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Modeling the physicochemical properties of orange beverage emulsion as function of main emulsion components using response surface methodology

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ABSTRACT

The effect of main beverage emulsion components namely Arabic gum (7–13% w/w), xanthan gum (0.1–0.3% w/w) and orange oil (6–10% w/w) on physicochemical properties of orange beverage emulsion was determined by using a three-factor central composite design (CCD). The reduced models with high R^2 ($\geqslant 0.80$) values and non significant ($p > .05$) lack of fit were significantly ($p < .05$) fitted to the experimental data, thus ensuring a satisfactory fitness of the regression models relating the response to independent variables. The quadratic effect of xanthan gum had a significant ($p < .05$) term in all reduced models. The independent variables had the most significant ($p < .05$) effect on turbidity loss rate and viscosity ratio. The overall optimum region resulted in the desirable orange beverage emulsion was predicted at a combined level of 13% (w/w) Arabic gum, 0.3% (w/w) xanthan gum and 10% (w/w) orange oil.

1. Introduction

Emulsions are dispersions of one liquid phase in the form of fine droplets in another immiscible liquid phase. The two immiscible phases are usually oil and water. The contact between oil and water molecules is energetically unfavorable (McClements, 1999). Thus, colloids and dispersions are inherently unstable systems, however, they can be considered as kinetically stable if their destabilization velocity is sufficiently low compared with the expected lifespan (Florence & Rieg, 1983). This may be explained by the fact that the change in the free energy during emulsion formation is usually positive and therefore emulsions are thermodynamically unstable system (Kontogiorgos, Biliaderis, Kiosseoglou, & Doxastakis, 2004). Kinetically stable emulsions can be formed by adding emulsifiers and/or thickening agents to overcome the activation energy of the system (Sun, Gunasekaran, & Richards, 2007). Polysaccharides are added as thickening agents so as to modify the rheological behavior of the continuous phase, thereby retarding or inhibiting the process of creaming (Kontogiorgos et al., 2004). Emulsification efficiency, droplet size and interfacial surface area which are directly linked to the stability of the system can be monitored by evaluating the emulsion turbidity (Williams, Phillips, & Randall, 1990).

The rheological behavior of an emulsion is a critical characteristic from both fundamental and applied point of view. Rheological measurements provide information on the physical stability, droplet size and distribution of the emulsion. Substantial changes in the rheology of the bulk phase, as induced by gelation or depletion phenomena, can be indicative of large changes in emulsion stability and droplet size and distribution (Tadros, 2004). The emulsion rheology is mainly governed by several factors such as the rheological properties of the continuous phase, average particle size and distribution, deformability, internal viscosity and droplet concentration and the nature of the particle–particle interaction (Barnes, 1994).

The characteristics of a suspension can be determined by understanding how individual colloids interact with one another. In fact, the relative magnitude of attractive (van der Waals, hydrophobic and depletion) and repulsive (electrostatic, steric and thermal flocculation) forces between the droplets can affect the rheological behavior, physical stability and optical characteristics of the emulsion systems. Stabilization of dispersions with polymers also occurs via two main mechanisms that depend upon the surface association of the polymer and particle (Ingersent, Klein, & Pincus, 1990): steric stabilization results from macromolecules adsorbed to the particle surface; and electrostatic stabilization which arises from the mutual repulsion between both particle's electrical

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double layer. Thus, the optimum stability of emulsion is provided by maximizing repulsive forces between emulsion droplets in order to prevent emulsion droplets from gathering into larger, faster settling agglomerates.

The physicochemical characteristics of oil-in-water emulsions such as beverage emulsion are important for designing of relevant equipment and apparatus, process control, handling, storage and shelf-life. In this work, a three-factor central composite design (CCD) was used to determine the effect of three main emulsion components namely Arabic gum content (7–13% w/w), xanthan gum content (0.1–0.3% w/w) and orange oil content (6–10% w/w) on the physicochemical emulsion properties of orange beverage emulsion. The main objective of present study was to determine an optimum level of the main emulsion components leading to (1) the highest magnitude of viscosity along with viscosity ratio, ζ-potential, electrophoretic mobility and (2) the least loss rate of turbidity. It should be noted that the other critical parameters such as turbidity, average particle size, polydispersity index, pH, density and flavor release of orange beverage emulsion have also been investigated in our other studies (Mirhosseini, Tan, Hamid, & Yusof, 2008, 2007). These optimization studies allow the manufacturers for the formulation of an optimum orange beverage emulsion with desirable emulsion properties.

2. Materials and methods

2.1. Materials

Arabic gum was provided by Colloids Naturals International Co. (Rouen, France). Xanthan gum was donated by CP Kelco (San Diego, CA, USA). Citric acid, sodium benzoate and potassium sorbate ($\geqslant$95%) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Valencia cold pressed orange oil was provided by Danisco (Cultor, Aarhus, Denmark).

2.2. Preparation of orange beverage emulsion

As demonstrated in our previous study (Mirhosseini et al., 2007), a representative orange beverage emulsion is usually composed of two phases: water phase and oil phase. To prepare the water phase, sodium benzoate, potassium sorbate and citric acid were sequentially dispersed in deionized water (60 °C) using a high shear blender (Waring blender 32BL80, Torrington, CT, USA). While mixing the solution, Arabic gum was gradually added to deionized water (60 °C) and mixed for 3 min to facilitate hydration. The Arabic gum solution was kept overnight at room temperature to fully hydrate (Buffo, Reineccius, & Oehlert, 2001). To prepare the water phase, xanthan gum solution was prepared separately by dissolving xanthan gum in deionized water and then mixed with Arabic gum solution by using a high speed blender. While mixing the water phase, the cold pressed orange oil was gradually added into water phase to provide an initial coarse emulsion. Fine emulsification (i.e. an small droplet size < 1 μm and narrow particle size distribution) was achieved by subjecting the pre-emulsions to prehomogenization using the high shear blender (Silverson L4R, Buckinghamshire, UK) for 1 min and then passed through a high pressure homogenizer (APV, Crawley, UK), for three passes (30, 28 and 25 MPa) (Mirhosseini et al., 2007).

