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# Recovery of cephalosporin C from aqueous solution using polymeric adsorbent

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Commercially available neutral polymeric sorbents are used for recovery of beta lactam antibiotic cephalosporin C (CPC), from aqueous solution. The objective of this work is to evaluate the separation of CPC from fermentation broth during purification process. The neutral forms of CPC are preferentially adsorbed onto the neutral sorbents. Adsorption of CPC was higher onto the aromatic (XAD 4) as compared to aliphatic ester sorbent (XAD 8). The kinetics of CPC adsorption on aromatic polymeric adsorbent has been investigated. Isopropyl alcohol solution was used to desorb CPC.

**Keywords**: Adsorption, Desorption, Cephalosporin C, Polymeric adsorbents
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Recovery and purification of bio-products require continuous research for the development of optimized and economic operation[1]. The world's largest antibiotic production is around $5\times10^7$ kg/year, from which $3\times10^7$ kg are represented by the group of β-lactams. The industrial recovery of these secondary metabolites from fermentation broth typically involves solvent extraction[2] although a range of highly water soluble antibiotics (e.g. erythromycin) require ion exchange[3]. In addition to ion exchange sorbents, neutral polymeric sorbents have become available and could be used for the fermentation recovery step. Nonionic sorbents have lower capacity of adsorption than ionic resins. However, they present better desorption performance and/ or easier regeneration that facilitates the operation in industrial cycles[4].

Cephalosporin C, a β-lactam antibiotic is an exception in that it is highly water soluble and nonextractable, yet it can be effectively adsorbed onto neutral aromatic sorbents. Thus although ion exchange recovery processes were developed[5], a significant amount of commercially produced CPC is still recovered using neutral aromatic adsorbents. For large scale operations, the most commonly used neutral polymeric adsorbents are copolymers of styrene (or ethyl vinyl benzene) and divinyl benzene.

Polymeric sorbents have been known to be more attractive than other adsorbents such as activated carbons and ion exchangers due to their regeneration characteristics[6,7]. Non ionic polymeric sorbents have lower adsorption capacities then ion exchangers reactivated carbons, but they have much better desorption or regeneration characteristics in industrial cyclic operations[8]. A mathematical kinetic model for the adsorption and desorption of cephalosporin C on Amberlite XAD-2 resin has been proposed[9]. The model can represent Langmuir, Freundlich or linear isotherms at equilibrium. The intrinsic kinetic parameters and adsorption isotherms as well as physical parameters such as the effective diffusivity and the external mass transfer coefficient were obtained at different temperatures and ethanol concentrations.

This paper reports the effect of *p*H, temperature and eluent on adsorption of CPC onto commercially available aliphatic (XAD 8) and aromatic (XAD 4) resins.

## Kinetic modeling

### *Adsorption*

In order to describe the adsorption of CPC on polystyrene resins a simple mechanism is adopted that can be represented as,

$$\text{resin} + \text{nCPC} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{complex} + \text{energy}$$

According to this reaction more than one CPC molecule is adsorbed (or attached) by a single active site. The mathematical kinetic model representing this proposition is

$$r_q ==== k_1 C^n (q_m - q) - k_2 q \qquad \ldots(1)$$

where, $k_1$ and $k_2$ are intrinsic kinetic constants, $C$ is the antibiotic concentration in the solution, $q$ is the antibiotic concentration adsorbed on the resin and $q_m$ is the maximum adsorption capacity of the resin. At equilibrium, this equation can be rewritten as

$$q_i = \frac{q_m C_i}{C_i + K_D} \quad \ldots(2)$$

where $K_D$ is the ratio $k_2/k_1$, and $q_i$ and $C_i$ are the concentrations at equilibrium. When the inequality $C^* << K_D$ holds, Eq. (2) takes the form of the Freundlich isotherm, according to Eq. (3),

$$q_i = Q_m C_i \, n \quad \ldots(3)$$

with $Q_m = q_m / K_D$

In this case, when n is greater than 1, it indicates that an unfavourable isotherm takes place. When n equals 1, the model indicates that one active site adsorb only one molecule of CPC. This is the case for so called monolayer adsorption, which is one of the assumptions taken into account in the Lagnmuir isotherm. Therefore, Eq. (2) becomes Eq. (4), the Langmuir model.

$$q_i = \frac{q_m C_i}{C_i + K_D} \quad \ldots(4)$$

#### *Desorption*

When the process of adsorption is at equilibrium, it can be displaced by changing process conditions such as temperature, solution composition, etc. The addition of some special components as an eluent, such as ethyl alcohol, in certain cases will increase desorption of the desired adsorbed components. In some extreme conditions it might bring about an irreversible desorption, depending on the eluent concentration. In order to describe desorption, a model similar to that for adsorption can be proposed.

