Original Communication

Optimization the formulation parameters in preparation of α-tocopherol nanodispersions using low-energy solvent displacement technique

Naghmeh Jaberi[1], Navideh Anarjan[1], and Hoda Jafarizadeh-Malmiri[2]

[1] Faculty of Engineering, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2] Faculty of Chemical Engineering, Sahand University of Technology, Tabriz, Iran

Abstract: α-Tocopherol is the main compound of vitamin E with great antioxidant activity. However, like other functional lipid bioactive compounds, it suffers from low bioavailability due to its low water solubility and liable chemical structure. A bottom-up procedure based on a solvent-displacement method was constructed for fabrication of α-tocopherol nanodispersions using response surface methodology (RSM). The effects of main formulation parameters, namely, weight ratio of emulsifier to α-tocopherol and volumetric percent of acetone to water on the average particle size (nm), polydispersity index, concentration of α-tocopherol loss (% w/w) and turbidity of the nanodispersions were evaluated and optimized to gain the most desirable nanodispersions (least particle size, polydispersity index, turbidity and highest α-tocopherol concentrations). Second order regression equations, holding quite high coefficients of determination (R^2 and adjusted $R^2 > 0.882$), were significantly (p-value < 0.05) fitted for predicting the α-tocopherol nanodispersion characteristics variations as functions of studied formulation parameters. A multiple optimization analysis offered 6.5 and 10% for weight ratio of Tween 20 to α-tocopherol and volume percent of acetone, respectively, as overall optimum values for studied parameters. Statistically insignificant differences between experimental and predicted values of studied responses, verified the satisfactoriness of presented models for explaining the response characteristics as a function of formulation parameters. Thus, the employed solvent-displacement technique may provide the most desired water dispersible α-tocopherol nanoparticles for several water-based foods, cosmetic nutraceutical formulations.

Keywords: nanodispersion, α-tocopherol, solvent displacement

Introduction

α-Tocopherol is one of the major abundant vitamin E active compounds. It can trap two peroxyl radicals causing lipid oxidation initiation and protect them against oxidative deterioration [1]. Furthermore, it is healthful for immune system, cardiovascular diseases and cancer prevention [2]. Therefore, as other bioactive functional lipid compounds, it is being widely used in numerous foods, cosmetic and pharmaceutical formulations, either for promoting their health effects, or preserving them against oxidative deteriorations, enhancing their quality by impeding the rancidity, production of off-flavor compounds, polymerization and other undesired reactions [1, 3]. However, like other functional lipid bioactive compounds, its uses are presently limited because of either its less chemical stability particularly against heat and oxygen or its water insolubility and low cellular uptake and bioavailability [3–5]. Therefore, incorporation of these compounds into various delivery systems has been received great interest to solubilize, protect, control release and increase their bioavailability [6]. The nano-sized delivery systems which decrease the particle size of active compounds to the nano-metric range (less than 500 nm), is a prevalent technique for the delivery of water insoluble components, as the dissolution rate is proportionate to the surface area. Likewise, the saturation solubility increases with decreasing their particle sizes [2, 5]. Nanodispersions are nano-sized colloidal delivery systems which can be attained via either top-down or bottom-up techniques [3]. The top-down methods which start with

larger solid particles are capable of producing fine particles through size reduction process and can be simply used in industrial scales. However, these procedures are time consuming and include high energy consumptions to the system leading to an extensive rise in their process costs [4, 7]. Contrary, the bottom-up methods assemble nano-sized particles by starting on the atomic level. Thus, better control over size, morphology and crystallinity of nanodispersion systems would be possible in this technique. Furthermore, less energy during the process is required in bottom-up which also known as nano-precipitation processes. In this process the water insoluble compound is dissolved in water soluble solvent such as ethanol or acetone and added to aqueous system containing dissolved suitable emulsifier or surface active biopolymers. After mixing two phases, supersaturation is occurred in system and the nucleation will be started by solvent evaporation process. Although controlled process and formulation parameters lead to production the fine nano and uni-sized particles, the particles can be grown through coagulation, condensation, or agglomeration under uncontrolled conditions [8].

Stabilizers play a key role in the creation of nanodispersions in aqueous solutions by reducing the interfacial tension between the lipid-based bioactive compounds and the aqueous phases, decreasing the energy extend needed to disrupt the droplets into smaller sizes. Furthermore, they prevent the coalescence of droplets by creating a protecting layer adjoining them [9]. Polyoxyethylene sorbitan monolaurate (Tween 20) is a non-ionic emulsifier that can be adsorbed quickly at the oil–water interface in nanodispersion systems, and shown good results in stabilization of small nano-sized organic particles for various applications [10]. This study motivated on earlier studies reported by Cheong et al., and targeted on replacing the top-down high energy process of α-tocopherol nanodispersions by a bottom-up low energy technique [11]. In our previous study the mixing parameters namely, mixing rate and time were optimized in order to obtain the α-tocopherol nanoparticles with least particle size, polydispersity and highest α-tocopherol concentration [12]. Then the evaporation parameters were optimized (unpublished data). Finally, in this study, the effects of formulation factors, namely, weight ratio of emulsifier to α-tocopherol and volume percent of acetone, on physicochemical characteristics of α-tocopherol nanodispersions were evaluated and optimized in order to get the most desirable nanoparticles. The GC analysis confirmed that the acetone was totally removed from the samples after evaporation step (unpublished data).

Therefore, the produced α-tocopherol nanoparticles are food grade and can be easily used in different food and pharmaceutical formulations without any toxicity due to presence of used solvent.

Materials and Methods

Materials

α-Tocopherol (95% w) were purchased from Sigma-Aldrich (Sigma-Aldrich Co. MO, USA). Polyoxyethylene sorbitan mono-laurate (Tween 20) and analytical or HPLC grade acetone, methanol, Hexane and acetonitrile were provided by Merck (Merck Co. Darmstadt, Germany) and Fisher Scientific (Leicestershire, UK), respectively.