2.3. Analytical methods

2.3.1. Turbidity loss rate (cloud stability index)

Emulsion breakage was monitored by measuring the absorbance (loss rate of turbidity) at 500 nm (Buffo et al., 2001) by means of a UV–visible spectrophotometer (Spectronic Genesys™ 10, GENEQ Inc., Montreal, Canada). In this technique, cloud stability is determined by measuring the turbidity as a function of time (Pearce & Kinsella, 1978). The readings were taken twice daily until absorbance fell to 10% of the initial absorbance value. The beverage emulsions were diluted to 0.25% (w/w) in a 10% sugar solution and stored in 1 l plastic bottles at room temperature before the absorbance reading. Absorbance vs. time data for each sample was fitted to a first-order model by the following equation (Labuza, Nelson, & Nelson, 1991):

$$\ln A = \ln A_0 - k_1 t \tag{1}$$

where t is the time, A the absorbance at time = t, A_0 the absorbance at time = 0 and k_1 the first-order rate constant. The emulsion stability end-point or "time to breakage" was computed from the above fitting equation at $A = 0.1A_0$. In this study, k_1 (first-order rate constant) was calculated as the loss rate of turbidity. The beverage emulsion involving the smaller k_1 value indicated the lower loss rate of turbidity, thus showing the higher cloud stability than the emulsion having the larger k_1 value. Daily absorbance values were the average of three consecutive readings.

2.3.2. Viscosity and fluid behavior

Immediately after the preparation of orange beverage emulsions, their viscosities were measured by means of a steady stress Brookfield viscometer (Brookfield DV-II+ Programmable Viscometer, Middleboro, MA, USA) equipped with the LV spindles. The measurement range of a Brookfield viscometer DV-II+ appears in the unit of cP or mPa s. To better understand the fluid behavior of beverage emulsions, viscosity readings were taken at rotational speeds of 2, 10, 20, 30, 50, 60 and 100 rpm (İbanoğlu, 2002). The measurement recorded at 60 rpm was considered as emulsion viscosity for the data analysis.

A common method for characterizing the fluid behavior is to determine the ratio of the fluid's viscosity as measured at two different spindle speeds (with the same spindle). These measurements are usually made at speeds that differ by a factor of 10 (for example 2 and 20 or 10 and 100 rpm) and then the viscosity ratio is calculated to judge the fluid behavior. In constructing the viscosity ratio, the viscosity value at the lower speed should be placed in the numerator, the one at the higher speed in the denominator. For pseudoplastic (shear-thinning) fluids, the ratio will exceed 1.0 as the degree of pseudoplastic behavior increases. Conversely, for dilatant (shear-thickening) fluids, the ratio will be less than 1.0 as the degree of dilatancy increases. The rheological behavior of emulsions approaches Newtonian character when the viscosity ratio would to close to 1.0. In this study, the viscosity ratio (10 rpm/100 rpm) was taken as a possible index for interpreting the fluid behavior of orange beverage emulsion. For each emulsion, the measurements were an average of two replicates.

2.3.3. ζ-Potential and electrophoretic mobility

The orange beverage emulsions were diluted (1:100) for the measurement of ζ-potential and electrophoretic mobility using a Malvern zeta sizer (Malvern series ZEN 3500, Malvern Instruments Ltd., Malvern, Worcester, UK). The velocity of a particle in an electric field is commonly referred to as its electrophoretic mobility. This is obtained by performing an electrophoresis experiment on the sample and measuring the velocity of the particles using laser Doppler velocimetry (LDV). Measurements of the electrophoretic mobility were carried out to evaluate the surface net charge around emulsion droplets (Hunter, 1981). To avoid multiple scattering effects, the emulsions were diluted with deionized water prior to analysis; then directly placed into the module. The measurement ranges of a Malvern zeta sizer appear in the units of mV and μm cm/Vs for ζ-potential and electrophoretic mobility, respectively. The measurements of ζ-potential and electrophoretic mobility were carried out immediately after emulsion preparation.

The measurements were reported as the average of three individual injections, with four readings made per injection. The averages of triplicate values obtained were taken as the response values for ζ-potential, and electrophoretic mobility.

2.4. Experimental design

Response surface methodology (RSM) was used to determine the effect of main emulsion components namely Arabic gum (7–13% w/w, x_1), xanthan gum (0.1–0.3% w/w, x_2) and orange oil contents (6–10% w/w, x_3) on turbidity loss rate (emulsion stability index) (Y_1), viscosity (Y_2), viscosity ratio (fluid behavior index) (Y_3), ζ-potential (Y_4) and electrophoretic mobility (Y_5) of orange beverage emulsion. Twenty beverage emulsions were prepared based on the CCD with three independent variables at five levels for each variable. Experiments were randomized in order to minimize the effects of unexplained variability in the actual responses due to extraneous factors. The center point was repeated six times to calculate the repeatability of the method (Montgomery, 2001). The matrix of CCD is shown in Table 1.

