Optimization of Calcium Alginate Preparation in Aqueous by Response Surface Methodology

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Abstract— In this study, a statistical software package and design of experiment were applied for the preparation of calcium alginate in aqueous. Alginate which is originates from polysaccharide brown algae with different type of uranic and manuronic chains were used as an intermediate, blended with calcium carbonate powder for macro size adsorbent preparation. Though adsorption has been an ideal choice in waste water purification, the needs to find an alternative source of adsorbent has received considerable interests recently. In this study, a central composite design was used to develop a model to predict and optimize the preparation condition of calcium alginate. Mathematical model equations were obtained from simulation programming. Analysis of variance (ANOVA) for viscosity and pH (responses) indicated that the model was adequate to fit the experimental data (p values, lack of fit, R^2adj). From the statistical parameters, it showed that the quadratic effects for both calcium carbonate and alginate powder were the most significant. Meanwhile, the correlation coefficient, R^2 for both independent variables (calcium carbonate and alginate) of 0.9974 and 0.9008 implied that the developed models were adequate to navigate the design space. The optimum preparation condition was carried out by compromising the independent factors and responses at different criteria. Finally, the optimum preparation condition for calcium alginate was obtained with 2.00 g of calcium carbonate and 10 % (w/v) that result in 38 cP of viscosity at pH 10, respectively.

Index Terms— Adsorbent, Alginate, pH, Statistical analysis, Viscosity.

I INTRODUCTION

Managing water pollution is one of the crucial challenges in current world due to rapid changes of product manufacturing and technological advancement that result in wide variation in industrial effluent. For instance, heavy metals byproducts from electroplating, paint, textile, mining and steel making activities carries significant amount of copper, leads, manganese and cadmium that poses a threat to human and environment. Generally, the discharge of industrial waste waters can be vary in terms of quantity and quality of heavy metals, organic and non organic matter, suspended particulates, to name a few. Therefore, discharge of untreated waste water has been a major concern to many stakeholders in order to safe guard environment and human health, particularly. If not properly and safely treated, waste water from industrial activities can be an impendent source that is very costly to remediate.

One of the available methods in physico-chemical is adsorption which offers better removal of heavy metals, high efficiency, high resistance, plentiful source of material and cost effectiveness [1]. Until now, various types of adsorbents have been discovered and tested for their efficiency in adsorption process including mineral deposits [2], agriculture wastes [3-5] and industrial byproducts [6, 7] The selection of these types of material mostly depending on the insoluble porous matrix and some available active groups that capable to reacts with polar and non polar pollutants [8].

Alginate based polysaccharides have been widely employed in biomedical and pharmaceutical fields mostly in drug delivery, wound dressings, and dental implants. In nano particles application, alginate has found their application in biological devices [9]. In environmental field, due to its gel forming ability, biocompatibility, non toxicity and biodegradability [10, 11], alginate has been used as catalysts and adsorbents for separation and purification in waste water treatment. However, the utilization of alginate in aqueous exposed their solubility which limits the adsorption properties for ionic pollutants specifically for the case of heavy metals ions. Therefore, an experimental work was carried out to modify alginate in aqueous blended with calcium carbonate powder to improve the adsorption properties. The preparation condition of the calcium alginate was tested towards viscosity and pH based on the mathematical equation developed from statistical software. "

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II EXPRIMENTAL SET UP

A MATERIALS

Principally, alginate has been recognized as an excellent polysaccharide in gelling system because of its unique physicochemical, thermal and rheological properties. It importance relies in hydrocolloid properties including their viscosity, pH, solubility and mechanical properties. In this work, sodium alginate (C6H7O6Na) powder was obtained locally and supplied by R&M Chemicals (Malaysia) with low molecular weight was preferred because it is widely used in many encapsulation processes [12]. Meanwhile, calcium carbonate powder was obtained from a limestone quarry wastes which is considered as a byproduct from the quarrying activity. The composition of alginate and calcium carbonate is listed in Table 1.

