Mixture Design Experiment on Flow Behaviour of O/W Emulsions as Affected by Polysaccharide Interactions

Nor Hayati Ibrahim, Yaakob B. Che Man, Chin Ping Tan, and Nor Aini Idris

Abstract—Interaction effects of xanthan gum (XG), carboxymethyl cellulose (CMC), and locust bean gum (LBG) on the flow properties of oil-in-water emulsions were investigated by a mixture design experiment. Blends of XG, CMC and LBG were prepared according to an augmented simplex-centroid mixture design (10 points) and used at 0.5% (wt/wt) in the emulsion formulations. An appropriate mathematical model was fitted to express each response as a function of the proportions of the blend components that are able to empirically predict the response to any blend of combination of the components. The synergistic interaction effect of the ternary XG:CMC:LBG blends at approximately 33-67% XG levels was shown to be much stronger than that of the binary XG:LBG blend at 50% XG level ($p < 0.05$). Nevertheless, an antagonistic interaction effect became significant as CMC level in blends was more than 33% ($p < 0.05$). Yield stress and apparent viscosity (at 10 s^{-1}) responses were successfully fitted with a special quartic model while flow behaviour index and consistency coefficient were fitted with a full quartic model ($R^2_{adjusted} \geq 0.90$). This study found that a mixture design approach could serve as a valuable tool in better elucidating and predicting the interaction effects beyond the conventional two-component blends.

Keywords—O/W emulsions, flow behavior, polysaccharide interaction, mixture design.

I. INTRODUCTION

MIXTURE of polysaccharide have been industrially utilized in emulsion-based food products due to their desirable interaction effect which provides better rheological properties of the products as well as cost effective [1]. Binary mixtures of xanthan gum (XG) and galactomannans (GMs) mainly guar gum (GG) or locust bean gum (LBG) are the most commonly manipulated for their synergistic effect which has been scientifically explained and proven in the literature. For

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examples, mixtures of 60% XG-40% LBG and 80% XG-20% GG have been demonstrated to exhibit a strong synergism in terms of viscosity and viscoelastic components [2]. Moreover, synergistic effect between XG and GMs on viscosity is also believed to play a major role in flavour retention within the polysaccharide matrix [3], [4]. Several models have been proposed to explain these synergisms and "junction zone" model referring to cooperative binding of both polysaccharides are generally accepted [5].

In reviewing the literature, very few works however have been carried out to investigate the effect of polysaccharide interactions (either synergism or antagonism) on the emulsion properties. In other words, the available studies only concern on the interaction within the native polysaccharide solutions rather than in model or real food systems. Additionally, information on the polysaccharide interactions other than XG-GMs is hardly found. It has been previously demonstrated in our study that the use of XG, carboxy methyl cellulose (CMC) and LBG could generally improve the rheology and stability of emulsions with partially crystallized fat droplets [6]. However, all of them showed a lack of functionality besides advantages on the overall emulsion characteristics. XG was found to provide an emulsion with a desirable gel character, but considerably unstable due to depletion flocculation and partial coalescence. Differently, CMC produced an emulsion with excellent storage stability yet exhibited a very weak viscoelastic property. With LBG, the emulsions demonstrated acceptable rheological properties and were found to be moderately stable upon storage. Based on these findings, binary or ternary blends of these polysaccharides in addition to common XG-GMs, shall be exploited in order to produce emulsions with desirable rheological properties as well as storage stability. With this in mind, mixture experiments along with non-linear modelling were used in the present study to investigate the polysaccharide interactions (synergism or antagonism) in a blend of two or more components. The approach has been shown to be very useful in predicting the extent of the interaction effects on the properties of model or real food systems [7], [8], [9]. Thus, the objectives of the present study were to investigate the interaction effects among XG, CMC and LBG on the flow properties of the emulsions as well as to fit an appropriate mathematical model to express each response as a function of the proportions of the blend components that are able to empirically predict the response to any blend of combination of the components.

World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:4, No:7, 2010

II. MATERIALS AND METHODS

A. Materials

Refined SBO was purchased from Moi Foods (Malaysia) Sdn. Bhd (Selangor, Malaysia). PKO was donated by Malaysian Palm Oil Board (MPOB, Selangor, Malaysia). Vinegar (7% acetic acid) and eggs were purchased from local supermarket from the same batch. XG, CMC and LBG were purchased from Sigma (Sigma-Aldrich, St. Louis, MO).

