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Synthesis, Sorption Properties of a New Chelating Ion-Exchange Resin containing β-Resorcylic Acid as the Functional Group and Its Biotechnological Application

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A new chelating ion-exchange resin containing β-resorcylic acid (2,4-dihydroxy benzoic acid) as the functional group, based on macroreticular polystyrene divinylbenzene (8% cross-linked, 18–44 mesh size) has been synthesized. The sorption properties of the chelating ion-exchange resin towards various divalent metal ions, *viz.*, Cu(II), Co(II), Ni(II), Cd (II), Pb(II) and Zn(II) are studied by a static batch equilibration technique, as a function of pH and time of equilibration

Key Words: Synthesis, Sorption, Chelating ion-exchange resin, β-Resorcylic acid, Biotechnological application.

INTRODUCTION

The incorporation of functional group into polymeric matrix is of interest in connection with trace concentration of heavy metal ions. Previously Eccles and Vernon[1] have synthesized a macroreticular chelating resin containing resorcinol and studied its properties with different metal ions. They suggested that the nitroso group produced from azo group and phenol group in the resin would appear to play an important role in the sorption of metal ions. Sugii *et al.*[2] synthesized a macroreticular polystyrene based chelating resin with nitrosoresorcinol as the functional group. Ghosh *et al.*[3] synthesized a macroreticular polystyrene based chelating resin with 1-nitroso-2-naphthol as the functional group and studied its sorption properties. Kapadia *et al.*[4] reported the synthesis of chelating ion-exchange resin from acetaldehyde-β-resorcylic acid (ACRA) using gel technique. Recently, the synthesis of some chelating resins with spacer, —CH_2—NH—C_6H_4, based on macroreticular chloromethylated polystyrene divinylbenzene containing 1-nitroso-2-naphthol as a functional group has been reported by Akerkar *et al.*[5]

The immobilization of enzymes on supports is a most important technique to increase productivity and life span of these biocatalysts. Conventionally, enzymes are immobilized on water insoluble supports such as polysaccharide derivatives, ion exchange resins, etc., and such systems have been applied in several manufacturing industries[6, 7]. Immobilized enzymes using insoluble material as carrier offer several advantages over free enzymes, including easy recovery, possibility for continuous operation, simplified downstream processing and sometimes enhanced stability. Roy *et al.*[8] have described the immobilization of β-amylase on polystyrene cation-exchange resin equilibrated with Al^{3+} ions (IR-120 Al^{3+}). Yamaguchi *et al.*[9] have used tannin resin as a support to immobilize α-amylase. Siso *et al.*[10] have described the enzyme encapsulation on chitosan microbeads. Iqbal *et al.*[11] studied the immobilization and stabilization of papain on chelating sepharose, a metal chelate regenerable carrier.

The present research paper describes the synthesis and sorption behaviour of a new chelating ion-exchange resin based on macroreticular polystyrene divinylbenzene containing β-resorcylic acid as the functional group. The resin then has been used for immobilization of enzymes, *viz.*, α-amylase and papain. Thus, the possibility of the synthesized chelating resin as an insoluble polymeric support for immobilized enzyme has been investigated.

EXPERIMENTAL

Chloromethylated polystyrene divinylbenzene (8% cross-linked, 18–44 mesh size) was supplied by Ion-Exchange India Ltd., Mumbai. All reagents used were of analytical grade. Double distilled water was used throughout the work. α-Amylase and papain (lyophilized powder) enzymes were obtained from Sigma Aldrich.

Each of the stock solutions of Cu(II), Co(II), Ni(II), Cd(II), Pb(II) and Zn(II) metal ions having a concentration of approximately 1 mg/cm^3, were prepared by dissolving the requisite quantities of the AR grade chlorides, sulphates and nitrates in distilled water. Standardization of the metal ion solutions was done volumetrically as per the standard methods reported in literature[12].

Synthesis of the chelating resin

Resin I. The starting material was an 8% cross-linked macroreticular choromethylated polystyrene divinylbenzene with 18–44 mesh size.

Resin II. To 5.0 g of resin I, 20 mL of 1,4-dioxane was added and the mixture was stirred for 24 h at room temperature to allow maximum swelling. To the thus preswollen resin I, 4.5 g β-resorcylic acid and 2.5 g anhydrous $ZnCl_2$ (freshly fused) were added. The resultant mixture was heated to 105–110°C and refluxed at this temperature for 11 h with stirring. The product was filtered off and washed with methanol using Soxhlet apparatus and dried under vacuum to yield resin II (colour: brown; yield: 5.6 g).

