

**STATISTICAL MODELING FOR PURIFICATION OF RIFAMPICIN  
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**ABSTRACT**

The aim of the present study is to develop a model to optimize batch experiments. The sorption data fitted with various isotherm models and Langmuir model was the best one with correlation coefficient of 0.999. Kinetic studies indicated that the process followed well pseudo first order model. Thermodynamic studies revealed that the process is exothermic and, spontaneous. Different concentrations of antibiotic were prepared and for the purification of rifampicin, the effect of dosage of adsorbent,  $p^H$ , temperature and contact time were observed.

The adsorbent used was Amberlite XAD-1180. The effect was analyzed by reading the absorbance at 425nm. The Desorption of the antibiotic was performed using organic solvents and was analyzed by studying the absorbance at 425nm. The modeling was done statistically by preparing a model, using central composite design by taking the parameters dosage of adsorbent,  $p^H$ , temperature and contact time as input factors. Analysis was done as per the runs and 3D plots were deduced. The experimental results coincide with the calculations of the model.

**KEYWORDS:** Biosorption, Rifampicin, Amberlite XAD-1180, Langmuir isotherm, Statistical modeling, RSM (Response surface methodology).

## 1. INTRODUCTION

Rifampicin is considered as one of the most effective antibiotics for the treatment of tuberculosis.<sup>[1]</sup> Apart from its application against pathogens of tuberculosis and leprosy, it has also been found to be effective against several pathogens including *Mycobacterium avium* and penicillin-resistant pneumococci. The antibiotics of the rifamycin class such as rifampicin, rifabutin and rifapentine have been employed on a global basis in a number of well established combination regimes for the clinical treatment of tuberculosis, leprosy, AIDS-related mycobacterial infections and many other enteric infections. Downstream processing is very important for any antibiotic industry.<sup>[2,3,4]</sup> These antibiotics will be produced in very minute quantities. Separation is a difficult task. Fluidized bed adsorption offers considerable potential for improved economic downstream processing of bioproducts.<sup>[5,6,7]</sup> It allows separating compounds from unfiltered fermentation broths with time economy and concomitant improvements in yield and quality. Its simplicity of operation, reduction in equipment, labor and solvent cost have boosted its application in several pilot and full scale operations.<sup>[8,9,10]</sup> Before going to fluidized bed initially the physical parameters should be optimized by conducting batch studies. For enhanced purification and adsorption of antibiotics several resins can be used in which the resin of primary interest is Amberlite XAD-1180, which is a polymeric adsorbent.<sup>[11]</sup> It is a non ionic, hydrophobic, cross-linked polymer which derives its adsorptive properties from its patented macroporous structure, high surface area, and the aromatic nature of its surface. This structure also gives this polymeric adsorbent excellent physical, chemical, and thermal stability enabling it to be regenerated and used through repeated cycles, in either column or batch modes. Its characteristic pore size distribution makes the polymeric adsorbent an excellent choice for the selective separation of a wide variety of large organic molecules from aqueous solutions or polar solvents such as plant extracts, antibiotics, and fermentation products.

In the present study, the adsorption capacity of Amberlite XAD-1180 was analysed as the function of parameters, the adsorption time, pH, adsorbent weight and temperature. In addition, equilibrium kinetic and thermodynamic studies were carried out. Various isotherm models including Langmuir and Freundlich were applied to fit experimental data. For better understanding and optimization of the adsorption, mathematical and statistical modeling can be performed using Response surface methodology. It explores the relationships between several input variables and one or more response variables. The method was introduced by G.

E. P. Box and K. B. Wilson in 1951. The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response by careful design of experiments.<sup>[12]</sup> The objective is to optimize a response (output variable) which is influenced by several independent variables (input variables). An experiment is a series of tests, called runs, in which changes are made in the input variables in order to identify the reasons for changes in the output response.

## 2. MATERIALS AND METHODOLOGY

The pure form/laboratory grade rifampicin and Amberlite XAD-1180 are acquired commercially. The standard curve of rifampicin is prepared by pasqualucci method spectrophotometrically using different concentration ranges.

