Immobilization of Lipase on Hydrogels: Structural Aspects of Polymeric Matrices as Determinants of Enzyme Activity in Different Physical Environments

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ABSTRACT: Well-defined and characterized polymeric matrices showing close chemical similarities but wide differences in water uptake and swellability in aqueous medium were used for lipase immobilization. Biphasic networks of 2-hydroxypropylcellulose (HPC) were synthesized with acrylamide (AAm), methacrylamide (MAAm), *N*-isopropylacrylamide (*N*-i-PAAm), and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA) and simultaneously crosslinked with *N*,*N*-methylene bisacrylamide in aqueous medium by using simultaneous γ-radiation technique. Lipase enzyme was produced from a mesophilic bacterial isolate (HBK-8) and was immobilized onto all the matrices by adsorption method. The activity of the immobilized enzyme was optimized for pH, temperature, and amount of crude enzyme and effect of dehydration. High relative activity for the immobilized enzymes was observed and loss of activity with time was minimal; reusability was found to be good. The activity of the immobilized enzyme was also observed to be good in both esterification and hydrolysis of esters. In the present study, lipase immobilization, hydrolysis of *p*-nitrophenyl palmitate, and optimum pH and temperature for substrate hydrolysis were evaluated for different matrices to study polymer structure and enzyme activity relationship in diverse physical environments. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 3135–3143, 2004

Key words: acrylamides; cellulosics; enzyme activity; hydroxypropyl cellulose; immobilized lipase; *p*-nitrophenyl palmitate

INTRODUCTION

Use of cellulosics and other biopolymers as support for immobilization of small molecules, proteins, and cells have received considerable attention in recent years. These supports have certain advantages over other materials such as low cost, ease of enzyme accessibility, hydrophilic character, and presence of hydroxyl groups on the surface capable of interaction with proteins. Enzyme immobilization on these supports is quick and apparently irreversible and provide nontoxic and biocompatible microenvironment conducive to the catalytic activity and stability of the enzyme. Hydrogels of natural polymers such as gelatin, chitosan, xanthan, agarose, and alginate were used conveniently in both the wet state and the dried state.[1,2] However, these supports suffer from low mechanical strength and easy microbial degradation.

Lipase is one of the most extensively investigated enzymes because of its ability in fat splitting, esterification, *trans*-esterification, and other reactions of industrial importance; therefore, many attempts have been made to immobilize it. Representative methods proposed for lipase immobilization include gel entrapment[3] and adsorption on membranes[4] or resins.[5] Lipase obtained from the porcine pancreas was immobilized on poly(acrylamide) beads partially hydrolyzed to active carboxylic groups.[6] Lipase hydrolyzes triglycerides and other carboxylic esters in aqueous medium[7] but lipases show good activity in hydrophobic organic solvents with limited water content,[8] where chemical equilibrium is shifted toward ester formation. Hydrolysis predominates in a water-rich environment, whereas esterification predominates at low water content. Reaction medium comprising organic solvents having low water activity as biphasic solvent systems consisting of water and water immiscible solvents, reverse micelle, or microaqueous systems shift thermodynamic equilibrium toward the ester synthesis.[9,10] In biphasic systems and in reverse micelle, enzyme is solubilized in water, whereas in microaqueous system, enzyme is in suspension in organic solvent. The nature of organic media in which the reaction is performed also influences lipase activity from both a kinetic and a thermodynamic point of view.[11,12]

To investigate the effect of structural aspects of the supports and that of the environmental conditions on the enzyme activity, an attempt was made to study immobilization of lipase onto chemically closely related polymeric networks based on hydroxypropyl

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cellulose (HPC), acrylamide (AAm), and its substituted forms such as methacrylamide (MAAm), *N*-isopropylacrylamide (*N*-i-PAAm), and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA), crosslinked with *N*,*N*-methylene bisacrylamide (*N*,*N*-MBAAm) and synthesized by simultaneous γ-ray initiation alone as monocomponent or as conjugate networks of HPC and different acrylamides. The conjugate networks are of special interest as HPC has surface-active properties and is known to act as good reinforcing agent because of the linear chains and AAm and substituted acrylamides have active adsorbent/anchor groups. Thus, a tailored combination of mechanical and chemical properties targeted those having interesting implications for lipase immobilization. HPC was synthesized from extracted cellulose and is 32% soluble in water at room temperature and has a low 15% degree of crystallinity. These structural aspects of reinforcing agent make the resultant network of biphasic nature as phase differentiation arose due to the partial solubility in the aqueous medium. Further, these networks have a wide spectrum of physical properties from polyelectrolyte behavior of poly(AAmPSA) to the thermosensitive nature of poly(*N*-i-PAAm) networks. Some of the networks used in this study were characterized for composition of acrylamide and cellulosic contents, water uptake/swellability in different swelling media as pure water, 0.5*N* HCl, 0.5*N* NaOH, and 5% NaCl solution, and also by FTIR, SEM, and thermal studies.[13,14] Enzyme activity was studied as a function of temperature, pH, and incubation time and amount and nature of solvent. Reproducibility of the enzyme activity was studied and the immobilized lipase was used as both hydrolase and esterase. The reported work has good potential to develop model bioreactors for synthesis and hydrolysis of useful esters.