Preparation of α-tocopherol nanodispersions

The aqueous phase composed of dissolved Tween20 in double deionized water was dispersed into acetone containing 0.5% w/w dissolved α-tocopherol, using a conventional mixer (VOSS Instruments LTD, Maldon, UK) at 380 rpm and 70 s [12]. The emulsifier (Tween 20) concentration in aqueous phase and acetone volume percentage were set as Table 1. The acetone was then removed from the system by an evaporation process using a rotary evaporator (Heidol ph 2000, Germany). At reduced pressure of 0.5 atm by rotating speed of 400 rpm during 13 min at 45 °C (unpublished data). The volume of samples was set at 50 mL by addition of water.

Determination of particle size and polydispersity

Mean particle size and size distribution of prepared nanodispersions were determined using a dynamic light scattering particle size analyzer (Nano Wave, Microtrac., Montgomeryvill, PA, US), on undiluted samples one day after sample preparation. Light scattering is a result of the interaction of nanoparticles with light in electric field. Dynamic light scattering is a non-destructive method for particle size measurement in suspension system in which it relates the Brownian movement speed of particles to their size, in certain viscosity and temperature using Stokes-Einstein equation. The polydispersity is also a dimensionless index of the width of the size distribution obtained from the cumulants analysis. It ranged from 0 (mono dispersed) to 1 (highly broad distribution) in used instrument [12].

Sample preparation for α-tocopherol determination

The α-tocopherol concentration measurements of nanodispersions were performed using a Shimadzu high pressure liquid chromatography system (HPLC, CT-10A VP,

Table 1. Central composite design 556 and response variables (experimental and predicted values) for α-tocopherol nanodispersions

Sample Number	Tween 20/α-tocopherol[1] (% w/w)	Acetone (% v)	Particle size (nm)		Polydispersity		α-tocopherol loss (% w)		Turbidity	
			Exp[1]	Cal[2]	Exp[1]	Cal[2]	Exp[1]	Cal[2]	Exp[1]	Cal[2]
1	10	28	10.98	18.99	0.195	0.2	14.96	13.84	0.005	0.005
2	5.5	50	31.30	32.49	0.282	0.279	1.33	1.23	0.237	0.240
3	5.5	28	53.80	48.34	0.215	0.207	10.33	10.75	0.060	0.602
4	2.32	12	47.23	51.42	0.187	0.215	17.50	17.01	0.456	0.396
5	5.5	28	46.80	48.34	0.222	0.207	10.99	10.75	0.059	0.602
6	5.5	28	45.80	48.34	0.218	0.207	10.88	10.75	0.058	0.602
7	5.5	28	49.80	48.34	0.211	0.207	11.33	10.75	0.060	0.602
8	1	28	57.10	50.91	0.215	0.214	20.11	20.93	0.231	0.312
9	8.68	12	15.42	9.89	0.327	0.351	6.11	6.95	0.016	0.014
10	5.5	28	45.40	48.34	0.209	0.207	10.22	10.75	0.058	0.602
11	8.68	43	38.70	32.35	0.155	0.161	8.15	8.98	0.033	0.034
12	5.5	5	26.02	26.57	0.378	0.349	5.55	5.37	0.187	0.019
13	2.32	43	33.10	37.12	0.298	0.308	9.86	9.27	0.406	0.039

[1]Experimental values of studied responses.
[2]Predicted values of studied response.

Shimadzu, Kyoto, Japan), equipped with SPD-10AV UV-Vis detector, a LC-10A pump system, and a CT-10A oven, at 295 nm. The separation was performed on a Nova-Pak® C18 (4 μm, 3.9 × 300 mm) waters PLC column, using an isocratic mobile phase of methanol: water (99:1 v/v) at 1 ml/min, injection volume of 40 μL and oven temperature of 40 °C. The sample extraction procedures prior to injection were carried out according to Anarjan et al. [12]. The calibration of peak area versus α-tocopherol concentration was linear in the concentration range of 0.05–0.5 mg/ml. All the results were expressed in mg/ml [11]. The concentration of α-tocopherol in each sample was measured after the evaporation process, and the loss (percentage) of α-tocopherol was obtained using equation 1 (eq. 2).

$$\alpha - \text{tocopherol loss}\,(\%\,\text{w/w}) = [(C^* - C)/C^*] \times 100 \quad (1)$$

where C and C* were the α-tocopherol content of samples after evaporation step and theoretical concentration of α-tocopherol, respectively. C* was different for each sample and calculated as:

$$C^* = z_1/(100 - z_2) \quad (2)$$

z_1 and z_2 were the α-tocopherol and acetone concentrations in each sample, in turn.

Turbidity measurements

A turbidity technique was used to describe the optical characteristics of the prepared nanodispersions. Spectrophotometers have a scale that reads in O.D. (absorbance) units. The turbidity at 600 nm was determined using a UV-visible spectrophotometer (Ultraspec pharamacia biotech 2000 England, Biochrom Ltd Cambridge, UK). Water was used as references for each treatment [13].

Experimental design

Response surface methodology (RSM) was used to find out suitable relationships between selected independent parameters, namely, weight ratio of emulsifier to α-tocopherol (x_1) and volume percent of acetone (x_2), on particle size (y_1), PDI (y_2), α-tocopherol loss percentage (y_3) and turbidity (y_4). Generation large amount of information based on small number of experiments and the opportunity of assessing the interaction effects between independent variables are some advantages of RSM over one factor at a time statistical process [12]. Central composite design (CCD) is the most prevalent design of experiment applied in RSM, which was used in experimental design of this study [4, 12]. Each independents parameters were studied at five levels, specifically, central points (x_1:5.5%, x_2:28%), level 1 (x_1:10%, x_2:50%), level −1 (x_1:1%, x_2:5%), level α (x_1:8.68%, x_2:43%), and level −α (x_1:2.32%, x_2:12%). α is the distance of star point from the center where, k is the number of factors. The α value was obtained from the equation $\pm\sqrt{K}$ and for K = 2 (two independent variables) it corresponded to 1.4142. The central point, which replicated five times for the assessment of pure error, was the point in which the independent parameters were set at their middle levels.

