Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 3, 2013

Research Article

PREPARATION AND EVALUATION OF OLIVE OIL NANOEMULSION USING SUCROSE MONOESTER

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Received: 03 Jun 2013, Revised and Accepted: 24 Jun 2013

ABSTRACT

In pharmaceutical view, nanoemulsion is one of the major dosage forms in delivering active ingredients to the target area which has attracted considerable attention in recent years for its application in personal care and cosmetic products also due to their ability to improve the penetration and permeation of active ingredients through the skin. The aim of this study was to formulate nanoemulsion and evaluating the effect of surfactants and storage on it. Nanoemulsions were formulated using sucrose monoesters as surfactants, glycerol and Olive oil by simple mixing, using heat to dissolve sucrose monoester in the glycerol then adding hot Olive oil to the surfactant mixture. Three different types of sucrose monoester (Laureate, Oleate and Palmitate) were used in the production of nanoemulsion and investigated their influence on nanoemulsion. The results revealed that, Sucrose Laureate produced nanoemulsion with good droplet size, polydispersity index and zeta potential compared to Oleate and Palmitate. It produced nanoemulsion with oil droplets size below 200 nm, low polydispersity below 0.2 and zeta potential lower than -40 mV. Stability study was conducted for the optimum formulations of nanoemulsion at different temperatures (4 °C, 25 °C and 40 °C) for six months. In conclusion, the optimum nanoemulsion formulations were very stable at 4 °C compared to 25 °C and 40 °C while at 25 °C nanoemulsion showed moderate stability but it was unstable at 40 °C, therefore, the ideal storage condition for nanoemulsion is 4 °C.

Keywords: Nanoemulsion; Sucrose monoester; Olive oil; Hydrophilic lipophilic balance; Stability.

INTRODUCTION

Nanoemulsions are submicron sized emulsions that are under extensive investigation as drug carriers for improving the delivery of therapeutic agents [1,2]. The small droplets sizes (in the range 20-200 nm), high solubilization capacity, high interfacial area, low viscosity, transparent or translucent appearance, and high kinetic stability, make nanoemulsions used for various applications [3-6]. Also nanoemulsions show a great promise for the future of cosmetics, drug therapies diagnostics, and biotechnologies [7]. Nanoemulsions were produced either by high energy emulsification methods or low energy emulsification methods. High energy emulsification methods involve high shear mixing, high-pressure homogenization or ultrasonification. While Low energy emulsification methods used the advantage of the physicochemical properties of the system which exploits phase transitions to produce nanoemulsion [8].

In the pharmaceutical field, nanoemulsions have been used as a drug delivery system through various systemic routes mainly: oral, topical and parenteral nutrition [9,10]. The main advantage of using nanoemulsions for the skin is its ability to improve the penetration and permeation of active ingredients through the skin without the need of incorporate penetration enhancer in the formulation [11-13]. Olive is the fruit of the Olive tree (*Olea europaea*) and belongs to the family *Oleaceae*. It consists of saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, oleic, stearic acid and palmitic acid [14]. Also it consists of phenolic compounds such as hydroxytyrosol, oleuropein and tyrosol and it exerts strong antioxidant activity and radical scavengers in preventing cancer [15]. Due to its high content of monounsaturated and polyunsaturated fatty acids, Olive oil is used in cosmetics and pharmaceutical products. Also it has been used in the treatment of chronic diseases such as diabetes, atherosclerosis, colon cancer, arthritis, asthma and hypertension [16-19]. Sucrose monoesters are low molecular weight emulsifiers. They consist of both hydrophilic part from hydroxyl head groups of sugar substituent (sucrose) and lipophilic parts from the tails of fatty acid [20]. They have unique emulsification property that tolerates any temperature variations, also they identified as non-ionic, biodegradable and nontoxic surfactants [21]. The aim of this research was to determine the impact of different surfactants on the preparation of Olive oil nanoemulsion and its stability over different storage conditions.

MATERIALS AND METHODS

Materials

Sucrose Laureate 1695, Oleate 1570 and Palmitate 1570 were supplied by Juhalim Biotech SDN BHD (Kuala Lumpur, Malaysia). Virgin Olive oil, glycerol were supplied by Sigma-Aldrich (USA).

