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Research Article
Extraction of Toxic Rhodamine B Dye by Using Organic Solvent: A Statistical Analysis

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Abstract
The extraction of Rhodamine B (RB) dye from aqueous solution by using soybean oil as organic solvent was investigated in this study. This study is aimed to found out the significant factors, which affect the efficiency of RB extraction using a full factorial design. A total of four factors (pH, organic to aqueous phase ratio, mixing time and mixing speed) were investigated. A full factorial design was applied and the results were analyzed statistically and only pH, organic phase to aqueous phase ratio and their second-order interaction influence the RB extraction significantly. The regression model for RB extraction was developed and the adequacy of the model was examined. The value of R^2_{adj} (98.57%) and R^2 (99.71%) were found that results showed the full factorial design is a very useful tool for the screening of factors.

Key words: Dye rhodamine B, soybean oil, liquid-liquid extraction, two-level factorial design

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nowadays, organic dyes are widely used in the industrial field, such as study and leather. Release such dyes will cause legislation problem and it is also a great challenge to the scientist (Forgacs *et al*., 2004). It is very difficult to remove such dyes by a conventional method because of the high stability against temperature, light, chemical and microbial attacks (Da Silva Lacerda *et al*., 2015). The removal of Rhodamine B (RB) by using liquid-liquid extraction has been reported, such as extraction and recovery of rhodamine B, methyl violet and methylene blue from industrial wastewater using D2EHPA as an extractant (Muthuraman and Tjoon, 2009) and facilitated transport of cationic dyes through a supported liquid membrane with D2EHPA as carrier (Hajarabeevi *et al*., 2009). Vegetable oils were used as organic solvent these years because of its nontoxic and environmental friendly characteristics. Several study have been reported by using vegetable oils as the organic solvent like di-(2-thylhexyl)phosphoric acid-coconut oil supported liquid membrane for the separation of copper ions from copper plating wastewater (Venkateswaran *et al*., 2007).

Factorial designs are very necessary to investigate the main effects and interactions of the factors on a response variable and they are widely used in experiments involving several factors. A very important special case of the factorial design is that the k factors of interest have only two levels. Because each replicate of these kind of designs have 2^k experimental runs, these designs are called 2^k factorial designs. A 2^k factorial design is very important in response surface design because it is a very useful tool to screen the factors at the start of a study and it is a basic building block used to create other response surface designs. The statistical model of a 2^k factorial design will include k main factor and k-factor interactions (Myers *et al*., 2009). Several research have been reported to use a 2^k factorial design for screen the effects of the factors, such as optimization and design of an ibuprofen-loaded nanostructured lipid carrier with a 2^3 full factorial design (Suto *et al*., 2015), quantitative and qualitative optimization of allergen extraction from peanut and Optimization of buffer and ionic strength using a full factorial experimental design (L'Hocine and Pitre, 2016) and coal fly ash alkalis content characterization by means of a full factorial design (Sanjuan and Argiz, 2016). This study is aimed to select a suitable vegetable oil as organic solvent for RB extraction and also to screen the effects of factors influencing the extraction.

MATERIALS AND METHDOS

Materials: Soybean oil with 100% purity was supplied by Soon Soon Oil Mill Sdn. Bhd., Malaysia. Rhodamine B (F and M chemicals), sulfuric acid (H_2SO_4) (Merk 98% purity), sodium hydroxide (NaOH) (Merk 99% purity) were used in the experiment.

Equipment: A digital viscometer (Brookfiled, LVDL-I Prime) was used to measure the viscosity of the organic solvent. An orbital shaker (Fisher Scientific, SSL1) was used to mix the aqueous and organic phases. A pH meter (Mettler Toledo, Deta 320) was used to measure the pH of aqueous phase before extraction. The concentration of rhodamine B is measured by a UV-visible spectrophotometer (Spekol 1200).

