ORIGINAL RESEARCH

Formulation development and optimization of palm kernel oil esters-based nanoemulsions containing sodium diclofenac

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Abstract: Response surface methodology was employed to study the effect of formulation composition variables, water content (60%–80%, w/w) and oil and surfactant (O/S) ratio (0.17–1.33), as well as high-shear emulsification conditions, mixing rate (300–3,000 rpm) and mixing time (5–30 minutes) on the properties of sodium diclofenac-loaded palm kernel oil esters-nanoemulsions. The two response variables were droplet size and viscosity. Optimization of the conditions according to the four variables was performed for preparation of the nano-emulsions with the minimum values of particle size and viscosity. The results showed that the experimental data could be sufficiently fitted into a third-order polynomial model with multiple regression coefficients (R^2) of 0.938 and 0.994 for the particle size and viscosity, respectively. Water content, O/S ratio and mixing time, quadrics of all independent variables, interaction between O/S ratio and mixing rate and between mixing time and rate, as well as cubic term of water content had a significant effect ($P<0.05$) on the particle size of nanoemulsions. The linear effect of all independent variables, quadrics of water content and O/S ratio, interaction of water content and O/S ratio, as well as cubic term of water content and O/S ratio had significant effects ($P<0.05$) on the viscosity of all nanoemulsions. The optimum conditions for preparation of sodium diclofenac nanoemulsions were predicted to be: 71.36% water content; 0.69 O/S ratio; 950 rpm mixing rate, and 5 minute mixing time. The optimized formulation showed good storage stability in different temperatures.

Keywords: response surface methodology, nanoemulsions, palm kernel oil esters, optimization, particle size, viscosity

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) belong to a group of drugs that are most commonly used for the alleviation of pain, fever, and inflammation. NSAIDs are nonselective cyclooxygenase inhibitors, which show a slightly preferential inhibitor activity for cyclooxygenase-2, while many of them prevent cyclooxygenase-1 and cause gastric damage.[1,2] Sodium diclofenac is commonly used and is especially effective in the controlling of severe conditions of inflammation and pain, musculoskeletal disorders, arthritis, and dysmenorrhea.

Although it is one of the best-tolerated classical NSAIDs, like other drugs in this category, it can cause gastrointestinal adverse effects.[1] In order to overcome the drug's adverse effects, high hepatic first-pass metabolism, and the short biological half-life, the development of a transdermal nanodelivery system like nanoemulsion is beneficial. Formulation as nanoemulsion is one of the nanoscience approaches that has been progressively considered in pharmaceutical science.[3]

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An emulsion is formed by the dispersion of one liquid, usually the oil phase in another immiscible liquid, water phase. In comparison to other oils, palm kernel oil esters (PKOEs) contain higher amounts of shorter chain esters, which can be applied in micro- and nanoemulsion systems as a carrier for actives. Conversely, palm oil esters contain longer chain esters and can be used as emollient in formulation. Furthermore, the higher iodine and saponification values, as well as lower slip melting point of PKOEs in comparison to other esters, provide some favorable properties for micro- and nanoemulsions in many applications.[4,5]

Nanoemulsions are a type of emulsion with particle sizes ranging from 20 nm to 500 nm. They may appear to be transparent or translucent mixtures of the oil phase, aqueous phase, surfactant(s), and cosurfactant(s) that create oil-in-water (O/W) or water-in-oil emulsions.[6] Due to their small particle size and very good stability, nanoemulsions have unique properties as they are protected against flocculation, coalescence, aggregation, and Ostwald ripening. Their physicochemical properties give them some advantages for their usage in many practical applications such as in personal care and cosmetics,[7] as well as in agrochemical,[8] food,[9] chemical,[10] and pharmaceutical applications.[11,12]

For nanoemulsion non-equilibrium systems, extrinsic energy is necessary. There are two main methods used to prepare nanoemulsions: condensation or low-energy methods, and dispersion or high-energy methods. In high-energy methods, the applied energy is achieved by high-shear stirring, high-pressure homogenizers, or ultrasound generators.[13] High-energy emulsification methods result in emulsions that have the highest level of homogeneous flow and adjustable control of droplet size; thus, they are suitable for industrial purposes. Furthermore, in these methods, the apparatus supply energy to produce the fine emulsions from a large variety of materials in the shortest time.[14] However, despite the formation mechanism, the properties of the prepared nanoemulsions are influenced by formulation compositions.[15–17]

In this work, formation of nanoemulsions in the PKOEs/lecithin (L):Cremophor® EL (Cr EL) (Sigma-Aldrich, St Louis, MO, USA)/water system was studied with respect to the effect of composition and its preparation variables. Optimization through an experimental design was carried out. The experimental design employed allowed the study of the effect of variables alone, as well as their interactions; which is contrary to the conventional approach used for experimentation.[18] This type of experiment evaluates the effect of one factor at a time, while the remaining factors are held constant.[6,18] The experimental design used in this study was response surface methodology (RSM), which is an efficient statistical and mathematical tool used for the modeling and analysis of processes that exist between the independent factors and their responses, and for optimization of the processes or products.[19–21]

For the transdermal delivery of nanoemulsions containing drugs, minimization of the particle size as well as reductions in viscosity exhibit faster release of active ingredients and enhance drug permeation.[22–24] Although it has been found that viscosity is important for the stability and efficiency of drug release, there are few reports on the effect of various factors on nanoemulsion viscosity, or its optimization in pharmaceutical research.

