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Preparation And Optimization Of Ibuprofen-Loaded Nanoemulsion Formulation

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***Abstract*— Nanoemulsion containing ibuprofen was developed. However, the composition and method to prepare nanoemulsion were not consistent and needed to be optimised. In this study, nanoemulsion containing a mixture of palm kernel oil esters (PKOE), ibuprofen, Tween 80 (T80), and water was modified from the previous report. It was prepared by a combination of two methods, including low energy and high energy emulsification methods. The composition of nanoemulsion was optimised by a Mixture of Experimental Design (MED), where PKOE, T80, and water were set as variables while droplet size was a response. A total of 15 run experiments were evaluated. An optimum formulation was validated, and the composition of 3.0 wt % of PKOE, 15.0 wt % of Tween 80, 2.0 wt % of ibuprofen and 80.0 wt. % of water with the droplet size of 97.26 nm was obtained. The formulation is stable in the storage at room temperature (25 ± 2 ºC) within 3 months against coalescence process. The polydispersity index and zeta potential of the optimized formulation were 0.271 and -19.8 mV, respectively.**

Keywords*— palm kernel oil esters; mixture experimental design; Ibuprofen; non-steroidal anti-inflammatory drug; nanoemulsion

I. INTRODUCTION

Nanoemulsion is an emulsion that fulfill the criteria in which the droplet size is not more than 200 nm [1]. It consists of two immiscible phases, the oil phase and aqueous phase, which stabilized by a surfactant and/or co-surfactant. Nanoemulsion is classified to oil-in-water (O/W) or water-in-oil (W/O) nanoemulsion. Surfactant plays a role in stabilizing the two immiscible phases, oil and aqueous phases. Surfactant could lower the surface tension and reduce the energy required. Besides, the surfactant in the nanoemulsion system was always related to the toxicity of the components when they are prescribed orally or topically . Non-ionic surfactants specifically Tween 80 is relatively less toxic and it is suitable for the formulation [2]. O/W nanoemulsions have captured the interest in delivery systems due to their ability to encapsulate the active ingredients in the pharmaceutical products and other products [3,4]. Nanoemulsions are clear colloidal systems [5-7] and allow the permeation of the drugs or active ingredients into skin layers, and it was being considered as efficient systems for dermal and transdermal drug delivery [8], make them suitable for the dermatological application. They are kinetically stable system and have smaller droplet size when compared to the conventional emulsion [9,10]. However, nanoemulsions are thermo-dynamically unstable [11]. Nanoemulsion is biocompatible, biodegradable, physically stable and relatively easy to produce on a large scale using proven technology [12, 13]. Nanoemulsion also has good stability to gravitational separation and aggregation and the ability to increase the bioavailibity of encapsulated active ingredients [14]. Besides, instabilities occurrence are also prevented due to the sterically protection given by the surfactants on their surface [15].

Nanoemulsion acts as a drug delivery tool because of their tiny droplet size capable of delivering drugs specifically to areas of disease and make it more efficacious and decrease their adverse side effects. A nano-sized emulsion can improve therapeutic outcomes by modifying drug distribution to the targeted area [16]. Preparation of nanoemulsion could be formed by high and/or low energy emulsification methods. High energy emulsification methods require large mechanical energy generated by high pressure homogenizer or ultrasound generators to produce nanoemulsions with fine droplets. In this method, the dispersion of two liquids (aqueous phase and oily phase) are achieved by forcing their mixture through a small inlet orifice at very high pressure (500 to 5000 psi), which subjects the product to intense turbulence and hydraulic shear resulting in extremely small particles of emulsion [17].

Low energy emulsification method is related to the chemical energy stored in the ingredients and produces the nanoemulsions almost spontaneously, thus have great attraction both in theoretical study and practical application. The spontaneous formation of nanoemulsion depends on the specific systems and environmental conditions, which changes the

interfacial properties of the nanoemulsion systems [18]. This can be achieved either by the phase inversion temperature method or by the phase inversion composition method in the spontaneous emulsification method.

