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Lipase immobilized on poly (vinyl alcohol) modified polysulfone membrane: application in hydrolytic activities for olive oil

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Abstract The advancement of membrane research closely relates to the activities of 'immobilization of enzymes'. The modification of polymeric membrane surfaces according to tailor-made specifications is considered an art and useful in this arena. In this study, lipase is immobilized on Polyvinyl alcohol photomodified Polysulfone (PS–PVA) membranes. The maximum immobilization (1.48 mg/cm^2) for PS–PVA membranes is achieved. The amount of immobilized lipase directly relates on the PVA content on the membrane. Scanning Electron Microscope and X-ray diffraction patterns show the evidences of lipase immobilization on membranes. The hydrolytic performances of lipase immobilized PS and PS–PVA–glu membranes for olive oil are studied. The free fatty acid (FFA %) and acid value (AV) parameters are determined by titrimetic analysis (1.53 and 3.04 for PS–PVA–glu) and compared with esterification GC-mass analysis data. The K_m and V_{max} values are 105 mM and 0.9 mM/min for lipase immobilized on PS–PVA–glu and 153.8 mM and 0.51 mM/min for lipase on PS. The reusability feature shows the lipase immobilized on PS–PVA–glu matrix have better stability (10.7% decrease) compared to lipase immobilized on PS matrix (33.3% decrease) after five cycles.

Keywords Lipase · Immobilization · Membrane · Modification · Olive oil

Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are of considerable importance in physiological and industrial arena [1, 2]. They play the key role in the metabolism

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of fats and catalyzing numerous reactions viz. hydrolysis, interesterification, esterification etc. In hydrolysis, the role of lipase is to catalyze the cleavage of carboxy ester bonds in mono, di, tri acyl glycerols to glycerol and fatty acids [3, 4]. Considering the applications, researchers are in a path to immobilize lipases on the solid matrix so that their mobility is restricted without loosing their activities. Immobilization is favored as it can easily control the enzymatic process, purity of the products and for its reusability feature [5, 6]. Moreover, there is the possibility of enzyme (i.e., lipase) in open form and capable of showing better activity in immobilized condition [7]. Among solid matrices, membranes (especially synthetic) are preferred for some advantages (viz. large surface area for immobilization, resistance to mechanical and chemical stresses and ease of preparation in different geometrical configurations).

There are various kinds of techniques to immobilize enzymes viz. entrapment (enzyme into the support matrix) [5, 8], covalent linkage (between enzyme molecule and support matrix) [3, 9] and adsorption (cross linking of enzyme molecules and onto the surface of the matrix) [6, 10]. In the present study, the adsorption and covalent attachment techniques are used to immobilize lipase on the asymmetric PS membrane. The modification of backbone polymers by certain monomers/polymers generates active functionalities on the membranes that show potentiality in different applications [11–14].

In this experiment, surface modification of Polysulfone (PS) membrane has generated the functionalities and capable to form covalent bond with immobilizing agent (glutaraldehyde) and consequently with lipase. Several techniques (viz. chemical treatment, photo-irradiation, high-energy radiation techniques) [15–21] can be employed for the surface modification. Photochemical technique is preferred here because of its simple nature and requirement of mild reaction conditions [22]. The relevance of this experiment can be correlated with the current trend of the studies are to find new modified membranes, which have biocompatibility and improved hydrophilicity and of efficient carriers of enzymes. It also shows the approach to get better immobilization of lipases employing covalent linkage compared to physical adsorption. The photomodification approach shows better method as there no other additives are involved to interfere with enzymatic process.

The hydrolysis of lipids and oils is an interesting process for the hydrolase enzyme (i.e., lipase) [23] as the process could help to overcome some of the drawbacks of high temperature process. Moreover, the lipase-immobilized membranes have the abilities to separate it out from the bulk phase (elimination of emulsification problems) and their large surface area of contact between two phases' oil and water are feasible. The hydrolytic activities (in different reaction parameters viz., temperature, pH, and substrate concentration) of immobilized lipases are compared. The free fatty acid (FFA %) and acid value (AV) are determined from the titremetic and GC-mass spectra. The kinetics of the hydrolytic reaction are analyzed and the parameters (K_m and V_{max}) from the Lineweaver–Burk and Hanes plot are determined.

Experimental

Materials

Polysulfone (Udel P-3500) (PS) (Solvay Advanced Polymers, USA), non-woven Polyester fabric (Filtration Sciences Corporation, USA) and dimethyl formamide (Qualigen, India) were used. PVA 14,000 (98–99% hydrolysed), glutaraldehyde, acacia powder from SD fine Chem, India, refined olive oil and BSA fraction V from SRL, India were procured. *Candida rugosa* lipase (EC 3.1.1.3) (M.P. Biomedicals, LLC, France), Folins reagent (Spectrochem, India) and Cu (II) chloride (Rankem, India) were used.

Techniques

The light induced experiment was carried out using the UV-lamp (Philips HPR-125 watt, Turnhout, Belgium). ATR-FTIR (Perkin Elmer Spectrum GX with a resolution ± 4 cm^{-1}, incident angle 45°) studies were performed to get the evidences of the newer functional groups, modified by PVA. X-ray diffractometer (X' PERT, Philips) (Cu Kα radiation as monochromator), scanning electron microscope (Leo, 1430UP Oxford instruments) were used to get the evidences of lipase immobilization on the membranes. The contact angles of PS and PS–PVA membranes (before and after immobilization) (in water) were measured by Tensiometer (DCAT 21, Data Physics, Germany). The instrumental parameters were set (motor speed 0.1 mm/sec and dipping length 5 mm).

