Solvent Free Biocatalytic Synthesis of Vinyl Monomers by Lipase Immobilized on Hydrogels

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ABSTRACT: Simple novel research plan has been employed to tailor supports for lipase immobilization. *N*-aminoethylacrylamide (*N*-AEAAm) and *N*-aminoethylmethacrylamide (*N*-AEMAAm) were crosslinked with *N*,*N*-methylene bisacrylamide (*N*,*N*-MBAAm) and consequently reacted separately with acrylic acid (AAc) and methacrylic acid (MAAc). The hydrogels thus formed, consist of both amide and carboxylic functional groups. These hydrogels were characterized by nitrogen analysis, SEM, FTIR and also by water uptake studies as a function of time, temperature, pH, and the presence of additives like sodium dodecyl sulfate (SDS) and cetyl trimethyl ammonium bromide (CTAB). These hydrogels are environmentally sensitive. In the presence of surfactants, these hydrogels show micellization and swell to the maximum at the critical micellar concentration (CMC) of the surfactants. Lipase was immobilized on these hydrogels and the one exhibiting the maximum activity was further used as biocatalyst to explore nonconventional green routes for the synthesis of some known and novel vinyl monomers. Yields obtained have been high and the immobilized lipase show high reusability. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 3200–3209, 2008

Key words: hydrogels; lipase immobilization; *N*-aminoethylacrylamide and *N*-aminoethylmethacrylamide; nonconventional routes; swelling; vinyl monomers

INTRODUCTION

Use of immobilized lipases has become increasingly popular for both ester hydrolysis and synthesis. The immobilized lipases offer several advantages those include: reusability, rapid termination of reactions, low cost product formation, and ease of separation.[1] Compared with the free form, immobilized lipases have been reported to show enhanced thermal and chemical stability. The product is formed in high yield and has purity.[2] Many interesting works have been reported recently on the use of immobilized lipases as biocatalyst in esterification, *trans*-esterification reactions, and enantioselective hydrolysis.

A correlation of the carbon chain lengths of acids and alcohols to the extent of reaction in esterification catalyzed by *Rhizomucor miehei* lipase involving acids (of carbon chain length C-2 to C-5) and alcohols (of carbon chain length C-1 to C-8) has been reported.[3] Biosynthesis of ethyl esters of short chain fatty acids using whole cell lipase from *Rhizopus chinensis* has been reported in nonaqueous phase.[4] In the lipase catalyzed esterification of disaccharides, the fatty acid chain length affects regioselectivity and initial specific reaction rate, and the later increase with the decreasing chain length of the acyl donor.[5] Synthesis of α-cyano-3-phenoxybenzyl alcohol in organic media was reported and diisopropyl ether was reported to be the most suitable solvent.[6] The enzymatic synthesis of 2-ethylhexyl palmitate has been reported with a maximum esterification degree of 91% by using immobilized lipase from *Candida* species.[7] Oleoyl ester of L-ascorbic acid was synthesized by using immobilized lipases in a series of solvents, such as ethanol, THF, pyridine, butanol, *t*-amyl alcohol, hexanol, octanol, and hexane; and tertiary amyl alcohol was reported to be the most suitable solvent from the view point of the substrate concentration and the enzyme activity.[8] Lipase catalyzed *trans*-esterification reactions in homogenous perfluorocarbon and hydrocarbon solvents enabled direct enantiomeric partitioning (95%) of the products by liquid–liquid separation.[9] Lipase catalyzed *trans*-esterification reaction has been reported for methyl 2-substituted 3-hydroxy-4-pentenoates[10] and poly- (ε-caprolactone).[11] Lipase catalyzed enantioselective *trans*-esterification of esters of 2-bromo-tolylacetic,[12] and enantioselective hydrolysis of methyl-2-chloropropionate[13] and D, L-menthyl benzoate to L-(−)-menthol has been reported.[14] However, there are two major concerns in the area of use of immobilized lipase. One, the activity of the immobilized lipase is low and most promising aspect of immobi-

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lized lipase as biocatalyst is its good performance in the organic solvents. These limitations can be improved by designing suitable supports for lipase immobilization. Poly acrylic acid (AAc) being hydrophilic with an active carboxylic group is a good choice to develop hydrogels for use as supports for lipase immobilization. Lipase immobilized onto poly (AAc) hydrogels has been reported for the conversion of chlorotrimethylsilane to chlorohydrin esters.[15] Combinatorial library of poly(AAc)-based supports and their evaluation as immobilization matrix for amperometric biosensors has also been reported.[16] Some of the poly(AAc)-based supports have been used for the adsorption of trypsin,[17] lysozyme,[18] and in biosensor applications.[19,20]

We have earlier reported use of functionalized polytetrafluoroethylene-co-perfluoroalkoxy vinyl ether films (teflon-PFA obtained from du Pont) and hydrogels of cellulosics as active supports for lipase immobilization.[21,22] In this communication we report use of novel polymeric supports for lipase immobilization to prepare some known and new vinyl monomers. Synthesis of monomers poses problems related to the generation and preservation of reactive vinylic double bond and the process and product both are often cost prohibitive. The high and harsh operating conditions require stringent protocol, otherwise the monomer formed may polymerize *in situ*. For example, synthesis of methyl methacrylate as synthesized conventionally is a sharp contrast to the present scheme. In one of the conventional route, it is obtained in a two step synthesis from hydrogen cyanide and acetone. This route just affords 47% atom economy and use of nonbenign chemicals and solvent are evident.[23] In view of the above, new protocols need to be developed for the preparation of most of the vinyl monomers through biocatalytic path. To develop an effective anchor for the lipase, we have used hydrogels based on AAc and methacrylic acid (MAAc). These monomers were reacted with ethylene diamine to form poly(*N*-aminoethylacrylamide) [poly(*N*-AEAAm)] and poly(*N*-aminoethyl methacrylamide) [poly(*N*-AEMAAm)]. These two amides were consequently polymerized, respectively, in the presence of AAc and MAAc as the second component and *N*,*N*-methylene bisacrylamide [*N*,*N*-MBAAm] was used as crosslinker. The resulting hydrogels possess diverse properties as compared with poly(AAc) and poly(MAAc), and are thus, expected to be of interest in lipase immobilization. These hydrogels are biocompatible and can be synthesized in any shape or size. SEM, nitrogen analysis, and FTIR have been used to establish structure–property relationship of these networks. To evaluate the suitability of different polymers for use as lipase support, swelling of these hydrogels was studied as a function of time, pH, and in the presence of two surfactants (SDS and CTAB).