### Equilibrium Studies

The experiments were carried out in 100mL Erlenmeyer conical flasks, at a constant agitation speed (160 RPM) with 25 ml solution with various antibiotic concentrations (1.6,1.5,1.4,1.3,1.2,1.1,1mg ml<sup>-1</sup>) and required amount of adsorbent (0.1to1 gm in 25ml) using orbital shaker. Initially the effect of contact time (5-70 min) on the sorption capacity of Amberlite XAD-1180 was evaluated. The equilibrium time obtained (60 min) was used in all experiments and the experiments were repeated thrice for all conditions of study.

### Analysis of concentrations of rifampicin

The concentrations of unadsorbed rifampicin was analyzed by reading the absorbance spectrophotometrically at 425nm. The desorption of the antibiotic was performed using organic solvents.  $C_e$  and  $q_e$  were then calculated and tabulated for subsequent analysis of the data. The rifampicin uptake ( $q_e$ ) was calculated using the general definition.<sup>[13]</sup>

$$q_e = \frac{V(C_T - C_e)}{M} \quad (1)$$

where  $q_e$  is the antibiotic uptake mg g<sup>-1</sup> adsorbent, V is the volume of antibiotic containing solution in contact with the adsorbent in ml,  $C_T$  and  $C_e$  are the initial and equilibrium (residual) concentration of antibiotic in the solution mg ml<sup>-1</sup>, respectively, and M is the amount of added adsorbent in g.

Rifampicin % of removal by Amberlite XAD-1180 was determined by equation 2 as follows:

$$R(\%) = \frac{C_T - C_e}{C_T} \times 100 \quad (2)$$

Where R is the percentage of rifampicin adsorbed by adsorbent.

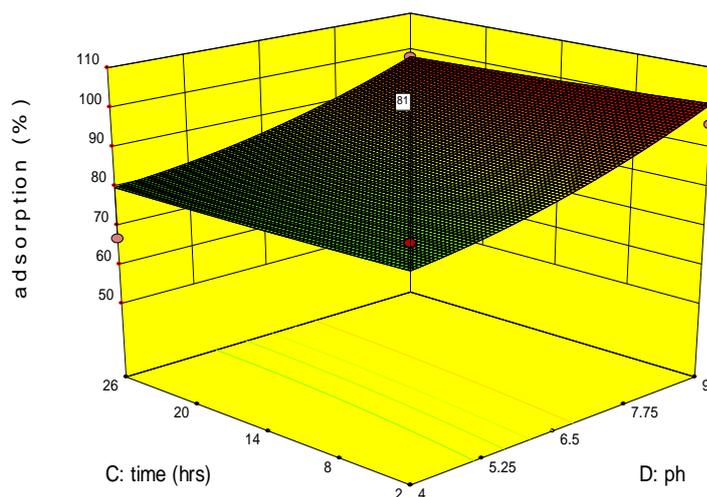
For RSM modeling of batch studies these parameters were taken as input factors and different runs were noted and performed. The desorption of adsorbent was done by taking 20ml of desorption solvent and 1gram of adsorbent with antibiotic. The desorption was analyzed by noting down the difference in absorbance prior to and after desorption.

### 3. RESULTS & DISCUSSIONS

The optimum parameters for the purification of antibiotic were determined both experimentally and statistically and the results were compared. The purification was completed with the desorption of antibiotic from the adsorbed solvent. The desorption was performed by using many organic solvents in which effective ones were found to be tetrahydrofuran and ethanol, wherein the desorption of antibiotic is found to be more than 90%. The optimization of batch studies was performed experimentally and then proceeds with comparing this data with the statistical modeling data.

