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Adsorption of Components of Enzymatic Synthesis of Ampicillin on Different Hydrophobic Resins

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Abstract

This work compared the performance of three hydrophobic resins for the adsorption of ampicillin (AMP), D-phenylglycine (PG), D-phenylglycine methyl ester (PGME), and 6- aminopenicillanic acid (6-APA). The influence of pH on adsorption efficiencies was assessed in the range of 4.5–8.5, at 4 and 25°C. The values at 4°C were slightly higher than those at 25°C. The adsorption efficiency of AMP and 6-APA decreased at higher pHs, for the three resins. An opposite behavior was found for PGME, and the pH did not affect PG adsorption efficiency. Isotherm models were fitted to experimental equilibrium data and the best models were discriminated.

Index Entries: Ampicillin; purification; adsorption; β-lactamics.

Introduction

Ampicillin (AMP) is one of the most widely used β-lactam antibiotics, with an annual production of 5.6 t *(1)*. The enzymatic production of semi-synthetic β-lactam antibiotics has acquired a great relevance in order to avoid the drawbacks of the conventional chemical processes, such as the high toxicity of some reagents or the requirement of a high number of synthetic steps *(2)*.

The economic viability of a biochemical process depends not only on the innovations achieved in reaction steps, but also on the innovations and optimization of downstream processes *(3)*.

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Adsorption on polymer resins is a current method used for the removal of chemicals and pharmaceuticals from dilute liquid mixtures *(4)*. Important advantages of these resins when used for such applications are their easy regeneration and high selectivity. Several investigators considered the use of these resins to recover the antibiotic cephalosporin C *(5,6)*. Grzegorczyk and Carta *(7)* have reported equilibrium data for the adsorption of penicillin-G by a number of porous polymeric adsorbents. Chaubal et al., *(8)* studied the equilibrium for adsorption of penicillin V, tetracycline, and cephalosporin C onto neutral polymeric adsorbents.

In the present study, the performance of the resins Amberlite® XAD-4, Amberlite® XAD-7, and Duolite® XAD-761 for the adsorption of AMP, D-phenylglycine (PG), D-phenylglycine methyl ester (PGME), and 6-aminopenicillanic acid (6-APA) was assessed. Temperature and pH effects were also investigated. Adsorption equilibrium data of the four components, for the three resins, were obtained and the respective isotherms discriminated.

Materials and Methods

Resins

Amberlites XAD-4 (polystyrene-divinylbenzene), XAD-7 (aliphatic ester), and Duolite XAD-761 (phenol-formaldehyde) were obtained from Rohm and Haas. AMP, PG, PGME, and 6-APA were obtained from Sigma (St. Louis, MO).

Preparation of Resin

The adsorbents were pretreated with methanol to remove any trace of ultraviolet-absorbing material. The beads were then washed with distilled water and dried at 30°C for 24 h.

Influence of Temperature and pH on Adsorption Efficiency

The effect of temperature and pH was evaluated in batch experiments. Preweighed amounts of hydrated adsorbent (0.25g dry basis, W) were placed in test tubes containing 5 mL (V) of a solution with a known initial solute concentration (C_0). The tubes were then placed in a thermostatic bath at the selected temperature and slowly stirred for a minimum of 2 h at 25°C and 24 h at 4°C. From preliminary experiments, these time spans were found to be sufficient to reach equilibrium. The compound equilibrium concentration (C^*) in the solution was determined by UV spectroscopy at 240 nm. Experimental equilibrium adsorption data were obtained by the same method. These data were correlated to linear, Freundlich, and Langmuir models. The amount of compounds adsorbed on the resin (q^*) was estimated by mass balance using Eq. 1:

$$q^* = \frac{\left(C_0 - C^*\right)}{W} \leftrightarrow V \tag{1}$$

The adsorption efficiency percent, %*A*e, was calculated as follows:

$$\%Ae = \frac{\left(C_0 - C^*\right)}{C_0} \times 100 \quad (2)$$

Determination of Dissociation Constants

The dissociation constants of PG, AMP, PGME and 6-APA were determined at room temperature using a Titrine pHstat Metrohm. The compounds (5–10 m*M*) were dissolved in water and titrated with 0.1 *N* NaOH or 0.1 *N* HCl. All pK_a and pK_b values were obtained from the average of three experiments. Isoelectric points (p*I*) were calculated as: $pI = (pK_a + pK_b)/2$.