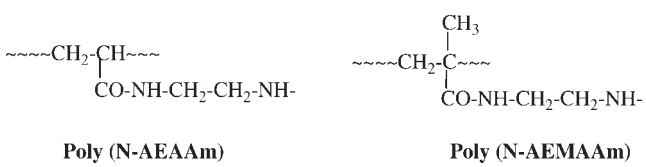
EXPERIMENTAL

Methods

Acrylic acid, methacrylic acid, ammonium persulphate, ethylene diamine, methanol, 1-propanol and 1-hexanol (S.D. Fine, Mumbai, India), *N*,*N*-methylene bisacrylamide, sodium dodecyl sulfate, and cetyltrimethylammonium bromide (Merck, Schuchardt, Germany), 1-nonanol, 1-dodecanol, (Across, Geel, Belgium) were used as received. Lipase was obtained from the Department of Biotechnology, Himachal Pradesh University, Shimla, India.

Amidation of acrylic acid and methacrylic acid

AAc (0.28 mol) and equimolar amount of ethylene diamine was mixed and allowed to stand for 30 min and the resultant amide was polymerized by heating at 60°C for 30 min in a controlled water bath in the presence of ammonium persulphate (1% of combined weight of AAc and amine used and predissolved in 2.0 mL of water). Poly(*N*-AEAAm) was precipitated by addition of cold water. It was filtered and dried in vacuum oven at 40°C. Poly(*N*-AEMAAm) was prepared in the similar manner from methacrylic acid. The structure of these polyamides is proposed as: poly(*N*-AEAAm) and (I) poly(*N*-AEMAAm) (II).



Poly (N-AEAAm) **Poly (N-AEMAAm)**

Preparation of hydrogels

Interpenetrating networks of poly(*N*-AEAAm)/poly-(AAc) and poly(*N*-AEMAAm)/poly(MAAc) were prepared by the following procedure. About 10.0 g of dried fine powder of poly(*N*-AEAAm) was dissolved in 20.0 mL of hot water. About 10.0 g of AAc was added to it along with *N*,*N*-methylene bisacrylamide [2.5% by weight of the total weight of the poly(*N*-AEAAm) and AAc) and ammonium persulphate (APS) 1% of the total weight of the monomer]. The reaction mixture was heated to 60°C for 30 min to ensure crosslinking. The hydrogel was treated with water in a soxhlet (to extract sol fraction), and dried at 30°C in a vacuum oven. The extraction and drying cycles were repeated to obtain constant weight. Poly(*N*-AEMAAm) was modified in the similar manner with MAAc.

Characterization of polymers

Different polymers were characterized by using SEM (taken on Jeol JSM-6100 scanning electron microscope); FTIR spectra (recorded on Perkin–Elmer and

Nicollete FTIR Spectrophotometers in KBr). Nitrogen analysis was carried on Carlo Erba EA-1108 which is equipped with a pneumatic auto sampler and a PC-based computer data system. The elements present are detected through a thermal conductivity detector.

Swelling studies

The hydrogels (0.1 g) were immersed in water in a temperature-controlled bath (accuracy ± 0.1°C). Water uptake was measured gravimetrically at different time and temperatures to optimize these two variables. All the hydrogels show equilibrium swelling at 480 min and at 35°C. The effect of pH and time on the swelling behavior of hydrogels was also studied in the presence of sodium dodecyl sulfate (SDS) and cetyl trimethyl ammonium bromide (CTAB). All the experiments were carried in triplicate. Percent swelling (Ps) of the hydrogels were calculated by applying following formula:

$$P_s = \frac{\text{Weight of the swollen hydrogel} - \text{Weight of the dry hydrogel}}{\text{Weight of the dry hydrogel}} \times 100$$

Immobilization of purified lipase

Purified lipase was immobilized on hydrogels by taking 10 g of the fine powdered matrix immersed in 50 mL of Tris-buffer (0.05*M*, pH 8.5) for 4 h, followed by filtration. Then the matrix was mixed with equal weight of the purified lipase and incubated overnight below 8°C. Lipase assay was performed by colorimetric method.[24] Solution of *p*-nitrophenol palmitate (*p*-NPP) (20 m*M*) was prepared in HPLC grade isopropanol. The reaction mixture comprised of 75 μL of *p*-NPP stock solution, 2–10 μL of free or immobilized enzyme (in mg) and Tris-buffer (0.05*M*, pH 8.5) to make final volume 3 mL. Appropriate control with a heat inactivated enzyme (5 min in boiling water bath) or SDS-treated enzyme (in duplicate) was included with each assay. The absorbance of *p*-nitrophenol released was measured at 410 nm (Shimadzu UV/visible spectrophotometer). Concentration of *p*-nitrophenol released from *p*-NPP was determined from a reference curve of *p*-nitrophenol. Lipase activity was defined as m*M* of *p*-nitrophenol released by 1 mL of free enzyme or 1 g of immobilized enzyme at 45 or 55°C for free or immobilized enzyme, respectively. Specific lipase activity was expressed as the μmole(s) of the *p*-nitrophenol released per min by 1 mg of the protein.