Methods

Formulation of nanoemulsion

Series of formulations containing various combinations of Olive oil as an oil phase with surfactant and glycerol as a liquid phase were used to produce nanoemulsion. A pseudo-ternary phase diagram was constructed based on three different types of surfactants combination with glycerol and oil separately at a constant temperature. Ternary phase diagram (A) consists of mixtures of oil, sucrose Laureate and glycerol while ternary phase diagram (B) consists of oil, sucrose Oleate and glycerol and ternary phase diagram (C) consists of combinations of oil, sucrose Palmitate and glycerol. The mixtures were then used to distinguish the effect of sucrose monoester on the emulsification and the production of nanoemulsion for Olive oil. The formulations were weighed based on ternary phase diagram using analytical balance (Meller Tolledo). Both of the phases, oil and glycerol, were heated separately at about 75 °C ± 2 °C using hot plate, then the surfactant was mixed with the hot glycerol using glass rod and stirred continuously until the surfactant was dissolved in the glycerol after that the hot oil was added to the mixture slowly and stirred for about 10 minutes to ensure its totally disappearance and the formation of an emulsion. A sample of the emulsion was dissolved in distilled water to form a diluted emulsion, the formulations were observed after being diluted with water and the droplets size of emulsion were measured in order to find the efficient region of emulsification. All experiments were carried out at room temperature of about 25 °C. The formulations that formed non-emulsified phases were not shown in the phase diagrams because they are out of the study scope. According to the ternary phase diagrams, nanoemulsion (NE) region was marked by the transparent and fine nano droplets, whereas macro-emulsion (ME) region was marked due to more whitening and isotropic solutions that might contain micelle solutions and coarse emulsion (CE) was the region of visibly cloudy dispersions even by visual observation.

Droplet size and zeta potential analysis

Droplets size and size distribution of emulsion system were determined using Malvern Mastersizer 2000 laser diffraction particle analyzer (Malvern instruments, UK). Photon correlation spectroscopy or dynamic light scattering, which is specialized in analysis of particle size of submicron, is a valuable technique which analyses changes in light intensity fluctuations initiated by Brownian motion. The temperature needs to be stable otherwise convection currents in the sample will cause non-random movements which will ruin correct size interpretation.

To observe the droplets size and size distribution, 250 µl of an emulsion was added to 300 ml of distilled water in a 500 ml beaker. A glass rod was used to induce gentle agitation in the mixture. The droplets size and size distribution of resultant emulsion were examined using Malvern Mastersizer 2000 laser diffraction particle analyzer. This step was conducted for the screening study of ternary phase diagram. Mean size and size distribution measurements were performed in triplicates. The Zeta potential of the selected nanoemulsion formulations were also performs by using Malvern Nano Zetasizer.

Stability of nanoemulsion formulations during storage

Droplets size, size distribution and zeta potential are among the most important characteristics for the evaluation of the stability of emulsion. Therefore, the effects of temperature and storage time were studied on the optimum formulations of nanoemulsion. The droplets size, size distribution and zeta potential were evaluated immediately after the production of the nanoemulsion and also after 1, 2, 4 and 6 months of storage under different temperatures 4, 25 and 40 °C.

RESULTS AND DISCUSSION

Effect of different surfactants combination on the formulation of nanoemulsion

Various formulations consist of Olive oil, surfactants and glycerol were studied for their potential ability to prepare nanoemulsion by stirring method. The ternary phase diagrams of such formulations were shown in figures (1-3). The Olive oil, surfactant and glycerol mixtures were divided into three different systems. System A (Oil, Sucrose Laureate and Glycerol), system B (Oil, Sucrose Palmitate and Glycerol) and system C (Oil, Sucrose Oleate and Glycerol).

