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ORIGINAL PAPER

Optimization of Mixing Parameters for α-Tocopherol Nanodispersions Prepared Using Solvent Displacement Method

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Abstract A bottom-up approach based on a solvent displacement technique was used for the production of α-tocopherol nanodispersions. Response surface methodology was utilized to study the effect of the mixing conditions of organic and aqueous phases, namely, mixing speed (1×100–6×100 rpm) and mixing time (30–150 s) on the average particle size (nm), polydispersity index and α-tocopherol concentration (mg/L) of the nanodispersions. Second order regression models, with high coefficient of determination values ($R^2 > 0.94$ and adjusted $R^2 > 0.79$), were significantly ($p < 0.05$) fitted for predicting the α-tocopherol nanodispersion characteristics as functions of mixing parameters. A multiple optimization procedure presented the optimum mixing speed and time as 3.8×100 rpm and 70 s, respectively. The statistically insignificant differences between experimental and predicted values of studied responses, verified the satisfactoriness of the models found for explaining the variation of produced nanodispersions, as a function of mixing conditions.

Keywords α-Tocopherol · Nanodispersions · Solvent displacement · Response surface methodology

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Introduction

Vitamin E, which is mainly composed of α-tocopherol, can entrap two peroxyl radicals responsible for lipid oxidation initiation, and protect them against oxidation and deterioration [1]. Furthermore, it has positive effects on cardiovascular diseases, cancer prevention and the immune system [2]. Therefore, as with other functional lipid bioactive compounds, it is being extensively used in several food, cosmetic and pharmaceutical products, either for their preservation or delaying oxidation and consequently increasing their quality by hindering the development of rancidity, off-flavor compounds, polymerization, reversion and other reactions that reduce the shelf life, nutritional value and sensory quality of the food products or promoting their health effects [1, 3]. However, as most other functional lipid compounds, its uses are currently restricted due to its low chemical stability especially against heat and oxygen, very poor water solubility and low bioavailability [4, 5]. Therefore, encapsulation and incorporation of these compounds have been receiving increased interest in order to solubilize, protect, control release and improve their bioavailability [6].

The nanosizing process which reduces the particle size of active compounds to the submicron range is a popular technique for the delivery of poorly water soluble compounds, since the dissolution rate of the compound is proportional to the surface area. Further, the saturation solubility of a compound also rises with reductions in their particle sizes [2]. Nanodispersions are submicron colloidal systems which can be prepared through either bottom-up or top-down approaches [3]. The top-down methods which start with larger solid particles are capable of producing fine particles and can be easily used on an industrial scale. However, these methods are time consuming and involve

high energy input to the system leading to a considerable increase in their process costs. In contrast, the bottom-up methods fabricate nanosized particles by starting at the atomic level. Therefore, they provide better control over size, morphology and crystallinity of nanodispersion systems in addition to consuming less energy during the process. In these methods, nanoparticles can be produced by either non-solvent addition to the system or solvent removal through evaporation [7]. The nanoprecipitation method is an easy and reproducible method involving the dispersion of preformed compounds, based on their interfacial deposition of following displacement of the semi-polar solvent miscible with water from the lipophilic solution [2]. After mixing the solution and antisolvent and generation of supersaturation, the nucleation will be started in the system through solvent an evaporation step. Under uncontrolled conditions the fine particles produced will be grow by coagulation, condensation, or agglomeration. Therefore, to obtain nanoparticles with a narrow size distribution it is necessary to create a high degree of supersaturation, uniform spatial concentration distributions in solutions and restriction of the growth of the particles [8].

Stabilizers play a major role in the formation of nanodispersions in aqueous solutions by decreasing the interfacial tension between the functional lipid compounds and the water phases, reducing the amount of energy required to disrupt the droplets and leading to smaller size droplets. Moreover, they form a protective coating surrounding the droplets thus preventing coalescence [9]. Polyoxyethylene sorbitan mono-laurate (Tween 20) is a non-ionic emulsifier that adsorbs quickly at the oil–water interface, and has shown good results in small particles for various applications [10]. This work focused on previous studies reported by Cheong *et al*., and aimed at replacing the top-down high energy fabrication of α-tocopherol nanodispersions by a bottom-up low energy preparation method [4]. The main objectives of this work were to obtain nanodispersions of α-tocopherol using a low energy solvent displacement technique and to study the effect of mixing variables, namely, speed and time on the mean particle size, size distribution (PDI) and α-tocopherol degradation of the obtained nanodispersions.

