The Influence of Charge Density and Steric Factor of Aminated Chloromethyl Polystyrene Homologs in Bilirubin Adsorption

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SYNOPSIS

Bilirubin, a bile pigment, was studied for its extent of adsorption on substituted amine–chloromethyl polystyrene by UV spectrophotometry. By performing simple displacement reactions on chloromethyl polystyrene with secondary and tertiary amines, the amount of charge density and steric factor on substituted nitrogen atom have been varied. Adsorption isotherms of bilirubin at 0°C by different amine–chloromethyl polystyrene homologs suggest the existence of electrostatic interaction of polymeric resin with bilirubin moiety. Results of adsorption of bilirubin to polymeric resin have shown that the extent of adsorption of bilirubin depends on the unit charge, and on the structure of the substituted amine–chloromethyl polystyrene. The effects of porosity of resin on bilirubin adsorption have also been discussed.

INTRODUCTION

Jaundice is often associated with an increased level of bilirubin in blood plasma.[1] The excessive concentration of bilirubin under certain circumstances can lead to serious physiological disorders.[2] Bilirubin, believed to be produced as a result of breakdown of old nonnucleated red blood corpuscles as shown in Figure 1, is a yellowish bile pigment consisting of four pyrrole rings with extensive conjugation. Currently, some of the techniques adopted in reducing the buildup of toxic bile pigments within the system are hemoperfusion and blood transfusion, which, by themselves, are not curative in nature.[3] Efforts have been made in the past to remove bilirubin by adsorbing onto receptors like choloestryamine,[4] oligopeptides,[5] and other related compounds.[6]

Literature presents contrasting and conflicting opinions about the mode of binding of bilirubin to amino group of macroporous polymeric resin. A coupling mechanism between amino group of the polymer and hydroxyl group of bilirubin molecule was initially proposed.[7] The modern thinking, however, suggests that the adsorption may be in the form of electrostatic attractions.[8] Additionally, there are reports available which suggest that the adsorption of bilirubin is dependent on time, temperature, pH, and the basic character of the polymer matrix.[3,4] The synthetic compound studied in this regard and found suitable was cholestyramine, a polymer containing tertiary nitrogen group.[9]

In this paper, we report the synthesis of aminated-chloromethyl polystyrene, prepared by substituting pendant groups like diethanol amine, dimethyl ethanolamine, and dimethylamine to chloromethyl polystyrene, as shown in Figure 2, and their adsorption behavior towards bilirubin. Also, polymer adsorbents with varying pore size have been tested to obtain a better understanding of the nature and extent of interaction prevailing when bilirubin is exposed to aminated-chloromethyl polystyrene.

EXPERIMENTAL

Materials

The starting material, chloromethyl polystyrene, used in the present synthesis was procured from Ion

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Journal of Applied Polymer Science, Vol. 42, 2483-2488 (1991)
 CCC 0021-8995/91/092483-06$04.00

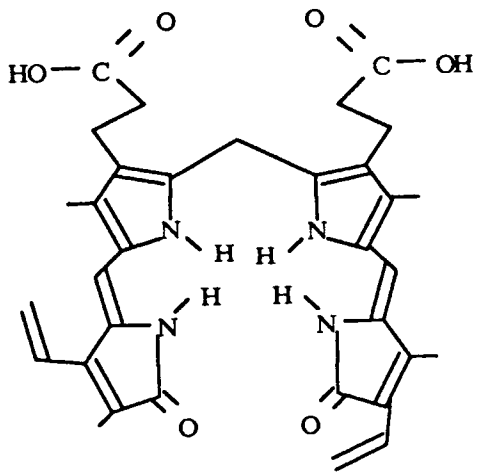


Figure 1 Structure of bilirubin.

Exchange (India) Ltd. Diethanol amine, dimethyl amine, and dimethyl ethanolamine were used as supplied. Dimethyl sulfoxide was used as the solvent. Bilirubin was obtained from Sigma Chemicals.