#### 3.1 Effect of contact time

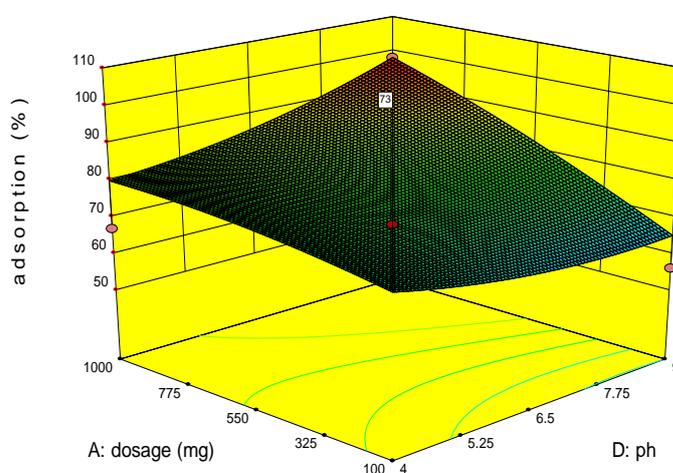
The effect of contact time on % adsorption of rifampicin was studied over a duration time period of 5 - 70 min, using 1g of Amberlite XAD-1180, 1 mg/ml of antibiotic solution at pH 7 and at temperature 30°C. The final concentration of antibiotic in solution and time showed (Fig. 1) that within 30 minutes of contact time 68 % of the antibiotic was removed from the solution and reached a maximum 96 % at 1 hr equilibrium time. Experimental results show a faster uptake at initial stages of contact, and subsequent slowing down as the equilibrium is approached. In the initial stages of contact, large number of vacant sites is available and hence the uptake is faster. The slowing down of metal uptake later is due to difficulty in occupying the remaining vacant sites.



**Fig. 1** 3-D plot of rifampicin adsorption by, Amberlite XAD-1180 (a) the effect of time and initial solution pH,

### 3.2 Effect of dosage of adsorbent

The effect of amount of XAD1800 added to the aqueous solution on the adsorption of antibiotic was shown in (Fig. 2). The % adsorption of antibiotic ranged from 54 to 96 % at a pH value of 7.

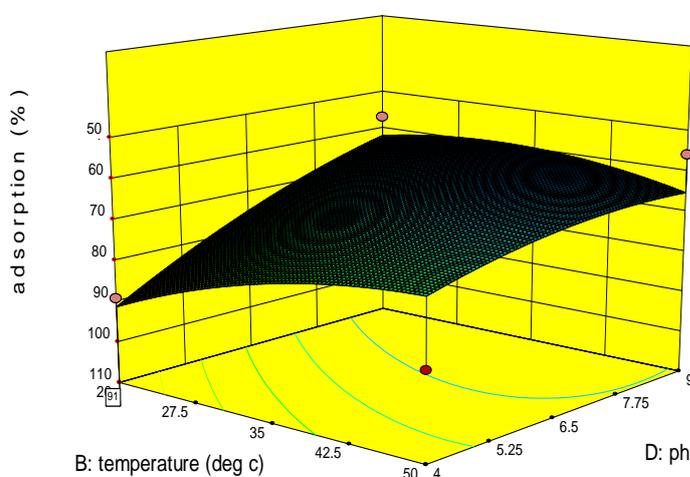


**Fig. 2.** 3-D plot of rifampicin adsorption by, Amberlite XAD-1180 (a) the effect of adsorbent dosage and initial solution pH.

### 3.3 Effect of pH

The effect of pH on the adsorption of rifampicin onto Amberlite XAD-1180 was studied in the pH range 5–9 and the results were presented in figure. It is found that the adsorption capacity is the highest at pH 7 which is the nearest to the pKa of Rifampicin. Increase in pH lower than pKa value result in increasing adsorption capacity. But, increase in pH higher than pKa value results decreasing adsorption capacity. This is postulate due to the electrostatics repulsion between antibiotic molecules and the surface of adsorbent. The uptake of Amberlite XAD-1180 was found to increase with the increase in pH up to 7.0, afterwards it decreased.<sup>[15]</sup> Fig.2 shows that an increase in initial pH from 5.0 to 7.0 resulted in adsorption of rifampicin from 72% to 96% and the adsorption decreased to 79% at pH 9 at an initial metal concentration of 1mg/ml. Optimum pH for antibiotic adsorption was found to be 7.0.

**3.4 Effect of Temperature:** All the experiments with rifampicin were conducted in the temperature range of 30-50°C.<sup>[16]</sup> The % removal of rifampicin by Amberlite XAD-1180 decreases from 96 to 56% with increase in temperature in the range 30-50°C at initial concentration of 1 mg ml<sup>-1</sup> as shown in the (Fig. 3) In most of the chemical reactions the temperature is expected to activate the process increasing the heat or mass transport processes. Sorption capacity of the XAD-1800 has decreased with increase in temperature; rise in temperature has a tendency to desorb the adsorbed metal ions from the interface to the solution.



