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SYNTHESIS AND SORPTION PROPERTIES OF A NEW CHELATING ION–EXCHANGE RESIN CONTAINING GALLIC ACID AS THE FUNCTIONAL GROUP AND ITS BIOTECHNOLOGICAL APPLICATION

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ABSTRACT

In the present work, a new chelating ion–exchange resin containing gallic acid (3,4,5–trihydroxy 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., Co (II), Pb (II), Ni (II),Cd (II), Cu (II) and Zn (II) are studied by a static batch equilibration technique, as a function of pH and time of equilibration.

Key words : Gallic acid, Sorption, Ion-exchange resin, Biotechnology

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 sorption of metal ions. Sugii and Ogawa[2] synthesized a macroreticular polystyrene based chelating resin with nitrosoresorcinol as the functional group. Ghosh and Das[3]. synthesized a macroreticular polystyrene based chelating resin with 1–nitroso–2–naphthol as the functional group and studied its sorption properties. Kapadia and Dalal[4]. reported the synthesis of chelating ion–exchange resin from acetaldehyde–gallic acid (ACGA) using gel technique. Recently, 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 and Hegde[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 β–amylase. Siso et. al. [10] have described the enzyme encapsulation on chitosan microbeads. Iqbal and Afaq[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 behavior of a new chelating ion–exchange resin based on macroreticular polystyrene divinylbenzene containing gallic acid as the functional group. Then the resin 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

Chemicals and reagents

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.

Metal ion solutions

Each of the stock solutions of Co (II), Pb (II), Ni (II),Cd (II), Cu (II) and Zn (II) metal ions having a concentration of approximately 1 mg/cm^3, were prepared by dissolving the requisite quantities of the A.R. 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 8 % cross–linked macroreticular choromethylated polystyrene divinylbenzene with 18–44 mesh size.

Resin II. To 5.0 g of resin I, 20 cm^3 of 1,4–dioxan was added and the mixture was stirred for 24 hrs. at room temperature to allow maximum swelling. To the thus preswollen resin I, 4.5 g gallic acid and 2.5 g anhydrous $ZnCl_2$ (freshly fused) were added. The resultant mixture was heated to 105 –110 ^oC and refluxed at this temperature for 11 hrs. 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 : 5.7 g)

Resin III. 5.5 g of Resin II was then added to 50 cm^3 of 1M sodium hydroxide solution containing 2.2 g of sodium nitrite. After cooling to 0^o C, 3.8 cm^3 of 40 % H_2SO_4 solution was added dropwise to the mixture with constant stirring for 4 hrs., while maintaining the temperature in the range, 0–5^o C. The product was filtered off, washed with methanol, 10 % HCl, water and again washed with methanol using Soxhlet apparatus, and finally dried under vacuum to give resin III. (Colour :Dark brown; yield : 5.8 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 in double distilled water for 48 hrs., then filtered off by suction and lightly pressed between filter papers to remove surface moisture. The resin was dried at 80^o C for 11 hrs. 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., Co (II), Pb (II), Ni (II),Cd (II), Cu (II) and Zn (II) were chosen. The metal ion uptake was studied by equilibrating 0.5 g of the dry resin with 25 cm^3 of metal ion solution (1 mg/cm^3) 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 cm^3 of buffered (from pH–2 to 12) metal ion solution (1 mg/cm^3). After equilibrating for 24 hrs., the mixture was filtered and the resin was thoroughly washed with distilled water. The sorbed metal ions were then completely eluted using 30 cm^3 of 2N HCl solution.

Metal ion uptake as a function of time of equilibration

0.5 g of the dry resin was added to 25 cm^3 of buffered metal ion solution (1 mg/cm^3) at an optimum pH of 7 to 11. 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 30 cm^3 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 cm^3 of acetate buffer (pH 4.6)/phosphate buffer

(pH 8.2), respectively. This matrix was then mixed with 10 cm^3 of α– amylase/papain solution (1 mg/cm^3) prepared in acetate buffer (pH 4.6) /phosphate buffer (pH 8.2) and stirred at room temperature for 3 hrs., 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 I.R. spectrum. The absorption band at 3441cm^{-1} is due to the presence of hydroxyl (–OH) group. The absorption band at 1712 cm^{-1} is attributed to the free carboxylic acid (–COOH) group. The presence of distinct absorption band at 1307 cm^{-1} corresponds to nitroso group. Microanalysis for the elements of the dried resin showed the following results:

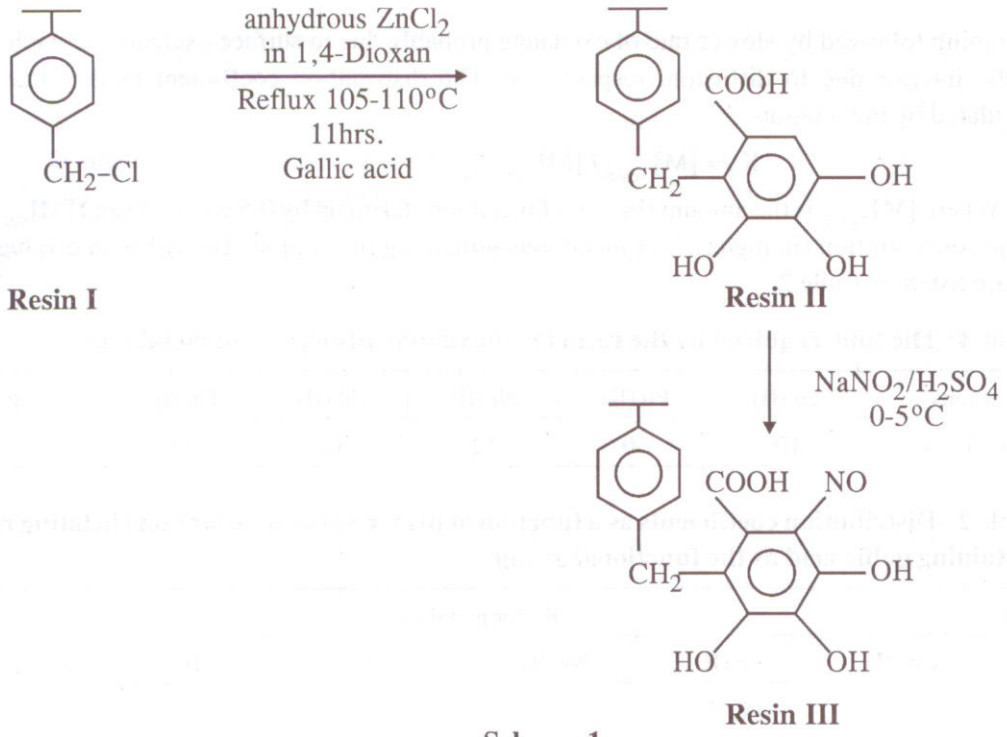
% C = 70.09, % H = 5.72, % N = 2.12.

The hydrogen ion capacity of the synthesized resin was found to be 3.32 meq/g. The water regain value of the synthesized resin is found to be 0.34 g/g.The sorption characteristics of thus synthesized chelating resin towards Co (II), Pb (II), Ni (II),Cd (II), Cu (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 equilibrium pH of the medium. For each metal ion, there is an optimum pH at which maximum intake of metal ion takes place. The optimum pH for Cu (II), Cd (II) and Zn (II) ions is pH 11, for Co (II) and Ni (II) ions, it is 10, and for Pb (II) ions, it is 7. The synthesized resin shows following affinity order for metal ions:

Co (II) >	Pb (II) >	Ni (II) >	Cd (II) >	Cu (II) >	Zn (II)
(96%)	(95%)	(94%)	(86%)	(78%)	(66%)

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

Thus, the time required for maximum adsorption of metal ions at their respective optimum pH was found to be in the range, 10–13 hrs. The sorption of cations was observed at higher pH i.e., 7–12. In strongly acidic solution, the carboxylic groups or phenolic groups remains 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