TABLE 1: Alginate and Calcium Carbonate Composition

Alginate ($C_6H_7O_6Na$)	
Specification	Content
Assay	91-106%
Moisture	Max 15%
Matter insoluble in water	Max 1%
Lost of ignition, LOI at 1100 °c	Max 25%
Molecular weight	85000
Viscosity; max at 2 g/L (used spindle no. 4), mPas	65
Calcium carbonate powder ($CaCO_3$)	
Elements	Content
C	20
CaO	75
SiO_2	3.4
Al_2O_3	1.1
Particle size	Passing 75 μm sieve aperture

B Calcium alginate preparation

The aqueous solution was prepared by first adding known volume of distilled water in 250 mL beaker and stirred at 85 °C for 15 min. Then, a known weight of alginate was added slowly with a constant stirring rate of 150 rpm for 10 minutes until homogeneous mixture was observed. Prior to the addition of calcium carbonate powder, the alginate solution was cool down to 50 °C to prevent thermal shock from occurring that would initiate lump formation between alginate and calcium carbonate. Subsequently, the mixture of alginate and calcium carbonate powder was stirred for 15 minutes and the measurement of viscosity and pH was carried out, respectively.

C Viscosity and pH measurement

The viscosity of the calcium alginate in aqueous was measured by using laboratory viscometer model DV II+Pro (Brookfield, USA). The spindle used during measurement

was based on the manufacturer recommendation of SC4-27. During the measurement, the aqueous temperature was increased to 80 °C for 15 minutes interval and the spindle speed was maintained at 20 rpm throughout the measurement process. Next, pH was measured by using Eutech 2700 pH meter (Thermo-Scientific, USA). The entire measurements were done in triplicates and the average values were used further in statistical analysis.

D Design of experiment

To achieve adequate and reliable measurements of interests, the response surface methodology (RSM) was used. RSM is a collection of mathematical and statistical technique for developing, improving and optimizing independent and dependent variables (5). It is normally used to identify the relative of several affecting factors in the presence of complex relationship. Above all, the application of RSM will increase product yields, reduce process variability, closer confirmation of the output response to nominal and target requirements and reduces trial and overall cost [13].

In this work, a central composite design (CCD) which is an efficient tool for sequential experiment was used to incorporate information from a properly planned factorial experiment. The CCD consists of 2k factorial or "cube" points where 'k' is the number of factors. 2k axial points fixed axially at a distance α, from the center to generate quadratic terms, and replicate tests at the center of experimental region (14). In addition, replicates of the tests are important as they provide an independent estimate of the experimental error. In this work, a CCD for 2 factors (alginate and calcium carbonate), with 5 replicates at the center resulting in total $2^2 + 2^2 + 5 = 13$ runs. A value of α = $[2^k]^{1/4}$ assures rotation of the CCD and equivalent to 1.414.

In order to narrower the independent variables ranges, a preliminary experiment was conducted prior to design of experimental runs to minimize the uncontrolled factors effects. It was found that the effective ranges of calcium lies between 2 to 10 g. Meanwhile, the effective alginate amount in aqueous preparation was found between 5 to 10 % (w/v). Generally, the viscosity and pH of calcium alginate relies on these preparation conditions knowing that increasing calcium carbonate dosage led to increase of pH due the precipitation of calcium ion. In addition, viscose solution tends to retard the formation of alginate beads when ejecting from injector nozzle. Therefore, these two independent factors have been identified as the key variables in the preparation of calcium alginate in aqueous. Simplified design summary for independent variables and responses in terms of coded factors is listed in Table 2. A complete CCD with 4 factorial points, 4 axial points and 5 replicates of the center point are given in Table 3.

TABLE 2: Independent Variables and Responses In Coded Term

Independent factors	Code	Unit	Range
CaCO$_3$	x_1	g	2 – 10
Alginate	x_2	% (w/v)	5 – 10
Responses	Code	Unit	Range
Viscosity	Y_1	cP	24 – 49
pH	Y_2	-	9.7 – 11.8