Since during experimental design the defined levels were set as axial points, at factorial points (levels −1 and +1), the

Table 2. Regression coefficients, R^2, adjusted R^2 (R^2-adj) and probability values for the final reduced models suggested for characteristics of α-tocopherol nanodispersions

Regression coefficients[a]	Particle size (nm)	Polydispersity	α-tocopherol loss (%w/w)	Turbidity
β_0 (constant)	44.874	0.1973	24.0089	0.77083
β_1 (main effect)	−1.4964	0.0386	−5.7800	−0.117680
β_2 (main effect)	1.1436	−0.0052	0.45	−0.018562
β_{11} (quadratic effect)	−0.6610	ns	0.3277	ns
β_{22} (quadratic effect)	−0.0370	0.0002	−0.0148	0.000343
β_{12} (interaction effect)	0.1865	−0.0014	0.0495	ns
R^2	0.896	0.943	0.984	0.910
R^2-adj	0.882	0.915	0.972	0.8381
p-value (regression)	0.002	<0.001	<0.001	<0.001

[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: weight ratio of tween 20 to α-tocopherol; 2: volume percent of acetone.
[ns] Not significant ($p > 0.05$).

Table 3. The significance probability (p-value, F-ratio) of regression coefficients for the final reduced models suggested for characteristics of α-tocopherol nanodispersions

	Main effects		Quadratic effects		Interaction effects
Response variables[a]	x_1	x_2	x_1^2	x_2^2	x_1x_2
Particle size (Y_1, nm)					
p-value	0.001	0.368	0.023	0.005	0.019
F-ratio	29.06	0.93	8.41	16.15	9.16
Polydispersity (Y_2)					
p-value	0.682[ns]	0.005	ns	<0.001	<0.001
F-ratio	0.18	14.26	ns	57.73	59.21
α-Tocopherol loss (Y_3, %w)					
p-value	<0.001	0.002	<0.001	<0.001	0.001
F-ratio	77.56	24.29	110.5	137.92	34.55
(Y4)Turbidity					
p-value	0.0022	0.049	ns	0.044	ns
F-ratio	10.876	6.703	ns	7.2029	ns

1: weight ratio of tween 20 to α-tocopherol, 2: volume percent of acetone.
[ns] Not significant (p-value > 0.05).

factors were set at their defined minimum and maximum levels. The positions of star points (−α, +α) were denoted by α as normalized levels ± α, which lied between the axial and central points. Thus, a total of 13 experiments, including four factorial points, four star points, and five central points were created by the software Minitab V.14 statistical package (Minitab Inc., PA, USA) (Table 1). All samples were fabricated in one day [1].

Statistical analysis

Second-order polynomial equations were used to describe the response variables as a functional of the independent variables, as follows:

$$y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 [14] \quad (3)$$

where y_i symbolizes the response variable, β_0 is the constant, β_i, β_{ii} and β_{ij} are the linear, quadratic and interaction coefficient, correspondingly. The coefficients of regression equations were determined using regression analysis. Analysis of variance (ANOVA) were also performed to estimate the significance of models and significant terms in selected model using p-value and F-ratio from the pure error obtained from replicates at the central point. The terms with p-values less than 0.05 were considered as statistically significant.

The final reduced models were attained after removing the insignificant terms from initial model (eq. 3). However, even with insignificant main effect of studied concentration parameters on some responses, they were reserved in final models due to their either quadratic or interaction significant (p-value < 0.05) effects (Tables 2 and 3) [4].

The Anderson-Darling normality tests were also performed for the responses' residuals (residual = predicted value – experimental value) and the results were shown in

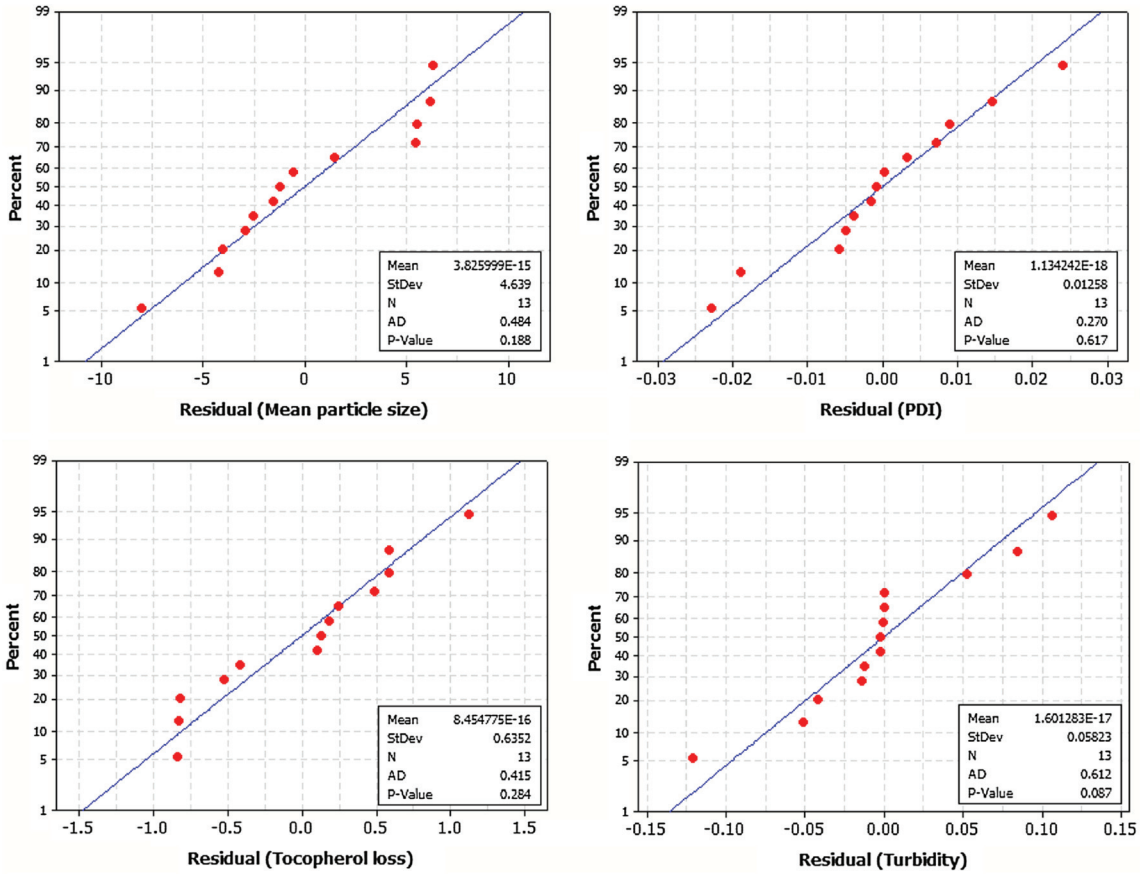

Figure 1. The responses residual probability plots for normality tests.