Resin III. 5.5 g of resin II was then added to 50 mL of 1 M sodium hydroxide solution containing 2.2 g of sodium nitrite. After cooling to 0°C, 3.8 mL of 40% H_2SO_4 solution was added dropwise to the mixture with constant stirring for 4 h, while maintaining the temperature in the range 0–5°C. The product was filtered off, washed with methanol, 10% HCI, water and again washed with methanol using Soxhlet apparatus, and finally dried under vacuum to give resin III (colour: dark brown; yield: 5.9 g).

Resin Characterization

Microanalysis. Elemental analysis of the synthesized resin was carried out at Microanalytical Department of UMICT (Mumbai).

Water regain. 1.0 g of the dry resin was allowed to stand up in double distilled water for 48 h, then filtered off by suction and lightly pressed between filter papers to remove surface moisture. The resin was dried at 80°C for 11 h after which its weight was recorded. The water regain value was calculated from the difference in the weights thus recorded.

Sorption of metal ions by batch operation

For studying the sorption properties of synthesized resin, six metal ions, *viz*., Cu(II), Co(II), Ni(II), Cd (II), Pb(II) and Zn(II) were chosen. The metal ion uptake was studied by equilibrating 0.5 g of the dry resin with 25 mL of metal ion solution (1 mg/mL) as a function of pH and time of equilibration.

Metal ion uptake as a function of pH

The dry resin (0.5 g) was added to 25 mL of buffered (from pH 2 to 12) metal ion solution (1 mg/mL). After equilibrating for 24 h, the mixture was filtered and the resin was thoroughly washed with distilled water. The sorbed metal ions were then completely eluted by using 30 mL of 2 N HCl solution.

Metal ion uptake as a function of time of equilibration

0.5 g of the dry resin was added to 25 mL of buffered metal ion solution (1 mg/mL) at an optimum pH of 7 to 11 respectively. After equilibrating for different intervals of time, the mixture was filtered off and the resin was thoroughly washed with double distilled water. The sorbed metal ions were completely eluted by using 30 mL of 2 N HCl solution.

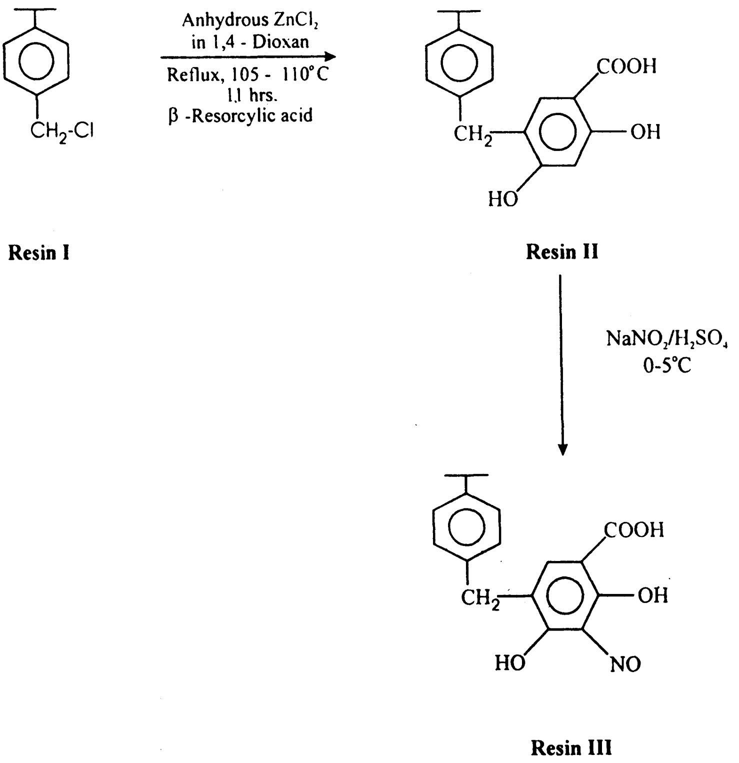
The amounts of metal ions present in the solution and retained on the resin were determined by chelatometric titration with EDTA.

