase diagram was plotted using Chemix School v3.50 software (Arne Standnes, Norway). This experiment was then repeated using Tween 80 and Tween 20.

Table 1. Composition of formulations modified with different concentration of xanthan gum

Code	Composition (% w/w)						
	RBO	T80	DeH_2O	Gly	PE	Di EDTA	XG
XG00	4	7	83.35	5	0.6	0.05	0.0
XG02	4	7	83.15	5	0.6	0.05	0.2
XG04	4	7	82.95	5	0.6	0.05	0.4
XG06	4	7	82.75	5	0.6	0.05	0.6
XG08	4	7	82.55	5	0.6	0.05	0.8
XG10	4	7	82.35	5	0.6	0.05	1.0

Note: RBO: rice bran oil; T80: Tween 80; DeH_2O: deionized water; Gly: glycerine; PE: phenoxyethanol; Di EDTA: disodium EDTA; XG: xanthan gum.

Formulation of RBO emulsion

The composition of the formulation was selected from Tween 80 ternary phase diagram. This formulation was prepared using low energy emulsification process. The aqueous phase which contained deionised water, Tween 80, glycerine, phenoxyethanol, disodium EDTA and hydrated xanthan gum were pre-combined until dissolved. Meanwhile, the oil phase was dominated by RBO. The oil phase was transferred into aqueous phase and was mixed at 200-300 rpm for 4 hour at 25°C using overhead stirrer (IKA-Werke, Germany). The mixture was then being homogenized at 10000 rpm for 5 minutes using high shear homogenizer (IKA T18 Ultra-Turrax, Germany). The compositions of the formulations were shown in Table 1.

Droplet size and zeta potential measurement

The droplet size measurements and zeta potential measurements of all formulations were quantified by zetasizer (Malvern, United Kingdom) after being formulated for 24 hours, to ensure the formulations' system has achieved equilibrium. Ten microlitre of samples were diluted with 10 mL deionised water and vortexed. All measurements were triplicated at 25°C.

Accelerated stability test

The accelerated stability test was conducted to predict the formulations stability at faster rate which introduced the formulations to an extreme condition. All prepared formulations were subjected to centrifugation test at 4000 rpm for 15 minutes at room temperature, 25°C. Then, the nature of the phase was observed.

pH measurement

All prepared formulations underwent pH measurement using pH meter (Delta 320, Mettler Toledo, Switzerland).

Rheological measurement

The rheology property of every formulation was measured by Kinexus Rotational Rheometer (Malvern Instruments Ltd). A stress/rate with a temperature controller was used to determine the rheological characterization of the formulations. The study were carried out using 4°/40 mm stainless steel cone and plate geometry with gap of 0.15 mm at 25°C ± 0.5°C. The shear rate was controlled at range 0.1-100 s^{-1} (Salim *et al*., 2012).

Results and Discussion

Ternary phase diagram

Figure 1 illustrates the ternary phase diagram system of RBO, surfactant, and water. Three regions were formed in each diagram which was isotropic (L), homogeneous (H), and multiphase (M) region. The isotropic emulsion system is one phase system that is transparent. It is also optically clear to the eyes and has similar characteristic to emulsion (Izquierdo *et al*., 2004). Opposite to the isotropic phase, the homogeneous system is a single phase system and it is opaque. In the meantime, the multiphase system has more than one phases and it is an instable system.

Ternary phase diagram of Tween 85 (Figure 1(a)) shows limited isotropic region was formed. The maximum RBO that was dissolved in isotropic region was 4% (w/w) when using 5% (w/w) Tween 85. In homogeneous milky region, the maximum oil that could be solubilised was 29% (w/w) when using around 35% (w/w) of Tween 85. The rest of this diagram consists of multiphase region. Meanwhile, the highest amount of RBO that could be dissolved in Tween 80 system was 10% (w/w) using as high as 78% (w/w) of Tween 80 that was shown in Figure 1(b). There was a slight increment in RBO solubilisation as the amount of Tween 80 was raised. Besides, in the homogeneous milky region, the maximum RBO that could be solubilised was 22% (w/w) using 32% (w/w) of Tween 80. The other part in this diagram was occupied by non-stable emulsions system. Subsequently, Figure 1(c) of Tween 20 ternary phase diagram shows that isotropic region was formed at the water rich apex. In this region, the maximum RBO that could be solubilized was only around 4% (w/w) using 18% (w/w) surfactant. The rest of this diagram was multiphase region.

