Poly(vinylbenzylchloride) Beads Grafted with Polymer Brushes Carrying Hydrazine Ligand for Reversible Enzyme Immobilization

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ABSTRACT: Poly(glycidylmethacrylate), p(GMA), brush grafted poly(vinylbenzyl chloride/ethyleneglycol dimethacrylate), p(VBC/EGDMA), beads were prepared by suspension polymerization and the beads were grafted with poly(glycidyl methacrylate), p(GMA), via surface-initiated atom transfer radical polymerization aiming to construct a material surface with fibrous polymer. The epoxy groups of the fibrous polymer were reacted with hydrazine (HDZ) to create affinity binding site on the support for adsorption of protein. The influence of pH, and initial invertase concentration on the immobilization capacity of the p(VBC/EGDMA-*g*-GMA)-HDZ beads has been investigated. Maximum invertase immobilization onto hydrazine functionalized beads was found to be 86.7 mg/g at pH 4.0. The experimental equilibrium data obtained invertase adsorption onto p(VBC/EGDMA-*g*-GMA)-HDZ affinity beads fitted well to the Langmuir isotherm model. It was shown that the relative activity of immobilized invertase was higher than that of the free enzyme over broader pH and temperature ranges. The K_m and V_{max} values of the immobilized invertase were larger than those of the free enzyme. After inactivation of enzyme, p(VBC/EGDMA-*g*-GMA)-HDZ beads can be easily regenerated and reloaded with the enzyme for repeated use. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 2661–2669, 2009

Key words: ATRP; polymer brushes; invertase; adsorption; immobilization

INTRODUCTION

A number of methods for reversible immobilization of enzymes via adsorption have been reported in the literatures.[1–3] However, the adsorption is generally not very strong and some of the adsorbed enzyme is desorbed during washing and operation. For this reason, reversible enzyme immobilization via adsorption requires a strong hydrophobic or ionic interaction between the enzyme and support.[4–6] Desorption of enzymes from fibrous polymer containing ion-exchange groups was found to require the use of denaturing conditions (under low pH and high ionic strength), but this desorption would be necessary after inactivation of the enzyme upon use.[5–7]

For reversible enzyme immobilization, the modification of support surface is mostly required to change the character of the base support surface from hydrophobic to hydrophilic in order to create selective absorptive surface for adsorption of protein.[8–10] Among the surface functionalization techniques, polymer brushes from surface-initiated polymerizations have been widely used to tailor the surface properties of substrates. Atom transfer radical polymerization (ATRP) is one of the most investigated methods of controlled graft polymerization. Among the various chemical functional groups that can be integrated into polymers, the epoxy group is attractive because it can be readily modified using various chemical reactions to introduce other moieties. For example, hydrazine as an affinity ligand has been incorporated on different matrices (membranes, beads and silica particles) to adsorption of different proteins via affinity interaction.[11–18] The hydrazine ligand offers several advantages over other ligands in terms of economy and ease of immobilization.

Invertase is a commercially important enzyme in the food industry, invert sugar from sucrose is produced by acid hydrolysis or by using the enzyme invertase (E.C.3.2.1.26.). The enzymatic process has advantages over acid hydrolysis because neither color nor by-products are obtained.[19]

In the present study, p(GMA) grafted and hydrazine functionalized p(VBC/EGDMA) beads was used for adsorption of invertase from aqueous medium. The hydrazine modified groups of the grafted polymer were used for the reversible immobilization of invertase. The invertase is a carboxylic group rich acidic glycoprotein. Therefore, the enzyme "invertase" was selected as a negatively charged protein to

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evaluate its immobilization on support coated with oppositely charged hydrazine. The immobilization of invertase affinity adsorption onto the hydrazine functionalized p(VBC/EGDMA-*g*-GMA) beads from aqueous solutions containing different amounts of enzyme at different pH was investigated in a batch system. The optimum pH and temperature for the free and immobilized enzymes, as well as operational stability in a continuous system, were investigated.

MATERIALS AND METHODS

Materials

Invertase (β-fructofuranosidase, EC 3.2.1.26, Grade VII from baker's yeast), glucose oxidase (GOD, EC 1.1.3.4. Type II from *Aspergillus niger*), peroxidase (POD, EC 1.11.1.7, Type II from horseradish), bovine serum albumin (BSA), o-dianisidine dihydrochloride, sucrose, glucose, ethyleneglycol dimethacrylate (EGDMA), glycidyl methacrylate (GMA), α′−α′-azobisisobutyronitrile (AIBN), bipyridine, hydrazine, 2-methyl pyrrolidone (MP), and CuBr were supplied from Sigma Chemical (St Louis, MO) and used as received. All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany).