Effect of external environment on the activity of immobilized lipase

Lipase activity was studied for the effect of some detergents on the activity of immobilized lipase by incubation of 50 mg of matrix in each of the detergent solution (10% w/v) for 20 min at 55°C. The effect of pH was studied on hydrogel matrix (1%) by varying the pH from 5.7 to 10. The effect of temperature in the range from 35 to 65°C by incubation in water bath under shaking (100 rpm) and the activity in the supernatant was estimated at specified intervals. The solvent effect on hydrolytic activity of the immobilized lipase was studied by incubating 50 mg of matrix in 1 mL of solvent for 20 min at 55°C.

Esterification reactions using immobilized lipase

The esterification of AAc and MAAc with methanol, 1-propanol, 1-hexanol, 1-nonanol and 1-dodecanol was carried out using immobilized lipase. The esterification reaction were carried in Mettler Autochem chemical reactor at 50°C using equimolar amounts (0.625 mol) of the reactants (AAc/MAAc and an alcohol) in the presence of 200 mg of the lipase immobilized matrix. The progress of the reaction was checked by alkali titration of 1 mL of the reaction mixture after every 30 min (up to 240 min). Because of ester formation, the reaction system formed two layers with time. On completion of the reaction, whole reaction mixture was treated with alkali to neutralize the residual acid. The total ester formed was separated from the upper layer of reaction system mixture and it was instantly polymerized using APS (1%) at 60°C for 30 min. Control experiments were carried in the absence of immobilized lipase for all the esterification reactions.

RESULTS AND DISCUSSION

Amidation of AAc and MAAc with a variety of amines is a simple acid base reaction resulting in the generation of amide functional group—an effective anchor for the protein adsorption. The reaction is facile even at low temperatures. Iizawa et al.[25] have reported the synthesis of thermosensitive poly(*N*-alkylacrylamide) gel by amidation of poly(AAc) gel. Hydrogels prepared by the present scheme are novel and not reported in literature. The characterization of the amides and crosslinked hydrogels was carried out by physical and chemical methods.

TABLE I
Nitrogen Analysis of Polymers

Polymer	Weight (mg)	%*N*
Poly (*N*-AEAAm)	2.910	10.6
Poly(*N*-AEAAm-*co*-AAc) -*cl*-*N*,*N*-MBAAm	1.804	8.72
Poly (*N*-AEMAAm)	1.980	8.12
Poly (*N*-AEMAAm-*co*-MAAc)-*cl*-*N*,*N*-MBAAm	2.100	7.03

Characterization of hydrogels

Nitrogen analysis

The percent of nitrogen in both the amides was analyzed. A comparison of the *N* present in a known weight of each polymer analyzed is given in Table I. Poly(*N*-AEAAm) has 10.6% *N*, while it is 8.72% *N* in poly(*N*-AEAAm-*co*-AAc)-*cl*-N,*N*-MBAAm. Similarly, in poly(*N*-AEMAAm) and poly(*N*-AEAAm-*co*-AAc)-*cl*-*N*,*N*-MBAAm, *N* was found to be 8.12 and 7.03%, respectively. From the lower %*N* in the hydrogels, it comes out that in the network amount of the poly-(AAc) and poly(MAAc) is higher than that of the amide. The amount of the acid was also higher in the feed.

FTIR spectra of polymers

FTIR spectrum of poly(*N*-AEAAm) shows peaks at 3372 cm^{-1} (*N*—H stretching), and 1645 cm^{-1} (C=O stretching of amide), suggesting full conversion of the AAc [Fig. 1.(a)], while its hydrogel with AAc [poly(*N*-AEAAm-*co*-AAc)-*cl*-*N*,*N*-MBAAm] has the respective peaks at 3364 and 1636 cm^{-1} [Fig. 1(b)]. In the spectrum of poly(*N*-AEMAAm) [Fig. 1(c)], these peaks appear at 3416 and 1646 cm^{-1} and in its hydrogel [poly(*N*-AEMAAm-*co*-MAAc)-*cl*-*N*,*N*-MBA Am] [Fig. 1(d)] these peaks appear at 3417 and 1640 cm^{-1}, apart from the usual peaks. The presence of the characteristics peaks in the respective polymers shows that the functionalization of AAc/MAAc by amidation, and later by the network formation with AAc/MAAc has taken place.

Scanning electron micrography

Scanning electron micrography (SEMs) of different polymeric hydrogels are shown in Figure 2(a,b). In the SEMs of both the hydrogels surface is seen continuous due to the film formation. It results from extensive crosslinking and that result from the reaction of residual amino groups of the amide and acid

