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Zahra Izadi [a] , Ali Nasirpour [b] & Ghasemali Garousi [a]

[a] Department of Biotechnology, College of engineering, Imam Khomeini International University, Qazvin, Iran

[b] Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

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Optimization of Phytosterols Dispersion in an Oil/Water Emulsion Using Mixture Design Approach

Zahra Izadi,[1] Ali Nasirpour,[2] and Ghasemali Garousi[1]

[1]*Department of Biotechnology, College of engineering, Imam Khomeini International University, Qazvin, Iran*
[2]*Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran*

Major problems related to enrichment of products with phytosterols are high melting temperature, chalky taste and low solubility in water phase. Dispersion of phytosterols in an emulsion was optimized using a mixture design with four components (phytosterols, emulsifier, soy oil, and water). It was found that the particle size of the dispersed phase decreased with the increase in emulsifier concentration. The appearance viscosity was increased with decreasing particle size. The stability of these emulsions could be correlated with the decrease in surface tension and particle size by using oil and emulsifier as components of oil phase.

Keywords Cholesterol, mixture design, optimization, phytosterol

1. INTRODUCTION

Phytosterols (plant sterols) are naturally occurring components of plants, especially seeds and oils. Common phytosterols include the unsaturated sterols such as β-sitosterol, stigmasterol, and campesterol and their saturated counterparts, campestanol, and sitostanol. The cholesterol-lowering properties of phytosterols have already been observed in humans in the early 1950s.[1] As a food ingredient or additive, phytosterols have been shown to reduce total cholesterol and low density lipoprotein (LDL) cholesterol in normo-cholesterolemic and hyperlipidemic populations.[2] The phytosterols are thought to displace cholesterol from bile acid micelles and/or co-precipitate cholesterol in the intestinal lumen, thereby limiting its uptake.[3] Intake of phytosterols and/or stanols at the level of 1.5–3.0 g/day has been documented to reduce blood LDL-cholesterol by 10%.[4,5]

Enriched products with this functional component have been recently presented on the markets. Major problems related to enrichment of products with phytosterols are high melting temperature, chalky taste and low solubility in water phase. Administering crystalline phytosterols, however, did not promote a significant decrease in serum cholesterol when compared to phytosterols dissolved in edible fat products.[6] Esterification of the phytosterols and stanols with long chain fatty acids increases their lipid solubility and facilitates their incorporation into foods[7] to levels as high as 10–20%.[8] A study that compared the effects of phytosterol esters and free phytosterols on beta-carotene and alpha-tocopherol found that phytosterol esters reduced the bioavailability more than plant free sterols.[9]

Free phytosterols exhibited greater activity than phytosterol esters. In an efficacy study on phytosterols, a daily consumption of 24 g of spread, containing 2–3 g of phytosterols esters, total serum cholesterol and LDL cholesterol were lowered by up to 6.4% and 10.1%, respectively. While the consumption of 1.6–2.0 g phytosterols or stanols per day resulted in reduction of total serum cholesterol and LDL cholesterol by 8–13%.[10] The increased activity of the free versus esterifies phytosterols is likely due to the availability of a terminal hydroxyl group in the former compounds for interaction with peroxide radicals.[11] Therefore, in this study, an oil/water (O/W) emulsion containing phytosterol was optimized in order to add this emulsion to dairy products. Furthermore, physicochemical properties of the emulsions were studied and optimized using mixture design approach.

