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DEVELOPMENT OF NANOEMULSION INCORPORATED WITH *Hibiscus sabdariffa* FOR COSMECEUTICAL APPLICATION

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ABSTRACT

Hibiscus sabdariffa (HS) has been reported to possess a crucial content of bioactive compounds, such as phenolic acids and flavonoids, therefore, HS was recognized as a source of antioxidants. Due to that, the development of nanoemulsion incorporated with HS appears promising for cosmeceutical application. This study is focused on the formulation of oil-in-water (O/W) nanoemulsions of HS to enhance the bioaccessibility of its active compounds. The influences of hydrophilic-lipophilic balance (HLB) value of surfactant and grapeseed oil (GSO) to olive oil (OO) ratio on the droplet size, zeta potential, PDI and stability of the nanoemulsions were investigated. The results showed that the smallest particle size was obtained at 145.9 nm with PDI = 0.388 and zeta-potential = -41.1 mV in the systems prepared using HLB value of 12 and 2:1 ratio of GSO to OO. Then the selected nanoemulsion which based on the lowest particle size (NE-F6, GSO:OO = 2:1, and HLB = 12) showed good stability over time and temperature without no phase separation, creaming or cracking was spotted. The pH value of the NE-F6 was obtained at 5.2.

Key words: *Hibiscus sabdariffa*, nanoemulsion, oil ratio, HLB value

INTRODUCTION

The cosmeceutical market in Asia seems to be one of the fastest growing markets. According to cosmetics and toiletries market overviews (2014), the market value of the Asia Pacific has increased to more than US$70 billion, which is the second highest market after the Western European market. As reported in 2013, Malaysian spent about US$407 million on cosmeceuticals and toiletries products and this demand was mainly met by imports (Azmi-Hassali *et al.,* 2015). Cosmeceuticals are not categorized and do not require Food and Drug Administration (FDA) approval before marketed (Chien-Hsing *et al.,* 2017). However, cosmeceutical must be safe for consumers and properly labeled. Companies and individuals that market cosmetics have a legal responsibility for the safety and labeling of their products (U.S. Food and Drug Administration). Upsettingly, there are 12 cosmeceutical products detected in Malaysia to contain mercury as the active ingredient (Rahman, 2013).

To address the above matter, the use of a plant-based active ingredient in cosmeceuticals may prove safer and attractive to the consumers. A myriad of plant extracts with functional properties such antioxidant and healing properties and moisturizing effect is reportedly available in Malaysia and other tropical regions (Wanjai *et al*., 2012; Soto *et al*., 2015). Among the vast number of plant extracts, *Hibiscus sabdariffa* (HS) or known

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as Roselle has been documented to contain high concentrations of polyphenols (Da-Costa-Rocha *et al*., 2014). A matter of fact, the calyces section of HS carry a high amount of the aforementioned compounds. Polyphenols are secondary plant metabolites that have important commercial and biological roles due to their antioxidant activity (Wittenauer *et al.,* 2015), hence it is no coincidence that the high number of researches on this group of compounds have been observed for the last two decades (Gourine *et al*., 2010).

In order to improve the bioavailability on human skin, increase in penetration of bioactive compounds especially plant extracts into human skin is undoubtedly necessary. Recent advances in nanotechnology and engineering prove to be effective in surging innovative formulation and product solutions. A new generation hybrid of nanotechnology and emulsion technology is able to manipulate the delivery of bioactive compound in achieving the best outcome of the treatment. Nanoemulsion is able to ensure these terms. Nanoemulsion is a mixture of two immiscible liquids forming a transparent or translucent dispersion of emulsion with droplet sizes ranging between 20-500 nm (Uso'n *et al*., 2004).

In the formulation of the nanoemulsion, surfactants played an important role as a stabilizing agent. Surfactants are surface-active agents that contain hydrophilic heads and hydrophobic tails. They can increase the stability of the system by decreasing the surface tension of a liquid and making it easier to spread. Among the surfactants, non-ionic surfactants are the least toxic and not easily ionized in aqueous solutions. They also improve the solubility of poorly water-soluble drugs. Besides, the type of oil and process conditions influenced the physicochemical properties of nanoemulsion (Einhorn-Stoll *et al*., 2002; McClements, 2011). The composition of the dispersed oily phase considerably influences the emulsion quality because of the different densities, viscosities and surface-active ingredients of the different types of oils (Einhorn-Stoll *et al*., 2002). These oily phases are in most cases used to dissolve bioactive compounds. Therefore, the objective of the present work was to explore the effect of Hydrophile-Lipophile Balance (HLB) and oil ratio (GSO:OO) to obtain stable HS oil-in-water (O/W) nanoemulsions with the minimum possible droplet size. Additionally, nanoemulsion stability over time, temperature and pH were also evaluated.