TABLE 3: Experimental Design and Results

Run	Design order		Results			
	CaCO$_3$, x_1	Alginate, x_2	Viscosity, Y_1 (cP)		pH, Y_2	
			Experiment	Predicted	Experiment	Predicted
1	-1	-1	24.00	24.37	10.40	10.20
2	+1	-1	40.00	40.63	9.70	10.03
3	-1	+1	38.00	37.87	11.30	11.67
4	+1	+1	45.00	45.13	10.40	10.37
5	-1.414	0	27.00	26.93	11.80	11.71
6	+1.414	0	44.00	43.57	9.00	9.34
7	0	-1.414	33.00	32.39	10.90	10.43
8	0	+1.414	45.00	45.11	11.30	11.54
9	0	0	49.00	48.60	11.40	11.54
10	0	0	49.00	48.60	11.80	11.54
11	0	0	49.00	48.60	11.80	11.54
12	0	0	48.00	48.60	11.40	11.54
13	0	0	48.00	48.60	10.40	10.20

III RESULTS AND DISCUSSIONS

A Model fitting

The most important parameters which affect the viscosity and pH of the calcium alginate are calcium carbonate and alginate dosage. In order to investigate the combine effects of these factors, experiments were conducted at different combination. Thirteen runs of experiments were carried out to evaluate the effects of these combinations and correlated based on the second-order polynomial model. Also, the suggested models for both responses were found to be quadratic as shown in Equations 1 and 2:

$Viscosity, Y_1 = +48.60 + 5.88x_1 + 4.50x_2 - 6.67x_1{}^2 - 4.92x_2{}^2 - 2.25x_1x_2$ (1)

$pH, Y_2 = +11.51 + 0.47x_1 + 0.39x_2 - 0.25x_1{}^2 - 0.8x_2{}^2 + 0.35x_1x_2$ (2)

where x_1 and x_2 are the calcium carbonate and alginate dosage. The coefficient with one factor is known as the effect of that particular factor, meanwhile the coefficient of two factors and the other with second-order terms known as the interaction between the two factors and quadratic effects. In addition, the positive and negative sign represents and synergistic and antagonistic effects, respectively (15).

B Models validation

To ensure satisfactory and adequate prediction to the real system of the fitted data, model validation was carried out. Also, the fitted model was validated for precise judgment as to avoid misleading conclusions. In this work, we used graphical and numerical methods as an ideal tool for model explanatory. A residual is defined as difference between an observed value and estimated value. Figure 1 shows residuals against the fitted values. Meanwhile, Figure 2 shows the residuals against observation data. The plot was drawn to evaluate any inconsistency or any drift for each observation of the residuals. It can be assumed that both residuals (viscosity and pH) of the models were randomly distributed and no obvious drift of the data models were found.

Next, normal probability plots of the models data were plotted to check for the normality. Additionally, if the residual plot lies approximately along a straight line, the normality assumption is satisfied. Any departure from a straight line indicates that a departure from a normal distribution of the residuals. In this study, it was observed that the residuals for both viscosity and pH were normally distributed and therefore the normality assumptions are satisfied and response variables are normally distributed. Plots of normal probability against residuals for viscosity and pH are shown in Figure 3.
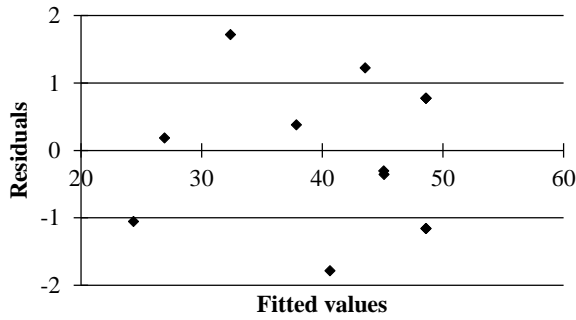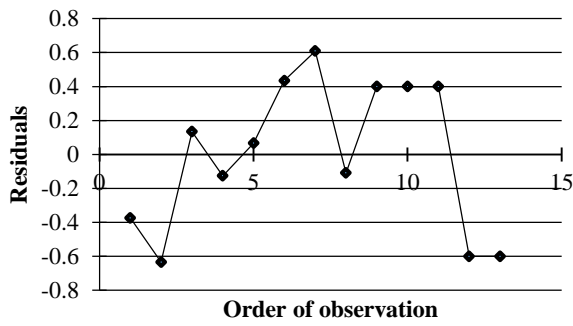
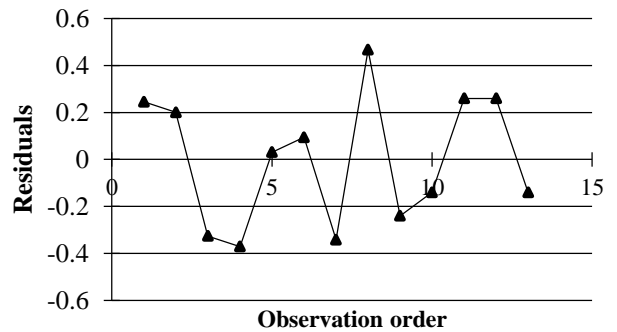**(a)**
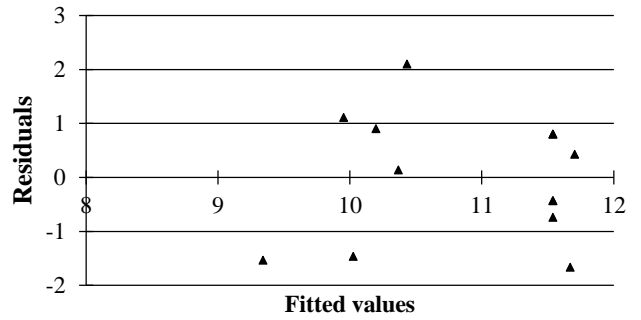
(b)

