Three-factor response surface optimization of nano-emulsion formation using a microfluidizer

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Abstract Emulsification of sunflower oil in water by microfluidization was studied. Response surface methodology (RSM) and the central composite design (CCD) were applied to determine the effects of certain process parameters on performance of the apparatus for optimization of nano-emulsion fabrication. Influence of pressure, oil content and number of passes on the disruption of emulsions was studied. Quadratic multiple regression models were chosen for two available responses, namely Sauter mean diameter (SMD) and Polydispersity index (PDI). Analysis of variance (ANOVA) showed a high coefficient of determination (R^2) value for both responses, confirming adjustment of the models with experimental data. The SMD and the PDI decreased as the pressure of emulsification increased from 408 to 762.3 bar for the oil content of 5 vol% and from 408 to 854.4 bar for the oil content of 13 vol%, and thereafter, increasing the pressure up to 952 bar led to increasing the both responses. The results implied that laminar elongational flow is the alternative disruption mechanism in addition to inertia in turbulence flow, especially at low treatment pressures. Both of responses improved with increase in number of passes from 2 to 4 cycles. The oil content depicted low effect on responses; however, interaction of this parameter with other regressors pointed remarkable impact. Also, the effect of pressure on Kolmogorov micro-scale was studied. The results implied that Kolmogorov equation did not take into account the over-processing and was applicable only for disruption of droplets in the inertial turbulent flow.

Keywords Nano-emulsion · Microfluidization · Droplet disruption · Emulsifier · Over-processing · Central composite design

Introduction

An emulsion is a system comprised of two immiscible liquids, one of which is dispersed as droplets throughout the other (Jafari et al. 2008; Becher 2001). Emulsions have found an essential place in formulated cosmetic, pharmaceutical, food, detergent and cleaning agent, lubricant, paint and polymer products (Kiljański 2004; McClements 2012).

Nano-emulsions are a type of emulsions with uniform and extremely small droplet size, in the range of 100–1,000 nm (Deen and Sajjadi 2013). Due to their characteristic size, nano-emulsions are optically transparent or milky. These properties together with low viscosity, high kinetic stability against creaming and a large interfacial area make nano-emulsions attractive in many different applications, such as foods, cosmetic and health care (Sadurní et al. 2005; Windhab et al. 2005).

The formation of these nano-emulsions requires extreme energy input via a homogenization process (Yu et al. 2012). The shear stress applied by the process must exceed the Laplace pressure, which is inversely proportional to droplet diameter (Bouchemal et al. 2004; Perrier-Cornet et al. 2005; Sadurní et al. 2005; Thorsen et al. 2001). According to Taylor's formula, very high shear rate (about 10^7 – 10^8 s⁻¹) is needed for preparation of such emulsions with small droplet size (Mason et al. 2006; Taylor 1934).

The rotor–stator and the high-pressure homogenizing processes are the most commonly utilized mechanical emulsification systems that produce emulsions (Santana et al. 2013). In all of these systems, droplets of the dispersed phase are disrupted under the action of shear or inertial forces in laminar

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or turbulent flow. In the case of rotor-stator, the liquid is fed into the colloid mill and flows through a confined gap (≈ 50 – $1,000\ \mu\text{m}$) between rotating and static disks. The very high rotating speed at which this apparatus operates eventually promotes the stretching and break-up of the dispersed-phase particles (McClements 2005; Jafari et al. 2008; Marie et al. 2002; Urban et al. 2006).

In high-pressure homogenization, the pressurized liquid is forced through a homogenizing nozzle that causes a sudden constriction in the flow. As the liquid passes through the nozzle, most of the applied pressure energy is converted into kinetic energy. This energy is also rapidly converted into heat because of viscous dissipation. The particle break-up is initiated by a combination of forces in turbulence and laminar elongational flow. The turbulence on the low pressure side of the nozzle (valve) is the dominant factor in disruption of the droplets (Marie et al. 2002; Dickinson and James 1998).

Microfluidizer is one of the most efficient systems for production of oil in water emulsions when compared to classical homogenizers. The process stream is delivered by a pneumatically powered pump that is capable of pressurizing the emulsions up to about 150 MPa. Apart from inertia in turbulent flow, laminar elongational flow and cavitation also contribute to droplet break-up (Wilking et al. 2011). Using the microfluidization process, mean droplet diameter of less than $0.2\ \mu\text{m}$ can be obtained. A microfluidizer has been used in the homogenization of milk and dairy model by many researchers (Olson et al. 2004; Strawbridge et al. 1995).

Response surface methodology (RSM) is a collection of statistical and mathematical techniques that has been suggested for studying the effect of several factors influencing the responses. The RSM approach involves simultaneously varying the relevant factors and carrying out a limited number of experiments. It uses a design of experiment to fit a mathematical model with experimental data by least square technique (Myers et al. 2002; Hajar et al. 2009; Royaei et al. 2012).

Using microfluidizer for emulsification process is well established. However, in previous studies the effect of one parameter on dispersion has been investigated while other parameters remain at constant value. The mechanisms that contribute to particle disruption and coalescence are not well known (Taylor 2003). Hence, the implementation of RSM to investigate the simultaneous effects of various parameters on the nano-emulsion formation is of interest (Jafari et al. 2007).

