Immobilization of Catalase on a Novel Polymer Support, Crosslinked Polystyrene Ethylene Glycol Acrylate Resin: Role of the Macromolecular Matrix on Enzyme Activity

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ABSTRACT: Crosslinked polystyrene ethylene glycol acrylate resin (CLPSER) was developed for the immobilization of the enzyme catalase by the introduction of a crosslinker, *O*,*O*′-bis(2-acrylamidopropyl) poly(ethylene glycol)$_{1900}$, to styrene. The crosslinker was prepared by the treatment of acryloyl chloride with *O*,*O*′-bis(2-aminopropyl) poly(ethylene glycol)$_{1900}$ in the presence of diisopropylethylamine. The resin was characterized with IR and ^{13}C-NMR spectroscopy. The catalytic activity of the catalase-immobilized system of CLPSER was compared with divinylbenzene-crosslinked polystyrene, ethylene glycol dimethacrylate crosslinked polystyrene, and 1,4-butanediol dimethacrylate crosslinked polystyrene systems. Crosslink levels of 2, 8, and 20 mol % were evaluated. Among these crosslinked systems, the 2 mol % system was found to be most suitable to support catalytic activity. When a long flexible hydrophilic poly(ethylene glycol) crosslink, introduced between the polystyrene (PS) backbone and functional groups was used for immobilization, the extent of coupling and enzyme activity increased. Depending on the nature of the support, the catalytic activity of the system varied. The hydrophilic CLPSER support was most efficient for immobilization compared to the other PS-based supports. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 8–19, 2005

Key words: catalysts; crosslinking; enzymes; supports

INTRODUCTION

The chemical immobilization of a protein on a synthetic polymer support and its application in the food, chemical, pharmaceutical, and agricultural industries opened a new era in biotechnology. Considerable attention has been focused on the preparation of immobilized enzymes in the past decades, and different kinds of support matrices for enzyme immobilization have been developed.[1–4] Catalases are an abundant enzyme in nature that decompose hydrogen peroxide to water and molecular oxygen.[5] The enzyme catalase is widely used in industry for the degradation of hydrogen peroxide after textile bleaching to prevent problems in subsequent dyeing[6] and for the elimination of the hydrogen peroxide added to sterilize milk in the dairy industry.[7] Polymer-supported catalases are used to remove hydrogen peroxide from reaction mixtures.[8] Catalase from bovine liver is a tetrameric enzyme with identical subunits, each containing a haem prosthetic group, and it has been used in several interesting reactions.[9]

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The immobilization of enzymes on insoluble polymer supports facilitates their recovery from a reaction mixture, but it can also slow down enzyme deactivation and make possible the exploitation of local concentrations of active sites.[10] Most immobilization strategies are designed by the covalent linking of a protein to a water-insoluble polymer matrix with the enzyme function performed in aqueous media or water–organic media.[11] The covalent binding method, based on the covalent attachment of protein to water-insoluble matrices, has been the most widespread and one of the most thoroughly investigated approaches to enzyme immobilization.[12,13] Synthetic carriers are the most common supports available for protein immobilization.[14–19] The physicochemical properties of polymeric supports affect the properties of immobilized enzymes and lead to easy separation and recyclability.

The physicochemical properties, chemical composition, morphology, hydrophilic–hydrophobic balance, distribution of the reactive group, pore size, and macroenvironment of the attached functional group in the polymer matrix affect the overall properties of immobilized enzymes.[20–22] The effect of factors such as spacer grouping, crosslink density, macroenvironment of the attached species, hydrophilic–hydrophobic balance of the system, and pore size have significant influence in polymer-supported reactions.[23] Among the various available methods, covalent binding is based on the covalent attachment of enzymes to

three-dimensional polymeric matrices, which reflects the physical properties of the support and the chemical nature of the attached functional groups in the reaction medium. Immobilization involves coupling between the functional groups present in the polymer support and the functional groups on the enzyme. Enzymes, as well as other proteins, maintain their structural conformation through intramolecular interactions. Enzymes in aqueous solution have both hydrophilic domains in contact with water and hydrophobic domains folded inside the molecules. The introduction of an organic solvent reduces the polarity of the medium surrounding the enzyme molecule; hence, hydrophobic domains are liable to disperse, resulting in the unfolding of the molecule. Furthermore, this permits the enzymes to be used repeatedly or continuously.

Our recent work has focused on the synthesis, immobilization, and catalytic activity comparison of supports with high- or low-flexible crosslinked polystyrene (PS) resins. Divinylbenzene-crosslinked polystyrene (PS–DVB), ethylene glycol dimethacrylate crosslinked polystyrene (PS–EGDMA),[24] 1,4-butanediol dimethacrylate crosslinked polystyrene (PS–BDDMA),[25] and the newly synthesized crosslinked polystyrene ethylene glycol acrylate resin (CLPSER)[26] system (2–20 mol %) were used for this investigation. The crosslinked PS resins were functionalized by chloromethylation, followed by amination and incorporation of the enzyme catalase to the support through carboxyl groups with tetramethyluronium hexafluorophosphate (HBTU) coupling, and proteins were estimated by Bradford's method. The kinetics of the catalase-immobilized ethylene glycol dimethacrylate (EGDMA), PS–BDDMA, and CLPSER systems were followed by the decomposition reaction of hydrogen peroxide. Catalytic studies were conducted at different times, temperatures, and pH values.

EXPERIMENTAL

Materials and methods

The enzyme catalase used was a commercially available sample (Sigma). Styrene, sorbitan monolaurate, and *N*-methyl imidazole were purchased from Aldrich (Sigma-Aldrich, St. Louis, MO). 1-Hydroxybenzotriazole (HOBt), 2-(1H-benzotriazol-1-yl)-1,1,3,3-hexafluorophosphate (HBTU), and diisopropylethylamine (DIEA) were purchased from Sigma. *O*,*O*′-bis(2-aminopropyl) poly(ethylene glycol)$_{1900}$ [$(NH_2)_2$PEG], *N*-(3-bromopropyl) phthalimide, and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Fluka (Sigma-Aldrich). All other chemicals and solvents high performance liquid chromatography (HPLC) grade were purchased from Merck and BDH (India). Fourier transform infrared spectra were recorded on a Bomem MB-series spectrometer (Canada) with KBr pellets. The ^{13}C-NMR measurements were recorded on a Varian Unity 400 instrument operating at 100 MHz.