The HLB value of Tween 85, Tween 80, and Tween 20 are 11.0, 15.0, and 16.7, respectively. Higher the HLB values make the surfactant more hydrophilic. By comparing ternary phase diagram of the three surfactants, Tween 80 has the largest isotropic region. Tween 80 has lower HLB value and longer fatty acid chain (oleic acid) compared to Tween 20 (lauric acid), thus could emulsify larger amount of RBO. Longer hydrophobic tail indicates more oil could be solubilised. Thus, longer the hydrophobic tail could efficiently improve the ability of a surfactant on reducing the interfacial tension of the oil (Niraula *et al*., 2004). Besides that, Tween 85 has lowest HLB value among surfactants used in this study and has oilier properties. This will ease the Tween 85 to dissolve in the internal oil phase, but it would however lead to insufficient numbers of polyoxyethylene chains available to form hydrogen bonds at the interface and the external phase of the system (Barakat *et al*., 2008). This may be the reason of less isotropic region formed in Tween 85 system. Therefore, Tween 80 system was chosen for further study. The selected point was marked with letter 'S' and red dot.

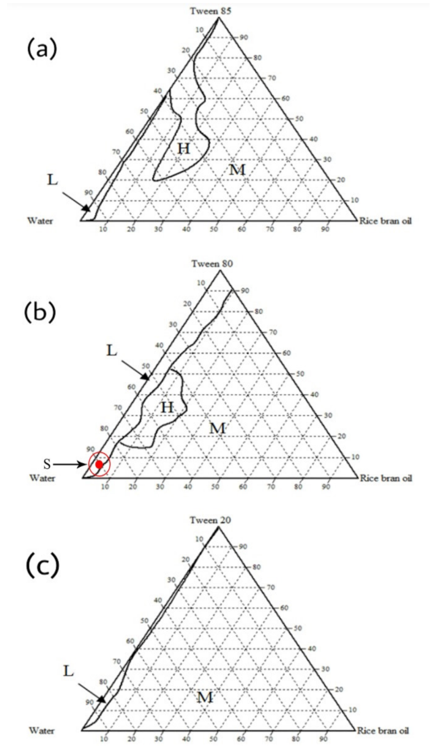

Figure 1(a-c). The phases obtained from the development of ternary phase diagram of Tween 85 (a), Tween 80 (b), and Tween 20 (c) (L: isotropic region; H: homogeneous region; M: multiphase region; S: selected point

Droplet size analysis

Figure 2 illustrated the mean droplet size and mean zeta potential of the formulations which were modified by the addition of different amounts

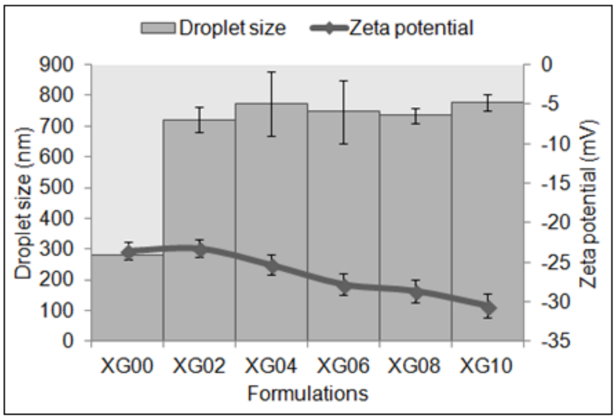

Figure 2. Effect of xanthan gum concentrations on droplet size (nm) and zeta potential (mV) of the formulations at 25°C

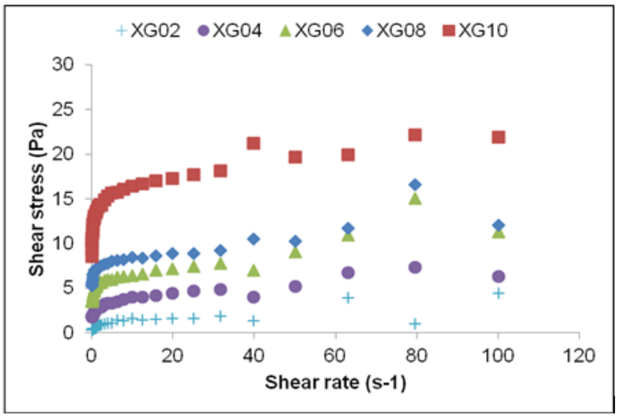

Figure 3. Flow behaviour of formulations modified with different concentrations of xanthan gum at 25°C