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Address correspondence to Ali Nasirpour, College of Agriculture, Department of Food Science and Technology, Isfahan University of Technology, Isfahan 84156, Iran. E-mail: ali.nasirpour@cc.iut.ac.ir

2. MATERIALS AND METHODS

2.1. Materials

Phytosterol, consisting of β-sitosterol (~40%), Stigmasterol (15 ~ 30%), Campsterol (15 ~ 30%), Brassicasterol

TABLE 1
Initial experimental area for formulations

Component	Minimum, % (wt/wt)	Maximum, % (wt/wt)
Emulsifier (A)	5	10
Phytosterol (B)	10	20
Water (C)	50	85
Oil (D)	0	20

(<10%), was a gift from Zhejiang Medicine Co., Ltd. (Xinchang Pharma, China). Emulsifier lactic acid esters of mono glycerides (LACTEM) were a gift from Poratus Co., (Poratus Co. Belgium). For preparation of O/W emulsions, cold pressed vegetable oil was purchased from local market, and double-distilled water was used. All chemicals, being of reagent grade, were used as such.

2.2. Methods

2.2.1. Formulation Design

Formulations were prepared by mixing four major components (emulsifier, phytosterol, water, and oil). Initial experimental range of components was chosen in order to cover a large area of formulations and presented in Table 1. Combination of major components of formulation was determined by D-optimal mixture design using Design-Expert 6.0.6 trial (Stat-Ease Inc., Minneapolis, MN, USA) software. Twenty formulations plus four replications were proposed by the software. All formulations and replications are presented in Table 2. In this study, formulations containing four components will be referred to as F-emulsifier/phytosterol/water/oil (by %w/w). For example, F10/10/60/20 means the formulation containing 10% emulsifier, 10% phytosterol, 60% water, and 20% oil.

2.2.2. Preparation of Emulsion

LACTEM and soy oil were used for dispersing phytosterol. The mixtures of oil, emulsifier, and phytosterol were heated to 130–140°C and oil phase was mixed for 2 minutes at 700 rpm, and then heated water was added to oil phase and mixing was continued for 4 minutes at 3000 rpm using a laboratory mixer (Heidolph, Germany) to achieve an emulsion with soft texture and white color.

2.3. Emulsion Analysis

2.3.1. Particle Size Distribution

Mean particle size and particle size distribution of emulsions were determined by static light scattering using a particle size analyzer (Mastersizer 2000 Ver. 5.22, Malvern, UK). Emulsion samples (0.05 g) were diluted with 150 ml 0.1% sodium dodecyl sulfate (SDS) solution in order to quench the interparticle connections and break up the floccules. The action of the SDS solution on the breakage of the floccules was confirmed by optical microscopy.

Particle size measurements were reported as the full particle size distributions, the surface-weighted mean diameter, $d_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$, and the volume-weighted mean diameter, $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$, where n_i is the number of particles with diameter d_i^3.

2.3.2. Determination of Surface Tension

The Du Nouy ring method (Didital Tensiometer, Germany) was employed to measure surface tension. All measurements were made at a temperature of 25 ± 0.5°C. Temperature control was maintained by a circulatory water bath. The samples were diluted to 1:1 ratio (40 g emulsion and 40 g distilled water). The ring is dipped into the solution whose surface tension is to be measured; then, it was pulled out. The maximum force needed to pull the ring through the interface is expressed as the surface tension in mN/m. The data presented are average of three measurements.

2.3.3. Emulsion Stability

For stability experiments, emulsion samples were poured in 10 ml glass graduated cylinders and subjected to centrifugation. A centrifugal force (g) of $4000 \times g$ was employed for 15 minutes at a temperature of 25 ± 0.5°C and this operation was repeated three times.

TABLE 2
Estimations of coefficients and probabilities of linear model for surface tension, serum separation and particle size

	Surface tension		Serum separation		Particle size	
Source	Coefficiet estimate	Probability	Coefficient estimate	Probability	Coefficient estimate	Probability
Model		<0.0001		0.0004		<0.0001
A	−94.26		−9.09		−20.10	
B	53.27		4.74		46.45	
C	52.53		−2.04		22.42	
D	45.02		1.19		6.85	

2.3.4. Rheological Behavior Determination

The viscosity of the emulsion samples (samples were mixed before analysis) was obtained using a rotational viscometer (Brookfield, RV-DVII, USA) with a spindle no.4 at 5°C and at rotation speed of 1.5 rpm.