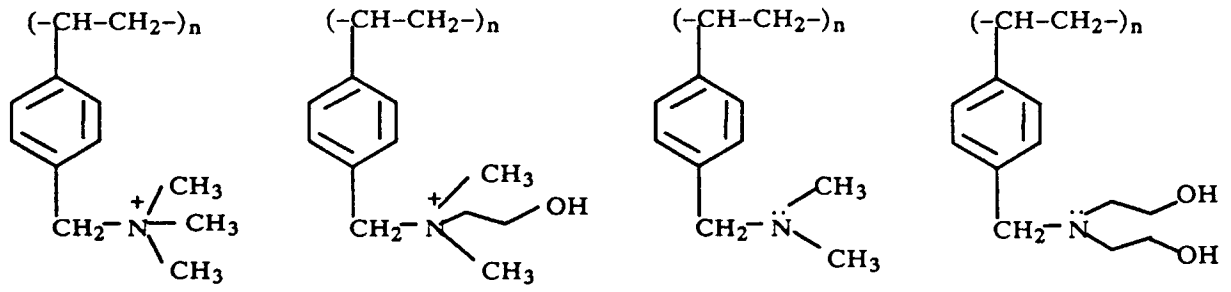
Synthesis of Substituted Chloromethyl Polystyrene by Amination

The chloromethyl polystyrene used was originally present in ethylene chloride. The resin was initially dried by pressing it with Whatman filter paper. It was washed with distilled water to remove traces of ethylene chloride and dried again. The washed and dried resin (25 g) was then placed in a three-necked round bottom flask fitted with a stirrer and a guard tube. Aminating reagent dimethyl amine or diethanol amine (50 mL), and dimethyl sulfoxide solvent were added to the reaction flask. The reaction was carried out at 40°C using an oil bath. After 6 h of stirring the reaction mixture, the excessive aminating agent was decanted along with the solvent. The aminated resin was then neutralized by adding dilute hydrochloric acid (0.5 *N*) drop wise to adjust the pH of the solution to approximately 7.[10,11] The resin was thoroughly washed with distilled water. Finally, the beads were warmed in water bath (60–70°C) for 15 min to remove traces of solvent and dried in a vacuum oven for 3 h to remove the moisture. The characterization of aminated resins was done using Infrared spectrophotometer (Perkin-Elmer 681). Also, the exchange capacity of the resins was determined.[12]

ADSORPTION STUDIES OF BILIRUBIN

Preparation of Bilirubin Stock Solution

A stock solution of bilirubin was prepared whenever required in order to minimize error introduced due to bilirubin photodegradation and oxidation.[13] Bilirubin was initially dissolved in minimal amount of 0.1 *M* NaOH solution and the volume was adjusted to a concentration of 0.5 mg/dL (pH = 7.7–7.8) by a buffer solution of 0.5 *N* K_2HPO_4/0.1 *M* NaOH. Using buffer medium and stock bilirubin solution, varying concentration of bilirubin solutions were prepared and Beer Lambert's law was verified regularly with different bilirubin concentrations.[14] The experimental flasks were wrapped with black carbon paper so as to minimize the photodegradation of bilirubin during adsorption studies. The flasks were placed in PVC container and kept in deep freeze to attain 0°C.



CMPS-1 CMPS-2 CMPS-3 CMPS-4

Figure 2 Structures of different aminated chloromethyl polystyrene (CMPS) where CMPS-1 is trimethylamine methyl polystyrene, CMPS-2 is dimethyl ethanolamine methyl polystyrene, CMPS-3 is dimethyl amine methyl polystyrene, and CMPS-4 is diethanolamine methyl polystyrene.

Adsorption Experiments

Experiments were carried out in the absence of oxygen at 0°C under inert atmosphere using nitrogen. Bilirubin solution was scanned using Shimadzu UV-260 spectrophotometer in the range 300–600 nm. The initial time required by bilirubin to equilibriate with polymeric resin was determined. Concentration changes of bilirubin with time was studied in the presence and absence of aminated-chloromethyl polystyrene. This was followed by measuring the absorbance of remnants of free bilirubin in the solution.

RESULTS AND DISCUSSION

The rate of reaction of an amine with chloromethyl polystyrene depends on the nucleophilicity of amine and also on the type of the solvent used.[10] Generally, aminated products of the type used in this study have been obtained in good yield with aprotic solvents like dimethyl formaldehyde and dimethyl sulfoxide.[15–17] Dimethyl sulfoxide was, therefore, chosen as one of the solvents for synthesizing these aminated resins.