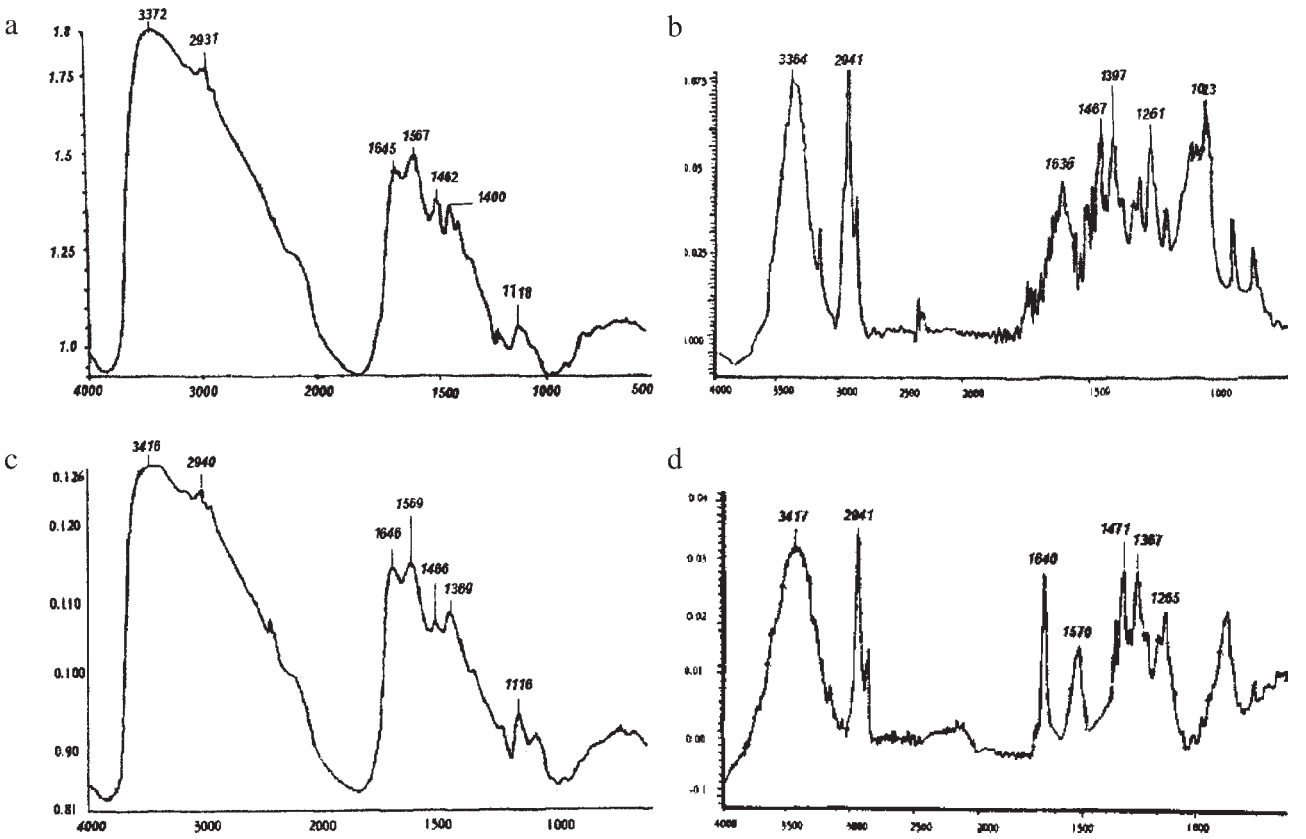


Figure 1 (a) FTIR spectrum of poly(*N*-AEAAm). (b) FTIR spectrum of poly(*N*-AEAAm-*co*-AAc)- *cl*-N, *N*-MBAAm. (c) FTIR spectrum of poly(*N*-AEMAAm). (d) FTIR spectrum of poly(*N*-AEMAAm-*co*-MAAc)-*cl*-N,*N*-MBAAm.

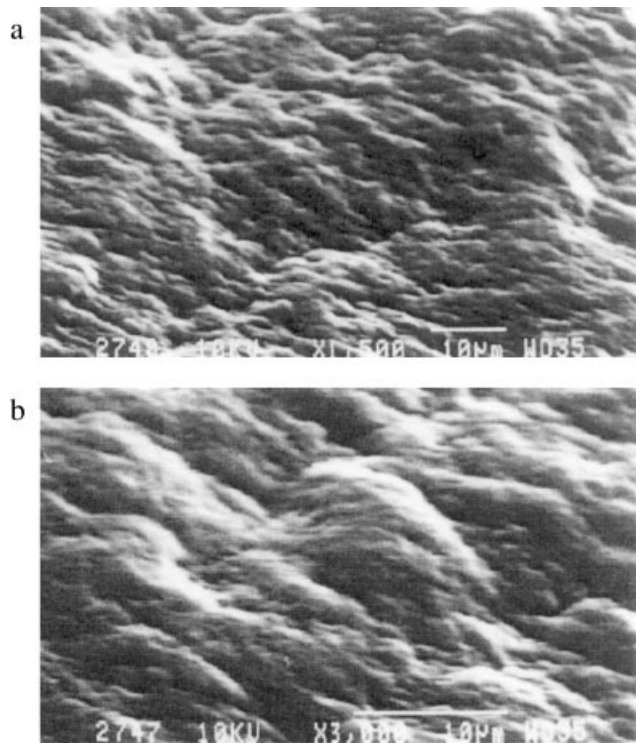


Figure 2 (a) SEM of poly(*N*-AEAAm-*co*-AAc)-*cl*-N,*N*-MBAAm. (b) SEM of poly(*N*-AEMAAm-*co*-MAAc)-*cl*-N,*N*-MBAAm.

moiety of AAc/MAAc during the network formation. The consequence is of importance as the resultant pore size of the hydrogel is small. Thus, lower swellability of these hydrogels in water is expected despite evident large polar adsorbent surface area.

Swelling studies

Despite being hydrophilic these hydrogels absorb less water, mainly due to the intense crosslinking and consequent small pore size of the networks. Apart from these structural aspects of the hydrogels, environmental factors also affect swelling behavior of these hydrogels.

Effect of time and pH

On variation of pH (4.0, 7.0 and 9.0) at 35°C, at pH 7.0 swelling increases with time [Fig. 3(a,b)]. At the low pH, shriveling of the hydrogels was observed, which results by the suppression of any ionized carboxylic groups from the strong hydrogen bonding and interpolymer complexation between side chains. Result is formation of a transient hydrogel of the hydrogen-bonded complex at the side chains as reported for the poly(AAc) graft copolymer.[26] Such complex formation makes the carboxylic functional groups less available for hydrogen bond formation with surrounding water. In the alkaline (pH 9.2), the hydrogels interact with the basic species of the medium, restricting the interaction of water molecules with those of acids, as a result the expected dipole–dipole interactions of carboxylic groups and water are suppressed.