Experimental procedure: The viscosity of organic solvent was measured by a viscometer. The pH of aqueous phase was adjusted by H_2SO_4 or NaOH solution to reach a certain pH value (3 or 5). A volume of 30 mL of RB solution with certain pH mixed with was mixed with soybean oil at a particular ratio (0.5 or 1) in a glass bottle. The bottle was shaken by an orbital shaker at a certain shaking speed (90 or 150 rpm) for a certain time (80 or 120 min). The mixture was then transferred into a phase separation funnel for phase separation. Finally, 10 mL of aqueous solution was taken to measure its concentration. The extraction of rhodamine B was presented by its percentage color removal in Eq. 1:

$$\text{Percentage Color removal} = \frac{(\text{Dye})_{\text{ini}} - (\text{Dye})_{\text{aq}}}{(\text{Dye})_{\text{ini}}} \times 100 \quad (1)$$

where, $(\text{Dye})_{\text{ini}}$ is the initial dye concentration in the aqueous phase and $(\text{Dye})_{\text{aq}}$ is dye concentration of aqueous phase after extraction. All runs were carried out in duplicate or triplicate at room temperature.

Design of experiment

Two-level factorial design: In this study, a 2^4 factorial designs were built to screen the effects of the factors. Total of 4 factors, namely pH, ratio of organic to aqueous phase (O/A ratio), shaking speed and shaking time were selected. A total of 21 runs were conducted at homogenous room temperature. Table 1 shows the factors and levels applied in 2^4 factorial designs.

Regression model: Multiple regression is a collection of statistical techniques, which is very useful to build the types of empirical models in response surface methodology. In general, the first-order regression model is represented in Eq. 2:

Table 1: Factors and levels applied in 2^4 factorial design

Factors	Symbols	Units	Level -1	Level +1
pH	A	-	3	5
O: A ratio	B	-	0.5	1
Shaking speed	C	rpm	90	150
Shaking time	D	min	80	120

$$Y = \beta_0 + \sum_{j=1}^{k}\beta_j\chi_j + \sum_{i<j}\sum\beta_{ij}\chi_i\chi_j + \varepsilon \quad (2)$$

In some situations, the curvature in the response function will not be adequately modeled by the first-order regression model. Hence, a second-order regression model is considered, which is given by the Eq. 3:

$$Y = \beta_0 + \sum_{j=1}^{k}\beta_j x_j + \sum_{i<j}\sum\beta_{ij}x_i x_j + \sum_{j=1}^{k}\beta_{jj}x_j^2 + \varepsilon \quad (3)$$

where, β_0 is the intercept of the plan, β_j is the regression coefficient, which represents the expected change in response per unit change in x_j when all the remaining independent variables x_i ($i \neq j$) are held constant, β_{jj} represents pure second-order or quadratic effects, β_{ij} is the interaction term, x_i and x_j are independent variables also called predictor variables or regressors and the term is the random error term. A second order regression model is widely used in response surface methodology as it is very flexible. It can take on a wide variety of functional forms and it often works well as an approximation to the true system (Montgomery, 2008).

Model adequacy checking: The adequacy of the model in this study fitting the observed data was examined by the ANOVA (analysis of variance) and the coefficient of determination (R^2). There are two reasons to examine the adequacy of a regression model. Firstly, it is to verify none of the least squares regression assumptions are violated and it also needs to examine the fitted model to ensure it provides an adequate approximation to the true system. In this study, R^2 measures the goodness of fitting a model and it is a measure of total variability in the data accounted for by a regression model. The value of R^2 is from 0-1 and it is given by the Eq. 4:

$$R^2 = \frac{SS_R}{SS_T} = 1 - \frac{SS_E}{SS_T} \quad (4)$$

where, SS_R is the residual sum of squares, SS_T is the total sum of squares and SS_E is the error sum of squares. The value of R^2 always increases when it is adding a variable to the model no matter the variable is significant or not. Hence, R^2_{adj} is prefer to use in the adequacy checking, which is given by Eq. 5:

$$R^2_{adj} = 1 - \frac{SS_E/(n-p)}{SS_T/(n-1)} = 1 - \frac{(n-1)}{(n-p)}(1-R^2) \quad (5)$$

where, n-p is the degree of freedom associated whit the error sum of squares, n-1 is the degree of freedom associated with total sum of squares. In general, the adjusted R_2 statistic will not always increase as variable are added to the model (Myers *et al*., 2009).

RESULTS AND DISCUSSION

Selection of vegetable oil: Different pH value from pH of 2-11 was investigated in this study. Table 2 shows the results of percentage color removal of RB, which has the highest value of percentage color removal (96%) at pH value of 6, loaded 150 mM tributyl phosphate (TBP) using corn oil as organic solvent. However, emulsion was observed at pH of 11 when corn oil alone as organic solvent without loading TBP, as well as soybean oil as organic solvent. No emulsion was occurred when TBP was loaded in the vegetable oil. This may because of the demulsification of TBP (Cao *et al*., 2016). Figure 1 shows the pH-extraction isotherms of RB extraction investigated with soybean oil and corn oil loaded with 150 mM TBP, respectively.