2.5. Statistical analysis

Response surface analysis was conducted to (1) determine regression coefficients and statistical significance of model terms and (2) fit the regression models to the experimental data aiming at an overall optimal region for all response variables studied. The behavior of the response surface was investigated for the response function (Y_i) using the polynomial regression equation. The generalized response surface model is given below:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ii} x_1 x_j \tag{2}$$

where Y is response calculated by the model; β_0 is a constant; β_i, β_{ii} and β_{ij} are linear, squared and interaction coefficient, respectively. Joglekar and May (1987) suggested that for a good fit of a model, R^2 should be at least 0.80. The corresponding variables will be more significant ($p < .05$) if the absolute t value becomes larger and the p-value becomes smaller. Only the significant ($p < .05$) independent variable effects were included in the reduced model. Therefore, the terms statistically found non-significant ($p > .05$) were dropped from the initial models and the experimental data was refitted to only significant ($p < .05$) independent variable effects in order to obtain the final reduced model. It should be noted that some variables were kept in the reduced model despite non-significance. For example, linear terms were also kept in the model if a quadratic or interaction term containing this variable was significant ($p < 0.05$). The experimental design matrix, data analysis and optimization procedure were performed using the Minitab v. 13.2 statistical package (Minitab Inc., State College, PA, USA).

Table 1
The matrix of central composite design (CCD)

Treatment runs	Blocks	Arabic gum	Xanthan gum	Orange oil
1	1	7.00	0.10	6.00
2	1	7.00	0.30	10.00
3	1	13.00	0.30	6.00
4*	1	10.00	0.20	8.00
5	1	13.00	0.10	10.00
6*	1	10.00	0.20	8.00
7	2	7.00	0.10	10.00
8	2	7.00	0.30	6.00
9	2	13.00	0.10	6.00
10*	2	10.00	0.20	8.00
11*	2	10.00	0.20	8.00
12	2	13.00	0.30	10.00
13*	3	10.00	0.20	8.00
14	3	14.90	0.20	8.00
15	3	10.00	0.20	4.73
16	3	10.00	0.20	11.27
17	3	10.00	0.04	8.00
18	3	5.10	0.20	8.00
19*	3	10.00	0.20	8.00
20	3	10.00	0.36	8.00

* Center point.

2.6. Optimization and validation procedures

The individual and overall optimization procedures were carried out to obtain the optimal levels of three independent variables (x_1, x_2 and x_3) leading to the desired response goals. An orange beverage emulsion would be considered an optimum product with the overall desirable goals. For graphical optimization, the reduced response models were expressed as three-dimensional (3D) surface plots to better visualize the interaction effect of main emulsion components on the physicochemical properties studied. It should be noted that the 3D plots were drawn by keeping one variable constant at the center point and varying the other two variables within the experimental range. A numerical optimization was also carried out by the response optimizer using the Minitab software for determining the exact optimum levels of individual and simultaneous multiple response optimizations leading to the desirable response goals. The adequacy of the regression equations was checked by comparing the experimental data with predicted values obtained from the equations.

3. Results and discussion

3.1. Fitting the response surface models to significant independent variables

Table 2 shows the experimental data obtained for the each response variable. The estimated regression coefficients of the response surface models, along with the corresponding R^2 values and lack of fit tests are given in Table 3. The significance of each term determined using the F-ratio and p-value are also shown in Table 4. As shown in Table 3, the significant ($p < .05$) response surface models with high R^2 value varied from 0.800–0.975 was obtained for all response variables studied. Therefore, the response surface models were accurately employed for predicting high variation percentage ($\geqslant$80%) of physicochemical emulsion properties as a function of main emulsion components studied. No indication of significant ($p > .05$) lack of fit was observed for the final reduced models, thus ensuring satisfactory fitness of the response surface model to the significant ($p < .05$) independent variable effects (Table 3). As also stated by Montgomery (2001), the response surface equations might be significant ($p < .05$) only in the studied independent variable levels. It may not be true beyond the selected ranges of the independent variables. Therefore, the response models may not be extrapolated beyond these ranges.

As shown in Table 4, the independent variables had the most significant ($p < .05$) effect on turbidity loss rate and viscosity ratio or flow behavior of orange beverage emulsion; while the results indicated that the ζ-potential was significantly ($p < .05$) affected only by the xanthan gum (Table 4). The main effect of xanthan gum showed the most significant ($p < .05$) effect on electrophoretic mobility (Table 4). As also shown in results (Table 4), the quadratic term of xanthan gum showed the significant ($p < .05$) effect on the changes of all response variables; thus the main and quadratic effects of xanthan gum should be kept in all final reduced models. Whereas the least significant ($p < .05$) variable effects on the variation of response variables were found to be the quadratic term of Arabic gum and interaction effect of Arabic gum and orange oil, respectively (Table 4).

Table 2
The experimental data obtained for the response variables (Y_j) (mean ± SD)