Materials and Methods

Materials

α-Tocopherol (95 g/100 g) and polyoxyethylene sorbitan mono-laurate (Tween 20) were purchased from Sigma-Aldrich (Sigma-Aldrich Co. Missouri, USA) and Merck (Merck Co. Darmstadt, Germany), respectively. Analytical and HPLC grade acetone, methanol and acetonitrile were provided by Fisher Scientific (Leicestershire, UK). Hexane was purchased from Dr. Mojallali (Dr. Mojallali Chemical Complex Co. Tehran, Iran).

Preparation of α-Tocopherol Nanodispersions

α-Tocopherol (0.5 %, w/w) was dissolved in acetone and dispersed into the aqueous phase containing (0.5 % w/w) of Tween 20 in double deionized water, using a conventional mixer (Voss Instruments LTD, Maldon, UK) according to the experimental design (Table 1) with an organic to aqueous phase ratio of 3:7. The acetone was then removed from the systems by vacuum rotary evaporation (Eyela Digital SB-1,000 water bath, Tokyo, Japan) with a pressure, temperature and rotation speed of 30 kPa, 60 °C and 50 rpm, respectively.

Characterization of α-Tocopherol Nanodispersions

Particle Size Analysis

The prepared α-tocopherol nanodispersions were characterized in terms of particle size and size distribution. Particle size and polydispersity index (PDI) measurements were performed using a dynamic light-scattering particle size analyzer (Nanotrac Wave, Microtrac, Montgomeryville, PA, US), on undiluted samples one day after the sample preparations. The dynamic light scattering technique measures the Brownian motion of particles in a system optically. According to Stokes–Einstein theory, particle motion is determined by the continuous phase viscosity, the temperature and the size of the particle [10]. Therefore, measurement of the particle motion in a system at a known temperature and viscosity, can lead to the particle size determination of the nanodispersions produced. PDI is a measure of the width of the distribution. It is a measure of the width of the distribution ranging from 0 (mono dispersed) to 1 (very broad distribution) [4].

Determination of α-Tocopherol Concentration

α-Tocopherol was completely extracted from the nanoparticles using hexane as the organic solvent. After complete extraction, its concentration was measured using a high pressure liquid chromatography system (HPLC, CT-10A VP, Shimadzu, Kyoto, Japan), equipped with an SPD-10AV UV–Vis detector, a LC-10AT pump system, and a CT0-10A oven. Quantitative measurement of α-tocopherol was done at 295 nm and the separation was performed on a Nova-Pak® C18 (3.9 × 300 mm) Waters HPLC column, using an isocratic mobile phase of methanol: water (99:1 v/v) at 1.0 mL/min. The oven temperature was set at 40 °C. The calibration of the peak area versus α-tocopherol

Table 1 Central composite design (CCD) and response variables for processing of α-tocopherol nanodispersions

Sample number	Speed (×100 rpm)	Time (s)	Particle size (nm)		PDI		α-Tocopherol concentration (mg/L)	
			Exp[a]	Pre[b]	Exp[a]	Pre[b]	Exp[a]	Pre[b]
1	1.7	48	76.9	74.2	0.442	0.434	458.0	460.3
2	1.7	132	82.1	79.4	0.228	0.249	228.5	231.9
3	3.5	90	63.4	61.6	0.353	0.336	341.8	342.7
4	5.3	48	103.5	103.6	0.120	0.119	442.9	447.1
5	3.5	90	61.3	61.6	0.349	0.336	341.8	342.7
6	3.5	90	61.0	61.6	0.362	0.336	347.1	342.7
7	3.5	30	85.5	86.8	0.305	0.316	499.1	496.1
8	3.5	90	62.1	61.6	0.305	0.336	345.8	342.7
9	3.5	150	56.8	58.1	0.510	0.481	159.4	155.0
10	3.5	90	60.1	61.6	0.312	0.336	346.8	342.7
11	6.0	90	88.3	87.6	0.280	0.264	326.8	326.4
12	1.0	90	78.4	81.8	0.289	0.284	356.7	359.1
13	5.3	132	58.3	58.4	0.508	0.535	192.8	198.0

[a] Experimental values of studied responses

[b] Predicted values of studied responses

concentration was linear in the concentration range of 50–500 mg/L. The results were expressed in mg/L.