The properties of nanoemulsions, as non-equilibrium systems, depend not only on composition variables but preparation variables such as emulsifying path, agitation or emulsification time. These variables can have a significant influence on the nanoemulsion final properties. The best properties for particular application, the minimum droplet size for better permeation, improving the stability of the nanoemulsion formulation where obtained through the optimization study [19]. Optimization of nanoemulsion formulation also had been done to obtain an optimum in the function for which the nanoemulsions are used. In this study, ibuprofen loaded nanoemulsion formulation was designed. The nanoemulsion composed of a mixture of palm kernel oil ester (PKOE), Tween 80, and water, was optimized using D-optimal mixture design (MED) for minimum droplet size. The advantages of using MED are reported to be used to evaluate multiple variables and the ability of the statistical tool to identify interactions. Therefore, it is less time consuming compared to studying one-variable at a time. Additionally, this experimental methodology generates a mathematical model which is presented in graphical form [20].

Non–steroidal anti-inflammatory drugs (NSAIDs) such as naproxen, ibuprofen and diclofenac are among the most frequently prescribed medications worldwide [21]. They are prescribed to reduce pain, inflammation, and also fever and are used for the long and short period of time management of various conditions, including osteoarthritis, rheumatoid arthritis, and musculoskeletal pain [22]. Ibuprofen was chosen in this study, as an active ingredient in the nanoemulsion formulation initially to reduce adverse side effects associated with the hepatic first-pass metabolism [23]. Ibuprofen was available for oral prescription for the treatment of rheumatoid arthritis [24]. Long therapy of the oral ibuprofen may cause ulceration, bleeding in the stomach or intestine. These risks can occur at any time without warning symptoms especially for older patients. The topical administration of ibuprofen may be useful for the patients as it reduces the adverse effects and applies relatively consistent drug levels in the site of action [25].

The formulation of nanoemulsion containing ibuprofen was previously developed through the addition of water into the oil phase. However, to make a stable nanoemulsion reproducible, a large number of factors must be controlled such as the selection an appropriate composition, controlling the order of addition of the components, and applying the right shear [20]. The objective of this study was to optimize the nanoemulsion formulation containing ibuprofen by using a mixture experimental design (MED) towards the droplet size. The physicochemical characteristics of the optimized nanoemulsion were then evaluated.

II. EXPERIMENTAL

A. Materials

Ibuprofen [2-(4-isobutylphenyl)-propionic acid] (99.8 %) was obtained from Eurochem (China). Palm kernel oil ester (PKOE) was prepared in the laboratory according to the method used by Gunawan *et al*. Fatty acids composition in PKOE are 0.5 % oleyl caproate (C24:1), 5.6 % oleyl caprate (C26:1), 5.9 % oleyl caprylate (C28:1), 54.1 % oleyl laurate (C30:1), 13.9 % oleyl myristate (C32:1), 6.2 % oleyl palmitate (C34:1), 1.2 % oleyl stearate (C36:1), 6.4 % oleyl oleate (C36:2) and 1.7 % oleyl linoleate (C36:3) [17]. The non-ionic surfactant, Tween 80 (polysorbate 80) was purchased from Merck (Germany). Deionized water from Milli-Q filtration system, EMD Millipore (Billerica, MA, USA) was used as the aqueous phase. All the chemicals were used as received.

B. Preparation of Nanoemulsion Containing Ibuprofen

Nanoemulsion was prepared by using a combination of low and high-energy emulsification technique. The composition was based on the experimental design stated in Table 2. Initially, Ibuprofen was dissolved in the PKOE and followed by the addition of Tween 80. Deionized water (10%) was added to the mixture and was homogenized using overhead stirrer (IKA RW16 Digital, Nara, Japan) at 300 rpm for 180 min. The remaining of deionized water was added dropwise and continuously homogenized to form an emulsion. It continues by homogenized using high shear homogenizer (IKA T25 Digital ULTRA-TURRAX, Germany) at 5000 rpm for 15 min. The final products were kept in a sample bottle at room temperature for further analysis. The optimum formulation was obtained by observing the formulation that produce a stable formulation with the smallest droplet size.

C. Experimental Design

The restrictions of component proportions are presented in Table 1. The technique used was Mixture Experimental Design (MED). From preliminary study, the lower and higher limits of the independent variables were determined. The independent variables were utilized to investigate the effect of PKOE (A), Tween 80 (B), and water (C) on the response variable (droplet size). A total of 15 run experiments were obtained. The contour and three-dimensional surface graphs were plotted to show the effect of independent variables on the response variable. The optimal compositions for the preparation of the nanoemulsion were chosen based on the condition resulting on minimum droplet size. Design – Expert software Version 7.0 (Stat Ease Inc., Minneapolis, MN, USA) was used to design the experiment, regression and graphical analysis.

