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MATHEMATICAL MODELLING OF BACKWARD EXTRACTION MIXED REVERSE MICELLE OF AMOXICILLIN BY SURFACE RESPONSE METHODOLOGY (RSM)

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ABSTRACT

One of important factor in reverse micelle extraction is backward transfer. It is important to investigate the favourable conditions for backward transfer from reverse micellar phase to an organic phase. The back extraction of amoxicillin was studied using mixed reverse micelle with combination sodium bis(2-ethylhexyl) sulfosuccinate (AOT) and TWEEN 85. Backward extraction was optimized via response surface methodology (RSM). For mathematical modelling, Central Composite Design (CCD) was used to studies the significant of independent variables: pH of stripping solution (5-8), KCl concentration (2.0 -16.0 g/L) and backward extraction time (5-35 minutes) on the response of the process. Optimized backward extraction for maximized final mass of amoxicillin extracted into aqueous were pH of stripping solution (6.58), backward time (19.8 minutes) and concentration of KCl (11.02 g/L) on the response of the process. Result showed that the experimental data was fitted well to a second-order polynomial model.

Keyword: Backward extraction, amoxicillin, mixed reverse micelle, response surface methodology

1.0 INTRODUCTION

Amoxicillin is a semi synthetic β-lactam antibiotic used for the treatment of bacterial infections such as bronchitis, gonorrhoea and pneumonia caused by Gram-positive and Gram-negative bacteria (Ahmadi *et al*., 2017). Amoxicillin is usually combined with β-lactamase inhibitor potassium clavulanate for commercial antibiotic drugs (Bejjani *et al*., 2016). In industry, amoxicillin is produce via a chemical coupling process by a β-lactam nucleus and appropriate acyl donors (Chuo *et al*., 2014). Therefore a powerful extraction method needed for separation by-product.

Reverse micelle extraction (RME) has been noticed as an attractive extraction tool for biological downstream processing (He *et al*., 2015). Reverse micelle formed when surfactant, oil and water are combining together under certain conditions and the driving forces of the process are steric, electrostatic and hydrophobic interactions between biomolecules and micelles (Hemavathi *et al*., 2010). Ding *et al.* (2016) described reverse micelle as the aggregation of surfactant in organic solution with an inner core of

water molecules dispersed in a continuous organic solvent phase. Biomolecules are able to solubilize into water core in reverse micelle and provide microenvironment and sheltered from organic medium hence maintaining the functional properties of biomolecules (Tonova *et al*., 2008; Xiao *et al*., 2013). RME have been recognized for its benefits since it able to operate in large-scale and continuous operation and it can be carried out by using the existing liquid–liquid extraction system (Kumar *et al*., 2011; Chen *et al*., 2006).

Reverse micelle consist two steps of extraction forward and backward extraction (Sun *et al*., 2009). In forward extraction, biomolecules solubilize in reverse micelle phase while for backward extraction the biomolecules will stripped back from reverse micelle solution (Leser *et al*., 1993). However, the backward extraction received less interest in the literature. A number of problems had been reported from previous researches regarding backward extraction (Mohd-Setapar *et al*., 2012; Mohd-Setapar *et al*., 2009; Sun *et al*., 2009; Ono *et al*., 1996), including need a sufficient amount of water to recover biomolecules (Sun *et al*., 2009) and easy to form emulsion between two phase (Lee *et al*., 2004) therefore, an improvement in backward extraction is necessary.

RSM is originally introduced by Box and Wilson (1951), which this method are provide a way to evaluate the effect of process variables and interactions on the response. Swamy and Muthukumarappan (2017) stated that RSM is an effective statistical approach to optimize a complex processes. Response surface methodology was employed in order to optimize the backward extraction of amoxicillin and enhance the recovery of amoxicillin. All data were implemented by CCD using STATISTICA 8.0 software.

