Research Article

Immobilization of a Few Enzymes on Different Chelating Resins Containing Sulphur as Donor Atom(S)

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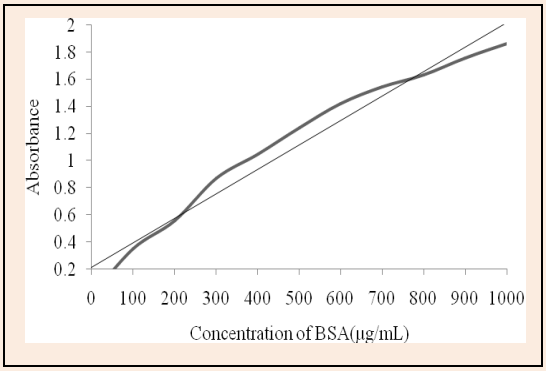
Abstract

In the present paper, five different enzymes viz., α-amylase, papain, lipase, urease and β-glucosidase were immobilized on three different chelating resins each containing, at least, a sulphur donor atom and the protein concentration was evaluated by Lowry et al. method. The chelating resins were prepared by suspension polymerization. The chelating resin (labelled R1) contains hexylthioglycolate group supported on poly-(styrene-divinylbenzene-methylmethacrylate), the chelating resin (labelled R2) contains diphenyldisulphide group supported on poly-(styrene-divinylbenzene-methylmethacrylate), and the chelating resin (labelled R3) also has diphenyldisulphide group supported on poly-(ethylacrylate-acrylonitrile-divinylbenzene). The maximum immobilization of each of the enzymes studied was observed in case of R2 having diphenyldisulphide group (with two sulphur atoms) supported on poly-(styrene-divinylbenzene-methylmethacrylate).



Keywords: Immobilization, chelating resins, α-amylase, papain, lipase, urease and β-glucosidase, Lowry et al. method

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Introduction

The term "immobilized enzymes" refers to "enzymes physically confined in a certain defined region of space with retention of their catalytic activities, and which can be used repeatedly and continuously" [1]. The immobilization technique is advantageous since it improves the stability of the biocatalyst and provides for its repeated use and the easy separation of the catalyst from reaction medium. Immobilized enzymes are widely used for a variety of applications in food, pharmaceutical, detergent, textile industries, and bioremediation etc. [2]. The enzymes are the potential catalyst works under mild temperature, pressure, pH and are used for the production of desired products without any intermediate products as contaminants. Since enzymes have various other limitations such as low stability and high sensitivity to the process conditions, these problems can be overcome by the use of immobilization techniques [3]. Enzyme immobilization frequently leads to improvement in operational stability of the enzymes.

B. M. Brena and F. Batista [1] have classified enzyme immobilization methods into irreversible enzyme immobilization and reversible enzyme immobilization methods. Irreversible enzyme immobilization includes covalent binding and entrapment. Reversible enzyme immobilization includes adsorption, ionic binding and affinity binding, and metal chelation.

A variety of inorganic solids such as alumina, silica [4], zeolites [5, 6] and mesoporous silicas [7–10] have been put to use for the immobilization of enzymes. Among all types of adsorbing materials put to use for enzyme immobilization, the ion-exchange resins are widely used. The present study deals with the immobilization study of α-amylase, papain, lipase, urease and β-glucosidase enzymes on three different chelating resins (synthesized in this

laboratory) each containing sulphur as donor atom(s). Each of the chelating resins contains either one or two sulphur atoms. The protein estimation was done using Lowry et al. method.

Experimental:

Chemicals and reagents:

Papain and urease enzymes were obtained from Loba Chemie and α-amylase, β-glucosidase and lipase enzymes from Sigma-Aldrich (India). All reagents used were of analytical grade. Double distilled water was used throughout the work.

Preparation of solutions:

(i) *Bovine Serum Albumin (BSA) standard protein solution:*

0.1 g of BSA was weighed, dissolved in double distilled water and made upto 100 mL in a volumetric flask in order to obtain stock solution containing 1000 µg/mL of BSA.

(ii) *Alkaline $CuSO_4$ solution:*

To 100 mL of 2% Na_2CO_3 in 0.1N NaOH, 2 mL of 0.5% $CuSO_4$ in 1% sodium tartarate was added to get alkaline $CuSO_4$ solution.