Table 1 : Restriction of component properties

Independent Variables	Range Composition (wt. %)	
	Lower value	Higher value
PKOE (A)	3.00	4.00
Tween 80 (B)	9.00	15.00
Deionized Water (C)	79.00	86.00

Note: ibuprofen was kept constant (2.0 wt. %)

D. Statistical Analysis

All data were statistically analysed, and the model was tested for analysis of variance (ANOVA) and R-squared (R^2). ANOVA results were based on confidences level, α = 0.05, and effectiveness of each variable should be determined according to the probability value (*P*-value). High *F*-value and low *p*-value show significant effects on the respective response variable [26,27].

III. Physicochemical Characterization of the Optimized Nanoemulsion Formulation

A. Droplet Size and Polydispersity Index Measurement

The mean droplet size and polydispersity index of the optimized nanoemulsions and was measured by dynamic light scattering (DLS). The measurements were conducted using a Zetasizer (Nano ZS, Malvern Instrument Ltd., UK). The mean hydrodynamic diameter (z-average mean) was calculated from the autocorrelation function of the intensity of light scattered from the particles. The software used was DTS Nano version 5.03, (Malvern Instruments Ltd.). All the samples were diluted with deionized water prior to measurement. Polydispersity indexes lower than 0.2 are ideal, as they indicated a narrow range of size distribution.

B. Zeta Potential Measurement

The rate of particle movement under the influence of an external oscillating electrical field with a voltage of 150V (electrophoretic mobility) was measured with a Zetasizer (Nano ZS, Malvern Instruments Ltd., UK). The measured electrophoretic mobilities were converted to zeta potentials by the instrument's software (Dispersion Technology Software, version 5.03, Malvern Instruments Ltd., UK) using the following equation.

$$U_e = \frac{2\varepsilon\zeta f(\kappa\alpha)}{3\eta}$$

where U_e is the electrophoretic mobility, ε is the dielectric constant, ζ is the zeta potential, η is the viscosity of the dispersant, and $f(\kappa a)$ is the Henry function. The Smoluchowski approximation, $f(\kappa a) = 1.5$, was used for high ionic strength media and the Hückel approximation, $f(\kappa a) = 1$, was used for low dielectric medium.

C. Stability Study

Each sample (5 mL) was subjected to centrifugation at 4000 rpm for 15 min in room temperature (25.0 ± 0.5 °C). Stability of the optimized nanoemulsion formulation was also determined by measuring the droplet size as a function of time at 25 °C.

a) Ostwald Ripening Analysis

Data on particle size for 3 months storage at room temperature was collected. Ostwald ripening is happen when the particle size of the nanoemulsion formulation was increased after certain period of time caused by the diffusion of the oil phase through the aqueous phase. The Lifshitz–Slesov–Wagner theory was used in determining the Ostwald ripening rate for the optimized nanoemulsion and the effect of storage temperature on this rate. The equation used for Ostwald ripening was:

$$\omega = \frac{dr^3}{dt} = \frac{8}{9}\left[\frac{C(\infty)\gamma V_m D}{\rho RT}\right]$$

where ω is the frequency of rupture per unit surface of the film, r the average radius of droplets over time, t storage time in seconds, C (∞) the bulk-phase solubility, V_m the molar volume of the internal phase, D the diffusion coefficient of the dispersed phase in the continuous phase, ρ the density of the dispersed phase, R the gas constant, and T the absolute temperature. Graphs of radii (nm^3) against storage time (seconds) at different temperatures were plotted and compared.

b) Coalescence Analysis

The coalescence rate analysis was carried out to determine the factor that affects the changes in droplet size over time. All the collected droplet size data within the three different storage temperatures were analyzed as the following equation.

$$\frac{1}{r^2} = \frac{1}{r_0^2} - \left(\frac{8\pi}{3}\right)\omega t$$

In this equation, r is the mean radius after a certain time, ω is the frequency of rupture per unit of the film surface, and r_0 the value at time t=0. A graph of ($1/r^2$) against time (seconds) was plotted to evaluate the coalescence rate. A linear relationship pattern was predicted for the coalescence rate.

IV. Results and Discussion

A. Nanoemulsion Formulation Containing Ibuprofen

The predicted and actual values of the effect of independent variables on the nanoemulsion containing ibuprofen were shown in Table 2. A linear model was fitted by the D-optimal design. The values obtained experimentally agreed with the predicted values.