Synthesis of poly(vinylbenzylchloride/ethyleneglycol dimetacrylate) beads

The cross-linked p(VBC/EGDMA) beads were prepared by suspension polymerization as described previously.[20] Briefly, vinylbenzylchloride (10.0 mL, 63.8 mmol), EGDMA (3 mL, 15.6 mmol), and AIBN (0.24 g, 1.42 mmol) were dissolved in toluene (15 mL). The resulting solution was dispersed in an aqueous medium, prepared by dissolution of PVA (0.5 g) in purified water (160 mL). The polymerization was carried out in a magnetically stirred reactor (250 mL) at 78°C for 8 h. The stirring rate was kept constant at 700 rpm during the polymerization process. After polymerization, the p(VBC/EGDMA) beads were transferred into water (1.0 L) and stirred magnetically for about 2 h. The beads were then filtered and washed with excess water and ethanol. They were subsequently dried in vacuum oven at 50°C. The beads were sieved and a proper size fraction (75–150 μm in diameter) was used for subsequent reaction.

Grafting of p(GMA) brushes by ATRP onto p(VBC/EGDMA) beads

Graft polymerization of glycidyl methacrylate was achieved through benzyl chloride initiation sites on the surface of the p(VBC/EGDMA) beads. A typical procedure is as follows: p(VBC/EGDMA) beads (5 g) were transferred into a magnetically stirred glass reactor (100 mL) and then the suspensions of the following chemicals [monomer GMA (20 mL, 0.15 mol), CuBr (0.432 g, 3.0 mmol), bipyridine (0.936 g, 6.0 mmol), and dioxane (10 mL)] were added. The suspension was purged with nitrogen for about 10 min and the reactor was sealed. Polymerization reaction was carried out at 65°C for 18 h. The grafted p(VBC/EGDMA-*g*-GMA) beads were filtered and transferred into a solution containing EDTA solution about 10% (w/v) and it was stirred continuously to remove copper ions for 24 h. The p(VBC/EGDMA-*g*-GMA) beads were filtered and washed sequentially with water (1.0 L) and ethanol (500 mL). The p(VBC/EGDMA-*g*-GMA) beads were dried in vacuum oven at 25°C.

Modification of p(VBC/EGDMA-*g*-GMA) beads with hydrazine

The p(VBC/EGDMA-*g*-GMA) beads about (10 g) was transferred into a reactor containing hydrazine (50 mL) and 2-methyl pyrrolidone (30 mL) at 0°C and the mixture was shaken at 150 rpm at room temperature for 24 h and at 90°C for 5 h. After cooling, the hydrazine modified p(VBC/EGDMA-*g*-GMA)-HDZ beads was transferred into purified water (500 mL) and washed sequentially with purified water (1.0 L) and methanol (25 mL). The modified p(VBC/EGDMA-*g*-GMA)-HDZ affinity beads were dried overnight under vacuum at 25°C for 24 h.

Characterization of p(VBC/EGDMA-*g*-GMA) beads

The grafting percentage (GP) was determined by calculating the percentage increase in weight using following equation:

$$GP = [(m_{gf} - m_0)/m_0] \times 100\% \quad (1)$$

where, m_0 and m_{gf} are the weights of the beads before and after grafting, respectively.

The average size and size distribution of the p(VBC/EGDMA-*g*-GMA) beads were determined by screen analysis performed by using molecular sieves. The amount of available surface functional epoxy groups content of the p(GMA) grafted p(VBC/EGDMA) beads was determined by pyridine-HCl method as described previously.[21] For the determination of the hydrazine content of the ion-exchange beads, sample (0.1 g) was left in contact with the mixture of HCl (1.0*M*, 30 mL), purified water 20 mL and 5 mL KI (0.1*M*) for 24 h with continuous stirring at room temperature. The consumed iodine was determined by titration with 0.1*M* sodium thiosulfate solution.

The FTIR spectra of the p(VBC/EGDMA), p(VBC/EGDMA-*g*-GMA), and p(VBC/EGDMA-*g*-GMA)-HDZ beads were obtained by using a FTIR spectrophotometer (FTIR 8000 Series, Shimadzu, Japan). The dry sample (about 0.01 g) mixed with KBr (0.1 g) and pressed into a tablet form. The FTIR spectrum was then recorded. The p(VBC/EGDMA) and p(VBC/EGDMA-*g*-GMA) beads were coated with a thin layer of gold under reduced pressure and their scanning electron micrographs were obtained using a JEOL (JSM 5600) scanning electron microscope.

Effect of pH and initial concentration of invertase on immobilization efficiency

Immobilization of invertase on the hydrazine functionalized p(VBC/EGDMA-*g*-GMA) beads was studied at various pH's, in either acetate (50 m*M*, pH 3.5–5.0), in phosphate buffer (50 m*M*, pH 6.0–6.5), or in Tris-HCl buffer (50 m*M*, pH 7.0–8.0).

To determine the immobilization capacity of hydrazine functionalized p(VBC/EGDMA-*g*-GMA) beads, the initial concentration of invertase was changed between 0.125 and 2.0 mg ml^{-1} in acetate buffer (5.0 mL, 50 m*M*, pH 4.0). All experiments were conducted in duplicates with 50 mg ion-exchange beads and initial concentration of invertase was 0.5 mg/mL in each set experiments. A calibration curve was prepared using invertase as a standard (0.1–2.00 mg ml^{-1}). The amount of invertase adsorbed onto beads was determined by subtracting the absorbance at 280 nm after adsorption from the value before adsorptions using UV/Vis spectrophotometer (Shimadzu, Tokyo, Japan, Model 1601).