Effect of surfactants

Water uptake studies were carried in the presence of two surfactants at 35°C and different time intervals from 30 min to 720 min at pH 7.0 [Fig. 3(c–f)]. SDS and CTAB affect water uptake by hydrogel by micelle formation.[27] Swelling increases with time. P_s increases dramatically in the presence of SDS, especially, at 8.1 m*M* and in case of CTAB also, the maximum P_s was observed at 0.92 m*M*. These are reported CMC values of the two surfactants at 25°C. The reported CMC values at 35°C for SDS and CTAB are respectively, 8.23[28] and 1.05.[29] The far higher swelling in the presence of SDS suggests that there is interaction of the $—CO_2H$ groups of the hydrogel with anionic surfactants. It is reported that in the presence of anionic surfactants, the solutions exhibit a dramatic increase in the solution viscosity around the CMC, which is attributed to the interpolymer crosslinking through the formation of mixed micelles involving the hydrophobes from different polymer chains and the surfactant molecules. The viscosity enhancement increases with the increase of hydrophobicity of the hydrophobe and decreases with the increasing AAc incorporation in the polymer.[30] As a result of increasing surfactant concentration in solution, the hydrophilic associates of the surfactant micelles with poly(acid) are formed. Macroscopic phase separation in poly(AAc) networks following the absorption of CTAB/chloride from the aqueous solutions has been reported.[31] At the critical aggregation concentration of the surfactant, micellar aggregates form on the polymer and interact with the alkyl side chains grafted on the hydrogel, leading to physical crosslinking and gelation.[32] On the other hand, the swelling behavior of the reference polymers, i.e., poly(AAc)-*cl*-*N*,*N*-MBAAm and poly- (MAAc)-*cl*-*N*,*N*-MBAAm is not affected by the presence of surfactants.[33] It is proposed that ethylene ($—CH_2\ CH_2—$) group in the hydrogels from the ethylene diamine, imparts adequate hydrophobic character for interaction of the hydrogels with the surfactant to enable micelle formation at the CMC. While in case of the reference, micelle formation is not possible due to higher hydrophilicity of the networks.