2.0 EXPERIMENTALS

Reagents and Materials

Analytical-grade amoxicillin, AOT and non-ionic TWEEN 85 surfactant were supplied from Sigma Aldrich and used without further purification. Isooctane and KCl were purchased from Merck, Germany. All chemicals used for the experiments were analytical grade. All aqueous solutions were prepared by using deionized. A stock solution of 5 gL^{-1} of amoxicillin was prepared by dissolving 5 g of the reagent in 1 L of deionized water in a volumetric flask.

Backward Extraction

For backward process, organic solution containing amoxicillin from the forward extraction process was reused. Equal volume (5mL) organic and aqueous solution was mixed together (Brandani *et al*., 1996) and the mixture was stirred for about 15 minutes at 350 rpm for extraction (Gaikaiwari *et al*., 2012). The steps for backward extraction are shown in Figure 1. Finally, the amoxicillin concentration in an aqueous phase after backward extraction was further analysed by using UV-Vis Spectrophotometer.

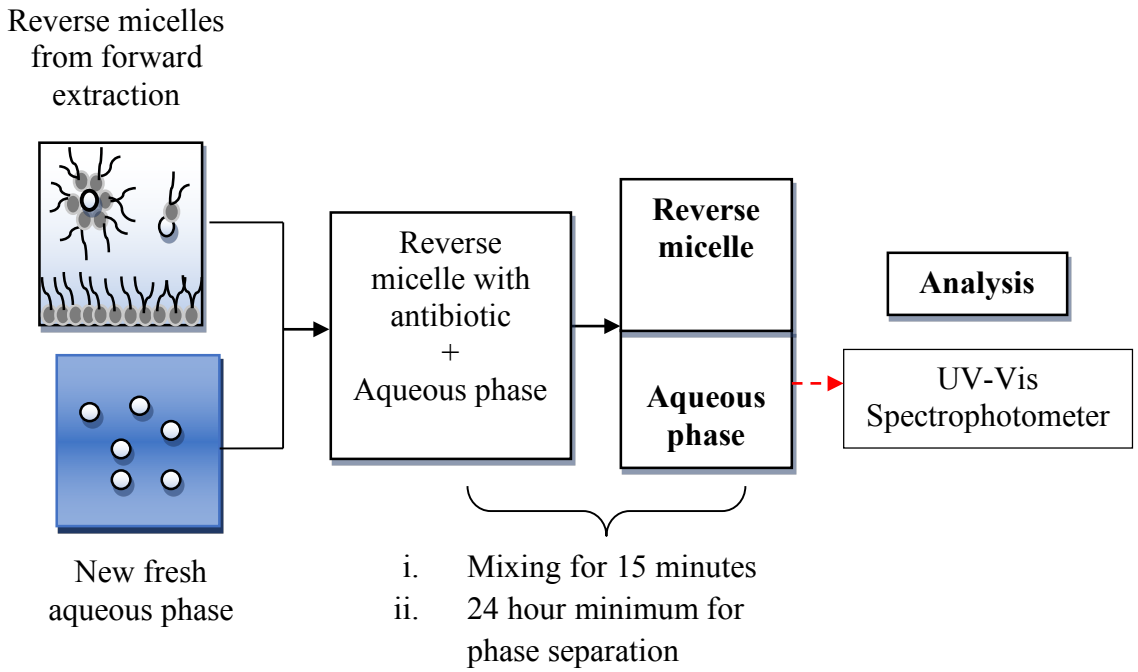

Figure 1: Flow chart of the experimental procedure for backward extraction

3.0 RESULT AND DISCUSSION

Model Fitting

The significance of the backward extraction model was examined by Fisher's F-test (Sun *et al*., 2009). The mathematical model represents the backward extraction of amoxicillin by mixed AOT/TWEEN 85 micelles in the experimental region considered here as follow:

For response final mass of amoxicillin extracted into aqueous, Y_1:

$$Y = -121.15077 + 28.03931\,X_1 - 2.12772\,X_1^2 + 4.28307\,X_2 - 0.19435\,X_2^2 + 2.28857\,X_3 - 0.05793\,X_3^2 \quad (1)$$

Where X_1 is stripping pH, X_2 is concentration of KCl (g/L) and X_3 is backward time (minute). The analysis of variance results is presented in Table 1 and the value of the coefficient determination (R^2) was identified as 0.94144. This implies that the data variation of 94.144% for the backward extraction of amoxicillin was attributed to the independent variables and only 5.856% could not be explained by using the model.