Experimental Design and Statistical Analysis

Response surface methodology (RSM) using a central composite design (CCD) with two independent variables, namely, mixing speed (1 × 100–6 × 100 rpm) and time (30–150 s), was applied to determine the least mean particle size (Y_1) and PDI (Y_2) and the highest α-tocopherol concentration (Y_3) of the nanodispersions. Due to advantages of RSM over classical one-variable-a-time optimization, such as the generation of large amounts of information from a small number of experiments and the possibility of evaluating the interaction effect between the variables on the response, it is a suitable procedure to assess the relationships between the studied responses and mixing variables and to optimize them in order to gain the desired characteristics of the product [11]. Each independent parameter was studied at five different levels, namely, central point (X_1: 3.5 × 100 rpm, X_2: 90 s), level −1 (X_1: 1 × 100 rpm, X_2: 30 s), level 1 (X_1: 6 × 100 rpm, X_2: 150 s), level $-\alpha$ (X_1: 1.7 × 100 rpm, X_2: 47 s) and level α (X_1: 5.3 × 100 rpm, X_2: 132 s). α value (1.4142) was obtained from the equation $\pm\sqrt{2}$ for $k = 2$ (two independent variables). Five replicates were performed at a central point for estimation of the pure error [1]. Therefore, a total of 13 experiments, including 4 factorial points (levels ± 1), 4 star points (levels ± α), and 5 central points were created using the software Minitab v. 14 statistical package (Minitab Inc., PA, USA) (Table 1). All experiments were carried out on 1 day.

A second order polynomial equation (Eq. 1) was used to express the mean particle size (Y_1), PDI (Y_2) and α-tocopherol concentration (Y_3) of the nanodispersions as a function of the studied mixing variables.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 \quad (1)$$

where Y is the response variable, β_0 is a constant, β_1, β_2 represent the linear terms, β_{11}, β_{22} correspond to the quadratic terms and β_{12} represents the interaction terms. The adequacy of model was examined accounting for the coefficient of determination (R^2) and adjusted the coefficient of determination (R^2-adj). To fit the second order polynomial equation, analysis of variance (ANOVA) was used to analyze the experimental data by multiple linear regressions, and the statistical significance of each regression term was evaluated using the. p value and t value from the pure error obtained from replicates at the central point. The p values lower than 0.05 were considered to be statistically significant. For the graphical analysis of the independent variable interactions, three dimension surface plots and two dimension contour plots were obtained from the fitted polynomial equations and used successfully [12]. The surface plots were used to explain clearly the interactive effects of the independent variables with the response variable [13]. It should be noted that the presented correlation is valid only within the range of variables investigated. Graphical and numerical multiple response optimizations were used to determine the optimum levels of mixing time and speed to attain the desired response goals [5]. In fact, optimal conditions for the mixing parameters were determined by evaluating the obtained surface plots with restrictions on the responses of a minimum value for mean particle size and PDI as well as a

Table 2 Regression coefficients, R^2, adjusted R^2 (R^2-adj) and probability values for the final reduced models

Regression coefficient[a]	Particle size (nm)	PDI	α-Tocopherol concentration (mg/L)
β_0(constant)	96.2466	0.872262	561.388
β_1(main effect)	−9.7445	−0.113720	−0.419
β_2(main effect)	−0.1985	−0.008714	−1.746
β_{11}(quadratic effect)	3.7021	−0.009989	–
β_{22}(quadratic effect)	0.0030	−0.000017	−0.005
β_{12}(interaction effect)	−0.1667	0.001996	−0.068
R^2	0.986	0.941	0.998
R^2-adj	0.976	0.792	0.989
Lack of fit	0.060	0.263	0.105
p value (regression)	0.000	0.001	0.000

[a] β_0 is a constant, β_i, β_{ii} and β_{ij} are the linear, quadratic and interaction coefficients of the quadratic polynomial equation, respectively. 1: mixing speed; 2: mixing time

maximum value of α-tocopherol concentration. Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies [13].

Results and Discussions

Fitting the Response Surface Models

The responses obtained from the experimental runs, which are shown in Table 1, were fitted to second order polynomial models by applying multiple regression analysis for studying two mixing parameters. Therefore, the estimated regression coefficients, either for initial or final reduced models, as well as the corresponding significance of regressions are given in Table 2.