There are a number of studies that examined the dominating factors that determine the particle size of nanoemulsions. The studies were performed by considering the concentration of the internal phase of the emulsion, as well as the concentration of the surfactant, the cosurfactant, or other compositions.[16,25,26] However, fewer investigations have been done on optimizing the processing parameters. It is advantageous to develop a model to study and optimize the processing conditions, such as time and rate in high-energy methods, to avoid spending extra time and energy, particularly in industrial applications.

The present work aims to study the critical factors involved in the processing conditions and formulation compositions that control the particle size and viscosity in nanoemulsions. The alternative aim is to optimize the conditions for the preparation of sodium diclofenac-loaded nanoemulsions with the smallest particle size and the lowest viscosity by RSM, as well as to study the optimized nanoemulsions under different storage temperatures.

Materials and methods
Materials
PKOEs were prepared in our laboratory. Soyabean L (Lipoid S75) was purchased from Lipoid GmbH (Ludwigshafen, Germany), and used without further purification. Cr EL and sodium diclofenac were obtained from Sigma-Aldrich and Santa Cruz Biotechnology (Dallas, TX, USA), respectively. Water used in this study was double-distilled water (Milli-Q deionized water; EMD Millipore, Billerica, MA, USA).

Construction of phase diagram
A pseudo-ternary phase diagram was constructed using different proportions of the PKOEs which were added to the mixture of L:Cr EL with a ratio of 60:40. An appropriate 5% (w/w) by weight of water was added to the samples; the altered weight

of the system was recorded. The mixtures were vortexed for 10 minutes using Vortex Mixer Model VTX-3000 L (LMS, Tokyo, Japan), and then centrifuged using centrifuge Z200A (Hermle, Wehingen, Germany) at 4,000 rpm for 15 minutes. The phase changes of the samples were determined visually through cross-polarized light. The above steps were done at room temperature. The phase diagram was constructed using the Chemix version 3.5 phase diagram plotter (Parallels® Software International, Inc, London, UK).

Nanoemulsion preparation

O/W nanoemulsions selected from the isotropic phase were prepared by continuous addition of the aqueous phase at 25°±2°C to the surfactants–oil mixture. The final concentration of sodium diclofenac in the nanoemulsions was 1.0 wt% dissolved in water as an aqueous phase. L was dissolved in the oil phase (PKOEs) at 50°C for 30 minutes. Cr EL was added to the mixture and homogenized under simple stirring. The aqueous phase was then added dropwise into the oil phase to attain an equilibrium state. The resultant emulsions were then homogenized by a high-shear stirrer (IKA®; RW 20 Digital, Nara, Japan). After the homogenization, the nanoemulsions were collected and kept overnight to equilibrate. Then, their particle size and viscosity were analyzed.

Particle size measurements

Sympatec GmbH Nanophox particle size analyzer (Clausthal-Zellerfeld, Germany) with Photon Cross Correlation Spectrometer (Sympatec GmbH) was used to determine the mean droplet size of the nanoemulsions at 25°C. The samples were diluted approximately 200 times with deionized water, and then were loaded into 1 cm^2 cuvettes in a thermostated chamber. The scattering intensity measurements were achieved at angles of 90°.

Viscosity measurement

Viscosity measurements were performed using Kinexus Rotational Rheometer (Malvern Instruments Ltd, Malvern, UK). The measurements were carried out at temperatures of 25.0°C±0.5°C at a 5-second^{-1} shear rate. Data points are the average of the two measurements for each sample.

Experimental design

A four-factor, five-level central composite rotatable design (CCRD) was used to determine the effect of water content (x_1) (60%–80%, w/w), oil and surfactant ratio (x_2) (0.17–1.33), mixing rate (x_3) (300–3,000 rpm), and mixing time (x_4) (5–30 minutes) on particle size (Y_1) and viscosity (Y_2) of the nanoemulsions. The following equation is used to calculate the total number of the designed experiments:

$$N = 2^k + 2k + n_0, \tag{1}$$

where k is the number of independent variables and n_0 is the number of repeated measurements at the center point.[27] A total of 30 experimental runs according to the CCRD were generated using the Design-Expert version 6.0.6 (State-Ease, Inc, Minneapolis, MN, USA); the experimental runs included 16 factorial points, eight axial points, and six center points. The center point was repeated six times to achieve a proper evaluation of the experimental error. The independent variables and their levels are shown in Table 1.