The enzyme leakage from the hydrazine functionalized beads was studied in enzyme operation conditions without adding substrate. The enzyme immobilized beads about (50 mg) were placed in a test tube containing 5 mL of three different kinds of enzyme operation medium: (i) pH 5.0, acetate buffer 50 m*M*; (ii) pH 6.0, phosphate buffer 50 m*M*; (iii) pH 7.0, phosphate buffer 50 m*M*, and shaken on a rotary shaker for 6 h. The amount of protein release into the medium was determined at 280 nm using UV/Vis spectrophotometer.

Activity assays of free and immobilized invertase

The activities of both free and immobilized enzyme were determined as described previously.[1] The activity-pH profiles of the free and immobilized invertase were studied in acetate buffer (50 m*M*) in the pH range 4.0–5.5 and in phosphate buffer (50 m*M*) in the range pH 6.0–8.5. The effect of temperature on the free and immobilized invertase was studied in acetate (50 m*M*, pH 5.5), and phosphate buffer (50 m*M*, pH 6.0), respectively.

The results of dependence of invertase activity on pH, temperature, and storage stability are presented in a normalized form with the highest value of each set being assigned the value of 100% activity. K_m and V_{max} values of the free enzyme were determined by measuring initial rates of the reaction with sucrose (30–300 m*M*) in acetate buffer (50 m*M*, pH 5.5) at 35°C.

K_m and V_{max} were calculated from the initial rate of the kinetic data. The activities of the free and the immobilized invertase were expressed in μmol glucose/min/mg of enzyme. Sucrose hydrolysis performance of the free and immobilized enzyme preparations was determined by measuring the glucose content of the medium according to a method described previously[22] using an UV/Vis spectrophotometer (Shimadzu, Model 1601, Tokyo, Japan), at 525 nm.

Reusability and storage stability of enzymes

To determine the reusability of the hydrazine functionalized beads for adsorption and cleaning of invertase was repeated six times by using the same affinity beads. Enzyme desorption were performed in a NaOH solution (1.0*M*). The enzyme adsorbed beads were placed in the cleaning medium while stirring at 100 rpm at 25°C for 2 h. The beads were removed from cleaning medium washed several times with acetate buffer (50 m*M*, pH 5.5) and were then reused in subsequence enzyme immobilization.

The storage stability of invertase was studied in wet states. The enzyme immobilized affinity beads were stored in phosphate buffer (50 m*M*) at 4°C. The activity of the immobilized invertase was determined as described above for a storage period of upto 2 months. The residual activity was defined as the fraction of total activity recovered after immobilization of invertase on the affinity beads compared with the same quantity of free enzyme.

RESULTS AND DISCUSSION

Characterization of p(VBC/EGDMA-*g*-GMA) beads

Poly(glycidyl methacrylate) grafting was achieved on the poly(VBC/EGDMA) beads by surface-initiated atom transfer radical polymerization. The grafting percentage – time plot is presented in Figure 1. As seen in this figure, an increase in the grafting time from 3 to 18 h leads to increase in grafting percentage on the poly(VBC/EGDMA) beads. The maximum epoxy group content of the beads was determined as 7.1 mmol/g. The amount of bound hydrazine on the p(VBC/EGDMA-*g*-GMA) beads was found to be 6.4 mmol/g beads by using the

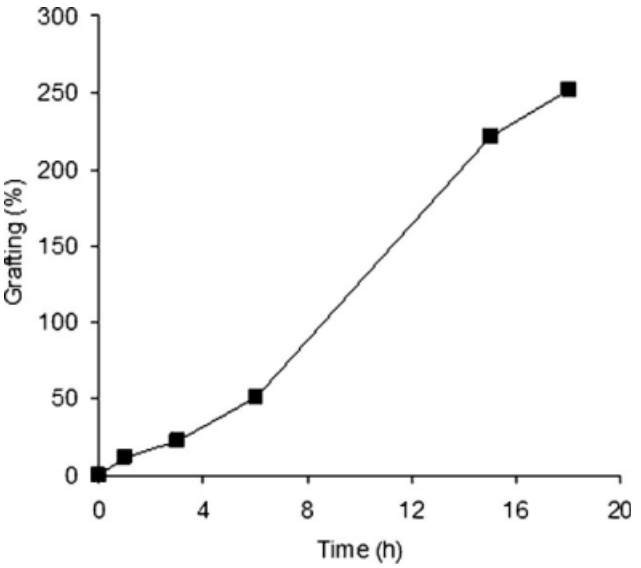

Figure 1 Grafting efficiency of p(GMA) on the p(VBC/EGDMA) beads versus time plot.

difference between initial and final iodine concentration in the reaction solution.

The schematic representation of the controlled growth of the grafted polymer brushes by atom transfer radical polymerization (ATRP) onto p(VBC/EGDMA) beads and the chemical modification of epoxy groups with hydrazine was illustrated in Figure 2.