Run	Block	Response variable				
		Turbidity loss rate (Y_1, Å/day)	Viscosity (60 rpm, Y_2, mPa s)	Viscosity ratio (Y_3)	ζ-Potential (Y_4, mV)	Mobility (Y_5, μm cm/Vs)
1	1	0.835 ± 0.007	11.4 ± 1.4	0.198 ± 0.008	−26.3 ± 0.43	1.960 ± 0.035
2	1	0.990 ± 0.021	83.5 ± 6.9	1.587 ± 0.052	−26.1 ± 0.82	2.046 ± 0.084
3	1	0.918 ± 0.021	76.0 ± 4.8	1.348 ± 0.201	−27.5 ± 0.69	2.157 ± 0.023
4	1	1.052 ± 0.035	45.5 ± 2.3	1.243 ± 0.045	−28.1 ± 0.75	2.205 ± 0.049
5	1	1.102 ± 0.035	49.0 ± 1.9	1.546 ± 0.083	−25.1 ± 1.26	1.966 ± 0.034
6	1	1.173 ± 0.050	40.0 ± 3.1	1.203 ± 0.107	−26.7 ± 0.67	2.155 ± 0.033
7	2	1.086 ± 0.007	16.2 ± 2.2	0.860 ± 0.031	−24.3 ± 0.77	1.903 ± 0.084
8	2	1.237 ± 0.050	78.5 ± 7.8	1.710 ± 0.075	−27.8 ± 1.23	2.120 ± 0.023
9	2	1.120 ± 0.028	27.5 ± 2.4	1.323 ± 0.402	−26.0 ± 0.35	2.038 ± 0.025
10	2	1.152 ± 0.021	52.0 ± 5.2	1.149 ± 0.096	−27.6 ± 0.62	2.161 ± 0.051
11	2	1.258 ± 0.028	54.4 ± 4.7	1.409 ± 0.108	−27.8 ± 0.76	2.178 ± 0.102
12	2	0.815 ± 0.042	114.0 ± 6.5	1.410 ± 0.302	−26.9 ± 1.07	2.136 ± 0.105
13	3	1.144 ± 0.021	55.0 ± 3.4	1.350 ± 0.074	−28.0 ± 0.79	2.195 ± 0.065
14	3	1.096 ± 0.050	80.1 ± 7.2	1.672 ± 0.010	−28.1 ± 2.43	2.199 ± 0.085
15	3	0.927 ± 0.007	54.5 ± 2.8	1.599 ± 0.096	−28.7 ± 1.02	2.252 ± 0.056
16	3	0.817 ± 0.042	73.0 ± 4.3	1.690 ± 0.303	−26.9 ± 0.27	2.106 ± 0.099
17	3	0.853 ± 0.205	14.4 ± 0.8	0.570 ± 0.025	−22.6 ± 0.14	1.852 ± 0.082
18	3	0.948 ± 0.014	31.2 ± 1.3	1.500 ± 0.403	−28.5 ± 1.28	2.092 ± 0.040
19	3	1.170 ± 0.014	64.0 ± 3.9	1.236 ± 0.081	−27.5 ± 0.63	2.155 ± 0.023
20	3	1.023 ± 0.021	134.0 ± 5.6	1.650 ± 0.108	−27.1 ± 1.35	2.139 ± 0.056

Table 3
Regression coefficients, R^2, adjusted R^2 and lack of fit for the final reduced models

Regression coefficient	Turbidity loss rate (Y_1, Å/day)	Viscosity (Y_2, mPa s, 60 rpm)	Viscosity ratio (Y_3)	ζ-Potential (Y_4, mV)	Mobility (Y_5, μm cm/Vs)
b_0	−1.613	24.951	−1.91	−20.82	1.568
b_1	0.067	−4.361	0.24	–	0.011
b_2	8.635	85.393	21.96	−57.62	3.919
b_3	0.394	−6.618	−0.19	–	–
b_1^2	–	–	–	–	–
b_2^2	−5.842	617.371	−11.30	117.75	−7.808
b_3^2	−0.021	–	0.02	–	–
b_{12}	−0.331	–	−0.98	–	–
b_{13}	–	1.035	–	–	–
b_{23}	−0.364	–	−0.59	–	–
R^2	0.819	0.974	0.930	0.800	0.853
R^2 (adj)	0.655	0.959	0.866	0.747	0.801
Regression (p-value)	0.009[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]
Lack of fit (F-value)	1.77	2.46	1.66	0.63	0.84
Lack of fit (p-value)	0.344[b]	0.248[b]	0.366[b]	0.653[b]	0.614[b]

b_i, the estimated regression coefficient for the main linear effects.
b_{ii}, the estimated regression coefficient for the quadratic effects.
b_{ij}, the estimated regression coefficient for the interaction effects.
1, arabic gum; 2, xanthan gum; 3, orange oil.

[a] Significant ($p < .05$).

[b] Not significant ($p > .05$).

Table 4
The significance of each independent variable effect indicated by using F-ratio and p-value in the final reduced models

Variables		Main effects			Quadratic effects			Interaction effects		
		x_1	x_2	x_3	x_1^2	x_2^2	x_3^2	x_1x_2	x_1x_3	x_2x_3
Turbidity loss rate (Y_1, Å/day)	p-value	0.009[*]	0.001[*]	0.002[*]	–	0.028[*]	0.004[*]	0.007[*]	–	0.032[*]
	F-ratio	10.45	23.43	16.92	–	6.65	13.47	11.59	–	6.23
Viscosity (Y_2, 60 rpm, mPa s)	p-value	0.181	0.260	0.113	–	0.004[*]	–	–	0.018[*]	–
	F-ratio	2.02	1.40	2.93	–	12.43	–	–	7.55	–
Viscosity ratio (Y_3, x/10x)	p-value	0.000[*]	0.000[*]	0.263	–	0.016[*]	0.048[*]	0.000[*]	–	0.040[*]
	F-ratio	43.35	51.54	1.41	–	8.46	5.06	34.45	–	5.58
ζ-Potential (Y_4, mV)	p-value	–	0.000[*]	–	–	0.000[*]	–	–	–	–
	F-ratio	–	45.40	–	–	32.23	–	–	–	–
Mobility (Y_5, μm cm/Vs)	p-value	0.024[*]	0.000[*]	–	–	0.000[*]	–	–	–	–
	F-ratio	6.37	52.22	–	–	35.24	–	–	–	–

x_1, x_2 and x_3 represent the main effect of Arabic gum, xanthan gum and orange oil, respectively.
x_1^2, x_2^2 and x_3^2 represent the quadratic effect of Arabic gum, xanthan gum and orange oil, respectively.
x_1x_2, x_1x_3 and x_2x_3 represent the interaction between Arabic gum and xanthan gum, interaction between Arabic gum and orange oil and interaction between xanthan gum and orange oil, respectively.

[*] Significant at $p < .05$.

