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Analytical Methods

Optimisation of conditions for the preparation of β-carotene nanoemulsions using response surface methodology

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Abstract

Response surface methodology (RSM) was used to optimise the conditions for preparing β-carotene nanoemulsions. The effects of β-carotene (0.2–1.8%, w/w) and emulsifier (6.9–13.1%, w/w) concentrations, the homogenization pressure (79.1–140.9 MPa) and temperature (34.5–65.5 °C) on the particle size and stability of the nanoemulsions were studied. Results showed that the experimental data could be adequately fitted into a second-order polynomial model with multiple regression coefficients (R^2) of 0.921 and 0.981 for the particle size and stability, respectively. Homogenization pressure and β-carotene concentration and the quadrics of β-carotene concentration ($P < 0.05$) had a significant effect on the particle size of the nanoemulsions. Homogenization temperature and pressure, β-carotene concentration, the quadrics of emulsifier concentration and the interactions between β-carotene and emulsifier concentrations and between homogenization temperature and emulsifier concentration ($P < 0.05$) had a significant effect on the stability of the emulsions. The optimum conditions for preparing β-carotene nanoemulsions were predicted to be: homogenization pressure, 129 MPa; homogenization temperature, 47 °C; β-carotene concentration, 0.82%; emulsifier concentration, 8.2%.

Keywords: β-Carotene; Nanoemulsions; Particle size; Stability; Response surface methodology

1. Introduction

β-Carotene is an important member of the carotenoid family of orange-coloured pigments found in many fruits and vegetables. As a retinol precursor with a high conversion rate, β-carotene provides a substantial proportion of the vitamin A in the human diet (Naves & Moreno, 1998; Omenn, Goodman, & Thornquist, 1996). In recent years, some researches have further shown that β-carotene, owning to its antioxidant activity, possesses important health-benefiting functions which might be helpful in the prevention of, and protection against, a number of serious health disorders such as cancer, cardiovascular disease, colorectal adenomas and so on (Albanes, 1999; Edge, McGarvey, & Truscott, 1997; Erhardt, Meisner, Bode, & Bode, 2003; Rock, 1997). For these reasons, there is a strong interest in using β-carotene and other carotenoids as functional ingredients in food products. However, β-carotene is insoluble in water and only marginally soluble in oil at room temperature. This makes it difficult to be incorporated in food formulations, while the crystalline form of β-carotene is considered to have poor bioavailability (Ribeiro & Cruz, 2005). Additionally, β-carotene is sensitive to oxygen, heat and light, which further limits its applications in food, nutraceutical and pharmaceutical products (Orset, Leach, Morais, & Young, 1999; Rodriguez-Huezo, Pedroza-Islas, Prado-Barragan, Beristain, & Vernon-Carter, 2004). To improve the solubility and bioavailability of carotenoids such as β-carotene, attempts have been made to incorporate them in the fine particles of oil-in-water (O/W) emulsions. Garti and co-workers (Amar, Aserin,

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& Garti, 2003; Spernath, Yaghmur, Aserin, Hoffman, & Garti, 2002), for example, have prepared food grade microemulsions containing carotenoids with considerable success.

In the last two decades, nanotechnology is rapidly emerging as one of the most active research fields with potential applications in many industries. For the food industry, the technology offers the potential to significantly improve the solubility and bioavailability of many functional ingredients including carotenoids, polyunsaturated fatty acids and numerous other compounds (Moraru et al., 2003). So far, however, some researches into the application of this technology in the food industry have been very limited and there are only a few publications that explored the use of this technology for preparing carotenoid emulsions. Tan and Nakajima (2005a, 2005b) successfully prepared β-carotene nanodispersions, using an emulsification and evaporation technique with high pressure homogenization. They further investigated the influence of six different polyglycerol esters of fatty acids as emulsifiers on the physicochemical properties of the nanodispersions and found that parameters such as homogenization pressure can significantly affect particle size and size distribution (Tan & Nakajima, 2005b). In our laboratory, we have found (unpublished data) that other parameters, such as homogenization temperature and β-carotene concentration, could also influence the physicochemical properties of β-carotene nanoemulsions.