Results and Discussion

Effect of Temperature

The results presented in Table 1 show that the adsorption efficiency of the compounds on the three resins slightly increased as temperature decreased. In most cases, adsorption efficiencies values obtained at 4°C were about 10% higher than those at 25°C, for all resins. However, this difference is too low to support a choice of a lower temperature for industrial operation, in view of the higher energy costs to work at 4°C. The resin XAD-4 performed best.

Effect of pH

As can be seen in Figs. 1–3, the pH has influenced in different ways the adsorption equilibrium (at 25°C) of the compounds for the hydrophobic resins that were tested. All *pK* values mentioned hereon were measured according to the methodology previously described.

AMP presents values for pK_a and pK_b of 2.66 and 7.24, respectively. The increment in pH above the isoelectric point (p*I*) of AMP (4.95) produces an increase in the net negative charge of the compound. In these conditions, the electrostatic interactions between AMP molecules and the aqueous mobile phase become higher than the hydrophobic interactions with the resins, reducing the adsorption capacity of the stationary phase. Futhermore, the increase in the net negative charge of AMP molecules will increase the repulsion among adsorbed molecules, and that effect may reduce the adsorption efficiency of the resins.

PGME does not present an amino acid behavior, since it possesses only the amine group (pK_b = 6.89). Hence, an increment in pH leads to an increase in the concentration of the ester neutral form; that is, more PGME molecules become neutral when the pH increases to about 6.89, which may cause a reduction of electrostatic interactions between PGME molecules and the mobile phase, improving the adsorption capacity of the resins. For the same reason, the repulsion among its molecules adsorbed on the resins may be reduced, increasing the adsorption efficiency of PGME molecules. This may explain the opposite behavior found for PGME and AMP.

Table 1
Effect of Temperature on Adsorption Efficiency (%*Ae*)
on Three Resins at pH 6.5 and 25°C

	XAD-4		XAD-7		XAD-761	
	25°C	4°C	25°C	4°C	25°C	4°C
PG	15	19	7	9	15	18
PGME	63	70	54	56	51	64
6-APA	19	20	10	10	17	25
AMP	76	79	32	48	49	56

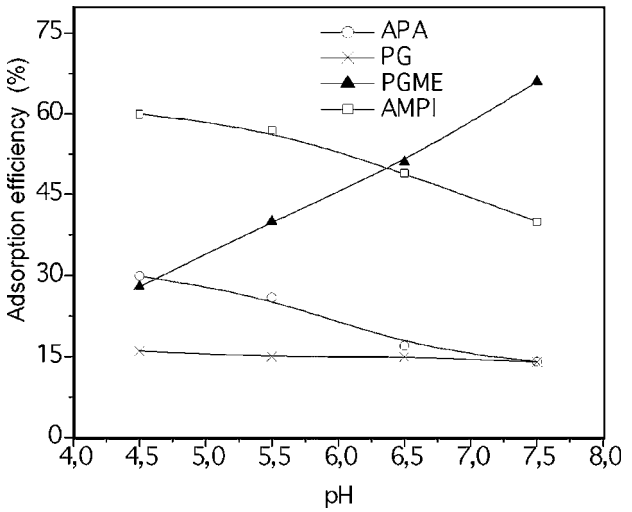

Fig. 1. Influence of pH on the adsorption efficiency (%*Ae*) of compounds on XAD-761 resin. T = 25°C.