EXPERIMENTAL

Methods

Lipase from bacterial isolates (Department of Biotechnology, Himachal Pradesh University, Shimla, India) was used without any purification. *p*-Nitrophenyl palmitate (*p*-NPP; Lancaster Synthesis, UK), 0.1*M* Tris–HCl buffer, pH 7.5 (0.1*M* Tris, 0.1*N* HCl, 0.4% Triton X-100, 0.1% gum acacia powder), 16.5 mM *p*-NPP (prepared in propane-2-ol), and 2–20 μg/mL *p*-nitrophenol (*p*-NP) were other analytical grade reagents used. Networks of hydroxypropylcellulose with different acrylamides [viz., AAm, MAAm, 2-acrylamido-2-propane sulfonic acid (AAmPSA), and *N*-isopropylacrylamide (*N*-i-PAAm)], crosslinked by *N*,*N*-MBAAm were synthesized by simultaneous γ-irradiation in aqueous medium[13,14] and networks synthesized at the optimum reaction conditions were used. These include three categories of matrices: crosslinked HPC, crosslinked acrylamides, and crosslinked networks of all of these acrylamides with HPC listed as:

Monoconjugate	Biconjugate
cl-HPC	—
cl-Poly(AAm)	HPC-*cl*-poly(AAm)
cl-Poly(MAAm)	HPC-*cl*-poly(MAAm)
cl-Poly(AAmPSA)	HPC-*cl*-poly(AAmPSA)
cl-Poly(N-i-PAAm)	HPC-*cl*-poly(N-i-PAAm)

Immobilization of lipase on polymeric matrices

The known weight of each matrix was washed with (0.1*M*) Tris–HCl buffer, pH 8.5. After two washings, matrices were dipped in 1.0 mL crude enzyme and incubated at 4°C for 24 h. After incubation for 24 h, matrices were washed and stored in (0.1*M*) Tris–HCl buffer, pH 8.5 at 4°C.

Assay procedure for immobilized lipase in aqueous medium

Lipase activity was determined by measuring *p*-NP released from *p*-NPP by using the modified spectrophotometric method of Winkler and Stuckmann.[15] Procedure for assay of lipase enzyme (HBK 8) and its proper characterization as optimum activity and effect of different conditions such as time of incubation, pH, and temperature has been reported in detail.[16] A mixture of *p*-NPP and (0.1*M*) Tris–HCl buffer, pH 8.5 (1 : 9 ratio), was incubated at 70°C for a few minutes until its turbidity disappeared. The mixture was cooled and reaction was carried out in a test tube containing 1.0 mL of substrate; 20.0 mg of immobilized enzyme was added and total volume was made 3.0 mL with (0.1*M*) Tris–HCl buffer (pH 8.5). Each test tube was incubated at 45°C for 20 min. Amount of *p*-NP released was measured at 410 nm. The corresponding concentration was determined from a *p*-NP standard graph. The control contained 1.0 mL of substrate, 2.0 mL (0.1*M*) Tris–HCl buffer, and 20.0 mg of matrix.

Effect of environmental conditions on immobilized enzyme activity

Different amounts of immobilized enzyme were added to the reaction mixture containing 1.0 mL of substrate [*p*-NPP, 16.5 mM and (0.1*M*) Tris–HCl buffer, pH 8.5, in 1 : 9 ratio] and final volume was made to 3.0 mL with Tris–HCl buffer. To study the effect of temperature, tubes were incubated at 25, 35, 45, and 55°C, for 20 min and observation was recorded at 410 nm as discussed earlier. After two washings in

(0.1*M*) Tris–HCl buffer, pH 8.5, the same procedures were repeated and absorbance was recorded at 410 nm. Effect of pH on immobilized enzyme in different matrices was studied in a similar way at pH (7.0, 7.5, 8.0, 8.5, 9.0, 9.5) and incubated at 45°C for 20 min. Effect of time of incubation on immobilized enzyme in different matrices was studied by variation of time of incubation at 45°C for 10, 20, 30, 40, and 50 min. Effect of dehydration of the matrices as a function of ethanol contents was also studied by immersion of matrices in 50, 60, 70, 80, and 90% ethanol.