of xanthan gum. The tested concentrations were 0.0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% (w/w) which were named XG00, XG02, XG04, XG06, XG08, and XG10, respectively. The droplet size of formulation XG00 was 287.7 nm and the rest of other formulations were around 750 nm. Increase in xanthan gum concentration to 0.2% (w/w) had increased the droplet size. However, further increase in the concentration exceeding 0.2% (w/w) did not showed much difference in formulations droplet size. The formulation without xanthan gum had however shown the lowest droplet size due to the molecules exhibiting perfect collision during Brownian motion which occurred for limited period of time (Ngan *et al*., 2014). Furthermore, without xanthan gum, the formulation system is very fluid and emulsification process is not affected by viscosity level. Thus, formulation without xanthan gum exhibited small droplet size. Meanwhile, the droplet sizes of formulations added with xanthan gum were larger. This may due to the nature of xanthan gum that possesses large molecular structure. This in turn, influenced the droplet size by forming the gel network in the formulations system. In addition, increasing the xanthan gum concentrations from 0.2% (w/w) to 1.0% (w/w) did not cause any difference in the formulations droplet size. This could possibly due to the differences in the concentration of xanthan gum between each formulation was too close. Besides that, xanthan gum helps to keep the droplets away from each other by forming steric stabilizing polymer-coated droplets because it has sufficient polymer chain to cover the surface of oil droplets thus maintaining the droplet sizes (Kumar *et al*., 2009).

Zeta potential analysis

Zeta potential is one of the key indicators for emulsion stability. Thus, it is important to measure the zeta potential of the prepared formulations. Figure 2 showed that all formulations have negative zeta potential values. Formulation XG00, XG02, XG04, XG06, XG08, and XG10 possessed zeta potential values of -23.53, -23.23 mV, -25.33 mV, -27.8 mV, -28.63 mV, and -30.5 mV, respectively. Increasing the concentration of xanthan gum in the formulations had increased the zeta potential value.

This may due to the anionic properties of xanthan gum. This particular property may lead to the shifting zeta potential value of the formulations to become more negative due to the interaction of negative charged carboxyl and sulphate group of xanthan gum with the negative surface charge of the formulations. Furthermore, increasing the xanthan gum content would increase the droplets negative surface charge, thus it would increase the zeta potential value of the formulations. In addition to that, the stability of emulsion can be predicted by its zeta potential value. The value of zeta potential greater than ± 25 mV is considered as stable emulsion, and lesser than that is otherwise (Mirhosseini *et al*., 2008). The increment of zeta potential value will increase the surface charge of the droplet. The surface charges could cause repulsive forces between droplets against flocculation and coalescence.

Stability analysis

All formulations were stable even after undergoing vigorous centrifugation process except for formulation XG00 and XG02 which consisted of 0.0% and 0.2% (w/w) xanthan gum, respectively. Both formulations showed phase separation after centrifugation process. Particularly, the existence of xanthan gum could reduce the interfacial tension and tend to form a cohesive interfacial film around the emulsion droplets thus improve the stability of the systems (Akhtar *et al*., 2011). It is concluded that, the instability of formulations XG00 and XG02 may be due to insufficient amount of xanthan gum present in

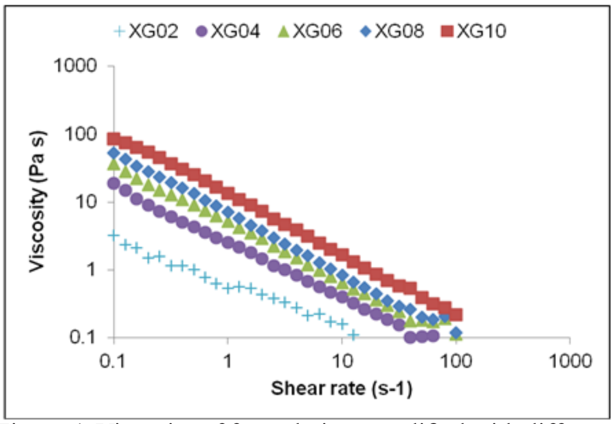

Figure 4. Viscosity of formulations modified with different concentrations of xanthan gum at 25°C

the system.

pH analysis

pH of formulation XG00, XG02, XG04, XG06, XG08, and XG10 were 4.5, 4.4, 4.7, 4.9, 4.5, and 4.4, respectively. It shows that xanthan gum had no effect on pH of formulations. Furthermore, pH values of all formulations were compatible with skin pH. The pH of the skin ranges from 4.0 to 5.6, and pH for topical formulation is frequently formulated in the range of 4 to 7 (Ashara *et al*., 2014).