Resin I **Resin II** **Resin III**

Scheme 1

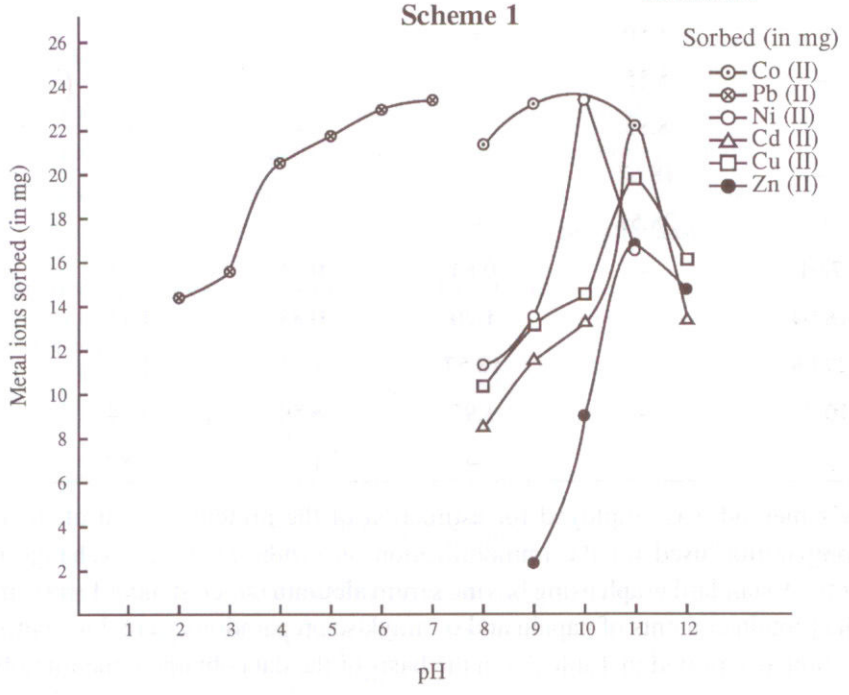


Fig. 1. Sorption behaviour of six metal ions between pH range, 2-12.

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].

$$K_d = [M]_{resin} / [M]_{solution} \quad ...(1)$$

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/cm^3) of metal ions remaining in solution. The calculated values of K_d are listed in Table 2.

Table 1: The time required by the resin for maximum adsorption of metal ions

Metal ions	Co (II)	Pb (II)	Ni (II)	Cd (II)	Cu (II)	Zn (II)
Time (hrs.)	10	10	12	12	11	13

Table 2 : Distribution coefficients as a function of pH for various cations on chelating resin containing gallic acid as the functional group

pH	K_d for metal ions					
	Co (II)	Pb (II)	Ni (II)	Cd (II)	Cu (II)	Zn (II)
2	–	1.45	–	–	–	–
3	–	1.80	–	–	–	–
4	–	5.55	–	–	–	–
5	–	8.83	–	–	–	–
6	–	18.67	–	–	–	–
7	–	28.51	–	–	–	–
8	7.01	–	0.84	0.51	0.71	–
9	18.04	–	1.20	0.83	1.11	0.10
10	29.08	–	18.57	1.11	1.39	0.55
11	10.02	–	1.97	6.59	3.74	1.98
12	–	–	–	1.15	1.80	1.42

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/cm^3) 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 average 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., Co (II), Pb (II), Ni (II),Cd (II), Cu (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.xD.F.)	Mean concentration of enzyme
α–Amylase	AF_1	0.745	1	1000	1000	1005 μg/cm^3
	AF_2	0.380	2	510	1010	
	AS_1	0.420	1	570	570	585 μg/cm^3
	AS_2	0.225	2	300	600	
Papain	PF_1	0.750	1	1010	1010	1025 μg/cm^3
	PF_2	0.385	2	520	1040	
	PS_1	0.365	1	490	490	585 μg/cm^3
	PS_2	0.260	2	340	680	

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.

AS_1/PS_1= Undiluted α–amylase/papain enzyme in the supernatant after adsorption.

AS_2/PS_2= 1 : 2 diluted α–amylase/papain enzyme in the supernatant after adsorption.

Table 4 : Immobilization capacity of the chelating resin

Chelating ion–exchange resin containing gallic acid as the functional group	α–Amylase			Papain		
	Adsorbed (mg)	Unadsorbed (mg)	Immobilized (mg)	Adsorbed (mg)	Unadsorbed (mg)	Immobilized (mg)
	10.05	5.85	4.20	10.25	5.85	4.40

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