Synthesis of *O*,*O*′-bis(2-acrylamidopropyl) poly(ethylene glycol)$_{1900}$ (Acr_2PEG)

Acryloyl chloride (1.6 mL, 2 equiv) was added dropwise to a mixture of $(NH_2)_2$PEG (20 g) and DIEA (3.1 mL) in dichloromethane (DCM) (25 mL), which was stirred at 0°C under a nitrogen atmosphere. Stirring was continued at 0°C for 1 more hour after the addition of acryloyl chloride. The salt that formed was filtered and washed with DCM (5 × 50 mL). The combined filtrate and washings were concentrated to dryness. Hydroquinone-stabilized ether (50 mL) was added to the residue, and the product was allowed to crystallize for 5 h at 0°C under mechanical stirring. The crude product was filtered off, washed with stabilized ether (5 × 50 mL), and dried (15 g, 75%). The crosslinker was dissolved in DCM, the solution was filtered, the filtrate was concentrated, and Acr_2PEG was again recrystallized from peroxide-free unstabilized ether. Acr_2PEG was obtained as a white powder, dried *in vacuo*, and stored at −20°C (yield = 15 g).

Synthesis of Acr_2PEG-crosslinked PS (CLPSER)

We removed inhibitors from styrene by washing it with 1% NaOH solution, followed by distilled water (2 × 50 mL), and drying it over anhydrous sodium sulfate. The polymerization reaction was carried out in a 1-L, double-welled cylindrical flask with a three-bladed propeller stirrer, a fixed counterblade, an inert gas inlet tube, a thermometer, and a condenser. The copolymer was synthesized in various ratios of crosslinker: 2, 8, and 20%. Heptane (120 mL) and CCl_4 (90 mL) were taken in the polymerization vessel and stirred at 70°C at a speed of 1500 rpm. Acr_2PEG (2.635 g) was dissolved in DMA (30 mL), thoroughly mixed with a required amount of styrene, benzoyl peroxide (0.2 g), ammonium peroxidisulfate (0.2 g), and sorbitan monolaurate [0.25 g in 1.25 mL of dimethylformamide (DMF)] and added to the reaction vessel. The reaction mixture was stirred at 1500 rpm, and the temperature was increased to 80°C. After 2 min, TEMED (1 mL) was added, and the polymerization was allowed to proceed under a nitrogen atmosphere for 3 h. The obtained copolymer was washed thoroughly with water (5 × 50 mL), ethanol (5 × 50 mL), benzene (5 × 50 mL), toluene (5 × 50 mL), and methanol (5 × 50 mL) and then Soxhleted with toluene, methanol, and acetone. The polymer was dried *in vacuo* over P_2O_5 to yield the dry polymer.

Chloromethyl resin

PS–DVB, PS–EGDMA and PS–BDDMA (2–20 mol %) and CLPSER (0.2 g) were made to swell in excess DCM in a round-bottom flask. After 1 h, the excess DCM was removed; chloromethyl methyl ether (1.2 mL) and 1*M* anhydrous $ZnCl_2$ in tetrahydrofuran (THF; 0.02 mL) were added, and the reaction mixture was heated at 50°C. After 3 h, the resin was filtered, washed with THF (3 × 50 mL), THF/H_2O (1:1, 3 × 50 mL), THF/3*N* HCl (1:1, 3 × 50 mL), THF (3 × 50 mL), methanol (3 × 50 mL), and ether (3 × 50 mL) and dried *in vacuo* over anhydrous P_2O_5. The chlorine capacity of the resin was 0.12 mmol of Cl/g as estimated by Volhardt's method.[27]

IR (KBr, cm^{-1}): 1702.12, 1440.28, 1418.64, 1242.34, 756, 700. ^{13}C-NMR (ppm): 47.138, 135.1.

Aminomethyl CLPSER resin

PS–DVB, PS–EGDMA, and PS–BDDMA 2–20 mol % and CLPSER (1 g) were made to swell in DMF for 1 h. The excess DMF was removed; potassium phthalimide (0.22 g, 1.2 mmol) dissolved in DMF (1 mL) was added to the resin, and the mixture was stirred at 120°C for 12 h. The resin was filtered and washed with DMF (5 × 50 mL), DCM (3 × 50 mL), THF (3 × 50 mL), and ether (3 × 50 mL). It was then dried *in vacuo*. The dried resin was swollen in distilled ethanol (20 mL) for 1 h; hydrazine hydrate (0.02 mL) was added, and the reaction mixture was refluxed at 80°C for 8 h. The resin was collected by filtration, washed with hot ethanol (5 × 50 mL), methanol (3 × 50 mL), and ether (3 × 50 mL), and dried *in vacuo*. The amino capacity of the resin was 0.11 mmol/g, as determined by the picric acid method.[28]

IR (KBr, cm^{-1}): 3100, 1702, 1541.18, 1520, 1480, 756, 700.

Swelling behavior

Solvent inhibitions of various resins were determined by a centrifuge method. A sample of resin of all of the crosslinked systems (1 g) was placed in a glass-sintered stick (G3), and the latter was immersed in the solvent for 1 h. The excess solvent was removed by placement of the stick in a centrifuge tube, which was centrifuged for 15 min at 2000 rpm. The stick and the contents were then weighed. A blank was also carried out with an empty stick. The data were expressed as the volume of the solvent absorbed by unit weight of dry resin (mL/g). In an alternative method, the resin (1 g) was placed in a syringe fitted with a sintered Teflon filter, and the solvent was poured from the top of the syringe. A constant suction, applied at the syringe outlet, generated a solvent flow. The suction was regulated to obtain a flow rate of 1 mL/min. The solvent was allowed to flow through the resin for 30 min. The syringe outlet was closed, and the resin was suspended in the solvent for 1 h. The swollen resin was compressed with the piston of the syringe, and the pressure was slowly released. The volume of the resin at this point was noted and related to the sample weight to obtain the resin swelling abilities. The experiment was repeated to ensure reproducible values.

Preparation of the carboxyl functional resin

Aminomethyl resins of 2–20 mol % PS–DVB, PS–EGDMA, PS–BDDMA, and CLPSER were swelled in DMF for 1 h. Succinic acid (2.3 mmol, 0.55 g), HOBt (2.3 mmol, 0.31 g), HBTU (2.3 mmol, 0.87 g), and DIEA (0.67 mmol, 0.086 g) mixtures in DMF (3 mL) were added to the swollen resin, and the mixtures were shaken occasionally for 1 h. The resin was filtered, washed with DMF (3 × 10 mL) and ether (3 × 10 mL), and dried *in vacuo*. The resin tested negative to the sensitive Kaiser test. Carboxyl capacities of the resins were determined.