Identifying the mechanisms involved in the formation of nano-emulsions using a microfluidizer were the major objectives of this study. Understanding the impact of the main factors on emulsification processes, disruption and coalescence mechanisms, has been therefore of great practical relevance. In this study, the molecular weight of the oil is large enough (about $876\ \text{g/mol}$) and the dispersed phase molecules

are essentially insoluble in the water to inhibit Ostwald ripening. RSM comprising a five-level, three-factor central composite design (CCD) was used in our work to evaluate the interactive effects and to obtain the optimum conditions for the formation of food nano-emulsion. The influence of homogenizing pressure, oil contents and number of passes through the microfluidizer with Tween 20 as emulsifier were studied. Application of RSM facilitated development of the mathematical model to describe quantitative mutual relationships existing between three independent variables. SMD and PdI were used as the response variables.

Material and methods

Chemicals

Sunflower oil (Cargill France, France) with the viscosity of $0.04\ \text{Pa}\cdot\text{s}$ at $20\ ^\circ\text{C}$ was used as dispersed phase, injected into distilled water that constitutes the continuous phase. Tween 20 (Polysorbate) with low molecular weight was supplied by Merck Co. (Germany). This non-ionic emulsifier was added to the continuous phase before the emulsification process.

The quantity of sunflower oil and Tween 20 in the final nano-emulsion was specified by response surface methodology.

Particle size distribution (PSD) measurement

Size distributions of oil droplets were determined by the dynamic laser light-scattering method. Dynamic light scattering (DLS), or photon correlation spectroscopy, is an established and popular technique for determining PSD. This technique is an ensemble measurement that quantifies the diffusion of particles as they undergo Brownian motion, and transforms this to hydrodynamic size and a size distribution using the Stokes-Einstein relationship.

The dispersions were characterized in terms of particle size and size distribution. Mean droplet size was determined by volume-space mean diameter (Sauter mean diameter), $d_{3,2}$ ($d_{3,2} = \sum n_i d_i^3 / \sum n_i d_i^2$). Particle-size analysis assessments were performed using a Zetasizer Nano ZS, (Malvern Instrument Ltd., UK) based on DLS. Refractive index and absorption values of 1.330 and 0.01, respectively, were used for the oil droplets to calculate volume and number size distributions as provided in the range of 0.4 to $10,000\ \text{nm}$. All samples were diluted with Acetate buffer (34 % v/v 0.2 M Sodium Acetate and 66 % v/v 0.2 M Acetic acid) to adjust to pH 4.4, in an effort to avoid multiple scattering effects. The PSD was measured after 2 h. During these 2 h samples were kept in refrigerator at $4\ ^\circ\text{C}$.

Emulsification process

Preparation of coarse emulsion with a rotor-stator system

Coarse emulsions were prepared by batch homogenization, using a rotor–stator device (ULTRA-TURRAX® T10, Germany). The oily dispersed phase was gradually added to the aqueous continuous phase in the presence of sufficient emulsifier. The circumferential rim speed was 22,000 rpm and the homogenization time was set to 5 min. These coarse emulsions were used as premix in the microfluidizer. The PSD for the coarse emulsion prepared by rotor-stator at 9 vol% of oil is shown in Fig. 1 (with Sauter mean diameter of 2.2 μm).

Preparation of nano-emulsion

Coarse emulsion was held at 4 °C for 2 h before homogenization to avoid aggregation of oily droplets. Then it was homogenized using a Microfluidizer M-110 L (Microfluidics International Corporation, Newton, MA, USA).

Droplet size and PSD were controlled in a microfluidizer by varying the processing pressure and the number of passes through the instrument.

The microfluidizer had an air-powered intensifier pump designed to supply the desired operating pressure at a constant rate through fixed-geometry microchannels within the interaction chamber.

The microfluidizer interaction chamber (the heart of the device) was a 75 μm -diameter ceramic Y type (F 20Y). In this chamber, the premixed emulsion accelerates to a high velocity due to the sudden sharp decrease in the pipe diameter and to the pressure release that leads to a tremendous shearing action. This phenomenon causes the disintegration of particles into smaller ones. The maximum flow velocity into the interaction chamber was estimated, by Bernoulli's law, depending on homogenization pressure and chamber size. The interaction chamber was placed in a 25 °C water bath for a long time and the discharge line was in contact with an open jacket cooling coil to cool the product nano-emulsion and prevent temperature increase in microfluidizer.

The coarse emulsion was passed through the microfluidizer, collected and immediately underwent this process again, up to the desired number of passes.

Experimental design

To determine the optimum conditions for emulsification of oil in water, the influencing factors were screened and the central composite design (CCD) was applied to establish the optimal levels of the significant factors and the interactions of such variables in such a process.