The adsorption efficiency of 6-APA presented the same behavior as AMP. 6-APA presents values for pK_a and pK_b of 2.47 and 4.93, respectively. In consequence, it has a low p*I* (3.7), so in the pH range used herein (4.5–8.5) 6-APA molecules always present a net negative charge. In this way, the increment of pH produces an increase in this net negative charge, causing the electrostatic interactions between 6-APA and the mobile phase molecules to be higher than the hydrophobic interactions with the resins, thus reducing the adsorption capacity of the stationary phase.

In the case of PG, the adsorption efficiency values showed a 15% boost with increasing pH. PG has a p*I* of 5.49, higher than AMP, and its amine pK_b group is 9.02 and its pK_a is 1.96. Thus, for the pH range studied, the concentration of PG molecules with charged amine groups (and therefore positively charged) diminished very slowly over the entire pH range.

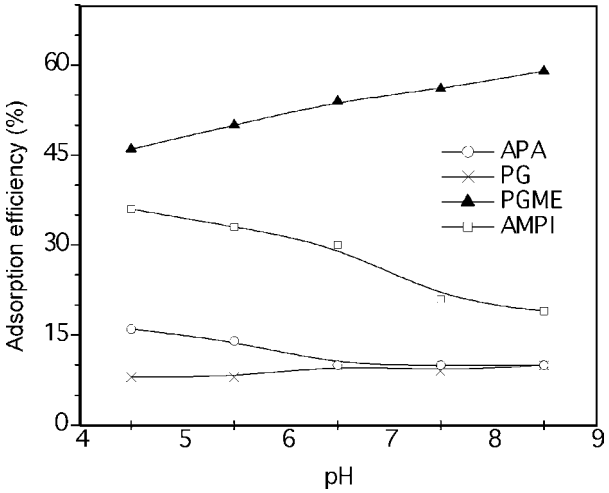

Fig. 2. Influence of pH on the adsorption efficiency (%*Ae*) of compounds on XAD-7 resin. T = 25°C

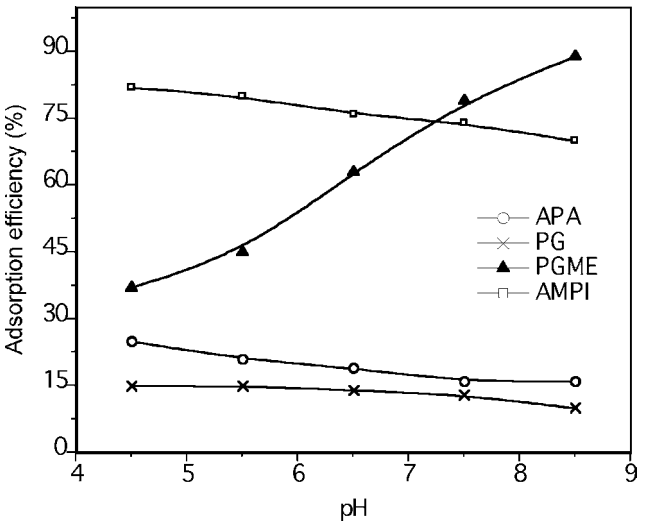

Fig. 3. Influence of pH on the adsorption efficiency (%*Ae*) of compounds on XAD-4 resin. T = 25°C.

Adsorption Isotherms

Batch experiments were carried out in a stirred tank in order to study the equilibrium of the adsorption of AMP, PGME, 6-APA, and PG on the hydrophobic resins XAD-4, XAD-7, and XAD-761. All experiments were carried out at 25°C and pH 6.5. The experimental data were fitted to the linear, Freundlich, and Langmuir models (Eqs. 3–5). The Lenberg-Marquardt algorithm for nonlinear least squares fitting was employed

Table 2
Best Adsorption Isotherm Models Fitted for Adsorption of AMP, 6-APA, PGME, and PG on Resins XAD-761, XAD-7, and XAD-4, with Respective Parameter Values of Models