Figure 1. As can be seen in this figure, the p-values of all residual probability plots were more than 0.05. Then the assumption of normal distribution for all responses residuals can be verified. Furthermore, the equality of variances were confirmed by F-tests and given p-values (0.85, 0.92, 0.88 and 0.06 for mean particle size, PDI, α-tocopherol loss and turbidity, respectively), which were higher than 0.05.

The adequacy of model was examined from coefficient of determination (R^2) and adjusted coefficient of determination (R^2-adj) values. Higher R^2 values (approximate to 1) corresponds to more ability to prediction the response variation based on independent parameters' changes. For the graphical analysis of the independent variable interaction, 3D surface plots of the regression models were used successfully [14].

The single and multiple optimizations were conducted to find optimum levels of two formulation parameters resulting in the desirable response variables purpose. In the numerical optimization, the exact best level of independent variables leading to the most desirable response variables was attained using response optimizer (Minitab V.14 statistical package). The graphical optimization was also performed using overlaid counter plots of independent factors in desired ranges of responses. The suitability of predicted models was proved by comparison of experimental data with calculated ones. Moreover, optimum sample was prepared and their response variables were compared with their predicted value, in order to check the validity of the models.

Results and discussions

Fitting models

Response-surface analysis offered empirically significant (p-value < 0.05) models to estimate the variations of particle size (p-value = 0.002), polydispersity (p-value < 0.001), α-tocopherol loss (p-value < 0.001) and turbidity (p-value = 0.009) as a function of emulsifier to α-tocopherol weight ratio and volume ratio of acetone to water. All regression coefficients, corresponding R^2 and R^2-adj, individual significance F-ratio and p-value of the independent variables are shown in Tables 2 and 3.

The high obtained coefficients of determinations (R^2 > 0.896, R^2-adj > 0.8381) (Table 2) have also shown that more than 83% of variability of the studied physicochemical characteristics of the prepared nanodispersions can be predicted by recommended models in this study.

In significance determination of terms, lower p-value and higher F-ratio corresponds to more significance of terms on studied response variations. Furthermore, it should be noted that the suggested models, might be significant (p-value < 0.05) only in studied ranges and may not be generalized outside of these ranges [12].

As shown in Table 3, the concentration parameters had significant (p-value < 0.05) effects on all selected characteristics of produced nanodispersions. The main effect of the weight ratio of emulsifier to α-tocopherol was the most significant (p-value < 0.05) on both particle size and turbidity of nanodispersions. On the other hand, the interaction effect of two parameters was the most significant on polydispersity variation and the quadratic effect of acetone to water (%v/v) was the most significant term on prediction of α-tocopherol loss changes. It also can be seen that the quadratic effect of aceton to water (%v/v) affected all selected responses significantly (p-value < 0.05).

Particle size

The mean particle size of prepared α-tocopherol nanodispersions was significantly (p-value < 0.05) described by a full quadratic regression model (R^2 = 0.896), because all main, quadratic and interaction effects of selected formulation parameters were significant on this response (Table 2).

The negative coefficients of both main and quadratic terms of emulsifier to α-tocopherol weight ratio indicated a decrease in particle size by increasing of this independent factor, especially at low levels. Furthermore, the positive main and negative quadratic coefficients of acetone to water also revealed the increases of particle size by increasing the acetone concentration up to certain levels. Further increases in acetone concentration caused the particle size to decrease (Table 2, Figure 2).

As previously stated, the most significant term on mean particle size variation was the main effect of Tween 20/α-tocopherol (w/w), because of its less p-value and higher F-ratio (Table 3). The negative interaction effect of the concentration parameters revealed that instantaneously increasing (or decreasing) of the independent variables led to production of bigger nanodispersions, compared to using high emulsifier and low acetone concentrations or contrariwise (Table 2 and Figure 2).

Consequently, the smallest particles could produce in middle to high levels of emulsifier to α-tocopherol weight ratio and low levels acetone to water volume percent.

As most emulsion system, nanodispersions are thermodynamically unstable but kinetically stable systems. Nanoemulsions or nanodispersions are transparent dispersion systems having great stability against destabilization mechanisms such as creaming, sedimentation, coalescence or flocculation. The exceptional stability of nanodispersions is attributed to their very small particle sizes and their Brownian motion enables an inordinate reduction of gravitational effects [14]. However, gravity is the main cause of sedimentation and creaming occurring during storage of microemulsions, the major cause of instability in nanodispersion systems is claimed to be the Ostwald ripening mechanism [4, 12, 15].

The formation of nanodispersions with small particle sizes is depended on the balance among the physical process of particle disruption, coalescence and the interfacial forces that tend to keep the particles together [4, 12]. Therefore, surface active molecules play key roles in the creation and stability of nanodispersions. The adsorbed emulsifier molecules decrease the interfacial tension between the phases and form a protective coat around the particles to prevent aggregation. By depressing the interfacial tension, less energy is necessary through homogenization process to splitting up the particles [15].

According to the results reported by Chu et al. [16] and Mainardes and Evangelista [17], an increase in concentration of emulsifier caused a decrease in particle size of dispersed phase in all emulsion systems. However, other researchers such as Lobo and Svereika [18] and Anarjan et al. [14], established two opposite effects for emulsifier concentrations on the particle size of dispersed phase in emulsions; an emulsifier-poor regime, in which particle size decreases with increasing emulsifier content, and emulsifier-rich regime, in which particle size does not depend on emulsifier concentration or grow with increasing the concentration of emulsifier [10]. As can be seen in Figure 2, at low solvent concentrations, particle size was decreased by raising the emulsifier concentration, which was expected due to stabilizing function of an emulsifier [10, 14]. However, this trend was soften and inversed by increasing the acetone concentration. These observations were in good agreement with our previous study reported by Anarjan et al. [14].