**Fig.3. 3-D plot of rifampicin adsorption by, Amberlite XAD-1180 (a) the effect of temperature and initial solution pH,**

For the statistical analysis the model is designed and runs are noted down. The runs were performed as experiments and the absorbance was tabled and compared. Further the 3D plots were deduced to study the effect of the parameters on adsorption.

### Final equation

$$\text{Adsorption} = 77.50 + 5.33*A - 0.25*B - 2.17*C - 2.17*D + 2.00*AB + 2.75*AC + 6.37*AD - 1.50*BC + 5.37*BD - 0.63*CD - 1.75*A^2 + 4.12*B^2 + 0.25*C^2 + 2.88*D^2$$

where A,B, C and D are the independent variables. The significance of each coefficient present in the equation determines by the F-values and values of probability >F. The results of the quadratic model in the form of ANOVA showed small probability value ( $P < 0.00001$ ) indicating the individual terms in the model have significant on the effect. The values of R-squared ( $R^2 = 0.9461$ ) and adjusted R-squared (Adj.  $R^2 = 0.9157$ ) are closed to 1, which is very high and indicates a high correlation between the observed and the predicted values. The predicted R-squared (Pred.  $R^2$ ) of 0.8891 is in acceptable agreement with the adjusted R-squared of 0.9157. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 21.34 indicates an adequate signal. Therefore, this model can be used to navigate the design space. The value of probability >F of less than 0.05 indicates that the model terms are considered to be statistically significant. The examination of the fit summary output revealed that the quadratic model is statistically significant for the response and therefore it will be used for further analysis.

### 3.4 Equilibrium isotherms

#### Langmuir Isotherm

The equation proposed by Langmuir is universally applicable to chemisorption with some limitations involving physical adsorption. This equation is applicable to the physical or chemical adsorption on solid surface with one type of adsorption active center. As long as the limitations are clearly recognized, the Langmuir equation can be used for describing equilibrium conditions for adsorption behaviour in different adsorbate-adsorbent systems or for varied conditions within any given system. Linear form of the Langmuir equation is given by:

$$\frac{C_e}{q_e} = \frac{1}{q_m b} + \frac{C_e}{q_m} \quad (3)$$

Where  $q_m$  (mg/g) is the maximum amount of the rifampicin per unit weight of adsorbent to form a complete monolayer on the surface. ' $C_e$ ' is the equilibrium concentration of the adsorbate in solution (mg/ml), and  $b$  is a constant which accounts for the affinity of the binding sites (ml/mg).  $q_m$  represents the limiting adsorption capacity when the surface is fully covered with antibiotic ions and helps in the evaluation of adsorption performance, particularly in cases where the sorbent did not reach its full saturation during contact. It is the most widely used simple two parameter equation (Langmuir, 1918) based on the assumptions, adsorption occurs on the surface of the adsorbent as a monolayer. There are fixed number of adsorption sites at equilibrium. From the plots between  $(C_e/q_e)$  and  $C_e$  the slope ( $1/q_m$ ) and the intercept ( $1/b$ ) can be calculated.