Fig. 1 Number distribution of coarse emulsion

A three-factor, three-level CCD including four replicates at the center point with 18 runs was employed. Tested variables (homogenizing pressure, oil content and number of passes) were denoted as x_1 , x_2 , and x_3 , respectively. Each of these variables was studied at three different levels, (−1, 0, +1) combining factorial points (−1, +1), axial points (−2, +2), and central point (0), as shown in Table 1 (the emulsifier content was 2 % wt for all the samples). The principle of RSM has been described by Montgomery with the objective of optimization of the response based on factors investigated (Montgomery 2008). The arrangement of CCD allows the development of an empirical second-order polynomial multiple regression model, which was considered as follows:

$$y = b_0 + \sum_{i=1}^k b_i x_i + \sum_{i=1}^{k-1} \sum_{j=2}^k b_{ij} x_i x_j + \sum_{i=1}^k b_{ii} x_i^2 + e \quad (1)$$

x_j are input variables that influence the response y , b_0 is the offset term, b_i is the linear effect, b_{ij} is the interaction effect, b_{ii} is the squared effect, and k is the number of variables. Analysis of variance (ANOVA) was conducted to determine the significance of the model. The quality of polynomial equation was checked by determination of the coefficient (R^2), and its statistical significance was studied using Fischer's F-test. The response surface and contour plots of the model-predicted responses were utilized to specify the interactive relationships between the significant variables.

Table 1 Independent variables and their levels for the central composite design used in the present study

Variable	Symbol	Coded levels ^a				
		−2	−1	0	+1	+2
Homogenizing pressure (bar)	x_1	136	408	680	952	1,224
Oil content (vol %)	x_2	1	5	9	13	17
Number of passes	x_3	1	2	3	4	5

^a For passage from actual variable level to coded variable level, the following equations were used: $x_1 = (\text{homogenizing pressure} - 680) / 272$; $x_2 = (\text{oil content} - 9) / 4$; $x_3 = (\text{number of passes} - 3)$

Design-Expert, version 8.0.7.1 (Stat-Ease Inc., Minneapolis, MN) was used for designing the experiments and for regression and graphical analysis of the obtained data.

Results

In this study two responses were considered in the response surface optimization; SMD and Pdl. For more accurate and detailed modeling and also observing the effects of every single factor on both SMD and Pdl, two separate RSM models were developed in emulsification system. Average size of oil droplets have been shown by SMD while the Pdl has been attributed to homogeneity and uniformity of emulsion. The emulsions with narrower distribution have smaller Pdl and vice versa. By varying pressure, oil content and number of passes, both monodisperse (Pdl<0.2) and polydisperse (Pdl>0.2) emulsions can be produced.

Response surface methodological approach for optimization of the Sauter mean diameter in a microfluidizer

The design matrix of tested variables and the experimental data are given in Table 2. Two basic responses, namely, SMD and Pdl have been also determined in Table 2. The mathematical equations for the Sauter mean diameter (SMD) and the Polydispersity index (Pdl) were developed as the sum of constant value, first-order effects (terms denoted by x_1 , x_2 , and x_3), interaction effects (terms denoted by x_1x_2 , x_1x_3 , x_2x_3) and second-order effects (terms denoted by x_1^2 , x_2^2 , and x_3^2).

The obtained results were analyzed by ANOVA to determine “goodness of fit” (i.e. to test a sample of data coming from a specific distribution such as normal distribution). Only those terms which were significant according to ANOVA were considered in the model. The regression equation which was a reduced quadratic model in terms of coded factors for the oil droplet size (Y_1) was as follows:

$$SMD(Y_1) = 185.32 - 10.39x_1 + 1.21x_2 - 5.69x_3 - 3.74x_1x_2 + 1.54x_2x_3 + 11.02x_1^2 - 1.07x_2^2 \quad (2)$$

In Fig. 2, values for SMD, predicted from the model have been plotted against those determined experimentally.

The adequacy of the model for SMD was checked using ANOVA, as shown in Table 3. The model fitted for the nano-emulsion production was significant by the F-test at the 5 % levels of significance. Validity of the F-test is based on the assumption that the residual is a random independent variable normally distributed with

constant variance (The “F-value” and the value of “Prob > F” for model were 256.75 and less than 0.0001, respectively). Estimation of the regression coefficients which is traditionally performed by the least squares method is actually a way to predict the measured response by minimizing the errors (Vining and Kowalski 2010).

The failure of the model was measured by the lack of fit test to represent data in the experimental domain at points which are not included in the regression analysis. The value of lack of fit for the polynomial regression (Eq. (2)) was not significant at the 5 % level ($P>0.05$), indicating the adequate predictability of the model (P-value for the model was 0.8158). The coefficient of determination (R^2) was 0.9945, indicating the proportion of variability in the data explainable by the model. Adjusted R^2 is used whenever a regressor variable is dropped from the model and was determined as 0.9906. The values of R^2 and adjusted R^2 in this study ensured a satisfactory adjustment of the model to the experimental data.

The coefficient of variation (CV) is calculated as the ratio of the standard error of estimate to the mean value of the observed response variable. In this study a value of 0.92 % was obtained for CV. The CV is a measure of the reproducibility of the suggested model suggested. As a general rule, CV less than 10 % indicates that the model is reproducible (Vining and Kowalski 2010). It seems, therefore, that the model presented in this study for the microfluidization of O/W is acceptable as a representation of the acquired data. The predicted residual sum of squares (PRESS) as a measure of fitness of the model to each point in the design was 97.75 (Table 3). In addition, the adequate precision value as a measure of the “signal to noise” ratio was found to be 64.189. Generally, a ratio greater than 4 is desirable and on this basis, the ratio obtained in this study may be regarded as an adequate ratio (Beg et al. 2003).