In such situations where multiple variables may influence the outputs, response surface methodology (RSM) is an effective technique for exploring the relationships between the response and the independent variables and optimising the processes or products (Baş & Boyacı, 2007; Giovanni, 1983; Vatsala, Saxena, & Rao, 2001). As a powerful statistical and mathematical tool, RSM has a major advantage over the one-factor-a time approach in that it allows the evaluation of the effect of multiple variables and their interactions on the output variables with reduced number of trials (Lee, Ye, Landen, & Eitenmiller, 2000; Liyana-Pathirana & Shahidi, 2005; Porretta, Birzi, & Vicini, 1995).

The objectives of the present study were to systematically investigate the influence of emulsifying conditions on the physicochemical properties of β-carotene nanoemulsions and to optimise the conditions for preparing β-carotene nanoemulsions with smallest particle size and greatest stability using RSM. The independent variables examined included the concentration of β-carotene and emulsifier, homogenization temperature and pressure.

2. Materials and methods

2.1. Materials

β-Carotene (30% in sunflower oil) was purchased from Xinchang Pharmaceuticals Co., Ltd. (Zhejiang, China). Medium chain triglyceride (MCT, C_8–C_{10}) was provided by Quest International (Givaudan Flavours Shanghai Ltd., Shanghai, China). Polyoxythylene sorbitan monolaurate (Tween 20) was purchased from Fucheng Chemical Reagents Factory (Tianjin, China). Ultrapure water, from a Milli-Q Plus system, was used in the preparation of β-carotene nanoemulsions.

2.2. Preparation of β-carotene nanoemulsions

Oil-in-water (O/W) emulsions were prepared using MCT containing β-carotene as the dispersed phase and Milli-Q water as the continuous phase. β-Carotene was first dissolved in MCT oil at 140 °C and the solution was mixed (10%, w/w) with Milli-Q water, containing Tween 20 at various concentrations as the emulsifier. The premix was homogenized using a high speed homogenizer (Model HD-1, Huayuanhang Experimental Equipment Factory, Beijing, China) at 5000 rpm for 10 min to form a coarse emulsion, followed by a two-stage high pressure homogenization (Model NS 1001L 2K, Niro-Soavi, Parma, Italy) at pre-determined homogenization temperature and pressure. The ratio of the first and second stage pressure was kept at 10 throughout the experiments. After homogenization, the emulsions were collected and their particle size and stability were analysed immediately.

2.3. Particle size analysis

The particle size of the nanoemulsions was determined by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK). The measurement was carried out at a fixed angle of 90° with the samples diluted approximately 1000 times with Milli-Q water. The particle size of the nanoemulsions was described by the cumulants mean (z-average) diameter.

2.4. Evaluation of nanoemulsion stability

The emulsion stability was evaluated using a Turbiscan Lab (Formulaction, France). The nanoemulsion sample was transferred to a glass cylindrical cell and analysed by a light beam emitted in near infrared (880 nm) wavelength which scanned the sample cell. Two synchronous optical sensors received, respectively light transmitted through the sample and light backscattered by the sample. The backscattering (BS) was directly dependent on the particle mean diameter. The sample in the cell was scanned every 30 min for 4 h at 55 °C. The change in BS (ΔBS) in unit time was taken as a measure of the instability of the nanoemulsions (IS) and the emulsion stability was calculated as the inverse of IS.

2.5. Experimental design

Response surface methodology (RSM) was used to study the effect of the independent variables: concentration of β-carotene (C_1), concentration of the emulsifier (C_2),

Table 1
Uncoded and coded independent variables used in RSM design

Symbol	Independent variable[a]	Coded levels				
		−1.55	−1	0	1	1.55
$X1$	C_1 (%)	0.2	0.5	1.0	1.5	1.8
$X2$	C_2 (%)	6.9	8	10	12	13.1
$X3$	T (°C)	34.5	40	50	60	65.5
$X4$	P (MPa)	79.1	90	110	130	140.9

[a] C_1, concentration of β-carotene; C_2, concentration of emulsifier; T, homogenization temperature; P, homogenization pressure.

homogenization temperature (T) and pressure (P), on the particle size (Y_1) and the stability (Y_2) of the nanoemulsions. The coded and uncoded independent variables used in the RSM design are listed in Table 1. The experiments were designed according to the central composite design (CCD) using a 2^4 factorial and star design with three central points as shown in Table 2. Individual experiments were carried out in random order.