Rheological study

Rheological study measures the flow behaviour and viscosity of formulation. Flow behaviour could provide information on type of the formulation. Figure 3 illustrates the flow behaviour of RBO emulsions with different xanthan gum concentrations. However, rheological study of formulation XG00 could not be measured accurately using cone and plate geometry due to its extremely liquid nature. Therefore, increase in xanthan gum concentrations lead to higher shear stress. All formulations (XG02 - XG10) did not possess zero value at zero shear rates. This likely suggested that the formulations were shear thinning non-ideal plastic-like material, with yield stress response. To paraphrase, the formulations behave like pseudoplastic material and only flow when applied stress exceeds its yield stress (Niraula *et al*., 2004). This situation and the graph curve in Figure 3 fits the Herschel-Bulkly model which is a non-Newtonian flow behaviour (Ng *et al*., 2014) with equation:

$$\sigma = \sigma 0 + k\acute{y}^n$$

The σ is shear stress, σ0 is the yield stress, k is consistency index, ý is shear rate and n is flow behaviour index. Higher yield stress indicates that the formulation has good stability towards deformation (Niraula *et al*., 2004).

In the meantime, viscosity of an emulsion is measured to gain information on its processing, handling, and optimum storing conditions. Hence, Figure 4 illustrates the viscosity of RBO emulsions with increasing concentrations of xanthan gum. The viscosity of formulation XG02 until XG10 was inversely proportional to the shear rate value. Increase in shear rate gradually decreased the viscosity level of formulations as the force that was applied to the formulations system had caused the droplets to move away from each other and initiated flowing. This phenomenon is non-Newtonian flow behaviour and classified as shear thinning behaviour. The viscosity results were consistent with the flow behaviour results which confirmed that the formulations were non-Newtonian and having shear thinning material property. Shear thinning behaviour is a desired characteristic in topical applications as this behaviour will keep the formulation stable and easily spread when applied onto the skin. The presence of xanthan gum increases the resistance of emulsions to flow thus increased the viscosity. This implies that a firm structure is formed to overcome the Brownian motion when more xanthan gum is used, and thus larger disruptive force is needed to make the formulation flows (Long *et al*., 2013).

Conclusions

The composition of the formulations was selected from Tween 80 ternary phase diagram. Results showed that the addition of xanthan gum in the formulations had influenced some properties of the system. As a result, the different concentrations of xanthan gum had shown some difference in droplet size among the formulations. But, xanthan gum did not affect the pH of the formulations. On the other hand, it increased zeta potential value when the concentration was increased. All of the formulations had remained stable after the centrifugation test except for formulation XG00 and XG02. Additionally, increase in xanthan gum also enhanced the flow behaviour and the viscosity of the formulations. Based on these results, it is concluded that xanthan gum plays a vital role in improving the physicochemical and rheological characterization of a formulation. Thus, it could be a functional important additive for emulsion development whether for foods, cosmetics, or medical applications.

Acknowledgements

The authors acknowledge BERNAS who had sponsored this research (grant no: 63727) and

Universiti Putra Malaysia for the Graduate Research Fellowship (GRF) for the student, Siti Hajar Saharudin. The authors also would like to thank Ms Nurul Farahin Abdul Wahab and Ms Noratiqah Abd Hamid for their guidance and help.

References

Akhtar, N., Khan, B., Khan, M., Mahmood, T., Khan, H., Iqbal, M. and Bashir, S. 2011. Formulation development and moisturising effects of a topical cream of aloe vera extract. World Academy of Science, Engineering and Technology 75: 172-180.

Ariviani, S., Anggrahini, S., Naruki, S. and Raharjo, S. 2015. Characterization and chemical stability evaluation of β-carotene microemulsions prepared by spontaneous emulsification method using VCO and palm oil as oil phase. International Food Research Journal 22 (6): 2432-2439.

Ashara, K. C., Paun, J. S., Soniwala, M. M., Chavada, J. R. and Mori, N. M. 2014. Micro-emulsion based emulgel: a novel topical drug delivery system. Asian Pacific Journal of Tropical Disease 4 (Suppl 1): S27-S32.

Barakat, Y., Gendy, T. and Ibrahim, V. 2008. Surfactants with natural hydrophobic tails HLB-CMC relationship of polyoxyethylenated cholesterol. Australian Journal of Basic and Applied Sciences 2 (3): 372-380.