For a better understanding of the importance of each independent variable and their interactions, the coefficients in the polynomial equation were plotted in Fig. 3, as they appeared in Eq. (2). It may be observed from this figure that homogenizing pressure and number of passes through the microfluidizer promote the emulsion disruption, while oil content imposes an undesirable impact on such a factor. The effect of the emulsification pressure is higher than those of oil content and number of passes through apparatus. The positive coefficient for the interactions between the oil content and pass number (x_2x_3) imply the undesirable effects of these interactions on the oil droplet diameter. The positive second-order coefficient of the pressure (x_1^2) and negative coefficient of the oil content (x_2^2) (which where x_1^2 is much greater than x_2^2) indicates presence of a minimum region for the emulsion size under conditions of the present work, which can be described by a parabola.

Table 2 Design Matrix of Experiments (actual and predicted values for the sauter mean diameter are also shown)

Std	run	Type	Coded independent variable levels			SMD (nm)	PdI
			x_1	x_2	x_3		
1	13	Factorial	−1	−1	−1	207.7	0.226
2	16	Factorial	1	−1	−1	192.5	0.18
3	2	Factorial	−1	1	−1	215.6	0.27
4	11	Factorial	1	1	−1	185.2	0.225
5	18	Factorial	−1	−1	1	192.6	0.221
6	3	Factorial	1	−1	1	180	0.15
7	5	Factorial	−1	1	1	206.4	0.201
8	17	Factorial	1	1	1	179.1	0.165
9	4	Axial	−2	0	0	250	0.29
10	1	Axial	2	0	0	209.6	0.206
11	10	Axial	0	−2	0	180	0.18
12	15	Axial	0	2	0	182.9	0.23
13	8	Axial	0	0	−2	198.7	0.227
14	6	Axial	0	0	2	174.6	0.145
15	9	Center	0	0	0	186.8	0.204
16	12	Center	0	0	0	182.2	0.206
17	7	Center	0	0	0	184.3	0.194
18	14	Center	0	0	0	186.9	0.205

Interpretation of residual plots

The model estimation is based on its ability to explain the experimental data. The residuals investigate how well the model predicts the observed data used in the estimation of the model. The quality of the statistical model analysis (i.e. there is some violation of the assumptions used for the model

estimation) depends on the information which could be extracted from the residuals (Hajar et al. 2009). The normal percent probability versus residuals is presented in Fig. 4a. The residuals fall on a straight line, indicative of the normal distribution of errors, therefore the adequacy of the least square fit was provided. Plot of the internally studentized residuals versus the predicted response values of the oil diameter (Fig. 4b) shows equal scatter of the points above and below the x-axis, indicating the residuals satisfy the independency and constant variance. This reveals that the suggested model is adequate in describing the dependency of the particle size on the selected regressors applied for the emulsification process. Figure 4c represents the plot of the internally studentized residuals versus the experimental run order. The plot shows a random scatter. This allows checking for hidden variables that may have influenced the response during the experimental runs (Myers et al. 2002).

Response surface methodological approach for optimization of the Polydispersity index in a microfluidizer

Similar to the RSM model for the SMD, the same procedure was applied on the second response variable, PdI. The reduced quadratic regression model in terms of coded factors for the rejection (%) (Y_2) was developed as follows:

$$PdI(Y_2) = 0.20 - 0.023x_1 + 0.011x_2 - 0.023x_3 + 4.5 \times 10^{-3}x_1x_2 - 0.012x_2x_3 + 0.011x_1^2 - 4.5 \times 10^{-3}x_3^2 \quad (3)$$

Fig. 2 Plot of the predicted versus measured yield of SMD from sunflower oil

Table 3 ANOVA and statistical parameters for response surface quadratic (Response: SMD)

Source	Sum of squares	Df	Mean square	F-value	p-value Prob>F
Model	5,707.98	7	815.43	256.75	<0.0001
x_1	1,728.48	1	1,728.48	544.25	<0.0001
x_2	23.28	1	23.28	7.33	0.0220
x_3	518.70	1	518.70	163.32	<0.0001
$x_1 x_2$	111.75	1	111.75	35.19	0.0001
$x_2 x_3$	18.91	1	18.91	5.95	0.0348
x_1^2	2,948.92	1	2,948.92	928.53	<0.0001
x_2^2	27.58	1	27.58	8.67	0.0146
Residual	31.76	10	3.18		
Lack of Fit	16.59	7	2.37	0.47	0.8158
Pure error	15.17	3	5.06		
Total	5,739.74	17			
R^2	0.9945				
R^2 adjusted	0.9906				
CV (%)	0.92				
PRESS	97.75				
Adequate Precision	64.189				

Subjected to $-\alpha < x_i < +\alpha$ for $i=1, 2, 3$. It is good to mention that only significant terms have been retained and the insignificant variables including interaction between homogenizing pressure and number of pass ($x_1 x_3$), and quadratic effect of oil content (x_2^2) were eliminated from the final model.

Similar to SMD results, the appeared coefficients in Eq. (3) were plotted in Fig. 5. According to the regression coefficients, homogenizing pressure and pass number were in a same order of significance with negative sign. Oil concentration was in less significance with an undesirable effect on distribution of oil droplets. The positive significant second-order coefficient of the pressure (x_1^2) assesses presence of a minimum region for the emulsion dispersion index under existing pressure conditions.