B. Preparation of Polysaccharide Blends

The XG, CMC and LBG dispersions (1.25%) were individually prepared by dispersing 1.25 g of dried polysaccharide powder in 100 g deionized distilled water with vigorous stirring at room temperature (25 ± 1 °C) by a magnetic stirrer. After a complete dispersion, the dispersion was heated at 80°C for 2 hours (or at room temperature for CMC) on a thermostated hot-plate, while continuously stirred. The dispersion was then cooled to room temperature and left overnight (to ensure a complete hydration) prior to blend preparation. Binary blends of XG:CMC, XG:LBG and CMC:LBG were prepared at 1/2:1/2 ratio. Ternary blends of XG:CMC:LBG were prepared at the following ratios: 2/3:1/6:1/6, 1/6:2/3:1/6, 1/6:1/6:2/3 and 1/3:1/3:1/3. Polysaccharide-polysaccharide interaction was previously proven to be stronger at 80°C rather than 25°C (room temperature) [10]. Thus, all blends were prepared at 80°C with continuous stirring for 30 min.

C. Emulsions Preparation

The XG, CMC and LBG dispersions (1.25%) were individually prepared by dispersing 1.25 g of dried polysaccharide powder in 100 g deionized distilled water with vigorous stirring at room temperature (25 ± 1 °C) by a magnetic stirrer. After a complete dispersion, the dispersion was heated at 80°C for 2 hours (or at room temperature for CMC) on a thermostated hot-plate, while continuously stirred. The dispersion was then cooled to room temperature and left overnight (to ensure a complete hydration) prior to blend preparation. Binary blends of XG:CMC, XG:LBG and CMC:LBG were prepared at 1/2:1/2 ratio. Ternary blends of XG:CMC:LBG were prepared at the following ratios: 2/3:1/6:1/6, 1/6:2/3:1/6, 1/6:1/6:2/3 and 1/3:1/3:1/3. Polysaccharide-polysaccharide interaction was previously proven to be stronger at 80°C rather than 25°C (room temperature) [10]. Thus, all blends were prepared at 80°C with continuous stirring for 30 min.

D. Flow Behavior Measurement

The flow behavior of the emulsions was determined by means of a controlled-stress rheometer (RheoStress 600, Haake, Karlsruhe, Germany) with a sand blasted cone sensor (C35/2° Ti; 35 mm diameter, 2° angle, 0.105 mm gap) and a measuring plate cover (MPC 35). The equipment was driven through the Haake software, Rheowin Job Manager Version 3.12. All samples were allowed at rest for 5 min after loading to allow temperature equilibration and induced stress to relax. The temperature was kept at 25°C during the measurement by a Haake circulating water bath and a universal temperature controller (UTC) with accuracy of ± 0.1°C. Flow curves were determined by using an increasing shear rate i.e. 1 - 100 s^{-1} within 2 min and then analyzed using the Rheowin Data Manager software Version 3.12. The experimental flow curves were modeled using Herschel-Bulkley model: $\tau = \tau_o + K \gamma^n$, where τ_o = yield stress (Pa), K = consistency coefficient (Pa.s^n), γ = shear rate (s^{-1}) and n = flow index ($n < 1$ corresponds to shear thinning behavior, $n > 1$ corresponds to shear thickening behavior, and $n = 1$ corresponds to Newtonian behavior). The choice of the model was determined by the software after considering the highest regression value of $R^2 \geq 0.98$. A measure of viscosity, η (Pa.s) was given for a shear rate of 10 s^{-1} for all samples. The rate was chosen as it felt within the shear rate range that clearly showed a distinctive shear-thinning degree among the emulsions.

E. Experimental Design and Statistical Analysis

Blends of XG, CMC, and LBG containing pure XG, CMC, LBG and blends thereof were prepared according to the augmented simplex-centroid mixture design with 10 points. The experimental domain consisted of different proportions of components of X_1 (XG), X_2 (LBG), and X_3 (CMC) between zero and one ($0 \leq X_i \leq 1$; $\sum X_i = 1$). The experimental domain was within an equilateral triangle (regular simplex). The vertexes of the simplex represented the pure components, the edges of the triangle represented the two-component blends, and points within the triangle represented the three-component blends. To allow error estimation, all blends were prepared in three independent replications, providing a total of 30 blends. The experimental design and the mean values of the flow responses of the emulsions, namely yield stress (σ_o), flow behaviour index (n), consistency coefficient (K), and apparent viscosity at 10 s^{-1} (η) are presented in Table I.

A one-way analysis of variance (ANOVA) with Tukey's Multiple Comparisons was applied on the data (n = 3) to determine a significant ($p < 0.05$) difference among the emulsions. Mixture regression analysis was performed to determine estimated coefficients and significance of the model terms, the F-test and coefficient of determinations (R^2). The results were initially fitted to all available mixture regression models of increasing complexity, from linear to full quartic. Model significance, significance of lack-of-fit and adjusted R^2 value were used to judge the adequacy of model fitness. The adjusted R^2 describes the proportion of variation in the responses that is explained by the model and the value has been adjusted for the number of terms in the model. Finally, the flow responses were found to be best fitted (due to insignificant of lack-of-fit and the highest adjusted R^2) with Scheffe's canonical special quartic or full quartic models [12] as follows;