$$\text{Complex} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{resin} + n\text{CPC} + \text{energy}$$

## Experimental Procedure

#### Method

Zn-CPC (sigma) and CPC produced by *Acremonium chrysogenum* (J.K. Pharmaceuticals) were used. *p*Ka values of cephalosporin C are 2.6, 3.1 and 9.8. Amberlite XAD 4 & XAD 8 (Hi media Ltd., Mumbai) were used for the adsorption of cephalosporin C. Amberlite XAD 4 (polystyrene divinyl benzene) and Amberlite XAD 8 (poly (methylacrylate ) had surface area 750 and 126 $m^2/g$ and average pore diameter of 5.0 and 20-60 mm, respectively (Manufacturers data).

Batch experiments were carried out to measure the progress of adsorption. 2.0 g of the resin with 500 mL aqueous solution of CPC of different concentrations (1 to 10 mmol/L) were shaken in a thermostat at different temperatures and *p*H in glass bottles. The *p*H of the adsorbate solution was adjusted by adding HCl or NaOH. The progress of adsorption was determined at different time intervals. After equilibrium, the supernatant was filtered. The clear solution was analyzed for cephalosporin C concentration by assay method[10] and confirmed by HPLC analysis at 260 nm. The amount of cephalosporin C adsorbed per gram of adsorbent, $q$ (mol/g) was calculated as:

$$q = V\Delta C / W$$

where, $\Delta C$ (mmol/L), is the change in the CPC concentration before and after the adsorption onto the resin. $V$ is the solution volume (L) and $W$ is the weight of the adsorbent (g).

#### Adsorption isotherm

In the present investigation the Langmuir equation as represented by Eq. (4) was used, where $q_m$ is the maximum capacity of the resin and $K_D$ is the Langmuir isotherm equilibrium constant.

## Results and Discussion

Adsorption isotherm plays a crucial role in predicting modeling procedures for analysis and designing of adsorption systems. Adsorption on synthetic adsorbents is generally driven by the dispersive forces between the adsorbate and the resin.

#### Effect of *p*H

The effect of resin's structural arrangement for CPC adsorption has been analyzed by taking XAD 4 (aromatic) and XAD 8 (aliphatic) resin for adsorption. Figure 1 shows $q/C_i$ for XAD 4 and XAD 8 at different *p*H. It infers a strong relationship between CPC adsorption on resin at different *p*H, the affinity being considerably low at high *p*H under which the cephalosporin C exist in dissociated forms. It was observed that CPC is adsorbed onto the aromatic sorbent with higher affinity compared to its

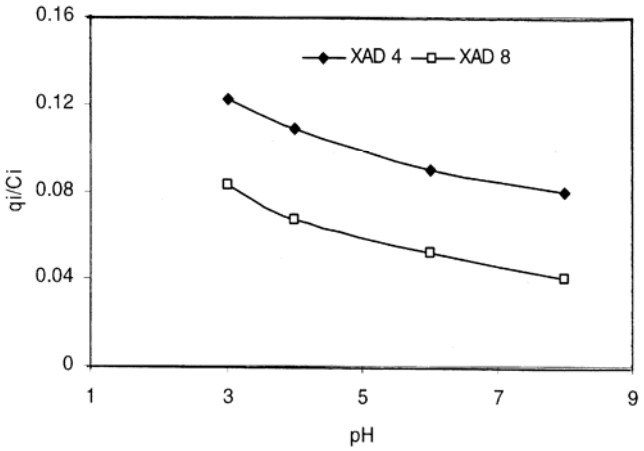


Fig. 1 — Binding capacity of different resins (XAD 4 & XAD 8) for CPC adsorption.

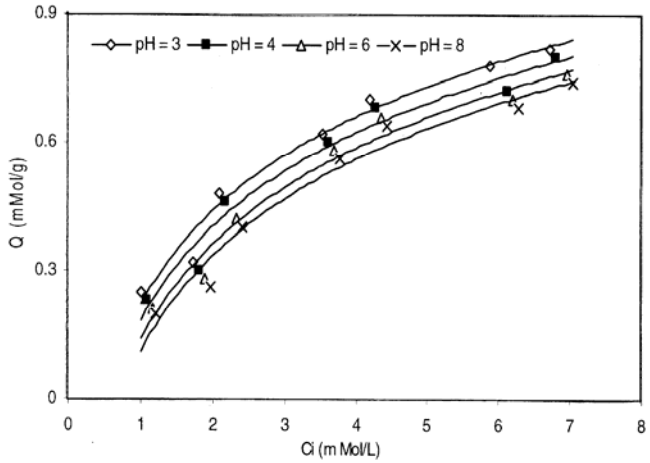


Fig. 2 — Non linear regression of the experimental data of the adsorption isotherm of CPC on XAD 4 resin, at different *p*H at 20°C.