Coupling of the carboxyl functional resins with the enzyme catalase

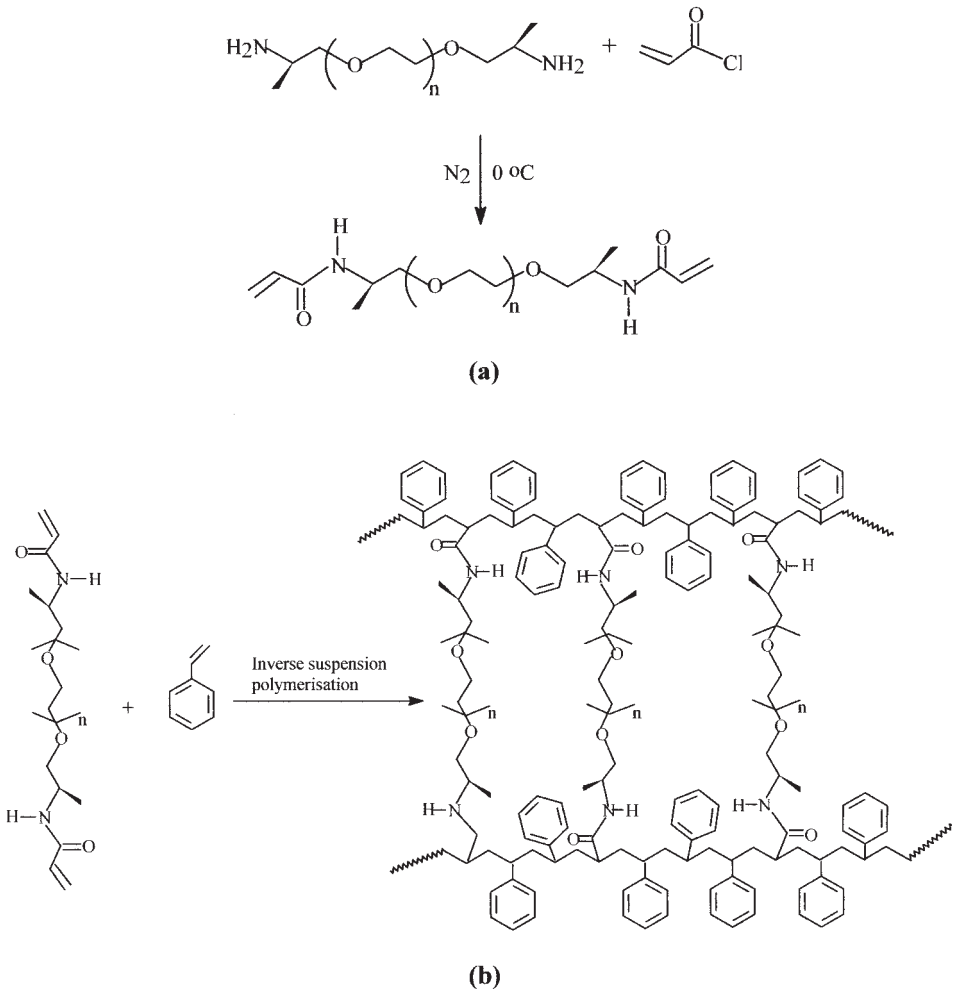
The enzyme catalase (20 mg) was dissolved in tris buffer (pH 7.2, 30 mL), and the clear solution was added to the carboxylate resin; coupled with HOBt (2.3 mmol, 0.31 g), HBTU (2.3 mmol, 0.87 g), and DIEA (0.67 mmol, 0.086 g) as mixtures in DMF; and then shaken for 1 h. The resin was filtered and washed with buffer until the filtrate was free from any unbound enzyme, as tested by the ninhydrin color reaction. The resin was dried *in vacuo*. The protein content of the bound support was calculated by Bradford's assay.

Catalytic activity study of the catalase-immobilized polymeric systems

A weighed quantity of the catalyst (10 mg) was stirred with 5 mL of 0.2*N* H_2O_2 in a thermostated reaction vessel at different temperatures. After a definite time, the insoluble polymer was collected by filtration, and the concentration of hydrogen peroxide (filtrate) was estimated by titration against a standardized potassium permanganate solution. The effects of conditions such as time, temperature, and pH on the catalytic decomposition of the hydrogen peroxide were also followed.

RESULTS AND DISCUSSION

The chemical nature of the monomers, the extent of crosslinking, and different experimental conditions of polymerization are generally the properties that govern the characteristics of polymer-supported species.



Scheme 1 Synthesis of (a) Acr_2PEG and (b) the CLPSER support.

This study explained the immobilization of the enzyme catalase on different PS crosslinks and their macromolecular activities. Poly(ethylene glycol) (PEG)-based polymeric supports are widely used in biochemical reactions because of their positive response to polar–nonpolar media. This investigation explained the immobilization method and its catalytic activity comparison of enzyme catalase on different crosslinked PS systems: CLPSER, PS–DVB, PS–EGDMA, and PS–BDDMA. Because the PS–DVB system was highly hydrophobic, one way to develop better results was to reduce the hydrophobicity through the introduction of flexible crosslinks. From this point of view, we studied the effect of the crosslinks on catalytic activity of the immobilized systems using hydrophobic DVB, semiflexible EGDMA, BDDMA, and more flexible polyethylene glycol units as crosslinkers to polystyrene. PS–DVB, PS–EGDMA, and PS–BDDMA (2, 8, and 20 mol %) were prepared by aqueous free-radical suspension polymerization at 80°C. The most flexible CLPSERs, at 2.8 and 20 mol %, were prepared by inverse suspension polymerization with the diacrylate of $(NH_2)_2PEG$ as crosslinker. The preparation of CLPSER involved two steps: the synthesis of Acr_2PEG, as shown in Scheme 1(a), and the synthesis of the CLPSER support, as shown in Scheme 1(b). Different crosslinks (2–20 mol %) prepared by inverse suspension polymerization are shown in Table I.

The crosslinks were synthesized by the treatment of $(NH_2)_2PEG$ with acryloyl chloride (Scheme 1). The polymer was synthesized initially by the bulk copolymerization of styrene and Acr_2PEG at various crosslinking degrees with a mixture of ammoni-

TABLE I
Preparation of CLPSER by Inverse Suspension Polymerization

CLPSER (mol %)	Amount of styrene (mL)	Amount of crosslinker (mL)	Yield (%)
2	13.5	0.42	80
8	12.7	1.69	78
20	11.0	4.22	75