Determination of viscosity of vegetable oils: Viscosities of vegetable oils (corn, soybean oils) were measured at room temperature in this study. Table 3 shows the density and viscosity obtained from this study, which has great similarity with those reported in the previous stusys (Esteban *et al*., 2012). The viscosity of soybean oil is relatively lower than corn oil and much higher than kerosene. Various organic solvent in the order of increasing viscosity are kerosene mixture of kerosene and soybean oil soybean oil corn oil, respectively. According to the percentage color removal by using corn oil and soybean oil as different organic solvent (Table 2), very similar result of percentage color removal was obtained. However, tributyl phosphate (TBP) can irritate the nose and throat when breathing and may be absorbed through the skin. It can also irritate the lungs causing coughing and/or shortness of breath, which is considered as hazard material (Pont, 2005). Soybean oil has relatively lower viscosity and it also has 2nd highest global consumption in the past decade was selected for further study alone as organic solvent.

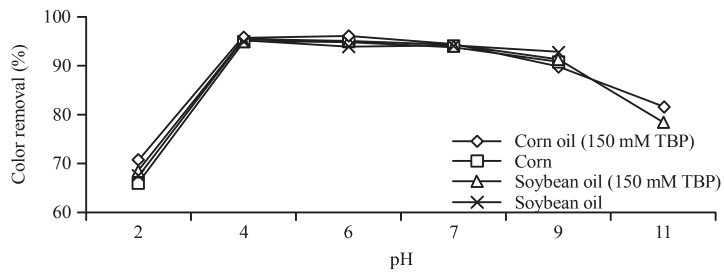

Fig. 1: pH-extraction isotherms of RB investigated with different vegetable oil

Table 2: Percentage extraction of RB by using corn oil and soybean oil as organic solvent

Corn Oil (Percentage color removal)			Soybean Oil (Percentage color removal)	
pH	TBP (150 mM)	Without extractant	TBP (150 mM)	Without extractant
2	70.8	66.1	68.5	67.5
4	95.7	95.0	95.4	95.0
6	96.0	94.9	95.1	94.0
7	94.2	94.0	94.4	94.1
9	89.8	90.8	91.4	92.9
11	81.5	Emulsion	78.5	Emulsion

Table 3: Density and viscosity of vegetable oils and kerosene measured at room temperatures

	Density (g cm^{-3})	Viscosity (m pa sec)
Corn	0.907	51.95
Soybean oil	0.907	45.16
Kerosene	0.762	00.59
Kerosene (10%)+soybean oil (90%)	0.894	32.99

Table 4: Design matrix of 2^4 full factorial design and percentage color removal measured

	Factors				
Run	A	B	C	D	Percentage color removal
1	5	0.50	90	120	91.9
2	4	0.75	120	100	92.7
3	3	0.50	150	80	86.6
4	5	1.00	90	120	95.0
5	3	0.50	90	80	86.6
6	3	1.00	90	120	91.1
7	4	0.75	120	100	92.1
8	3	1.00	90	80	90.8
9	3	0.50	90	120	86.5
10	3	1.00	150	80	91.9
11	5	1.00	150	80	95.4
12	5	1.00	150	120	94.7
13	4	0.75	120	100	92.7
14	5	0.50	150	120	92.0
15	5	1.00	90	80	95.7
16	3	1.00	150	120	91.5
17	5	0.50	150	80	91.7
18	4	0.75	120	100	92.0
19	4	0.75	120	100	92.1
20	3	0.50	150	120	85.4
21	5	0.50	90	80	91.7

Screening of factors influencing percentage color removal: Rhodamine B dye waste water with concentration of 0.1 g L^{-1} was used in the liquid-liquid extraction process. Total of 21 runs with 2 replications of 2^4 full factorial design with 4 factor (pH (A), O/A ratio (B), shaking speed (C) and shaking time (D)) were carried out in homogenous condition and the results are shown in Table 4. Percentage color removal ranged from 85.4% (run 20) to 95.4% (run 15).