Fig. 3 Coefficients from the response surface as appeared in Eq. (2). The error bars show 95 % confidence levels (SMD response)

The ANOVA table for the reduced quadratic (Eq. (3)) model has been summarized in Table 4. According to the obtained data for F-value, P-value, R^2 , R^2 adjusted, and “adequate precision” term, it was revealed that the developed RSM model was valid from statistical point of view for prediction of oil distribution in microfluidizer.

Discussion

Effects of the significant factors

Mean plot of variables in Figs. 6 and 7 demonstrates the effects of independent variables while holding the rest of variables at zero levels. In fact, the fitted model for the emulsification with microfluidizer in this study is useful in showing the direction in which one particular variable changes in order to minimize Sauter mean diameter.

As it can be observed in Fig. 6, an increase in oil content (x_2) leads to an increase in the SMD. At constant energy density (e.g. emulsification pressure), droplet diameter was enhanced with increasing oil content. There are a number of possible reasons: (1) higher oil content leads to higher emulsion viscosity, and thereby droplet disruption would be more difficult; (2) there may be insufficient emulsifier amounts present to completely cover the newly formed droplets; and (3) the rate of collision frequency and coalescence frequency is increased. As it can be seen in Fig. 6, Oil content has less effect than other parameters in the current experimental range.

Fig. 4 The residual plots for SMD of nano-emulsion using microfluidizer, according to the CCD. **a** Normal probability; **b** residuals versus predicted SMD; **c** residuals versus experimental run order

According to Fig. 7, the effect of oil concentration on PdI response is more significant than SMD response. By increasing oil content, the number of oil droplets is increased and so droplets have more chance for escaping from high shear zone. This leads to enhancement in width of droplets distribution.

The pressure provides the energy needed for emulsification. Increased pressure levels relate to an increase in laminar and turbulent shears through small eddies (Kolmogorov

theory). The use of higher pressures represented more energy and more turbulence, and induced a higher disruption rate and so smaller particles must be produced (Marie et al. 2002) (as long as there is sufficient emulsifier to cover new interface and re-coalescence is prevented as much as possible). Under a given set of emulsification conditions, there is a certain size below which SMD cannot be reduced with increasing emulsification pressure. Therefore emulsifying the system any

Fig. 5 Coefficients from the response surface as appeared in Eq. (3). The error bars show 95 % confidence levels (PdI response)

longer would be inefficient (McClements 2005; Jafari et al. 2008), or sometimes shows an increase in SMD, and is referred to as over-processing.

As it might be observed in Fig. 6, emulsification pressure (x_1) has a bi-effect. With increasing mechanical energy (emulsification pressure) up to 0.47 (coded value), the SMD was significantly improved. At this point, the rate of disruption by mechanical energy is equal to particle coating by emulsifier. After the minimum, increasing pressure level has a negative effect on SMD. This phenomenon is called over-processing which is attributed to the re-coalescence of new droplets.

The same behavior was depicted in Fig. 7. By increasing pressure level from -1 to $+1$, the slope of PdI response was

decreased. According to Kolmogorov theory, pressure has direct impact on turbulent shear. Particles were imposed to smaller eddies that lead to narrower dispersion. Unlike the SMD response, the minimum point was shifted toward coded pressure of $+1$ for the PdI response. The particle distribution is more resist against pressure variation, so over-processing moved to higher pressure condition.

Since there are spatial and temporal inhomogeneities in the pulsed microfluidic flow, it is generally necessary to recirculate the emulsion through the region of high shear (Mason et al. 2006). The effect of number of passes (x_3) through the microfluidizer can also be seen in Figs. 6 and 7. As it can be observed in these figures, the mean droplet diameter and polydispersity reduced through successive passes in the interaction chamber. By increasing the pass number, oil droplets have sufficient time to be exposed to high shear zone, resulting in smaller and narrower particle size. The residence time of energy dissipation of the device must be longer than the time required for droplet disruption. Recirculating the emulsion through the microfluidizer, increases the residence time of emulsification and reduces the width of particle distribution.

Interpretation of response 3D surface plots as related to the emulsification in the microfluidizer

In Figs. 8 and 9, the response surface plots for both responses (SMD and PdI) are shown. The three-dimensional (3D) response surface is a graphical representation of the data. It would be useful for evaluation of the individual and cumulative effect of the independent variable and mutual interaction

Table 4 ANOVA and statistical parameters for response surface quadratic model (Response: PdI)

Source	Sum of squares	Df	Mean square	F-value	p-value Prob>F
Model	0.023	7	3.247 e-3	68.25	<0.0001
x_1	8.372 e-3	1	8.372 e-3	176.00	<0.0001
x_2	2.116 e-3	1	2.116 e-3	44.48	<0.0001
x_3	6.724 e-3	1	6.724 e-3	141.35	<0.0001
$x_1 x_2$	1.62 e-4	1	1.620 e-4	3.41	0.0948
$x_2 x_3$	1.104 e-3	1	1.104 e-3	23.22	0.0007
x_1^2	2.919 e-3	1	2.919 e-3	61.37	<0.0001
x_3^2	4.991 e-4	1	4.991 e-4	10.49	0.0089
Residual	4.757 e-4	10	4.757 e-5		
Lack of Fit	3.83 e-4	7	5.471 e-5	1.77	0.3448
Pure error	9.275 e-5	3	3.092 e-5		
Total	0.023	17			
R^2	0.9795				
R^2 adjusted	0.9651				
CV (%)	3.33				
PRESS	1.935 e-3				
Adequate precision	32.350

Fig. 6 Mean plot of tested factors for SMD response. **a** the effect of pressure; **b** the effect of oil content; **c** the effect of pass number

between the predictor and the dependent variable. The response surface analyzes the geometric nature of the surface and the significance of the coefficient of the polynomial equation. Following this approach of data presentation, the optimum points would be obtained. In general, main variables with the largest absolute coefficients in the fitted model have been used for the axes of the plots to account for curvature of the surfaces.