Table 1: Analysis of variance (ANOVA) for the final mass of amoxicillin extracted into aqueous phase in backward extraction

$F_{calculated}$= 250.0394 $F_{tabulated}$= 4.64	R^2=0.94144; Mean Square of Residual=2.042278				
Factors	**Sum of Square**	**Degrees of freedom**	**Mean of Square**	**F-statistic**	**P-value**
(1) pH of Stripping Solution (Linear)	1.0858	1	1.0858	0.5317	0.478840
(1) pH of Stripping Solution (Linear)	30.5469	1	30.5469	14.9573	0.001942
(2) KCl Concentration (Linear)	104.7639	1	104.7639	51.2976	0.000007
KCl Concentration (Quadratic)	147.1839	1	147.1839	72.0685	0.000001
(3)Backward Extraction Time (Linear)	0.6230	1	0.6230	0.3050	0.590115
Backward Extraction Time (Quadratic)	226.4520	1	226.4520	110.8821	0.000000
Error	26.5496	13	2.0423		
Total Sum of Square	453.3695	19			

From the calculation, the statistical significance of the quadratic model was revealed by using the *F*-test since the $F_{calculated} >> F_{tabulated}$ was indicated in ANOVA table, therefore, implying that the model was significant at the 1% significance level. This suggests that the backward model accurately represented the data in the experimental region and the second-order term was sufficient to express the data.

Influence of Independent Variables

In order to observe the significance of various effects on the process parameter on the response, the regression analysis was carried out by using statistical software. Table 2 indicates the magnitude of *P*-values and it clearly shows that the quadratic and linear term for all process variables have significant effect on the amoxicillin recovery at 5% significant level except for linear pH of stripping solution and linear backward extraction time since the value of p is 0.590115 and 0.478840 respectively ($p>0.05$). In addition, the positive sign of coefficient for X_1, X_2 and X_3 for Y_1 showed linear effects to the final mass of amoxicillin extracted into aqueous phase in backward extraction.

Table 2: Significance of regression coefficients for responses Y_1

Coefficients	Response Y_1 Values	*P*-Values
Constant	-121.15077	
X_1	28.03931	0.478840
X_2	4.28307	0.000007
X_3	2.28857	0.590115
$X_1 X_1$	- 2.12772	0.001942
$X_2 X_2$	- 0.19435	0.000001
$X_3 X_3$	- 0.05793	0.000000

As stated before, anionic surfactants (in this research is AOT), the maximum extraction efficiency had been observed at pH of stripping solution above the pI value (4.7). As the pH was increased, the amount of amoxicillin recovery would increase through the result of electrostatic repulsion between surfactant head group and amoxicillin molecules. Meanwhile, for KCl concentration variables, Hemavathi *et al.* (2010) clarified that the efficiency of backward extraction increases when salt concentration is raised, due to the decrease of reverse micelles size, hence proteins are easily to expel out from the micelles (in their case). In addition, by increasing the backward extraction time (between 5 until 15 minutes), it was found that the amount of amoxicillin was enhanced because the amoxicillin molecules had enough time to be in contact with the aqueous solution and transfer back from reverse micelle core. A Pareto chart in Figure 2 also can be used to graphically summarize and display the relative importance of the independents variables on the amoxicillin recovered in backward extraction.