Biotechnological Application

Immobilization of (α-amylase/papain) enzyme on the chelating resin: 0.5 g of the synthesized chelating resin was washed with double distilled water. The thoroughly washed resin beads were finally equilibrated with 10 mL of acetate buffer (pH 4.6)/phosphate buffer (pH 8.2) respectively. This matrix was then mixed with 10 mL of α-amylase/papain solution (1 mg/mL) prepared in acetate buffer (pH 4.6)/phosphate buffer (pH 8.2) and stirred at room temperature for 3 h and the supernatants were collected. The amount of enzyme immobilized on the chelating resin was estimated from the difference between the amount of enzyme (α-amylase/papain) in the enzyme-buffer solution before and after immobilization. Protein estimation was done according to Lowry *et al.*[13]

RESULTS AND DISCUSSION

The chelating resin in this work was synthesized from choromethylated polystyrene divinylbenzene (8% cross-linked, 18–44 mesh size) through the steps shown in **Scheme-1**. The synthesized resin was characterized by IR spectrum, which exhibits a strong broad band at 3437 cm^{-1} due to the presence of hydroxyl (—OH) group. The absorption band at 1615 cm^{-1} is attributed to the carboxylic acid (—COOH) group. The presence of distinct absorption band at 1219 cm^{-1} corresponds to nitroso group.



Scheme-1

Microanalysis for the elements of the dried resin showed the following results:

(%) C = 71.72, H = 5.69, N = 2.42.

The hydrogen ion capacity of the synthesized resin was found to be 3.48 meq/g. The water regain value of the synthesized resin was found to be 0.49 g/g.

The sorption characteristics of the synthesized chelating resin towards Cu(II), Co(II), Ni(II), Cd (II), Pb(II) and Zn(II) metal ions have been investigated by static batch operation, over the pH range, 2–12, as a function of pH and time of equilibration. The sorption of metal ions on chelating resin at various pH values was investigated, and the results are shown in Fig. 1. The metal ion uptake was found to be dependent on the equilibrium pH of the medium. For each metal ion there is an optimum pH at which the maximum intake of metal ion takes place. The optimum pH for Cu(II), Cd(II) and Zn(II) ions is 11, for Co(II) and Ni(II) ions it is 10 and for Pb(II) ions it is 7. The synthesized resin shows the following affinity order for metal ions:

Cu(II)	>	Co(II)	>	Ni(II)	>	Cd (II)	>	Pb(II)	>	Zn(II)
(97%)		(96%)		(95%)		(93%)		(91%)		(90%)

The time required for maximum adsorption of metal ions studied was found to be as presented in Table-1.

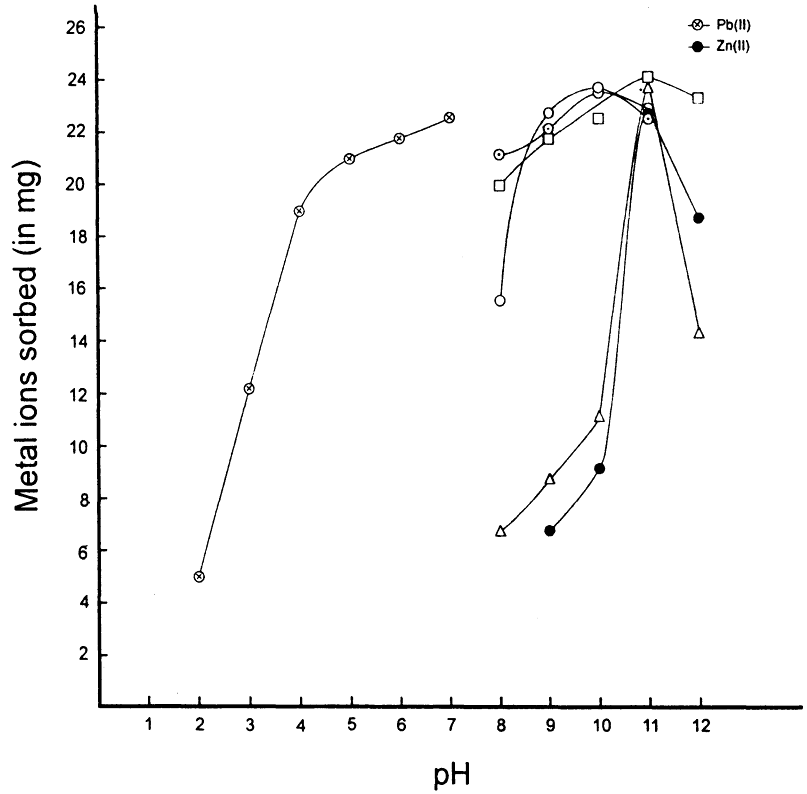


Fig. 1. Sorption behaviour of six metal ions between pH range 2–12

TABLE-1
TIME REQUIRED BY THE RESIN FOR MAXIMUM ADSORPTION OF METAL IONS

Metal ions	Cu(II)	Co (II)	Ni(II)	Cd (II)	Pb(II)	Zn(II)
Time (h)	12	14	14	12	13	14