2.3.5. Statistical Analysis

Mixture design was used to study the effect of added four components on rheological, particle size, surface tension, and stability of O/W emulsion. When working with four components, the experimental domain corresponded to a tetrahedron (quadrangular). All possible mixtures could be identified by a point in this space, with vertices corresponding to the pure components. The experimental results were analyzed using Expert-Design 6.0.6 (Stat-Ease Inc., Minneapolis, MN, USA). An analysis of variance (ANOVA) was performed to determine the statistical significance of the fitted models. The canonical form of a mixture model for four components takes the form of the following "interaction model"

$$Y = Ax_1 + Bx_2 + Cx_3 + Dx_4 + ABx_1x_2 + ACx_1x_3 + ADx_1x_4 + BCx_2x_3 + BDx_2x_4 + CDx_3x_4 + e$$

A, B, C, D: Component

AB, AC, AD, BC, BD, CD: Interactions between components.

2.3.6. Optimization

A desirability function method was used for optimization of responses. The method finds the desired targets for each factor and response. All independent factors were kept within range while the responses, target and in range, were either minimized, maximized. The numerical optimization finds a point that maximizes the desirability function. The characteristic of a target may be changed by adjusting the weight or importance.[12] In this study, desirability functions were developed for the criteria of maximum viscosity, minimum surface tension, and particle size.

3. RESULTS AND DISCUSSION

3.1. Particle Size

3.1.1. Modeling of Particle Size Distribution

The objective of using mixture design is to understand the effect of mixture components on measured properties of the formulations. In this type of design, the sum of the components should be equal to 1 or 100. Therefore, by increasing one component content in formulation, other components should be decreased. Analysis of variance of emulsions particle size is given in Table 2. This shows that a linear model is well attuned to the experimental data of particle size response. A linear model was found to explain the effect of each component on particle size (Equation (1)).

$$\text{Particle size} = -99.86\,A + 90.27\,B + 21.63\,C - 22.85\,D \quad [1]$$

where A, B, C, and D are emulsifier, phytosterol, water and oil weight percentages in formulation, respectively. As it is clear in this equation, coefficient of each model term explains the effect of the model term on particle size. While emulsifier and oil had negative sign, phytosterol and water had positive sign. This means that emulsifier and oil had a decreasing effect and phytosterol and water had an increasing effect on particle size of the emulsions.

It is obvious from Table 2 that all components had significant effects on particle size. Coefficient estimate or regression coefficient for each model term (Table 2) was estimated by the least square method from the results given by the experiments and represents the expected change in the response per unit change in this term when all remaining terms of model are held constant. This is a measure of the effect of this model term relative to other terms in the model. Therefore, the effect of various model terms on response variables can be compared by this coefficient directly. It can be seen from Table 2 that coefficient estimation for phytosterol (B) is the highest and for the emulsifier (A) the lowest than other terms of model.

3.1.2. Influence of Formulation Composition on Particle Size

Particle size distribution of the emulsion particles was determined after preparation. Table 3 shows particle size distribution of diluted emulsion. As results show, the mean particle size of samples is between 10.187 and 33.121 μm. Figure 1a shows counter plots of predicted particle size for the mixtures containing 69% water. Particle size of the emulsions depends on percentages of emulsifier, phytosterol, and oil. As can be seen in Figure 1a and Equation (1), by increasing the emulsifier and oil content in the emulsions, particle size decreased and by increasing the phytosterol content in the emulsions, particle size increased. Phytosterol has the highest effect on increasing of particle size. This might be because phytosterols are oil soluble components and in formulations containing low concentration of emulsifier and oil, they are not soluble and might be in crystal form, and resulted in increasing particle size of emulsions. As in formulation containing low concentration of oil and emulsifier (emulsifier and soy oil were used for dissolving and dispersing phytosterols), by increasing phytosterol content in the emulsions, particle size increased, because of the concentration oil and insufficiency of emulsifier for solubility of phytosterols.