Table 2: Matrix of D-optimal MED; variables parameter and a response

Run	Independent variables			Response (Droplet size)	
	A, PKOE content (wt. %)	B, T80 content (wt. %)	C, water content (wt. %)	Predicted (nm)	Actual (nm)
1	3.685	9.000	85.315	58.45	64.38
2	3.500	12.000	82.500	157.40	146.59
3	3.500	12.000	82.500	149.90	146.59
4	3.999	13.459	80.542	134.8	153.94
5	4.000	9.898	84.102	63.37	58.48
6	3.000	12.043	82.957	86.54	134.71
7	3.000	14.601	80.399	86.54	93.96
8	4.000	15.000	79.000	165.30	165.55
9	3.000	14.601	80.399	101.90	93.96
10	3.450	12.645	81.905	165.30	150.26
11	4.000	11.276	82.724	29.14	106.75
12	3.500	12.000	82.500	137.80	146.59
13	3.000	9.000	86.000	122.80	118.86
14	3.010	11.073	83.916	132.20	137.52
15	4.000	12.375	81.625	131.80	134.99

B. Analysis of Variance (ANOVA) and Model Fitting

Table 3 showed that the response was suitable for linear model with *F*-value and *p*-value of 29.68 and 0.0010, respectively. It was indicated that the model was significant, which revealed the good correspondence between predicted and actual values.

Table 3 : ANOVA fitting linear model equation for a response (droplet size)

Source	Mean square	F-value	p-value	Significance
Model	2589.92	29.68	0.001	Significant
Linear Mixture	944.95	10.83	0.0152	
AB	1047.44	12	0.0179	
AC	447.05	5.12	0.073	
BC	1844.03	21.13	0.0059	
Residual	87.25	-	-	
Lack of fit	61.34	0.59	0.6094	Not significant
Pure error	104.52	-	-	

Abbreviation : ANOVA, analysis of variance

Table 4 shows the regression coefficient for the response (droplet size) which indicated that the predicted R^2 was in good agreement with the adjusted R^2. Figure 1 represents the comparison of predicted and actual values of the droplet size of nanoemulsion. The signal to noise ratio gave an adequate signal, thus the model was used to navigate the design space. Figure 2 shows the normal plot of residual of the design. The design was accepted when residuals follow an approximately straight line. Based on the figure, the plot of residual has some scatters, but has a definite pattern.

Table 4 : Regression coefficient for the response (droplet size)

	Response (droplet size)
Standard deviation	9.34
PRESS	2010.48
R^2	0.9674
Adjusted R^2	0.9348
Predicted R^2	0.8498
Adequate Precision	15.521

Abbreviation: PRESS, predicted residual error sum of squares.

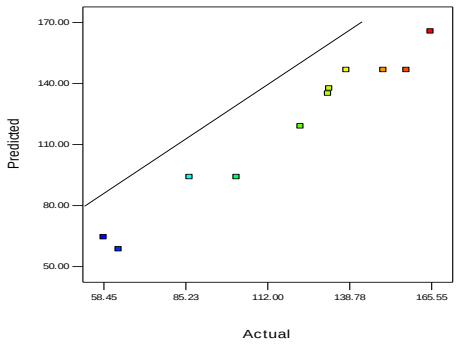

Figure 1: Comparison of predicted and actual values of droplet size of nanoemulsion

Using Design-Expert software Version 7.0, a quadratic model was fitted to the experimental results. The final obtained model to predict the droplet size nanoemulsions in terms of the actual factors of mixture components could be expressed in the following equation.