	XAD-761		XAD-7		XAD-4	
Compound	Isotherm	Parameter	Isotherm	Parameter	Isotherm	Parameter
AMP	Freundlich ($r^2 = 0.9984$)	$K_F = 21.0$ $n = 0.66$	Linear ($r^2 = 0.9991$)	$K_H = 11.8$	Langmuir ($r^2 = 0.9988$)	$q_m = 376.2$ $K_L = 5.45$
6-APA	Langmuir ($r^2 = 0.9986$)	$q_m = 190.0$ $K_L = 42.1$	Linear ($r^2 = 0.9980$)	$K_H = 2.4$	Linear ($r^2 = 0.9992$)	$K_H = 5.2$
PGME	Langmuir ($r^2 = 0.9992$)	$q_m = 590.2$ $K_L = 21.4$	Freundlich ($r^2 = 0.9992$)	$K_F = 15.5$ $n = 0.81$	Langmuir ($r^2 = 0.9998$)	$q_m = 1184$ $K_L = 39.4$
PG	Linear ($r^2 = 0.9976$)	$K_H = 4.4$	Linear ($r^2 = 0.9986$)	$K_H = 2.5$	Linear ($r^2 = 0.9995$)	$K_H = 4.0$

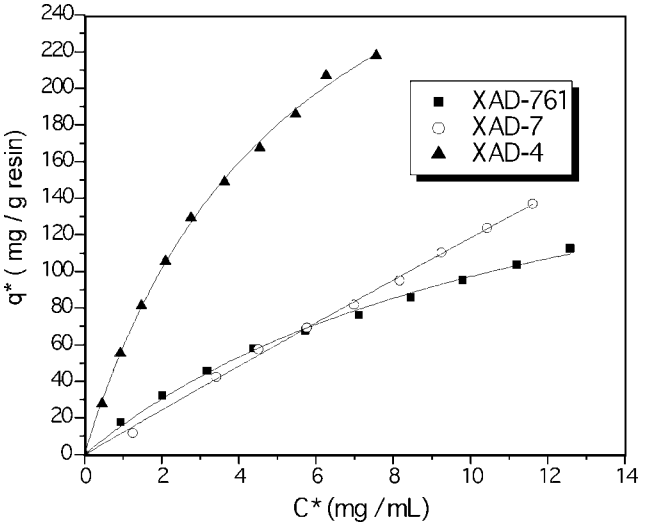

Fig. 4. Adsorption isotherms of AMP on the three resins: (○, ▲, ■) experimental data; (——) fitting with models.

(using the software Microcal Origin 6.0). An example of the fitting of the three tested isotherm models is shown in Fig. 4, for AMP. Tables 2 contains the parameters of the best model fitted for each compound, on the three resins. To discriminate which isotherm was the "best model," two criteria were used: the minimum sum of the squares of the residues, and a nonbiased distribution of these residues:

$$q^* = \frac{q_m C^*}{K_L + C^*} \tag{3}$$

$$q^* = K_F C^{*n} \tag{4}$$

$$q^* = K_H C^* \tag{5}$$

in which K_L (mg/mL) and q_m (mg/g) are constants of the Langmuir equation, K_F (mL/mg) and n are constants of the Freundlich equation, and K_H (mL/mg) is the constant of the linear equation.

In Fig. 4 we provide a comparison of the adsorption isotherms of AMP for the three adsorbents. As can be seen, the adsorption capacity of XAD-4 was much greater than for XAD-7 and XAD-761. For example, when the equilibrium concentration in the solution was 6 m*M*, the amount of AMP adsorbed was approx 190 mg/g for XAD-4, being only 65 mg/g for both XAD-7 and XAD-761. Table 2 presents the parameters corresponding to the best model fitted for all the tested compounds, on each resin.

It can be observed from the results in Table 2 that for 6-APA, XAD-4 resin reached higher adsorption capacity values than XAD-7 and XAD-761. Using the fitted models, it is possible to calculate that at a concentration of 15 m*M*, the amounts of 6-APA adsorbed would be approx 75, 45, and 30 mg/g, for XAD-4, XAD-7, and XAD-761 resins, respectively.