MATERIALS AND METHODS

Preparation of plant extracts

An amount of 1000 g of calyces of HS was air-dried for one week at room temperature and ground using an electric grinder to a mesh size of 1 mm. Sample of HS powder of 10 g was transferred into a beaker containing 100 mL of solvent and soaked for 24 h, filtered and the supernatant was vacuum evaporated to obtain pure HS. The extract of HS was stored at 4°C until further analysis.

Preparation of nanoemulsion

Oil-in-water (O/W) nanoemulsion was prepared using a spontaneous emulsification method. The oil phase of nanoemulsion contained a mixture of grape seed oil (GSO) and olive oil (OO). Meanwhile, an aqueous phase contained deionized water, xantham gum, and the nonionic surfactant. The effect of Hydrophile-Lipophile Balance (HLB) ranging from HLB of 6-14 and the series of oil ratio (GSO:OO), of (2:1, 1:1, 1:0, 0:1 and 1:2) were investigated while other components were fixed. The calculation of HLB was shown in the equation below. Total weight of the nanoemulsion was 100% (w/w) including the HS extracts and phenonip as the antimicrobial agent. The nanoemulsion was prepared by heating the aqueous phase to 60°C followed by the stepwise addition of the required amount oil phase to the mixture with constant stirring at 2400 rpm for 2 h. The appearance of the physical stability of nanoemulsion was observed. The particle size, zeta-potential, and polydispersity index (PDI) were measured immediately using dynamic light scattering (Malvern Nano ZS90, Malvern, UK).

$$HLB_{mix} = \frac{\text{wt\% of surfactant A}}{100} \times (HLB_A - HLB_B) + HLB_A$$

Stability studies of nanoemulsion

pH measurement

The analysis was carried out in three replicates using a method defined by Abd. Gani *et al*. (2010) to determine the pH value of the optimized nanoemulsion using a Delta 320 pH meter (Mettler-Toledo, Schwerzenbach). The standard buffer solution of both pH 4.01 and pH 7.00 were used for calibration purpose of the pH meter.

Thermodynamic stability

Apparently thermodynamic stability of nanoemulsion with the minimum particle was determined by observing phase separation (instability) as a function of storage time. Samples (10 g) in screw cap glass tubes were kept at 5°C, 25°C (RT) and at 60°C for six months and periodically observed for phase separation.

RESULTS AND DISCUSSION

Effect of HLB value on droplet size, zeta potential and poly-dispersity index (PDI) of nanoemulsion

The HLB value has been proven to be very useful in choosing the excellent type of emulsifier for any given oil phase. Sagitani (1981), suggested that a desirable surfactant HLB value was a key factor for the formation of an emulsion with small droplets. The HLB system predicts the best emulsifier is acquired when the HLB values of the emulsifier and oil are matched (Gullapalli & Sheth, 1999). Spans and Tweens are well-known non-ionic sorbitan alkanoates and ethoxylated sorbitan alkanoates, with a wide range of HLB values which are appropriate for tuning the HLB (Ganem-Quintanar, 1998). The effects of the HLB value on the particle size, zeta potential and PDI of nanoemulsion is tabulated in Table 1. It can be seen the smallest particle size was obtained at 177.8 nm with PDI = 0.414 and zeta-potential = -40.5 mV in the systems prepared using mixed surfactant of Tween 80 and Span 20 that gives the HLB value 12. Meanwhile, the vast boom increase of particle size was observed with decreases in the HLB value, as in this experiment the largest particle was found at 297.2 nm (PDI= 0.320 and zeta-potential = -38.2 mV) with HLB value 6. This impact has frequently been explained as being the result of increased emulsifier adsorption around the oil-water interface of a droplet and decreased interfacial tension in the system, both of which in favor of the formation of nanoemulsions with smaller droplets (Lamaallam *et al*., 2005). This observation agrees with empirical data which suggest that minimum droplet size and maximum emulsion stability is obtained for O/W emulsions when using surfactants with an HLB number within the range 10–12 (McClements, 2005). Besides, in another report, HLB value between 11 to 16 are optimum for stabilization of oil-in-water emulsions. Dai *et al.* (1997) observed that the emulsifier molecular structure had a significant effect on the final emulsion droplet size. Thus, the results suggest an important influence of the optimized HLB values on the nanoemulsion preparation.