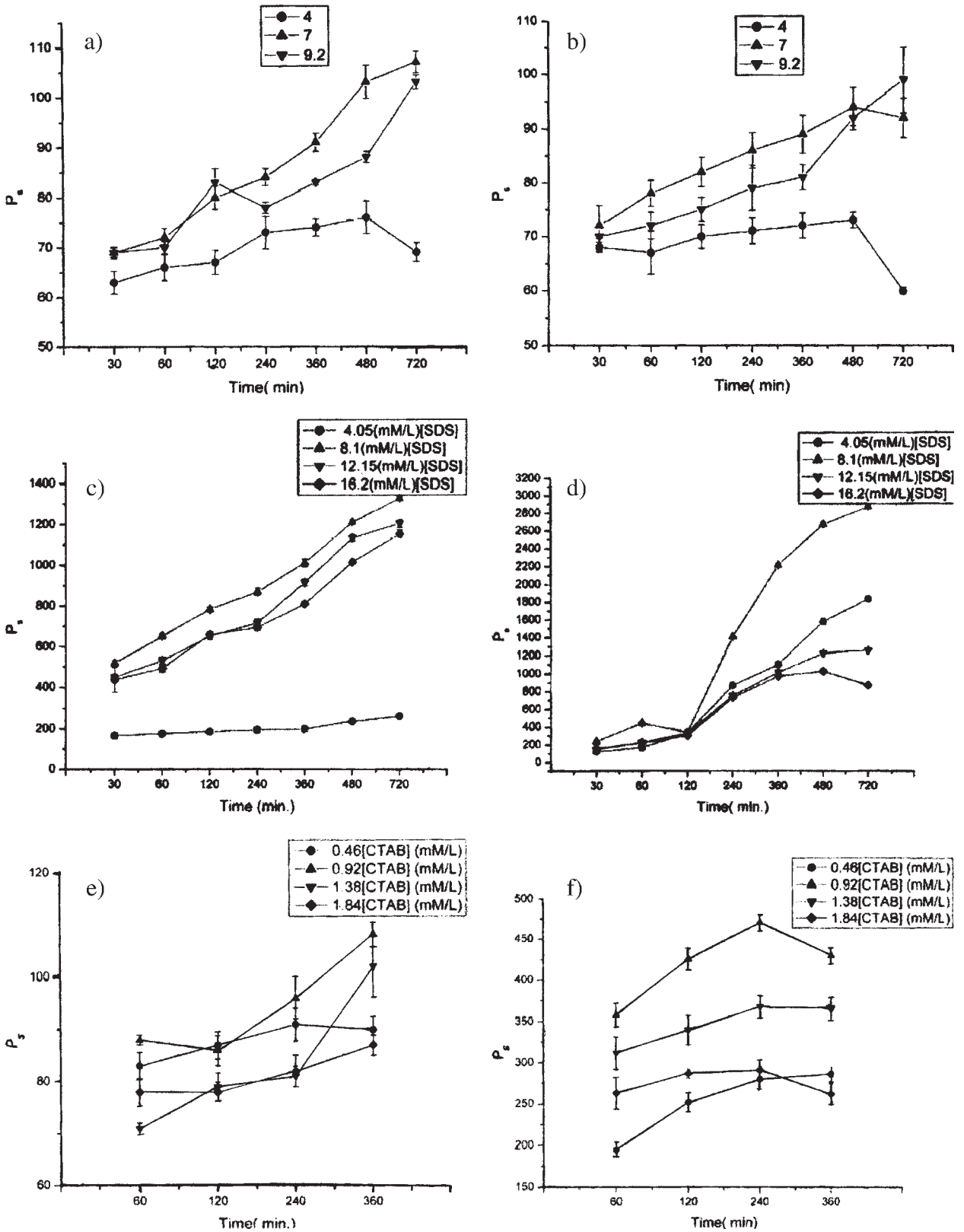


Figure 3 (a) P_s of poly(*N*-AEAAm-*co*-AAc)-*cl*-N,*N*-MBAAm as a function of time at 35°C. (b) P_s of poly(*N*-AEMAAm-*co*-MAAc)-*cl*-N,*N*-MBAAm as a function of time at 35°C. (c) P_s of poly(*N*-AEAAm-*co*-AAc)-*cl*-N,*N*-MBAAm as a function of time and SDS at 35°C. (d) P_s of poly(*N*-AEMAAm-*co*-MAAc)-*cl*-N,*N*-MBAAm as a function of time and SDS at 35°C. (e) P_s of poly(*N*-AEAAm-*co*-AAc)-*cl*-N,*N*-MBAAm as a function of time and CTAB at 35°C. (f) P_s of poly(*N*-AEMAAm-*co*-MAAc)-*cl*-N,*N*-MBAAm as a function of time and CTAB at 35°C.

TABLE II
Immobilization Characteristics of Hydrogels

Hydrogel series	Protein binding efficiency (%)	Total activity increase/decrease efficiency (%)	Adsorption coefficient (mg of protein/g of hydrogel)
Poly(*N*-AEAAm-*co*-AAc)-*cl*-*N*,*N*-MBAAm	95.5	+9.5	525
Poly(AAc)-*cl*-*N*,*N*-MBAAm	88.8	+2.2	520
Poly(*N*-AEMAAm-*co*-MAAc)-*cl*-*N*,*N*-MBAAm	90.7	+2.9	500
Poly(MAAc)-*cl*-*N*,*N*-MBAAm	85.5	−2.0	500

Total activity and protein binding efficiency

Total % activity increase/decrease, % protein binding efficiency and adsorption coefficients for different matrices are shown in Table II. Poly(*N*-AEAAm-*co*-AAc)-*cl*-*N*,*N*-MBAAm shows maximum activity, protein binding efficiency and adsorption coefficient at pH 5.7 and 55°C. Hence, it was selected for use in the further studies. The lipase activity of this hydrogel/support is understandable from the structural aspects and the forgone discussion on its water uptake behavior, especially, in the presence of the surfactants, and the understandable compatibility with lipase. Hence, formation of micelle with lipase is also proposed. The immobilization though is a surface phenomenon, yet the interactions between lipase and support are strong enough, as is evident from the loss of only 50% activity even after the tenth cycle.

Effect of immobilization conditions on lipase activity

The free lipase shows maximum activity of 38.2 U with protein concentration of 0.264 μg/mL in both at 48-h post inoculation at pH 8.5 [Fig. 4(a)], and hence, lipase is of alkaline nature and it shows higher activity at lower pH. The lipase protein was optimally precipitated at 60% (w/v) ammonium sulfate saturation. The precipitates were reconstituted in minimum volume of 0.05*M* Tris buffer and specific activity 279.1 U/mg was extensively dialyzed against the same buffer. The dialyzed enzyme shows an activity of 168 U/mg. On immobilization on poly(*N*-AEAAm-*co*-AAc)-*cl*-*N*,*N*-MBAAm under standardized conditions, the immobilized lipase possessed optimal catalytic activity in *n*-hexane at pH 5.7 and 55°C under assay conditions [Table III and Fig. 4(b)]. The activity increase with temperature shows that lipase molecule unfolds and at the higher temperatures and its conformational stability increases. The half-life (50%) of the immobilized preparation was observed even after tenth cycle of reuse. Lipase activity decreases in the presence of detergents (Table IV). The surfactant and lipase both being compatible with the hydrogel/support competes for active binding on the adsorption sites. However, due to the bigger size of lipase it is less adsorbed compared with the surfactant molecule, hence the observed results.

Kinetics of monomers synthesis using immobilized lipase

The extent of water uptake behavior observed with different surfactants makes these hydrogels suitable

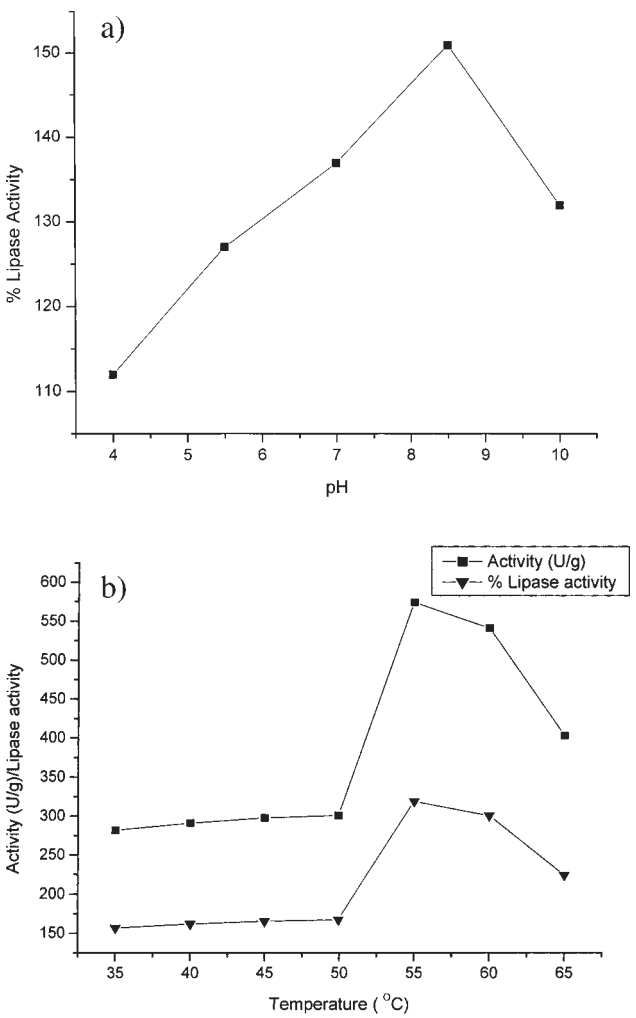


Figure 4 (a) Effect of pH on the activity of immobilized lipase. (b) Effect of temperature on the activity of immobilized lipase.