$$Y_i = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{1123} X_1^2 X_2 X_3 + \beta_{1223} X_1 X_2^2 X_3 + \beta_{1233} X_1 X_2 X_3^2$$

or

$$Y_i = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{12(1-2)} X_1 X_2 (X_1 - X_2) + \beta_{13(1-3)} X_1 X_3 (X_1 - X_3) + \beta_{23(2-3)} X_2 X_3 (X_2 - X_3) + \beta_{1123} X_1^2 X_2 X_3 + \beta_{1223} X_1 X_2^2 X_3 + \beta_{1233} X_1 X_2 X_3^2 + \beta_{12(1-2)}^2 X_1 X_2 (X_1 - X_2)^2 + \beta_{13(1-3)}^2 X_1 X_3 (X_1 - X_3)^2 + \beta_{23(2-3)}^2 X_2 X_3 (X_2 - X_3)^2$$

World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:4, No:7, 2010

where Y_i is the predicted rheological response, β_1, β_2, and β_3 are the estimated coefficient of each linear effect terms, $\beta_1\beta_2$, $\beta_1\beta_3$, $\beta_2\beta_3$, $\beta_{12(1-2)}$, $\beta_{13(1-3)}$, $\beta_{23(2-3)}$, $\beta_{12(1-2)}^2$, $\beta_{13(1-3)}^2$, and $\beta_{23(2-3)}^2$ are binary interaction effect terms, β_{1123}, β_{1223}, and β_{1233}, are ternary/quaternary interaction effect terms. Significance of lack-of-fit and R^2 value were used to judge the adequacy of model fitness. The models were then reduced (if any), leaving only significant terms [13]. Once the estimated model equation for each response was obtained, contour and surface plots were generated. The plots show how a response relates to three components based on the model equation. Two new ternary blends were chosen from the interior of the simplex and the blends were used to prepare two new emulsions. The blends (n = 3) were randomly chosen from the interior of the simplex. Finally, the model was validated by comparing the mean responses of these emulsions with the respective value predicted by the model equations. The experimental design, data analysis, contour and surface plots were developed using a Minitab (Release 14) (Minitab Inc., USA) statistical software package. The level of confidence used was at $\alpha = 0.05$.

III. RESULTS AND DISCUSSION

A. Effect of Binary Blends

This study was purposely designed to investigate the effect of XG, CMC and LBG interactions on the rheological properties of the emulsions. Thus, the discussion focused on the interaction effects relative to the pure effects of these polysaccharides. The evolution of viscosity (η) as a function of shear rate (γ) of the emulsion containing pure XG, CMC, LBG and their binary blends (at 1/2:1/2 ratio) are demonstrated in Fig. 1. In our previous paper, the effect of pure polysaccharides on the flow properties of the same emulsions has already been discussed [6]. All XG, CMC and LBG have been found to cause a typical shear thinning behaviour of the emulsions. Of all, XG could provide the highest yield stress giving the best suspension ability of the emulsion containing it. In the present study, all emulsions prepared with polysaccharide blends were also expected to exhibit a similar shear thinning behaviour yet differences in specific flow responses. As depicted in Table I, emulsions with binary blends of XG:CMC (code B) and XG:LBG (code F) exhibited a shear-thinning behaviour with a close value of yield stress. Significant differences ($p < 0.05$) were observed in their flow behaviour index (n) and consistency coefficient (K). The XG:CMC emulsion was found to be more shear-thinning ($n = 0.388$) as opposed to the XG:LBG emulsion ($n = 0.524$). This is evident in Fig. 2, showing a relatively slow drop in viscosity with increasing shear rate of the latter emulsion.

Of two combinations, only the XG:CMC emulsion showed a significant ($p < 0.05$) difference from the emulsion containing pure XG (code A), particularly in their n and K values. This reveals that, interactions between XG and CMC or XG and LBG at the defined component ratio did not seem to greatly affect the flow properties of the emulsions. Differently, a shear-thinning behaviour without a yield stress was observed for CMC:LBG emulsion. The flow properties of this emulsion were more close to that of pure CMC emulsion rather than that

of pure LBG emulsion. Apart from that, all binary blends were not significantly different in terms of their viscosity.

TABLE I
AN AUGMENTED SIMPLEX CENTROID DESIGN WITH TEN RATIOS OF XANTHAN GUM (X_1), CARBOXYMETHYL CELLULOSE (X_2) AND LOCUST BEAN GUM (X_3) BLENDS AND FLOW RESPONSES OF THE RESPECTIVE EMULSION