The three different systems showed different behavior in producing the nanoemulsion. As a comparison between them, system (A) shown in figure 1, which comprised of sucrose Laureate as non-ionic surfactant, produced larger region of nanoemulsion compared to other systems due to its good emulsification properties. On the other hand, figure 2 showed the ternary phase diagram of system (B) containing sucrose Palmitate with bad nanoemulsion properties producing small region of nanoemulsion compared to other systems and less emulsification properties compared to sucrose Laureate. However, the ternary phase diagrams of system (C) containing sucrose Oleate shown in figures 3 has better nanoemulsion region compared to system (B) with moderate emulsification properties. Sucrose laureate showed the best emulsification properties compared to sucrose Palmitate and Oleate, which may be due to its good miscibility properties. Same findings were stated by Szuts et al. [22], who mentioned that sucrose Laureate was good in preparing solid dispersion due to its good miscibility properties in water compared to sucrose Palmitate and sucrose Stearate.

In general, all of the surfactants produced nanoemulsion formulations with simple stirring. The capability of producing nanoemulsion was due to the temperature used to dissolve the sucrose ester in the glycerol. The heat treatment of the formulations may lead to changes in the molecular characteristics of the surfactant. Therefore, sucrose ester becomes progressively dehydrated during heating because it is non-ionic surfactant with a hydrophilic head group. For that reason, the surfactant molecules will have changes in the interfacial tension, packing, and oil/water solubility during heating. Same results were shown in previous studies on non-ionic surfactants that produced micro-emulsions and nanoemulsions formulations by the help of these changes facilitated at higher temperatures [23-26]. In addition, a kinetic energy barrier in the oil-glycerol-surfactant system prevents it from moving from an emulsion to nanoemulsion at ambient temperature. But as the temperature was raised this kinetic energy barrier was reduced, which helped in changing from one state to another. Same results were stated by Rao and McClements, [26], who used oil-water-surfactant to produce micro-emulsion and nanoemulsion.

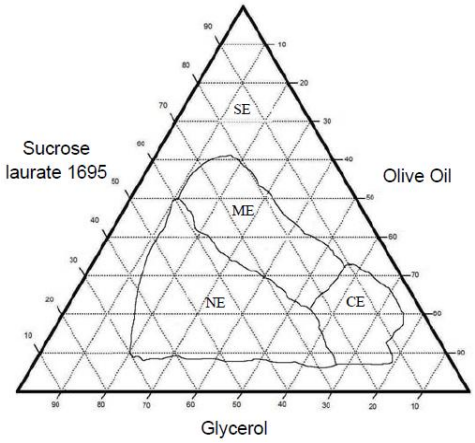

Fig. 1: System A; Olive oil, Sucrose Laureate and Glycerol.

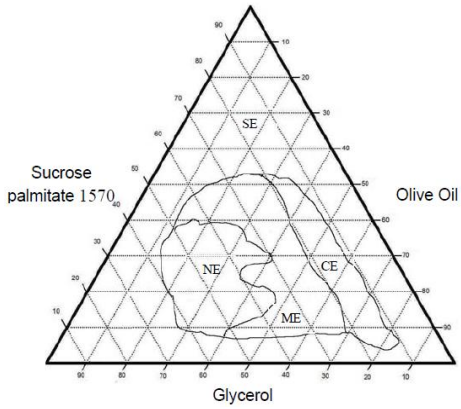

Fig. 2: System B; Olive oil, Sucrose Palmitate and Glycerol.

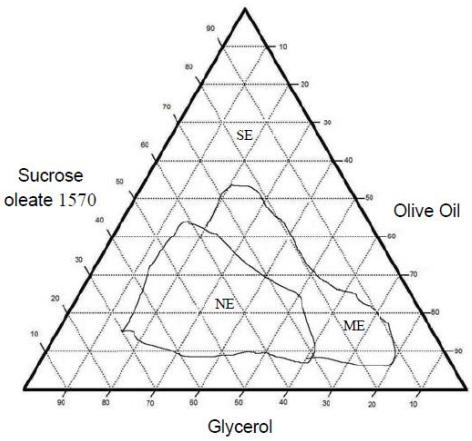

Fig. 3: System C; Olive oil, Sucrose Oleate and Glycerol.