TABLE III
Effect of Organic Solvents on Activity of Immobilized Enzyme (Tris Buffer 0.05*M*, pH 8.5, 20 min, 55°C)

	A_{410}	Lipase activity (%)
Alcohols		
Methyl alcohol	0.583	106.7
Ethyl alcohol	0.611	112.0
n-propyl alcohol	0.964	188.0
Iso-propyl alcohol	1.002	192.0
n-butyl alcohol	0.618	113.0
iso-butyl alcohol	0.669	123.0
iso-amyl alcohol	0.524	96.4
n-octyl alcohol	0.609	123.0
m-cresol	0.580	107.0
Decyl alcohol	0.781	131.0
1-dodecanol	0.610	112.0
Cyclohexanol	0.600	110.0
Hexanol	0.608	111.0
None	0.544	100
Alkanes		
Cyclohexane	0.872	138.0
n-hexane	1.496	292.0
1-chloro-2,3-epoxypropane	0.382	50.0
Hexadecane	0.994	192.0
n-nonane	0.785	132.0
Octadecane	1.004	195.0
None	0.544	100.0

supports for use as biocatalysts. The immobilized lipase was used to synthesize a number of esters with reactive vinyl functionality. In the present case, not only the use of nonbenign chemicals and solvents is avoided, but very high reactant conversion (85–98%) has also been achieved. It has been observed that the extent of esterification decreases with an increase in the chain length of the alcohol both with AAc and MAAc [Fig. 5(a,b)]. The extent of esterification for AAc and MeOH is 97.36% within 4 h, whereas it is 72.22% for AAc and 1-propanol, 62.5% for AAc and 1-hexanol, 66.67% for AAc and 1-nonanol, and 53.67% for 1-dodecanol in the same time. The nature of the acid also plays important role, as the extent of esterification is more with AAc than with MAAc for all the alcohols. For example, the extent of esterification for MAAc and MeOH is 77.27% in 4 h whereas it is 70% for MAAc and

TABLE IV
Effect of Detergents on Immobilized Enzyme Activity (Tris Buffer 0.05*M*, pH 8.5, Detergent 10% w/v, 20 min, 55 °C)

Detergent tested	A_{410}	Residual activity (%)
Tween-20	0.378	83.6
Tween-80	0.380	84.1
C-TAB	0.359	79.4
SDS	0.133	64.8
Triton X-100	0.257	56.9
None	0.452	100.0

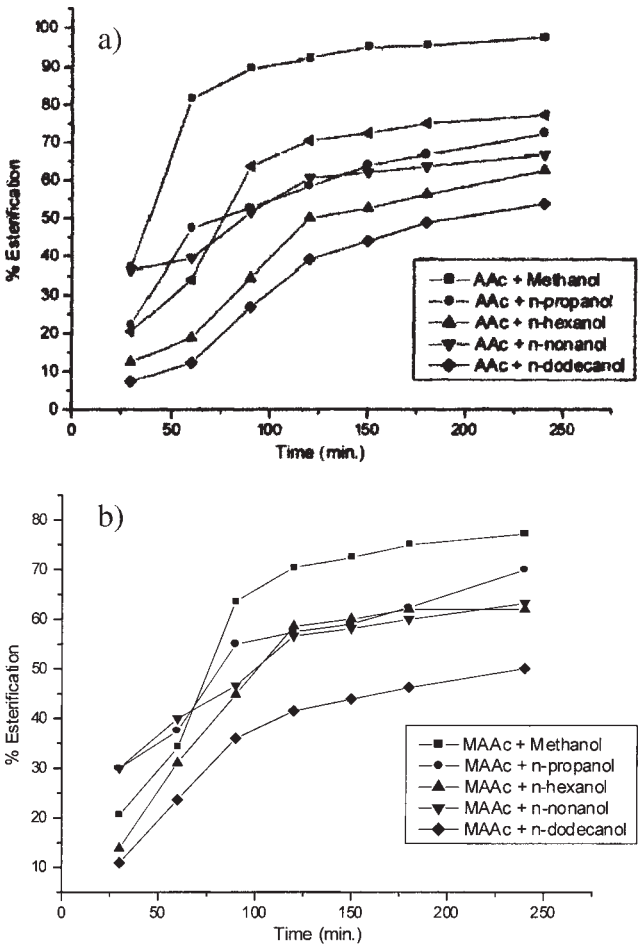


Figure 5 (a) Effect of the alkyl chain length of alcohols in the esterification of AAc using immobilized lipase on poly(*N*-AEAAm-*co*-AAc)-*cl*-N,*N*-MBAAm. (b) Effect of the alkyl chain length of alcohols in the esterification of MAAc using immobilized lipase on poly(*N*-AEAAm-*co*-AAc)-*cl*-N,*N*-MBAAm.