Porometric studies were carried out to characterize the unmodified PS membrane (thickness 40–45 μm casted on polyester fabric $\sim$90 μm) by Capillary Flow Porometer (Porous Materials Inc, USA, Model 1500 AEX), considering the pores as capillaries. The details were described elsewhere [24]. The characterization of the PS membrane was determined in terms of bubble point pressure 0.41 MPa and diameter at maximum pore size distribution 0.066 μm.

The pure water permeability of the membranes were tested and compared at 0.19 MPa by Sepa-200 dead end filtration arrangement. The permeability and photo-irradiation arrangements were sketched in our previous experiments [14, 25].

Protein estimation was done by UV–Vis Spectrophotometer (Varian, Carry 500Scan, USA). The standard method using Folin Ciocalteu's Phenol reagent (2 N) was employed to get the amount of immobilized lipase on membranes [26].

GC-mass analysis of fatty acid esters, produced in hydrolytic reaction

The titremetric analysis of free fatty acids (produced from hydrolysis) was done by sodium hydroxide solution. The conversion of free fatty acid to its methyl esters was done for the GC-mass analysis. AOCS Official method Ce 2-66 [27] (described later) was followed for the conversion. The analysis was based on fatty acid methyl ester mixtures 18919-1AMP C4-C24 (Sigma Aldrich, USA). GC mass QP 2010 (Shimadzu) using RTX-5 column (length 30 m, id. 0.25 mm, film thickness 0.25 μ) was used. Injector and detector temperatures were set at 240 and 200 °C,

respectively. Oven temperature program was 40 °C (3 min)–5 °C/min–240 °C (40 min). Helium carrier gas was used. The column flow rate was kept at 1 ml/min. Samples (0.2 μl) were injected in split (1:30) mode.

Methods

Preparation of asymmetric Polysulfone membrane and their modification

PS solutions were prepared (in dimethyl formamide, 15% w/w) through slow dissolution 5–6 h at 40–45 °C. The viscous polymer solutions (in DMF) were spread into a thin film on the non-woven polyester fabric (1 m) and immediately immersed in non-solvent medium (water), mixed with sodium lauryl sulfate. The membrane formation process was done using a proto-type casting machine. The membranes were kept in the gelation bath for at least 3 h to complete the phase inversion process. The membranes were washed and dried.

Two different concentrations (0.5 and 2% in water) of PVA were spread on to PS membranes (asymmetric side) fitted on glass tray for different duration. The details of the experimental conditions are in Table 1. The membrane samples were placed at 20 cm distance from the light (300–400 nm). The experiment was carried out at ambient temperature. The radiation density flux on all the membrane surface area was assumed constant in each run. The solutions from the membrane surface were decanted and photo-irradiated 10 min at ambient conditions. PVA concentrations were measured by formation of PVA–Cu(II) complex. The absorption maximum was observed at 623 nm. The concentrations of PVA solutions (pre and post dipping of the membranes) were determined by relating the standard curve of PVA–copper complex in alkaline medium. The total amount (mg) and amount per unit area (mg/cm^2) on the membranes were calculated from the difference of concentrations of PVA (pre and post dipping the membranes).

Table 1 Pure water permeability of membranes and variation of amount of lipase immobilization with amount of PVA

Membranes	Concentration of PVA (%)	Dipping time (min)	Amount of PVA on the surface (mg/cm^2)	Pure water permeability ($lm^{-2}h^{-1}$) before immobilization	Amount of lipase immobilized (mg/cm^2)
PS virgin[a]	–			82.4	0.29
Memb-I	0.5	10	0.65	46.8	0.63
Memb-II	0.5	30	1.06	31.6	0.82
Memb-III	0.5	60	1.12	15.1	0.87
Memb-IV	2	10	1.7	8.6	0.77
Memb-V	2	30	4.6	4.6	1.48
Memb-VI	2	60	4.8	3.3	1.47

[a] Without glutaraldehyde

Immobilization of lipase on the membranes

The immobilization of lipase on membranes with or without immobilizing agent (glutaraldehyde) was carried out. At first, membranes (modified and unmodified of area 9.1 cm^2) were impregnated in aqueous solution of glutaraldehyde for 4 h. The samples were removed from glutaraldehyde. Then, lipase solution (10 ml) [(2 mg/ml; 150 LU/ml for olive oil substrate) in 0.1 M of phosphate buffer at pH 7] was spread. It was kept under shaking condition for 12 h at 10 °C. Membranes were taken out from lipase solution and washed with deionized water to remove loosely adhered lipase from the membrane surface. The immobilization of lipase was done without using glutaraldehyde by the same manner. The immobilization was done on the asymmetric or modified surface of the membranes.

The amount of lipase immobilized on the membrane was estimated using the method proposed by Lowry et al. [26]. The standard curve at 750 nm was plotted using BSA standard protein. The amount of immobilized lipase was determined from the difference of lipase concentration in the solution before and after immobilization.