Thus, the time required for maximum adsorption of metal ions at their respective optimum pH was found to be in the range, 12–14 h. The sorption of cations was observed at higher pH, *i.e.*, 7–12. In strongly acidic solutions, the carboxylic groups or phenolic groups remain only partially dissociated because of which the adsorption of cations on the chelating resin is less. On the contrary, in neutral or alkaline medium, these groups get ionized with ease, leading to the formation of chelate rings with metal ions. Thus, the adsorption of metal ions was observed at higher pH. The resin thus synthesized in the present work shows rapid rate of exchange in the beginning followed by slower rate of exchange probably due to surface exchange or exchange in the interior due to diffusion respectively. The distribution coefficient (K_d) values are calculated by the relation[14]

$$K_d = [M]_{resin}/[M]_{solution}$$

where $[M]_{resin}$ is the amount (in mg) of metal ions taken up by 0.5 g of resin and $[M]_{solution}$ is the concentration (in mg/mL) of metal ions remaining in solution. The calculated values of K_d are listed in Table-2.

TABLE-2
DISTRIBUTION COEFFICIENTS AS FUNCTION OF pH FOR VARIOUS CATIONS ON CHELATING RESIN CONTAINING β-RESORCYLIC ACID AS FUNCTIONAL GROUP

pH	K_d for metal ions					
	Cu(II)	Co (II)	Ni(II)	Cd (II)	Pb(II)	Zn(II)
2	—	—	—	—	0.25	—
3	—	—	—	—	1.03	—
4	—	—	—	—	3.54	—
5	—	—	—	—	6.37	—
6	—	—	—	—	8.83	—
7	—	—	—	—	13.75	—
8	4.18	6.73	1.70	0.35	—	—
9	7.22	19.25	11.61	0.51	—	0.37
10	10.62	29.08	22.90	0.78	—	0.57
11	38.95	11.36	10.30	15.36	—	9.77
12	6.30	—	—	1.32	—	2.96

Lowry's method was employed for estimation of the protein content of the commercial enzymes preparation used for the immobilization on synthesized ion exchange resin in the present study. A standard graph using bovine serum albumin (stock standard, 1000 μg/mL) was plotted. The protein contents of papain and α-amylase preparations were done before and after immobilization as reported in Table-3. On the basis of the data obtained, the immobilization of synthesized ion-exchange resin was determined, as reported in Table-4. The synthesized chelating resin shows high immobilization capacity for α-amylase and papain.

Thus, the chelating resin synthesized in the present study can be used for the separation of various divalent metal ions, *viz.*, Cu(II), Co(II), Ni(II), Cd(II), Pb(II) and Zn(II), as well as useful as an insoluble polymeric support for immobilization of enzymes.

TABLE-3
PROTEIN ESTIMATION BY LOWRY'S METHOD

Enzyme	Unknown enzyme sample	O.D. at 750 nm	Dilution factor (D.F.)	Protein concentration (V) from graph	Concentration of unknown (V × D.F.)	Mean concentration of enzyme (μg/cm^3)
α-Amylase	AF$_1$	0.745	1	1000	1000	1005
	AF$_2$	0.380	2	510	1010	
	AS$_1$	0.340	1	460	460	460
	AS$_2$	0.175	2	230	460	
Papain	PF$_1$	0.750	1	1010	1010	1025
	PF$_2$	0.385	2	520	1040	
	PS$_1$	0.325	1	440	440	470
	PS$_2$	0.185	2	250	500	

AF$_1$/PF$_1$ = undiluted α-amylase/papain enzyme in the solution before adsorption.
AF$_2$/PF$_2$ = 1 : 2 diluted α-amylase/papain enzyme in the solution before adsorption.
$_1$/PS$_1$ = undiluted α-amylase/papain enzyme in the supernatant before adsorption.
AS$_2$/PS$_2$ = 1 : 2 diluted α-amylase/papain enzyme in the supernatant before adsorption.

TABLE-4
IMMOBILIZATION CAPACITY OF THE CHELATING RESIN

Chelating ion-exchange resin containing β-resorcylic acid as the functional group	α-Amylase			Papain		
	Adsorbed (mg)	Unadsorbed (mg)	Immobilized (mg)	Adsorbed (mg)	Unadsorbed (mg)	Immobilized (mg)
	10.05	4.60	5.45	10.25	4.70	5.55

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