Mainardes and Evangelista [17] also found that an increase in the organic phase concentration led to a slight decrease in particle size; contrary, Anarjan et al. [14] and Chu et al. [16] reported an increase in particle size with increasing organic phase concentrations. The results of this study presented a growth in particle size of nanodispersions by increasing the solvent phase, at high emulsifier concentrations. However, this effect was inversed at low levels of emulsifier concentrations (Figure 2). After evaporation of solvent, at high acetone samples, the concentrated system was more prone to particle coalescence through bridging

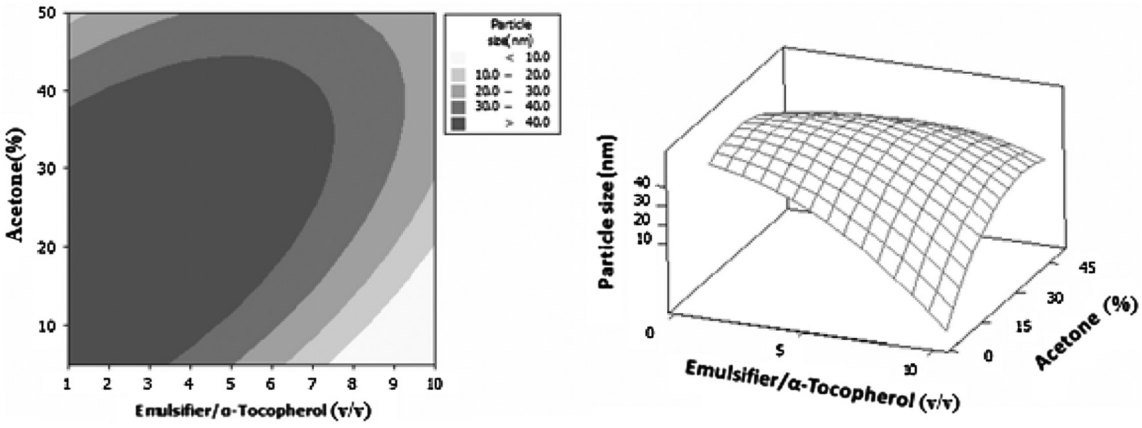

Figure 2. Contour (a) and response surface (b) plots for mean particle size of α-tocopherol nanodispersions as function of significant ($p<0.05$) interaction effects between weight ratio of Tween 20 to α-tocopherol and volume percent of acetone.

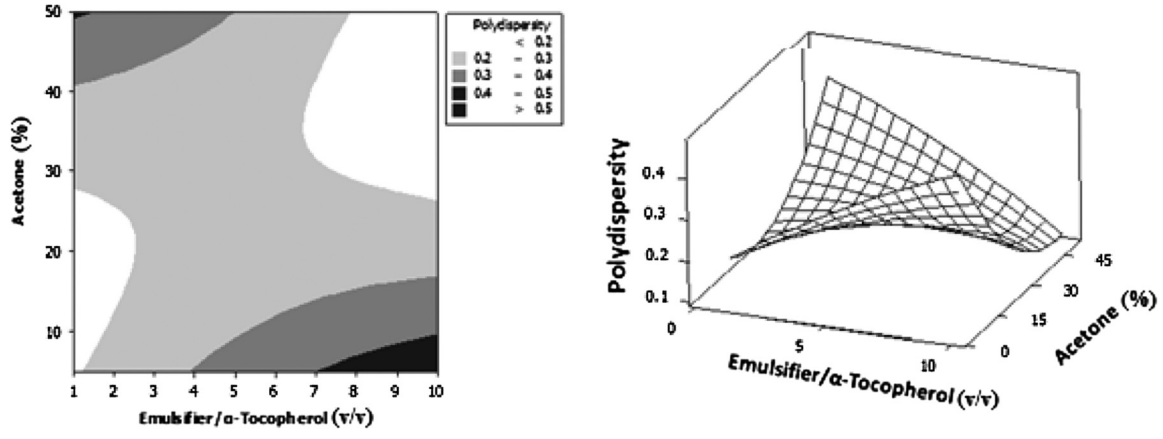

Figure 3. Contour (a) and response surface (b) plots for polydispersity of α-tocopherol nanodispersions as function of significant ($p<0.05$) interaction effects between weight ratio of Tween 20 to α-tocopherol and volume percent of acetone.

flocculation, depletion flocculation, and other mechanisms, particularly at high emulsifier contents [14].

The individual optimum optimization procedure showed that the minimum particle size (< 20 nm) would be obtained by 10% w/w emulsifier (to α-tocopherol) and 28% v/v acetone (to water).

Polydispersity

The result showed that concentration variables had also significant (p-value < 0.05) effects on the polydispersity changes (Tables 2 and 3). Thus the polydispersity of produced nanodispersions could be described as a function of emulsifier to α-tocopherol weight ratio and volume percent of acetone. The final reduced model showed a relatively high coefficients of determination ($R^2 = 0.943$), revealed that more than 94% of polydispersity behavior of system could be predicted by presented regression model.

It was shown that the single and quadratic effects of emulsifier concentration in studied system were not significant on polydispersity value of produced nanodispersions. However, its interaction with acetone content was significantly negative on this response variation (Tables 2, 3). The negative interaction effects as well as the negative single and positive quadratic effects of solvent content indicated that using simultaneously less (or high) concentrations of emulsifier and solvent would lead to production of less polydisperse nanodispersions. As shown in Figure 3, at low emulsifier concentrations, an increase in the organic phase concentration increased the polydispersity, while at high emulsifier concentrations, it operated inversely.