Hydrolytic activities of free and immobilized lipase

The hydrolytic reaction of olive oil using free and immobilized lipase was carried out. At first, the reaction mixture of 5 ml olive oil emulsion (olive oil + gum acacia + sodium benzoate and 5 ml 0.1 M phosphate buffer) was prepared [28]. Free lipase (1 ml) or lipase bound membranes in to the reaction mixture was added. The reaction conditions were kept at 37 °C and pH 8. Acetone–methanol (1:1) mixture was added to cease the reaction. The amount of free fatty acid released during hydrolysis was measured by titrimetric determination using 0.025 N NaOH solution. The effects of various reaction parameters viz. pH, temperature on hydrolysis at different substrate concentrations were studied.

Esterification of released fatty acid

The further evidences of released fatty acid were also proved by esterification. 4 ml ethanolic NaOH (0.5 N) solution was added to the hydrolytic mixture (5 ml) and two immiscible layers viz. aqueous and organic phase were formed. Free fatty acid (mainly oleic acid) in aqueous phase and tri, di and mono glycerides in the organic phase were present in the mixture. The aqueous phase was evaporated to its mass and BF_3-methanol reagent was added to it employing AOCS Official method Ce 2-66 [27].

Results and discussion

Preparation of asymmetric membranes and their modification

Asymmetric membrane formation is recognized as a phase separation process in which a homogeneous polymer solution is transformed in to a two-phase system i.e.,

a dense polymer rich phase, which forms the membrane structure and a polymer poor phase that forms the membrane pores. The entire phase separation process is due to dimethyl formamide (solvent)/nonsolvent (water) exchange during the gelation step. This structure formation process is often referred as a wet phase inversion process [29–31]. Modification of PVA is done by using environment benign photo-irradiation technique. It is favored over the chemical method to avoid traces of other chemical compounds (initiators) on the membrane. The evidences of PVA onto PS membrane are evaluated by FTIR–ATR spectroscopy [32, 33].

The most important ν_{OH} band (for the PS–PVA membrane) in 3,100–3,600 cm^{-1} is observed in Fig. 1b, c, d. The peak intensity increases with the presence of polyvinyl alcohol where as the peak is absent for the virgin PS membrane (a). The other characteristic peaks at 2,941 cm^{-1} ($-CH_2$), 1.427 cm^{-1} (O–H group coupled to CH_2 stretching) and 1,093 cm^{-1} to the C–O bond in the spectra (b, c, d) are also observed.

The contact angle decrease suggests that the modified surfaces have developed hydrophilicity. The mean contact angle of the modified membranes is low (viz. for Memb-V 81.37° containing 4.6 mg/cm^2 PVA) compared to the virgin PS membrane 84.48°. The pure water permeabilities of the membranes are in ensemble (Table 1). It shows that modified membranes are of low water permeabilities compared to PS virgin membrane and the order follows with the PVA contents on the membrane. The water permeability order is as follows (PS virgin > Memb-I > Memb-II > Memb-III > Memb-IV > Memb-V > Memb-VI). The low water permeabilities signify the partial pore blocking due to the modification and the permeability decreases as the content of PVA increases on the membrane.

Immobilization of lipases

The asymmetric nature of the virgin PS membrane facilitates the simple adsorption of lipase. As glutaraldehyde results strong binding and probability of enzyme, leakage is low compared to simple adsorption [24], the adsorption and crosslinking of lipase by glutaraldehyde on PS (PS–glu) membrane has showed higher immobilizing trend with respect to simple adsorption without glutaraldehyde. The

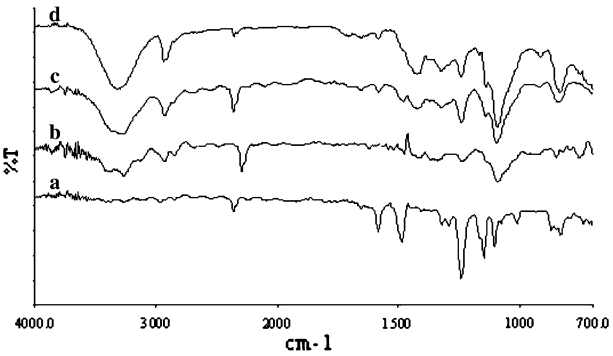


Fig. 1 FTIR–ATR spectra of membranes **a** PS virgin, **b** Memb-IV, **c** Memb-V and **d** Memb-VI

glutaraldehyde technique is different from simple adsorption. The attachment of lipase with –CHO groups of glutaraldehyde that is already attached to membrane surface. The asymmetric character of the membrane also helps to anchor the glutaraldehyde. The amount of lipase immobilization on PS–PVA membranes is higher (0.57 mg/cm^2) than hydrophobic PS virgin membranes (0.29 mg/cm^2). There is the possibility of covalent attachment of lipase with the carbonyl group possessed inherently (presence of polyvinyl acetate) or may be from photo oxidation of PVA apart from adsorption. The occurrence contradicts the general trend that the hydrophilic surface hinders the immobilization. However, the lipase immobilization on PVA modified surface is better than PS, but it is less compared to PS–glu (0.72 mg/cm^2). In case of PS–PVA–glu membranes one –CHO functionality of bi-functional glutaraldehyde binds with –OH of PVA attached to PS membranes and $-NH_2$ functional group of lipase in the other end. Thus, it establishes the covalent attachment of lipase with the membrane matrix through the bridging of PVA–glutaraldehyde similar to report by Araujo et al. [34]. The order is as follows PS–PVA–glu (Memb-V) > PS–glu (2.5%) > PS–PVA (without glutaraldehyde) > PS virgin (Table 2). The reaction with PVA and glutaraldehyde is as in Scheme 1.