Code	Component Proportion			Flow Responses [a]			
	X_1	X_2	X_3	σ_0 (Pa)	n	K (Pa.s^n)	η (Pa.s) at 10 s^{-1}
A	1	0	0	2.808 [d]	0.467 [cd]	1.884 [cd]	0.833 [cd]
B	1/2	1/2	0	3.253 [d]	0.388 [e]	3.452 [b]	1.180 [cd]
C	0	1	0	0.092 [e]	0.487 [c]	2.517 [c]	0.782 [d]
D	0	1/2	1/2	0.000	0.585 [b]	2.030 [c]	0.781 [d]
E	0	0	1	0.568 [e]	0.670 [a]	2.250 [c]	1.109 [cd]
F	1/2	0	1/2	3.540 [d]	0.524 [bc]	1.953 [c]	0.697 [d]
G	2/3	1/6	1/6	14.740 [a]	0.408 [de]	4.255 [a]	2.570 [a]
H	1/6	2/3	1/6	0.606 [e]	0.583 [bc]	1.536 [d]	0.788 [d]
I	1/6	1/6	2/3	5.545 [c]	0.512 [bc]	2.532 [c]	1.377 [c]
J	1/3	1/3	1/3	11.818 [b]	0.555 [bc]	2.252 [c]	1.991 [b]
Standard deviation range				0.021–0.535	0.001–0.080	0.008–0.352	0.013–0.514

[1] Fitted with Herschel-Bulkley model ($R^2 > 0.97$): $\sigma = \sigma_0 + K\gamma^n$; σ_0, yield stress; n, flow index; K, consistency coefficient; η, apparent viscosity.
[a-e] Means with the same superscript within the same column are not significantly different at $\alpha = 0.05$. Means are reported from three independent experiments (n = 3).
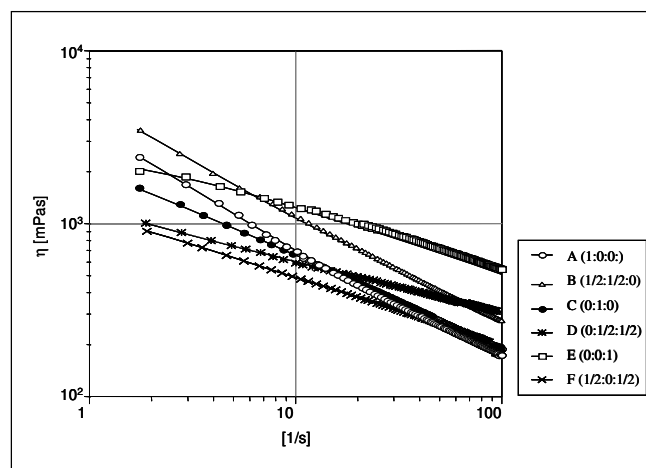

Fig. 1 Rheogram of emulsions representing the evolution of viscosity (η) as a function of shear rate (γ). A, C and E, represent emulsions with pure xanthan gum (XG), carboxymethyl cellulose (CMC) and locust bean gum (LBG), respectively. B, D and F, represent binary blends (at ratio 1/2:1/2) of XG:CMC, CMC:LBG and XG:LBG, respectively.

Higher yield stress and viscosity (although the values were not statistically significant from that of pure XG emulsion) obtained for the XG:LBG emulsion compared to pure XG and LBG emulsions, could undoubtedly be related to a synergistic interaction between XG and LBG in the emulsion aqueous continuous phase. Similarly, Makri and Doxastakis [14] reported higher viscosity for bean protein-stabilized emulsions containing 50% XG and 50% LBG compared to those with pure LBG or XG. Generally, an intermolecular interaction may have occurred through the association of the locust bean's unsubstituted (i.e. galactose deficient) region, with xanthan

World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:4, No:7, 2010

helix backbone (a cellulose backbone of β-[1⟶4]-*D*-glucopyranose glucan) [3], [4]. This association particularly involved the hydrophobic junction zones of both polysaccharides. These junction zones are anticipated to be formed due to hydrogen bonding between water and hydroxyl groups of both polysaccharides [15].

B. Effect of ternary Blends

Considering ternary blends of XG:CMC:LBG, yield stress value was found to be immensely increased in the emulsions containing higher proportion of XG (code G, $\sigma_0 = 14.74$ Pa) and same proportion of all polysaccharides (code J, $\sigma_0 = 11.82$ Pa) (Table I). Thus, it is worth mentioning that the suspending ability of the emulsion could be efficiently improved by incorporating these ternary blends. The viscosity of both emulsions was also significantly ($p < 0.05$) higher than that of pure XG emulsion. On the other hand, with higher proportion of LBG, the emulsion exhibited a small yet significant ($p < 0.05$) increase in the yield stress value (code I, $\sigma_0 = 5.54$ Pa). These three emulsions similarly demonstrated a strong shear-thinning as their viscosity gradually dropped with increasing shear rate (Fig. 2).
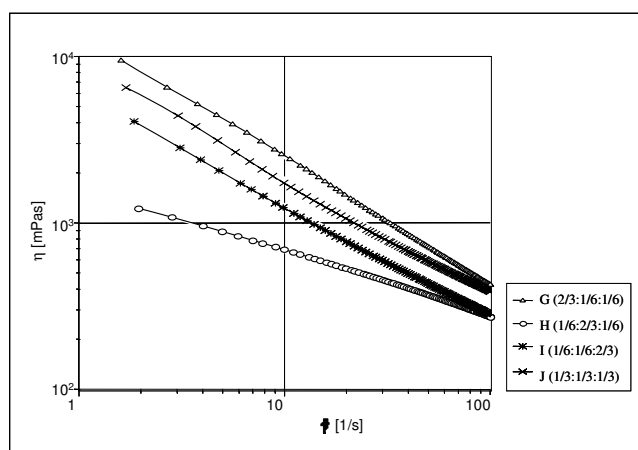