Previous researchers have reported various results for the effect of emulsifier concentrations on polydispersity; Chu et al. [16] and Mainardes and Evangelista [17] recounted that the polydispersity of nanodispersions was reduced by increasing the emulsifier content. Anarjan et al. [14] reported inverse trend for variation of

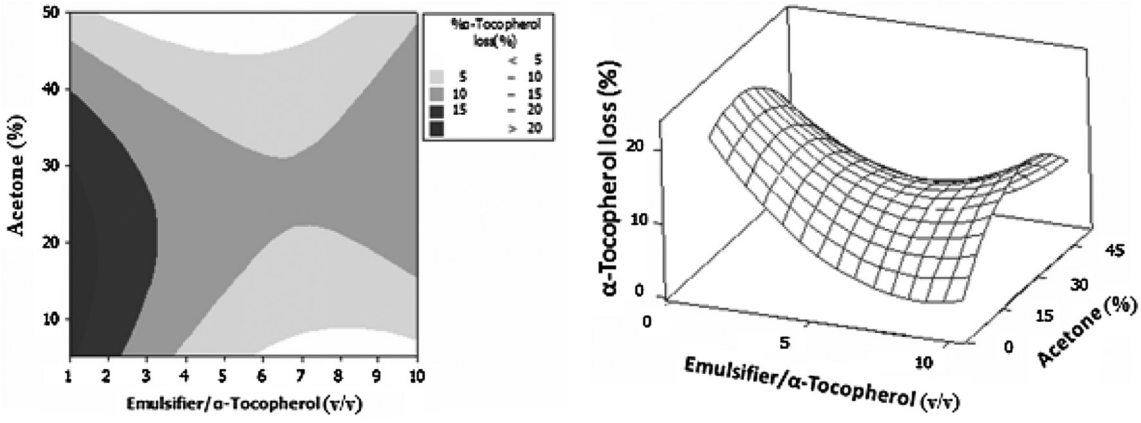

Figure 4. Contour (a) and response surface (b) plots for α-tocopherol loss (%w/w) of freshly produced nanodispersions as function of significant ($p<0.05$) interaction effects between weight ratio of Tween 20 to α-tocopherol and volume percent of acetone.

polydispersity by emulsifier content. Moreover, they observed less variations of this response by changes the solvent content, which was not agreed to our results. The observed difference may be related to selected dissimilar nanoparticle formation technique in these two works. It was shown that in high energy techniques, homogenization parameters played important role on homogeneity of systems, while in low energy techniques phase separation parameters affects more considerably the homogeneousness of nanodispersions [19]. In solvent displacement process, less acetone content, at less emulsifier concentrations caused a rapid supersaturation and less crystal growth, resulting uniform particle formation [14, 19]. However, decreasing the polydispersity with increasing the solvents content at high emulsifier concentrations has not explained yet.

The individual optimum concentrations for the production of more uniform and uni-sized nanodispersions were predicted to be in 8.68% weight ratio of emulsifier to α-tocopherol and 43% the volume percent of acetone.

α-tocopherol loss

As shown in Tables 2 and 3, the variation of α-tocopherol loss in produced nanodispersions was significantly (p-value < 0.05) fitted to nonlinear second-order regression equation with relatively high coefficient of determination (R^2 = 0.984). All single, quadratic and interaction effects of selected independent variables were significant on α-tocopherol loss of nanodispersions during mixing and evaporation processes.

As clearly observed in Table 3, the emulsifier concentration affected α-tocopherol loss more pronounced compared to acetone content (less p-value and higher F-ratio). The negative main and positive quadratic effects of emulsifier to α-tocopherol ratio revealed that increasing the emulsifier content caused an increase in chemical stability of nanodispersions. However, further increase of emulsifier content affected this response, reversely. On the other hand, increasing the acetone content of system caused a decrease in α-tocopherol content of nanodispersions, and this effect was moderated at high levels of acetone concentrations.

The reduction in α-tocopherol loss with increasing emulsifier concentration (Figure 4) was due to protective effect of the emulsifier against lipid oxidation of the active compound by modifying the particle interface characteristics [10]. Increasing the α-tocopherol loss of nanodispersions by increasing the solvent content is related to needed severer evaporation condition (higher time or temperature) for solvent removal process, leading to more degradation of α-tocopherol that is sensitive to heat, light and oxygen exposure [4, 12, 14].

The individual optimization predicted that using Tween 20 to α-tocopherol with weight ratio of 5.5 and 50% acetone can produced the α-tocopherol nanodispersions with the least α-tocopherol loss.

Turbidity

One of the main differences between nanodispersions and conventional dispersions are their transparency, which is related to extent of their light scattering [7]. However, there has been a lack of studies describing the appearance of these systems. The nanodispersions with less turbidity were desired in most applications such as fortifying the food formulations such as clear fruit juices [7, 18]. Turbidity of produced α-tocopherol nanodispersions were also significantly (p-value < 0.05) fitted to nonlinear polynomial regression model like other studied response variables with acceptable high coefficient of determination (R^2 = 0.910). As shown in Tables 2 and 3, the main effect of emulsifier to α-tocopherol ratio (w/w) on turbidity of nanodispersions

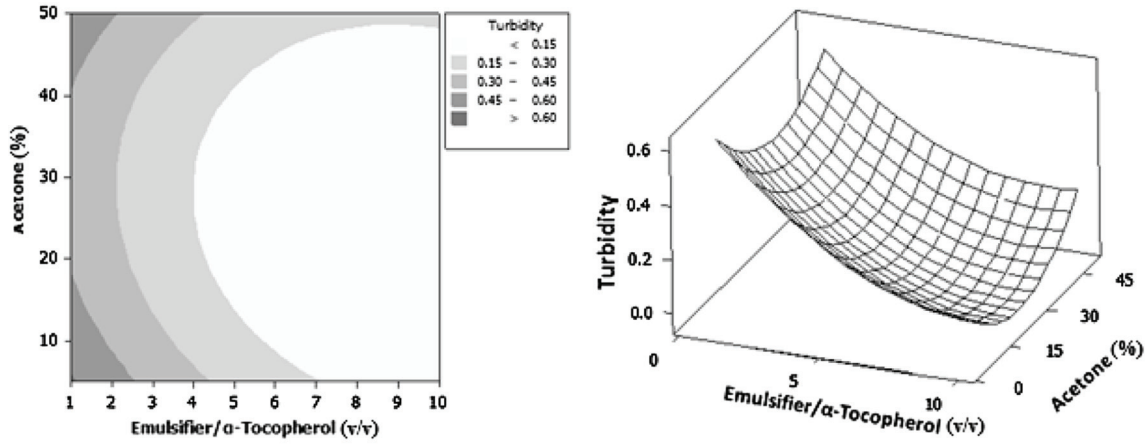

Figure 5. Contour (a) and response surface (b) plots for turbidity of freshly produced α- tocopherol nanodispersions as function of significant ($p<0.05$) interaction effects between weight ratio of Tween 20 to α-tocopherol and volume percent of acetone.

was significantly negative. Therefore, the increasing of emulsifier content caused the turbidity to decrease (Figure 5). Furthermore, the negative main and positive quadratic effects of used acetone volume percent showed that increasing the solvent phase caused a decrease in turbidity up to certain value. Further increase in this formulation parameter affected the turbidity, inversely.