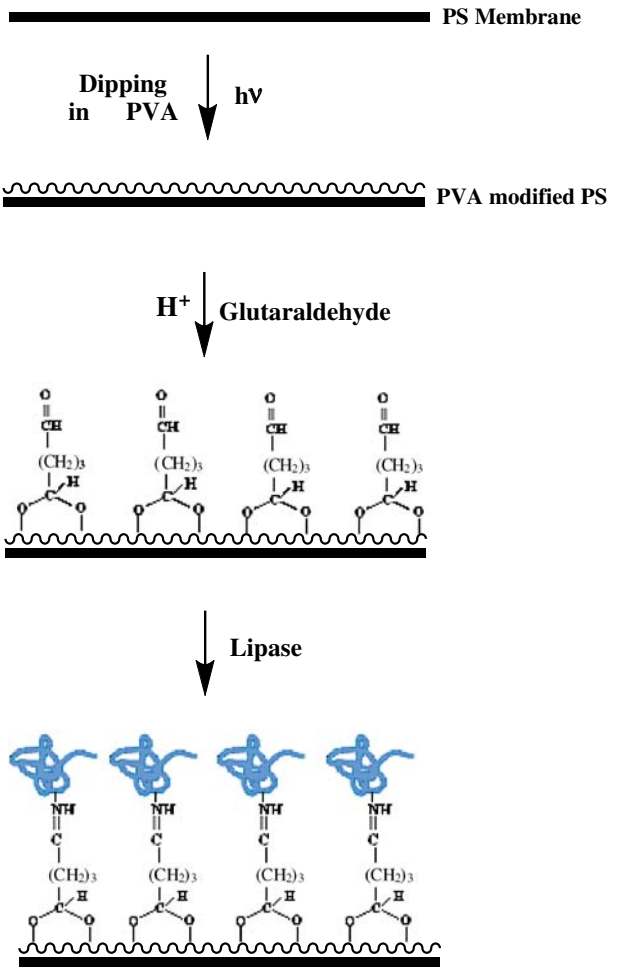
The amount of PVA on PS increases with dipping time and concentration (Table 1). The amount of immobilization depends on PVA content. The saturation amount of lipase immobilization is appeared at 30 min and PVA (2%). The glutaraldehyde concentration is influenced by the immobilization for the virgin PS as well as PS–PVA membranes (2%, 30 min dipping time) and expected to follow the similar trend for other PS–PVA membranes. The immobilized amount is reached its saturation with 2.5% glutaraldehyde (Table 2).

Evidences of immobilization from analytical tools

The immobilization of lipases is proved by different analytical tools. Figure 2 shows the evidences of immobilization by X-ray diffraction. The peaks in XRD patterns of virgin PS membranes are due to polyester fabric, used as support. The XRD patterns of lipase immobilized membranes have showed that there is the development of another peak due to lipase. There is no indication of developing peak for PS–PVA–glu

Table 2 Variation of amount of lipase immobilization, using different concentration of glutaraldehyde

Membranes	Glutaraldehyde used (concentration, %)	Amount of lipase immobilized (mg/cm^2)
PS (virgin)	0	0.29
	1	0.63
	2.5	0.72
	5	0.71
PS–PVA (2%, dipping time (30 min)	0	0.6
	1	1.38
	2.5 (Memb-V)	1.48
	5	1.44



Scheme 1 Steps of lipase immobilization on PS–PVA using glutaraldehyde

membrane. The XRD pattern (of lipase immobilized PS–PVA–glu) is shown greater peak intensity with respect to lipase immobilized PS membrane as the content.

The evidence of immobilization is visually observed from the micrographs (Fig. 3a, b). It is seen that distinct spots of protein aggregates throughout membrane surface 3b (Membrane V of maximum content of lipase). The decrease in contact angle of membranes (81.37–79.08° refer to Memb-V) suggests that the lipase immobilized surface is relatively hydrophilic compared to the parent one (unimmobilized).

Hydrolytic activities of free and immobilized lipases

This is related to the conversion of triglycerides in to the constituent fatty acid and glycerol in presence of water. Lipases are capable of hydrolyse acylglycerides. The

