Immobilization of Invertase onto Crosslinked Poly(*p*-chloromethylstyrene) Beads

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ABSTRACT: Invertase was immobilized onto poly(*p*-chloromethylstyrene) (PCMS) beads that were produced by a suspension polymerization with an average size of 186 μm. The beads had a nonporous but reasonably rough surface. Because of this, a reasonably large external surface area (i.e., 14.1 m^2/g) could be achieved with the proposed carrier. A two-step functionalization protocol was followed for the covalent attachment of invertase onto the bead surface. For this purpose, a polymeric ligand that carried amine groups, polyethylenimine (PEI), was covalently attached onto the bead surface by a direct chemical reaction. Next, the free amine groups of PEI were activated by glutaraldehyde. Invertase was covalently attached onto the bead surface via the direct chemical reaction between aldehyde and amine groups. The appropriate enzyme binding conditions and the batch-reactor performance of the immobilized enzyme system were investigated. Under optimum immobilization conditions, 19 mg of invertase was immobilized onto each gram of beads with 80% retained activity after immobilization. The effects of pH and temperature on the immobilized invertase activity were determined and compared with the free enzyme. The kinetic parameters K_M and V_M were determined with the Michealis–Menten model. K_M of immobilized invertase was 1.75 folds higher than that of the free invertase. The immobilization caused a significant improvement in the thermal stability of invertase, especially in the range of 55–65°C. No significant internal diffusion limitation was detected in the immobilized enzyme system, probably due to the surface morphology of the selected carrier. This result was confirmed by the determination of the activation energies of both free and immobilized invertases. The activity half-life of the immobilized invertase was approximately 5 times longer than that of the free enzyme. © 2002 John Wiley & Sons, Inc. J Appl Polym Sci 83: 1268–1279, 2002

Key words: chloromethylstyrene; suspension polymerization; enzyme immobilization; invertase; glycoenzyme; polyethylenimine; crosslinking; functionalization of polymers; macroporous polymers; polyimines

INTRODUCTION

One of the most important research areas in enzyme immobilization technology is on the production of carriers, and much effort has been dedicated toward their development.[1,2] Glycoenzymes are an important family that is widely used in immobilization studies. Hydrophilic support materials precoupled with Concanavalin-A (Con-A) have been extensively investigated for the immobilization of these enzymes.[3–13] Invertase has been one of the most widely used glycoenzymes in immobilization studies because of the commercial

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importance of sucrose hydrolysis. Yeast invertase was coupled with high yields onto Con-A–agarose.[5] A fixed reactor of immobilized invertase onto Con-A–sepharose was successfully operated without a significant loss in the enzyme activity.[6] Iqbal and Saleemuddin reported a significant improvement in the stability of yeast invertase by immobilization onto Con-A–sepharose.[7] The same researchers also investigated the activity and stability of various invertase preparations immobilized on sepharose matrices carrying different amounts of precoupled Con-A.[8] The immobilizations of other glycoenzymes, glucose oxidase, S1-nuclease, RNase T2, and β-D-glucosidase, onto Con-A carrying hydrophilic supports have also been investigated.[9–13]

Another biospecific route tried in the immobilization of glycoenzymes has been the preparation of insoluble complexes of these enzymes with Con-A. This method has been applied for the immobilization of glucose oxidase, amyloglucosidase, yeast invertase, β-glucosidase, and β-galactosidase.[14–17] The insoluble complex of Con-A and invertase showed an enhanced stability over the soluble enzyme.[17]

Various attempts have also been made for the immobilization of invertase by physical entrapment or covalent attachment.[18–29] Polyvinyl alcohol films under electrochemical polarization and carboxymethylcellulose-gelatine gels crosslinked by different chromium salts have been used for the physical entrapment of invertase.[18,19] Different synthetic supports (i.e., granular dimer acid-co-alkyl polyamine, poly(ethylene-*g*-acrylic acid and periodate-activated sepharose beads) have also been tried as alternative matrices for the immobilization of invertase by covalent attachment.[20–22]