The scheme of the central composite design is listed in Table 2. A third-order polynomial equation was used to represent the particle size (Y_1) and viscosity (Y_2) of the nanoemulsions as a function of the independent variables as follows:

$$Y = a_0 + \sum_{i=1}^{4} a_i x_i + \sum_{i=1}^{4} a_{ii} x_i^2 + \sum_{i=1}^{4} a_{iii} x_i^3 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} a_{ij} x_i x_j$$
$$+ \sum_{i=1}^{3} \sum_{j=i+1}^{4} a_{iij} x_i^2 x_j + \sum_{i=1}^{3} \sum_{j=i+1}^{4} a_{ijj} x_i x_j^2 \tag{2}$$
$$+ \sum_{i=1}^{2} \sum_{j=1+1}^{3} \sum_{k=j+1}^{4} a_{ijk} x_i x_j x_k + e$$

where Y is the dependent variable, a_0 is a constant; a_i, a_{ii}, a_{iii} and a_{ij} are the linear, quadratic, cubic, and interactive coefficient, respectively; and e is the error of the model.

Statistical analysis

The experimental data were analyzed to fit the third-order polynomial equation to all the independent variables. Analysis of variance (ANOVA) and R^2 (coefficient of determination) statistics were performed to evaluate the significant differences between the independent variables. Nonsignificant terms ($P > 0.05$) were removed from the initial model to achieve a significant model. Then, the experimental

Table 1 Range of variables and their levels used in RSM design

Independent variables	Unit	Coded levels				
		−2	−1	0	1	+2
Water	% w/w	60	65	70	75	80
O/S ratio	–	0.166	0.457	0.75	1.039	1.33
Stirring rate	rpm	300	975	1,650	2,325	3,000
Stirring time	minutes	5	11.25	17.5	23.75	30

Abbreviations: RSM, response surface methodology; O/S, oil and surfactant; % w/w, percentage weight/weight; rpm, revolutions per minute.

Table 2 Scheme of the central composite design: independent, predicted, and experimental values of the response variables

Experiment number	Water (x_1) (%)	O/S (x_2)	Mixing rate (x_3) (rpm)	Mixing time (x_4) (minutes)	Particle size (Y_1) (nm)		Viscosity (Y_2) (Pa·s^{-1})	
					Experimental	Predicted	Experimental	Predicted
1	65	0.46	975	11.25	64.81	64.99	90.49	89.00
2	75	0.46	975	11.25	87.44	89.81	4.818	5.12
3	65	1.04	975	11.25	98.78	96.03	13.97	14.78
4	75	1.04	975	11.25	115.38	118.18	2.203	−0.25
5	65	0.46	2,325	11.25	80.91	87.61	80.49	83.51
6	75	0.46	2,325	11.25	110.43	112.42	1.318	3.86
7	65	1.04	2,325	11.25	89.28	96.57	5.97	9.29
8	75	1.04	2,325	11.25	114.46	118.72	0.654	−1.51
9	65	0.46	975	23.75	71.67	75.03	92.78	93.53
10	75	0.46	975	23.75	116.62	110.54	15.341	9.65
11	65	1.04	975	23.75	111.72	106.07	23.79	19.31
12	75	1.04	975	23.75	145.15	138.92	0.847	4.28
13	65	0.46	2,325	23.75	83.28	81.69	88.96	87.04
14	75	0.46	2,325	23.75	106.83	117.2	4.197	7.40
15	65	1.04	2,325	23.75	92.54	90.64	12.11	12.82
16	75	1.04	2,325	23.75	127.33	123.49	0.473	2.02
17	60	0.75	1,650	17.5	70.93	69.52	128.47	128.29
18	80	0.75	1,650	17.5	154.17	152.76	0.144	−0.037
19	70	0.17	1,650	17.5	95.52	88.28	39.96	39.78
20	70	1.33	1,650	17.5	121.19	125.61	4.23	4.05
21	70	0.75	300	17.5	85.94	93.34	15.32	19.91
22	70	0.75	3,000	17.5	110.76	100.53	17.12	12.17
23	70	0.75	1,650	5	94.57	84.56	5.62	3.85
24	70	0.75	1,650	30	92.18	99.37	10.51	11.91
25	70	0.75	1,650	17.5	76.62	79.6	9.22	11.98
26	70	0.75	1,650	17.5	81.21	79.6	11.13	11.98
27	70	0.75	1,650	17.5	75.53	79.6	13.34	11.98
28	70	0.75	1,650	17.5	85.28	79.6	15.031	11.98
29	70	0.75	1,650	17.5	76.55	79.6	12.105	11.98
30	70	0.75	1,650	17.5	82.39	79.6	11.07	11.98

Abbreviations: O/S, oil and surfactant; rpm, revolutions per minute.

data were refitted to check the variation of data around the fitted model (lack of fit).[28]

For better visualization of the effect of the independent variables on the response, surface response, and contour plots of the fitted polynomial regression equations were generated. The optimal conditions for producing the desired nanoemulsion formulations were generated using the software's numerical optimization function.