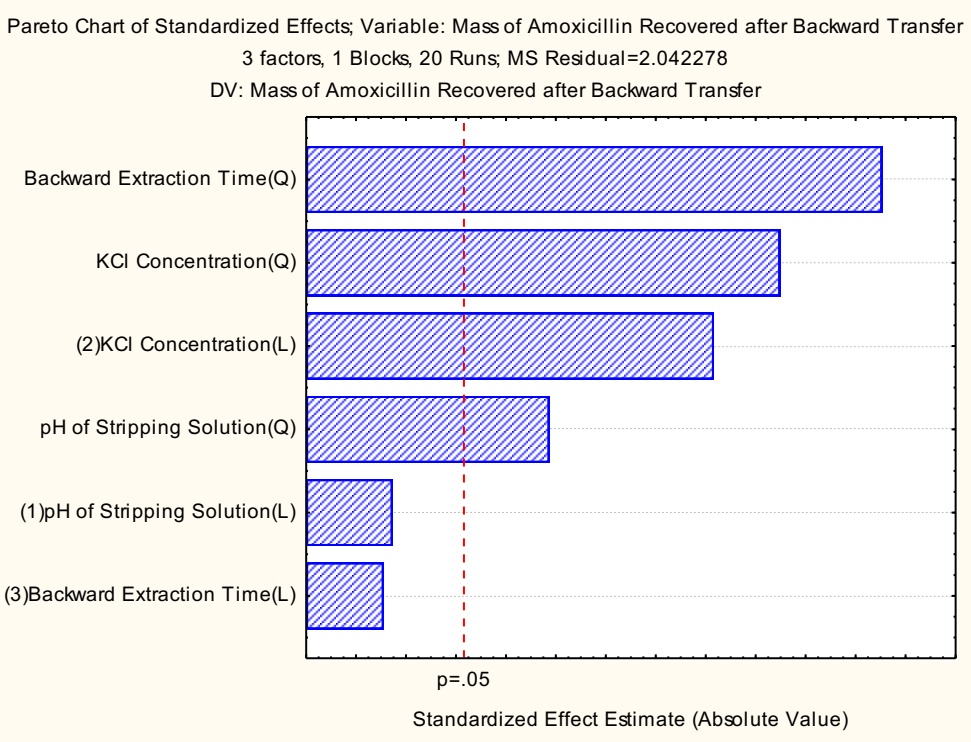

Figure 2: Pareto chart indicating the significance level for each parameter for response *Y*

The contributing factors in descending order are identified as quadratic backward extraction time > quadratic KCl concentration > linear KCl concentration > quadratic pH of stripping solution. Therefore, the most contributing factors in order to maximize the recovery of amoxicillin in backward extraction are the backward extraction time and KCl concentration. However from another point of view if the time extraction was too long, it might have increased the chance for the amoxicillin to be in contact with the iso-octane solvent, leading to the damage the amoxicillin and affecting the performance of backward transfer.

Response Surface and Contour Plot

The response surface methodology was applied for backward extraction of amoxicillin in order to develop, improve and optimize the processes and was used to evaluate the relative significance of several affecting factors. The contour plots of response surface described by the regression model were developed to demonstrate the effects of the independent variables and effects of each independent variable on the response *Y* as shown in Figure 3-5.

The surface plot presents the final mass of amoxicillin extracted into aqueous phase response as a function of two factors and keeping another one as constant. At a definite pH of stripping solution, the recovery of amoxicillin increased with the increasing KCl concentration. However, the aqueous pH had a positive linear effect on the amoxicillin extracted at low pH level.

When the pH of the stripping aqueous phase exceeded the pI of amoxicillin (pI=4.7), the final mass of amoxicillin increased because of the electrostatic interaction between the amoxicillin and AOT/TWEEN 85, where the reverse micelles were weakened and more amoxicillin molecules were released from the reverse micelles into the aqueous phase. In addition, Zhao *et al.* (2010) asserted that high aqueous pH more than pI combined with increased salt concentration might have destabilized the reverse micelles resulting in higher back transfer efficiency.

However, a further increase in the pH resulted in the decrease of the amount of amoxicillin because of the structure damage of amoxicillin. As reported by Ono *et al.* (1996), that when the value of pH was increased during the extraction of haemoglobin using dioleyl phosphoric acid (DOLPA) (in their case), the backward extraction rate dramatically increased and almost reached 90% at a pH of 8.0 and then declined with further increase in the pH. This result also was found similar with the results obtained by Zhou *et al.* (2012) and Goto *et al.* (1998).