Suspension polymerization is a useful tool in the preparation of beaded support materials suitable for enzyme immobilization by physical entrapment or covalent attachment.[30–33] Covalent enzyme immobilization methods usually involve reactive functional groups on the bead surface. These groups can be incorporated into the bead structure by the use of functional monomers during the polymerization.[30,31] Then, enzymes can be covalently attached onto the polymeric beads after the activation of surface functional groups (i.e., hydroxyl, carboxyl, or amine groups) by the appropriate agents. The polymeric supports with the functional groups with a direct reaction ability against the amine group have been widely utilized in the covalent attachment of enzymes. Functional monomers, such as *p*-chloromethylstyrene (CMS), epoxypropyl-methacrylate, *N*-acryloxysuccinimide, and acrolein, can be considered in this class.[34–38] Poly(*p*-chloromethylstyrene) (PCMS) beads have attracted attention especially as a carrier matrix for biotechnological applications because of their capability to directly bind amine groups. Several polymerization procedures have been proposed for the production of CMS based beads with different bulk and surface properties.[39–41] We also developed some polymerization procedures for the synthesis of PCMS carriers both in the form of large-size polydisperse particles and uniform latex particles.[42–44] In our previous studies, we have investigated the DNA-binding properties of proposed materials.[43,44]

In this study, PCMS beads were produced by the suspension polymerization of CMS. A two-step derivatization route was developed for the functionalization of the produced beads. For this purpose, a polymeric ligand carrying amine groups, polyethylenimine (PEI), was covalently attached onto the bead surface. Next, the free amine groups of PEI were activated by glutaraldehyde (GA). A model glycoenzyme, invertase, was covalently attached onto the bead surface via the direct chemical reaction between aldehyde and amine groups.[45,46] The appropriate enzyme binding conditions and batch-reactor performance of the immobilized enzyme system were investigated.

EXPERIMENTAL

Materials

The monomer CMS (CMS, 98% purity), the crosslinker ethylene glycol dimethacrylate (EGDMA), and the stabilizer poly(vinyl alcohol) (87–89% hydrolized; M_r = 85,000–146,000) were supplied from Aldrich Chemical Co. (Milwaukee, WI) and used as received. The initiator 2,2′-azobisisobutyronitrile (AIBN) was obtained from BDH Chemicals Ltd. (Poole, England). PEI (molecular weight = 25,000) was supplied from Aldrich. Invertase (EC 3.2.1.26, Grade V), GA (Grade II, 25% aqueous solution), and Biuret reagent were purchased from Sigma Chemical Co. (St. Louis, MO). The substrate sucrose was supplied from the Ankara Sugar Plant (Ankara, Turkey). Distilled–deionized water was used in all experiments.

Production of PCMS Beads

Crosslinked PCMS beads were prepared by a suspension polymerization method. The detailed

preparation procedure was described elsewhere.[43] The proposed method was obtained by the combination of the principles of the polymerization method proposed by Nonaka et al.[41] and the principles of suspension polymerizations investigated in our previous studies.[47–50] CMS (2.5 mL), EGDMA (0.75 mL), and AIBN (0.06 g) were dissolved in cyclohexane (3.6 mL). The resulting homogeneous phase was dispersed in the aqueous medium prepared by dissolution of PVA (0.1 g) in water (40 mL). The polymerization was carried out in a magnetically stirred batch reactor (100 mL) at 78°C for 6 h. The stirring rate was kept constant at 300 rpm during the polymerization. After the polymerization period, PCMS beads were extensively washed with ethanol and then with water to remove the diluent and any possible unreacted monomer. The beads were screened in the presence of water and a proper size fraction (i.e., 147–297 μm) was isolated.