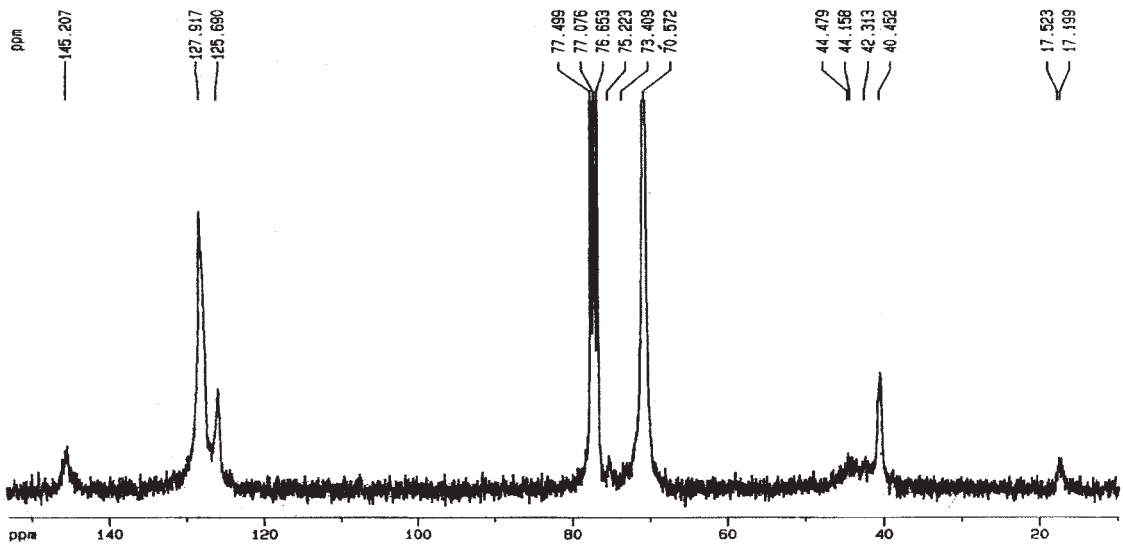


Figure 1 Gel-phase ^{13}C-NMR spectrum of CLPSER in $CDCl_3$.

umperoxodisulfate and benzoyl peroxide as radical initiators. The CLPSER resins were characterized by IR and ^{13}C-NMR spectroscopy. The IR spectrum, apart from the usual PS peaks, showed sharp peaks at 1701.48 cm,$^{-1}$ corresponding to amide-I; 1539.78 cm^{-1}, corresponding to amide II; and 1104.05 cm^{-1}, corresponding to the crosslinker. ^{13}C-NMR of the polymer gel in $CDCl_3$ showed an intense peak at 127.917 ppm, corresponding to the aromatic PS carbon; a sharp peak at 70.572 ppm, corresponding to C—O—C carbon of the crosslinker; a peak at 40.452 ppm, corresponding to the backbone methylene carbon of the polymer; and a peak at 145.207 ppm, corresponding to C-3 of the styrene (Fig. 1).

Functionalization of the polymer supports

The chloromethyl group was incorporated in the benzene ring of the PS–DVB, PS–EGDMA, PS–BDDMA, and CLPSER resins by chloromethylation with chloromethyl methylether in the presence of anhydrous zinc chloride as a catalyst. The chlorine capacity was determined by Volhardt's method. In the CLPSER system, the IR (KBr) spectrum showed additional bands at 1418.64 cm^{-1} for C—Cl stretching and 1242.34 cm^{-1} for H—C—Cl vibrations.^{13}C-NMR gave additional peaks at 47.138 ppm for the methylene carbon of the chloromethyl group and a small peak at 135.1 ppm for C_6 of the PS ring. The chloromethyl resin was converted to aminomethyl resin by Gabriel's phthalimide reaction. The amino capacity measurement showed that the conversion was quantitative. The amino capacity of the various crosslinked PS–DVB, PS–EGDMA, and PS–BDDMA systems is given in Table II.

In all of the crosslinked systems, as the crosslinking increased, the amino capacity of the system decreased.

Swelling characteristics of the resins

The swelling of the crosslinked polymer is a measure of its solvation in a given solvent. Solvation is one of the necessary conditions for chemical reactions in a gel phase, a useful parameter to explain the capability of the resin as a support in different chemical reactions. For maximum accessibility of reactive functional groups in the resins to reactants and solvents, the polymer matrix should swell extensively in the solvent medium. Swelling measurements of the resin were carried out in a cylinder by the measurement of its dry volume and its volume in excess solvent. An increase in crosslinking and rigidity of the resin resulted in decreases in the swelling and efficiency of the resin. The swelling behavior of the 2–20 mol % DVB, EGDMA, BDDMA, and CLPSER resins was followed for this investigation. The swelling behavior of the resin showed that as the crosslinking increased from 2 to 20%, the swelling also decreased, except for CLPSER. The studies were conducted in polar–nonpolar solvents, as shown in Table III. In polar solvents,

TABLE II
Amino Capacities of the PS–DVB, PS–EGDMA, PS–BDDMA, and CLPSER Systems

Crosslink density	PS–DVB (mmol/g)	PS–EGDMA (mmol/g)	PS–BDDMA (mmol/g)	CLPSER (mmol/g)
2	1.7	0.9	1.1	0.75
8	1.2	0.7	0.6	0.3
20	0.7	0.3	0.2	0.11

TABLE III
Swelling Studies of Different Crosslinked Systems [Volume of Solvent (mL)]

Polymer system	Crosslinks	DCM	Water	DMF	Benzene	Toluene	Dioxane	Water–DMF	Water–dioxane
CLPSER	2	9.3	6.5	7.7	7.2	6.1	5.9	7	6.2
	8	9.6	7.4	8.1	7	6	6.7	7.9	7
	20	10.4	9.8	9.3	6.4	5.7	7.8	9.5	8.8
PS–DVB	2	5.5	1	3.4	6.8	7.2	0.5	1.4	0.5
	8	6.0	0.2	3.2	7.9	8.1	0.4	1	0.3
	20	6.8	0	2.7	8.3	8.5	0.4	0.8	0.2
PS–EGDMA	2	7	1.4	3.6	4.4	4.7	1.2	2.0	1.3
	8	6.4	2.1	3.9	3.9	3.9	2	2.6	1.9
	20	6	2.9	4.2	3.4	3.1	2.8	3.1	2.7
PS–BDDMA	2	7.8	1.6	6.3	4.9	5.1	1.1	3.8	0.7
	8	7.6	2.4	7	4.3	4.5	1.7	4.7	1.8
	20	7	3.2	7.5	3.6	3.8	2.1	5.1	2.3

the rigid PS–DVB, PS–EGDMA, and PS–BDDMA systems exhibited low swelling, which increased as the polarity decreased. However, the CLPSER system exhibited high swelling in the polar medium and in the nonpolar medium. In the CLPSER system, at 20% crosslinking, the resin had high swelling in polar and nonpolar media due to its flexibility and hydrophilicity. The high degree of swelling may have resulted in complete stretching of the poly(ethylene oxide) crosslinking present in the polymer because most of the reactive sites in the polymers were located in the interior part of the resin; this may have enhanced the range of chemistry that could be performed on the resin bound functional groups.