When light passes through nanodispersions, light scattering in the visible region by α-tocopherol controls their turbid appearance. The degree of light scattering depends on particle size, particle concentration and the refractive index difference between the particles continuous phase. The free emulsifier content of system affects the refractive index of phases [7, 20]. It was shown that a colloidal dispersion with particle size less than 100 nm looks transparent or semi-transparent depending on either particle size and concentration or the concentration of free emulsifier molecules. Thus, one way to reduce the turbidity is to reduce the particle size [20]. The comparison between the particle size and turbidity changes of nanodispersions in current research (Figures 2 and 5) showed that generally the nanodispersions with less particle size were less turbid. These results are in reasonable agreement with previous researches [7, 20]. Decreasing the turbidity with increasing the emulsifier concentration can also be related to decrease of differences between refractive indices of two phases in produced nanodispersions. Unlike previous studied it was not shown any linear relationship between particle size and turbidity of produced nanodispersions [20].

Optimization of Concenteration Parameters for the Production of α-tocopherol Nanodispersions

The α-tocopherol nanodispersions would be considered as best product if it hold the smallest mean particle size, polydispersity, α-tocopherol loss (% w/w) and turbidity. Therefore, an overlaid contour plot as a graphical optimization approach was used to find the optimum region for selected formulation variables in order to produce the most desirable α-tocopherol nanodispersions (Figure 6).

The best ranges for selected independent variables to gain the optimum product were shown as white colored area in Figure 6. It can be seen that the most desirable α-tocopherol nanodispersions would be obtained at weight ratio of emulsifier to α-tocopherol ranges around 5–7 and the volume percent of acetone about 10 to 45.

Numerical multiple optimization was also performed to find the exact optimum levels of studied mixing variables. The results predicted 6.5 and 10 for emulsifier to α-tocopherol weight ratio and acetone volume percent, respectively, as optimum levels for production the best

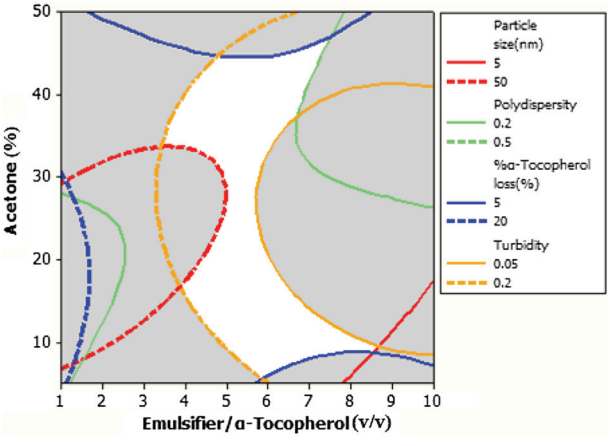

Figure 6. Overlaid contour plot of particle size, polydispersity, α-tocopherol loss and turbidity with acceptable levels as function of weight ratio of Tween 20 to α-tocopherol and volume percent of acetone.

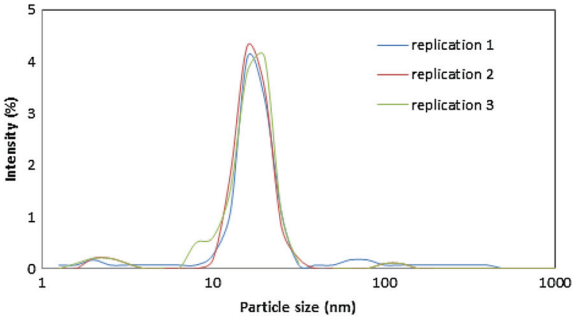

Figure 7. Particle size distribution for optimum suggested α-tocopherol nanodispersions (Prepared with 6.5 and 10 of tween 20 to α-tocopherol weight ratio and acetone volume percent, respectively.

nanodispersions with 27 nm, polydispersity of 0.329, 6.52% α-tocopherol loss (% w/w) and turbidity of 0.1099.

The particle size distribution of prepared triplicated optimum nanodispersions were presented in Figure 7. It can be seen that the suggested optimum nanodispersions had mono-modal distribution with good consistency of replications. Moreover, the insignificant differences found between the predicted and experimental values of the best sample were re-confirmed the suitability of final reduced models fitted by the RSM for all responses.

The adequacy of the presented regression models obtained was checked by plotting the experimental values versus predicted ones. For all responses the linear plots with intercepts of zero and slope of and high coefficients of variations ($R^2 > 0.896$) confirmed the similarity of predicted and experimental values (Figure 8). Therefore the adequacy of the models was confirmed. The overall closeness between the predicted and experimental values of the responses could also be concluded from the p-values of t-test analysis between them (p-value = 1.00 for all four responses).

Stability evaluation of prepared optimum α-tocopherol nanodispersion over time

The mean particle size, polydispersity, α-tocopherol loss and turbidity of the optimum α-tocopherol nanodispersion were monitored over four weeks of storage at 4 °C to evaluate its physical and chemical stabilities. The results were reported in Table 4. While no significant changes were