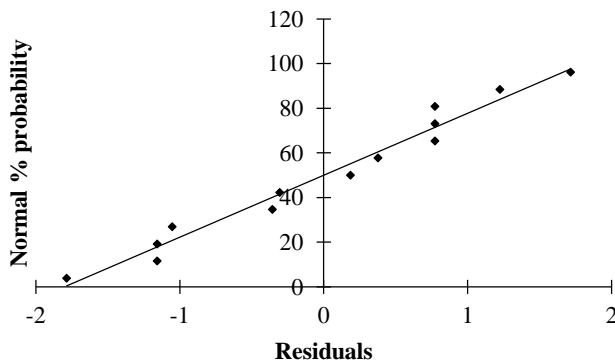Figure 1: Plot of residuals against fitted values for a) Viscosity and b) pH
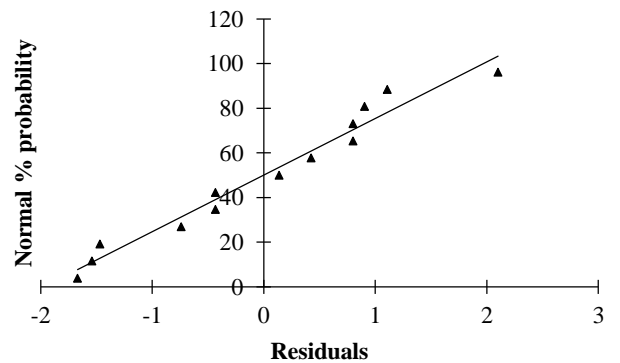

(a)

(b)

Figure 2: Plot of residuals against order of observation for a) Viscosity and b) pH

(a)

(b)

For numerical analysis, the developed models were then checked by employing the coefficient of determination (R^2) and adjusted R^2 (R^2adj). R^2 indicates how fit a set of data points distribute in a statistical model. In contrast, R^2adj modifies the R^2 by taking into account extra explanatory variable in the model. To obtain this, the sum of squares (SS), number of experiment (n) and the number of predictor or terms (p) were used and calculated as follows:

$$R^2 = 1 - \frac{SS_{residual}}{SS_{model} + SS_{residual}} \tag{3}$$

$$R^2_{adj} = 1 - \frac{n-1}{n-p}(1 - R^2) \tag{4}$$

The calculated R^2 values for both viscosity and pH for respective models were higher than 90%, corresponds to 0.9974 and 0.9008. In addition, the R^2adj for both responses were found close to R^2 (0.9955 and 0.8299) and conforms satisfactory adjustment of the quadratic models towards the experimental data. Analysis of variance (ANOVA) was then carried out to analyze the differences between group means and variation. As demonstrates in Table 4, low probability values of less than 0.0001 and 0.0021 for viscosity and pH explained that the regression were significant to the quadratic models.