adsorption onto the aliphatic sorbent. This is quite similar to earlier reports[11]. This also suggests that structural and morphological parameters of the polymeric resins determine their adsorption intensity for the amphoteric CPC molecule. It is implied that sorbent surface chemistry affects the adsorption equilibrium and a specific solute sorbent interaction involving a π-electron on the sorbent surface may dominate the adsorption of CPC on polymeric resin. This can also be correlated with the observed results that the aromatic adsorbents (XAD 4) have higher adsorption intensity than the aliphatic sorbent (XAD 8), which lacks π-electron.

Equilibrium adsorption data of CPC on XAD 4 at different *p*H are shown in Fig. 2. It is observed that the absorption decreases with increase in *p*H. Due to amphoteric nature of cephalosporin C the difference in amount adsorbed might be due to the change of its ionic form which is strongly dependant on the solution *p*H. The reduction in adsorption capacity of CPC on non ionic macroporous resins with increase of *p*H has been interpreted from a model incorporating hydronium ion concentration and dissociation constant attributable to both the carboxylic and amino functional group. At low *p*H, CPC becomes hydrophobic and the hydrophobic interaction of un-dissociated CPC are greater than for the corresponding ionic form that exist at high *p*H. Thus, the observation of high $q/C_i$ at low *p*H implies the probable role of hydrophobic interaction on the adsorption process. Adsorption equilibria for CPC can not be represented by a simple isotherm as there is an interaction of various forms at a given *p*H (ref. 12). Hence, the adsorption equilibrium for cephalosporin C at low *p*H values may be encountered by a competitive multi component adsorption model. In the *p*H range of 5-8, the negatively charged cephalosporin C is obliviously predominant while under *p*H values of 2-5, either positively or negatively charged forms coexist with its zwitter-ion (neutral) form.

As discussed earlier, only the neutral form of CPC is capable of adsorbing onto the neutral sorbent, the adsorption can be described by,

$(CPC)_{neutral}$+unbound adsorption sites=adsorbed CPC

It has been also discussed that, in the aqueous phase, the concentration of the neutral form of CPC ($C_n$) is related to the total concentration ($C$) and the *p*H by the Henderson- Hasselbach relationship:

$$C_n = \frac{C}{(1+10^{pH-\ pK_a})}$$

This relationship is used to convert the total aqueous phase concentration to the concentration of the neutral form ($C_n$). As suggested earlier $q'/C_n$ is constant, which can be used as a quantitative measure of adsorption equilibrium.

**Effect of temperature**

The temperature effect on the adsorption equilibrium has been evaluated by measuring the adsorption at three different temperatures at a constant *p*H of 3 (Fig. 3). It was found that adsorption decreases as there is increase in temperature. The adsorption equilibria were interpreted from Langmuir

isotherm. Table 1, shows the Langmuir constants for CPC adsorption at different temperature.

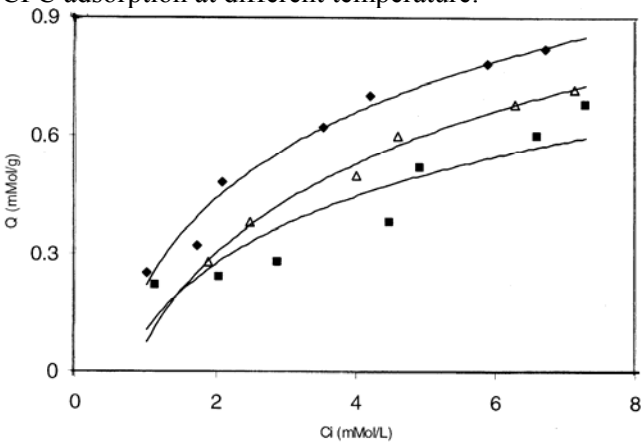


Fig. 3 — Non linear regression of the experimental data of the adsorption isotherm of CPC on XAD 4 resin, at different temperature ◆ 20°C, ■ 30°C, △ 40°C.

Table 1 — Equilibrium isotherm parameters according to the Langmuir model

| T (K) | $q_m$ (mmol CPC/g resin) | $K_D$ |
|---|---|---|
| 293 | 2.3 | 8.1 |
| 303 | 1.8 | 8.5 |
| 313 | 1.6 | 9.4 |

**Effect of solvent**

Adsorption of CPC onto XAD 4 was also observed in the presence of methanol and IPA (Fig. 4) to select an appropriate eluent for CPC desorption. It was observed that presence of IPA favours less adsorption than methanol.