The results showed in Table 2 also demonstrate that for PGME, XAD-4 resin presented the best performance in the adsorption of the compound. For the sake of comparison, the calculated amount of PGME adsorbed on the resin, in equilibrium with 15 m*M* PGME in liquid phase, would be approx 320, 230, and 140 mg/g for XAD-4, XAD-761, and XAD-7, respectively. The results for PG, given in Table 2 indicate similar adsorption capacities for the three tested resins. The calculated amount of PG adsorbed on XAD-4 and XAD-761 was about 11 mg/g, while on XAD-7 it was 7 mg/g (all values were taken at an equilibrium concentration in the solution of 3 m*M* PG).

Because PG presents low solubility, the range of concentrations used to determine its equilibrium isotherms was very low. This may explain why linear isotherms were obtained for equilibrium adsorption data of PG, for all resins.

Comparison of the adsorption isotherms of 6-APA, PG, PGME, and AMP for the different resins shows that the highest adsorption capacities were attained for XAD-4, for all compounds. Higher selectivity was achieved using XAD-4 resin, too: AMP/PGME = 2.2 (at pH 4.5), AMP/PG = 7.0 (at pH 8.5), and AMP/6-APA (4.5 (pH between 7.5 and 8.5).

Conclusions

The effects of temperature and pH on the adsorption efficiency of 6-APA, PGME, PG, and AMP on polymeric hydrophobic resins were studied. An improvement in the adsorption efficiency of about 10% was obtained at 4°C, compared to the results obtained at 25°C, for all compounds on the three resins tested. This enhancement of adsorption capacity, however, was not enough to overcome the higher energy costs that the industrial operation at low temperature would impose.

Adsorption was affected by the solution pH, as expected, reflecting the interactions among polymer resin, adsorbates, and mobile phase. PGME adsorption increased at high pH values, whereas AMP, and 6-APA presented an opposite behavior, for all resins used. No significant effect of pH on the adsorption of PG was observed.

The equilibrium isotherms of 6-APA, PG, PGME, and AMP were determined in a batch stirred tank. A linear isotherm described satisfactorily PG experimental equilibrium data for all resins. The same model described well 6-APA and AMP adsorption on XAD-7 and 6-APA on XAD-4. Other equilibrium data were satisfactorily represented by nonlinear models (Freundlich and Langmuir).

The batch tests allowed screening of adsorbents on the basis of adsorption capacity. The results presented demonstrate that the hydro-

phobic resins can be used in the downstream process of the enzymatic synthesis of ampicillin.

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References

1. Ospina, S., Barzana, E., Ramírez, O.,T., and López-Munguía, A. (1996), *Enzyme Microb. Technol.* **19,** 462–469.
2. Hernández-Justiz, O., Terreni, M., Pagani, G., García, J. L., Guisán, J. M., Fernández-Lafuente, R. (1999), *Enzyme Microb. Technol.* **25,** 336–343.
3. Wheelwright, S. M. (1987), *Bio/Technology* **5(8),** 789.
4. Doulia, D., Rigas, F., and Gimouhopoulos, C. (2001), *J. Chem. Technol. Biotechnol.* **76,** 83–89.
5. Kirkby, N. F., Slater, N. K. H., Weisengerger, K. H.; Addo-Yobo, F. and Doulia, D. (1986), *Chem. Eng. Sci.* **41(8),** 2005–2016.
6. Casillas, J. L., Martinez, M., Addo-Yobo, F., Aracil, J. (1993), *Chem. Eng. J. Biochem. Eng.* **52(3),** B71–B75.
7. Grzegorczyk S. and Carta, G. (1996), *Chem. Eng. Sci.* **51,** 819–826.
8. Chaubal, M. V., Payne, F. G., Reynolds, C. H., and Albright, R. L. (1995), *Biotech. Bioeng.* **47,** 215–226.