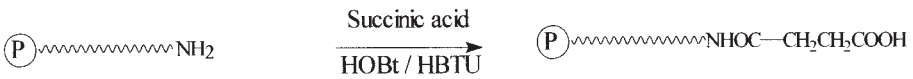
Catalase immobilization on various crosslinked polymeric supports

Carboxyl group is introduced to amino-functional of PS–DVB, PS–EGDMA, PS–BDDMA, and CLPSER resins using succinic acid and coupling reagent HOBt/HBTU/DIEA. The resin tested negative to the Kaiser test. The carboxyl functionalization of the amino polymer is represented in Scheme 2. The carboxy-functional PS–DVB, PS–EGDMA, PS–BDDMA, and CLPSER were treated with the enzyme catalase with tris buffer (pH 7.2) with HOBt/HBTU coupling, as shown in Scheme 3. After the complete reaction of immobilization, the resin was washed several times with free tris buffer to remove unbound enzyme. The filtrate, together with washings, were collected, and the enzyme concentration in it was measured with Bradford reagent at 590 nm. The amount of catalase bound on different polymeric supports was in the range 8–63 mg/g of polymer.

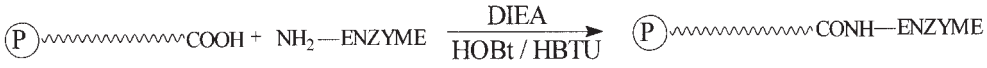
Macromolecular characteristics of the catalytic activity of the immobilized catalase enzyme

Effect of the nature of support

This study explained the catalytic activity, immobilization yield, and nature of binding of the enzyme catalase on the PS–DVB, PS–EGDMA, PS–BDDMA, and CLPSER systems. The nature of the support had a significant role in catalytic activity; it was found to vary by the changing of the hydrophilic/hydrophobic nature of the solid support. This study explained the catalytic activity of various 2 mol % crosslinked systems. The catalytic activity decreased in the order CLPSER > PS–BDDMA > PS–EGDMA > PS–DVB, as shown in Figure 2. In the PS system, as the crosslinking agent was changed from divinylbenzene (DVB), ethylene glycol dimethacrylate, BDDMA, and PEG, a considerable difference in the catalytic activity, immobilization yield, and a variation in activity were also observed. In the PS–DVB system, the catalytic activity and immobilization yield were very low compared



Scheme 2 Covalent attachment of the enzyme catalase on CLPSER.



Scheme 3 Covalent attachment of the enzyme catalase on CLPSER.

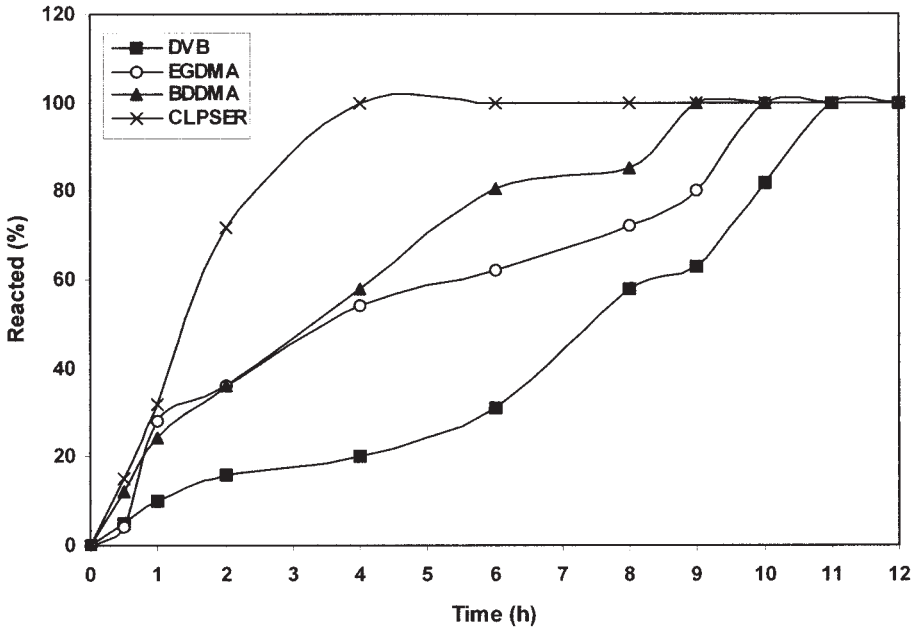


Figure 2 Catalytic activity of the PS–DVB, PS–EGDMA, PS–BDDMA, and CLPSER systems.

with other systems. For the 2% PS–DVB system, the enzyme content was 14 mg/g of polymer. However, for CLPSER, the enzyme content was 63 mg/g of polymer. However, for the 2% PS–EGDMA and PS–BDDMA system, the binding of the enzyme was 24 and 30 mg/g, respectively, as shown in Table IV. The results specifically indicated that the flexible crosslinking agent CLPSER had a vital role in the catalytic activity, enzyme binding, and yield. The long flexible hydrophilic PEG unit acted as spacer grouping between the polymer matrices, reducing the steric effect and increasing the hydrophilicity of the CLPSER, making it more compatible with the enzyme. The high swelling characteristics of CLPSER increased the availability of immobilized enzyme to the reaction medium. We also believe that the PEG spacer in CLPSER helped to overcome the physicochemical incompatibility of the resin with enzyme binding, which has been observed as one of the major problems associated in the PS–DVB resin. The highly hydrophobic PS–DVB system that showed only a minimum increase in swelling compared to other crosslinked systems resulted least distortion of the enzyme leading to reduced catalytic activity. However, in the CLPSER system, due to the flexible crosslinker PEG, the enzyme was easily distorted to the reaction medium, which led to high activity. For 2% PS–EGDMA and PS–BDDMA, as the hydrophilicity of the crosslinker increased from EGDMA to BDDMA, the catalytic activity also increased. These results show that the hydrophilic supports were more efficient in bringing about immobilization reactions, and they had more affinity toward the substrates.

TABLE IV
Effect of the Nature of Supports on Enzyme Binding in the Different Polymeric Systems

Crosslink density	PS–DVB (mg/g)	PS–EGDMA (mg/g)	PS–BDDMA (mg/g)	CLPSER (mg/g)
2	14	24	30	63
8	12	20	26	48
20	8	16	18	30

Effect of crosslinking

The extent of crosslinking had a significant role in catalytic activity. This article discusses catalase-immobilized PS–DVB, PS–EGDMA, PS–BDDMA, and CLPSER systems with 2, 8, and 20% crosslink densities. In all of the PS-crosslinked systems, we observed that as the crosslink density increased, the uptake of enzyme and the catalytic activity decreased; the catalytic activity decreased in the order: $2 > 8 > 20$ mol %. This was due to the rigid nature of the polymer matrix at its high crosslink densities. As the crosslink density increased, the rigidity of the system increased, and this caused a decrease in the availability of active sites in the polymer matrix. However, in the CLPSER system, as the crosslinking percentage of PEG units increased, the flexibility of the system increased, causing high swelling, but due to steric effects, the high crosslink density showed the least reactivity. Because of its high hydrophilic character, it exposed the catalytic site very easily to the reaction medium compared with the other systems. This was the reason for its high enzyme binding and catalytic activity, as shown in Figure 3.