The effect of various HLB values on the formulation and the capability to achieve nanoemulsion were also studied. As the degree of sucrose esterification increased and/or the fatty acid chain length increased, the sucrose ester HLB value will be reduced. Oil in water dispersion required HLB value between 9 to 18, so the selection of the optimum HLB value of emulsifying agent depends on its hydrophilicity [27]. System (A) containing sucrose Laureate with

high HLB value (HLB 16) was chosen as a non-ionic surfactant to produces large region of nanoemulsion formulations with smaller oil in water droplets and good stability. The ability of sucrose Laureate to form nanoemulsion with small droplets size and good stability was due to its good droplets entrapment and stabilization efficacies which is explained by the low amounts of di-, tri-, and polyLaureates (20%) and higher amount of monoLaureate (80%) as well as the lauric acid short chain length. While in system (B) and system (C) sucrose Oleate (HLB 15) and sucrose Palmitate (HLB 15) were used respectively, both of the surfactants showed smaller nanoemulsion region compared to system A, that's due to their low HLB value compared with sucrose Laureate and less amount of monoesters (70%). Leong et al. [27], stated that sucrose Laureate was better than sucrose Palmitate, Stearate and Oleate in preparing Phytosterol nanodispersions having small particles size below 100 nm. Therefore, it could be concluded that sucrose Laureate as non-ionic surfactant having high HLB value (HLB 16) is good in forming nanoemulsions with small droplets with high stability.

Influence of oil and surfactants ratios on droplets size and size distribution

The droplets size and size distribution (uniformity) were studied for all formulations at the preliminary investigation using Mastersizer Malvern Instrument. The figures (1-3) showed different nanoemulsion regions when different surfactants have been used. Their droplets size and size distributions were varied. System (A) containing sucrose Laureate as surfactant led to the formation of oil droplets with the smallest size and size distribution compared to other systems (B) and (C). For Olive oil in system (A) the smallest droplet size was 129 nm with 0.189 uniformity. But system (B) containing sucrose Palmitate showed significant higher droplets size 319 nm and size distribution 0.394 compared to system (A). While system (C) consists of sucrose Oleate showed better results compared to system (B) but still not as good as system (A). Its droplet size was 241 nm and 0.348 uniformity.

The optimum nanoemulsion formulations for Olive oil from system (A) having droplets size below 200 nm were shown in table 1. In addition, sucrose Laureate led to the production of nanoemulsion formulations with various oil concentrations between 36 to 50%. From the ternary phase diagram it can be observed that when the oil concentration in the formulation increased from 20% to 50%, their droplets size was decreased and form more stable formulations. This attributed to the fact that sucrose ester will be bounded to the surfaces of the oil droplets when the viscosity of the formulations was increased and therefore will be less available to participate in the formation of the emulsion. The same findings were reported by Murakami et al. [28] and Rao and McClements, [26], who stated that the viscosity has an influence in forming stable colloidal dispersion formulation with small droplets size using sucrose ester as non-ionic surfactant. But as the oil concentration goes up more than 50% the droplets size were increased and separation happened due to less amount of surfactant to form colloidal dispersion and stabilize the system.

Table 1: The optimum nanoemulsion formulations from the ternary phase diagram measured by Mastersizer.

Formulation	Sucrose Laureate (%)	Glycerol (%)	Olive oil (%)	Droplet size (nm) ± SD	Uniformity ± SD
A	19.2	44.8	36.0	189 ± 1.4	0.296 ± 0.006
B	15.0	35.0	50.0	169 ± 0.8	0.478 ± 0.007
C	25.6	38.4	36.0	145 ± 1.3	0.257 ± 0.003
D	20.0	30.0	50.0	129 ± 1.2	0.189 ± 0.008
E	32.0	32.0	36.0	168 ± 0.9	0.279 ± 0.001
F	25.0	25.0	50.0	153 ± 2.1	0.245 ± 0.005

All data are presented as mean ± SD, (n= 3).

In general, the formulations B, D and F that containing 50% oil concentration showed smaller droplets size with good uniformity compared to formulations A, C and E which contain 36% oil concentrations. Also in a comparison between the formulations with 50% oil it was found that formulation B contain 15% surfactant has larger droplet size and uniformity compared to formulations D with 20% surfactant and F with 25% surfactant. The high uniformity for formulation B which was higher than 0.3 makes it unstable due to Ostwald ripening when compared to other formulations which have uniformity below 0.3. In the other hand, both formulation D and F showed almost similar droplet size and uniformity. However, formulation D will be better than F because it has less amount of surfactant. Therefore, it can be concluded that the nanoemulsion formulation which contains 20% sucrose Laureate 1695 and 50% oil will produce a stable nanoemulsion with small droplet size and uniformity.