Stability study

Stability of the optimized nanoemulsion formulation was studied by determining the changes in particle size and surface charge of the formulation through storage at 4°C and 25°±1°C after its preparation.

Zeta potential analysis

The surface charge of the nanoemulsion formulation was measured using the Zetasizer Nano Series from Malvern Instruments at 25°C. The nanoemulsion formulations were diluted to the proper concentration with deionized water. A folded capillary electrophoresis cell was used to determine the surface charge. The zeta potential was calculated by measuring the electrophoretic mobility of the dispersed particles in a charged field.

Results and discussion
Pseudoternary phase diagram and variable screening

Figure 1 depicts the pseudoternary phase diagram of PKOEs/ L:Cr EL (60:40)/water system at 25°C±2°C. The resulting phases observed were the isotropic phase, homogenous phase, two-phase region, and three-phase region. The major isotropic phase was located at the water-rich corner in the pseudoternary phase diagram at 50% of water and above. The levels of the variables, such as water content and the O/S ratio, were selected with regards to their ability to produce
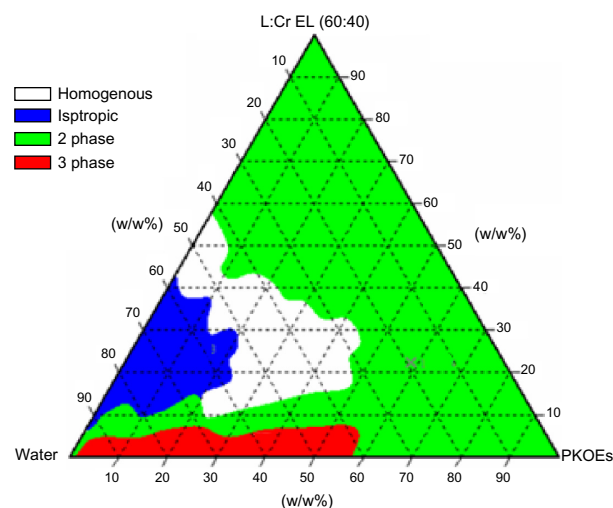
Figure 1 Pseudo-ternary phase diagram of PKOEs/L:Cr EL (60:40)/water.
Note: Cremophor® EL (Sigma-Aldrich, St Louis, MO, USA).
Abbreviations: PKOEs, palm kernel oil esters; L, lecithin; Cr EL, Cremophor® EL.

the isotropic phase. A preliminary study was carried out to evaluate the levels of the other two independent variables (mixing rate and mixing time).

Fitting the models

The response surface models were applied to predict the variation of the average particle size and viscosity as a function of the emulsion compositions and preparation variables of sodium diclofenac-loaded nanoemulsions. Table 2 presents the particle size and viscosity values of the nanoemulsions which resulted from all the experiments.

The coefficients of the cubic polynomial equation were calculated via the experimental data and were used to predict the particle size and viscosity values of the nanoemulsions. The predicted values showed a satisfactory agreement with the experimental ones gained from the RSM design. Fitting the data to various models and their subsequent ANOVAs illustrated that the aliased cubic model had higher coefficients for the responses of particle size and viscosity. The response surface analysis showed that the third-order polynomial response model that was applied for viscosity had a higher coefficient value ($R^2 = 0.994$) when compared to the response surface model for particle size ($R^2 = 0.938$).

Table 3 depicts the regression coefficients, F-value, and P-value of the four factors and their comparative importance on particle size and viscosity. In the regression equation, a positive value illustrates an efficacy level, which advocates that the optimization was due to a synergistic effect, while an opposing effect or an inverse relationship between the factor and the response were expressed as negative values.[29] A larger F-value and a smaller P-value for each term in the models would demonstrate the effect of the higher degree of significance on the dependent factors.[3]

The coefficient estimates and respective P-values shown in Table 3 indicated that all the four independent variables (water content [x_1], O/S ratio [x_2], mixing rate [x_3], and mixing time [x_4]) on the variation of viscosity and all the independent variables (except mixing rate on the variation of particle size) had significant effects ($P < 0.05$). Upon closer observation, in the linear term of the cubic polynomial model,

Table 3 Analysis of variance of the regression coefficients of the fitted cubic equations for the particle size (Y_1) and viscosity (Y_2) of the nanoemulsions