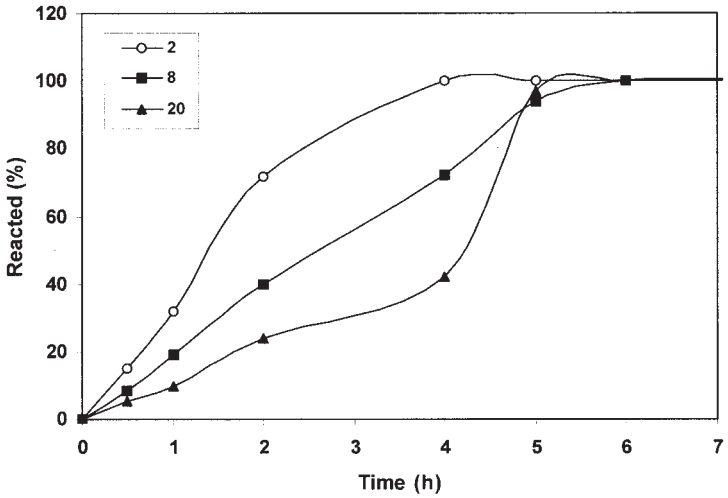


Figure 3 Catalytic activity of the 2–20 mol % CLPSER system.

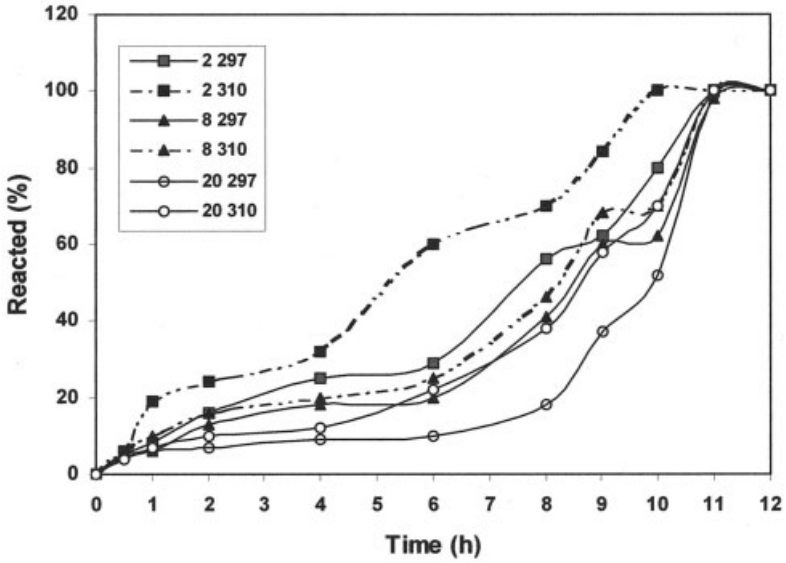


Figure 4 Catalytic activity of the PS–DVB system at different temperatures.

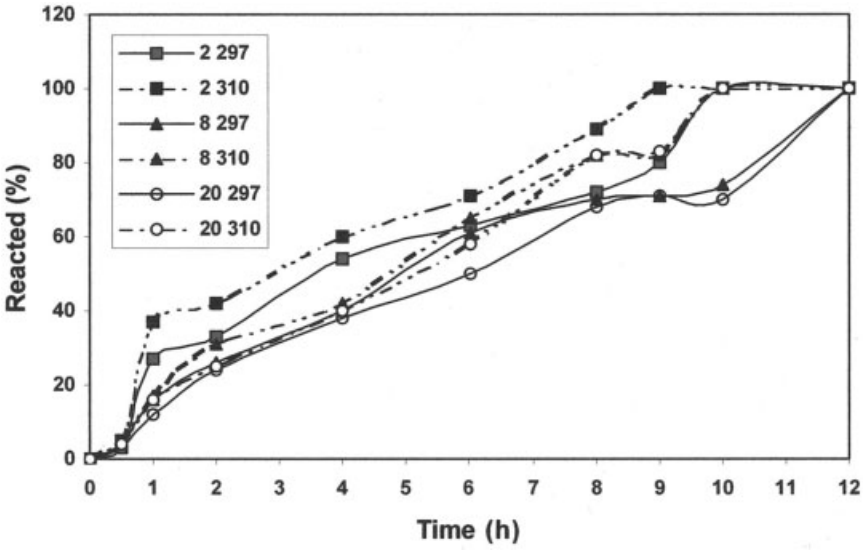


Figure 5 Catalytic activity of the PS–EGDMA system at different temperatures.

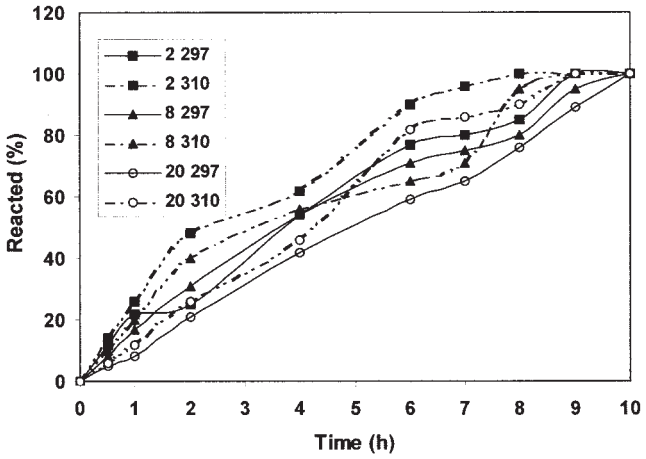


Figure 6 Catalytic activity of the PS–BDDMA system at different temperatures.