Normal probability plot and pareto chart of standardized effect: Figure 2 shows the normal plot of the standardized effects of the factors and their interaction terms. All of the effects that lie along the line are negligible, where as the large effects are far from the line. The significant effects that emerge from this analysis are the main effect of A (pH), B (O/A ratio) and their interaction term AB. The parato plot (Fig. 3) displayed the absolute value of the effect of terms, which are considered to be important in this design. A reference line is drawn to indicate that the factors, which extend past this line are potentially important (Antony, 2003).

Regression model for percentage color removal: The estimated effects, coefficients (Coef), standard deviation (SE Coef), t-statistics (t-value) and probability (p-value) of the regression model for percentage color removal is shown in Table 5. A regression model for percentage color removal is presented as in Eq. 6:

$$\text{Percentage color removal} = 91.15+2.3563A+2.1062B-0.0062C -0.1437D-0.4188AB-0.0563AC-0.0312AD+0.1188BC -0.0438BD-0.1062CD-0.2063ABC-0.1937ABD+ 0.1187ACD+0.0188BCD-0.0312ABCD \quad (6)$$

where, A is pH, B is O/A ratio, C is shaking speed and D is shaking time. The negative sign of the coefficient indicates their antagonistic effect and the positive sign donates a synergistic effect. At a 5% significant level, the p-value of a term is larger than 0.05 indicate the term is insignificant and the p-value of a term is smaller than 0.05 indicates the term is significant. After deducting the insignificant terms as p-values are larger than 0.05, the regression model is reduced as in Eq. 7:

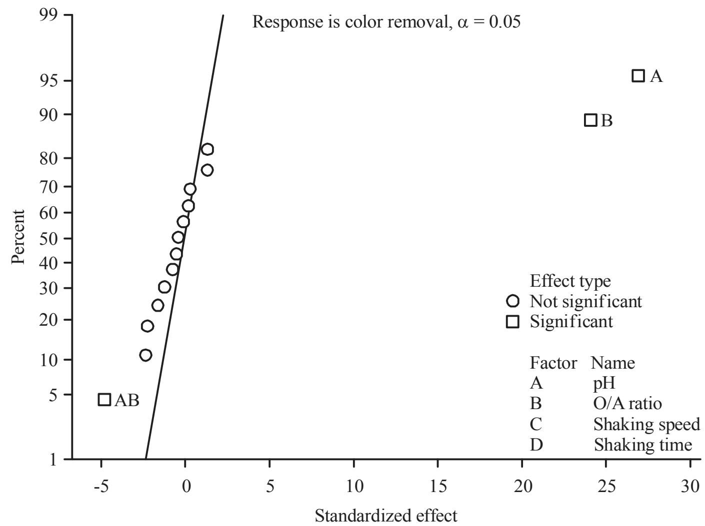

Fig. 2: Normal plot of the standardized effects for percentage color removal

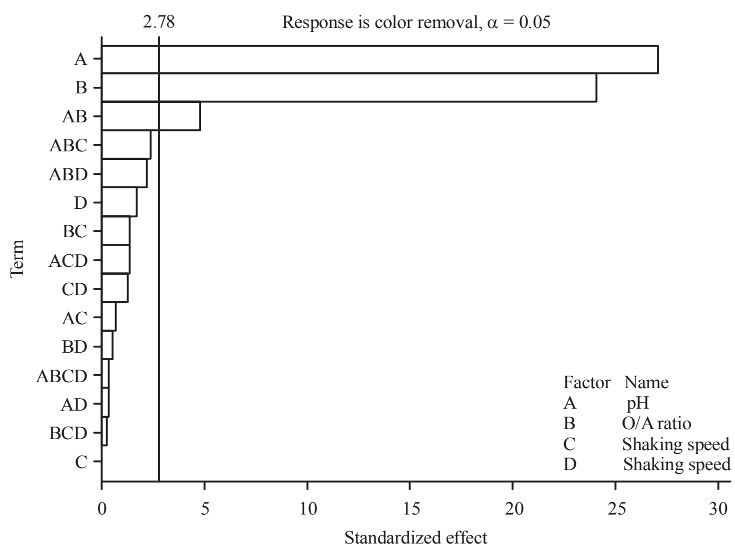

Fig. 3: Pareto chart of the standardized effects for percentage color removal

$$\text{Percentage color removal} = 91.15+2.3563A+2.1062B-0.4188AB \quad (7)$$

The positive sign of pH (A) and O/A ratio (B) incline to enhance the percentage color removal, while their interaction term pH sign O/A ratio (AB) attend to reduce it. The value of regression coefficient is larger, the degree of significant is larger. The sequence of the significant main and interaction effects of influence on percentage color removal was found to be A>B>AB.