TABLE 3
Surface tension, serum separation, particle size, particle size distribution, and viscosity responses of the formulations

Formulation[a]	Surface tension (mN/m)	Serum separation (ml)	D[4,3][b] (μm)	Size distribution (μm)	Viscosity (at 1.5 rpm)(cP)
F6.25/17.5/61.25/15	33.45 ± 1.49	4 ± 0.25	22.637	2.655–32.968	78500
F10/10/80/0	31.25 ± 1.32	1.2 ± 0.15	12.723	4.225–23.979	233500
F5/15/70/10	51.70 ± 1.76	0.5 ± 0.30	20.057	2.456–25.930	118000
F5/10/65/20	49.20 ± 0.70	0 ± 0	15.470	1.721–34.619	125000
F10/20/70/0	33.60 ± 1.34	2 ± 0.25	24.161	4.567–32.452	45500
F10/10/60/20	27.35 ± 1.75	0 ± 0	10.187	1.448–24.710	285000
F10/20/50/20	28 ± 0.49	0 ± 0	15.119	2.425–33.150	183200
F7.5/15/57.5/20	40.30 ± 0.49	0.2 ± 0.32	13.905	2.071–30.634	186200
F5/10/85/0	53.10 ± 1.15	3.7 ± 0.30	24.218	4.254–29.497	26800
F5/20/55/20	50.60 ± 1.60	0 ± 0	15.625	2.549–32.859	122700
F7.5/10/72.5/10	35.30 ± 0.61	1 ± 0.2	14.187	2.471–30.234	183500
F5/15/80/0	54.50 ± 1.28	4.2 ± 0.2	32.826	4.793–34.147	21400
F10/10/80/0	32 ± 1.35	1.4 ± 0.2	12.999	4.075–24.191	188000
F10/15/75/0	32.20 ± 1.39	1.7 ± 0.25	23.353	4.640–31.503	58500
F6.25/12.5/76.25/5	43.90 ± 1.65	2.5 ± 0.35	12.640	2.527–26.179	282500
F10/20/60/10	29.80 ± 0.41	0.2 ± 0.25	19.705	2.007–27.979	121500
F5/10/65/20	50.10 ± 0.40	0 ± 0	15.253	1.925–34.359	127800
F6.67/20/66.67/6.7	38.7 ± 1.30	3 ± 0.20	24.999	3.214–36.624	23500
F5/20/75/0	55.5 ± 1.11	6.7 ± 0.36	33.121	4.503–34.157	18600
F5/20/55/20	51.10 ± 1.65	0 ± 0	15.240	2.849–32.459	133400
F10/15/65/10	29.80 ± 1.20	0.1 ± 0.2	15.620	2.149–33.159	124600
F10/20/50/20	27.20 ± 1.06	0 ± 0	15.211	2.125–33.459	152800
F10/10/60/20	28.25 ± 0.40	0 ± 0	10.690	1.948–23.710	288000
F8.75/15/71.25/5	38.50 ± 1.06	2 ± 0.15	12.148	2.681–24.115	283000

[a]Formulations (F) indicate the content (%, wt/wt) of emulsifier/phytosterol/water/oil.
[b]Weight mean of volume diameter of microcapsules per μm.