Fig. 2 Rheogram of emulsions representing the evolution of viscosity (η) as a function of shear rate (γ). G, H, I, and J, represent emulsions with ternary blends of xanthan gum, carboxymethyl cellulose and locust bean gum (XG:CMC:LBG) at ratios 2/3:1/6:1/6, 1/6:23:1/6, 1/6:1/6:2/3, and 1/3:1/3:1/3, respectively.

On the other hand, the flow properties of an emulsion with higher proportion of CMC (code H) almost similar to that of emulsion with pure CMC. This observation revealed that, large contribution to the emulsion flow properties could be obtained from CMC when its proportion exceeded the proportion of XG or LBG. In relation to this, it is reasonable to consider that the presence of CMC at ~33% in ternary blend had contributed to another synergistic effect. It is likely that synergistic interaction might have been occurred between the unsubstituted region of LBG and the CMC cellulose backbone (β-(1⟶4)-*D*-glucopyranose polymer). The mechanism is believed to be similar to the one proposed for XG-LBG. Moreover, as anionic polymer, CMC offers a good compatibility with most water-soluble nonionic gums [15] like LBG that could enhance the interaction. Previously, Zhang and Kong [17] and Robert,

Elmore, Langley and Bakker [18] reported similar interaction in CMC-guar gum and CMC-hydroxypropyl guar gum solutions, respectively. It is also believed that, the carboxymethyl deficient region of CMC could interact with the cellulose backbone of XG (since both polysaccharides contain hydroxyl groups to form hydrophobic junction zones). These effects were pronouncedly observed in the emulsion containing ~67% XG, ~16% CMC and ~16% LBG. Consequently, a strong polymer complex might have occurred in the system that led to more complex network structure, reflecting a high elastic modulus. From these observations and explanations, we proposed the occurrence of a ternary synergism of XG-CMC-LBG in addition to binary synergisms (XG-LBG and CMC-LBG) in our ternary blends. In a different view, CMC might have formed its own network interpenetrating with the network from XG-LBG synergism that finally resulted in more complex network structure as previously suggested to be occurred in a ternary blend of XG, LBG and methylcellulose [19]. However, this effect seemed not to be important when the level of CMC exceeded ~33%. As a result, we did not observe synergism in the emulsion containing XG:CMC or CMC:LBG binary blends.

C. Fitted Regression Models, Contour and Surface Plots

To empirically predict the flow responses as functions of the proportions of the blend components, mixture regression analysis was applied on the experimental data. The design used in this study supports the fitting of the special quartic model [12] and initially, we intended to fit all responses to this model. However, after the statistical analysis, we found a significant lack-of-fit for the models fitted to some of the responses. On the other hand, those responses were adequately described by a more complex model of full quartic. Table II presents the results of fitting special quartic model to σ_0 and η responses whilst full quartic model to *n* and *K* responses. In addition, Table III provides the terms in both equations that are able to describe related responses.

The *F*-statistic on individual model terms indicated that several terms in both equations could be deleted since those terms are unnecessary (insignificant probability, $p > 0.05$). Consequently, deleting those terms resulted in a higher insignificant lack-of-fit. Particularly for full quartic model fitting (*n* and *K*), removing those terms allowed a lack-of-fit test. Thus, Table III only provides the terms in both equations that significantly ($p < 0.05$) describe related responses. It is clear that, both fittings finally provided no lack-of-fit (with p-value of 0.117-0.767) and high adjusted coefficient of determinations (with R^2 of 0.8906-0.9967). The R^2 values were found to be essentially high and the variances found in all responses were explained well by the models.