Synthesis of *p*-nitrophenyl benzoate (*p*-NPB) in organic phase

Organic phase mixture (10.0 mL) contains dioxane (1.0 mL) and hexane (9.0 mL), (*p*-NP : benzoic acid) in 1 : 1*M* ratio. Lipase (100 mg) immobilized on HPC-*cl*-poly(AAm) matrix dehydrated with 90% ethanol was transferred to organic phase (10.0 mL) and incubated at 45°C for 30 min. Poly(AAm) matrix (100.0 mg) without enzyme was taken as control in 10.0 mL of organic phase. Progress of reaction was monitored by observation of reduction in the concentration of *p*-NP. One hundred micrograms of control and test sample, diluted in hexane, was mixed with 2.9 mL of Tris–HCl buffer, pH 8.5 to develop color of the unreacted *p*-NP. The absorbance of control and test sample was observed after every 30 min by following the method mentioned earlier.

Effect of solvent composition on synthesis of *p*-NPB

To study the effect of solvent composition, following the same procedure as discussed above, benzoic acid and *p*-NP were mixed and dissolved in different flasks containing different amounts of hexane and dioxane (total volume, 25.0 mL). After every 30 min, samples were withdrawn and diluted in the solvent mixture (same as used for synthesis) and after that 100 μL of diluted sample was added to 2.9 mL of Tris–HCl buffer, pH 8.5 for developing the color of *p*-NP. Absorbance was taken at 410 nm.

Hydrolysis of *p*-NPB

Water (10.00 mL) was added to *p*-NPB in the reaction mixture to form two layers. One hundred milligrams of crosslinked poly(AAm) and crosslinked poly-(MAAm) immobilized matrices without dehydration was used for hydrolysis. After incubation for 30 min, samples were withdrawn and diluted in the solvent mixture (same as used for synthesis). After that, 100 μL of diluted sample was added to 2.9 mL of Tris–HCl buffer, pH 8.5; release of *p*-NP in the solution mixture indicates hydrolysis of synthesized *p*-NPB.

RESULTS AND DISCUSSION

It is known that immobilization enhances conformational stability of lipase and proteins and immobilized enzyme becomes more resistant toward denaturation and can be used at higher temperatures as a result of enhanced conformational stability. Thus, higher optimum temperature, broader pH range nature, and composition of medium are also expected to be shifted from those observed for free enzyme and applicability of immobilized systems is expected to be wider. Because the spectrum of water uptake/swellability of these hydrogels and their swelling environment responses is very broad, these properties as manifestation of the inherent structural aspects affect enzyme activity in a very defined way and it has been observed to be so.

Effect of environmental factors on enzyme activity

The results of the effects of substrate concentration, temperature, pH, and time of incubation on activity of the free enzyme are presented in Table I.[16] It follows that enzyme activity increases until 0.8 mM of the substrate and was found to be constant thereafter until the substrate concentration reaches 1.0 mM. Highest enzyme activity was observed at 45°C, pH 8.5, and 20 min time of incubation and effect on enzyme activity in the immobilized form as a function of environmental factors such as pH, temperature, and time of incubation is sharp and pronounced.

Effect of polymeric networks on activity of immobilized lipase

Activity of the immobilized enzyme (59.79) was observed maximum in crosslinked HPC. It is ascribed to the fact that HPC has surface-active properties and thus the compatibility of lipase and support is established. Activity of lipase in other matrices though lesser than the crosslinked HPC is comparable and in crosslinked poly(*N*-i-PAAm), crosslinked poly(AAm), and crosslinked poly(MAAm), whereas it was observed to be far less in the crosslinked poly(AAmPSA). In the network series, the order of activity follows as HPC-*cl*-poly(*N*-i-PAAm) > HPC-*cl*-poly(AAm) > HPC-*cl*-poly(MAAm) $\gg$ HPC-*cl*-poly(AAmPSA). However, other results show that chemical composition of polymeric matrices have a significant effect on the enzyme activity, as polymeric matrix having active sulfonic acid groups, those are known to self-ionize as poly(AAmPSA) is itself a polyelectrolyte that possibly causes denaturation of the enzyme. Hence, lower enzyme activity was observed in the case of poly(AAmPSA)-based matrices. Network formation of substituted amides with HPC affects enzyme activity, as it remains more or less the

TABLE I
Effect of Running Concentrations of Substrate (*p*-NPP), Time of Incubation, *p*H, and Temperature on Activity of Free Enzyme

[*p*-NPP] (mM)	Enzyme activity (Units/MI)
0.1	02.52
0.2	05.29
0.3	11.33
0.4	17.22
0.5	21.53
0.6	34.04
0.7	48.39
0.8	53.04
0.9	52.24
1.0	52.06

Time (minutes)	Enzyme activity
5	17.37
10	26.37
15	41.96
20	52.45
25	50.25
30	51.25

Assay *p*H	Enzyme activity
4.5	0.00
5.0	0.00
5.5	0.00
6.0	13.28
6.5	19.06
7.0	21.28
7.5	26.60
8.0	35.91
8.5	56.01
9.0	41.59
9.5	14.97