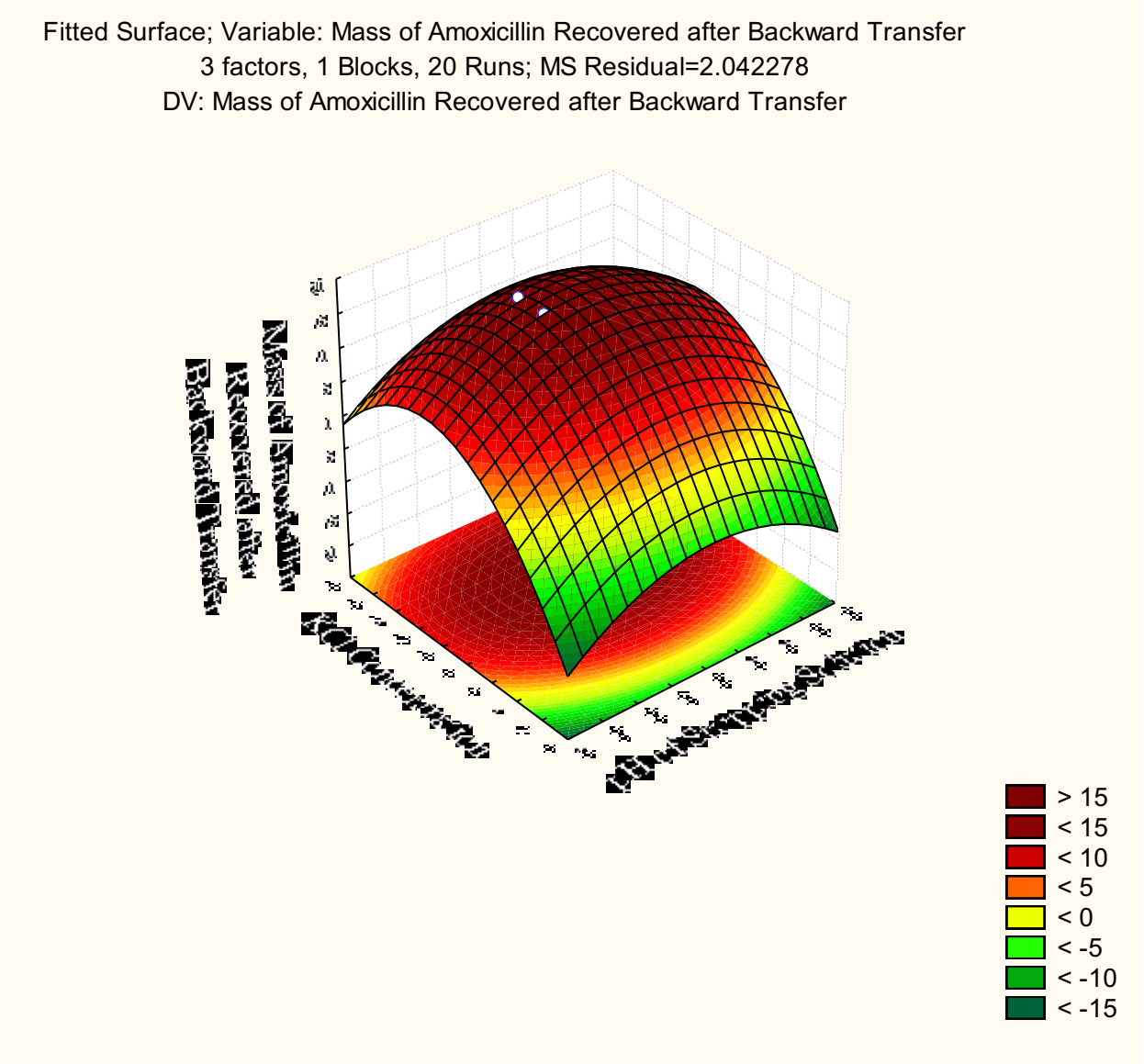

Figure 3: Response surface plots of aqueous stripping pH and KCl concentration on amoxicillin recovery at 20 minutes backward extraction time