As presented in Table III, significant interaction terms in the special quartic model of the yield stress and viscosity generally indicated that the emulsion rheology will be affected not only by the individual polysaccharides, but also by the binary interactions between XG-CMC, XG-LBG, or CMC-LBG as well as ternary interactions among all polysaccharides. However, there will be no significant effect of binary interactions of CMC-LBG on yield stress and viscosity and XG-LBG on the viscosity of the emulsions. Besides, there will

World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:4, No:7, 2010

be no significant effect of ternary interactions of XG-CMC-LBG with higher LBG proportion on the. As a result, all insignificant interaction terms were removed from the respective models. Thus, reduced special quartic models for these responses are shown by the following equations;

$$\sigma_0 = 2.8X_1 - X_2 + 0.5X_3 + 7.5X_1X_2 + 7.7X_1X_3 + 947.7X_1^2X_2X_3 - 357.4X_1X_2^2X_3 + 148.5X_1X_2X_3 \quad (1)$$

$$\eta = 0.76X_1 + 0.75X_2 + 1.03X_3 + 1.76X_1X_2 + 137.0X_1^2X_2X_3 - 54.35X_1X_2^2X_3 \quad (2)$$

Considering the interaction terms in equations (1) and (2), the highest positive coefficient (e.g. 947.7 for σ_0) confirmed that the strongest synergism could be obtained by using a ternary blend of XG:CMC:LBG at higher XG proportion. On the other hand, the highest negative coefficient (e.g. 357.4 for σ_0) supported the fact that the strongest antagonist effect could result from a ternary blend of XG:CMC:LBG with higher proportion of CMC. These effects can be clearly visualized in the pattern of the respective contour and surface plots. One can observe a similarity of the contour pattern between yield stress (Fig. 3) and viscosity (Fig. 4). The corresponding surface plots similarly demonstrated a maximizing synergistic effect at increasing level of XG. This similarity can be expected since these two flow responses are interrelated to each other. By definition, yield stress is the stress level that required to initiate flow and is related to the internal forces of the material that must be overcome before flow occur and viscosity is a measured of flow resistance of the material [20]. Based on the definitions, both are basically related to the original internal forces of the material that have been preserved before the structure deformation or flow occurs. One can notice a limitation of equation (1) in estimating the data for the points fall below the contour lines of $\sigma_0 = 0$. This is due to the fact that the estimated value would be < 0, which is not true for the real yield stress. However, the equation is applicable to predict future data from the points of interest that are located beyond the constraint lines of $\sigma_0 = 0$.

For both yield stress and viscosity responses, the contour and surface plots showed synergistic effect of ternary mixtures in a large area that maximized in between the points of ternary mixtures with higher XG and similar polysaccharide proportion (centre of the simplex), which far away from the CMC corner. Inversely, antagonistic effects can be observed within the large area in the simplex, near the points of pure CMC, binary mixture of CMC:LBG and ternary mixture of XG:CMC:LBG with higher LBG. It should be mentioned that, a simultaneous substitution of XG and LBG by CMC will result in larger decrease in these responses than a simultaneous substitution of XG and CMC by LBG. In future, the upper synergistic region should be of interest in order to focus on the combinations that could give optimum rheological properties and to a certain extent, a high stability. The lower region with binary/ternary combinations approaching a higher level of CMC should be a constraint due to undesirable effects (i.e. low yield stress, low viscosity, low elastic modulus, and more viscous nature relative

to elastic nature). It is also expected that, new mathematical model equations that could be derived from this approach will not demonstrate any limitations in predicting future data within this new experimental domain.

TABLE II
ANALYSIS OF VARIANCE OF MODEL FITS

Source [a]	Degree of freedom	Adjusted sum of square	Adjusted mean square	F	p
Fitted with special quartic model					
σ_0					
Model	7	703.948	100.564	1267.36	< 0.001
Lack-of-fit	2	0.261	0.130	1.76	0.198*
Pure error	20	1.485	0.074		
η at 10 s^{-1}					
Model	5	10.0614	2.01229	52.41	< 0.001
Lack-of-fit	4	0.2736	0.06839	2.11	0.117 *
Pure error	20	0.6479	0.03239		
Fitted with full quartic model					
n					
Model	8	0.197536	0.024692	30.51	< 0.001
Lack-of-fit	1	0.000076	0.000076	0.09	0.767*
Pure error	20	0.016919	0.000846		
K					
Model	8	17.75552	2.21944	84.89	< 0.001
Lack-of-fit	1	0.02584	0.02584	0.99	0.332*
Pure error	20	0.52322	0.02616		

[a] σ_0, yield stress; n, flow index; K, consistency coefficient; η, apparent viscosity. * Not significant at 95% confidence interval ($p > 0.05$).

TABLE III
ESTIMATED REGRESSION COEFFICIENTS (REDUCED TERMS) AND ADJUSTED COEFFICIENT OF DETERMINATION (R^2) OF MODEL FITS

Term	Fitted with full quartic model [a]			
	σ_0	η at 10 s^{-1}	n	K
X_1	2.8	0.76	0.467	1.85
X_2	-0.0	0.75	0.489	2.52
X_3	0.5	1.03	0.672	2.21
X_1X_2	7.5	1.76	-0.360	5.08
X_1X_3	7.7	*	-0.182	*
X_2X_3	*	*	*	-1.33
$X_1X_2 (X_1-X_2)$	-	-	-2.581	32.74
$X_1X_3 (X_1-X_3)$	-	-	1.495	-7.67
$X_1X_1X_2X_3$	947.7	137.89	5.903	-28.91
$X_1X_2X_2X_3$	-357.4	-54.35	*	*
$X_1X_2X_3X_3$	148.5	*	*	*
$X_1X_2 (X_1-X_2)^2$	-	-	-1.780	24.22
Adjusted R^2	0.9967	0.8986	0.8906	0.9586

[a] σ_0, yield stress; n, flow index; k, consistency coefficient; η, apparent viscosity. * Estimated coefficients were not provided since they were not significant at 95% confidence interval ($p > 0.05$).