1-propanol, 62.06% for MAAc and hexanol, 63.33% for MAAc and nonan-1-ol, and 50.14% for MAAc and 1-dodecanol. The effect of the carbon chain of alcohols on the extent of reaction is as expected. (i) It is well established mechanistic aspect of the esterification reactions that lower the carbon chain of the (reactant) alcohol and acid, the higher is the extent of reaction due to the electronic reasons. (ii) All the alcohols are soluble in both the acids, though the solubility decreases with the chain length of alcohols. In case of the higher alcohols, with the progress of the reaction, phase separation was observed as along with the ester formed unreacted alcohol preferentially and successively partition. (iii) The lower conversion in case of the MAAc compared with AAc, is explained as $—CH_3$ group in MAAc offers steric hindrance and formation of the transition state is not been stabilized to the extent as is case with AAc.

Characterization of acrylates and methacrylates

Evidence of ester formation has been provided by FTIR of the ester and further by polymerization of the monomer. FTIR spectra show that the monomer has its vinyl double bond intact (around 1640 cm^{-1}) and C=O stretching of the ester bond appears around 1735 cm^{-1}, while free carboxylic and hydroxyl groups have not been observed. FTIR spectrum of methyl methacrylate (prepared from MAAc and methanol) shows peaks of importance as: 1733.4 cm^{-1} (C=O stretching of ester), 1147.8 cm^{-1} (symmetric stretching of O—C—O grouping of ester), 1637.5 cm^{-1} (C=C stretching of methacrylic group), along with other usual peaks. FTIR spectra of *n*-propyl methacrylate (prepared from MAAc and propan-1-ol) shown has peaks at 1726.2 cm^{-1} (C=O stretching of ester), 1187.5 cm^{-1} (symmetric stretching of O—C—O grouping of ester), 1634.1 cm^{-1} (C=C) stretching of methacrylic group), and its polymer shows prominent peaks at 1731.0 cm^{-1} (C=O stretching of ester), 1149.9 cm^{-1} (symmetric stretching of ester group), while the peak near 1640 cm^{-1} (C=C stretching of methacrylic group) is absent after polymerization. FTIR spectra of *n*-hexyl acrylate (prepared from AAc and *n*-hexyl alcohol) shows peaks at 1727.2 cm^{-1} (C=O stretching of ester), 1192.3 cm^{-1} (symmetric stretching of ester group), 1636.1 cm^{-1} (C=C) stretching of methacrylic group), while the later has peaks at 1738.0 cm^{-1} (C=O stretching of ester), 1165.4 cm^{-1} (symmetric stretching of O—C—O grouping of ester), and the peak near 1640 cm^{-1} (C=C stretching of methacrylic group) is absent. FTIR spectrum of *n*-hexyl methacrylate (prepared from MAAc and 1-hexanol), shows peaks at 1725.6 cm^{-1} (C=O stretching of ester), 1172.2 cm^{-1} (symmetric stretching of ester group), 1637.6 cm^{-1} (C=C) stretching of methacrylic group), and its polymer shows prominent peaks at 1729.9 cm^{-1} (C=O stretching of ester), 1151.3 cm^{-1} (symmetric stretching of OC—O group of ester), while the peak near 1640 cm^{-1} (C=C stretching of methacrylic group) is absent after polymerization. In the FTIR spectrum of *n*-nonanyl acrylate and its polymer peaks at 1727.3 cm^{-1} (C=O stretching of ester), 1188.5 cm^{-1} (symmetric stretching of ester group), 1637.0 cm^{-1} (C=C) stretching of methacrylic group), while the later shows prominent peaks at 1737.6 cm^{-1} (C=O stretching of ester), 1166.5 cm^{-1} (symmetric stretching of ester group), while the peak near 1640 cm^{-1} (C=C stretching of methacrylic group) is absent. In the FTIR spectra of *n*-nonanyl methacrylate and its polymer, the monomer shows peaks at 1731.5 cm^{-1} (C=O stretching of ester), 1166.3 cm^{-1} (symmetric stretching of ester group), 1638.0 cm^{-1} (C=C) stretching of methacrylic group), while its polymer shows prominent peaks at 1729.9 cm^{-1} (C=O stretching of ester), 1150.5 cm^{-1} (symmetric stretching of ester group), while the peak for C=C stretching is absent. FTIR spectra of *n*-dodecyl acrylate (prepared from AAc and 1-dodecanol) shows prominent peaks at 1735.9 cm^{-1} (C=O stretching of ester), 1178.7 cm^{-1} (symmetric stretching of group of ester), 1636.8 cm^{-1} (C=C) stretching of methacrylic group).

CONCLUSIONS

It follows from the foregone discussion that these hydrogels are smart or intelligent gels, as these respond quickly to even small changes in their external environment. Some inferences are drawn from the results of the water uptake behavior of these hydrogels, especially, their interaction with surfactants. One, water interaction is reduced due to the intensive crosslinking despite the presence of highly water interacting carboxylic and amide groups. Two, a large adsorbent area containing amide groups is present in these networks. Three, substantial hydrophobic character is present due to the incorporation of $—CH_2CH_2—$ groups from the amine. Four, considerable surfactant interactions and consequent micellization has been observed. All these four inferences make these matrices ideal supports for the lipase immobilization. The use of these supports for the immobilization of lipase to synthesize vinyl esters/monomers is an important step in the direction of clean synthetic tools and processes. The anticipated benefits of the present scheme are detailed as: (i) use of simple benign operating conditions was followed against the usual use of strong acids. (ii) Separation of water is not required as the hydrogel is good water sorbent; hence driving force for esterification is far higher. (iii) No solvent and auxiliary chemical was required, even most of the effective esterification by lipase are reported in organic solvents like hexane. (vi) High yields with high purity were obtained. (vii) And the scheme could be extended for the synthesis of other systems from other unsaturated substrates where the reactivity of the double bond is a concern. Thus, it follows that the hydrogels as lipase supports have been suitably tailored to immobilize lipase with high conformational stability and repeatability for use as biocatalyst to synthesize vinyl esters in high yield by following total green route. hydrogel is good water sorbent; hence driving force for esterification is far higher.

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