A second-order polynomial equation was used to express the particle size (Y_1), and the stability (Y_2) of the nanoemulsions as a function of the independent variables as follows:

$$\begin{aligned} Y_i = {} & a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + a_{11}X_1^2 + a_{22}X_2^2 \\ & + a_{33}X_3^2 + a_{44}X_4^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{14}X_1X_4 \\ & + a_{23}X_2X_3 + a_{24}X_2X_4 + a_{34}X_3X_4, \end{aligned} \tag{1}$$

where Y_i represents the response variables, a_0 is a constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interactive coefficients, respectively. The coefficients of the response surface equation were determined using Statgraphics Centurion XV (StatPoint, Inc., 2005).

2.6. Statistical analysis

Experimental data was analysed by multiple regressions to fit the second order polynomial equation to all independent variables. Analysis of variance (ANOVA) was performed to evaluate significant differences between independent variables. To visualise the relationships between the responses and the independent variables, surface response and contour plots of the fitted polynomial regression equations were generated using Statgraphics Centurion XV.

3. Results and discussion

3.1. Fitting the models

The particle size and stability values of the β-carotene nanoemulsions obtained from all the experiments are given in Table 2. The experimental data was used to calculate the coefficients of the quadratic polynomial equations, which were used to predict the values of particle size and stability

Table 2
Predicted and experimental values of particle size and emulsion stability of β-carotene nanoemulsions obtained from the central composite experimental design

Experiment number	C_1[a] (%)	C_2 [a] (%)	T[a] (°C)	P[a] (MPa)	Particle size (nm)		Emulsion stability	
					Experimental	Predicted	Experimental	Predicted
1	0.5	8	40	90	154	151	1.41	1.47
2	1	10	65.5	110	137	146	0.952	0.761
3	1.77	10	50	110	155	154	1.54	1.615
4	1.5	12	40	90	172	170	0.606	0.504
5	1.5	8	60	90	170	167	2.08	2.25
6	1	10	50	79.1	177	174	0.862	0.872
7	1.5	12	40	130	130	137	0.541	0.698
8	1.5	12	60	130	137	135	0.513	0.458
9	1.5	8	60	130	142	138	2.63	2.71
10	1.5	12	60	90	166	170	0.472	0.357
11	0.5	12	40	90	136	143	0.394	0.339
12	1	10	50	141	129	134	1.25	1.18
13	1	6.91	50	110	140	149	3.125	3.01
14	1	13.1	50	110	147	141	0.333	0.392
15	0.23	10	50	110	121	124	0.658	0.525
16	1	10	34.5	110	159	153	0.980	1.12
17	1	10	50	110	147	150	1.15	1.02
18	0.5	12	40	130	122	121	0.448	0.289
19	0.5	8	40	130	135	135	1.64	1.78
20	0.5	12	60	130	121	119	0.350	0.402
21	0.5	8	60	130	127	125	1.35	1.47
22	1.5	8	40	130	150	146	3.57	3.37
23	0.5	12	60	90	143	142	0.336	0.545
24	0.5	8	60	90	147	143	1.39	1.25
25	1	10	50	110	153	150	0.971	1.02
26	1	10	50	110	153	150	0.847	1.02
27	1.5	8	40	90	168	174	2.86	2.83

[a] C_1, concentration of β-carotene; C_2, concentration of emulsifier; T, homogenization temperature; P, homogenization pressure.