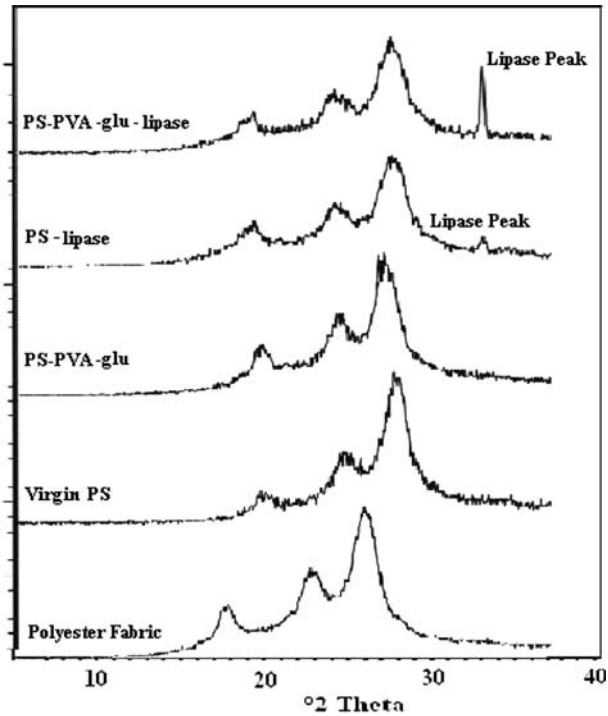


Fig. 2 XRD pattern of membranes

hydrolytic activity of biocatalytic membranes is expressed in terms of units of lipase activity per unit membrane area (U/cm^2). The immobilized *Candida rugosa* lipases on virgin PS and PS–PVA–glu (Memb-V) membranes from different loaded membranes are chosen for their hydrolytic activities.

The activity yield (%) for the hydrolysis (as shown in Eq. 1) is studied by varying pH of the hydrolytic reaction from 5 to 9 and depicted in Fig. 4. The optimum pH for both the immobilized lipase is 8. The pattern is similar for both the membranes, but the activity yield (%) for the PS–PVA–glu is higher than PS virgin membrane.

$$\text{Activity yield } (\%) = \frac{\text{Hydrolytic activity of immobilized lipase} \times 100}{\text{Total hydrolytic activity of free lipase}} \quad (1)$$

Figure 5 shows the variation of activity with the temperature (10–50 °C). The activity yield shows its maximum at 37 °C for both the membranes. The effect of substrate concentrations on the activities of immobilized lipase for hydrolysis is presented in Fig. 6. The pattern is similar and found maximum at 150 mM substrate concentration for both membranes. The activity yield slightly decreases above 150 mM. This may be due to drop in the rate of lipolysis at higher concentration of substrate and enzyme inhibition [35].

The parameters (free fatty acid (FFA) and acid value (AV)) are determined from the following expression. The FFA is the percentage by weight of free acid groups

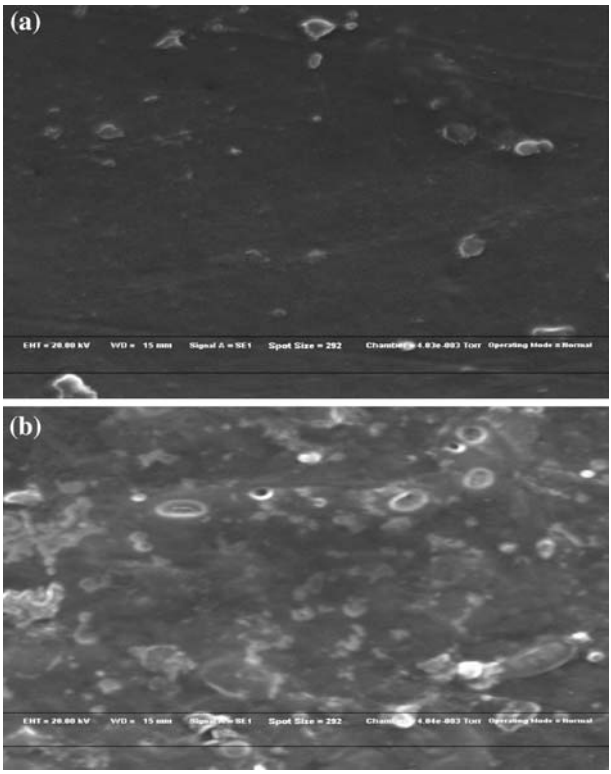


Fig. 3 Scanning Electron Micrograph of membranes **a** PS–PVA, **b** PS–PVA–glu lipase

in the oil where as AV is the weight (in mg) of alkali required neutralizing free acid groups in oil. The parameters are expressed by the following expression (Eqs. 2, 3) [27].

$$\text{FFA}(\%)\ (\text{as oleic acid}) = \frac{\text{Volume of NaOH (ml)} \times \text{Strength (in normality)} \times 28.2}{\text{weight of sample (gms)}} \tag{2}$$

(Eq. wt. of oleic acid is 28.2)

$$\text{Acid value (AV)} = \%\,\text{FFA (oleic acid)} \times 1.99 \quad [36]. \tag{3}$$

Table 3 features the %FFA (normalized), AV and peak area (normalized) of oleic acid methyl ester (from GC-mass). The normalization is based on olive oil (%FFA = 0.176, peak area 1988064) (i.e., by dividing other values with the value of olive oil). The normalization is done to simplify the values. The normalized value shows that the maximum %FFA for free lipase where as in bounded condition it is lowered. The acid value also shows the same trend. Moreover, it is also seen that the parameters (viz. AV and %FFA) are of higher value for the immobilized lipases of