To further investigate the model adequacy, lack of fit for each of the models was carried out. Principally, the lack of fit describes the variation in the data to the fitted model. In the case that the model does not fit the data sufficiently, the lack of fit will be significant. As can be seen from Table 4, the lack of fit of 0.395 and 0.1084 for viscosity and pH indicates that the lack of fit was not significant which suggests that the model was capable to describe the data well. Moreover, adequate precision measures the signal to noise ratio for both viscosity and pH of 61.540 and 9.592 (data not shown) which more than 4 implies that the model can be used to navigate the design space.

C Optimization analysis

To obtain an optimum preparation condition for calcium alginate, response optimizer was employed. However, it is crucial to analyze the relationship between predictors and responses prior to optimization for each model and carried out first. The analyses were carried out by means of Fisher 's 'F' and Student 't' tests. Generally, an F-test is used to test for more than one coefficient or joint hypotheses, whereby, t-test is used whenever the hypotheses test is concern to one coefficient at a time. The p values associated with t- test explain the significance for each factor and the interaction between them. As the magnitude of t increases, the values of p become smaller which corresponds to more significance of the coefficient term. As can be seen from Table 5, linear, quadratic and interaction terms for predictors were found to be significant for viscosity measurement with p values less than 0.000. It can be considered that all the coefficients gave

ultimate effect in determining the optimum condition for viscosity.

Meanwhile, quadratic effect of calcium carbonate gave least significant effect for determining pH with p values of 0.110 followed by interaction between calcium carbonate and alginate (0.095). In addition, linear effect of calcium carbonate (0.008), alginate (0.020) and quadratic (alginate, 0.001) were found to have significant impact from the coefficient of regression for pH, respectively.

TABLE 4: ANOVA for Viscosity and pH

Statistical parameter	Degree of free-dom	Sum of square	Mean square	Prob.>F	Remarks
Viscosity					
Model	5	884.88	176.98	< 0.0001	Significant
x_1	1	276.61	276.61	< 0.0001	
x_2	1	161.74	161.74	< 0.0001	
x_1^2	1	309.95	309.95	< 0.0001	
x_2^2	1	168.73	168.73	< 0.0001	
$x_1 x_2$	1	20.25	20.25	0.0001	
Lack of fit		1.15	0.34	0.3953	Not significant
pH					
Model	5	8.36	1.67	0.0021	Significant
x_1	1	1.79	1.79	0.0078	
x_2	1	1.19	1.19	0.0197	
x_1^2	1	0.44	0.44	0.1105	
x_2^2	1	4.75	4.75	0.0005	
$x_1 x_2$	1	0.49	0.49	0.0950	
Lack of fit				0.1084	Not significant

Figure 4 shows the three dimensional mesh wire plots for viscosity and pH. The plots enable graphical visualization to understand the relationship between independent variables and responses. From the figure, increasing amount of alginate and calcium carbonate led to increase in viscosity. In contrast, little dosing of alginate and calcium carbonate reduced viscosity from 37 to 21 cP due to the leaching of calcium carbonate and alginate compared to water's density. This condition is undesirable because low viscosity will only retard the formation of calcium alginate in cross-link solution. Therefore, the interest region for viscosity was fixed at 37 cP to ensure pourability of calcigum alginate mixture. For the case of pH, its proven that lower dosing of calcium carbonate and alginate results in low pH. Generally, precipitation of alkaline ions from calcium will increase hydroxide ions. Therefore, increasing the amount of calcium

carbonate results in increase the pH values. Meanwhile, it is worth to mention that alginate plays least effect to the pH of the aqueous because dissolution of uranic and manuranic acid in alginate was limited to the amount of hydrophobic agents presence in the aqueous. In this case, water, as the main dissolving mediator was capable to overcome the acidic condi tion of alginate and produces bases aqueous condition.