Droplet size, polydispersity index and zeta potential analysis

Different formulations of nanoemulsion optimized by ternary phase diagram are presented in table 2, measured by Malvern Zetasizer to determine their droplets size, polydispersity index (PdI) and zeta potential. Overall, all formulations showed small mean droplets size between 111 to 163 nm, also it was observed from the table that the droplets size were decrease with an increase in the oil concentration the reason as mentioned in the previous section, also all the formulations showed low PdI in a range between 0.157 to 0.422. In addition zeta potential was between -28.5 to -40.2 mV.

Table 2: The optimum nanoemulsion formulations measured by Malvern Zetasizer.

Formulation	Sucrose Laureate (%)	Glycerol (%)	Olive oil (%)	Droplet size (nm) ± SD	PdI ± SD	Zeta Potential (mV) ± SD
A	19.2	44.8	36.0	163 ± 0.6	0.261 ± 0.002	-32.7 ± 1.1
B	15.0	35.0	50.0	148 ± 1.2	0.422 ± 0.004	-28.5 ± 1.9
C	25.6	38.4	36.0	133 ± 1.6	0.226 ± 0.003	-35.7 ± 1.2
D	20.0	30.0	50.0	111 ± 1.9	0.157 ± 0.002	-40.2 ± 1.7
E	32	32.0	36.0	149 ± 0.9	0.238 ± 0.003	-34.5 ± 1.4
F	25.0	25.0	50.0	134 ± 1.1	0.227 ± 0.001	-34.8 ± 0.9

All data are presented as mean ± SD, (n= 3).

In general, all the formulations have high negative value of zeta potential charge above -30 mV, which indicates that nanoemulsion formulations are stable [29]. Formulation B showed slightly low zeta potential charge and high PdI. The high PdI may lead to instability of the formulation upon storage because this formulation has low amount of surfactant which is not enough to make it stabilized. In summary, formulations containing 50% oil showed smaller droplets size and zeta potential compared to formulations containing 36% oil. Also in a comparison between the formulations containing 50% oil, formulation D with the optimum amount of surfactant showed the smallest droplets size, lowest PdI and the best zeta potential. Also it is known that large amount of surfactants may cause skin irritation, therefore, formulation D was chosen as an optimum formulation compared to other nanoemulsion formulations.

Influence of different storage temperatures on the droplets size, polydispersity index and zeta potential

One of the most important characteristics used to evaluation of the stability of an emulsion system are droplets size, size distribution and zeta potential. Therefore, the droplets size parameters and the surface electrical charge (zeta potential) were evaluated immediately after the production of nanoemulsion and over six months (1, 2, 3, 4, 5 and 6 months) of storage at three different temperatures (4, 25 and 40 °C), the evaluation was carried for the formulations which have droplets size below 200 nm and when separation was not visible. The measurement of the droplets size initially and during storage gives an indication on the system stability [30-32].

Nanoemulsion formulations were stored at three different temperatures to test the system under anxiety condition. In general nanoemulsion formulations for Olive oil as shown in figures (4-6), remained in their nano-size range below 200 nm when stored at 4 °C. Also The polydispersity index remain in the same magnitude as the values obtained immediately after production and the droplets still showing negative charge at their surface < -30. An exception was in formulation B, which showed an increment in the size above 200 nm and instability, because the initial reading for this formulation showed high polydispersity index above 0.3, and low negative zeta potential, below -30 mV, therefore, it was unstable during storage. However, when nanoemulsion formulations were stored at 25 °C (Figures 7-9), there were a slightly significant increase in the droplets size above 200 nm. Also at 40 °C (Figures 10-12), all the formulations showed a significant precipitation with increase in the droplets size, polydispersity index and decrease in their negative zeta potential.