The fitted model indicated that homogenizing pressure (x_1) and the number of passes (x_3) through the microfluidizer were the major independent variables affecting oil disruption and dispersion, according to Eqs. (2) & (3) and Figs. 3 and 5.

It can be seen from Fig. 8a that pressure had a very important effect on disruption. At low pressures (about

Fig. 7 Mean plot of tested factors for Pdl response. **a** the effect of pressure; **b** the effect of oil content; **c** the effect of pass number

408 bar) SMD enhanced with increasing oil volume content from 5 to 13 %. This is attributed to re-coalescence of formed droplets during emulsification. Since the concentration of Tween 20 was too high (2 % wt), its effect on the droplet size has been discarded.

Coalescence frequency (W) depends on collision frequency (C) and probability of coalescence (P):

$$W = PC \quad (4)$$

where C is the number of collisions per unit time and volume, which itself is a function of other factors such as dispersed

Fig. 8 Surface plots for SMD. **a** as a function of homogenizing pressure and oil content; **b** as a function of oil content and number of passes

phase concentration, viscosity of the continuous phase, droplet diameter, power input, etc.; and P (the coalescence probability) is a function of some other parameters including droplet size, power density, etc. (Tesch and Schubert 2002).

At higher oil contents, extreme shear which drives droplets close to each other, overcomes the disjoining pressure and causes the droplets to merge. This phenomenon is known as shear-induced coalescence (Leal 2004). Hence, collision frequency and coalescence probability were increased, which eventually leads to an increase in coalescence frequency.

As can be observed in Fig. 8a, by increasing pressure from 408 to 952 bar, the effect of oil content has disappeared and SMD was independent of oil content. The pressure increase seemed to mask the oil content effect. This behavior was previously observed by Flourey et al. (2002).

Pressure was the dominant factor affecting disruption of oil in this range. The plot showed a clear minimum within the experimental pressure range studied (Fig. 8a). This minimum moved from 762.3 to 854.4 bar, with increasing oil content from 5 to 13 %. The turbulence intensity was suppressed by increasing oil content, so higher pressure was needed for disruption of higher dispersed phase concentration. It was also found that the value of SMD did not fall steeply when the pressure changed slightly from its optimum value. This means that the emulsification of oil in microfluidizer will be robust to slight fluctuation in pressure during operation.

Combination of inertial forces in turbulent flow (in the interaction chamber) and laminar elongational flow at the inlet of the interaction chamber are predominantly responsible for disruption in microfluidizer.

Fig. 9 Surface plots for Pdl. **a** as a function of homogenizing pressure and oil content; **b** as a function of oil content and number of passes

The following function was proposed by Walstra for both laminar and turbulent flows:

$$\frac{\tau_{ADS}}{\tau_{COL}} \approx \frac{6\pi e \Gamma \varphi}{d C_S} \quad (5)$$

where τ_{ADS} is emulsifier adsorption time on the surface of droplets; τ_{COL} is collision time between droplets; j is dispersed phase volume fraction; d is droplet diameter; G is excess surface concentration of the emulsifier; and C_S is the concentration of emulsifier (Walstra 2002). The balance between droplet disruption and re-coalescence strongly depends on the emulsification energy. Between newly formed droplet and its nearby neighboring droplets, surfactants adsorb onto the created interface to prevent its re-coalescence. According to Eq. (5), the fresh interface would not be completely covered and re-coalescence would be observed if the timescale of surfactant adsorption is longer than the timescale of collision. This means although the energy input during emulsification has been increased (up to 952 bar), the resulting emulsions have bigger SMD rather than expected smaller sizes because of over-processing phenomenon. The value of optimum pressure depends on oil content.

The evolution of the SMD as a function of the number of passes was shown in Fig. 8b. As the number of passes increased, the mean droplet diameter decreased. Passing the nano-emulsion through the region of extreme shear multiple times, decreased the SMD mainly because all droplets eventually felt the highest peak shear rate.

The response surface plot for Pdl is depicted in Fig. 9. It is evident from Fig. 9a that at low oil concentration, the Pdl decreases continuously by increasing the pressure. At higher oil content, the effect of homogenizing pressure becomes less important. The interaction effect of oil content and number of passes is shown in Fig. 9b. At higher oil content, the effect of pass number is more significant. These results for pass number are in contrast with the results of pressure. At high oil levels, increasing the pressure has not important effect on oil disruption but recirculation of emulsion through the apparatus increases the uniformity of emulsion significantly. So it is true that pressure and pass number are the most affecting parameters for monodispersity of oil droplet, but interaction of these parameters with oil content play a major role in the result.