In view of the effect of charge and steric aspects of the polymer on bilirubin adsorption, the charge density and the substituent groups of the polymer were varied to build an effective adsorbent for attracting bilirubin moiety. The amines with different substituents include diethanol amine, dimethyl ethanolamine, and dimethyl amine. These have been considered for synthesizing the aminated chloromethyl polystyrenes. By tailoring the amine pendant groups of the polymer, the influence of polymer environment on bilirubin adsorption has been assessed.

Characterization

The para-substituted aminated polymers were characterized by Infrared spectroscopy. Figure 3 is a representative spectrum of methyl derivative amine methyl polystyrene (CMPS-3). A representative spectrum of hydroxyl derivative amine methyl

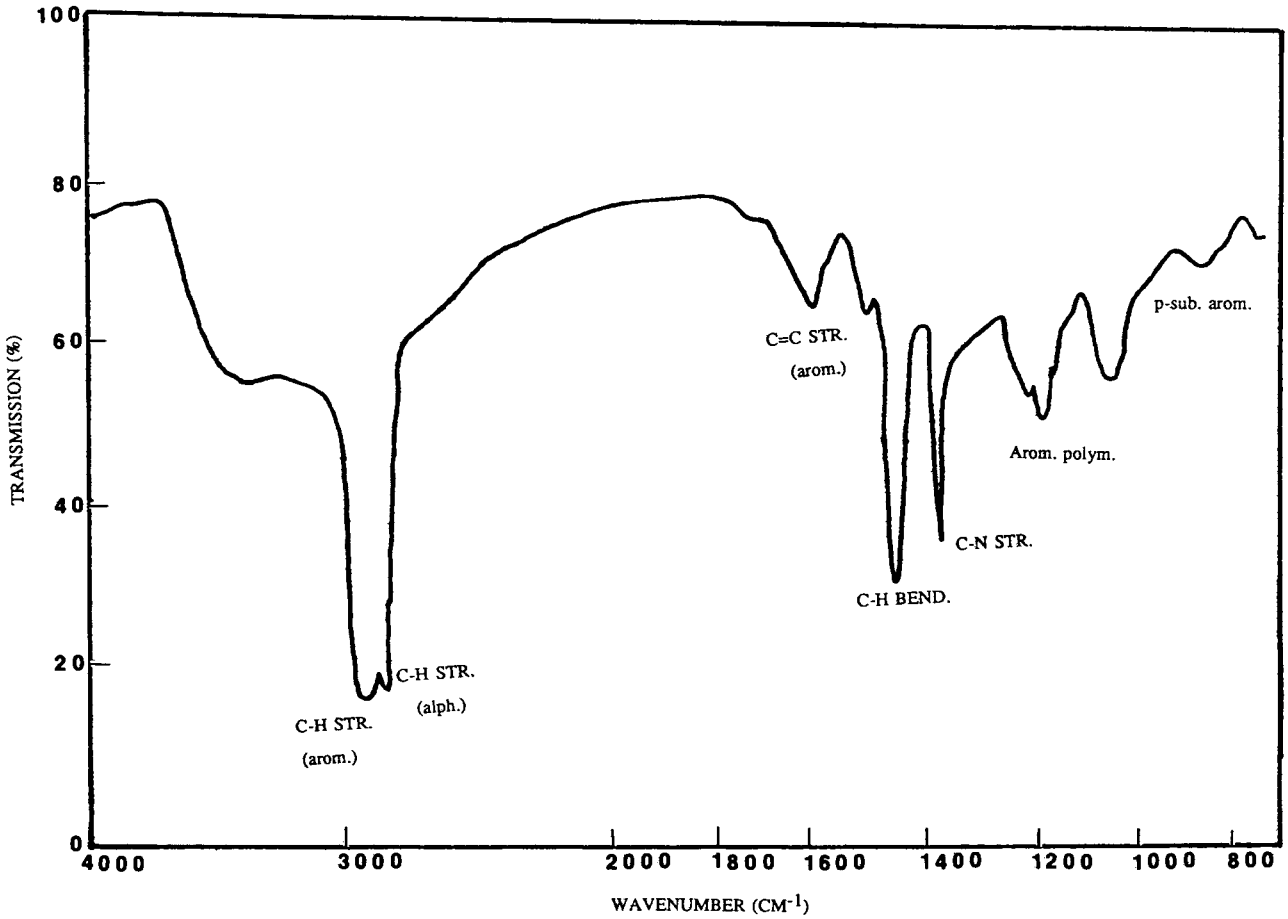


Figure 3 IR spectrum of dimethyl amine methyl polystyrene.