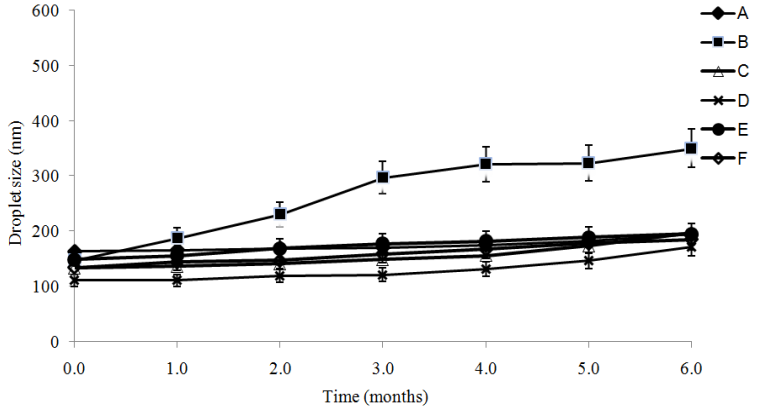

Fig. 4: Droplet size measurement of nanoemulsion after production and subjected to different storage duration: 1, 2, 3, 4, 5 and 6 months of storage at 4 °C.

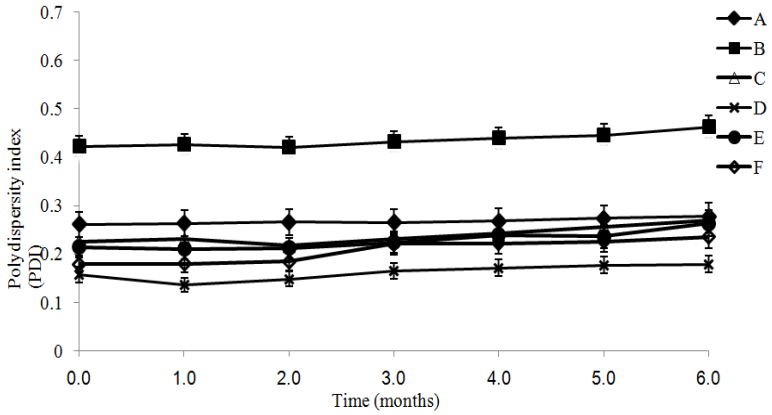

Fig. 5: Polydispersity Index (PdI) measurement of nanoemulsion after production and subjected to different storage duration: 1, 2, 3, 4, 5 and 6 months of storage at 4 °C.

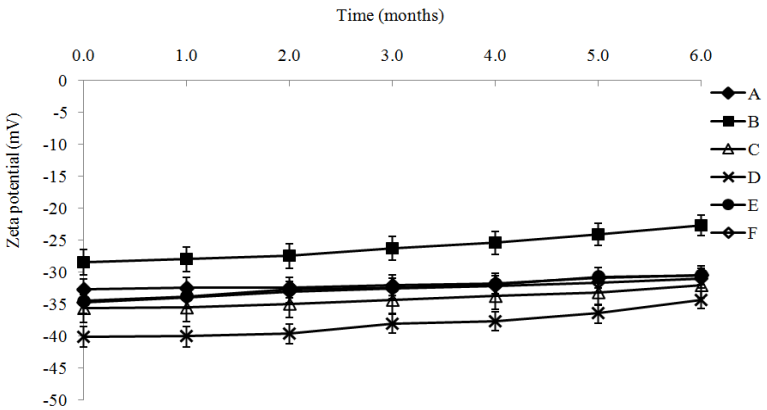

Fig. 6: Zeta potential measurement of nanoemulsion after production and subjected to different storage duration: 1, 2, 3, 4, 5 and 6 months of storage at 4 °C.

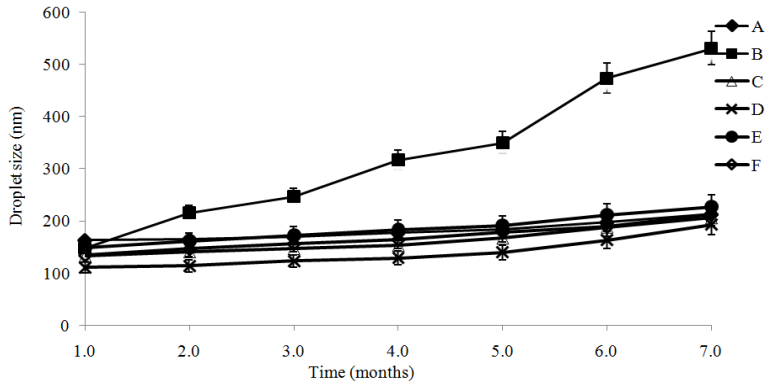

Fig. 7: Droplet size measurement of nanoemulsion after production and subjected to different storage duration: 1, 2, 3, 4, 5 and 6 months of storage at 25 °C.