FTIR spectra of the p(VBC/EGDMA), p(VBC/EGDMA-*g*-GMA), and hydrazine functionalized p(VBC/EGDMA-g-GMA) beads are presented in Figure 3. The peak at 1485 cm^{-1} is the characteristic absorption peak of the aromatic ring of the p(VBC/EGDMA), p(VBC/EGDMA-*g*-GMA), and hydrazine functionalized p(VBC/EGDMA-g-GMA) beads [Fig. 3(a–c)]. The absorption in the FTIR spectrum at 1722 cm^{-1} is due to carbonyl (C=O) stretching vibrations. The symmetric and asymmetric vibrations of the epoxy rings are observed at 1253 and 905 cm^{-1}, respectively [Fig. 3(a)]. Among the characteristic vibrations of both EGDMA and GMA is the methylene vibration at ~2935 cm^{-1}. The FTIR spectra of hydrazine functionalized p(VBC/EGDMA-*g*-GMA) beads had absorption bands different from that of the p(VBC/EGDMA-*g*-GMA) beads at 3243 and 1653 cm^{-1} corresponds to the -NH_2 stretching vibration and N—H deformation, respectively, are due to the incorporation of the amino groups of hydrazine on the polymer structure [Fig. 3(c)]. The

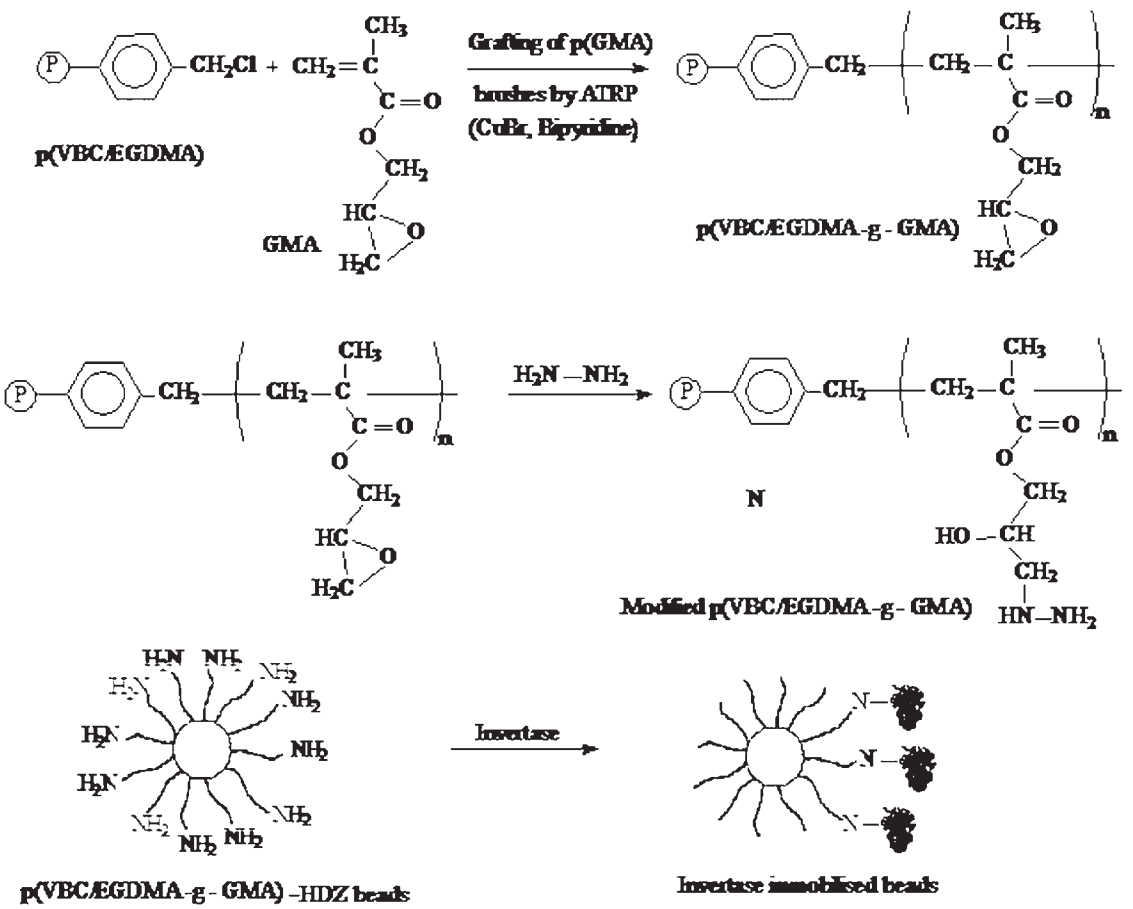

Figure 2 Schematic representation of experimental protocols.