The effect of aqueous stripping pH and backward extraction time on the final mass of amoxicillin extracted at 10 minutes is shown in Figure 4. Again, there was an optimal value for the aqueous stripping pH to obtain the highest amoxicillin recovery. Lower or higher values than this one would lead to the decrease of amoxicillin recovery. From the contour plot, it shows that the amoxicillin recovery had a positive linear backward time. However, the negative quadratic effect also became significant when further increase of backward time. This was reflected in the plateau of the final mass of amoxicillin for the extraction times of over 30 minutes. Further increase in backward extraction time would result in little change in the final mass of amoxicillin, indicating that the extraction had achieved equilibrium.

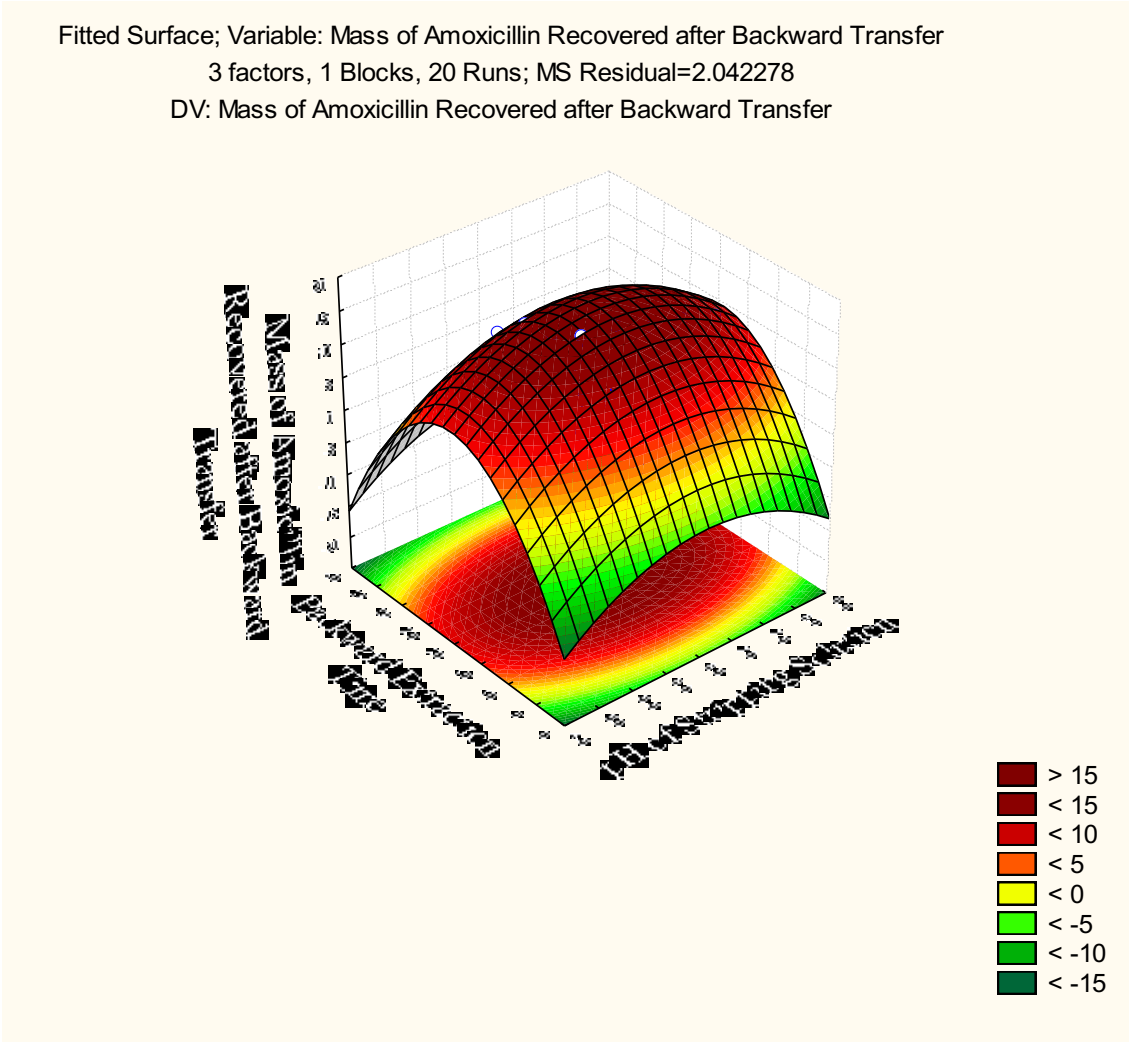

Figure 4: Response surface plots of aqueous stripping pH and backward extraction time on amoxicillin recovery at 12 g/L of KCl concentration

Figure 5 illustrated the KCl concentration and backward extraction time effect on amoxicillin recovery at pH 7. KCl concentration had a positive linear effect on the oil yield at low KCl concentration. According to previous report, the backward extract on the higher KCl concentration favoured the back transfer of bio-molecules (Lakshmi and Raghavarao, 2010) due to the destabilization of the reverse micelles (Zhao *et al*., 2010) and size exclusion effect. With an elevation of the KCl concentration, however, the negative quadratic effect of the KCl concentration on the amoxicillin recovery also became important. This was probably because when the KCL concentration was too high, the hydrophobic interactions, aggregation and precipitation became dominant during the backward extraction, hence decreasing the final mass of amoxicillin (Nandini and Rastogi, 2009). At a certain KCl concentration, there was an optimal value for backward extraction time to achieve the highest amoxicillin recovery. This is because due to both of the negative quadratic effects of backward extraction time on the amoxicillin recovery which had also become important