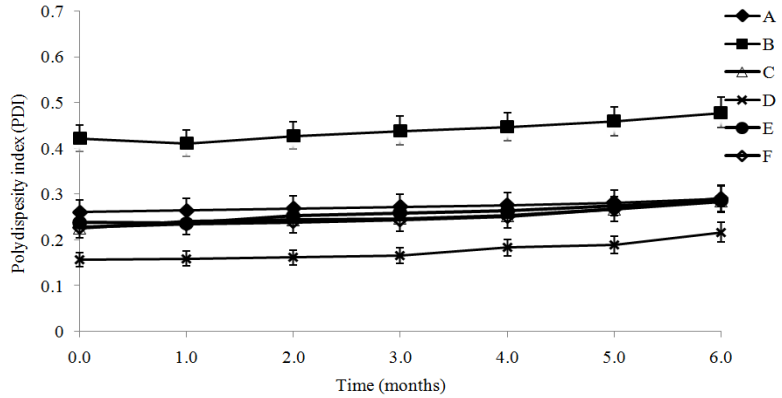

Fig. 8: Polydispersity Index (PdI) measurement of nanoemulsion after production and subjected to different storage duration: 1, 2, 3, 4, 5 and 6 months of storage at 25 °C.

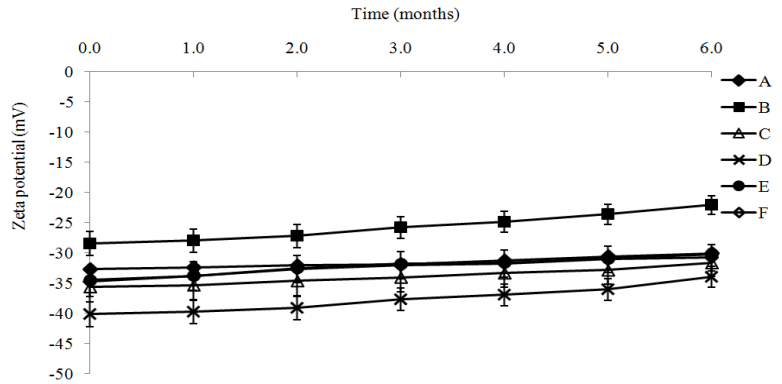

Fig. 9: Zeta potential measurement of nanoemulsion after production and subjected to different storage duration: 1, 2, 3, 4, 5 and 6 months of storage at 25 °C.

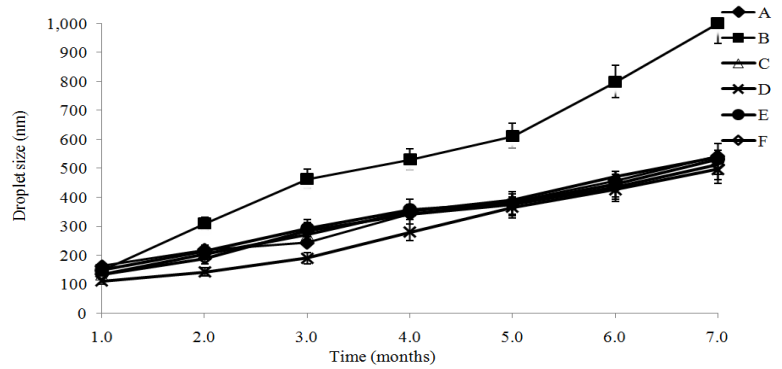

Fig. 10: Droplet size measurement of nanoemulsion after production and subjected to different storage duration: 1, 2, 3, 4, 5 and 6 months of storage at 40 °C.