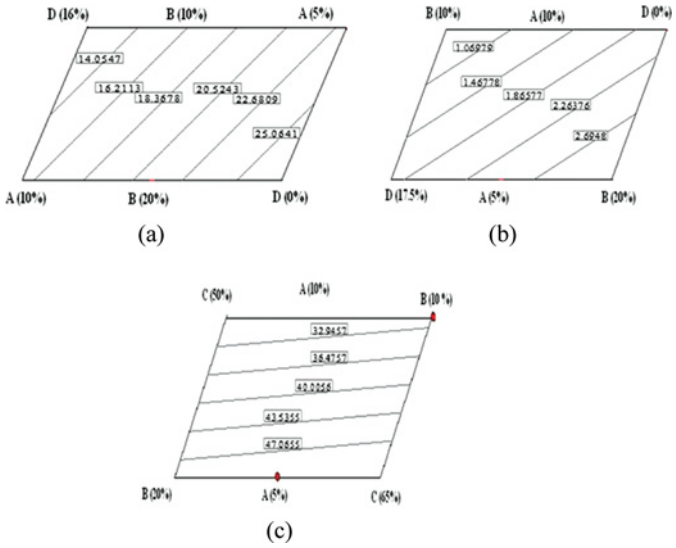

FIG. 1. Counter plots of predicted responses of the formulations: a) particle size, b) emulsion stability of formulations, c) surface tension. (Figure available in color online.)

By comparing each pairs of the formulations (2, 6), (5, 7), (9, 17), (14, 21), (19, 20), and (3, 12) it can be concluded that at the same concentration of the emulsifier and phytosterol, increasing oil in formulations leads to decrease in particle size. This conforms to coefficients of Equation (1).

In the formulations containing low concentration of emulsifier and high amount of phytosterol, coalescence of droplets affects measured size of emulsions. Comparing formulations 9 and 19 shows that at low concentration of emulsifier and oil, increasing phytosterol causes the increase in particle size, but at low concentrations of phytosterol, increasing emulsifier to the maximum level, leads to the decline of particle size (formulations (2, 9) and (4, 6)).

At minimum concentration of oil (0%) and same concentration of the emulsifier, by increasing phytosterol from 10% to 20%, an increase in particle size was seen (formulations 2, 5, and 14) and (9, 12, and 19) but at same concentrations of oil and phytosterol, by increasing emulsifier from 5% to 10%, particle size was decreased. This is the case for formulations of (2, 9), (4, 6), (3, 21), and (7, 10).

At maximum concentration of oil (20%) and constant concentration of emulsifier, by increasing phytosterol from 10% to 20%, particle size of samples were increased (formulations 4, 10 and 6, 7). Although, this increase depends on oil content of the formulations; as oil quantity in a formulation decreased, in the same content of phytosterols, particle size was increased. Based on these result and Equation (1), it can be concluded that emulsifier has highest effect on decreasing particle size in O/W emulsions. This is probably because the emulsifiers decrease interfacial tension of O/W interface and create a thin layer around oil droplet which prevents coalescence of droplets. Niraula et al.[13] showed the same results for effect of emulsifier on particle size and reported that the particle size of the dispersed phase decreased with the increase in both the bulk surfactant concentration and length of the alkyl tail of the surfactant. Therefore, oil volume fraction and emulsifier concentration significantly affected the particle size. As the oil volume fraction and emulsifier concentration increased, the particle size decreased. This observation has also been reported by previous researchers.[14]

The particle size in F10/10/60/20 was smaller than other formulations (see Table 3). By increasing the emulsifier and oil contents to maximum level in formulations, particle size decreased. Formulations containing a large quantity of oil and emulsifier (e.g., F10/10/60/20) had smaller particle size compared with those containing less emulsifier and oil (e.g., F5/20/75/0).

3.2. Influence of Formulation Composition on Emulsion Stability

The effect of oil, emulsifier, phytosterol, and water content on emulsion stability after production was studied. Emulsion stability was followed by changes in average droplet size and rheology. Results of serum separation (emulsion stability) of the formulations are given in Table 3. Analysis of variance shows that a linear model is well attuned to the experimental data of emulsion stability. Figure 1b shows counter plots of predicted emulsion stability for the mixtures containing 67.5% water. As can be seen, emulsion stability depends on percentages of emulsifier, phytosterol and oil in the formulations.