Process optimization

As stated earlier, in this study two responses (SMD and Pdl) were considered in the response surface optimization. In the

case of multiple response processes, the desirability function approach has been widely applied for process optimization in which its basic principles have been described in details in the literatures concerning RSM (Pasandideh and Niaki 2006). Based on this theory, it is necessary to find out the optimal point as a compromise between lower Sauter mean diameter and polydispersity index values. In the present case, both responses should be minimized in which belongs to the larger-the-best (*LTB-type*) desirability function form. The optimum conditions for the emulsification of sunflower oil in a microfluidizer with three controllable variables were obtained using the above mentioned technique. The developed optimization results have been summarized in Table 5. The optimum conditions for the emulsification process were obtained by using numerical optimization feature of Design Expert 8.07.1 Software. The numerical optimization function of the Design Expert combines the individual desirabilities into a single number and then looks for the greatest desirability (desirability ranges from zero to one for the response). “In range” possibility was used in the present study for the predictors. Under the optimum conditions, the value of responses for SMD and Pdl were 174.8 nm and 0.19, respectively.

Validation of the statistical model was determined by performing the confirmation run. The predicted responses for SMD and Pdl were determined as 174.8 nm and 0.19, while the average value of the experimental results was 175.6 and 0.19 (less than 0.5 % error). As it is apparent, the experimental data was quite close to that predicted from the model, indicating the validity of the model presented.

Kolmogorov equation: application to water jet emulsifying process

The Weber number (We) of a droplet is the ratio of its kinetic energy due to turbulent fluctuations (E_v), to the energy due to the interfacial tension (E_s):

$$We = \frac{E_v}{E_s} = \frac{\rho_c u^2 d}{\gamma} \quad (6)$$

where ρ_c is the density of the continuous phase, u^2 is the mean-square spatial fluctuation in the liquid velocity over a distance d (drop size) and γ is the interfacial tension (Abismai et al. 2000). The breakup of a drop occurred when the Weber number reaches a critical value, We_c ($We_c \approx 1$), which corresponds to the maximum stable drop size, d_{max} . In the turbulent isotropic regime, according to Kolmogorov's theory u^2 is function of the average power dissipated per unit of mass, ϵ :

$$u^2 = (\epsilon d)^{2/3} \quad (7)$$

Table 5 Optimum condition for formation of nanoemulsion in a microfluidizer by minimization of SMD and Pdl

Variable	Limits		Goal
	Lower	Upper	
x_1	408	952	In range
x_2	5	13	In range
x_3	2	4	In range
SMD (nm)	174.6	250	Minimize
Pdl	0.145	0.29	Minimize
Hemogenizing pressure (bar)	Oil content (vol%)		Number of pass
856.5	5		4

Resolving Eq. (6) for $d=d_{\max}$ yields:

$$d_{\max} = C_1 \left(\frac{\rho_c}{\gamma} \right)^{-3/5} \varepsilon^{-2/5} \quad (8)$$

where C_1 is a constant generally of the order of unity. The energy dissipation rate was correlated to power density (E) using the following relation:

$$E = \varepsilon \rho_c \quad (9)$$

By substituting Eq. (9) into Eq. (8) the diameter of the largest particle that cannot be disrupted in the emulsion obtained:

$$d_{\max} = CE^{-2/5} \gamma^{3/5} \rho_c^{-1/5} \quad (10)$$

The power density E (W.m^{-3}) is equal to ratio between the pressure release for a fluid, that is assumed to be incompressible, and the pressure release time as proposed in Eq. (11):

$$E = \frac{\Delta P_{\text{turb}}}{t_{\text{rel}}} \quad (11)$$

where ΔP_{turb} is pressure drop in turbulent flow in chamber and t_{rel} is pressure release time. The pressure change in chamber is calculated according to following equation:

$$\Delta P_{\text{turb}} = \Delta P_{\text{tot}} - (\Delta P_{\text{noz}} + \Delta P_{\text{ent}}) \cong \Delta P_{\text{tot}} \quad (12)$$

Where ΔP_{tot} is the total pressure loss, which has to be determined experimentally, ΔP_{noz} is the pressure loss due to friction with the nozzle in turbulent flow and ΔP_{ent} is the sudden enlargement in cross section area immediately after

the nozzle. The pressure losses due to friction and sudden enlargement are negligible in compare with total pressure loss.

Volumetric flow rate is only function of treatment pressure and chamber nozzle size ($75 \mu\text{m}$), so it can be obtained for each pressure level experimentally. The nozzle velocity was calculated from volumetric flow rate and cross section area of the nozzle, which has been used for calculation of Reynolds number of the chamber.

The Reynolds number was calculated using Eq. (13) to determine the flow regime:

$$\text{Re} = \frac{\rho v_{\max} d_{\text{ch}}}{\mu} \quad (13)$$

where d_{ch} is nuzzle diameter ($75 \mu\text{m}$).

The effect of treatment pressure on disintegration of oil droplet according to Kolmogorov equation was illustrated in Table 6. The Kolmogorov equation did not take into account volume fraction of phases and number of circulations. Experimental results showed that oil content had less significant effect on the disruption of emulsions, so the assumption of Kolmogorov equation is approximately acceptable. However treatment pressure and number of passes were the most important parameters in breakup of oil droplets which the last one was not considered in Kolmogorov equation.

The breakup frequency increases with the increase in the pressure, emulsion Reynolds and Weber number. Since, the inertia force increases by increasing the water sheet Reynolds number or Weber number which is greater than the surface tension force, the emulsion stability and disruption of oil decreases. So Reynolds and Weber number are calculated in Table 6.