Y = -6167.3918A – 36840.2981B + 16.61495C + 9.3911AB +6.1159AC + 36229.4848BC

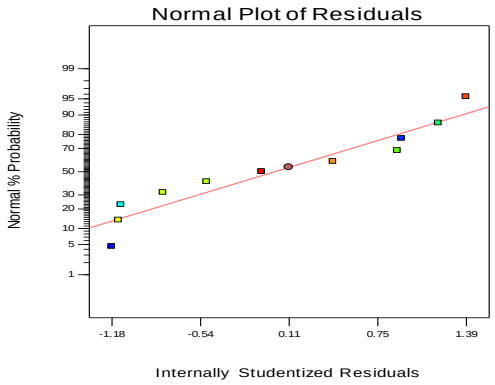

Figure 2: Normal plot of the residuals

C. Response Surface Analysis

Figure 3 shows the three-dimensional surface (3D) plots illustrated the effect of three variables on the droplet size of nanoemulsion. Based on the surface plot, when the amount of oil (PKOE) increased, the droplet size was increased. The increased amount of PKOE resulted would increase the viscosity of the formulation which causes an increase in flow resistance and restriction on the droplet break-up process thus causing the formation of larger droplet size [28, 29]. However, the droplet size was decreased when the amount of Tween 80 increased. This could be due to the surfactant used, Tween 80, which able to stabilize both the oil phase and aqueous phase. The increase in droplet size could happen due to incomplete coverage of surfactant on the new form droplet. This coverage limitation can lead to an increasing of the droplet size of an emulsion [30]. Tween 80 which is a nonionic surfactant was selected since they are common excipient and solubilizing agent used in the pharmaceutical industry, and are generally regarded as safe, and are biocompatible to be applied on the skin [31].

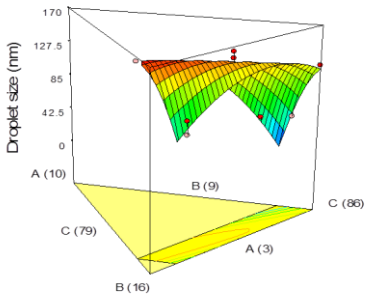

Figure 3: The 3D surface illustrated the effect of three variables: A (PKOE), B (Tween 80) and C (Water) against the droplet size of nanoemulsion, where ibuprofen was kept constant (2 wt. %).

D. Optimization of the Formulation

Table 5 shows five random formulations with different weight percentage and the actual values of droplet size were compared with the predicted value to verifying the model. Based on the results, the actual values were found to be close with the predicted values, which show an excellent fitness of the model generated. The resulting responses were compared to the predicted values by calculating the residual standard error (RSE) as following equation.

$$\text{RSE } \% = \frac{\text{Actual value} - \text{Predicted value}}{\text{Predicted value}} \times 100\%$$

The interactions between the variables were investigated and gave the optimum nanoemulsion formulation containing ibuprofen. The optimum ibuprofen–loaded nanoemulsion formulation was indicated as a composition of 3.0 wt.% PKOE, 15.0 wt. % Tween 80, 2.0 wt. % ibuprofen and 80.00wt.% water. Based on the optimum formulation, the values of droplet size of actual and predicted were 97.260 nm and 83.163 nm, respectively. The desirability of the optimum formulation was 1.00 and it was regarded to be acceptable (the range should be 0.80 to 1.00). The final product was transparent liquid. The addition of xanthan gum as a thickening agent (2 wt. %) was used to construct the nanoemulsion-based cream for improving the viscosity of nanoemulsion for topical administration.

Table 5 : Validation sets and optimum condition of nanoemulsion formulation containing ibuprofen

	Variables parameter (wt. %)			Droplet size (nm)		RSE (%)
	A	B	C	Actual	Predicted	
Validation						
V1	0.60	2.40	16.60	138.06	134.96	2.30
V2	0.75	3.00	15.85	151.71	145.09	4.50
V3	0.66	2.40	16.54	141.87	145.76	2.70
V4	0.70	3.00	15.90	137.65	140.28	1.87
V5	0.70	1.80	17.10	83.79	85.00	1.42
Optimum Formulation						
O1	0.60	3.00	16.00	97.26	83.16	16.90

E. Stability study

The droplet size is essential for the topical nanoemulsion formulation, as the size decreases, the surface area will increase and resulting in better adsorption. All nanoemulsion formulations prepared based on the optimum condition, were stable at room temperature for 3 months. There were no phase separation formed after the samples were centrifuged. Stability of the formulation can be defined as the capability of the formulation in a specific system to remain within its physical appearance within the specified limits, the time of the storage and use [32,33]. This stability was achieved due to the steric stabilizing effect of the non-ionic surfactant (Tween 80). The droplet in the nanoemulsion formulation protected by the formation of a bulk steric carrier from colliding to each other and cause flocculation. Figure 4 (a) showed that the optimized formulation was stable at room temperature (25 ºC) within 3 months, as the droplet size has no increment and maintains its range within 90 - 120 nm).

Coalescence happened when the particle size become large. This situation occur due to the fractured of the films of aqueous phase. However, the collected particle size data produce a non-linear pattern, which indicated no coalescence phenomena as it does not agree with the Equation 3. Therefore, the increment in particle size over time was not caused by coalescence [34].