Formulations containing 0% oil (results are presented in Table 3) were not stable during stability test and serum separation was observed in these samples rapidly. Phytosterol is in solid state at ambient temperature (melting temperature of phytosterol is about 138 to 154°C) and in these cases the samples were a dispersion of phytosterol in water. Therefore, by centrifuging the samples, the water phase was separated easily. By adding oil to the formulations and because of the presence of emulsifier, oil, and phytosterol are dispersed in water phase. Moreover, phytosterol is oil soluble component and addition of oil caused solubilization of phytosterol. Formulations containing oil were stable during the test and serum separation was not observed. Therefore, the soy oil has an important effect on emulsion stability. Presence of emulsifier in the formulations act as surfactant and prevent the coalescence and flocculation of lipid particles.

At constant levels of emulsifier, by increasing dispersed phase (oil and phytosterol), emulsion stability was reduced. This is probably because of the emulsifier creating a stable thin layer around oil droplet that prevents coalescence of droplets. Therefore, at constant levels of emulsifier, by increasing dispersed phase, emulsifier content is not enough to create a protective layer around the dispersed phase and the resulting layer does not have sufficient strength and therefore, emulsion stability decreases. Moreover, emulsifiers, by reducing particle size, increase emulsion stability. In the formulations containing 5% emulsifier (F5/10/85/0, F5/15/80/0, and F5/20/75/0), serum separation was 3.7, 4.2, 6.7 ml, respectively. In formulations containing 10% emulsifier (F10/10/80/0, F10/15/75/0, F10/20/70/0), serum separation was 1.2, 1.7, 2 ml, respectively. In these formulations, at constant oil levels and emulsifier, by increasing phytosterol, serum separation was increased. Therefore, as Figure 1b shows, phytosterol adversely affects stability of emulsions.

3.3. Influence of Formulation Composition on Surface Tension

The surface tension of the formulations is given in Table 3. The results of surface tension were analyzed by mixture design and the results of ANOVA and contour plot of predicted results are presented in Table 2 and Figure 1c.

A linear model was found to explain the effect of each component on surface tension (Equation (2)). The coefficients of the linear model, estimated by the least squares method from the results given by the experiment, are presented in Equation (2).

$$\text{Surface tension} = -3.461A + 0.753B + 0.732C + 0.518D \quad [2]$$

Equation (2) shows the importance of each component. Based on this equation, the effect of emulsifier on surface tension is more important than other components. Emulsifier is adversely affected surface tension while phytosterol, water and oil increased surface tension (Figure 1c and Equation (2)). It is obvious from Table 2 that all components had significant effects on surface tension. It can also be seen from Table 2 that coefficient estimation for phytosterol (B) is higher and for emulsifier (A) lower than other terms of model.

Formulations containing a large quantity of emulsifier (e.g., F10/10/60/20; F10/20/50/20) had lower surface

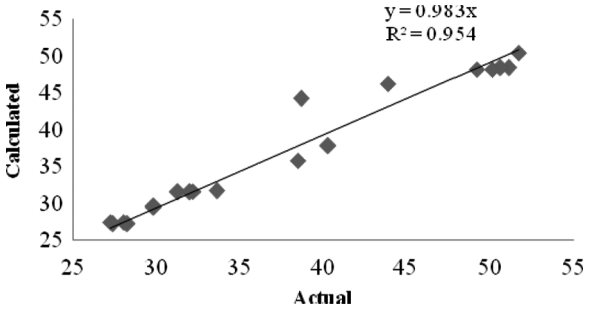

FIG. 2. Correlation between actual and calculated data for validation of linear model for surface tension.