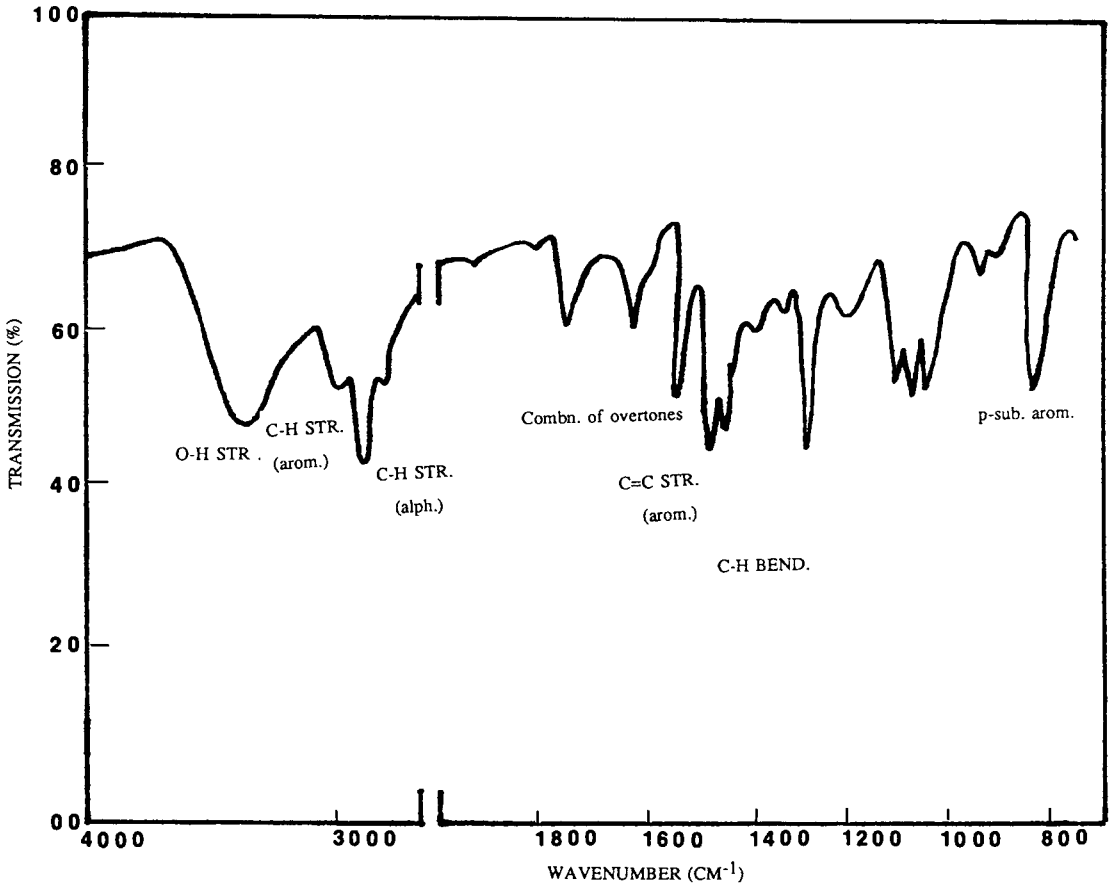


Figure 4 IR scan of diethanol amine methyl polystyrene.

polystyrene (CMPS-4) is shown in Figure 4. The peak corresponding to 1300–1370 cm^{-1} is characteristic of the C—N stretching in the secondary or tertiary amino group. The exchange capacity of the aminated polymer was noted to be 750 meq/mL.

Adsorption Isotherms and Factors Affecting Binding Interaction

Figure 5 represents the bilirubin adsorption isotherms obtained at 0°C for polymer adsorbents having pendant groups of varying basicity. The adsorption data are estimated to have an uncertainty of ±7%. Kinetic studies of bilirubin adsorption on aminated polymer reveal that there is an initial increase in bilirubin adsorption. However, with time the magnitude of bilirubin adsorption is found to be dependent on the charge density of the nitrogen of the aminated polymer and its chemical structure. Of the series of homolog polymers studied, bilirubin adsorption was found to be the highest in the case of trimethyl amine methyl polystyrene (CMPS-1) while diethanol aminated methyl polystyrene (CMPS-4) adsorbed the least. Furthermore, a comparison of the two adsorption isotherms as shown in Figure 5 suggest that the behavior of a polymeric resin towards bilirubin is not only dependent on the charge density of the substituted nitrogen atom, but also on the structure of the polymer.