The stability of the nanoemulsion can be investigated by plotting r^3 vs storage time for determining the rate of Ostwald ripening. Figure 4 (c) showed that the droplet size of the nanoemulsion increased slowly during storage at 25°C resulting in low Ostwald ripening effect. Ostwald ripening was a process in which the particles in the nanoemulsion system underwent expansion to become larger. This phenomenon happened due to the oil-particle diffusion through the continuous phase (water). Particles in the nanoemulsion system absorbed energy from their surroundings [35].

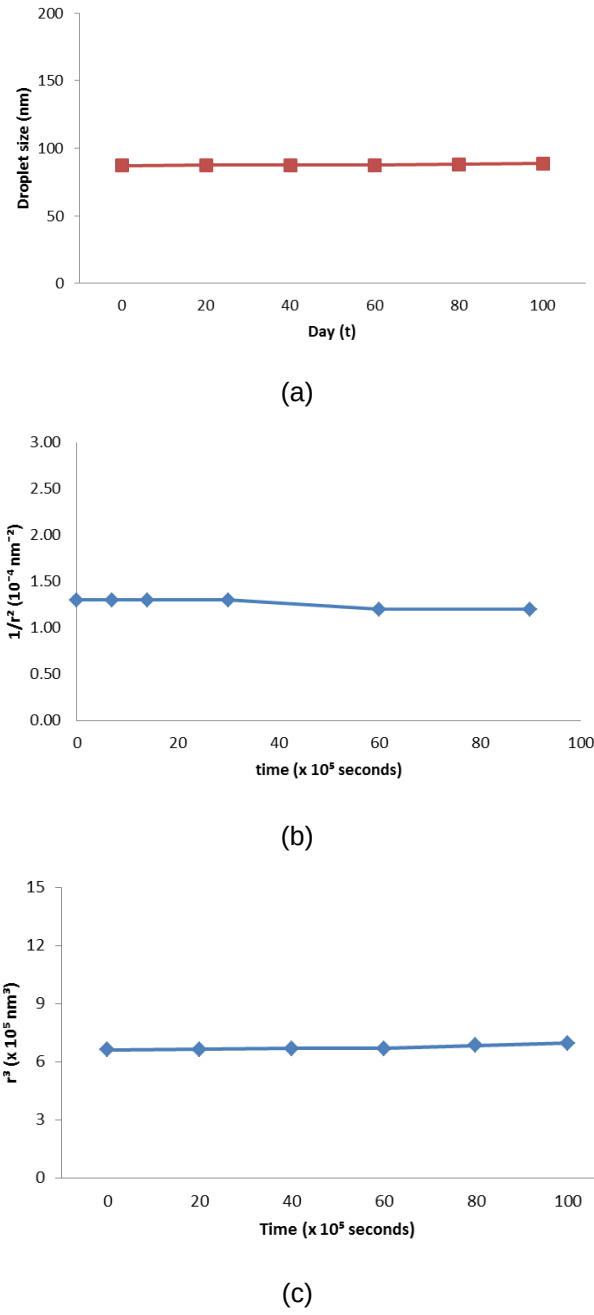

Figure 4: (a) the droplet size of the optimized formulation within 3 months at room temperature, (b) graph of $1/r^2$ vs storage time (seconds), (c) Graph of r^3 vs storage time (seconds).

V. CONCLUSION

The finding showed that the ibuprofen–loaded nanoemulsion formulation was successfully prepared by a combination of low and high-energy emulsification techniques. The composition was well-optimized by a Mixture Experimental Design (MED) with the droplet size of less than 200 nm and good PDI and zeta potential values. The result was well-fitted linear model with low RSE value. The optimized formulation was physically stable when stored at room

temperature within three months, where no phase separation was observed. Here, further studies are needed to evaluate the permeation of ibuprofen and to determine their anti-inflammatory and anti-microbial activities of the optimized nanoemulsion formulation.

VI. REFERENCES

[1] Abolmaali S. S., Tamaddon A. M., Farvadi F. S., Daneshamuz S. and Moghimib H. (2011). Pharmaceutical Nanoemulsions and Their Potential Topical and Transdermal Applications. Iranian Journal of Pharmaceutical Sciences. 7 (3): pp 139-150.