PEI Attachment onto the PCMS Beads

It is known that chloromethyl groups present on the PCMS beads are reactive to amino groups.[40] Then, PEI was covalently attached to the PCMS beads with the direct reaction between chloromethyl and amine groups. The detailed procedure and the mechanism of the reaction were given elsewhere.[43] PCMS beads (1 g) were dispersed in the aqueous medium prepared by dissolution of PEI (3 g) in water (30 mL). We conducted the reaction at 50°C for 6 h by shaking the medium at 100 cpm. At the end of this period, PCMS beads were extensively washed with water to remove the physically bound PEI from the PCMS beads.

GA Activation of PEI-Carrying PCMS Beads

PEI-carrying PCMS beads were activated with GA by methods described in the literature.[45,46] For this purpose, free amine groups of covalently attached PEI chains present on the surface of PCMS beads were reacted with GA. PEI-carrying PCMS beads (1 g) were dispersed in a buffer solution (30 mL) with a pH of 8.5 and containing GA (2.5% by weight). We conducted the activation at room temperature (i.e., 22°C) for 1 h by shaking the medium at 100 cpm. The beads were extensively washed with water to remove unbound GA.

Immobilization of Invertase

GA-activated PCMS beads were immediately put into the invertase solution (25 mL) with a pH of 4.7. In these experiments, invertase concentration was varied between 0.5 and 2.75 mg/mL (based on total protein content of invertase). We conducted the enzyme immobilization at 4°C for 24 h by shaking the medium at 100 cpm. The amount of immobilized protein was found by the determination of initial and final protein concentrations in the immobilization medium by the Biuret method. PCMS beads were extensively washed with pH 4.7 buffer to remove physically adsorbed enzyme. However, no significant invertase release from the beads was detected after completion of the washing. Invertase-immobilized PCMS beads were stored at 4°C in a buffer solution at pH 4.7. To determine the optimum immobilization conditions, the effects of GA concentration, immobilization pH, and initial enzyme concentration on the immobilized amount of invertase were investigated.

Determination of Invertase Activity (U)

U was defined as the amount of hydrolized sucrose per unit time (μmol of sucrose hydrolyzed/min). Enzymatic activity experiments both by free and immobilized invertase were performed in a stirred batch reactor. We determined U by following the concentration of inverted sugar by the dinitrosalicylic acid (DNS) method.[51] A typical procedure utilized for the determination of immobilized U was as follows: By means of a special pipette, a certain amount of invertase-immobilized PCMS beads or free invertase (0.5 mg based on total protein content of invertase) were added into a batch reactor containing 15 wt % sucrose solution (30 mL) with a pH of 4.7. The hydrolysis reaction was conducted at 55°C with a stirring rate of 200 rpm. The concentration of inverted sugar was determined against to the time by DNS method.

RESULTS AND DISCUSSION

Characterization of PCMS Beads

A detailed physical characterization of the PCMS beads was given elsewhere.[43] In the suspension polymerization, the yield of spherical beads was 87 wt % based on the mass of monomer initially charged to the reactor. Figure 1 shows a scanning electron miscroscopy (SEM) photograph of the typical surface morphology of the plain PCMS beads. As seen here, the PCMS beads had a non-

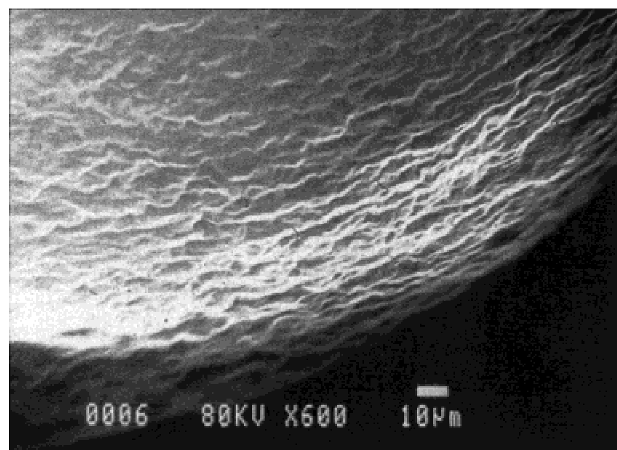

Figure 1 SEM photograph of the typical surface structure of plain PCMS beads.