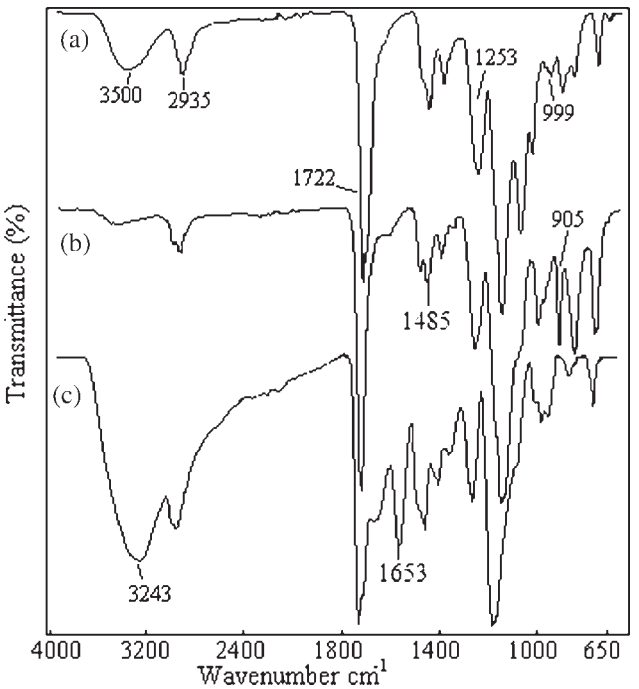

Figure 3 The FTIR spectra: (a) p(VBC/EGDMA); (b) p(VBC/EGDMA-*g*-GMA); and (c) p(VBC/EGDMA-*g*-GMA)-HDZ beads.

appearance of the new peaks evidenced the successful modification on the p(VBC/EGDMA) beads.

The surface morphology of the p(VBC/EGDMA-*g*-GMA) beads are exemplified by scanning electron microscopy (SEM) micrographs in Figure 4. The p(VBC/EGDMA) beads have spherical form with a smooth surface. Fibrous polymer grafted beads can be suitable matrices for of enzyme due to their intrinsically high specific surfaces, providing the quantity and accessibility of the interaction sites for high immobilization capacity.[17,18]

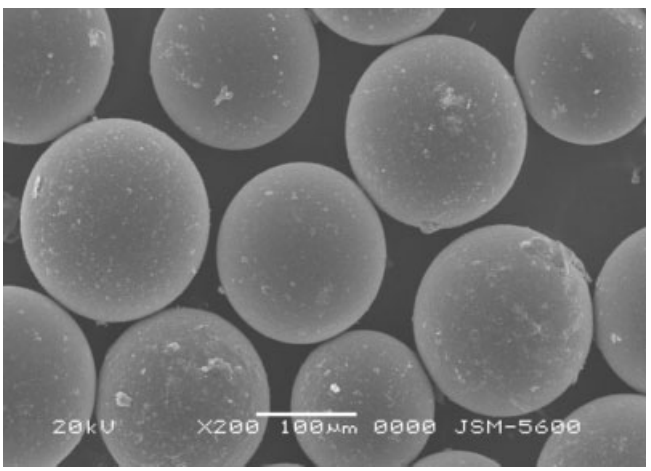

Figure 4 SEM micrograph the p(VBC/EGDMA-*g*-GMA) beads.

Immobilization of invertase on the hydrazine functionalized beads

To investigate the effects of pH on the invertase immobilization efficiency affinity beads, the medium pH was changed between pH 3.5 and 8.0. Figure 5 shows the effect of pH on the amount invertase adsorbed on affinity beads. As seen in this figure, the electrostatic interaction between invertase and hydrazine functionalized affinity beads was found to be maximum at around pH 4.0 and gave the highest immobilization capacity. Proteins have no net charge at their isoelectric points, and therefore the maximum adsorption from aqueous solutions is usually observed at their isoelectric points. The isoelectric pH of invertase is around 4.2. In the present study, the maximum adsorption was observed at pH 4.0. The resulting invertase adsorption at pH 4.0 may be due to suitable conformation of invertase molecules on hydrazine functionalized beads surface. Specific interactions (electrostatic and hydrogen bonding) between invertase and affinity beads at pH 4.0 may result from both primary, secondary amino groups and hydroxyl groups of functionalized brush polymer and amino acid side chains and carbohydrate moieties of the invertase molecule. It should also be pointed out that the hydrazine ligand is a weak ion-exchange ligand (primary and secondary amines) and therefore can not be used at pH-values higher than about 9.0.

Figure 6 shows the effect of initial concentration of invertase on the immobilization efficiency of the affinity beads at 25°C. From the equilibrium adsorption observations, it can be concluded that the amount of invertase adsorbed by the affinity beads increased with increasing invertase concentration in the medium. As presented in Figure 6 with increasing enzyme concentration in solution, the amount of

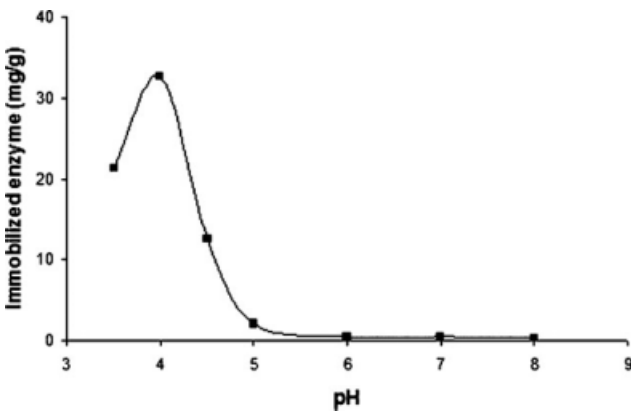

Figure 5 Effects of pH on invertase immobilization efficiency on the hydrazine modified p(VBC/EGDMA-*g*-GMA) beads. Experimental conditions: Temperature 25°C. Initial concentration of invertase (5 mL, 0.5 mg/mL); Amount of hydrazine modified beads 50 mg; Contact time: 2 h.