On the basis of these observations it can be concluded that low temperature and absence of any organic solvent facilitates CPC adsorption onto XAD 4, whereas, high temperature and IPA leads to less adsorption rather it facilitates desorption.

**Adsorption kinetics**

The kinetics of CPC adsorption was analyzed by measuring CPC concentration in the fluid phase with the time. Adsorption experiments were performed at various stirring speeds and it showed almost constant adsorption at all speeds. It may be concluded that external mass transfer played a insignificant role and could, therefore, be neglected for further evaluation. The adsorption of CPC on XAD 4 determined at 300 rpm exhibits a rapid initial uptake followed by a relatively slow approach to equilibrium. The kinetics of reaction may be considered as first order processes. The fast initial step may be correlated with adsorption in the easily accessible mesopore of the particle and

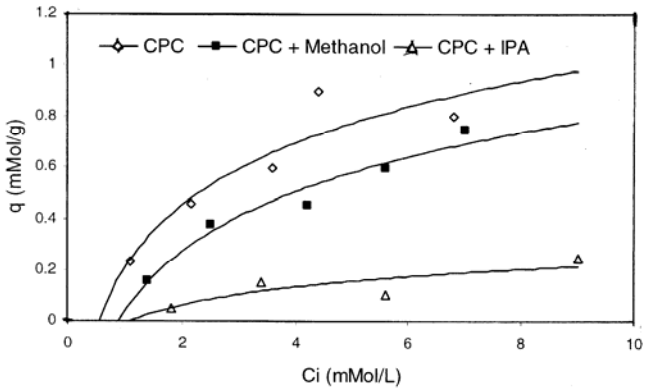


Fig. 4 — Non linear regression of the experimental data for CPC adsorption in the presence of organic solvent.

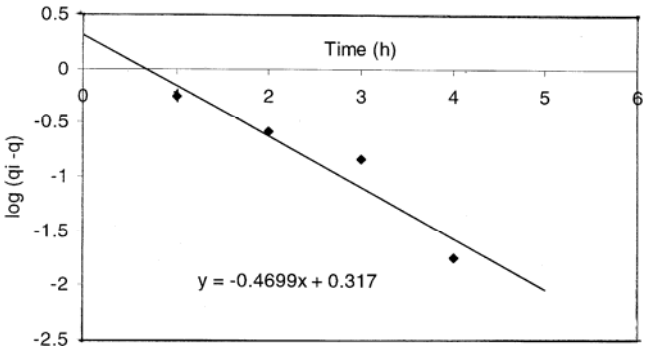


Fig. 5 — Lagergren plot for adsorption of cephalosporin C on XAD 4.

the slow step to adsorption in the micropore of the microsphere typical of the XAD 4 resin. The rapid uptake is consistent with low external mass transfer at short times whereas the slower uptake at long time is consistent with a high intraparticle mass transfer resistance. However this needs to be further evaluated using the internal mass transfer kinetics.

The adsorption kinetics was examined using first order reaction rate and may be defined by Lagergren equation,

$$\log(q_i - q) = \log q_i - (k_{ads}/2.303)\, t$$

The present study only evaluates and excludes the external mass transfer. A reasonably good linearity is observed in the plot of log ($q_i - q$) versus time (Fig. 5), indicating that first order kinetics well represent the adsorption before attainment of equilibrium. The value of the adsorption rate constant was found to be $3\times10^{-4}$ s typical of a slow adsorption process, which may be due to internal mass transfer limitations[13].

## Conclusion

The adsorption of cephalosporin C on XAD 4 and XAD 8 is strongly dependent on the aqueous phase

*p*H and this dependence is typical of the behaviour predicted by a neutral species adsorption model. Low temperature and absence of any organic solvent favours adsorption. Langmuir models represent the adsorption isotherm. The adsorption onto XAD 4 appears to be driven by enthalpic interaction, presumably involving the delocalized π-electrons on the surface of the polymeric sorbents and the CPC molecules. The rate of adsorption onto the XAD 4 appears to be controlled by both internal mass transfer and particle diffusion. However, a more elaborate model is necessary to develop better description for dynamic adsorption process.

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## Nomenclature

$C$ : Bulk concentration ($mmolL^{-1}$)
$\Delta C$ : Change in solute concentration ($mmolL^{-1}$)
$C_i$ : Equilibrium liquid phase concentration ($mmolL^{-1}$)
$K_D$ : Langmuir isotherm constant ($mmolg^{-1}$)
$k_{ads}$ : Adsorption rate constant
$q_m$ : Langmuir constant ($mmolg^{-1}$)
$q_i$ : Equilibrium solid phase concentration ($molg^{-1}$)
V : Volume of the solution (L)
W : Weight of the solid adsorbents (*g*)

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