Model adequacy checking: The adequacy of the regression model for percentage color removal was analyzed by ANOVA (analysis of variance) at 5% significant level and the degree of freedom (DF), adjusted sum of squares (Adj SS), mean sum of squares (MS), F-statistics (F-value) and probability (p-value) are shown in Table 6. The high value of F and low value of p indicates the significance of the terms pH, O/A ratio and their interaction term. The R^2 value of the model is 99.71% and this indicates that only 0.29% of total variability is not explained by the regression model. The value of R^2_{adj} is 98.57% and the deviation between R^2 and R^2_{adj} is only 1.17%. The small value of deviation means there is less chance that the insignificant terms have been included in the model (Myers *et al.*, 2009).

Table 5: Estimated effects and coefficients of the regression model

Term constant	Effect	Coefficient	SE Coef	t-value	p-value
		91.1563	0.0873	1043.92	0.000
A	4.7125	2.3563	0.0873	26.98	0.000
B	4.2125	2.1062	0.0873	24.12	0.000
C	-0.0125	-0.0062	0.0873	-0.07	0.946
D	-0.2875	-0.1437	0.0873	-1.65	0.175
AB	-0.8375	-0.4188	0.0873	-4.80	0.009
AC	-0.1125	-0.0563	0.0873	-0.64	0.555
AD	0.0625	0.0312	0.0873	0.36	0.739
BC	0.2375	0.1188	0.0873	1.36	0.245
BD	0.0875	-0.0438	-0.0873	-0.50	0.643
CD	-0.2125	-0.1062	0.0873	-1.22	0.291
ABC	-0.4125	-0.2063	0.0873	-2.36	0.077
ABD	-0.3875	-0.1937	0.0873	-2.22	0.091
ACD	0.2375	0.1187	0.0873	1.36	0.245
BCD	0.0375	0.0188	0.0873	0.21	0.840
ABCD	-0.0625	-0.0312	0.0873	-0.36	0.739

A: pH, B: O/A ratio, C: Shaking speed and D: Shaking time

Table 6: ANOVA of the regression model for percentage color removal

Source	DF	Adj SS	MS	F-value	p-value
Model	16	170.139	10.6337	87.16	0.000
Linear	4	160.142	40.0356	328.16	0.000
A	1	88.831	88.8306	728.12	0.000
B	1	70.981	70.9806	581.81	0.000
C	1	0.001	0.0006	0.01	0.946
D	1	0.331	0.3306	2.71	0.175
2-Way interactions	6	3.309	0.5515	4.52	0.083
AB	1	2.806	2.8056	23.00	0.009
AC	1	0.051	0.0506	0.41	0.555
AD	1	0.016	0.0156	0.13	0.739
BC	1	0.226	0.2256	1.85	0.245
BD	1	0.031	0.0306	0.25	0.643
CD	1	0.181	0.1806	1.48	0.291
3-Way interactions	4	1.512	0.3781	3.10	0.149
ABC	1	0.681	0.6806	5.58	0.077
ABD	1	0.601	0.6006	4.92	0.091
ACD	1	0.226	0.2256	1.85	0.245
BCD	1	0.006	0.0056	0.05	0.840
4-Way interactions	1	0.016	0.0156	0.13	.739
ABCD	1	0.016	0.0156	0.13	0.739
Error	4	0.488	0.1220		
Total	20	170.627			

A: pH, B: O/A ratio, C: Shaking speed and D: Shaking time

CONCLUSION

Soybean oil was selected as the most suitable organic solvent because of its lower viscosity compared to corn oil. Screening of 4 factors (pH, O/A ratio, shaking speed and shaking time) influencing the percentage color removal by soybean oil as organic solvent using a two-level factorial design reveals that only pH, O/A ratio and their interactions are considered as significant terms. A second order regression model was developed and the adequacy of the model was examined. The value of R^2_{adj} (98.57%) and R^2 (99.71%) were determined. The results indicate that the two-level factorial design is a very useful tool for screening of factors and evaluate their effects at the start of the study.

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