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

Variable[a]	Particle size (Y_1)			Emulsion stability (Y_2)		
	Regression coefficient	*F*-value	*P*-value	Regression coefficient	*F*-value	*P*-value
a_0	179.653			2.69726		
Linear						
a_1	71.652	44.62	0.0000	0.0299466	76.60	0.0000
a_2	7.9483	3.31	0.0939	−0.291728	442.20	0.0000
a_3	−0.657024	2.51	0.1393	−0.0230783	8.12	0.0146
a_4	−0.928955	77.06	0.0000	0.0107051	6.30	0.0274
Quadric						
a_{11}	−17.8915	5.17	0.0421	−0.171804	0.15	0.7025
a_{22}	1.0	1.22	0.2906	0.00840686	27.61	0.0002
a_{33}	0.05	0.02	0.8841	0.000348207	0.39	0.5440
a_{44}	−0.2625	0.84	0.3787	0.00222906	0.00	0.9481
Interaction						
a_{12}	−0.543465	0.36	0.5589	0.010923	41.91	0.0000
a_{13}	0.0875	0.02	0.8830	0.00128894	3.70	0.0786
a_{14}	−0.034375	2.49	0.1405	−0.000446649	1.77	0.2083
a_{23}	−0.00292799	1.11	0.3134	−0.0000364019	5.38	0.0388
a_{24}	−0.001875	0.68	0.4245	0.000123822	3.70	0.0785
a_{34}	0.00449307	0.05	0.8254	−0.0000623051	0.25	0.6247
R^2	0.9208			0.9810		

[a] a_0 is a constant, a_i, a_{ii} and a_{ij} are the linear, quadratic and interactive coefficients of the quadratic polynomial equations, respectively.

of the emulsions (Table 2). The predicted values agreed well with the experimental ones obtained from the RSM design. Analysis of variance (ANOVA) showed that the resultant quadratic polynomial models adequately represented the experimental data with the coefficients of multiple determination (R^2) for the responses of particle size and stability values being 0.921 and 0.981, respectively. This indicates that the quadric polynomial models obtained were adequate to describe the influence of the independent variables studied on the particle size and stability of the nanoemulsions.

Analysis of variance (ANONA) was used to evaluate the significance of the coefficients of the quadric polynomial models (Table 3). For any of the terms in the models, a large *F*-value and a small *P*-value would indicate a more significant effect on the respective response variables (Quanhong & Caili, 2005). Thus, the variable with the largest effect on the particle size of the nanoemulsion was the linear term of pressure, followed by the linear term of β-carotene concentration ($P < 0.001$); the other two linear terms (emulsifier concentration and homogenization temperature) did not show a significant effect ($P > 0.05$). The quadric term of β-carotene concentration also had a significant effect ($P < 0.05$) on the particle size of the nanoemulsions, however, the effect of the other three quadric terms was insignificant ($P > 0.05$). Furthermore, none of the interactive terms had a significant effect ($P > 0.05$) on the particle size of the nanoemulsions.

For the stability of the emulsions, the variable having the largest effect on this response was the linear term of emulsifier concentration, followed by the linear term of β-carotene concentration and the interaction between β-carotene and emulsifier concentrations ($P < 0.001$). The quadric term of emulsifier concentration ($P < 0.001$), the linear terms of homogenization temperature and pressure and the interaction between homogenization temperature and emulsifier concentration ($P < 0.05$) also had a significant effect on the stability of the nanoemulsions, while the effect of the remaining terms was insignificant ($P > 0.05$) (Table 3).

3.2. Analysis of response surfaces

To visualise the effect of the independent variables on the dependent ones, surface response and contour plots of the quadric polynomial models were generated by varying two of the independent variables within the experimental range while holding the other two constant at the central point. Thus, Figs. 1 and 3 were generated by varying the concentration of β-carotene and the emulsifier in the emulsion while holding homogenization temperature and pressure at 50 °C and 110 MPa, while Figs. 2 and 4 were generated by varying the homogenization temperature and pressure while maintaining the β-carotene and emulsifier concentrations at 1.0% and 10.0%, respectively. In general, the particle size increased with an increase in the concentration of β-carotene. However, the effect of emulsifier concentration on the particle size was insignificant ($P > 0.05$). When the concentration of β-carotene was less than 1.0%, an increase in the emulsifier concentration up to 10% had little effect on the particle size; but further increases in the concentration resulted in evident decreases in the particle size of the emulsion (Fig. 1).