Effect of time and temperature

The catalytic activity of PS systems with different crosslinks was studied for this investigation with hydrogen peroxide as the reaction medium. The decomposition reaction of hydrogen peroxide was used as the model reaction to explain the catalytic activity of the immobilized enzyme. A definite amount of enzyme-bound resin was added to the reaction substrate, and the evolved oxygen was found volumetrically with permanganometry. PS–DVB, PS–EGDMA, PS–BDDMA, and CLPSER (2, 8, and 20 mol %) resins were used for this study. These investigations were followed at 35°C (308 K) and 24°C (297 K). Catalytic activity indicated that a more flexible resin of a rich hydrophilic nature had good catalytic activity compared with PS–DVB like rigid crosslinked systems. The PS–DVB system at two different temperatures is shown in Figure 4. As the temperature increased, the reactivity of the system also increased. Because of the easy accessibility of the catalytic site in the low crosslinked system, the 2 mol % system was more reactive compared to the other systems. In the DVB-crosslinked system, the reactivity was less because of its low swelling in polar solvents; as the crosslinking increased, the rigidity in the matrix increased. However, in 2–20 mol % EGDMA and BDDMA, due to the presence of semiflexible crosslinkers, the entire system became partially hydrophilic, and this caused increase in catalytic activity. As the temperature increased, the catalytic activity also increased, as shown in Figures 5 and 6. When these two crosslinked systems were compared, the BDDMA-crosslinked system exhibited good activity compared with EGDMA due to its variation in hydrophilicity. In BDDMA, EGDMA, and PEG crosslinked systems, as crosslinking density increases, the catalytic activity decreases of steric reasons. The flexibility of the PEG crosslinker allowed easy permeation of the reagent into the interior part of the matrix compared with other crosslinker percentage, as shown in Figure 7. The kinetic study of the 2 mol % DVB, PS–EGDMA, PS–BDDMA, and CLPSER systems was followed by the decomposition reaction of hydrogen peroxide. The activation energy required for the decomposition of hydrogen peroxide decreased in the order DVB > EGDMA > BDDMA > CLPSER, as shown in Table V. The low activation energy of the CLPSER system compared with the DVB and other systems was due to its easy accessibility of the catalytic site to the substrate molecule. When all of the crosslinked systems were compared, the CLPSER system was more efficient than other three systems.

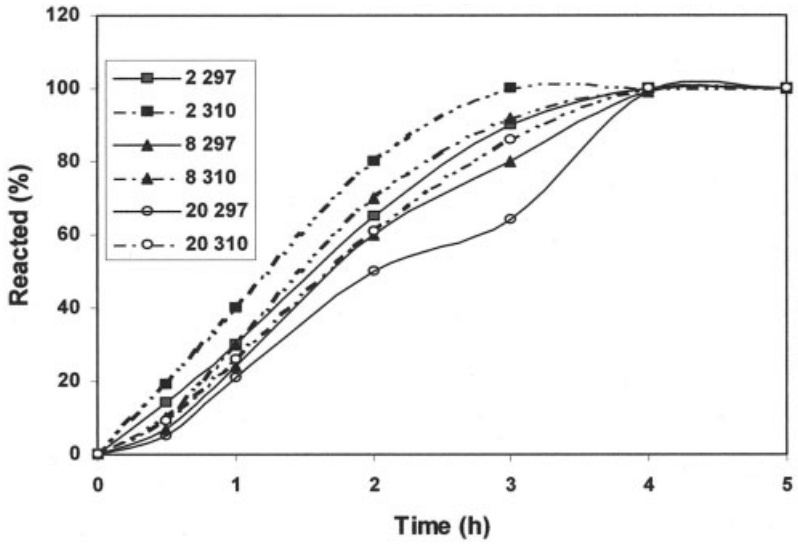


Figure 7 Catalytic activity of the CLPSER system at different temperatures.

TABLE V
Kinetic Parameters of the PS–DVB, PS–EGDMA, PS–BDDMA, and CLPSER Systems

Crosslinking agent	Crosslink density	ΔE (k'J/mol)	A (s^{-1})
DVB	2	35.89	5.2×10^{-2}
	8	56.85	5.6×10^{-2}
	20	57.23	9.4×10^{-2}
EGDMA	2	24.37	1.2×10^{-2}
	8	32.45	4.2×10^{-2}
	20	42.56	4.5×10^{-2}
BDDMA	2	21.60	8.9×10^{-3}
	8	23.65	2.5×10^{-2}
	20	35.35	3.5×10^{-2}
CLPSER	2	7.25	2.3×10^{-3}
	8	11.23	4.5×10^{-3}
	20	21.20	3.9×10^{-3}

Effect of the nature of solvents, ph, and thermal stability

Cosolvents. The catalytic activities of the different crosslinked systems of enzyme-bound polymers were studied by the variation of the solvents in the reaction medium. The studies revealed that the nature of the solvent strictly influenced the catalytic activity of the polymer-bound species. Various cosolvent were studied to explain the catalytic activity, and the water–DMF system was found to be more active. In water–DMF, the CLPSER system showed high catalytic activity compared with the other PS–DVB, PS–EGDMA, and PS–BDDMA systems in aqueous medium alone, as shown in Figure 8. The enhanced catalytic activity of the catalase-immobilized CLPSER system was related to the easy accessibility of the substrate to the polymer-bound enzyme. In water–DMF, the hydrophobicity of the polymer matrix became minimized, and the substrate could easily interact with the polymer-bound enzyme. In CLPSER, the polymer consisted of a flexible PEG crosslinker part and hydrophobic styrene monomer units. The enzyme was covalently immobilized to the styrene part, and when the system interacted with the substrate in the water–DMF cosolvents, the enzyme-bound styrene part exposed more compared with water system alone. For the completion of the reaction in the water medium, CLPSER took 4 h, but in the water–DMF cosolvent, it only took 2 h, as shown in Figures 2 and 9. In the other PS-based systems, PS–DVB, PS–EGDMA, and PS–BDDMA, the catalytic activity increased in water–DMF compared with the water system due to the expansion of the styrene moiety in the polymer matrix.

The effect of pH on the activity of free and catalase-immobilized CLPSER systems was followed at 35°C. The study was followed at different pH values (3–12) of the hydrogen peroxide solution. Tests of the catalase-immobilized CLPSER and free catalase systems were conducted, and the catalase-immobilized CLPSER system showed optimum catalytic activity at pH 7.2 and the least activity for pH values of 3 and 12. The high reactivity at pH 7.2 is due to the easy protonation compared with the other pH values, as shown in Figure 10.

The effect of temperature on the thermal stability of free and immobilized catalase is shown in Figure 11. The immobilized enzyme on the CLPSER system had a higher thermal stability than the free catalase because of the reduction of conformational flexibility in the immobilized enzyme. The activities of the free and immobilized enzyme decreases with increase in tem-

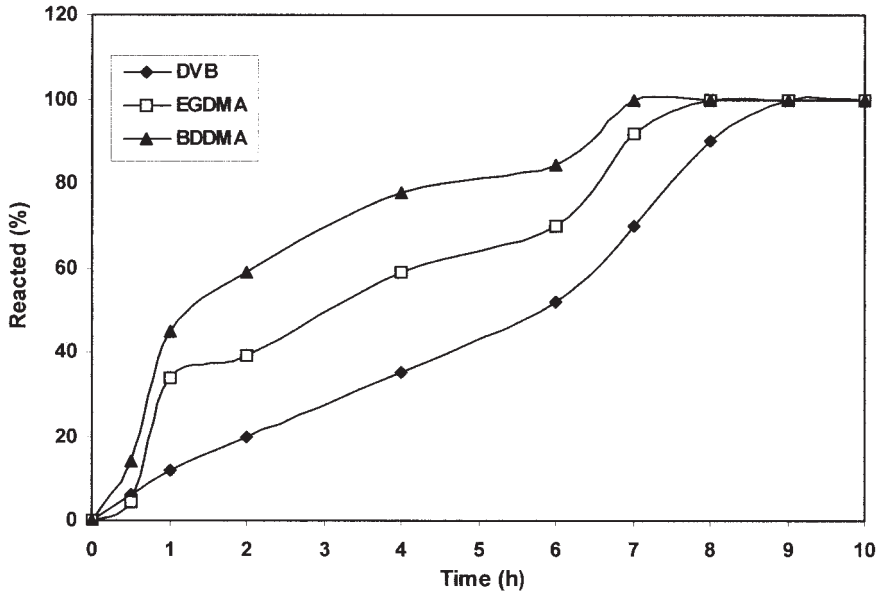


Figure 8 Catalytic activity of the 2 mol % PS–DVB, PS–EGDMA, and PS–BDDMA systems in water–DMF.