After fitting with a full quartic model, it was generally found that interaction terms had significant effect on flow behaviour index and consistency coefficient of the emulsions (Table III). Nonetheless, there was no significant binary interactions effect of XG-LBG and CMC-LBG on consistency coefficient and flow behaviour index, respectively. Furthermore, ternary interaction effect of XG-CMC-LBG on these responses was only significant at higher XG proportion. Consequently, the following equations are the simplified full quartic models for these responses, in which all insignificant interaction terms have been removed;

World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:4, No:7, 2010

$n = 0.467X_1 + 0.489X_2 + 0.672X_3 - 0.360X_1X_2 - 0.182X_1X_3 - 2.581X_1X_2(X_1-X_2) + 1.495X_1X_3(X_1-X_3) + 5.905X_1^2X_2X_3 - 1.780X_1X_2(X_1-X_2)^2$ (3)

$K = 1.85X_1 + 2.52X_2 + 2.21X_3 + 5.08X_1X_2 - 1.33X_2X_3 + 32.74X_1X_2(X_1-X_2) - 7.67X_1X_3(X_1-X_3) - 28.91X_1^2X_2X_3 + 24.22X_1X_2(X_1-X_2)^2$ (4)
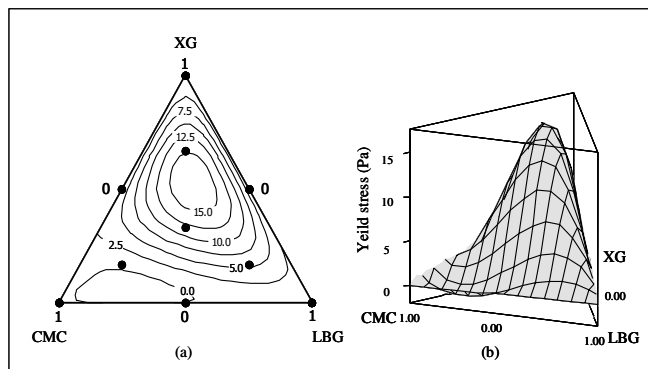

Fig. 3 Contour (a) and surface (b) plots for yield stress (σ_o) response fitted by a special quartic model. XG, xanthan gum; CMC, carboxymethyl cellulose; LBG, locust bean gum.
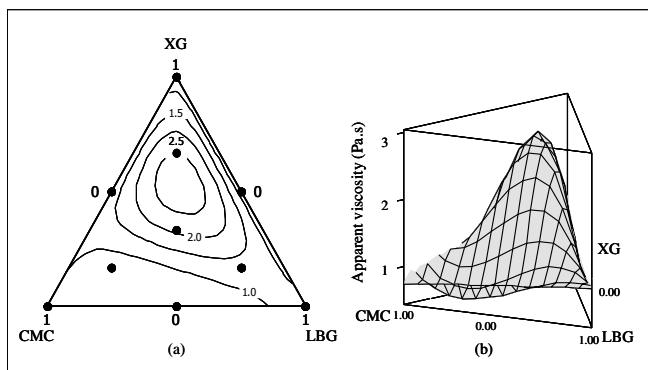

Fig. 4 Contour (a) and surface (b) plots for apparent viscosity (η) (at 10 s^{-1}) response fitted by a special quartic model. XG, xanthan gum; CMC, carboxymethyl cellulose; LBG, locust bean gum.

The aforementioned interaction effects on both responses were clearly demonstrated by their contour and surface plots (Figs. 5 and 6). As observed in the previous responses, the strongest synergistic effect on flow behaviour index was contributed by a ternary interaction of XG-CMC-LBG at higher XG level. It was evident by the highest positive coefficients of 5.905 in equation (3). From Fig. 5, it can be seen that the emulsions were predicted to be less shear-thinning with high *n* values for ternary combinations in the middle region of the simplex as well as binary combinations of XG:LBG and CMC:LBG. However, the antagonist effect on the flow behaviour index will be important as it surface response obviously demonstrated a minimizing effect. From equation (3), the strongest antagonistic effect was expected to result from the binary interaction of XG:CMC at extra low CMC level with the highest negative coefficient (2.581).
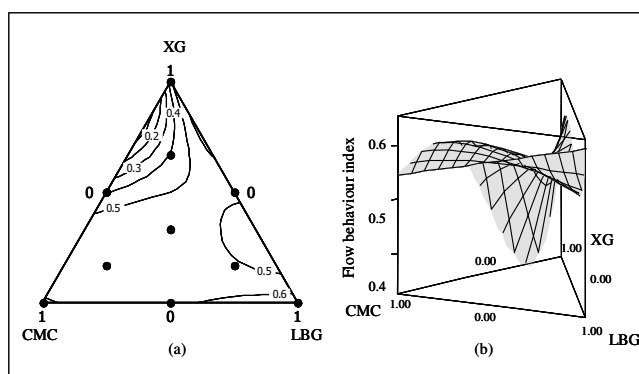