Temperature (°C)	Enzyme activity
25°C	13.54
30°C	18.42
35°C	25.55
40°C	32.23
45°C	54.27
50°C	47.12
55°C	27.23

same as observed in single-component matrices. Incorporation of more hydrophobic poly(*N*-i-PAAm) does not alter the enzyme adsorption activity of HPC as the quantum of activity of enzyme immobilized on HPC-*cl*-poly(*N*-i-PAAm) is the same as that of the crosslinked HPC. However, in other cases, there is significant reduction of enzyme adsorption and these results are in line with earlier reported studies.[17] Network formation is useful in the present case as the mechanical strength and the shape of the matrices are not affected as compared to the single-component acrylamide(s) network.

Enzyme immobilization and reusability

The matrices with immobilized enzyme were used many times and activity is presented in Table II. Loss of activity is rapid for crosslinked poly(AAm) and less for crosslinked HPC, crosslinked poly(*N*-i-PAAm), and crosslinked poly(MAAm) or their conjugate networks. The order for retention of activity can be formulated as crosslinked poly(*N*-i-PAAm) > crosslinked poly-(MAAm) ≈ crosslinked HPC > crosslinked poly(AAm). Crosslinked poly(AAmPSA) lost all activity after first use. Apart from the behavior of poly(AAmPSA), reasons for which has been given earlier, the better activity behavior of other matrices is explained on the basis of increase in hydrophobicity of the substituted acrylamide component. Reactivity profile of the conjugate matrices is formulated as HPC-*cl*-poly(*N*-i-PAAm) > HPC-*cl*-poly(*N*-MAAm) > HPC-*cl*-poly(AAm) > HPC-*cl*-poly(AAmPSA).

Effect of temperature on immobilized lipase enzyme activity

Temperature variation at low content of the water increases enzyme activity[18] and in the present case enzyme activity also increases when temperature variation was made from 25 to 45°C and after that it stabilizes at higher temperature (55°C) only in the case of HPC-*cl*-poly(AAm). Temperature variation has a pronounced effect on enzyme activity for crosslinked HPC, crosslinked poly(AAm), and crosslinked poly-(MAAm) (Fig. 1). Maximum activity was observed at 45°C; activity decreased at higher temperatures. On increasing temperature from 45 to 55°C, reduction in the activity of immobilized enzyme was not significant as compared to that of the crude enzyme, which was reduced to almost to one-half of that observed at 45°C. The highest enzyme activity for all the matrices was observed at 45°C but for crosslinked poly(*N*-i-PAAm), where maximum activity was observed at 35°C and thereafter, it decreases. The reusability of immobilized enzyme in the case of the conjugate matrices was observed better at the optimum temperature evaluated in the case of respective matrices as observed for the reusability of HPC-*cl*-poly(*N*-i-PAAm) at 35°C, which was better than crosslinked poly(*N*-i-PAAm). Such behavior of poly(*N*-i-PAAm)-based matrices can be ascribed to its lower critical solution temperature (LCST = 32.5°C), as above this temperature these gels undergo sharp volume transition by releasing the absorbed water. Higher activity of crosslinked HPC-*cl*-poly(*N*-i-PAAm) at 35°C is ascribed to an increase in the LCST of poly(*N*-i-PAAm) due to the network formation.[14]

TABLE II
Reusability of Immobilized Enzyme in Hydrolysis of *p*-NPP[a]

Repeatability (after washing)	Backbone	Activity (g/min)	(%) Cumulative loss of activity
First use	HPC-*cl*-poly(MAAm)	6.7	—
	HPC-*cl*-poly(AAm)	9.9	
	cl-Poly(MAAm)	5.7	
	cl-Poly(AAm)	5.5	
Second use (after 3 days of first use)	HPC-*cl*-poly(MAAm)	5.7	14.93
	HPC-*cl*-poly(AAm)	8.1	18.18
	cl-Poly(MAAm)	5.4	5.26
	cl-Poly(AAm)	4.9	10.90
Third use (after 3 days of second use)	HPC-*cl*-poly(MAAm)	5.5	17.91
	HPC-*cl*-poly(AAm)	7.4	25.25
	cl-Poly(MAAm)	4.7	17.54
	cl-Poly(AAm)	4.3	21.81
Fourth use (use after 3 days of third use)	HPC-*cl*-poly(MAAm)	4.9	26.86
	HPC-*cl*-poly(AAm)	7.1	28.28
	cl-Poly(MAAm)	4.4	22.80
	cl-Poly(AAm)	3.9	29.09
Fifth use (after 3 days of fourth use)	HPC-*cl*-poly(MAAm)	4.7	29.85
	HPC-*cl*-poly(MAAm)	4.7	29.85
	HPC-*cl*-poly(AAm)	6.7	32.32
	cl-Poly(MAAm)	4.0	29.82
	cl-Poly(AAm)	3.5	36.36

[a] Activity of immobilized enzyme is defined as microgram of p-nitrophenol released per gram of wet matrix per minute under assay conditions.