porous but reasonably rough surface. For the spherical PCMS beads, the specific surface area was 14.1 m^2/g by the BET measurement.[43] The rough character of the bead surface probably provided a high specific surface area. PCMS beads obtained by the proposed suspension polymerization procedure were dominantly collected in the size range of 88–354 μm.[43] The average bead size was 186 μm, as determined by screen analysis.[43] PCMS beads in the size range of 147–297μm were isolated and used as carriers for the immobilization of invertase.

The synthesis of PCMS beads with different functional groups can be accomplished by the covalent binding of ligands such as $NH_2(CH_2)_nX$, where X can be CH_2NH_2, CH_2OH or COOH.[41] In this study, a polymeric ligand (i.e., PEI) was selected for the introduction of amine functionality onto the surface of PCMS beads. PEI was attached covalently onto the bead surface via a direct chemical reaction that took place between amine groups of PEI and chloromethyl groups of PCMS beads. The number–density of amine functionality on the PEI chain was reasonably high. In contrast to a small (i.e., short chain) ligands, the flexibility of free amine groups on a long-chain polymeric ligand attached onto the particle surface should be higher. The steric hindrance effect could be reduced by the selection of a ligand with a longer chain, such as PEI. The variation of equilibrium PEI adsorption capacity of PCMS beads by the initial PEI concentration was defined in our previous study.[43]. Based on these results, the plateau value of equilibrium PEI adsorption capacity was obtained as approximately 200 mg of PEI/g of beads with the initial PEI concentrations higher than 5.0 wt %.[43] After covalent attachment of PEI with the initial PEI concentration of 10 wt %, the free amine content of PCMS beads was 0.875 meq/g. A reaction scheme for the covalent binding of PEI onto the PCMS beads was given elsewhere.[43] Based on the proposed mechanism, PEI could react with the chloromethyl groups via either primary or secondary amine groups. However, the possibility of the reaction being conducted via secondary amine groups was higher relative to that of primary amines.[43]

Determination of Appropriate Immobilization Conditions

GA is one of the most widely used bifunctional agents for enzyme immobilization. It has been known to be an extremely effective agent in enzyme immobilization studies despite the fact that major questions still exist with regard to its mechanism of action.[2] To create free aldehyde groups on the bead surface, the residual amino groups on the PEI-attached PCMS beads were activated by GA. In this part, PEI-attached PCMS beads were activated with different GA concentrations ranging between 0.5 and 5.0 wt %, and the activated beads were treated with an aqueous buffer solution (pH 4.7) that contained invertase (2 mg of protein/mL). The variation of the immobilized amount of invertase on the PCMS beads by the GA concentration is given in Figure 2. Based on the observed behavior, the appropriate GA concentration was 2.5 wt %.

It is well known that enzymes are amphoteric molecules with a large number of acidic and basic groups. The charges on these groups vary according to the their dissociation constants and with the pH of their environment. This affects the total net charge of the enzyme and the distribution of charge on its exterior surface in addition to the reactivity of the active groups.[52] Therefore, the optimum pH for the immobilization of invertase onto PCMS beads was determined. Then, invertase was immobilized onto the PCMS beads at different pH values ranging between 4 and 7. The variation of the immobilized amount of invertase by the immobilization pH is shown in Figure 3. Here, the effect of immobilization pH on the relative activity of immobilized invertase was also included. All activity determinations of the immobilized invertase samples obtained by different immobilization pH values were performed at pH