Fig. 5 Contour (a) and surface (b) plots for flow behaviour index (*n*) response fitted by a full quartic model. XG, xanthan gum; CMC, carboxymethyl cellulose; LBG, locust bean gum.
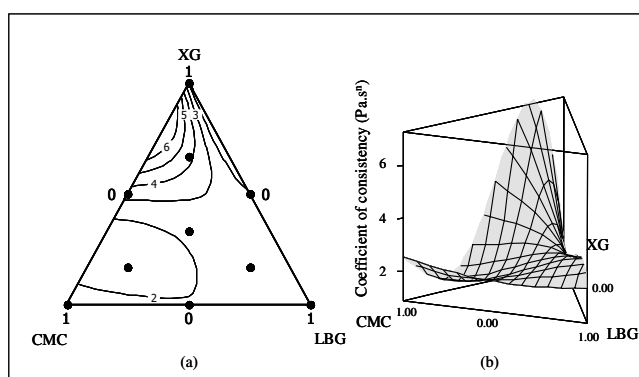

Fig. 6 Contour (a) and surface (b) plots for consistency coefficient (*K*) response fitted by a full quartic model. XG, xanthan gum; CMC, carboxymethyl cellulose; LBG, locust bean gum.

C. Model Validation

To validate the model equation of each response, two new ternary blends at ratios 1/2:1/5:3/10 (50% XG, 20% CMC and 30% LBG) and 3/10:1/5:1/2 (30% XG, 20% CMC and 50% LBG) were chosen. Two new emulsions were prepared with these blends and their rheological responses were determined and the results are displayed in Table IV. An actual mean of each response was compared to the respective predicted value calculated using the respective equation model. The model is said to be valid when the actual mean response either matched well with the fitted value or fell within the range of the predicted value at 95% confidence interval [21]. As shown in Table IV, the actual mean for each response fell within the range in which the estimated mean response for the new combinations are expected to fall. Thus, both special and full quartic models were experimentally checked as good and valid.

This study showed the importance of the interactions among XG, CMC and LBG in affecting the flow properties of the prepared emulsions. The synergistic interaction effect of the ternary XG:CMC:LBG blends at approximately 33-67% XG levels appeared to be much stronger than that of the binary XG:LBG blend at 50% XG level. Nevertheless, an antagonistic interaction effect became important as CMC level in blends

World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:4, No:7, 2010

was more than 33%. The use of a mixture design with regression modelling was shown to be a valuable tool in better elucidating these interaction effects. The approach provided more information on the extent of the interaction effects in the whole region of the simplex since we can predict any synergism or antagonism for many combinations within the experimental domain, beyond the points originally provided by the experimental design.

TABLE IV

PREDICTED VALUE FOR RESPONSES OF EMULSIONS WITH NEW COMPONENT PROPORTIONS AS COMPARED TO ACTUAL VALUE

Proportions [a]			Predicted values [b]			Actual values [c]
X_1	X_2	X_3	Fit	SE of Fit	95% CI	
Predicted by a special quartic model						
σ_0 (Pa)						
1/2	1/5	3/10	16.85	0.15	(16.53, 17.17)	17.03 ± 0.06
3/10	1/5	1/2	11.29	0.15	(10.97, 11.61)	11.14 ± 0.12
η (Pa.s) at 10^{-1}						
1/2	1/5	3/10	2.76	0.11	(2.54, 2.98)	2.63 ± 0.04
3/10	1/5	1/2	1.91	0.06	(1.78, 2.04)	1.86 ± 0.01
Predicted by a full quartic model						
n						
1/2	1/5	3/10	0.51	0.02	(0.48, 0.54)	0.50 ± 0.03
3/10	1/5	1/2	0.52	0.012	(0.49, 0.55)	0.49 ± 0.01
K (Pa.s^n)						
1/2	1/5	3/10	3.06	0.09	(2.87, 3.24)	2.95 ± 0.06
3/10	1/5	1/2	2.52	0.08	(2.14, 2.89)	2.39 ± 0.14

[a] X_1, xanthan gum; X_2, carboxymethyl cellulose; X_3, locust bean gum
[b] SE, standard error; CI, confidence interval.
[c] Data are presented as mean ± standard deviation, from three independent determinations (n = 3). σ_0, yield stress; n, flow index; K, consistency coefficient; η, apparent viscosity at 10 s^{-1}.

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