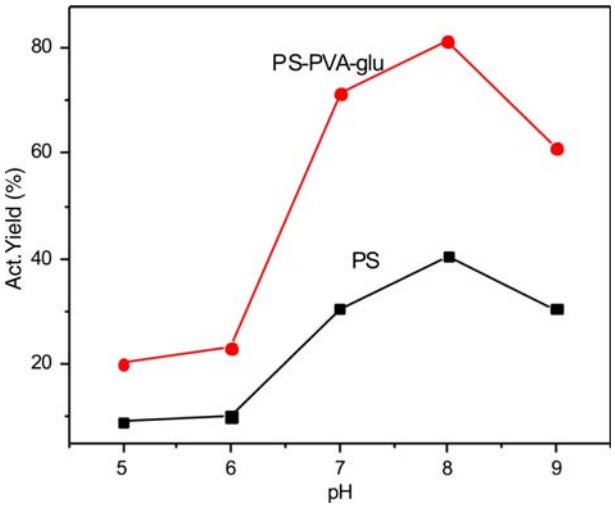


Fig. 4 Effect of pH on the activity of immobilized lipase

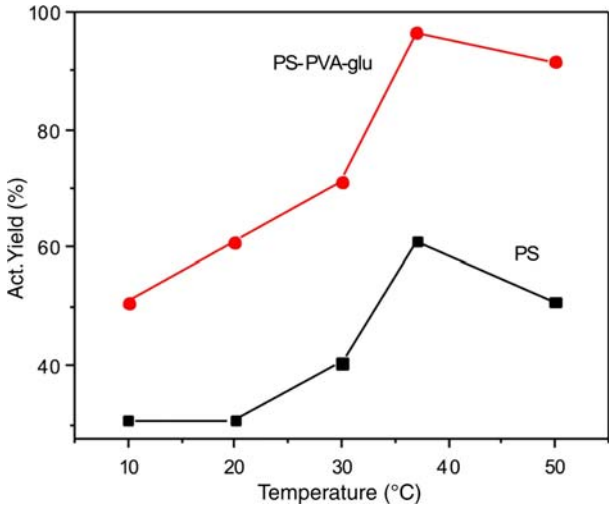


Fig. 5 Effect of temperature on the activity of immobilized lipase

PS–PVA–glu membranes compared to immobilized lipases on PS membrane, as the amount of immobilization.

The esterification data from GC-mass spectra shows similar trend to %FFA and AV. The GC-mass spectra for methyl esters for immobilized as well as free lipases are displayed in the Fig. 7. The GC-mass spectra show the presence of different esters (viz. from palmitic acid, linoleic acid). Considering oleic acid methyl ester as the parent one, the peak area for the immobilized and free lipases is normalized with respect to olive oil before hydrolysis. The normalized data support the previous

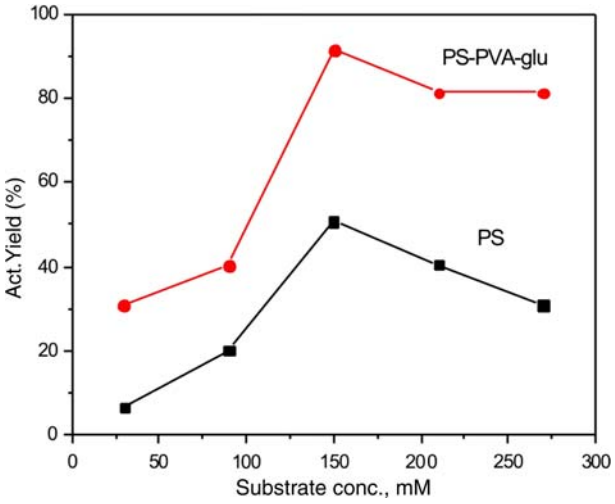


Fig. 6 Effect of substrate concentration on the activity of immobilized lipase

Table 3 Titrimetric and gas chromatographic values for hydrolysis as well as esterification of free lipase and lipase immobilized two membranes (PS virgin and PS–PVA–glu)

Membrane	%FFA (normalized) [A]	Acid value (AV)	Peak area of oleic acid methyl ester (from Fig. 8) (normalized) [B]	[A]/[B]
Olive oil	1	0.35	1	1
Free lipase	13.06	4.58	12.95	1.01
PS–PVA–glu	8.69	3.04	9.28	0.94
PS	5.22	1.83	5.15	1.01

normalized %FFA as higher esterification is possible for more amount of FFA in the system [A] and [B] in the column in Table 3). The normalized data of peak areas show almost similar data as %FFA of lipase immobilized membrane to olive oil, which suggests that esterification, is almost 100% for the free fatty acid.