The reduced models were obtained after removing the insignificant terms. However, the insignificant main effect of the studied parameters should be kept in the model due to their either quadratic or interaction significant ($p < 0.05$) effects (Tables 2, 3) [5]. F ratio and p value of each term in suggested models, which provides their significance determinations, are also shown in Table 3. It should be considered that in significance determination of terms, lower p value and higher F ratio corresponds to a higher significance of a term on studied response variations. Moreover, the suggested models might be significant ($p < 0.05$) only in the studied independent variable levels and they may not be extrapolated outside these ranges [14].

Table 3 The significance probability (p value, F ratio) of regression coefficients in final reduced second order polynomial models

Main effects	Main effects		Quadratic effects		Interacted effects
	x_1	x_2	x_1^2	x_2^2	x_1 x_2
Mean particle size (Y_1, nm)					
p value	0.005	0.095[a]	0.000	0.000	0.000
F ratio	12.57	3.13	146.26	31.87	63.92
PDI (Y_2)					
p value	0.013	0.001	0.035	0.036	0.000
F ratio	12.04	42.38	7.34	7.18	62.88
α-Tocopherol concentration (Y_3, mg/L)					
p value	0.879[a]	0.000	[a]	0.001	0.039
F ratio	0.03	85.75	[a]	29.52	6.06

[a] Not significant ($p > 0.05$)

Since the coefficients of determinations (R^2 and adjusted R^2) are good measure for overall model performance, their obtained relatively high values confirmed the suitability of suggested models. Furthermore, attained non-significant lack of fits for suggested models ensured their adequate fitness to the independent variable effects (Table 2). As shown in Table 3, the mixing parameters had significant ($p < 0.05$) effects on all studied characteristics of produced nanodispersions. While the main effect of mixing speed showed the most significant ($p < 0.05$) effect on mean particle size variation of system, the mixing time is the most effective on the changes of chemical stability and α-tocopherol concentration of produced nanodispersions. It was also shown that the interaction effects of mixing parameters had significant ($p < 0.05$) effect on the changes of all response variables.

Mean Particle Size and PDI

Mean particle sizes of the obtained α-tocopherol nanodispersion ranged from 56.8 to 103.5 nm (Table 1). In all cases, a mono-modal size distribution was obtained. Therefore, it is possible to prepare α-tocopherol nanodispersions in moderate mixing conditions without applying high energy to the system via any high shear or high pressure homogenization.

The resulted regression coefficients and their p values and F ratios revealed that whereas the main effects of mixing variables had a negative effect on mean particle size variations of the system, their quadratic terms affected this response positively (Table 3). It means that increasing either mixing speed or time, at their lower levels, led to a small decrease in mean particle size of the system, due to their lower F ratios of main effects, while at high levels, the mean particle size of the system was increased

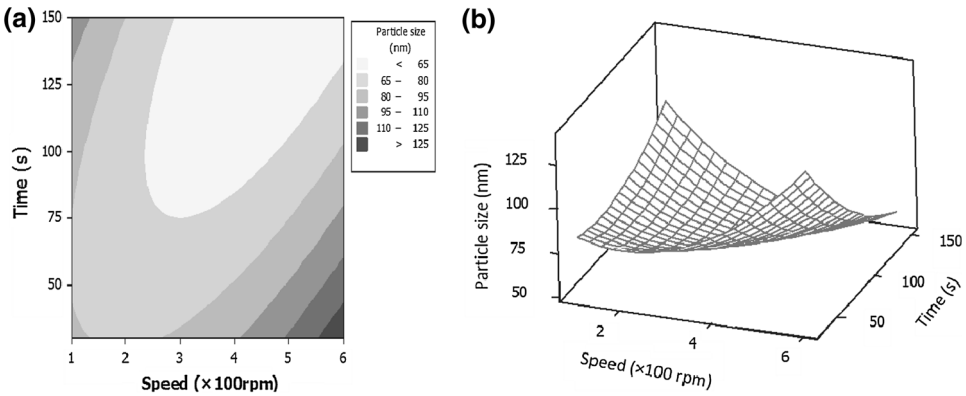

Fig. 1 Contour (**a**) and response surface (**b**) plots for mean particle size of α-tocopherol nanodispersions as function of significant ($p < 0.05$) interaction effects between mixing speed and time