[2] Ferreira LM, Marcondes Sari MH, Cervi VF, Gehrcke M, Barbieri AV, Zborowski VA, Ruver Beck RC, Nogueira CW, Letícia Cruz L (2016). Pomegranate seed oil nanoemulsions improve the photostability and in vivo antinociceptive effect of a non-steroidal anti-inflammatory drug. Colloids and Surfaces B: Biointerfaces. 144: pp 214–221.

[3] Campani V, Biondi M, Mayol L, Cilurzo F, Pitaro M, De Rosa G (2016). Development of nanoemulsions for topical delivery of vitamin K1, International Journal of Pharmaceutics. 511: pp 170–177.

[4] Saberi AH, Fang Y, McClements DJ (2013). Fabrication of vitamin E-enriched nanoemulsions: factors affecting particle size using spontaneous emulsification. Journal of Colloid Interface Science. 391: pp 95–102.

[5] Singh Y, Meher JG, Raval K, Khan FA, Chaurasia M, Jain NK (2017). Nanoemulsion: concepts, development and applications in drug delivery, Journal Control Release: 252: 28–49.

[6] Qian C, McClements DJ (2011) Formation of nanoemulsions stabilized by model foodgrade emulsifiers using high-pressure homogenization: factors affecting particle size, Food Hydrocolloids. 25: pp 1000–1008.

[7] Mayer S, Weiss J, McClements DJ (2013) Vitamin E-enriched nanoemulsions formed by emulsion phase inversion: factors influencing droplet size and stability. Journal of Colloid Interface Science. 402: pp 122–130

[8] Schwarz JC, Weixelbaum A, Pagitsch E, Löw M, Resch GP, Valenta C (2012). Nanocarriers for dermal drug delivery: influence of preparation method, carrier type and rheological properties. International Journal of Pharmaceutics. 437: pp 83–88.

[9]Cécile J,Raphaëlle S.,Christelle H.S, Didier P., Julien M., Fernando L.C., Chrystel F.(2019). O/W Pickering emulsions stabilized by cocoa powder: Role of the emulsification process and of composition parameters. Food Research International. 116 : pp 755-766

[10] Bushra R, Aslam N (2010). An overview of clinical pharmacology of ibuprofen. Oman Medical Journal. 25(3): pp 155-161.

[11] Carreau A, Hafny-Rahbi BE, Matejuk A, Grillon C, Kieda C (2011). Why is the partial oxygen pressure of human tissues a crucial parameter Small molecules and hypoxia. Journal of Cell Molecular Medicine. 15: pp 1239–1253.

[12] Zainol S, Basri M, Basri HB, Shamsuddin AF, Abdul-Gani SS, Karjiban RA, & Abdul-Malek E (2012). Formulation optimization of a palm-based nanoemulsion system containing levodopa. International Journal of Molecular Sciences. 13(10): pp 13049-13064.

[13] Li, J.; Nie, S.; Yang, X.; Wang, C.; Cui, S.; Shenyang, WP (2008). Optimization of tocol emulsions for the intravenous delivery of clarithromycin. International Journal of Pharmaceutics. 356: pp 282–290.

[14] McClements DJ (2012). Nanoemulsions versus microemulsions: terminology, differences, and similarities. Journal Soft matter. 8(6): pp 1719-1729.

[15] Tien BY, Naim MN, Zakaria RA, Bakar NF, Ahmad NO, Lenggoro WU (2019). Stabilisation of Emulsified Agarwood oil in an Aqueous System Using Non-Ionic Surfactant. InKey Engineering Materials. 797: pp. 186-195.

[16] Fryd MM. and Mason TG (2012). Advanced nanoemulsions. Annual Review of Physical Chemistry, 63: 493-518.

[17] Gunawan ER, Basri M, Rahman MBA, Salleh AB, Rahman RNZA (2005). Study on response surface methodology (RSM) of lipase-catalyzed synthesis of palm-based wax ester. Enzyme and Microbial Technology. 37: pp 739–744.

[18] Gutiérrez JM, González C, Maestro A, Solè IMPC, Pey CM, & Nolla J (2008). Nano-emulsions: New applications and optimization of their preparation. Current Opinion in Colloid & Interface Science, 13(4): pp 245-251.

[19] Jafari SM, He Y, Bhandari B (2007). Effectiveness of encapsulating biopolymers to produce sub-micron emulsions by high energy emulsification techniques, Food Research International. 40: pp 862-873.