CMPS-1 is a resin having one unit positive charge. The presence of this unit positive charge introduces an electrostatic interaction between the polymer and bilirubin, a negatively charged species. Replacement of one of the methyl groups of trimethyl aminated methyl polystyrene by the ethanol group led to a drop in the bilirubin adsorption. The incorporation of the ethanol group into the CMPS-1 had resulted in a decrease in the binding affinity of bilirubin towards CMPS-2. This observation is consistent with the earlier reports of the mode of attachment of bilirubin to polymer.[2,8] The results available in the literature indicate that there is a strong interaction between COO^- group of bilirubin molecule and NR^{3+} group of the polymer. However, the adsorption behavior of bilirubin to polymer is not well discussed with regard to the influence of

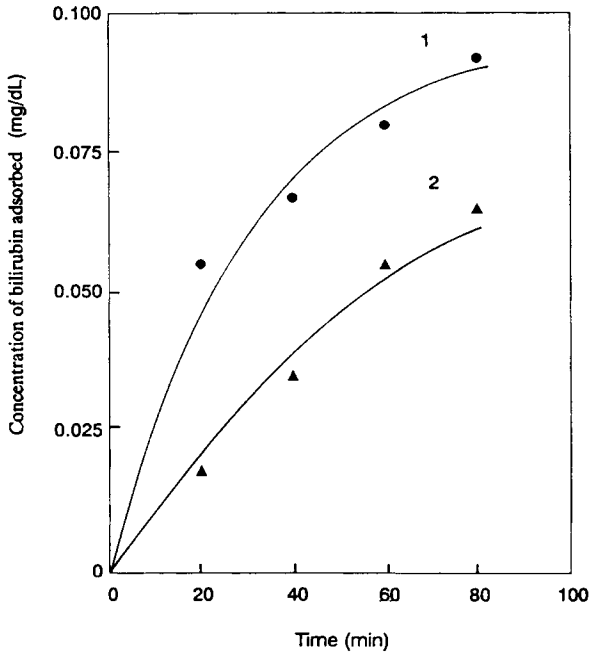


Figure 5 Kinetic studies of bilirubin adsorption on various polymers: (1) trimethylamine methyl polystyrene (CMPS-1) and (2) dimethyl ethanolamine methyl polystyrene (CMPS-2).

both the charge character and the steric factors of the polymer adsorbate. Recent experimental work[2] suggests that the unit positive charge and steric factor of pendant groups play a role in stacking of bilirubin to immobilized oligopeptides containing lysine. The current study indicates the relevance of steric factor and charge of polymer in bilirubin adsorption by substituting various pendant groups to aminated methyl polystyrene.

The isotherms of bilirubin adsorption by CMPS-3 and CMPS-4 are shown in Figure 6. The difference in binding behavior of bilirubin to CMPS-1, CMPS-2, CMPS-3, and CMPS-4 are thought to be reflected in their chemical structure. CMPS-3 and CMPS-4 lack quarternary nitrogen atom; hence there is no unit positive charge present on these polymers. Therefore, these polymers may not favor bilirubin sorption to the extent of CMPS-1 and CMPS-2. Such an effect is clearly evident by correlating data of Figures 5 and 6. On the other hand, there is some striking difference in the bilirubin adsorption behavior of CMPS-3 and CMPS-4. This can be correlated to the existence of steric effect in substituted polymers. CMPS-3 has two methyl groups attached to it, whereas CMPS-4 has two ethanol groups linked to the main chain. The presence of two ethanol groups within a single repeating unit of polymer could hinder the process of bilirubin adsorption. Thus, the concentration of bilirubin adsorbed was found to be the lowest among the series of compounds considered. This confirms our earlier observations drawn from Figure 5, i.e., both steric factor and the unit charge of the polymer play a key role in the adsorption process of bilirubin moiety.