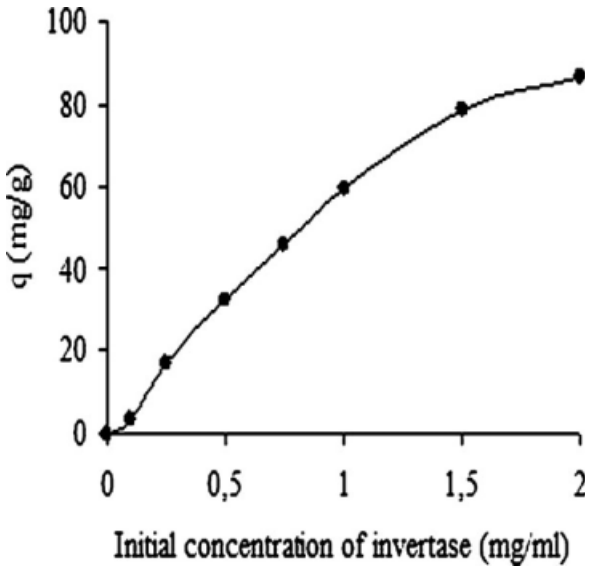

Figure 6 Effects of initial concentration of invertase on the immobilization capacity via adsorption on the hydrazine functionalized p(VBC/EGDMA-*g*-GMA) beads. Experimental conditions: Temperature 25°C; Initial concentration of invertase (5 mL, between 0.125 and 2.0 mg/mL); pH 4.0 (50 m*M* acetate buffer); Amount of hydrazine modified beads 50 mg; Contact time: 2 h.

invertase adsorbed per unit area by affinity beads increases almost linearly up to 1.0 mg/mL. At enzyme concentration above 1.0 mg/mL, it still has some certain adsorption action for invertase, as shown by the curve in Figure 6. This could be explained by saturation of hydrazine modified groups with the adsorbed invertase molecules, which achieve maximum immobilization capacity. The maximum invertase immobilization onto affinity beads was found to be 86.7 mg/g. The specific interactions between invertase and the hydrazine functionalized beads can result from the co-operative effect of different mechanisms such as specific interaction (i.e. hydrogen bonding) caused by the ketoamine, carbonyl groups, and carbohydrate moiety of the invertase with the hydrazine groups of the beads.[23,24] These results indicated that there was a relationship between the surface interaction groups of invertase with the hydrazine modified groups of the beads.

The enzyme leakage from hydrazine functionalized beads was studied in the different enzyme operation conditions as described above. Any measurable enzyme leakage was not observed under all these studied conditions.

Adsorption isotherm

The adsorption isotherm was obtained from batch experiment at different temperatures. The corresponding transformations of the equilibrium data for invertase gave rise to a linear plot, indicating that the Langmuir could be applied in these systems and described by the equation:

$$q = q_m C/(K_d + C) \quad (2)$$

where, C is the equilibrium concentration of protein in solution, q is the equilibrium amount of protein adsorbed on the beads, and q_m is the maximum adsorption capacity of the beads. K_d (k_2/k_1); (i.e., the ratio of reverse and the forward rate constants) is the dissociation constant of the ligand/surface interaction. K_d has dimension of concentration, and the protein binding is stronger when it is smaller.

The range of invertase concentration selected for the adsorption isotherm study was 0.1–2.0 mg/mL. The maximum capacity (q_m) data for the adsorption of invertase was obtained from the experimental data. As shown in Figure 7, the plot of C_{eq} versus C_{eq}/q_{eq} yielded a straight line, revealing the adsorption of invertase obeyed the Langmuir adsorption isotherm. From the slope and intercept, the values of q_m and K_d were found to be 109.8 mg/g and 2.52 $\times$ $10^{-6}M$, respectively. The correlation coefficient (R^2) was 0.992.

The effect of pH and temperature on the free and immobilized enzyme activity

The effect of pH on the activity of the free and immobilized invertase for hydrolysis of sucrose to glucose and fructose was examined in the pH range 4.0–8.5 at 35°C. As seen from Figure 8, the hydrolysis reaction has maximum activity for free and immobilized enzymes at pH 5.0 and at pH 6.0,

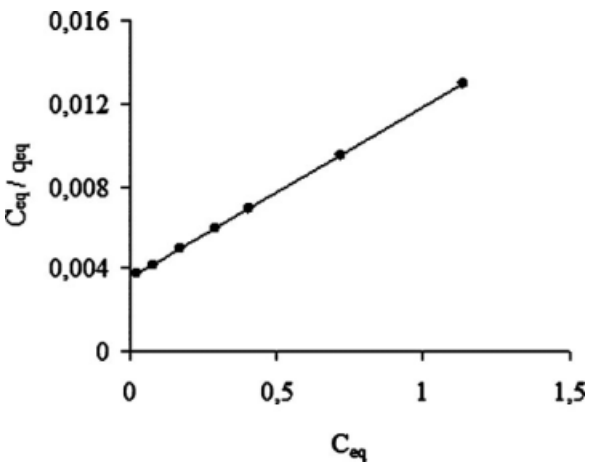

Figure 7 Langmuir isotherm plot for adsorption of invertase on the hydrazine functionalized p(VBC/EGDMA-*g*-GMA) beads. Experimental conditions: Temperature 25°C; Initial concentration of invertase (5 mL, between 0.125 and 2.0 mg/mL); pH 4.0 (50 m*M* acetate buffer); Amount of hydrazine modified beads 50 mg; Contact time: 2 h.