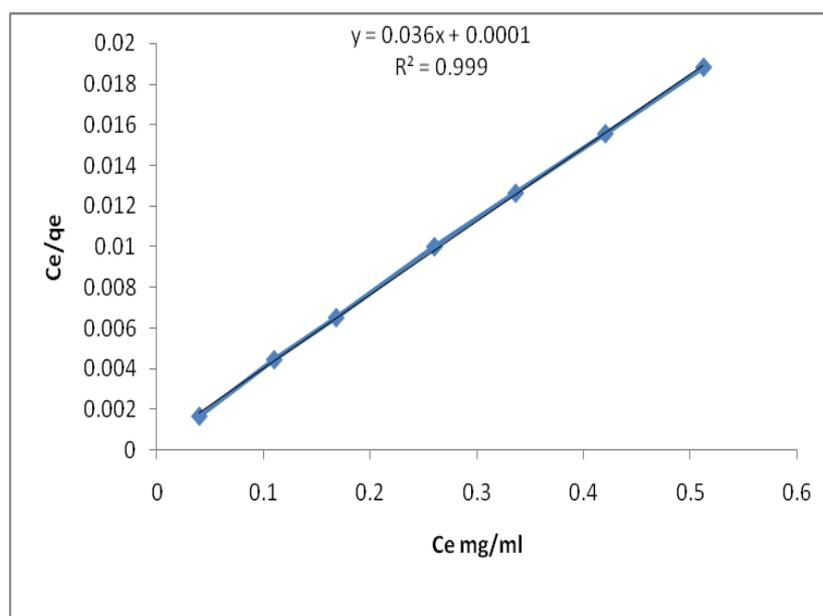


Fig.4 Langmuir plot for the adsorption of rifampicin by Amberlite XAD-1180 at pH 7,30<sup>0</sup> C. The Langmuir constant can be used to determine the suitability of the adsorbent to adsorbate by using dimensionless parameter  $R_L$  (Hall separation factor) can be calculated by:

$$R_L = \frac{1}{1 + bC_0} \quad (4)$$

The dimensionless factor value is  $0 < R_L < 1$  indicates that favorable for adsorption and  $R_L > 1$  indicates un-favorable for adsorption. Whereas  $R_L$  is 1 and  $R_L$  is 0 indicated that the linear and irreversible adsorption respectively.

The linearized Langmuir adsorption isotherms of rifampicin using Amberlite XAD-1180 obtained at different temperatures and adsorption constants evaluated (fig.4) with correlation

coefficients (Table 1).  $C_e / q_e$  vs  $C_e$  plot yielded a straight line with  $R^{2[1]}$  indicating the sorption data could be best represented by the Langmuir model. The higher adsorption capacity,  $q_m(\gg 1)$  indicated the strong electrostatic force of attraction. From the value of  $b$ , a dimensionless parameter  $R_L$  at different initial metal ion concentrations was calculated and the values are shown in Table 1.

### 3.5. Freundlich Isotherm

An adsorption isotherm was proposed by Boedecker (Dabrowski, 2001) which was later modified by Freundlich (Freundlich, 1926). The Freundlich adsorption equation expressed as:

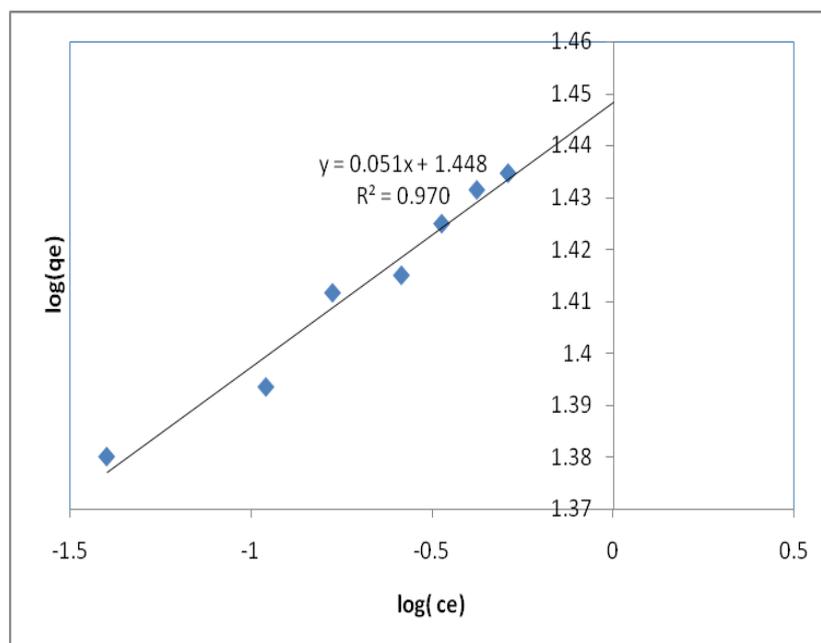
$$q_e = K_f C_e^{\frac{1}{n_f}} \quad (5)$$

Taking logarithm on both sides,

$$\ln q_e = K_f + \frac{1}{n_f} \ln C_e \quad (6)$$

where ' $q_e$ ' is equilibrium adsorption capacity ( $\text{mg g}^{-1}$ ), ' $C_e$ ' is the equilibrium concentration of the adsorbate in solution ( $\text{mg/ml}$ ), ' $K_f$ ' and  $n_f$  are constants related to the adsorption process such as adsorption capacity and intensity respectively. This empirical model has shown best fit for non-ideal sorption on heterogeneous surfaces as well as multilayer sorption. The Freundlich isotherm is also more widely used but provide no information on the monolayer adsorption capacity, in contrast to the Langmuir model. Freundlich isotherm has been derived by assuming an exponentially decaying sorption site energy distribution.