(iii) Folin reagent:

The commercially procured reagent was diluted in 1:2 ratio with double distilled water just before use.

Chelating resins:

Three chelating resins (as described below) were synthesized in this laboratory [11, 12 and 13] and were made use for enzyme immobilization:

R1= Hexylthioglycolate supported on poly-(styrene-divinylbenzene-methylmethacrylate),
R2= Diphenyldisulphide supported on poly-(styrene-divinylbenzene-methylmethacrylate and
R3= Diphenyldisulphide supported on poly-(ethylacrylate-acrylonitrile-divinylbenzene).

Method for immobilization of enzymes on the chelating resins:

0.5 g of each of the synthesized resin beads were washed with double distilled water. The thoroughly washed resin beads were successively equilibrated with 10 cm^3 of acetate buffer (pH 4.6) or phosphate buffer (pH 8.2) respectively. The beads were then mixed with a 10 mL aliquot of α-amylase/papain, trypsin, urease, β-glucosidase and lipase solution (1 mg/cm^3) prepared in acetate buffer (4.6) or phosphate buffer (8.2) respectively and stirred at room temperature for 12 hours.

Finally, the supernatant from each sample was collected. The amount of enzyme immobilized on each of the chelating resins was estimated from the difference between the amount of enzyme (α-amylase/papain, urease, β-glucosidase and lipase) in the enzyme buffer solution before and after immobilization. Protein estimation was done according to Lowry *et al.* method [14].

Spectrophotometric determination of protein:

Various dilutions using the standard BSA (1000 µg/mL) solution were prepared and 5 mL of alkaline $CuSO_4$ solution was added into each of them. The solutions were mixed well and were allowed to stand at room temperature for 10 minutes. Then, 0.5 ml of Folin reagent was added into each solution and the mixtures were allowed to stand for 30 minutes. Finally, the absorbance (A) of the solutions was measured at 750 nm using UV-Visible spectrophotometer (Systronics, model-118) which have been recorded in Table 1. A graph of absorbance versus protein concentration was plotted as depicted in Figure1 which was used for the determination of the concentration of protein in the unknown samples.

Table 1 Protein estimation by Lowry et al. method

Concent-ration (µg/mL)	Stock solution (mL)	Double distilled water (mL)	Total Volume (mL)	Alkaline $CuSO_4$ solution (mL)		Folin reagent (mL)		Absorbance (750 nm)
100	0.1	0.9	1.0	5.0		0.5		0.201
200	0.2	0.8	1.0	5.0		0.5		0.335
300	0.3	0.7	1.0	5.0		0.5		0.581
400	0.4	0.6	1.0	5.0		0.5		0.892
500	0.5	0.5	1.0	5.0		0.5		1.051
600	0.6	0.4	1.0	5.0		0.5		1.340
700	0.7	0.3	1.0	5.0		0.5		1.533
800	0.8	0.2	1.0	5.0		0.5		1.792
900	0.9	0.1	1.0	5.0		0.5		2.001
1000	---	---	1.0	5.0		0.5		3.412
Blank	---	---	1.0	5.0		0.5		
Unknown1 (undiluted)	---	---	1.0	5.0		0.5		As per the Tables 2, 3 and 4
Unknown 2 (diluted in 1:2 ratio)			1.0	5.0		0.5		

Incubate at room temperature for 10 minutes | **Incubate at room temperature for 30 minutes**

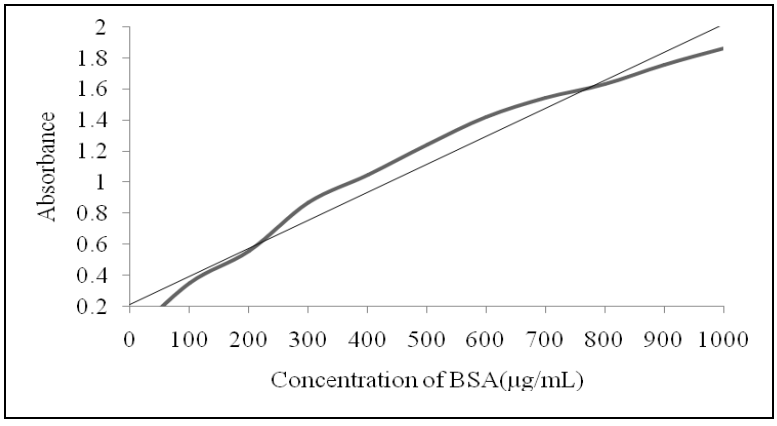

Figure 1 Protein estimation by Folin-Lowry method

Results and Discussion:

The determination of protein contents of α-amylase, papain, urease, β-glucosidase and lipase were carried out before and after immobilization on chelating resins (R1, R2 and R3) as reported in Tables 2 – 4, using Lowry's *et al.* method.

Table 2 Protein estimation by Lowry's method in respect of R1

Enzyme	Unknown enzyme sample	Absor-bance (750 nm)	Dilution Factor	Protein concentration (V) from graph	Concentration of unknown (V x D.F.)	Mean concentration of enzyme	% Adsorption
α-Amylase	AF1	0.259	1	180	180	210	52.38
	AF2	0.188	2	120	240		
	AS1	0.042	1	80	80	100	
	AS2	0.010	2	60	120		
Papain	PF1	0.286	1	140	140	110	68.18
	PF2	0.068	2	40	80		
	PS1	0.098	1	50	50	35	
	PS2	0.029	2	10	20		
Urease	UF1	0.436	1	210	210	165	51.51
	UF2	0.122	2	60	120		
	US1	0.164	1	90	90	85	
	US2	0.075	2	40	80		
β-Glucosidase	GF1	0.880	1	410	410	405	50.61
	GF2	0.415	2	200	400		
	GS1	0.464	1	220	220	210	
	GS2	0.219	2	100	200		
Lipase	LF1	0.426	1	210	210	195	69.23
	LF2	0.198	2	90	180		
	LS1	0.126	1	60	60	70	
	LS2	0.064	2	40	80		

Table 3 Protein estimation by Lowry's method in respect of R2

Enzyme	Unknown enzyme sample	Absorbance (750 nm)	Dilution Factor	Protein concentration (V) from graph	Concentration of unknown (V x D.F.)	Mean concentration of enzyme	% Adsorption
α-Amylase	AF1	0.259	1	180	180	210	54.76
	AF2	0.188	2	120	240		
	AS1	0.039	1	70	70	105	
	AS2	0.041	2	70	140		
Papain	PF1	0.286	1	140	140	110	72.72
	PF2	0.188	2	40	80		
	PS1	0.085	1	40	40	30	
	PS2	0.021	2	10	20		
Urease	UF1	0.436	1	210	210	165	54.54
	UF2	0.122	2	60	120		
	US1	0.137	1	70	70	75	
	US2	0.061	2	40	80		
β-Glucosidase	GF1	0.880	1	410	410	405	59.25
	GF2	0.415	2	200	400		

	GS1	0.353	1	170	170	165	
	GS2	0.164	2	80	160		
Lipase	LF1	0.426	1	210	210	195	71.79
	LF2	0.198	2	90	180		
	LS1	0.101	1	50	50	55	
	LS2	0.048	2	30	60		

Table 4 Protein estimation by Lowry's method in respect of R3

Enzyme	Unknown enzyme sample	Absorbance (750 nm)	Dilution Factor	Protein concentration (V) from graph	Concentration of unknown (V x D.F.)	Mean concentration of enzyme	% Adsorption
α-Amylase	AF1	0.259	1	180	180	210	50.00
	AF2	0.188	2	120	240		
	AS1	0.039	1	70	70	95	
	AS2	0.012	2	60	120		
Papain	PF1	0.286	1	140	140	110	63.63
	PF2	0.068	2	40	80		
	PS1	0.086	1	40	40	40	
	PS2	0.031	2	20	40		
Urease	UF1	0.436	1	210	210	165	48.48
	UF2	0.122	2	60	120		
	US1	0.148	1	80	80	80	
	US2	0.069	2	40	80		
β-Glucosidase	GF1	0.880	1	410	410	405	48.94
	GF2	0.415	2	200	400		
	GS1	0.416	1	200	200	200	
	GS2	0.210	2	100	200		
Lipase	LF1	0.426	1	210	210	195	64.10
	LF2	0.198	2	90	180		
	LS1	0.119	1	60	60	60	
	LS2	0.053	2	30	60		