Effect of pH on immobilized lipase enzyme activity

pH affects enzyme activity by affecting stability of the immobilized enzyme and it was observed that activity increases with an increase in the pH at a first instance up to pH 8.5 and thereafter decreases (Fig. 2) and follows the order of crosslinked poly(MAAm) > crosslinked HPC > crosslinked poly(*N*-i-PAAm) > crosslinked poly(AAm). Maximum activity was observed at pH 8.0 and then decreases slightly at pH 8.5; the decrease was drastic at pH 9.0. At pH 7.5, activity of the immobilized enzyme decreased to 75% of the optimum value, whereas for crude enzyme activity, it decreases more. Thus, we can conclude that immobilization of enzyme led to the stabilization of pH ranging from almost 7.5 to 8.5, hence, broadening the functioning range of pH of enzyme activity.

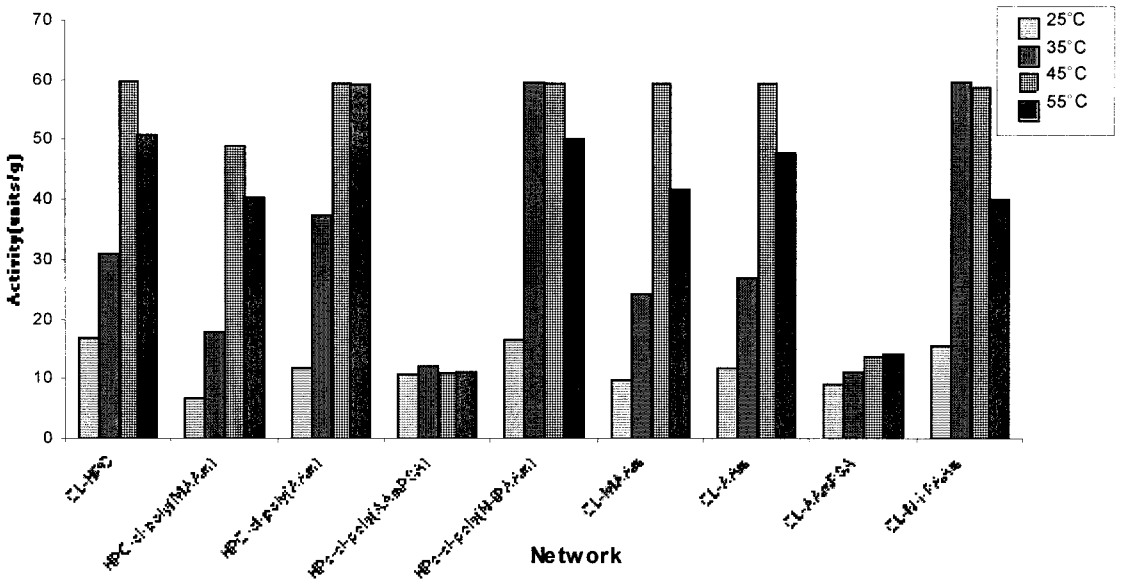

Figure 1 Effect of temperature on immobilized enzyme activity (time of incubation = 20 min, pH = 8.5).

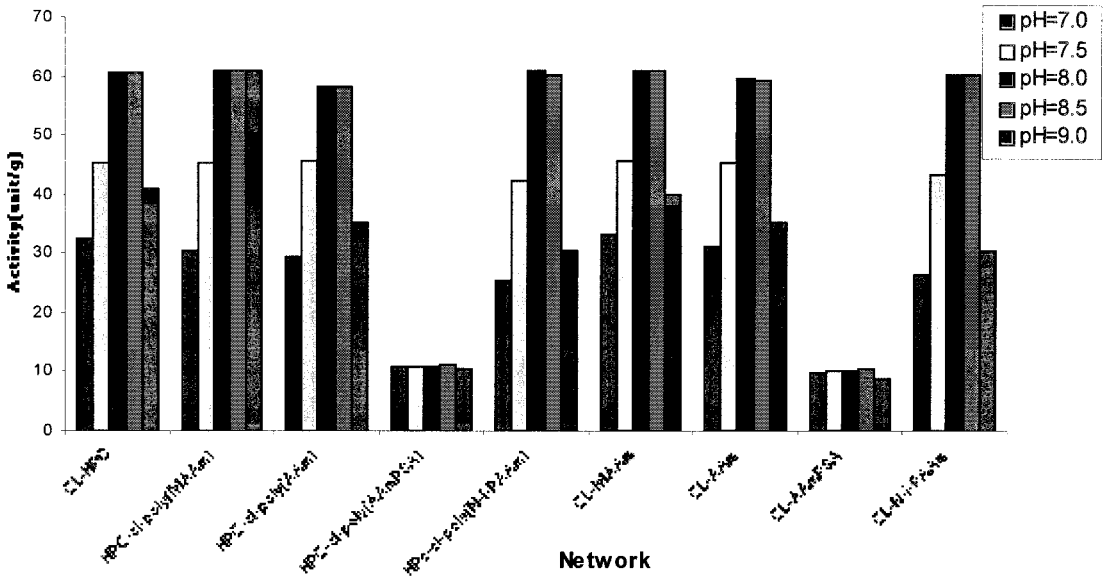

Figure 2 Effect of pH on activity of immobilized enzyme (time of incubation = 20 min, temperature = 45°C).