The plot in Fig. 5 gives the isotherm drawn for adsorption of rifampicin on to Amberlite XAD-1180. The coefficient of determination for this case is 0.97 and the values of  $n_f$  and  $K_f$  (Table 2) are found to be 19.6 and 28.26 at  $30^\circ \text{C}$ . Freundlich constant  $n_f$  between 1 and 10 indicates a trend more favorable for adsorption by Amberlite XAD-1180. This is also suggestive that rifampicin could well be separated from its aqueous solution with high adsorption capacity.



**Fig.5** Freundlich plot for the adsorption of rifampicin by Amberlite XAD-1180 at pH 7, 30°C.

**Table 1.** Langmuir and Freundlich isotherm model parameters for rifampicin adsorption on Amberlite XAD-1180

Temp. (°K)	$q_m$ (mgg <sup>-1</sup> )	B (mlmg <sup>-1</sup> )	$C_0$ (mgml <sup>-1</sup> )	$R_L$	$R^2$	$K_F \{(\text{mgg}^{-1})(\text{mgml}^{-1})^n\}$	$n_f$	$R^2$
303	27.78	359.95	1	0.003	0.999	28.05	19.6	0.97

### 3.6 Adsorption Kinetic Models

Several kinetic models have been proposed earlier to identify the mechanism of solute adsorption from aqueous solution onto the adsorbent. They are Pseudo first order/Lagergren kinetic model, First order reversible kinetic model, Ritchie' second order kinetic model and Pseudo second order kinetic model. In the present study Pseudo first order and Pseudo second order kinetic models have been attempted to fit the present adsorption data.

#### Pseudo-first-order/Lagergren kinetic model

The Pseudo-first-order or Lagergren kinetic rate equation for the adsorption of liquid-solid system was derived based on solid adsorption capacity. It is one of the most widely used adsorption rate equations for adsorption of a solute from a liquid solution.<sup>[17]</sup>

The pseudo first order kinetic equation can be expressed as:

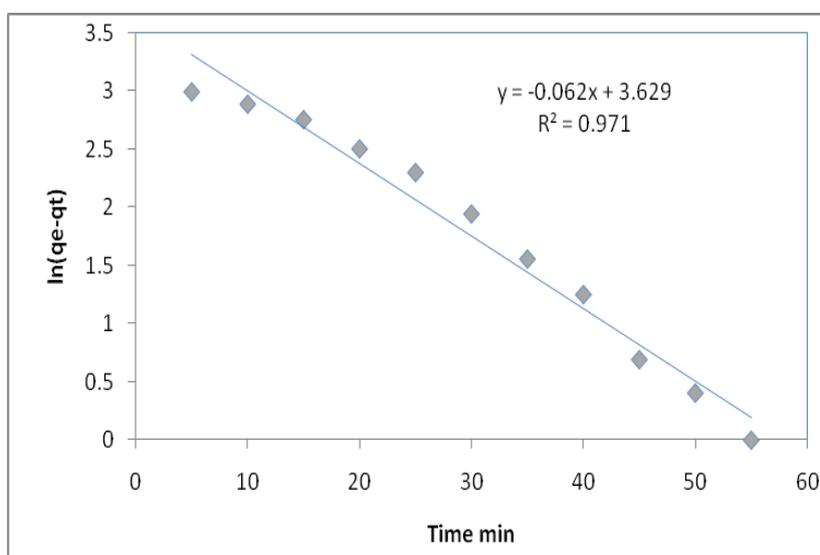
$$\frac{dq}{dt} = k_1(q_e - q_t) \quad (7)$$

Where ' $q_e$ ' is the amount of solute adsorbed at equilibrium per unit mass of adsorbent (mg/g), ' $q_t$ ' is the amount of solute adsorbed at any given time ' $t$ ' and ' $k_1$ ' is the rate constant. By using the boundary conditions and simplifying, the equation 7 yields.

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (8)$$

' $k_1$ ' can be computed from the slope of the linear plot between  $\ln(q_e - q_t)$  vs. ' $t$ ' for different adsorption parameters such as pH, temperature, adsorbate concentration, adsorbent dose, and particle size.