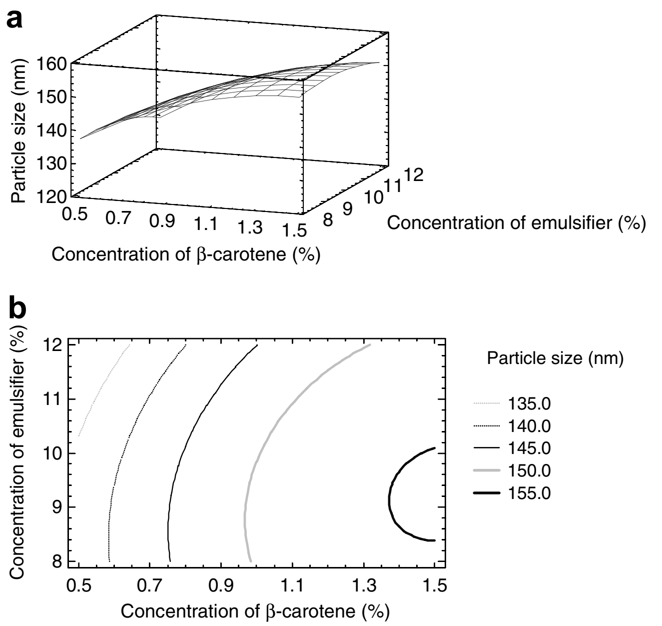

Fig. 1. Response surface (a) and contour (b) plots of the particle size of the nanoemulsions as a function of the concentration of β-carotene and emulsifier at the homogenization temperature and pressure of 50 °C and 110 MPa, respectively.

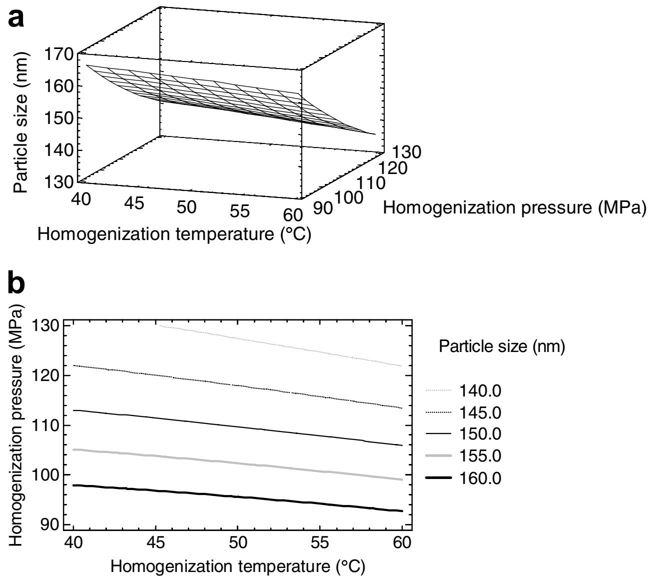

Fig. 2. Response surface (a) and contour (b) plots of the particle size of the nanoemulsions as a function of the concentration of homogenization temperature and pressure at the β-carotene and emulsifier concentrations of 1.0% and 10.0%, respectively.

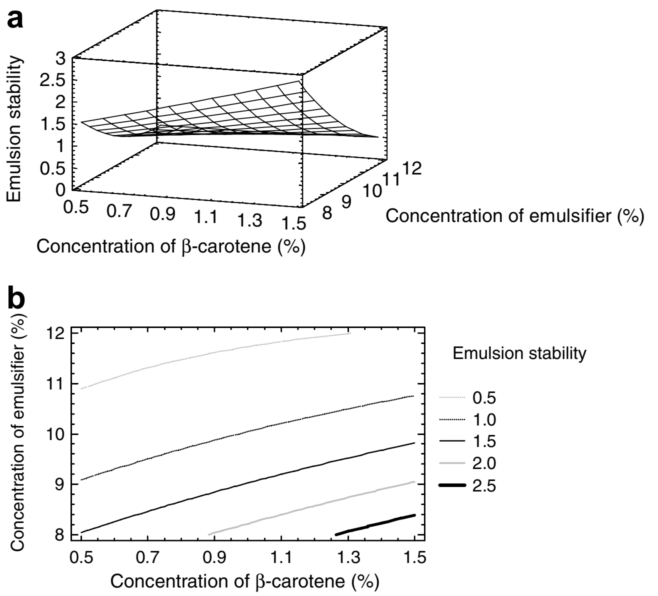

Fig. 3. Response surface (a) and contour (b) plots of the stability of the nanoemulsions as a function of the concentration of β-carotene and emulsifier at a homogenization temperature and pressure of 50 °C and 110 MPa, respectively.