Variable	Particle size (Y_1)			Variable	Viscosity (Y_2)		
	Regression coefficient	**F-value**	**P-value**		**Regression coefficient**	**F-value**	**P-value**
a_0	−4,320.13898			a_0	+11,219.36229		
x_1	+207.80312	22.64	0.0002	x_1	−416.29749	288.6	<0.0001
x_2	−10.30760	39.2	<0.0001	x_2	−703.86072	367.77	<0.0001
x_3	+8.83918E-003	1.45	0.2455	x_3	−0.031135	7.45	0.0148
x_4	−6.60740	6.17	0.0244	x_4	+1.33749	8.08	0.0118
x_{11}	−3.26544	31.98	<0.0001	x_{11}	+5.23226	386.23	<0.0001
x_{22}	+80.73313	24.04	0.0002	x_{22}	−303.54830	14.01	0.0018
x_{33}	+9.51509E-006	9.67	0.0067	x_{33}	+2.22586E-006	2.34	0.1458
x_{44}	+0.079144	4.92	0.0414	x_{44}	−0.026230	2.39	0.142
x_{12}	−0.45747	0.13	0.7202	x_{12}	+11.82917	392.75	<0.0001
x_{14}	+0.085580	2.15	0.1623	x_{13}	+3.13574E-004	1.48	0.2407
x_{23}	−0.028106	9.14	0.0081	x_{34}	−5.88000E-005	0.082	0.7788
x_{34}	−9.45926E-004	4.78	0.044	x_{111}	−0.022432	31.27	<0.0001
x_{111}	+0.017052	4.09	0.0602	x_{222}	+148.33734	53.15	<0.0001
Model		18.71	<0.0001	Model		217.03	<0.0001
Lack of fit		4.56	0.0533	Lack of fit		3.86	0.0737

the O/S ratio had the most significant effect ($P>0.05$) on the variability of both responses that followed by water content. All quadratic terms had significant effects on the particle size. The quadratic term of water content had the most significant effect on the particle size and viscosity; however, the effect of mixing rate and mixing time were not significant for viscosity. The interaction between the O/S ratio and the mixing rate and the interaction between mixing rate and mixing time in addition to cubic term of water had significant effects ($P<0.05$) on the particle size of nanoemulsions. For the viscosity variation, interactions of water content and the O/S ratio and the cubic term of water content and the O/S ratio were significant on the response. However, the remaining terms showed insignificant effects ($P>0.05$).

The final third-order polynomial equations of the model (based on the coded values) for particle size and viscosity are as follows, respectively:

$$\text{Particle size} = +79.17 + 12.33\ X_1 + 9.42\ X_2 + 1.83\ X_3$$
$$+ 3.75\ X_4 + 7.85\ X_1^2 + 6.85\ X_2^2 + 4.23\ X_3^2$$
$$+ 3.10\ X_4^2 - 0.62\ X_1x_2 + 2.63\ X_1x_4$$
$$- 5.50\ X_2x_3 - 4.00\ X_3x_4 + 2.17\ X_1^3, \qquad (3)$$

$$\text{Viscosity} = +11.98 - 20.87\ X_1 - 23.55\ X_2 - 1.94\ X_3$$
$$+ 2.02\ X_4 + 13.04\ X_1^2 + 2.48\ X_2^2 + 1.01\ X_3^2$$
$$- 1.02\ X_4^2 + 17.21\ X_1x_2 + 1.06\ X_1x_3$$
$$- 0.25\ X_3x_4 - 2.80\ X_1^3 + 3.66\ X_2^3. \qquad (4)$$

Response surface analysis

Surface response and contour plots of the cubic polynomial model were produced to visualize the effect of the independent factors on the dependent ones. To achieve this purpose, two of the independent variables were varied within the experimental range, while the other parameters were held constant at the central point. Figures 2 and 4 were generated by varying the water content and the O/S ratio in the nanoemulsions, while the mixing rate and mixing time were held constant at 1,650 rpm and 17.50 minutes, respectively. Figures 3 and 5 were generated by varying the mixing rate and the mixing time while holding the water content and the O/S ratio at 70% and 0.75, respectively.

As shown in Figure 2, the particle size increased with increases in the water content. Increasing the water eliminates the rigid film and leads to the coalescence of the droplets and increases in particle size.[21] The results showed that increasing the O/S ratio from 0.17 to 0.5 resulted in a decrease in droplet size, but further increases in the O/S ratio resulted in increases in the particle size of the nanoemulsions. Formation of emulsions is influenced by an equivalency between two main mechanisms, namely droplet disruption and droplet coalescence.[30] At higher O/S ratios, there was not enough surfactant for the existence of oil or water content. In this manner, surfactant concentration is low enough to adsorb onto the surface of the newly formed droplets, and it can thus break down the oil droplets to smaller sizes during the emulsification process.[21] The surfactant concentration could control the total droplet surface area, the rate of droplet dispersion, and coalescence.[21,31] This result is in agreement with the results of Chanana and Sheth.[32]