considerably by increasing the mixing time or speed, because of the higher F ratios of the quadratic effects. Then, it can be concluded that the positive effects of mixing time and speed on increasing the particle size of system were greater than their negative effects. The interaction effect of mixing parameters also was found to be significant ($p < 0.05$) on mean particle size of system (Fig. 1). As clearly observed in Fig. 1, simultaneously increasing (or decreasing) of the time and speed of the mixer could lead to production of nanodispersions with a lower mean particle size, compared to using a mixer with high speed and less time (or low speed and more time). Consequently, the minimum values for mean particle size of produced nanodispersions fell in medium to high levels of mixing speed and high levels of mixing time.

The mixing process in liquid antisolvent precipitation causes supersaturation followed by nucleation and growth. Therefore, mixing and precipitation influence the particle formation process [8]. Since the mixing process involves the macromixing (occurrences on a crystallizer scale), mesomixing or turbulent mixing (consists of a large scale mass transfer of a solution) and micromixing (involves molecular diffusion) of two phases, precipitation is composed of nucleation and growth processes [8]. Increasing the mixing time (especially at medium levels of mixing speed) led to providing sufficient time in order for all three mentioned mixing stages and good contacting of particles and stabilizer molecules to occur. However, further increases in mixing time (especially at low levels of mixing speed) caused the prevailed of the mixing time compared to the precipitation time. Therefore, the particle formation was controlled by the mixing process, and the metastable zone was crossed very slowly. Then the accelerated particle growth will lead to the production of large crystals [8, 15]. On the other hand, increasing the mixing speed (up to certain levels) could enhance the mixing process, as well. Consequently, the supersaturation is accelerated and the metastable zone is crossed quickly, leading to a large number of nuclei and precipitation of ultrafine particles [8, 16]. However, production of smaller particles due to further increases in mixing time could offer them a greater tendency to aggregate, due to their higher susceptibility to Brownian motion, leading to a greater chance of collision, coagulation and aggregation [8, 17], which could also be observed in the results of present study, especially at low and middle levels of mixing time.

The individual optimum optimization procedure indicated that the minimum mean particle size ($Y_1 = 52.8$ nm) would be obtained by the homogenization process at 4.70×100 rpm for 150 s.

The results also demonstrated that the mixing variables had significant ($p < 0.05$) effects on the PDI (Y_2) variations (Tables 2, 3). Thus, the PDI changes could also be explained as being a function of speed and time of the using mixer. The results shown that the PDI of produced nanodispersions could be correlated significantly ($p < 0.05$) to all main, quadratic and interaction effects of the mixing parameters. Therefore, all effects were kept in the final prediction model for PDI. However the negative signs of all main and quadratic regression coefficients presented the generally inverse changes of PDI by both mixing speed and time, but the positive sign of interaction coefficient provided complicated variation of this response with studied mixing parameters. Thus, as shown in Fig. 2, at less mixing time, increasing the mixing speed caused a significant decrease in PDI, but at a long mixing time, this effect was vice versa. Furthermore, since the changes of PDI with mixing time at slow mixing speeds were not considerable, at high mixing speeds, increasing the mixing time would increase the PDI of produced nanodispersions.

Table 3 also indicated that after the interaction effect of mixing speed and time, which had the highest significant effect (lower p value and higher F ratio) on PDI changes among all, the single effect of mixing time was the second important variable in PDI variations. It could be also concluded that main and interaction effects of the studied parameters had a more significant effect on PDI in comparison to their quadratic effects. Decreasing the PDI by