Effect of time of incubation on immobilized lipase enzyme activity

Incubation period for the immobilized enzyme decreases from 30 to 20 min in the case of the crude enzyme (Fig. 3). Such a change in the incubation time to realize optimum activity on immobilization is a function of accessibility of substrate to the immobilized enzyme as compared to the free enzyme. The local concentration of substrate near the enzyme is different from the actual concentration of the substrate in the solution. These processes depend on the rate of diffusion or partitioning capability of the substrate between the matrices and the solution phase, and hence, enzyme requires more time to come in contact with the substrate and incubation time required is more in the case of the immobilized enzyme. From these observations as broadening of the pH, temperature range, and increase in the time of incubation indicate (1) that enzyme is stabilized in a varied and more unnatural and incompatible environment and (2) that most of the enzyme is adsorbed on and not entrapped in the matrices.

Applications of the immobilized enzymes

The lipase-immobilized supports were used to synthesize and hydrolyze an aromatic ester as discussed below.

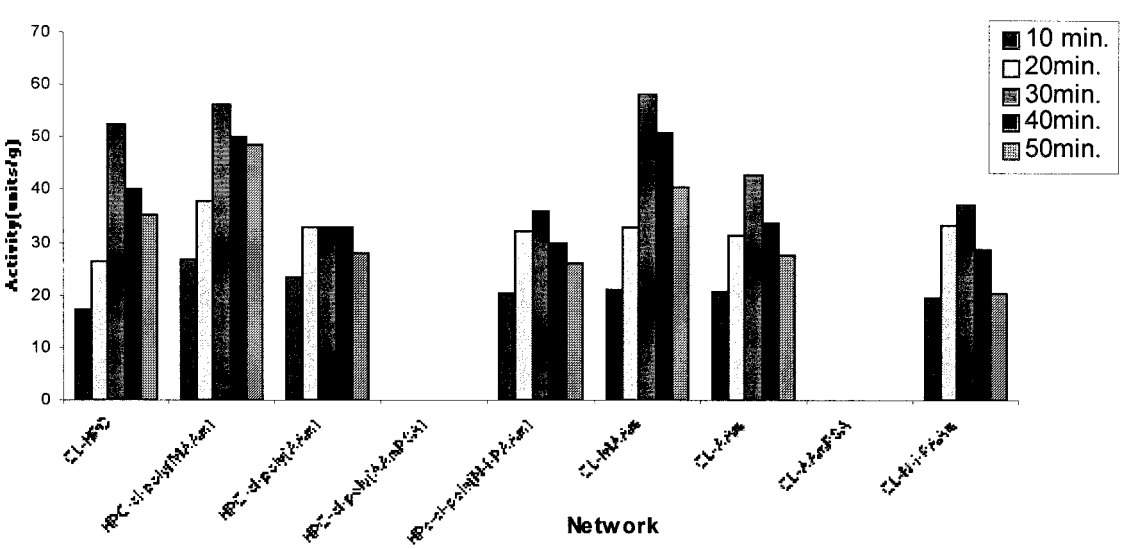

Figure 3 Effect of time of incubation on activity of immobilized enzyme (pH = 8.5, temperature = 45°C).