3.1.1. Turbidity loss rate (cloud stability index)

As shown in Table 2, the positive loss rate of turbidity indicated that the turbidity of orange beverage emulsion decreased during storage. Previous researchers (Dłużewska, Stobiecka, & Maszewska, 2006; Ray, Johnson, & Sollivan, 1983) also reported the decrease in the turbidity of emulsions and beverages during their storage. Ray et al. (1983) described that this phenomenon can be interpreted by the aggregation of oil droplets and the changes in refractive index of oil phase and aqueous phase. Dłużewska et al. (2006) explained that the decrease of turbidity of beverage emulsion in its diluted form could be caused by loss of polysaccharides molecules for external layers of film formed on the interfacial surface, which led to the change in refractive index of disperse phase. The results indicated that the main effects of independent variables showed a significant ($p < .05$) positive effect on the variation of turbidity loss rate (Y_1); while the interaction effects of independent variables had a significant ($p < .05$) negative effect on the turbidity loss rate (Y_1) (Tables 3 and 4).

As shown in Table 4, all independent variable effects except for the quadratic effect of Arabic gum and its interaction with orange oil were found to be significant ($p < .05$) on the variation of turbidity loss rate (Y_1). The most significant ($p < .05$) effect on turbidity loss rate (Y_1) was shown to be the main effect of xanthan gum followed by the main and quadratic effects of orange oil. As mentioned earlier, the turbidity loss rate was studied as an indicator for evaluating the cloud stability. Therefore, the results suggested that these variables should be considered as significant ($p < .05$) primary factors affecting the cloud stability of orange beverage emulsion. Previous researchers (Taherian, Fustier, & Ramaswamy, 2006) also reported that the cloud stability was significantly ($p < .05$) governed by the oil phase concentration. The significant ($p < .05$) quadratic and interaction effects of independent variables on turbidity loss rate demonstrated that the variation of turbidity loss rate (Y_1) (or cloud stability) could be explained as a nonlinear function of main emulsion components studied (Table 4). Fig. 1(a and b) clearly exhibited a nonlinear relationship between the significant ($p < .05$) interaction effect of independent variables and turbidity loss rate (Y_1). Thus, the present study offered that the interaction effects between emulsion components should be considered as important parameters affecting the cloud stability of orange beverage emulsion.

The individual optimization procedure exhibited that an orange beverage emulsion containing 13% (w/w) Arabic gum, 0.3% (w/w) xanthan gum and 10% (w/w) orange oil was predicted to provide the highest cloud stability (the minimum loss rate of turbidity, $Y_1 = 0.784$ Å/day); whereas combined level of 7% (w/w) Arabic gum, 3% (w/w) xanthan gum and 7.11% (w/w) orange oil was estimated to result in the least cloud stability (the highest turbidity loss rate, $Y_1 = 1.189$ Å/day). As indicated in our previous study (Mirhosseini et al., 2007), the highest concentration levels of main emulsion components led to the least polydispersity index (PDI). Thus, it could be concluded that the optimum region which resulted in the narrow size distribution (low PDI) was predicted to provide the least loss rate of emulsion turbidity (the highest cloud stability).

As mentioned earlier, high concentrations of both hydrocolloids were predicted to provide the least loss rate of turbidity (the highest cloud stability). This observation may be explained by the positive effect of hydrocolloids on the emulsion viscosity and/or the presence of active sites in the structure of hydrocolloids. As demonstrated by Williams et al. (1990), Arabic gum consists of three distinct fractions include: a high molecular mass arabinogalactan plus protein complex (AGP); a glycoprotein (G1) and a lower molecular mass fraction, an arabinogalactan polysaccharide, which is protein-deficient (AG). The AGP fraction is mostly responsible for the emulsifying properties: the hydrophilic carbohydrate blocks are linked to the protein chain that strongly adsorbs at o/w interface promoting emulsion stability (Williams et al., 1990). In fact, the formation of a thick, sterically stabilizing layer around emulsion droplets enables the flavor oil emulsion to be stabilized both as a concentrate and as a diluted form.

3.1.2. Viscosity

In most cases, the viscosity of orange beverage emulsions decreased when the spindle speed was increased. This may be explained by the fact that as the shear rate increased, the droplet–droplet interaction was deformed and eventually disrupted which resulted in the size reduction of the flocs thereby decreasing the viscosity (Peamprasart & Chiewchan, 2006). This observation corresponded to the non-Newtonian pseudoplastic behavior (or shear-thinning) of orange beverage emulsions. As shown in Table 3, the viscosity (60 rpm) was directly proportional to the main and quadratic effects of xanthan gum, and interaction effect of Arabic gum and orange oil. As shown in Table 4, the viscosity (60 rpm, Y_2) of orange beverage emulsions was significantly ($p < .05$) influenced by the quadratic effect of xanthan gum and interaction effect of Arabic gum and orange oil. The quadratic term of xanthan gum had the most significant ($p < .05$) effect on viscosity reported at 60 rpm (Table 4).

As shown in Fig. 1(c), the viscosity (60 rpm) of orange beverage emulsions increased with increasing the proportion of orange oil. The results also indicated that the emulsion viscosity significantly ($p < .05$) increased when the xanthan gum content was increased (Table 3). The positive correlation between the emulsion viscosity and xanthan gum concentration has also been reported in previous study (Sun et al., 2007). As stated by Hemar, Tamehana, and Singh (2001), xanthan gum has high molecular weight molecules (~3,000,000 Da) composed of a 1-4-linked β-D-glucose backbone with three side-chains: a D-glucuronosyl unit between two D-mannosyl units. When xanthan gum is dispersed in water system, its complex molecules form complicated aggregates through hydrogen bonds and polymer entanglement. In the ordered conformation, the side-chains fold back around the main chain to give a structure analogous to a double helix. Because of these highly ordered networks and entanglements, xanthan gum provides a high viscosity at low shear rate. The chemical structure of xanthan gum also composed of a large number of free carboxyl groups. The presence of these active sites in the xanthan gum structure causes a great water absorption capacity followed by increasing the viscosity.