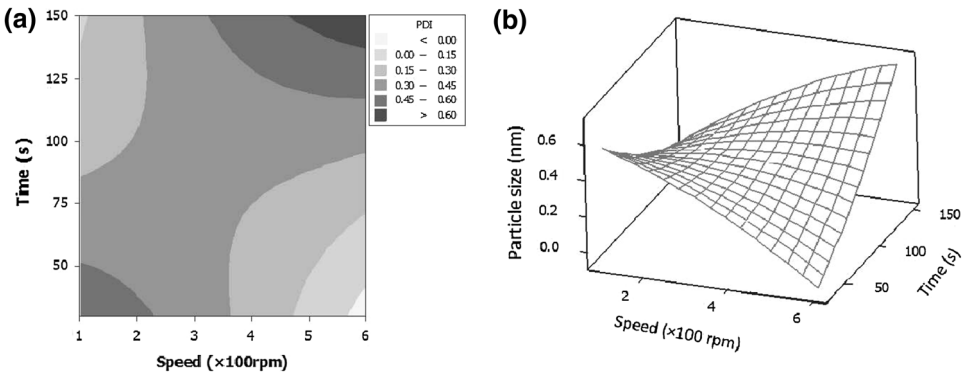

Fig. 2 Contour (**a**) and response surface (**b**) plots for PDI of α-tocopherol nanodispersions as function of significant ($p < 0.05$) interaction effects between mixing speed and time

increasing the mixing speed can be related to provided well macro, meso and micro mixing of organic and aqueous phases and fine disruption the bonds holding the particle together (interfacial forces < disruptive forces) [4], while rising the PDI with increasing the mixing speed/time or resulting energy input of system (especially at higher levels of mixing time/speed), could be observed due to the formation of a broad tail in the distribution function caused by some re-coalescence of the newly created particles during the solvent shifting process or removal of the solvent from the system [5]. Consequently, it can be concluded that over-processing conditions which can occur by supplying more energy to the system due to high levels of speed or time play important role in preparation of homogenous nanodispersion systems. It seems that the over processing may lead to poor stabilization of the newly formed droplets due to the high rate of re-coalescence of newly formed droplets [18].

The individual optimum conditions indicated that the minimum PDI ($Y_2 = 0.101$) was predicted as being obtained by a mixing speed and time at 5.5 × 100 rpm and 50 s, respectively.

Since the particle size and PDI varied quite reversely with each other, mild mixing of the organic and aqueous phases is required during the nanodispersions preparation procedure to obtain very fine droplets. These results were in good agreement with the results of previous researches [4, 5, 19].

α-Tocopherol Retention

The results shown that the α-tocopherol loss arose from either mixing or evaporation stages due to its exposure to light, temperature and oxygen. The experimental values of α-tocopherol content of produced nanodispersions were best fit to a reduced quadratic model by multiple regressions after excluding the non-significant terms (quadratic effect of mixing speed). However the main effect of mixing speed was retained in the model because of its significant interaction effect ($p < 0.05$) with mixing time. The regression coefficients of the suggested model for α-tocopherol concentration of nanodispersions are shown in Table 2. The R^2, adjusted R^2 and lack of fit for the obtained model were found to be 0.998, 0.989 and 0.105, respectively. Therefore, it has been shown that the variation of α-tocopherol content in nanodispersions could be well predicted by studied mixing parameters. As clearly observed in Table 3, the mixing time affected the α-tocopherol content of systems more significantly as compared to mixing speed. Therefore, mixing time is considered to be the most vital parameter in the determination of this response. As shown in Fig. 3, despite the relatively insignificant effect of mixing speed, the α-tocopherol was mostly degraded by increasing the mixing time. Increasing the mixing time corresponded to more exposure of samples to environmental light and oxygen, which can cause a higher degradation rate of α-tocopherol in the produced dispersed systems. The results of the present study proved the relatively good stability of α-tocopherol after less than 75 s of mixing at all studied speeds. A slight decrease in the α-tocopherol content of nanodispersions by increasing the mixing speed which is mostly seen at high levels of mixing time can be related to the relatively high sheer force and created heating, prompting the degradation of α-tocopherol in the nanodispersion [4, 5, 20]. As clearly shown, the lowest levels of speed (1 × 100 rpm) and time (30 s) of mixing resulted in the highest α-tocopherol concentration (502.2 mg/L).

Optimization of Processing Parameters for the Production of α-Tocopherol Nanodispersions

The α-tocopherol nanodispersions would be considered an optimum product if the process resulted in the smallest mean particle size and PDI along with the highest α-tocopherol content. Therefore, an overlaid contour plot as a graphical optimization approach was used to find the optimum region for mixing variables in order to produce α-tocopherol nanodispersions with the minimum particle size and PDI and the maximum α-tocopherol content (Fig. 4).