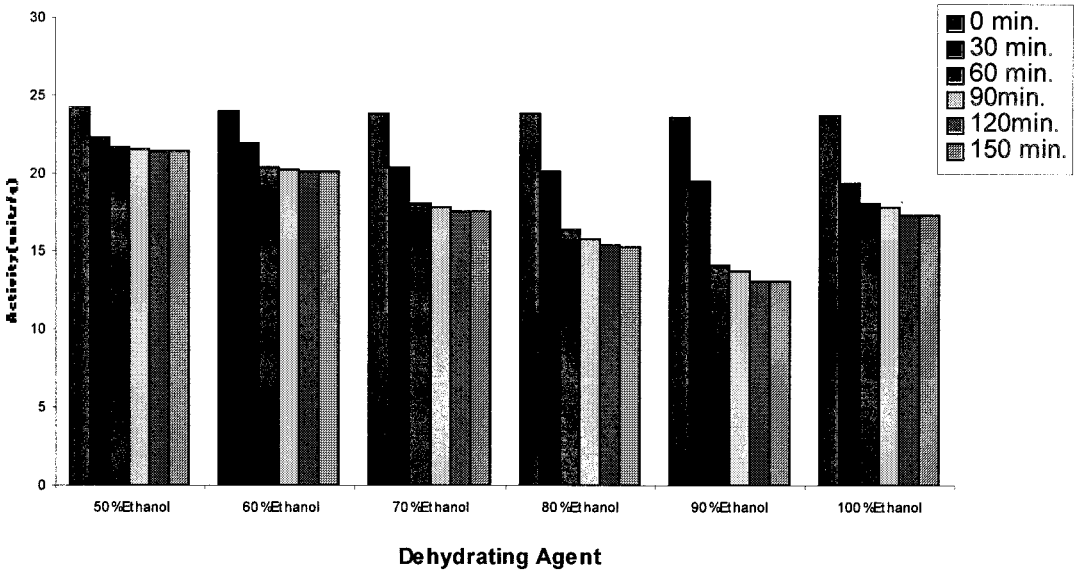

Figure 4 Effect of dehydration on activity of immobilized enzyme [network used = HPC-*cl*-poly(AAm), pH = 8.5, temperature = 45°C].

Synthesis by immobilized enzyme: Effect of dehydration and solvent composition

Amount of water is a key parameter in a nonconventional or in organic medium. A small amount of water molecules is absolutely essential to obtain sufficient enzyme conformational flexibility for enzyme activity. However, at higher water content, aggregation of solid particles induces diffusional limitation of substrates and a reduction in the reaction rate is observed. The optimum amount of water needs to be added in the reaction medium. This requirement depends upon initial water content of the solid and liquid phase and on solvent polarity, substrate, and several other physicochemical parameters. Therefore, an optimum amount of water cannot be predicted and should be determined for each reaction mixture. Solvent hydrophobicity and water content in biphasic systems influence both enzyme stability and equilibrium displacement in ester synthesis. Effect of dehydration (Fig. 4) and solvent composition (Fig. 5) was studied for optimization of synthetic conditions. The dehydrated matrix can remove water produced in the reaction itself more efficiently as swelling capacity of the matrix is enormous and such characteristics of the poly-

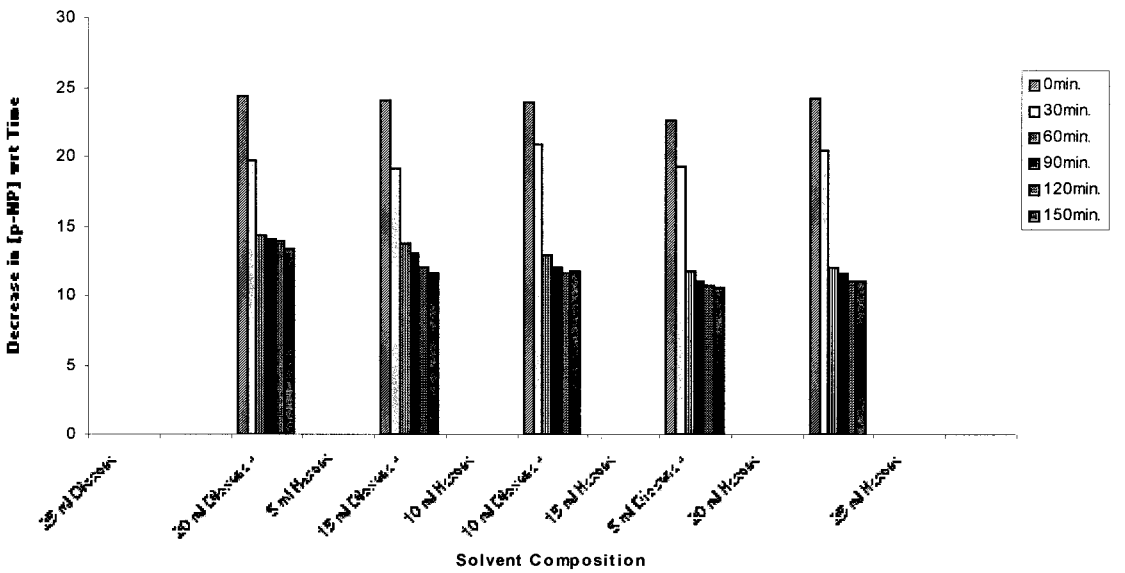

Figure 5 Effect of solvent composition on activity of immobilized enzyme [network used = HPC-*cl*-poly(AAm), pH = 8.5, temperature = 45°C].