Increasing the homogenization pressure resulted in significant decreases ($P < 0.05$) in the particle size (Fig. 2), which agreed with the findings of Tan and Nakajima (2005b). This result is expected as higher pressures would produce greater shear forces and turbulence, which in turn would result in reductions in the particle size of the emulsions (Floury, Desrumaux, & Lardières, 2000; Prajapati, Jana, & Upadhyay, 2004). Temperature may also influence the particle size of an emulsion because it could affect the viscosity of, and the interfacial tension between, the oil and aqueous phases (Floury, Desrumaux, Axelos, & Legrand, 2003). However, the effect of homogenization temperature on the particle size was found to be relatively minor, as a rise in homogenization temperature resulted in only a slight decrease in the particle size of the emulsions (Fig. 2).

The stability of the nanoemulsions increased with the elevation of the concentration of β-carotene and decreased with the increase in the concentration of emulsifier in our present experimental condition (Fig. 3). These results showed an opposite trend to the effects of the two parameters on the particle size of the emulsion (Fig. 1), which could be explained by the smaller particles having greater surface areas and surface energy, which would lead to coalescence more easily to a certain extent (Friberg, Larsson, & SbjÖlom, 2004).

Response surfaces showing the effect of homogenization pressure and temperature on the stability of the emulsions were given in Fig. 4. The stability of the emulsions increased with an increase in homogenization pressure but decreased with a rise in homogenization temperature (Fig. 4). This result was consistent with the finding of Dybowska (2000).

3.3. *Optimisation of conditions for preparing β-carotene nanoemulsions*

The conditions for the preparation of β-carotene nanoemulsions would be considered optimum if the particle size

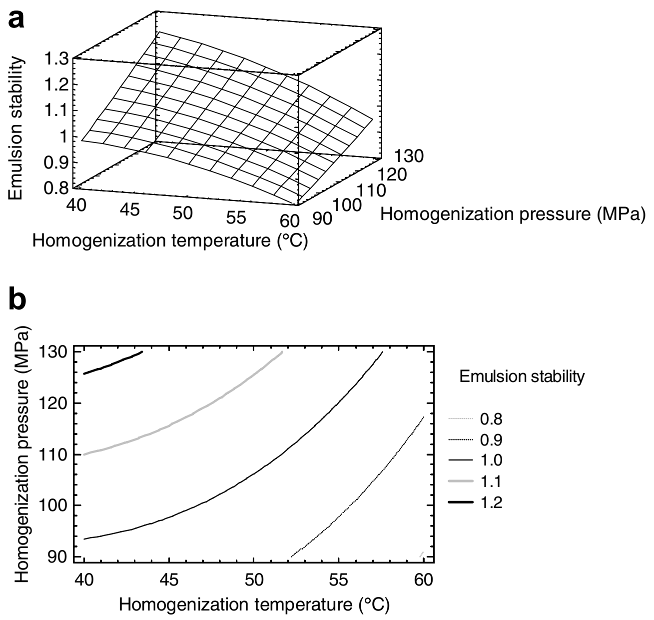

Fig. 4. Response surface (a) and contour (b) plots of the stability of the nanoemulsions as a function of the concentration of homogenization temperature and pressure at the β-carotene and emulsifier concentrations of 1.0% and 10.0%, respectively.