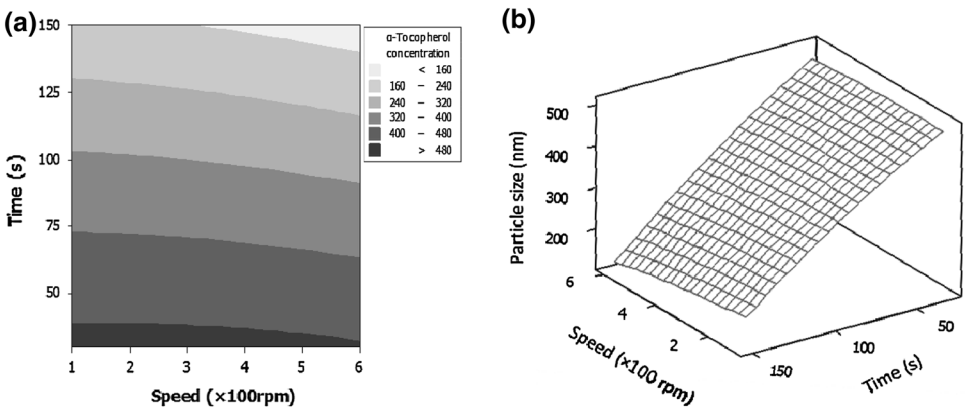

Fig. 3 Contour (**a**) and response surface (**b**) plots for α-tocopherol concentration of freshly produced nanodispersions as function of significant ($p < 0.05$) interaction effects between mixing speed and time

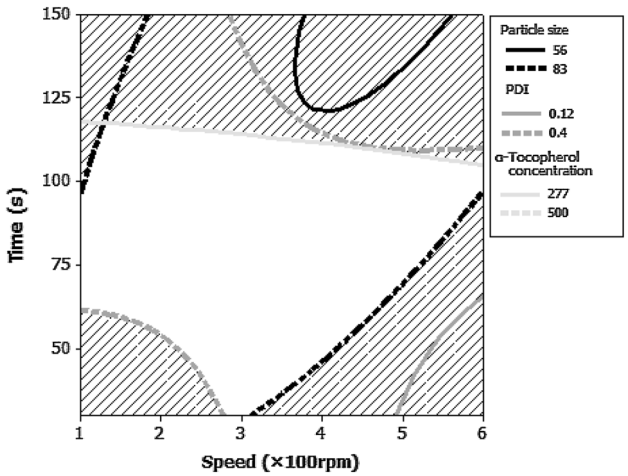

Fig. 4 Overlaid contour plot of particle size, PDI, α-tocopherol concentration with acceptable levels as function of mixing speed and time

The indicated white colored area in Fig. 4, indicated the desired mixing speed and time levels to get the optimum nanodispersion products. The third quartiles of mean particle size and PDI were considered as their accepted higher levels. The first quartile of α-tocopherol content were also picked as its desired low level. As clearly observed in Fig. 4, the most desirable products were obtained in the mixing of two organic and aqueous phases at mixing speed of less than 4.5 × 100 rpm for 25–80 s.

Numerical multiple optimization was also used to find the exact optimum levels of studied mixing variables. The results also showed that the mixing conditions with a speed of 3.8 × 100 rpm for 70 s in preparation of α-tocopherol nanodispersions would give the most desirable products with 69.3 nm, PDI of 0.301 and α-tocopherol content of 396.1 mg/L.

The adequacy of the regression equations obtained was checked by plotting the experimental values *versus* predicted ones by the final reduced models. As shown in Fig. 5, the linear plots obtained with intercepts of zero and a slope of 1, as well as their high R^2 values (>0.96) confirmed the adequacy of the models. Furthermore, the overall closeness between the predicted and experimental values of the responses could be concluded from the p values of t-test analysis between them (1.00 for all three responses). Moreover, three α-tocopherol nanodispersions were prepared according to the recommended optimal levels by numerical multiple optimization and characterized in terms of studied physicochemical properties. The measured experimental values for the average particle size, PDI and α-tocopherol concentration of these three nanodispersion samples were 73.92 ± 5.58 nm, 0.297 ± 0.009 and 422.5 ± 30.0 mg/L, respectively. The size distributions of these samples were also shown in Fig. 6. As clearly can be seen in Fig. 6, the size distribution of the produced optimum α-tocopherol nanodispersions are monomodal. The monomodal size distributions was favored in compared to polymodal distributions, because the polymodal particle distributions can speed up the Oswald ripening of particles and decrease the physical stability of nanodispersion systems. Therefore, the obtained monomodal nanodispersion systems would have better long term stability than the polymodal ones. The insignificant differences found between the predicted and experimental values of the optimum suggested sample was reconfirmed by the adequacy of the final reduced models fitted by the RSM for studies responses.