Kinetic parameters of the hydrolytic reaction

Linear forms of Michaelis–Menten equation provide interesting insights into the kinetics of the enzyme in determining the values of K_m and V_{max}. Here, it is tried to fit the data into Lineweaver–Burk and Hanes plot [37–39]. V_{max} and K_m are in ensemble (Table 4) from the respective slope and intercept of the corresponding equations (Eq. 4, Eq. 5). Figure 8 and 9 show the Lineweaver–Burk and Hanes's plot. It shows more or less similar values from both the plots and trend is similar. The co-relation co-efficient is better suggests that the data is more fitted in Lineweaver plot. The K_m values for the membranes follows the order i.e., lipase

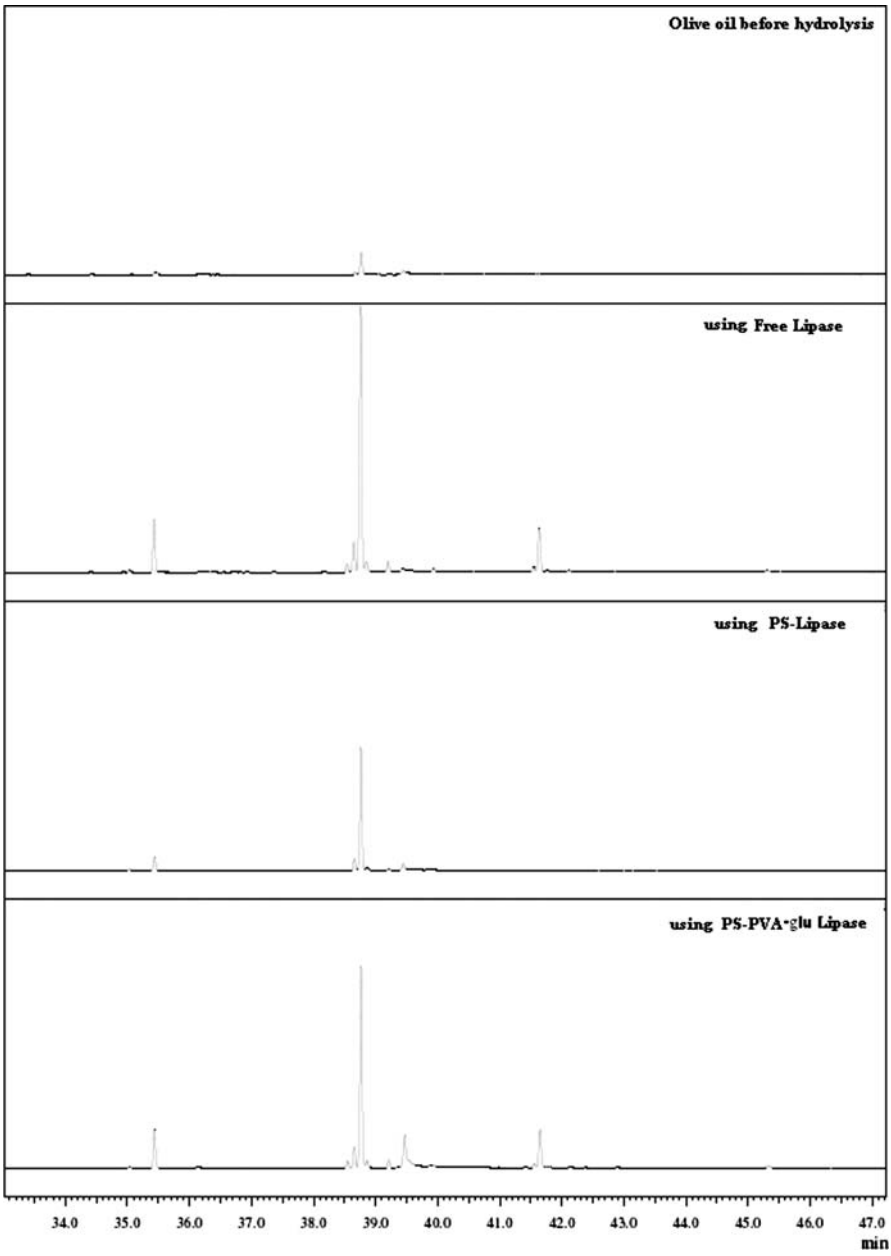


Fig. 7 GC-mass spectra of methyl esters from olive oil

immobilized PS–PVA–glu membrane < lipase immobilized PS membrane. The lower K_m value signifies greater affinity i.e., lower concentration of substrate needs to achieve a given rate.

Table 4 Kinetic parameters V_{max} and K_m for lipase immobilized PS and PS–PVA–glu membranes for Lineweaver and Hanes plot

Equation	For free lipase		For lipase immobilized on PS		For lipase immobilized on PS–PVA–glu	
	V_{max} (mM/min)	K_m mM	V_{max} (mM/min)	K_m mM	V_{max} (mM/min)	K_m mM
Lineweaver–Burk	3.1	80	0.51	153.8	0.90	105
Hanes	3.1	75	0.51	145	0.91	110

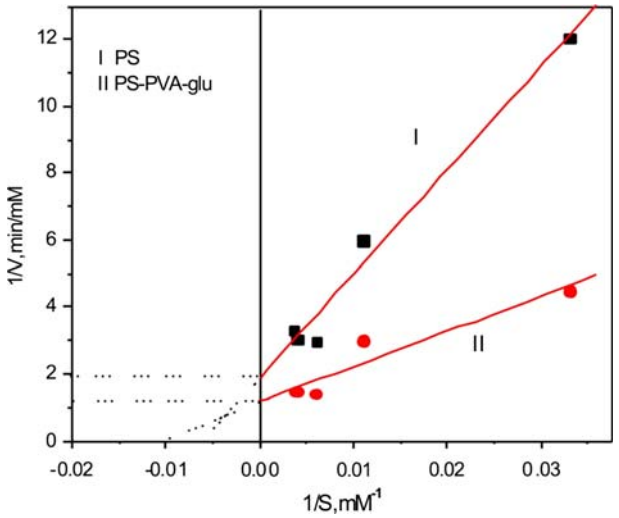


Fig. 8 Lineweaver–Burk Plot of immobilized lipases on membranes [R (co-relation co-efficient) for PS 0.99 and PS–PVA–glu 0.96]

Lineweaver–Burk equation:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \tag{4}$$

Hanes equation:

$$\frac{S}{V} = \frac{S}{V_{max}} + \frac{K_m}{V_{max}} \tag{5}$$

where S (mM) is concentration of substrate, V is the initial velocity of reaction, V_{max} (mM/min) is the saturation velocity and K_m (mM) is Michael's–Menten constant.