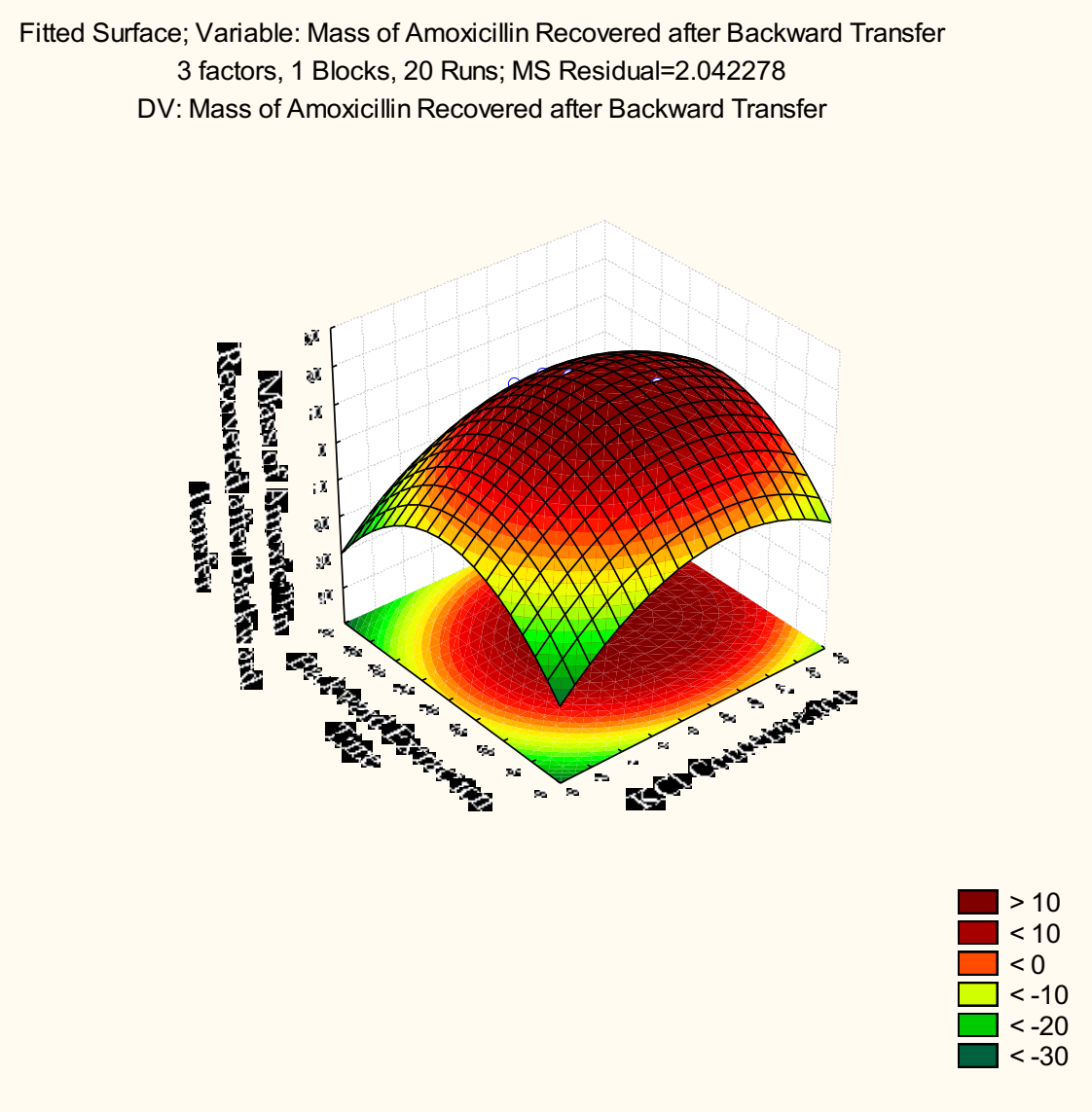

Figure 5: Response surface plots of KCl concentration and backward extraction time on amoxicillin recovery at pH 7

In order to optimize the processing conditions for final mass of amoxicillin extracted into aqueous phase, the first partial derivatives of the regression model were equated to zero according to $\delta Y/\delta X_1$, $\delta Y/\delta X_2$, $\delta Y/\delta X_3$ respectively:

$$\frac{\delta Y}{\delta X_1} = 28.03931 - 4.25544\, X_1 = 0 \quad (2)$$

$$\frac{\delta Y}{\delta X_2} = 4.28307 - 0.38870\, X_2 = 0 \quad (3)$$

$$\frac{\delta Y}{\delta X_3} = 2.28857 - 0.11586\, X_3 = 0 \quad (4)$$

The optimal condition for backward extraction of amoxicillin by using mixed AOT/TWEEN 85 was determined. The experimental value was compared with the predicted in order to determine the validity of the model. From the model, the stationary point giving a maximum backward transfer of amoxicillin by using mixed reverse micelle of AOT/TWEEN 85, following the critical values as the pH of stripping solution, X_1 = 6.58, KCl concentration, X_2 = 11.02 g/L and backward extraction time, X_3 = 19.8 min. The predicted and experimental value at optimum conditions is shown in Table 3.

Table 3: Predicted and experimental value at optimum conditions

Model	Optimum Conditions			Predicted Value (mg)	Experimental Value (mg)
	pH of Stripping Solution	KCl Salt Concentration (g/L)	Backward Extraction Time (min)		
	X_1	X_2	X_3	Y_{pre}	Y_{exp}
Y_1, (g/L)	6.58	11.02	19.8	17.42	17.25

The predicted final mass of amoxicillin in backward in these conditions by using STATISTICA analysis was 177.42 mg (91.68%), while the experimental value for these conditions was 17.25 mg (90.79%). Therefore, the results indicated that the experimental value was found to be in agreement with the predicted one. The extraction method by using mixed reverse micelle can be considered as a novel method since the recovery of amoxicillin was able to reach up to 90%.

4.0 CONCLUSIONS

The objective of this study was to provide mathematical model which would be able to describe mixed reverse micelle amoxicillin effectively. Based on the results, the experimental data was fitted well mathematical models. Model exhibited R^2 value of 0.94144 with a significant lack of fit. The optimum conditions were obtained by experimental are closed with mathematical modelling with the predicted final mass of amoxicillin was 177.42 mg (91.68%), while the experimental value was 17.25 mg (90.79%).

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