Figure 3 depicts the interaction between mixing rate and mixing time on the particle size of nanoemulsions. Although, the mixing rate was not significant ($P>0.05$) on particle size, the interaction of mixing rate and mixing time showed a significant effect. Increasing the applied power of high-shear stirring increases the shear force which, in turn, decreases the droplet size of the emulsion.[21] However, the
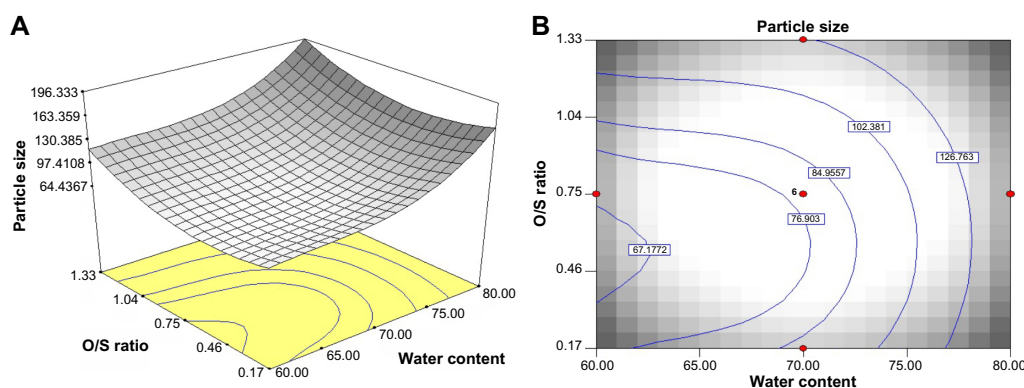

Figure 2 Response surface and contour plots of the particle size of the nanoemulsions showing the interaction between two parameters (water content and O/S ratio) at the mixing rate of 1,650 rpm and 17.50 minutes.
Notes: (**A**) Response surface; (**B**) contour plots.
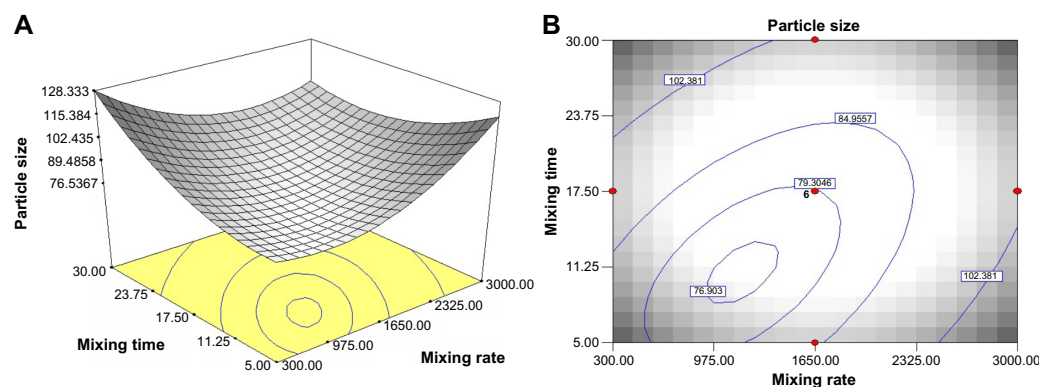**Abbreviation:** O/S, oil and surfactant.

Figure 3 Response surface and contour plots of the particle size of the nanoemulsions showing the interaction between two parameters (mixing rate and mixing time) at the water content level of 70% and O/S ratio of 0.75.
Notes: (**A**) Response surface; (**B**) contour plots.
Abbreviation: O/S, oil and surfactant.

results depicted that the smallest particle size of 76 nm could be obtained at the mixing rate of 1,200 rpm and at the time of 10 minutes. Further increases in particle size were observed at higher mixing rates, which could create some destabilization mechanisms such as coalescence and sedimentation, resulting in a larger final particle size. A similar result was observed between emulsion droplet size and mixing rate for nanoemulsions consisting of Tween 20/Span 20 and liquid paraffin.[6] The formation of larger droplets during longer mixing times (>10 minutes) could be ascribed to the effect of over-processing of the emulsification, which would lead to coalescence. A similar result was obtained for emulsification of the nanoemulsions using an ultrasonic method.[21,33]

Figure 4 shows the response surface and contour plot of interaction between water content and the O/S ratio on the nanoemulsion viscosity. It was found that the viscosity of the nanoemulsions decreased with increases in water content, and it also decreased with increases in the O/S ratio at water content levels less than 75%. The viscosity

of nanoemulsions is a function of their ingredients such as the surfactant, water, and oil, and their concentrations. Increasing the water content leads to a decrease in viscosity.[34] Decreases in viscosity by increasing the O/S ratio could be due to decrease in the hydrodynamic interaction between the droplets. Increasing the O/S ratio results in an increase in the droplet size; thus, the mean separation distance between the droplets increases, and this then results in decreases in viscosity.[32,35,36]