Effect of oil ratio (GSO:OO) on droplet size, zeta potential and poly-dispersity index (PDI) of nanoemulsion

The formation of stable nanoemulsions often depends on identifying an oil phase composition that optimizes each nanoemulsion formation and its stability (McClements *et al*., 2012; Kentish *et al*., 2008). The series ratio of GSO to OO (GSO:OO) ranging from 2:1, 1:1, 1:0, 0:1 and 1:2 and HLB kept constant at 12 have been studied for its influenced on the particle size, zeta-potential and PDI. Table 2 shows the particle size and PDI reduced when the ratio of GSO in the oil phase increased from 0:1 to 2:1, with small droplets and narrow distributions being fashioned when the oil phase

Table 1. Effect of HLB value of surfactant on the particle size, zeta potential, and PDI of nanoemulsion incorporated with HS extracts

Formulation	HLB_{mix}	Size (nm)	Zeta-potential (mV)	Polydispersity Index (PDI)
F1	6	297.2	-38.2	0.320
F2	8	259.8	-42.9	0.368
F3	10	254.5	-38.0	0.340
F4	12	177.8	-40.5	0.414
F5	14	192.7	-22.6	0.320

Table 2. Effect of oil ratio, GSO to OO on the particle size, zeta potential, and PDI of nanoemulsion incorporated with HS extracts

Formulation	GSO:OO	Size (nm)	Zeta potential (mV)	Polydispersity Index (PDI)
F6	2:1	145.9	-41.1	0.388
F7	1:1	250.2	-34.5	0.276
F8	1:0	164.0	-37.2	0.415
F9	0:1	204.9	-32.5	0.490
F10	1:2	175.4	-39.6	0.660

Table 3. Stability study on storage time and temperature of nanoemulsion incorporated with HS extracts

Day (s)	1			15			30			60			90			180		
Temperature (°C)	3	25	45	3	25	45	3	25	45	3	25	45	3	25	45	3	25	45
Stability	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

(√) – Stable

contained about 66% GSO and 33% OO. When the GSO concentration was further increased, there was once a steep decrease in mean particle diameter and PDI. Briefly, the oil ratio phases that led to the smallest droplet sizes, 145.9 nm had been a ratio of 2:1, it was discovered that previously, flavor oils have been proven to be more suitable for forming nanoemulsions with small droplets than triacylglycerol oils, and so one would expect the droplet size to decrease with increasing flavor oil content (Davidov-pardo *et al*., 2015). A ratio of 1:0, the mean particle diameter was greater at 250.2 nm and the PDI was 0.490 followed by zeta potential = -32.5 mV, therefore, higher levels of OO were not used.

Stability study of nanoemulsion

pH measurement

The study was carried out with the smallest particle size of nanoemulsion (NE-F6). The pH value of the NE-F6 was obtained at 5.2 at 26.5°C showing it slightly acidic. The pH between 4 and 7 was frequently chosen for the aqueous phase of a dermal formulation. This is due to the fact the pH of the skin surface is around 5.5 that have been reported by Martinez- Pla *et al.* (2004). Thus the pH value of the formulation intended for skin application should have pH close to this range. Consequently, this F6 is favorable for topical application and less irritation to the skin surface. Meanwhile, in a different study performed by way of Blaak *et al.* (2011) reveals that good skin integrity was achieved when the skin is treated with o/w emulsions that have a pH value of 3.5 to 4.5.

Storage time and temperature

The apparent thermodynamic stability of NE-F6 on storage at a range of temperature (3°C, 25°C and 45°C) for up to three months was determined from the physical appearance and the particle size during storage at various temperatures. Table 3 shows that F6 was significantly stable during storage at all temperature where no phase separation, creaming or cracking was spotted which indicated that this system had excellent thermal stability. This stability is due to the difference in density between the oil and aqueous phase. In addition to that, this observation shows the active ingredient is degrading or uniformity distributed (Noor *et al*., 2013).

CONCLUSION

Owing to the viable of nanoemulsions incorporated with HS extracts in cosmetic products, an intensive study was performed in order to point out the role of physicochemical properties of HLB value of surfactants and the oil ratio on nanoemulsion size distribution, zeta potential, and PDI. The formulation that provides the smallest particle size of 145.9 nm was obtained under HLB value of 12 with 53% of Tween 80 and 47% of Span 20, whilst the ratio of grapeseed oil to olive oil was 2:1 in the oil phase system. The study was carried out with the smallest particle size of nanoemulsion (NE-F6) showed desirable stability when stored at 4°C, 25°C and 45°C up until 180 days with pH 5.2.

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