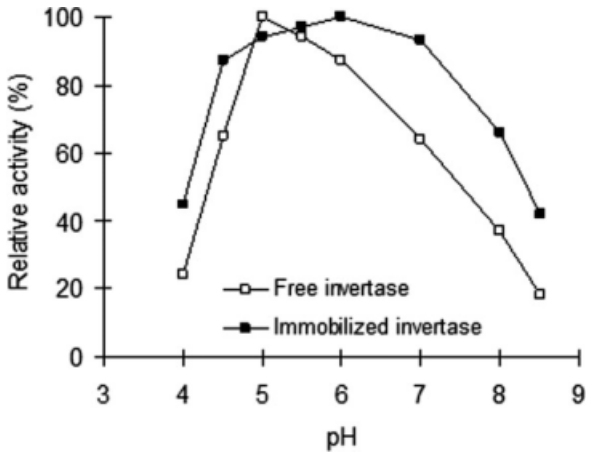

Figure 8 Effect of pH on the free and immobilized invertase activity: the relative activities at the optimum pH were taken as 100% for free and immobilized invertase. Experimental conditions; pH: 4.0–8.5; Temperature: 35°C.

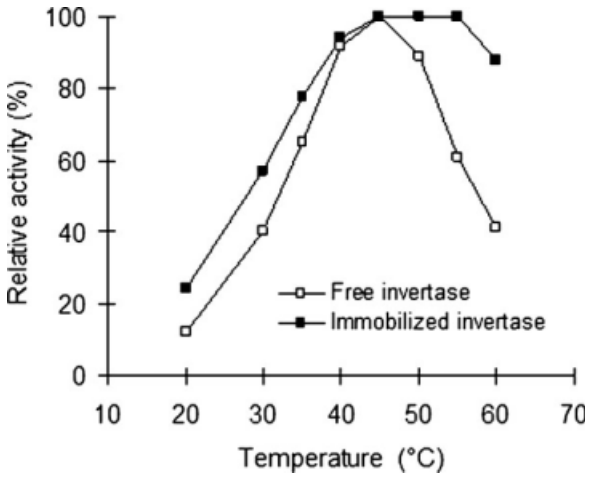

Figure 9 Effect of temperature on the free and immobilized invertase activity; the relative activities at optimum temperature were taken as 100% for free and immobilized invertase. Experimental conditions; pH: 5.5 (acetate buffer, 50 m*M*); Temperature: 35°C.

respectively. This shift may depend on the immobilization method as well as the basic character of the support material. The pH profiles of the immobilized invertase display strongly improved stability of the optimum pH value, in comparison to that of the free form, which means that the immobilization method preserved the enzyme activity.

Effect of temperature on the relative activity of free and immobilized invertase for affinity beads is shown in Figure 9. The immobilized enzyme showed an optimum reaction temperature between 45 and 55°C, whereas free enzyme had an optimum temperature about 45°C. As was evident from the data, the immobilized enzyme possessed a better heat-resistance than that of the free enzyme. The immobilization of invertase on the fibrous polymer via electrostatic interaction might also reduce the conformational flexibility and may result in higher activation energy for the molecule to reorganize the proper conformation for the binding to substrate sucrose.[25–28]

Kinetic parameters

Kinetic parameters K_m and V_{max} for the sucrose hydrolysis were calculated from Lineweaver-Burk plots at constant temperature and pH while varying the substrate concentration. K_m values were calculated as 17 and 23 m*M* for free and immobilized enzyme, respectively (Table I). The change in the affinity of the enzyme to its substrate is probably caused by structural changes in the enzyme introduced by the immobilization procedure. The K_M value of the immobilized invertase was increased compared to free counterpart, possibly due to the diffusional limitation imposed on the flow of substrate and product molecules from of the fibrous layers of the grafted polymer. This may be explained by the fact that fibrous polymer chains of the beads should be covered with adsorbed invertase, resulting a sticky web like coating over the fibrous polymer surface. Thus, these layers may prevent the diffusion of the substrate and/or products molecules from the inner layer of the polymer brushes where invertase was immobilized. The V_{max} value of immobilized enzyme was estimated from the data as 104 U/mg adsorbed proteins onto affinity beads. As expected, the K_m and V_{max} values were significantly affected after immobilization on to the affinity beads (Table I).

The efficiency factor, η, can be calculated from the maximum reaction rates of the immobilized enzyme over that of its free counterpart. From this calculation, composite fibers enzyme system provided an efficiency factor of 0.70 for the immobilized enzyme.