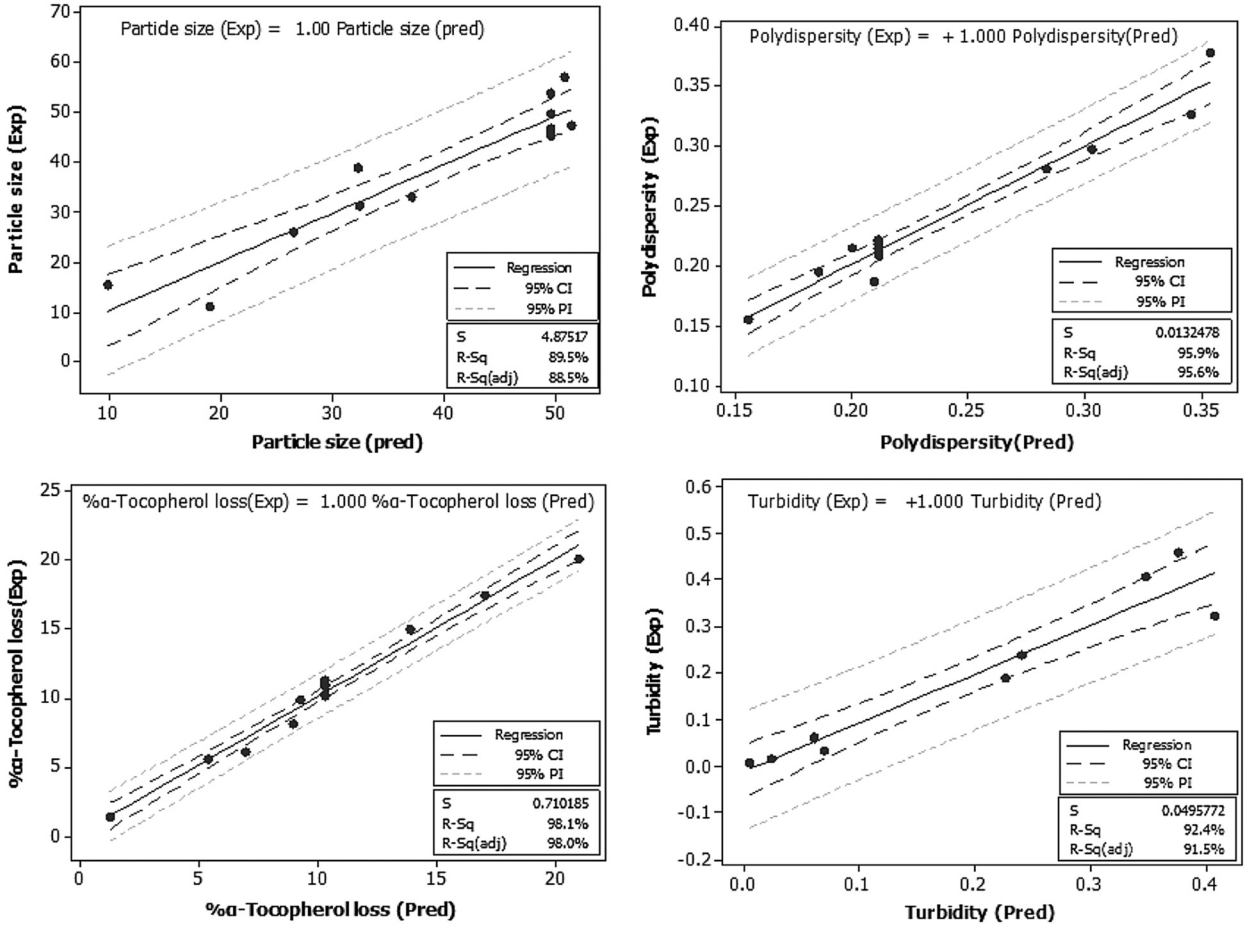

Figure 8. Fitted line plot between the experimental and predicted values of particle size, polydispersity, α-tocopherol loss (%w/w) and turbidity.

Table 4. The changes on characteristics of optimum α-tocopherol nanodispersions during four week storage at 4 °C

	Fresh sample	After 2-week storage	After 4-week storage
Mean particle size (nm)	29±5.5[a]	26±6.2[a]	31±4.5[a]
Polydispersity	0.33±0.080[a]	0.36±0.095[a]	0.38±0.055[a]
α-tocopherol loss (% w/w)	5.9±1.12[a]	7.9±0.90[ab]	9.5±1.05[b]
Turbidity	0.105±0.008[a]	0.108±0.005[a]	0.107±0.010[a]

Values are mean ± standard deviation (n = 3).
[a–b] Different letters indicate statistically difference (p-value < 0.05) between response values in which comparison tests were performed between similar responses of each row (storage time).

observed for mean particle size, polydispersity and turbidity of optimum α-tocopherol nanodispersion, the α-tocopherol loss of sample were increased significantly (p-value < 0.05) during 4 weeks storage. Thus, no coalescence occurred in the optimum nanodispersion over studied storage time. Consequently, the physical stability of sample could be confirmed. But, the significant (p-value = 0.015) decrease in α-tocopherol content (α-tocopherol loss) of the prepared nanodispersion revealed its limited chemical stability during storage. α-Tocopherol is sensitive to light and oxygen like carotenoids [1, 7]. Therefore, the presence of light and oxygen in storage environment caused the α-tocopherol loss in studied nanodispersions. Consequently, either any modification of atmosphere such as storage under nitrogen or storage at dark room can preserve the α-tocopherol content of prepared nanodispersions, considerably [7, 14].

Conclusion

RSM was used in this study to acquire empirically significant (p-value < 0.05) models predicting the most important physicochemical characteristics of α-tocopherol nanodispersions, namely, mean particle size, polydispersity, α-tocopherol loss and turbidity, as a function of emulsifier to α-tocopherol weight ratio and volume percent of acetone. The results revealed the usefulness of CCD for studying the effects of the main formulation parameters on the selected responses and optimizing them with the intention of getting the most desirable nanodispersions with minimum particle size, polydispersity, α-tocopherol loss and turbidity. Therefore, second order polynomial regression models were offered to express the correlations between selected the most important concentration variables and nanodispersions' characteristics. It was demonstrated that using Tween 20 to α-tocopherol with the weight ratio of 6.5 and acetone to water with the volume percent of 10 would provide the α-tocopherol nanodispersions with minimum particle size (27 nm), polydispersity (0.329) and α-tocopherol loss (6.52%) and turbidity (0.1099). No significant differences between the experimental and predicted values of responses confirmed the suitability of models. Therefore, the bottom-up solvent-displacement technique could successfully produce the water dispersed α-tocopherol nanoparticles with the most desirable characteristics (minimum particle size, PDI, α-tocopherol loss and turbidity) for e.g. water-based food, cosmetic and pharmaceutical uses.

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Publication ethics
This work didn't include human subjects or animals.

Navideh Anarjan, PhD
Assistant Professor
Tabriz Branch-Islamic Azad University
Pasdaran Highway
Tabriz
Iran
anarjan@iaut.ac.ir