The plot (Fig. 6) of  $\ln(q_e - q_t)$  vs. ' $t$ ' of Eq.<sup>[8]</sup> gave a linear relationship from which  $k_1$  value was determined. The rate constant and the correlation coefficient for *Pseudo-first-order kinetic model* were calculated and summarized in Tables 2. These values showed that the pseudo-first order kinetic plot fits well the adsorption data.. This kinetic model can be aptly proposed to predict the kinetics of adsorption of rifampicin on Amberlite XAD-1180.



**Fig.6. First order kinetics for the adsorption of rifampicin by Amberlite XAD-1180**

**Table 2** Kinetic parame

ters for rifampicin adsorption on Amberlite XAD-1180

$q_e$ (mg/gm)	$K_2$ (g/mg-min)	$R^2$
37.67512	0.062	0.971

**Pseudo-second-order kinetic model:** In view of the above the fitness of the sorption data was tested using pseudo- second- order reaction model .The pseudo-second-order reaction model could be expressed by the rate expression as.<sup>[17]</sup>

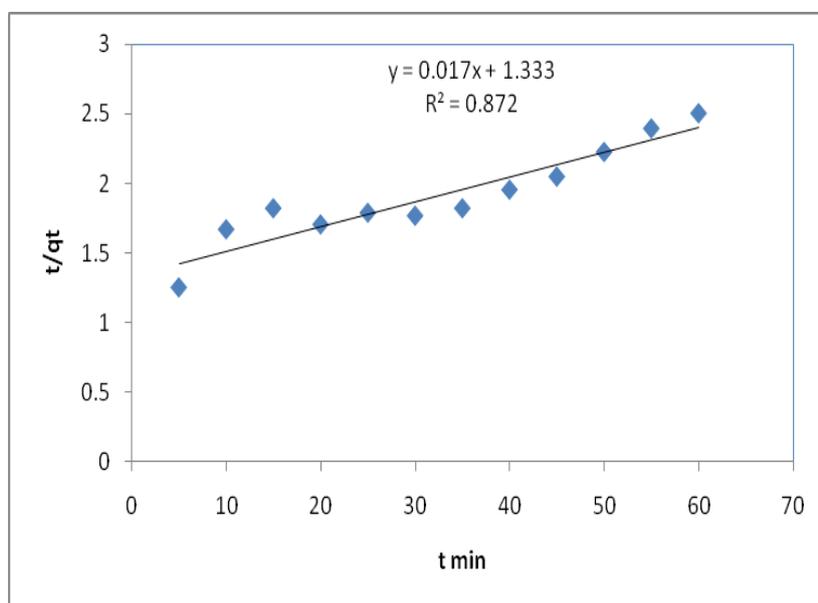
$$\frac{dq}{dt} = k_2(q_e - q_t)^2 \quad (9)$$

On integration for boundary conditions when  $t=0$  to  $t>0$  and  $q_t=0$  to  $q_t>0$  and further simplifications, equation 9 becomes,

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (10)$$

$K_2$  ( $\text{g mg}^{-1} \text{ min}^{-1}$ ) is the second-order reaction rate equilibrium constant  $q_t$  ( $\text{mg/g}$ ) is the antibiotic uptake at any time  $t$ .  $q_e$  is equilibrium antibiotic uptake ( $\text{mg/g}$ ).

The plot (Fig. 7) of  $t/q_t$  versus  $t$  of Eq.<sup>[10]</sup> gave a non linear relationship with correlation coefficient 0.872 implies that the pseudo-second order model is not fit compared to pseudo-first order model in explaining the kinetics.



**Fig.7 Second order kinetics for the adsorption of rifampicin by Amberlite XAD-1180**

### 3.7. Thermodynamic parameters

Gibbs free energy  $\Delta G$  is the basic criterion for deciding whether the chemical process does occur/proceed or not. The spontaneity of the reaction can also be judged by the sign and magnitude of  $\Delta G^\circ$ . A negative sign for  $\Delta G^\circ$  is an indicative of the spontaneity of any chemical process. To design any chemical process system one should have the knowledge of changes that are expected to occur during