The individual optimum region led to the desirable emulsion viscosity (the highest value, $Y_2 = 118$ mPa s) was estimated to be achieved by a set level of 13%, 0.3% and 10% (w/w) for Arabic gum, xanthan gum and orange oil, respectively. From the optimization results, a combination of high content of main emulsion components resulted in the largest magnitude of emulsion viscosity. This may be interpreted by the reason that the presence of large number of particles induced by high concentration of emulsion components increased the resistance to the flow which hence resulted in an increase in the apparent viscosity. On the other hand, Dłużewska et al. (2006) also reported that the increase of oil phase concentration led to significant ($p < .05$) increase in apparent emulsion viscosity. They explained this observation by the fact that with the addition of Arabic gum, the increase of oil phase induced an increase in average droplet size and considerable extension of droplet size distribution.

3.1.3. Fluid behavior

As reported in Table 2, the viscosity ratio of orange beverage emulsions showed that orange beverage emulsions behaved between Newtonian and pseudoplastic in the most cases. Previous researchers (Peamprasart & Chiewchan, 2006; Suzuki, Maeda,

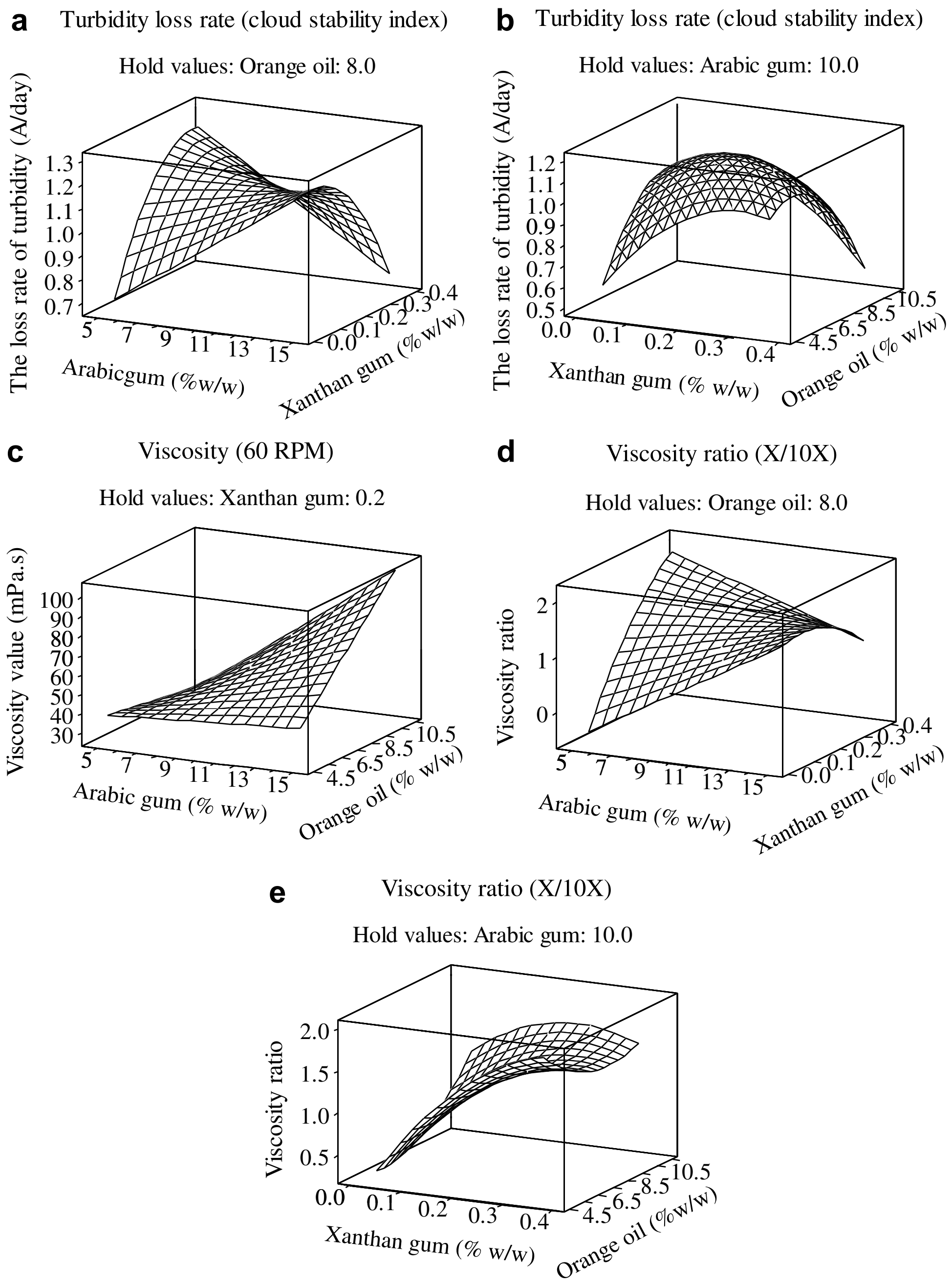

Fig. 1. Response surface plots showing the significant ($p < .05$) interaction effects of Arabic gum, xanthan gum and orange oil on turbidity loss rate (a and b), viscosity (c) and viscosity ratio (d and e).

Matsuoka, & Kubota, 1991) investigated the effect of oil content or emulsifier concentration on the rheological properties of o/w emulsions. They also found that the rheological behavior ranged between Newtonian and pseudoplastic. Dłużewska et al. (2006) also found that Arabic gum-based beverage emulsion behaved as a non-Newtonian pseudoplastic fluid. Sun et al. (2007) similarly described that the emulsions with little or no xanthan gum (0 and 0.02 wt%) behaved like a Newtonian fluid; while the emulsions containing relatively higher xanthan gum concentration ($\geqslant$0.05 wt%) exhibited as a shear-thinning fluid. Previous study (Coia & Stauffer, 1987) also described that the o/w emulsions containing various polysaccharides behaved as pseudoplastic fluid.