TABLE 5: Estimated Regression Coefficient for Viscosity and pH

| Parameter	Viscosity, Y_1				pH, Y_2			
	Estimated coefficient	Standard error	T-value	Prob.>T	Estimated coefficient	Standard error	T-value	Prob.>T
Constant	48.600	0.2591	187.540	0.00	11.5400	0.1622	71.136	0.000
x_1	5.880	0.2049	28.702	0.00	0.4725	0.1283	3.684	0.008
x_2	4.496	0.2049	21.947	0.00	0.3859	0.1283	3.009	0.020
x_1^2	-6.675	0.2197	-30.382	0.00	-0.2513	0.1375	-1.827	0.110
x_2^2	-4.925	0.2197	-22.417	0.00	-0.8263	0.1375	-6.008	0.001
x_1x_2	-2.250	0.2897	-7.766	0.00	0.3500	0.1814	1.930	0.095
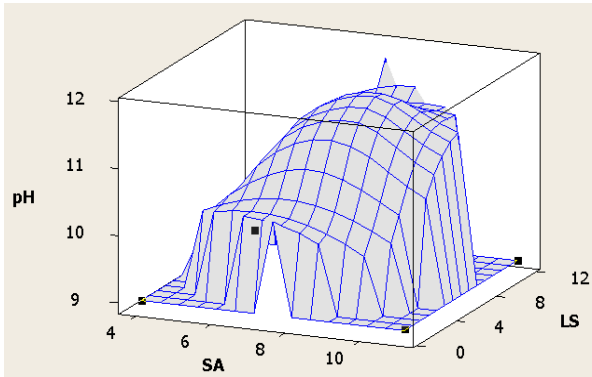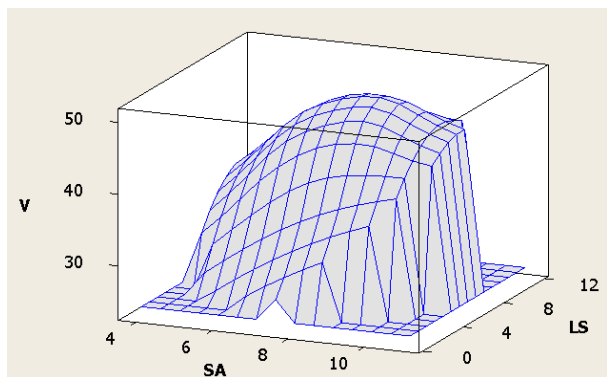
Figure 4: 3 dimensional wire plots for a) viscosity and b) pH

An optimum preparation condition for calcium alginate in aqueous was determined based on the numerical optimization process. To obtain this, combination of two responses were compromised subject to optimization parameters. Principally, a viscous condition and relatively high pH are desired to obtain an optimum preparation condition. This process is crucial in order to reduce the number of experimental runs when the original designs contain more points. In addition, the target goal for independent variables (calcium carbonate and alginate) were fixed in the range while the responses (viscosity and pH) were fixed at 37 cP and 10, respectively. The software searches for a combination of input variables levels that would jointly optimize a set of responses by satsifying the requirements for each response in the set. Finally, after obtaining composite desirability for each response, the globla solution for each of the preparation conditions was obtained sucssfully at 2 g of calcium carbonate and 10% of alginate that results in 37 cP of viscosity and pH 10. To conforms with suggested preparation conditions from the software, three replicates of experiments were carried out for Calcium carbonate and alginate. As shown in Table 6, the viscosity and pH obtained from the additional experiments are close to those predicted from the model

which indicates that the RSM was the ideal tool for optimizing the preparation conditions of the calcium alginate.

TABLE 6: Replication of Suggested Preparation Condition

	Viscosity	pH
Predicted values	37	10
Replicate 1	35	10
Replicate 2	36	11
Replicate 3	35	10
Error (%ave)	4.5	3.3

V CONCLUSION

This study has demonstrated that the utilization of RSM and design of experiment has successfully obtained the optimum preparation conditions of calcium carbonate and alginate for calcium alginate. A statisical modelling with CCD with fixed values of independent variables managed to produce a high correlation coefficient from two quadratic models. Normal probability plots for both responses were found normally distributed and successfully explained by the

ANOVA. F inally, additional experiments have shown that relatively small error arised from the replicates indicates that RSM and CCD can be used for modelling and optimizaing the calcium alginate preparation conditions.

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