Conclusion

A response surface methodology (RSM) was used in this study to obtain empirically significant ($p < 0.05$) models predicting the mean particle size, PDI and α-tocopherol retention of the prepared nanodispersions, as a function of mixing speed and time of organic (solvent) and inorganic (aqueous) phases. The results show the usefulness of CCD for studying the effects of the processing conditions on the physicochemical properties of α-tocopherol nanodispersions and to optimize them in order to get the most desirable

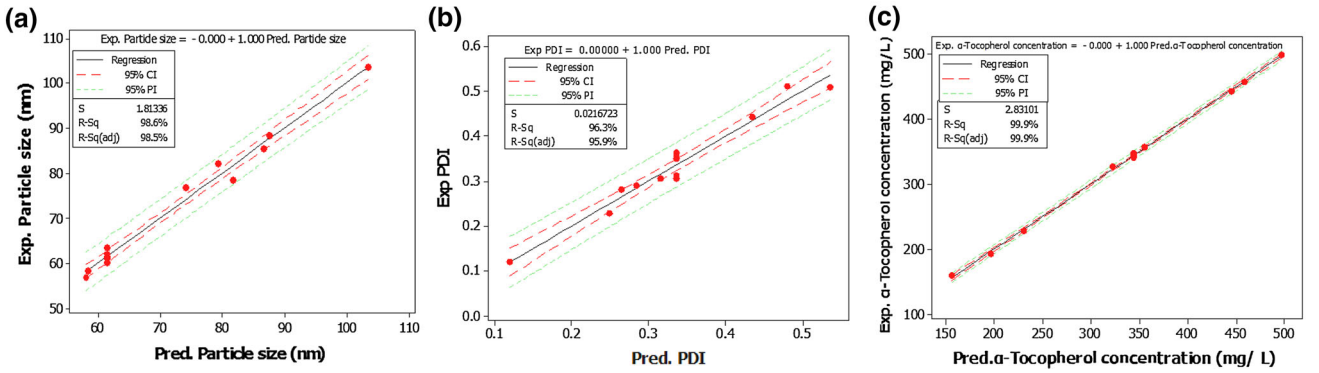

Fig. 5 Fitted line plots between the experimental and predicted values of **a** mean particle size, **b** PDI and **c** α-tocopherol concentration

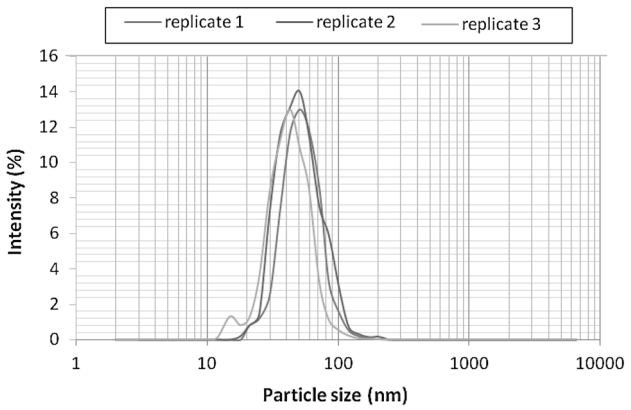

Fig. 6 Particle size distribution for three replicates of the resulting optimum α-tocopherol nanodispersions (produced under mixing conditions of 3.8 × 100 rpm for 70 s)

nanodispersions with minimum particle size and PDI and maximum incorporated active compound retention. The results of this study showed the significant ($p < 0.05$) dependency of physicochemical characteristics of nanodispersions on mixing parameters. Therefore, second order polynomial models were presented to express the correlation between the mixing conditions and the nanodispersion characteristics. Moreover, it was demonstrated that mixing the organic phase containing α-tocopherol and the aqueous phase containing Tween 20 as emulsifier and stabilizer, for 70 s in 3.8 × 100 rpm would give the α-tocopherol nanodispersions with minimum particle size and PDI and maximum chemical stability of α-tocopherol (α-tocopherol content).

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