Among the orange beverage emulsions, the sample (no. 7) seemed to behave as a Newtonian fluid, as its viscosity ratio was close to 1.0. On the other hand, two orange beverage emulsions (nos. 1 and 17) exhibited a different fluid behavior from the other orange beverage emulsions. This observation was taken by the observation that their viscosities increased when the spindle speed was increased (viscosity ratio < 1). This behavior corresponds to the dilatant (or shear-thickening) fluids. The most significant ($p < .05$) variable effect on viscosity ratio (Y_3) was found to be the main effect of xanthan gum followed by the main effect of Arabic gum (Table 4). The viscosity ratio was significantly ($p < .05$) influenced by most of independent variable effects (Table 4). This observation clearly demonstrated that the rheological behavior of orange beverage emulsion was significantly ($p < .05$) governed by the concentration of emulsion components especially xanthan gum and orange oil contents.

As shown in Fig. 1(d and e), the variation of viscosity ratio (or rheological properties) of orange beverage emulsion could be explained as a nonlinear function of the main components of orange beverage emulsion. The optimization results indicated that combined level of 7% (w/w) Arabic gum, 0.3% (w/w) xanthan gum and 10% (w/w) orange oil was estimated to result in the optimum rheological behavior (the highest viscosity ratio, $Y_3 = 1.78$). Conversely, the orange beverage emulsion formulated by 7% (w/w) Arabic gum, 0.1% (w/w) xanthan gum and 6% (w/w) orange oil was predicted to provide the weakest pseudoplastic behavior (the least viscosity ratio, $Y_3 = 0.39$).

3.1.4. ζ-Potential

As shown in Table 2, ζ-potential values (Y_1) varied from −22.6% to −28.7%. As reported in previous study (Leiberman, Reiger, & Banker, 1989), an absolute value, less than or greater than 25 mV is indicative of flocculated and deflocculated emulsions, respectively. Therefore, the prepared orange beverage emulsions

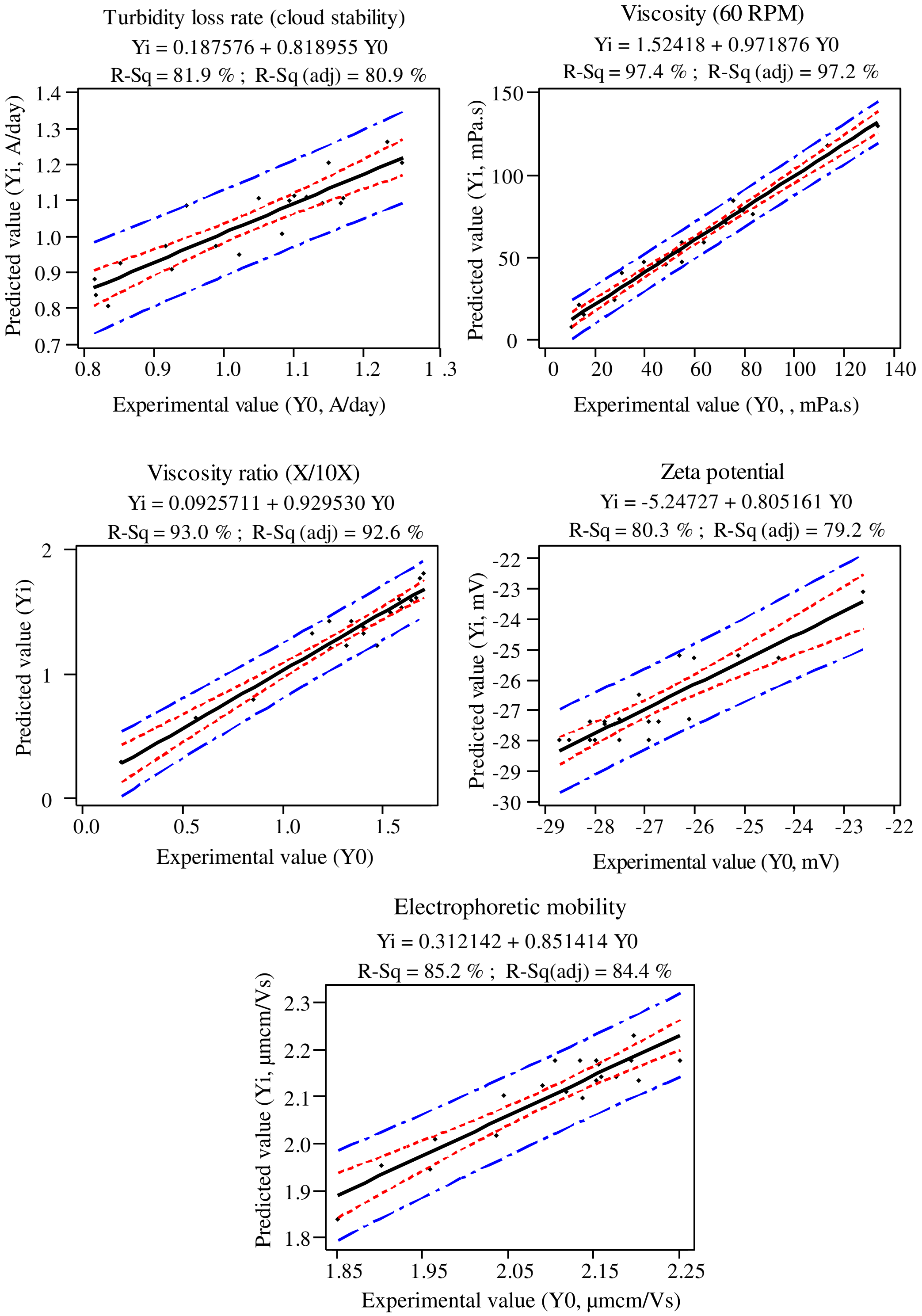

Fig. 2. Fitted line plots showing the closeness between the experimental (Y_0) and predicted values (Y_1).

appeared to be in deflocculated form in most cases (Table 2). The results exhibited that the ζ-potential of orange beverage emulsions was found to be negatively charged. This may be explained by the fact that xanthan gum is negatively charged ionic polysaccharides (Sun et al., 2007). As shown in Table 3, the magnitude of negatively charged ζ-potential (Y_4) was directly proportional to the xanthan gum concentration. The results indicated that the reduced ζ-potential model was significantly ($p < .05$) fitted to only the main and quadratic effects of xanthan gum (Table 4).