Where,
AF1/PF1/UF1/GF1/LF1 = Undiluted α-amylase/ papain/ urease/β-glucosidase/ lipase enzymes in the soluition before adsorption,
AF2/PF2/UF2/GF2/LF2 = 1:2 diluted α-amylase/ papain/ urease/β-glucosidase/ lipase enzymes in the soluition before adsorption,
AS1/PS1/US1/GS1/LS1 = Undiluted α-amylase/ papain/ urease/β-glucosidase/ lipase enzymes in the soluition after adsorption, &
AS2/PS2/US2/GS2/LS2 = 1:2 diluted α-amylase/ papain/ urease/β-glucosidase/ lipase enzymes in the soluition before adsorption.

Figure 2 depicts the results pertaining to adsorption of various enzymes using three chelating resins (R1, R2 and R3). In general, it is clearly noticeable that the % adsorption for papain and lipase is much higher than for β-glucosidase, α-amylase and urease in cases of all the three resins (R1, R2 and R3). It was also found that among all the enzymes, the adsorption of lipase was maximum i.e., 69.23% and 64.10% in case of resins R1 and R3 respectively. Similarly, for R2, the adsorption of papain was found to be maximum i.e., 72.72%. Among all five enzymes viz., α-amylase,

papain, urease, β-glucosidase and lipase, the adsorption of urease was found to be minimum i.e., 54.54% by R2 in comparison to R1 and R3. The order of % adsorption of enzymes with respect to R1 and R3 was found to be:
Lipase > papain > α-amylase > urease > β-glucosidase

And the order of % adsorption of enzymes with respect to R2 worked out to be:
Papain > lipase > β-glucosidase > α-amylase > urease

Thus, it emerges that the performance of R2 was found to be somewhat better as compared to both R1 and R3 in respect of immobilization of the commercial enzymes studied.

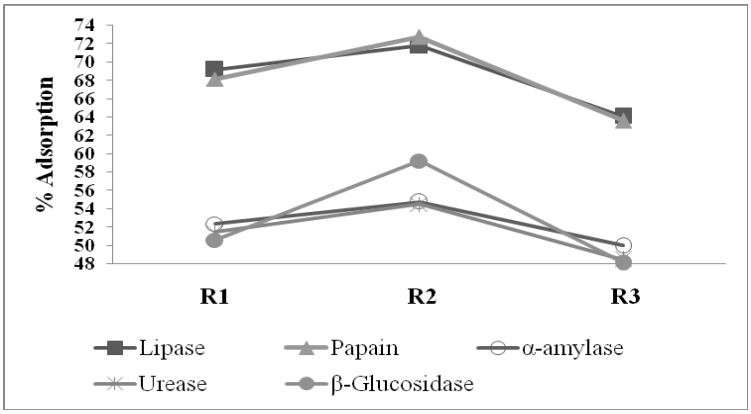

Figure 2 Enzyme immobilization by R1, R2 and R3

Conclusions

Immobilization of enzymes viz., α-amylase, papain, urease, β-glucosidase and lipase was studied with three different chelating resins (R1, R2 and R3) synthesized in this laboratory, each containing sulphur as donor atom(s). The protein estimation before and after immobilization was done using Lowry *et al*. method. The following pertinent conclusions have been drawn:

- The % adsorption for papain and lipase is much higher than for β-glucosidase, α-amylase and urease in cases of all the three resins (R1, R2, and R3).
- The order of adsorption of enzymes with respect to R1 and R3 was found to be:
  Lipase > papain > α-amylase > urease > β-glucosidase
- and, the order of adsorption of enzymes with respect to R2 worked out to be:
  Papain > lipase > β-glucosidase > α-amylase > urease

- Among three chelating resins, R2 containing diphenyldisulphide group (having two sulphur atoms) supported on poly-(styrene-divinylbenzene-methylmethacrylate) showed the most promising potential with regard to the five enzymes studied for their immobilization.

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