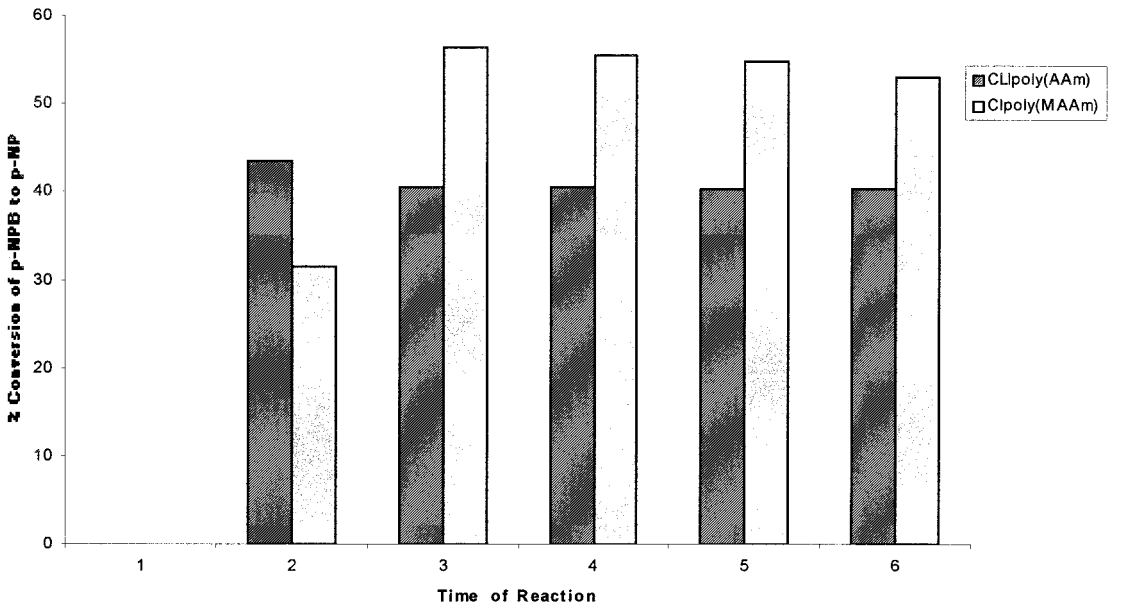

Figure 6 Hydrolysis of *p*-nitrophenyl benzoate by immobilized enzyme (pH = 8.5, temperature = 45°C).

meric matrix should be of interest in the ester synthesis where self-removal of water by the matrix should shift equilibrium in favor of the ester. It was observed that matrices dehydrated with 90% ethanol were found to be the best for ester synthesis. In such a system, amount of water in the reaction mixture is minimum, which is just enough to maintain the tertiary structure of the enzyme. It is further supported by the fact that on dehydration with 100% ethanol enzyme activity is lowered due to the conformational changes in the structure of enzyme as has also been reported by other workers.[18] It is also reported that hydrophobicity of organic solvent greatly influences the activity of biocatalyst acting in biphasic water/organic system. Characteristics of the organic phase were determined by log *P* values of such solvents. Dioxane and hexane were used separately and as mixtures in different ratios. It was observed that in hexane % conversion, and hence, the enzyme activity, was better. Such conclusions have also been reported earlier.[19] On the basis of higher log *P* values for hexane (3.5) as compared to dioxane, solvents with log *P* value less than 2.5 distort the water layer around the enzyme, which is required to maintain the tertiary structure. Hence, % conversion increases with an increase of hexane in the solvent composition (Fig. 5).

Hydrolysis of *p*-nitrophenyl benzoate in hexane

Hydrolysis of *p*-nitrophenyl benzoate was studied as a function of release of *p*-NP with time (Fig. 6). Product concentration increased with an increase in time up to 120 min. After that, it decreased in the case of the crosslinked poly(MAAm) and remained the same in the case of crosslinked poly(AAm). The conversion factor was 52.9% in crosslinked poly(MAAm) and 40.19% in crosslinked poly(AAm). Similar observations were made earlier that the structure of matrix strongly affects both hydrolysis and esterification and as observed in the present study hydrophilic matrix gives better results in esterification reactions, while the hydrophobic nature of the matrix is better in hydrolysis.[20]

CONCLUSION

From the foregone discussion it is concluded that activity of the immobilized enzyme on different polymeric supports studied depends on the structural aspects of supports. The contribution of hydrophobicity of the acrylamides and the surfactant properties of the HPC are useful in exhibition and retention of high-enzyme activity. The immobilization takes place by adsorption as is evident from the broadening of the effect of pH and temperature range of the immobilized enzyme. The thermosensitivity of poly(*N*-i-PAAm) exhibited in high activity of the immobilized enzyme has special significance with respect to the usability of this matrix at lower temperature range. The effect of the individual constituents of the networks/supports on the activity of the enzyme and effect of dehydration as brought about by the present study should contribute to the existing knowledge of tailoring of the polymeric supports and biotechnological techniques and these results can be useful in the development of an effective bioreactor for both the ester synthesis and the hydrolysis.

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