As also shown in Table 4, the independent variables had the least significant ($p < .05$) effect on ζ-potential (Y_4) as compared to the other response variables (Table 4). The results indicated that the interaction between independent variables had no significant ($p > .05$) effect on the negatively charged ζ-potential (Table 4). On the other hand, the final reduced model fitted for ζ-potential exhibited the least R^2 as compared to the other reduced models. Thus, it was concluded that the changes of ζ-potential (Y_4) was most probably influenced by the other factors such as pH, the dielectric constant of the medium, the viscosity of medium, particle size and distribution. The individual optimum region resulted in the largest magnitude of negatively charged ζ-potential ($Y_4 = -28.71$ mV) was estimated to be obtained by combined level of 7% (w/w) Arabic gum, 0.24% (w/w) xanthan gum and 6% (w/w) orange oil; while the least magnitude ($Y_4 = -24.43$ mV) of negatively charged ζ-potential was predicted to be achieved by a combined level of 7% (w/w), 0.1% (w/w) and 10% (w/w) of Arabic gum, xanthan gum and orange oil, respectively.

3.1.5. Electrophoretic mobility

As shown in Tables 3 and 4, the main effects of Arabic gum and xanthan gum exhibited a significant ($p < .05$) positive effect on the electrophoretic mobility (Y_5); while the variation of electrophoretic mobility was negatively influenced by the concentration of xanthan gum. Among the independent variable effects, the main effect of xanthan gum followed by its quadratic effect had the most significant ($p < .05$) effect on the electrophoretic mobility (Y_5) (Table 4). It was found that an orange beverage emulsion containing 11.76% (w/w) Arabic gum, 0.25% (w/w) xanthan gum and 6% (w/w) orange oil was predicted to result in the optimum electrophoretic mobility (the highest value, $Y_5 = 2.22$ μm cm/Vs); while the least mobility value ($Y_5 = 1.9$ μm cm/Vs) was estimated to be provided by a set level of 7% (w/w) Arabic gum, 0.1% (w/w) xanthan gum and 10% (w/w) orange oil.

3.2. Optimization procedure for predicting an optimum orange beverage emulsion

The multiple optimization procedure was carried out to determine an optimum set level of independent variables leading to the desirable response goals. An orange beverage emulsion would be considered an optimum product if the criteria applied for the optimization resulted in (1) the highest viscosity simultaneously along with viscosity ratio, negatively charged ζ-potential and electrophoretic mobility as well as (2) the least loss rate of turbidity. From the multiple response optimizations, the overall optimum region was achieved by a combined level of 13% (w/w) Arabic gum, 0.3% (w/w) xanthan gum and 10% (w/w) orange oil. The corresponding response values predicted for viscosity (60 rpm), viscosity ratio, turbidity loss rate, electrophoretic mobility and ζ-potential of optimum orange beverage emulsion were estimated to be 100, 1.383, 0.81, 2.15 and −27.35, respectively.

3.3. Validation of the final reduced models

The adequacy of the response surface models was checked by the comparison of experimental and predicted values. No significant difference ($p > .05$) was found between the experimental and predicted values (data not shown). As shown in Fig. 2, the overall closeness between those variables exhibited the adequacy of response surface models employed for predicting the variation of physicochemical emulsion properties as function of main emulsion components. The desirable orange beverage emulsion containing the predicted optimum formulation was practically prepared and then evaluated in the terms of physicochemical emulsion properties studied. Under the optimum condition, the corresponding experimental values for viscosity (60 rpm), viscosity ratio, turbidity loss rate, electrophoretic mobility and ζ-potential of the optimum orange beverage emulsion were 109, 1.396, 0.792, 2.26 and −27.79, respectively. The corresponding response values obtained from the experimental data and those ones predicted by the models were observed to be close together. No significant ($p > .05$) difference between the experimental and predicted values verified the adequacy of final reduced models fitted by RSM.

4. Conclusions

In this study, RSM was employed to study the main and interaction effects of main emulsion components on the turbidity loss rate (cloud stability index), viscosity, fluid behavior, ζ-potential and electrophoretic mobility of orange beverage emulsion. In general, CCD was shown to be a useful experimental design to provide the response surface models for explaining the variation of five response variables as function of three independent variables. The response surface analysis offered the significant ($p < .05$) relationship regression equations with high R^2 (>0.8) and non-significant ($p > .05$) lack of fit. This observation demonstrated a satisfactory adjustment of the response surface models fitted to the experimental data. The results clearly exhibited that the xanthan gum content should be considered as a primary factor for the pre-formulation of orange beverage emulsion in terms of physicochemical properties studied. In general, the orange beverage emulsion containing high concentration of xanthan gum ($\geqslant 0.24\%$ w/w) was suggested to provide all individual and overall desirable goals. In the present study, the orange beverage emulsions behaved between Newtonian and pseudoplastic fluid in most cases. This observation could be due to the effect of xanthan gum concentration on the rheological behavior. The present study indicated that the cloud instability of beverage emulsion could be controlled by an appropriate proportion of main emulsion components.

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