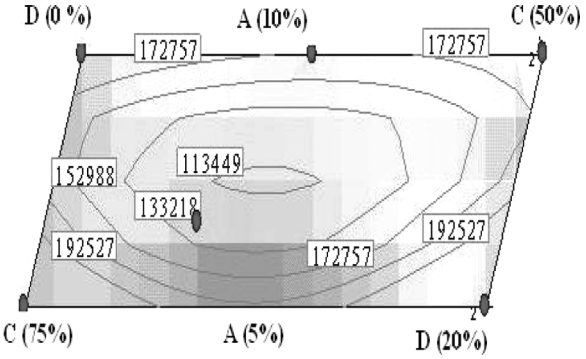

FIG. 3. Counter plots of predicted viscosity of the formulations.

tension compared to those containing less emulsifier (e.g., F5/10/85/0; F5/20/75/0; F5/15/80/0) (see Table 3). Base on Table 3 and Equation (2), emulsifiers reduce the surface tension. The effect of emulsifier on surface tension depends on emulsifier nature and its concentration. It can be transported to the interface where it lowers surface tension and forms surface protective layer.

Niraula et al.[13] showed that the presence of surface active agent helps reduce the tension (interfacial free energy) at the interface and thus, renders some degree of stability to the resulting emulsion system.

Figure 2 shows correlation between calculated and predicted data for final validation of surface tension models; R-squared coefficient was 0.95 for surface tension responses.

TABLE 4
Estimations of coefficients and probabilities of special cubic model for viscosity of the formulations

	Viscosity	
Source	Coefficient estimate	Probability[a]
Model		<0.0001
AB	1.597E + 007	0.0779
AC	−5.677E + 006	0.1111
AD	−7.098E + 006	0.0512
BC	5.319E + 006	0.0002
BD	6.883E + 006	<0.0001
CD	5.701E + 005	0.1104
ABC	−3.086E + 007	0.0093
ABD	−3.104E + 007	0.0062
ACD	7.055E + 006	0.0257
BCD	−6.376E + 006	0.0002

[a]*P* values less than 0.0500 indicate the model terms are significant; *P* values greater than 0.1000 indicate the model terms are not significant.

A: emulsifier; B: phytosterol; C: water; D: oil.

3.4. Influence of Formulation Composition on Viscosity

Viscosity of the formulations is given in Table 3. Results of viscosity were analyzed by mixture design. The results of ANOVA and contour plot of predicted results are presented in Table 4 and Figure 3. Emulsion viscosity was measured at 1.5, 3, 5, 10, 15, 20, 30 rpm. In most cases, the viscosity of the formulations decreased when the spindle speed was increased. Viscosity of the formulations was compared at 1.5 rpm spindle rotational speed.

A special cubic model was found to explain the effect of each component on viscosity (Equation (3)).

$$\begin{aligned}\text{Viscosity} = {} & -87429.189\,A - 5.112\,B - 19195.636\,C \\ & - 90129.854\,D + 49024.035\,AB + 2563.574\,AC \\ & - 6781.163\,AD + 7941.083\,BC \\ & + 16674.054\,BD + 1129.675\,CD \\ & - 719.790\,ABC - 724.022\,ABD \\ & + 164.549\,ACD - 148.70749\,BCD\end{aligned}$$

Emulsion components had a significant effect on viscosity of the samples. While interactions between emulsifier with other components (oil, water and phytosterol), also

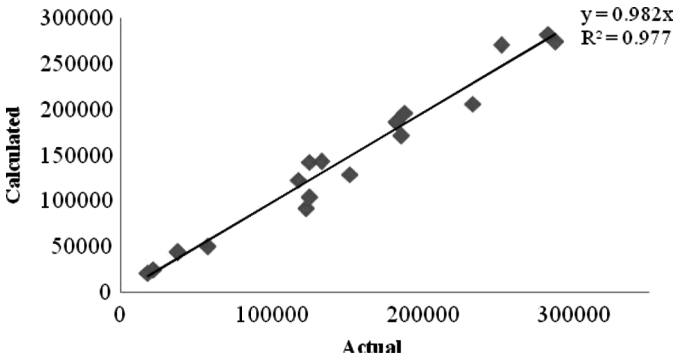

FIG. 4. Correlation between actual and calculated data for validation of special cubic model of formulations viscosity.