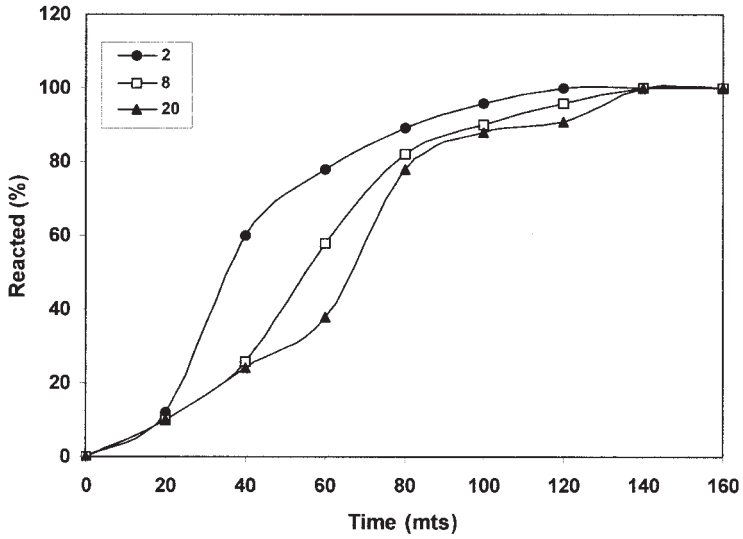


Figure 9 Catalytic activity of the 2–20 mol % CLPSER system in water–DMF.

perature for the initial 2 h of preincubation. At 50°C, the free catalase lost about 55% of its activity, whereas the CLPSER-immobilized catalase lost about 35% of its activity after 2 h of preincubation. These results suggest that the thermostability of the catalase-immobilized CLPSER system became significantly higher than that of the free catalase at higher temperatures. The immobilization of catalase on CLPSER was supposed to preserve the tertiary enzyme structure, and it protected the enzyme from any conformational changes that have affected the macromolecular environment of the polymer support.

CONCLUSIONS

This investigation suggested that the CLPSER system was very suitable to explain the catalytic activity of the enzyme-immobilized system. The nature and extent of the crosslinking agent had a significant role in this study. The catalytic activity of the CLPSER system was high compared with the PS–DVB, PS–EGDMA, and PS–BDDMA systems. The optimum hydrophilic–hydrophobic balance of the resin showed a very high degree of swelling in a broad range of polar and nonpolar solvents. From the foregoing results, we concluded that for the efficiency of the method to be employed for the immobilization of an enzyme on a synthetic polymer support, the yield of immobilization and catalytic activity depend on a number of factors, such as the nature of the macromolecular matrix. The catalytic activity of the immobilized enzyme is affected by the nature of the support and it decreased in the order CLPSER > PS–BDDMA > PS–EGDMA > PS–

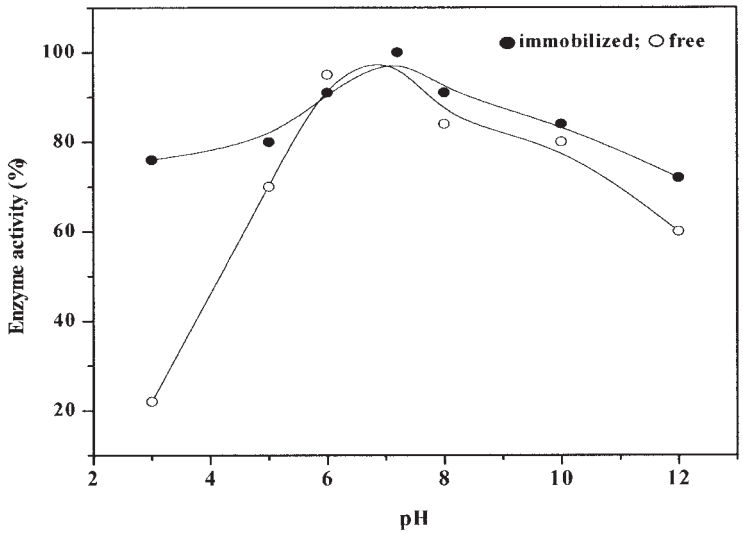


Figure 10 Effect of pH on the catalytic activity of free catalase and the catalase-immobilized CLPSER system.

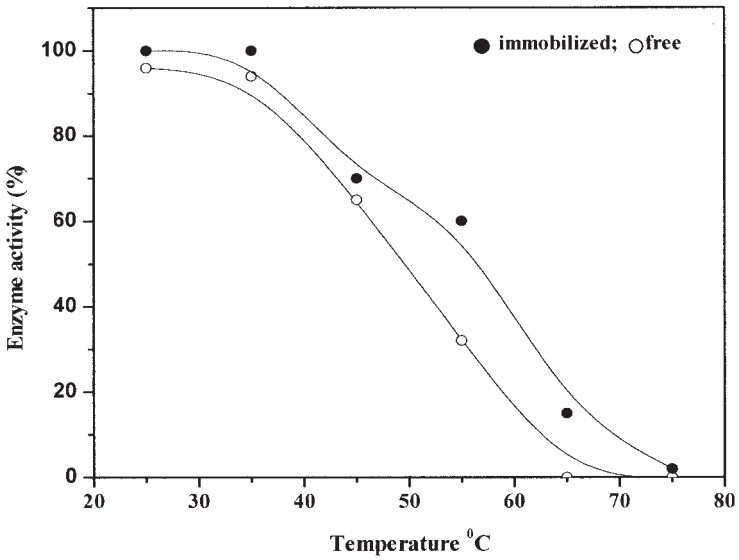


Figure 11 Thermal stability of free catalase and the catalase-immobilized CLPSER system.

DVB. The nature of the cosolvents, the extent of crosslinking, and the pH of the reaction medium had vital roles in the activity. This study revealed that among the available systems, the CLPSER system was most suitable to explain the bioactivity in polar–nonpolar environments.

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