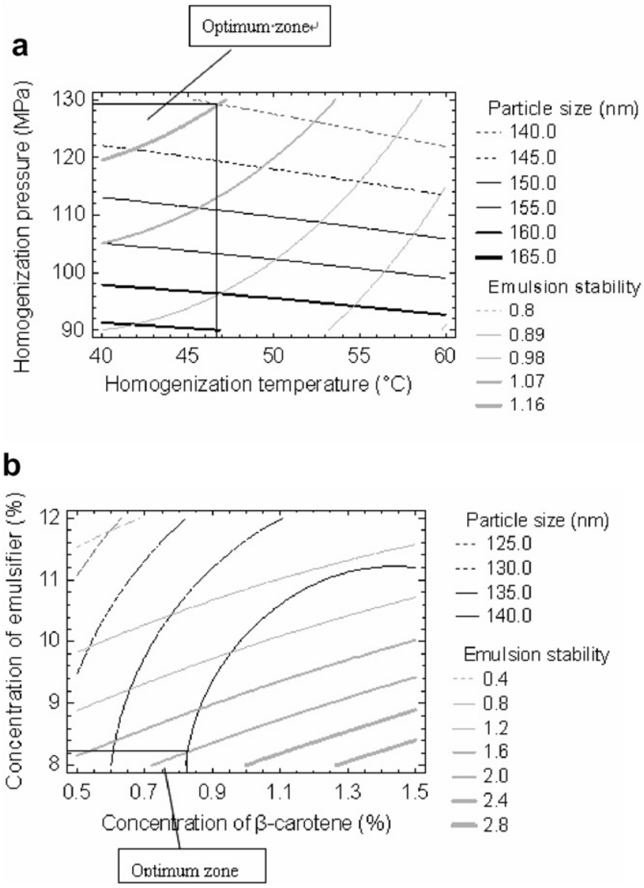

Fig. 5. Superimposed contour plots for the particle size and stability of β-carotene nanoemulsions as a function of homogenization pressure and temperature at a fixed β-carotene and emulsifier concentration of 1.0 and 10.0% (a) and as a function of β-carotene and emulsifier concentration at a fixed homogenization pressure and temperature of 129 MPa and 47 °C (b), respectively.

and the stability of the nanoemulsion attained the smallest and largest possible values, respectively. Optimisation was carried out by first superimposing the contour plots for the particle size and the stability of the nanoemulsion as a function of homogenization pressure and temperature at a fixed β-carotene and emulsifier concentration of 1.0 and 10.0%, respectively (Fig. 5a). This resulted in an optimum zone in which every point would represent a combination of homogenization pressure and temperature that would give minimum and maximum values for the particle size and the stability of the nanoemulsion, respectively. For practical considerations, the point representing the lowest possible combination of pressure and temperature within the optimum zone would be preferred over other combinations. For the principle of this, the point at 129 MPa and 47 °C were selected as the fixed values to generate the contour plots for β-carotene and emulsifier concentrations as shown in Fig. 5b. This produced another optimum zone, in which every point would represent a combination of β-carotene and emulsifier concentrations that would give minimum value for the particle size and maximum value for the stability of the nanoemulsion. In this case, however, it would be preferable to have the β-carotene concentration as high as possible (to produce a product with a high β-carotene concentration) while the emulsifier concentration as low as possible (to save cost), within the boundary of the optimum zone. When both conditions could not be met simultaneously, priority was given to a higher β-carotene concentration. Therefore, from the principles mentioned above, the point at the β-carotene and emulsifier concentrations of 0.82% and 8.2%, respectively, was chosen as the optimum conditions, along with the optimum homogenization pressure and temperature of 129 MPa and 47 °C, respectively.

4. Conclusion

The current study showed that the second-order polynomial model was sufficient to describe and predict the responses of the particle size and stability of β-carotene nanoemulsions, to changes in emulsifying conditions within the experimental ranges. The independent parameters of homogenization pressure and β-carotene concentration and the quadrics of β-carotene concentration had a significant effect on the particle size of the nanoemulsions. Concurrently, the independent variables of β-carotene concentration, homogenization temperature and pressure, the quadrics of emulsifier concentration and the interactions between β-carotene and emulsifier concentrations and between homogenization temperature and emulsifier concentration had a significant effect on the stability of the nanoemulsions. The graphical optimisation method was adopted to find the best emulsifying conditions and it was predicted that the optimum conditions for preparing β-carotene nanoemulsions would be: homogenization pressure of 129 MPa; homogenization temperature of 47 °C; β-carotene concentration of 0.82% and emulsifier (Tween 20) concentration of 8.2%.

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