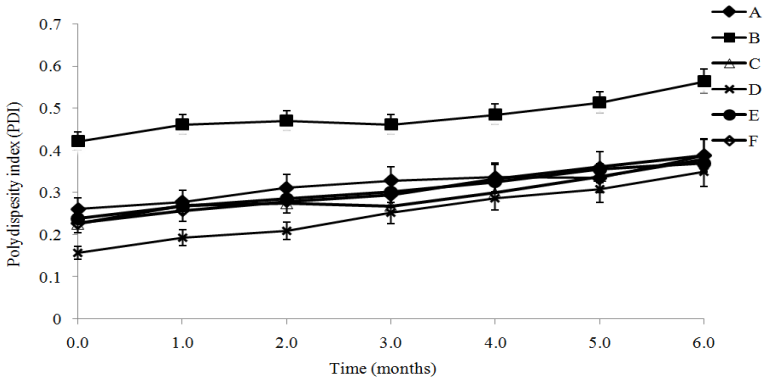

Fig. 11: Polydispersity Index (PdI) measurement of nanoemulsion after production and subjected to different storage duration: 1, 2, 3, 4, 5 and 6 months of storage at 40 °C.

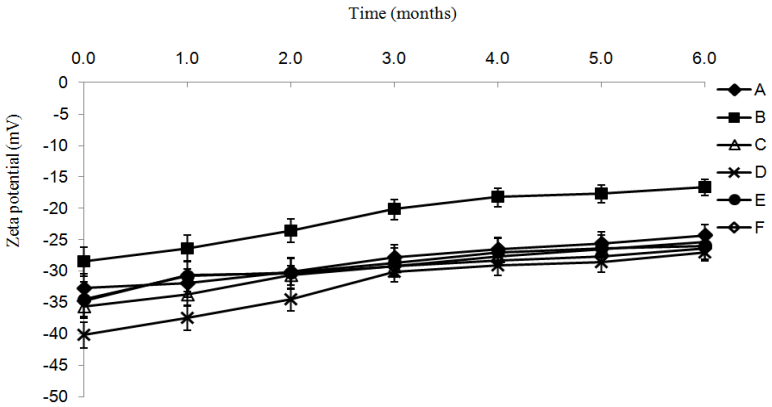

Fig. 12: Zeta potential measurement of nanoemulsion after production and subjected to different storage duration: 1, 2, 3, 4, 5 and 6 months of storage at 40 °C.

It is postulated that when the temperature was increased, the oil droplets movement in the emulsion was also increased. Then the droplets will be forced to make physical contact and due to their low negative zeta potential their mutual repulsion is overcome, which cause higher tendency to flocculation and coalescence [33,34]. In addition, the significant difference between the formulations stability stored at 25 °C and those stored at 40 °C, may be due to the change in the optimum curvature of the surfactant monolayer and the type of emulsion structure formed because of the dehydration of the hydrophilic surfactant head group at high temperatures. The Same findings were reported by Rao and McClements, [26], who studied the Influence of storage temperature on the colloidal dispersions of lemon oil using sucrose Palmitate as non-ionic surfactant.

The differences between the evaluated parameters were not significant at 4 °C of storage for six months, which means that the systems were physically stable under these storage conditions. Over the period of six months the mean droplets size remained lower than 200 nm for all the formulations except formulation B as mentioned above. The relatively small, nano-size droplets distribution displayed a satisfactory long term stability (PdI<0.300). After six months of storage at room temperature, all nano-size droplets still revealed a negative charge on their surface (zeta potential <-30 mV), but their size were more than 200 nm. In a conclusion, in order to keep nanoemulsion formulations stable, they need to be stored at 4 °C.

CONCLUSION

In conclusion, Nanoemulsion was prepared using sucrose monoester as surfactant blended with glycerol and Olive oil. The nanoemulsion properties were affected by the nature and the concentration of sucrose monoester surfactant. Sucrose Laureate surfactant showed better nanoemulsion properties with small droplets size, low polydispersity index and high negative zeta potential value compared to sucrose Oleate and Palmitate. In addition, the properties of nanoemulsion were affected by different storage conditions. Nanoemulsion stored for six months under different temperatures, showed good stability when stored at 4 °C compared to 25 °C and 40 °C.

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