TABLE 5
Solutions for optimum condition by the desirability function method

Solution number	Emulsion components				Responses				
	Emulsifier	Phytosterol	Water	Oil	Viscosity	Surface tension	Particle size	Serum separation	Desirability
1	10.00	10.00	68.39	11.61	148435	29.07	11.18	0.43	0.76
2	10.00	20.00	56.08	13.92	172349	28.78	17.01	8.96	0.72
3	10.00	14.82	64.25	10.93	138530	29.31	14.79	0.49	0.69
4	7.58	20.00	52.42	20.00	179382	37.63	17.25	0.0008	0.64
5	7.60	19.26	53.14	20.00	164198	37.54	16.72	6.11	0.63

interactions between oil and water had no significant effect, other interactions had a significant effect on viscosity of emulsions (Table 4).

Figure 4 shows correlation between calculated and predicted data for final validation of viscosity models; R^2 coefficient was 0.97 for viscosity responses.

Viscosity of the emulsion samples was strongly influenced by the particle size, which, in turn, was affected by oil content and surfactant concentration. The viscosity of emulsions as a function of particle size is given in Table 3. The viscosity of emulsion samples was increased by decreasing particle size. This might be because there is an increase in particle size, number of the particles per unit volume of emulsion decreases, and mean distance between particles increases. Therefore, the particles become more mobile and show low resistance to flow. Maximum viscosity of samples was observed for F10/10/60/20 (containing 10% emulsifier, 10% phytosterol, 60% water, and 20% vegetable oils), because in this formulation, particle size was the minimum.

Niraula et al.[13] reported similar results for the effect of particle size of the dispersed phase on emulsion viscosity.

3.5. Optimization

As noted earlier, phytosterol had the highest positive coefficient estimate and played the most important role in increasing response variables of surface tension and particle size. On the other hand, increasing surface tension and particle size is highly related to phytosterol content in the formulations. Emulsifier had the highest negative coefficient estimate and played the most important role in decreasing response variables of surface tension, particle size and serum separation (increasing of emulsion stability). On the other hand, decreasing surface tension, particle size and serum separation is highly related to emulsifier content in the formulations. Emulsifier, oil, and water interactions had the highest positive coefficient estimate and played the most important role in increasing response variable of viscosity, emulsifier and oil interactions had the highest negative coefficient estimate and played the most important role in decreasing response variable of viscosity (Table 4).

The optimum condition for enrichment of yogurt with phytosterol was determined to obtain maximum viscosity, minimum surface tension, and particle size. Numerical optimization carried out for the emulsion components of enriched yogurt obtained the best quality yogurt. To perform this operation, Design Expert software, version 6.0.6, was used for optimization of the responses. The desired targets for each response were chosen to adjust the shape of its particular desirability function. Table 5 shows three software-generated optimum conditions of variables with the predicted values of responses. Four solutions for optimum condition were different, as they had desirability of 0.76, 0.72, 0.69, and 0.64. Among them, solution 1 was selected as the optimum condition of fortified yogurt production (Table 5). This solution has maximum desirability and minimum particle size.

4. CONCLUSIONS

Mixture design involving an experimental design and regression analysis was used to evaluate the effects of emulsifier, phytosterol, oil and water on viscosity, particle size, emulsion stability and surface tension acceptability of the formulations. Composition of formulations had a significant effect on viscosity, emulsion stability, surface tension, and particle size. The soy oil has an important effect on emulsion stability. Moreover, phytosterol is oil soluble component and addition of oil caused solubilization of phytosterol. By increasing the quantity of emulsifier and oil in the formulations, the particle size of emulsions would decrease to the extent that it can lead to increasing emulsion viscosity. The formulations were optimized by numerical optimization method (desirability function).

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