Figure 5 depicts the interaction of mixing rate and mixing time on viscosity of the nanoemulsions. The increase in mixing rate led to a decrease in viscosity. However, viscosity increased with an increase in mixing time. The decrease in the viscosity with an increase in the mixing rate could be due to the production of the larger particle sizes, which were due to coalescence and the effect of over-processing the emulsification with higher shear force.[21] As mentioned before, increases in particle size and increases in the mean separation distance between the droplets could result in decreases in viscosity.
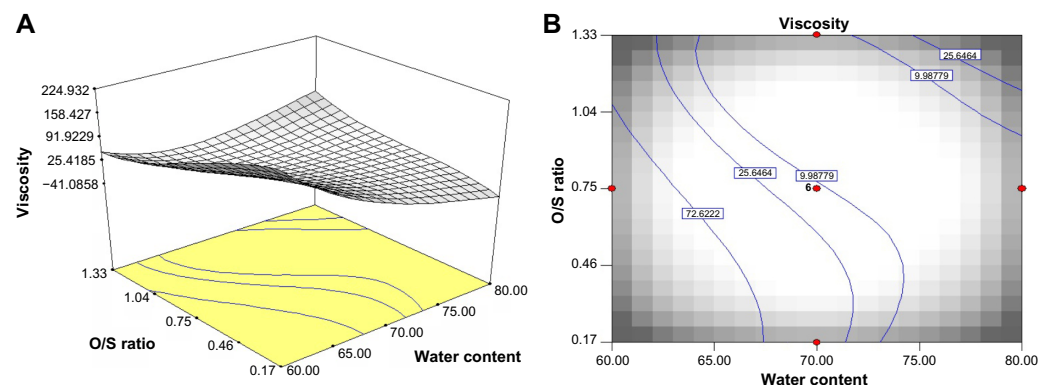

Figure 4 Response surface and contour plots of the viscosity of the nanoemulsions showing the interaction between two parameters (water content and O/S ratio) at the mixing rate of 1,650 rpm and the mixing time of 17.50 minutes.
Notes: (**A**) Response surface; (**B**) contour plots.
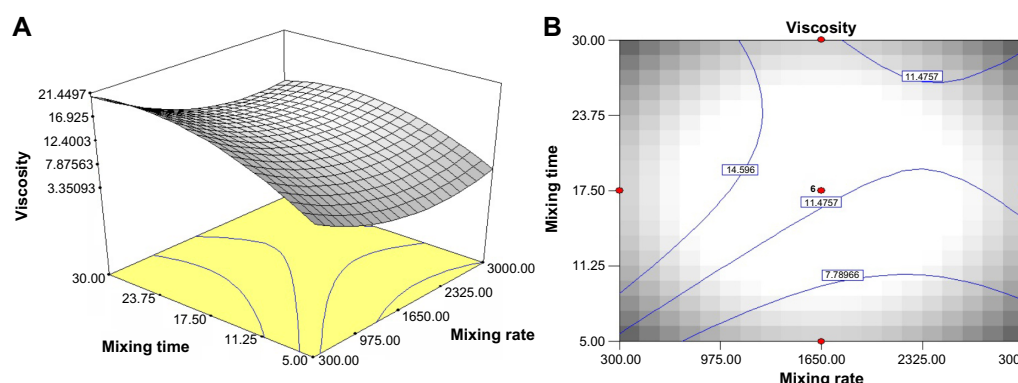**Abbreviation:** O/S, oil and surfactant.

Figure 5 Response surface and contour plots of the viscosity of the nanoemulsions showing the interaction between two parameters (mixing rate and mixing time) at the water content level of 70% and the O/S ratio of 0.75.
Notes: (**A**) Response surface; (**B**) contour plots.
Abbreviation: O/S, oil and surfactant.

Optimization of conditions and model validation

The optimum conditions for preparing sodium diclofenac-loaded nanoemulsions would be considered if the minimum values of the particle size and viscosity were achieved. Optimization was performed by the software numerical optimization function. The optimum sodium diclofenac-loaded nanoemulsion was achieved by the combined level of 71.36% water, 0.69 O/S ratio, a mixing rate of 950 rpm, and a mixing time of 5 minutes. Based on the optimum formulation, the predicted values for the responses of average particle size and viscosity were computed to be 78.11 nm and 5.49 Pa·s^{-1}, respectively. Table 4 shows the comparison between the experimental and predicted data obtained from the CCRD final reduced models. To check the competence of the response surface equations, predicted and experimental values of the responses were compared. The sodium diclofenac nanoemulsion was prepared under the optimum combination levels, and the experimental values showed the average particle size and viscosity of 79.85 nm and 5.04 Pa·s^{-1}, respectively. The proximity of the experimental and predicted values demonstrated the high validity and adequacy of the model.