The results of Table 6 are categorized into three pressure levels namely low, medium and high pressure. In all of cases, Weber number is high enough to ensure that the inertia force overcomes the surface tension force, because of high velocity of emulsion in a narrow nozzle.

At low pressure levels (136 and 408 bar) the Kolmogorov micro-scale showed higher mean diameter than the experimental data. The difference was more observable at 136 bar. The Reynolds number at 136 bar was about 2390. At this Reynolds number the transition behavior is dominant and so Kolmogorov equation is not applicable. Laminar elongational flow is responsible for particle disruption in this pressure. At 408 bar the turbulent conditions ($\text{Re} > 4,000$) was reached but the extreme of turbulence was not sufficient to make it the dominant procedure for breakup of the droplets. At this pressure, fabrication of nano-emulsion was related to combination of laminar elongational flow and turbulent shear flow. By increasing the pressure, differences between the predicted data and experimental data decreases and the impact of turbulent shear becomes more important.

Table 6 Results of calculations for Kolmogorov equation (Eq. (12))

Pressure (bar)	$V_{\max}$ (m/s)	Re^a	We^b	Power density ($W\ m^{-3}$)	$d_{\max}$ (nm) ^c by Eq. (12)	$d_{\max}$ (nm) by experiment	Error (%)
136	115	2,390	698	1.56×10^{11}	527.7	250.2	110.9
408	199	4,140	1,084	8.13×10^{11}	273	205.7	32.7
680	257	5,347	1,331	1.75×10^{12}	200.9	183.6	9.4
952	304	6,327	1,522	2.90×10^{12}	164.2	184.9	−11.2
1,224	345	7,174	1,683	4.23×10^{12}	141.2	208.6	−32.3

^a The Reynolds number was calculated using: $Re = \frac{\rho v_{\max} D}{\mu}$

^b The Weber number was calculated using: $We = \frac{\rho v_{\max}^2 d}{\gamma}$

^c For calculation of $d_{\max}$ the interfacial tension of $10\ mN\ m^{-1}$ were used

At medium pressure level (680 and 952 bar) the results of SMD for Kolmogorov equation were in good agreement with experimental data. The difference between the theoretical and experimental results reached 10 % for both of the pressures. At these pressures, turbulent shear flow is responsible for oil disruption. The obtained optimum condition was in this pressure range. As the pressure increased, the theoretical diameters were lower than the experimental ones.

And finally at high pressures (about 1,224 bar), the smaller droplet diameter was predicted by theoretical model. Combination of breakup and coalescence contribute to final droplet size. The balance between these processes is in majority of important. As mentioned in experimental results, increasing the pressure to higher level continuously was not the practical way for reducing the diameter of oil particles. Despite the ability of pressure in enhancement of globule disruption, it has promoting effect on coalescence of droplets. At these pressures, inertia force is extremely higher than surface tension force, so breakup frequency increases significantly. On the other hand the Kolmogorov eddy-dimension did not consider the over-processing and coalescence of droplets, so the results of this model were significantly less than experimental data.

Conclusions

A microfluidizer with a 75- μ m-diameter ceramic Y-type interaction chamber was utilized for formation of nano-emulsion in the presence of Tween 20 stabilizer. Response surface methodology (RSM) and the Central composite design (CCD) were successfully applied for modeling and optimization of nano-emulsion production. The effects of operating factors including homogenizing pressure, oil content and number of passes through the microfluidizer on the Sauter mean diameter (SMD) and Polydispersity index (PdI) were examined by RSM. The developed models revealed that

increasing the pressure and the number of passes promoted both of responses while oil content showed opposite sense. To assess the quality of the predicted models, residual analyses were performed. The results indicated that the statistical optimum strategy was an effective method for optimization of the emulsification parameters.

The most significant and effective parameters on the SMD and the PdI were found to be homogenizing pressure and number of pass. Interaction between pressure and oil content was relatively significant for SMD response, while interaction between oil content and number of passes played a major role in PdI response. By increasing the pressure, minimum regions in both responses were observed which attributed to positive coefficients of squared effect of pressure in the fitted models. In the presence of sufficient amount of emulsifier, increasing the pressure leads to an increase in laminar and turbulent shears and induce a higher disruption rate. But with intensive increase in pressure, over-processing was observed which is attributed to the re-coalescence of newly formed droplets. The effect of oil content on the PdI was more significant than the SMD. For PdI response, high values of oil content masked the effect of homogenizing pressure and promoted the effect of pass number. Recirculating the emulsion through the microfluidizer increased the uniformity and homogeneity of emulsion. The results revealed that laminar elongational flow and inertia in turbulent flow contributed to droplet disruption.

Finally, the minimal response was predicted and confirmed experimentally. The obtained optimal point by using 2 wt% emulsifier was found to be pressure=856.5 bar, oil content=5 vol% and number of pass=4, where SMD and PdI of 176.1 nm and 0.163 were achieved, respectively.

The ability of Kolmogorov equation for prediction of emulsion size was validated. The results revealed that Kolmogorov equation is applicable only for disruption of oil droplets in turbulent flow. This equation could not predict

over-processing and so, could not take into account the coalescence of droplets in turbulent flow.

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