chemical reaction. The rate and extent of changes are more informative in the design of process equipment. In view of the above, analysis has been carried out on the effect of thermodynamic parameters on the adsorption of rifampicin on Amberlite XAD-1180. The Thermodynamic parameters such as changes in standard free energy change  $\Delta G^\circ$ , Enthalpy  $\Delta H^\circ$ , Entropy  $\Delta S^\circ$  for any given adsorption process could be determined from the Equations.<sup>[18]</sup>

$$\Delta G^0 = -RT \ln K_c$$

where  $\Delta G^0$  is the free energy change, expressed as J/mol.  $K_c$  is the apparent equilibrium constant for the process.  $K_c$  can be derived from:

$$K_c = \frac{C_{ad}}{C_e}$$

' $C_e$ ' is the equilibrium concentration of the adsorbate in solution (mg/L)

$C_{ad}$  is concentration of metal ion adsorbed mg/L at equilibrium

$$\log\left(\frac{C_{ad}}{C_e}\right) = -\frac{\Delta H^0}{2.303RT} + \frac{\Delta S^0}{2.303R}$$

$\frac{C_{ad}}{C_e}$  can be defined as 'adsorption affinity'.

The enthalpy changes ( $\Delta H$ ) and entropy changes ( $\Delta S$ ) for the adsorption process of rifampicin Using Amberlite XAD-1180 were obtained from the plot of  $\log\left(\frac{C_{ad}}{C_e}\right)$  drawn against  $1/T$  was shown in Fig. 8. The calculated thermodynamic data were compiled in Table 4. The negative value for  $\Delta G^0$  indicates the spontaneity of biosorption process. The negative  $\Delta H^0$  values indicated exothermic nature of the adsorption. The negative value of  $\Delta S^0$  suggested a decrease in the randomness at solid/solution interface during the adsorption of rifampicin on to Amberlite XAD-1180

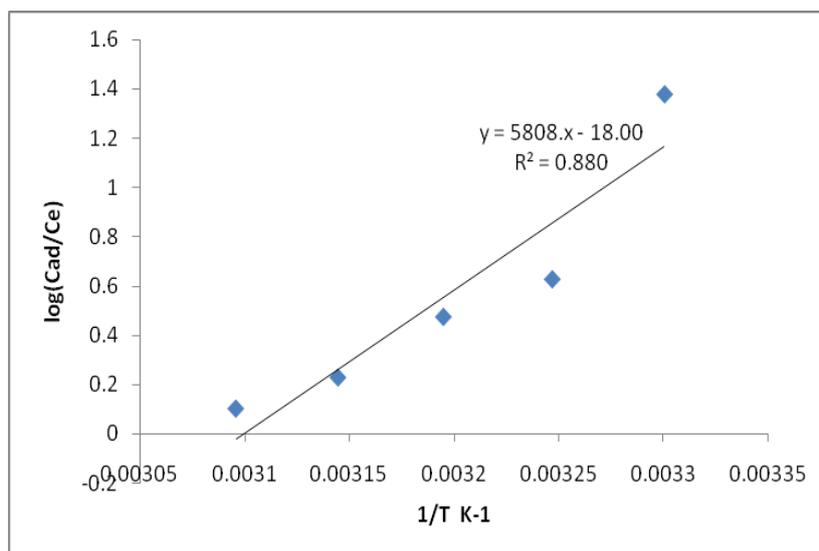


Fig.8. Plot of Thermodynamic parameters .

Table 4 Thermodynamic parameters for rifampicin adsorption on Amberlite XAD-1180

Temperature (K)	$\Delta H^0$ (KJ/mol)	$\Delta S^0$ (J/mol K)	$\Delta G^0$ (KJ/mol)
303	-111.207	-344.65	-6.777

## CONCLUSION

1. The experimental optimization along with the statistical modeling gives us the optimum conditions for the effective and enhanced purification of the antibiotic. This optimum conditions can be used in laboratory scale as well as industrial scale.
2. Both Langmuir and Freundlich equilibrium isotherm models proved to be good fits for the experimental data of rifampicin adsorption on to Amberlite XAD-1180 and the kinetics could be described by a first-order kinetic model.
3. Free energy change ( $\Delta G^0$ ) with negative sign reflects the feasibility and spontaneous nature of the process. The negative enthalpy values indicate exothermic nature and negative entropy value point towards decrease in randomness at solid liquid interface.

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