In addition to the study of effect of influence of charge density and steric factor of polymer on bilirubin adsorption, the role of porosity of polymer is considered. It is a well-known fact that the microporous resin provides a larger surface area for binding of substrates, whereas the macroporous resins do not. Figure 7 shows kinetic results obtained on performing preliminary adsorption experiments on two identical resins (CMPS-2) with varying porosity. It was noticed that the microporous resin provides a greater amount of bilirubin binding active sites, and this has led to an increase in the concentration of bilirubin adsorption to polymer.

CONCLUSIONS

By tailoring the pendant groups of the aminated chloromethyl polystyrene, the current study has indicated qualitatively the importance of charge and

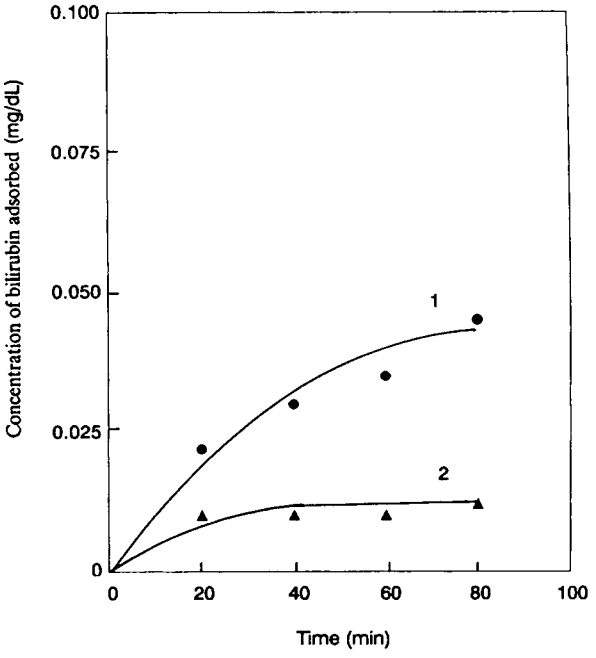


Figure 6 Kinetic studies of bilirubin adsorption on various polymers: (1) dimethylamine methyl polystyrene (CMPS-3) and (2) diethanolamine methyl polystyrene (CMPS-4).

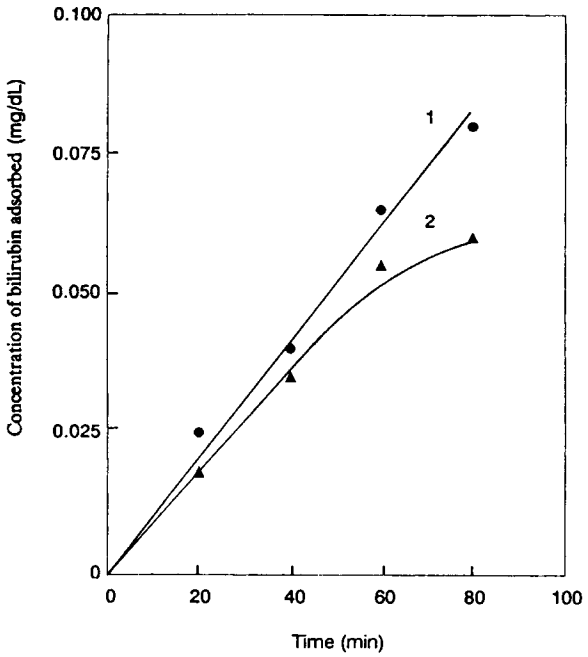


Figure 7 Kinetic studies of bilirubin adsorption on dimethyl ethanolamine methyl polystyrene (CMPS-2) and (1) microporous and (2) macroporous resins.

the steric effect of polymer on amount of bilirubin adsorbed. Of the homolog-aminated polymers, the quarternary polymer having a unit positive charge and carrying minimal steric effect provided the highest adsorption, whereas the nonquarternary polymer with no unit positive charge and a strong steric effect showed the least sorption. The results obtained with microporous resin show greater adsorption of bilirubin.

One of the authors, D. Raghavan, thanks the Department of Chemistry, Indian Institute of Technology, Bombay for its support. The authors wish to thank Dr. P. Pai, Works Manager, Ion Exchange Ltd., Bombay, India for providing them the resin samples and to Dr. G. R. Gokhale at Tata Cancer Research Center, Bombay, India for his interest and advice.

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Received January 15, 1990
Accepted August 31, 1990