[20] Masoumi HRF, Basri M, Samiun WS, Izadiyan Z, & Lim CJ (2015). Enhancement of encapsulation efficiency of nanoemulsion-containing aripiprazole for the treatment of schizophrenia using mixture experimental design. International Journal of Nanomedicine, 10: pp 6469-6476.

[21] McGettigan P. and Henry D (2013). Use of Non-Steroidal Anti-Inflammatory Drugs That Elevate Cardiovascular Risk: An Examination of Sales and Essential Medicines Lists in Low-, Middle-, and High-Income Countries. PLoS Medicine, 10(2).

[22] Scheiman JM and Hindley CE (2010). Strategies to Optimize Treatment With NSAIDs in Patients at Risk for Gastrointestinal and

Cardiovascular Adverse Events. Clinical Therapeutics, 32(4) pp 667-677.

[23] Supakanya W., Amaraporn W., Katie M., Janani R. (2018). A Comprehensive Review of Non-Steroidal AntiInflammatory Drug Use in The Elderly. Aging and Disease, 9(1): pp143-150.

[24] Makhmalzadeh, B.S., Torabi, S., & Azarpanah, A. (2012). Optimization of Ibuprofen Delivery through Rat Skin from Traditional and Novel Nanoemulsion Formulations. Iranian Journal of Pharmaceutical Research, 11 (1): pp 47-58.

[25] Mason TG, Wilking JN, Meleson K, Chang CB and Graves SM (2006). Nanoemulsions: formation, structure, and physical properties. Journal of Physics: Condensed Matter. 18(41): pp 635–666.

[26] Musa SH, Basri M, Fard Masoumi HR, Roghayeh AK, Abd Malek E, Basri H, Shamsuddin AF (2013). Formulation optimization of palm kernel oil esters nanoemulsion-loaded with chloramphenicol suitable for meningitis treatment. Colloids and Surfaces B: Biointerfaces. 112: pp 113 – 119.

[27] Chang Y, McLandsborough L, McClements DJ (2015). Fabrication, stability and efficacy of dual-component antimicrobial nanoemulsions: essential oil (thyme oil) and cationic surfactant (lauric arginate). Food Chem, 172: pp 298–304.

[27] Ngan CL, Basri M, Tripathy M, Karjiban RA, Abdul-Malek E. (2015) Skin intervention of fullerene-integrated nanoemulsion in structural and collagen regeneration against skin aging. European Journal of Pharmaceutical Sciences. 70 : pp22–28.

[28] Cheah HY, Kiew LV, Lee HB, Japundzic-Zigon N, Vicent MJ, Hoe SZ, & Chung LY (2017). Preclinical safety assessment of nano-sized contructs on cardiovascular system toxicity: A case for telemetry, Journal of Applied Toxicology. 37(11): pp 1268-1285.

[29] Arbain N, Salim N, Wui WT, Basri M, Abdul Rahman MB. Optimization of Quercetin loaded Palm Oil Ester Based Nanoemulsion Formulation for Pulmonary Delivery. Journal of Oleo Science : 2018 67(8) pp933-940.

[30] Ochekpe NA, Olorunfemi PO, Ngwuluka NC (2009). Nanotechnology and drug delivery part 2: nanostructures for drug delivery. Tropical Journal of Pharmaceutical Research. 8(3): pp 31-37.

[31] Zouboulis AI, Avranas A (2000). Treatment of oil-in-water emulsions by coagulation and dissolved-air flotation. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 172(1-3): pp 153-161.

[32] Tang SY, Shridharan P, Sivakumar M (2013). Impact of process parameters in the generation of novel aspirin nanoemulsion- comparative studies between ultrasound cavitation and microfluidizer. Ultrasonics Sonochemistry, 20(1): pp 485-497

[33] Author links open overlay panel Veronica G. Carla D.,Mattia G.,Sacchetti Lilia N.,Paola P.(2016) Role of olive oil phenolics in physical properties and stability of mayonnaise-like emulsions. Food Chemistry. 213 : pp 369-377.

[34] Studart AR, Shum HC, Weitz DA (2009). Arrested coalescence of particle-coated droplets into nonspherical supracolloidal structures. The Journal of Physical Chemistry B. 113(12): pp 3914-3919.

[35] Musa SH, Basri M, Masoumi HR, Shamsudin N, Salim N (2017). Enhancement of physicochemical properties of nanocolloidal carrier loaded with cyclosporine for topical treatment of psoriasis: in vitro diffusion and in vivo hydrating action. International Journal of Nanomedicine. 12: pp 2427-2441.