Bernardi, D. S., Pereira, T. A., Maciel, N. R., Bortoloto, J., Viera, G. S., Oliveira, G. C. and Rocha-Filho, P. A. 2011. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: *in vitro* and *in vivo* assessments. Journal of Nanobiotechnology 9 (44): 1-9.

Burlando, B. and Cornara, L. 2014. Therapeutic properties of rice constituents and derivatives (*Oryza sativa* L.): A review update. Trends in Food Science & Technology 40: 82-98.

Cicero, A. and Derosa, G. 2005. Rice bran and its main components: potential role in the management of coronary risk factors. Current Topics in Nutraceutical Research 3 (1): 29-46.

Hoed, V. V., Depaemelaere, G., Ayala, J., Santiwattana, P., Verhe, R. and Greyt, W. 2006. Influence of chemical refining on the major and minor components of rice brain oil. Journal of American Oil Chemists' Society 83 (4): 315-321.

Izquierdo, P., Esquena, J., Tadros, T. F., Dederen, J. C., Feng, J., Garcia-Celma, M. J., Azemar, N. and Solans, C. 2004. Phase behavior and nano-emulsion formation by the phase inversion temperature method. Langmuir 20: 6594-6598.

Juliano, C., Cossu, M., Alamanni, M. C. and Piu, L. 2005. Antioxidant activity of gamma-oryzanol: mechanism of action and its effect on oxidative stability of pharmaceutical oils. International Journal of Pharmaceutics 299: 146-154.

Kumar, V., Wang, L., Riebe, M., Tung, H. and Prud'homme, R. K. 2009. Formulation and stability of itraconazole and odanacatib nanoparticles: governing physical parameters. Molecular Pharmaceutics 6 (4): 1118-1124.

Long, Z., Zhao, Q., Liu, T., Kuang, W., Xu, J. and Zhao, M. 2013. Influence of xanthan gum on physical characteristics of sodium caseinate solutions and emulsions. Food Hydrocolloids 32: 123-129.

Mirhosseini, H., Tan, C. P., Hamid, N. S. A. and Yusof, S. 2008. Effect of Arabic gum, xanthan gum and orange oil contents on ζ-potential, conductivity, stability, size index and pH of orange beverage emulsion. Colloids and Surfaces A: Physicochemical and Engineering Aspects 315: 47-56.

Ng, S. P., Lai, O. M., Abas, F., Lim, H. K. and Tan, C. P. 2014. Stability of a concentrated oil-in-water emulsion model prepared using palm olein-based diacylglycerol/virgin coconut oil blends: Effects of the rheological properties, droplet size distribution and microstructure. Food Research International 64: 919-930.

Ngan, C. L., Basri, M., Tripathy, M., Roghayeh, A. K. and Abdul-Malek, E. 2014. Physicochemical characterization and thermodynamic studies of nanoemulsion-based transdermal delivery system for fullerene. The Scientific World Journal 2014: 1-12.

Niraula, B., King, T. C., Chun, T. K. and Misran, M. 2004. Rheology properties of glucopyranoside stabilized oil-water emulsions: effect of alkyl chain length and bulk concentration of the surfactant. Colloids and Surfaces A: Physicochemical and Engineering Aspects 251: 117-132.

Oluremi, O., Solomon, A. and Saheed, A. 2013. Fatty acids, metal composition and physico-chemical parameters of Igbemo Ekiti rice bran oil. Journal of Environmental Chemistry and Ecotoxicology 5 (3): 39-46.

Oppermann, A. K. L., Renssen, M., Schuch, A., Stieger, M. and Scholten, E. 2015. Effect of gelation of inner dispersed phase on stability of (w1/o/w2) multiple emulsions. Food Hydrocolloids 48: 17-26.

Patel, M. and Naik, S. 2004. Gamma-oryzanol from rice bran oil: a review. Journal of Scientific and Industrial Research 63 (7): 569-578.

Rezaee, M., Basri, M., Rahman, R. N. Z. R. A., Salleh, A. B., Chaibakhsh, N. and Karjiban, R. A. 2014. Formulation development and optimization of palm kernel oil esters-based nanoemulsions containing sodium diclofenac. International Journal of Nanomedicine 9: 539-548.

Salim, N., Basri, M., Rahman, M. B., Abdullah, D. K. and Basri, H. 2012. Modification of palm kernel oil esters nanoemulsions with hydrocolloid gum for enhanced topical delivery of ibuprofen. International Journal of Nanomedicine 7: 4739-4747.

Sun, C., Gunasekaran, S. and Richards, M. P. 2007. Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. Food Hydrocolloids 21: 555-564.