Reusability in hydrolytic activities

The reusability feature is studied five times for the hydrolytic reaction of olive oil. The hydrolytic activity at optimum condition (pH 8, temp 37 °C, reaction time

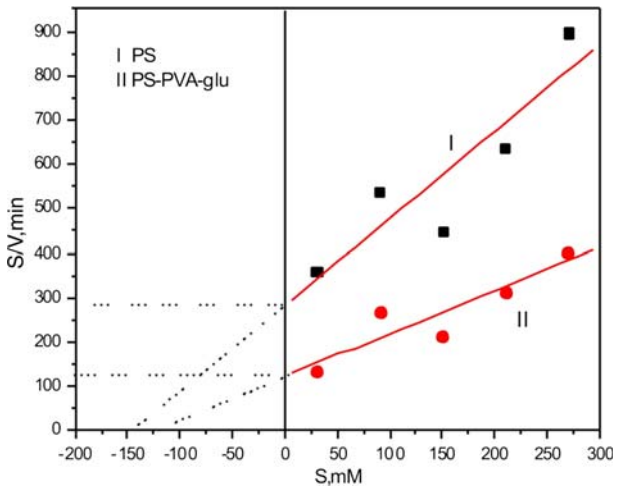


Fig. 9 Hanes Plot of immobilized lipases on membranes [R (co-relation co-efficient) for PS 0.9 and PS–PVA–glu 0.91]

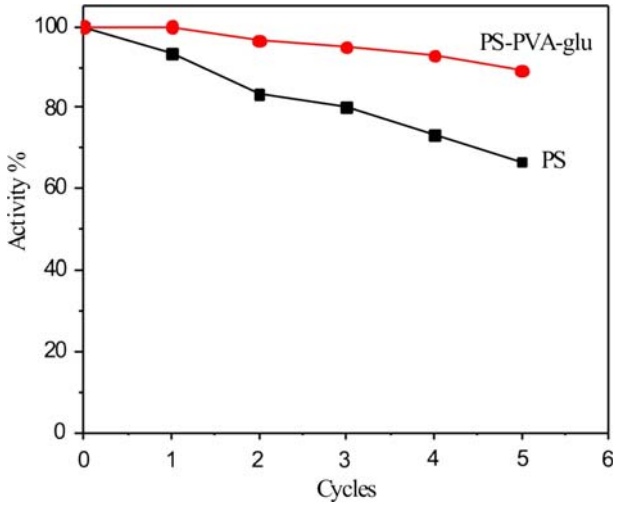


Fig. 10 Reusability of lipase immobilized PS and PS–PVA–glu membranes

30 min) is compared for lipase immobilized PS and PS–PVA–glu (Memb-V) membranes. The initial activity is considered as 100% for both the membranes.

After studying the hydrolytic activity, post treatment of both the membranes is done by washing three times with 0.1 M phosphate buffer (pH 7.0). The activity of the immobilized lipase decrease slowly during the repeated use (Fig. 10). It is seen that lower decrease in activity (10.7%) for Memb-V compared to 33.3% decrease for lipase immobilized on PS membrane after five cycles. It suggests the stability of the lipase with the PS–PVA–glu matrix.

Conclusions

The study details the experiments of lipase immobilization on PVA modified PS membranes. Photo-irradiation technique was employed to modify the surface. FTIR, contact angle and pure water permeability results showed the evidences of the modified surface. The PVA content on the membranes depended on dipping time in PVA solution.

XRD, SEM results proved the immobilization of lipase. The amount of immobilization varied as follows: PS–PVA–glu > PS–glu > PS–PVA > PS. The covalent approach with glutaraldehyde for PS–PVA–glu membrane was resulted better immobilization than simple adsorption. The total hydrolytic activity of lipase on PS–PVA–glu was higher than PS due to higher amount of lipase. The influencing factors (e.g., pH, temperature and substrate concentration) of hydrolytic reaction were evaluated. The maximum hydrolytic activity was at pH 8, temperature 37 °C and 150 mM. PS–PVA immobilized membrane liberated more free fatty acid (FFA) compared to PS. The acid values from two methods (titrimetric and GC) showed the similar trend. The corresponding data fitted into two linear equations (Lineweaver–Burk and Hanes plot). The hydrolytic reaction rate (V_{max}) of lipase on PS–PVA–glu membrane was higher compared to lipase on PS. The lower K_m value for lipase immobilized PS–PVA–glu than PS membrane signified greater affinity to olive oil. The reusability feature showed the better stability of the PS–PVA–glu–lipase matrix.

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