TABLE I
Kinetic Parameters for the Free and Immobilized Invertase

Form of enzyme	K_m (m*M*)	V_{max} (U/mg enzyme)	Efficiency factor $\eta = v_{immob}/v_{free}$	Catalytic efficiency (V_{max}/K_m)
Free	17	148	–	8.71
Immobilized	23	104	0.70	4.52

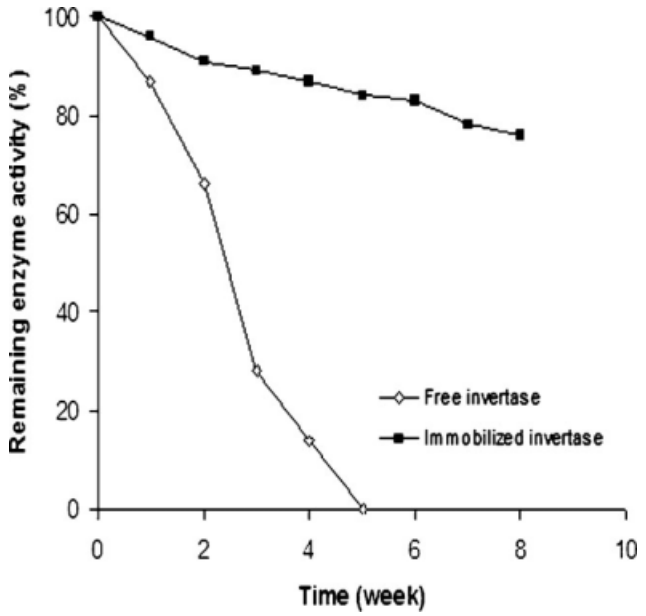

Figure 10 Storage stabilities of the free and immobilized invertase. Storage conditions: pH: 6.0 (phosphate buffer, 50 m*M*); Temperature: 4°C.

A catalytic efficiency, which is reflected by the ratio of V_{max} over K_m was found to be different values for the free and immobilized enzyme. The catalytic efficiency of invertase was decreased upon immobilization from 8.71 to 4.52 fold upon immobilization (Table I).

Storage stability of the invertase preparations

The free and the immobilized invertase preparations were stored at 4°C in the wet states. The activity loss of the immobilized invertase was about 27% in the 2 month storage period. The free enzyme lost all its activity within 5 week. Thus, the immobilized invertase exhibits higher storage stability than that of the free form (Fig. 10). The higher stability of the immobilized invertase could be attributed to the prevention of denaturation as a result of multipoint interaction of invertase molecules on the hydrazine functionalized polymer chains. On the basis of this observation, p(GMA) grafted and hydrazine functionalized beads should provide a stabilization effect, minimizing possible distortion effects imposed from aqueous medium on the conformational structure of the immobilized enzyme. The generated multipoint ionic interactions between enzyme and hydrazine functional groups should also convey a higher conformational stability to the immobilized enzyme. Thus, the affinity beads and the immobilization method provide higher shelf life when compared with that of its free enzyme.[29–33]

Cleaning of the beads for reuse in enzyme immobilization

The cleaning of adsorbed invertase after inactivation from the affinity beads was achieved under alkaline condition. The invertase adsorbed on the hydrazine functionalized p(VBC/EGDMA-*g*-GMA) beads was placed within the cleaning medium containing 1.0*M* NaOH. The adsorption–desorption cycle of invertase was repeated six times by using the same affinity beads. The immobilization capacity of the affinity beads did not change significantly after six times use in the repeated use of the support after regeneration of the affinity beads in 1.0*M* NaOH for 2 h. The sixth adsorption–desorption cycle of invertase, the amount of immobilized enzyme (83.5 mg/g beads) was about 3.6% lower than that of the first use (86.7 mg protein/g beads). This indicates that the prepared hydrazine functionalized beads were of high stability in repeated utilization.

CONCLUSION

Fibrous materials are among the most suitable solid supports for immobilization of enzymes because of their intrinsically high specific surfaces, providing the quantity and the accessibility of the active sites necessary for high reaction rates and conversions. In this study, the functional hydrazine groups carrying fibrous polymer grafted p(VBC/EGDMA) beads were used for the immobilization of invertase. The desired amount of enzyme can be loaded on the beads by changing the initial concentration of enzyme in the immobilization medium. The optimal pH was broadened for the immobilized enzymes. The optimum temperature was about 45°C for the free invertase, on the other hand, and this value was increased up to 55°C for the adsorbed enzyme. The Michaelis–Menten constants for the free enzyme and immobilized enzyme were 17 and 23 m*M*, and V_{max} values were found to be 148 and 104 U/mg protein, respectively. The storage stability of the immobilized invertase was also increased at 4°C with respect to the free enzyme. The results show that the immobilized invertase has improved properties compared to the free counterpart. After inactivation of enzyme upon use, the adsorbed enzyme can be removed from the affinity beads with 1.0*M* NaOH. The regenerated affinity beads can be reused for the reversible immobilization of same or different enzyme.

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