Stability study

Figure 6 shows the changes in average droplet size and surface charge of the optimized sodium diclofenac nanoemulsions during storage at 4°C and 25°C. A slight increase in average droplet size was observed during the storage period of 90 days (Figure 6A). In the study, the variation of droplet size ranged from 79.85–93.42 nm at 4°C and was from 79.85–85.62 nm at 25°C. Two main processes controlled the final particle size of an emulsion: droplet break-up and coalescence.[30] The good stability of the nanoemulsion formulation could be due to the steric stabilizing effect of the nonionic emulsifier (L and Cr EL), which formed a thick steric barrier around the particles to prevent flocculation and coalescence. The existence of some free emulsifier in the micellar form plays a basic role in preventing coalescence after emulsification and storage, which results in improved emulsion stabilization.[3] The increase in viscosity of the continuous phase is the other factor that could improve the stability of the emulsion by restraining the Brownian movement of the droplets in the disperse phase.[3]

Despite of the low viscosity of the optimized nanoemulsion (as a desired condition for optimization of formulation), the result of particle size and surface charge measurements indicated that the optimized nanoemulsion presented good physical stability throughout the 90 days of the storage period. In essence, it is hypothesized that the oil–water interfaces that are formed are in a stable state against coalescence, which is achieved through surfactant adsorption. Sufficient amounts of L and Cr EL could cover the interface formed in the breakup prior to coalescence.

Figure 6B shows the zeta potential values of the optimized formulations during the storage time. The droplets

Table 4 Optimum condition for the preparation of sodium diclofenac nanoemulsion

Optimal conditions				Particle size (nm)		Viscosity (Pa·s^{-1})	
Water (%)	O/S	Mixing rate (rpm)	Mixing time (minutes)	Experimental	Predicted	Experimental	Predicted
71.36	0.69	950	5	79.85	78.11	5.04	5.49

Abbreviations: O/S, oil and surfactant; rpm, revolutions per minute.
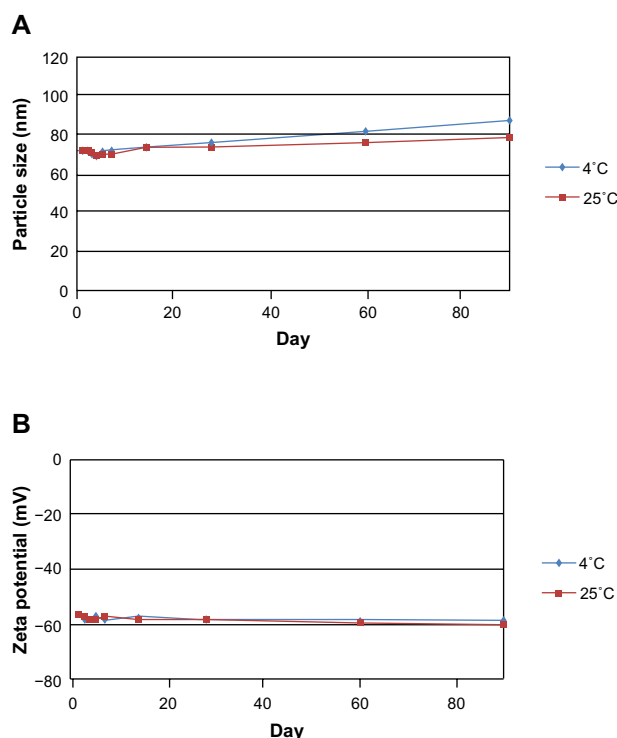
A

B

Figure 6 Storage stability of the nanoemulsions at different temperatures.
Notes: (**A**) Average droplet size of sodium diclofenac nanoemulsions during storage time at 4°C and 25°C; (**B**) average zeta potential values of sodium diclofenac nanoemulsions during storage time at 4°C and 25°C.

of the prepared sodium diclofenac-loaded nanoemulsions were found to be negatively charged. The zeta potential was approximately –57 to –58 and –57 to –60 at 4°C and 25°C, respectively. A slight increase in the absolute zeta potential was observed during the storage time.

The negative charges on the droplets could be contributed from the adsorption of sodium diclofenac in the aqueous phase at the oil–water interface. It has been reported that an increase in the surface charge can improve the stability of emulsions. This is due to increases in the repellent force between the droplets that resulted in higher stability against flocculation and coalescence.[37]

Conclusion

The study shows that the third-order polynomial model was effectively applied to explain and predict the responses of the particle size and viscosity of sodium diclofenac-loaded nanoemulsions. The independent variables of water content, O/S ratio and mixing time, the quadrics of all variables and interaction between the O/S ratio and the mixing rate, and between mixing rate and time, and the cubic term of water content had significant effects on the particle size of the nanoemulsions. Concurrently, the linear effect of all independent variables, quadrics, and cubics term of water content and the O/S ratio, as well as the interaction between water content and the O/S ratio had significant effects on the viscosity of the nanoemulsions. The numerical optimization method was applied to attain the best emulsifying conditions, and the optimum conditions for preparing the nanoemulsion formulation was predicted to be: water content of 71.36%; O/S ratio of 0.69; mixing rate of 950 rpm; and a mixing time of 5 minutes. The optimized formulation showed good stability over time.

Disclosure

The authors report no conflicts of interest in this work.

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