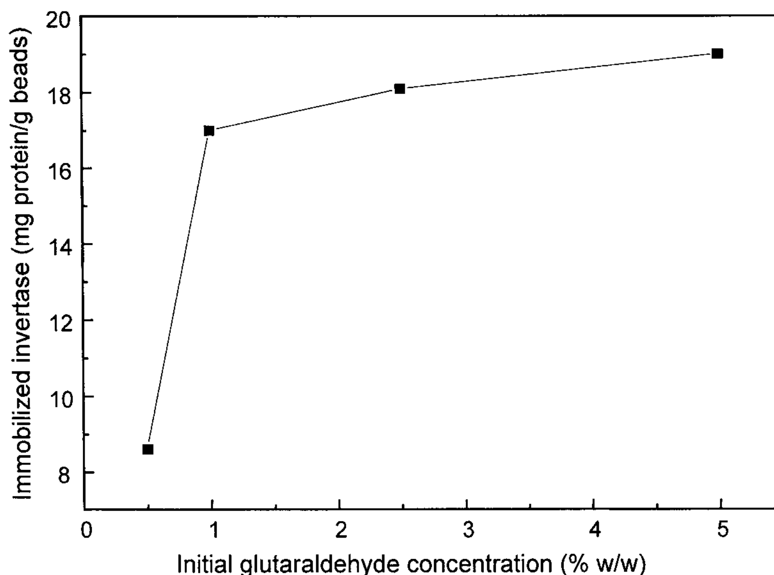

Figure 2 Variation of the immobilized amount of invertase on the PCMS beads by the GA concentration [GA activation conditions: 1 g of PCMS beads in 30 mL of solution, pH 8.5, room temperature (22°C), 100 cpm; invertase immobilization conditions: initial invertase concentration = 2 mg of protein/mL, 1 g of PCMS beads in 25 mL of solution, pH 4.7, 4°C, 24 h, 100 cpm].

4.7. The relative activity was defined as the ratio of activity of immobilized invertase obtained by a certain immobilization pH to the activity of immobilized invertase prepared at the immobilization pH of 4.7. The immobilization pH that provided the highest enzymatic activity under se-

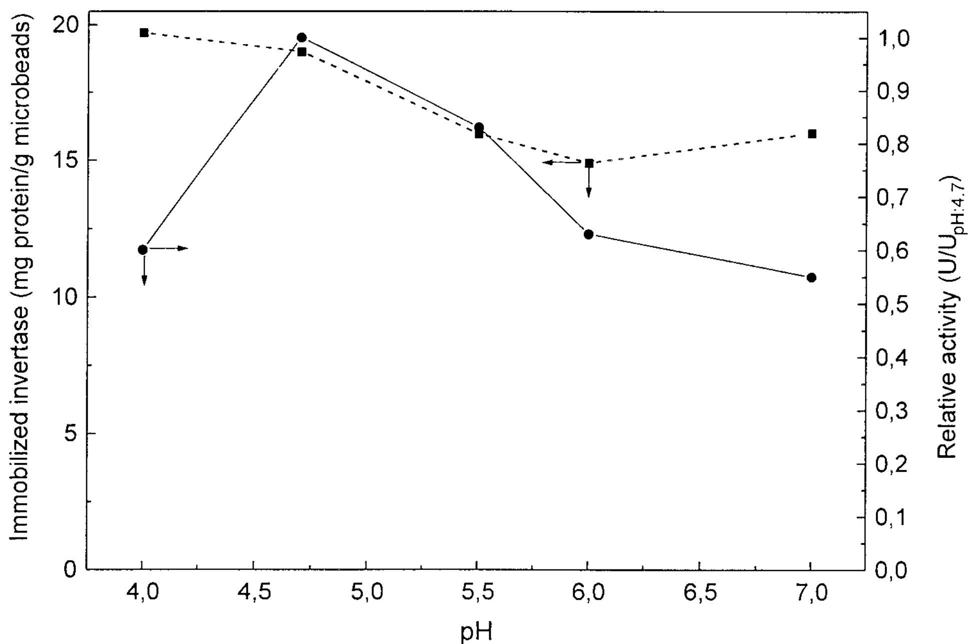

Figure 3 Variation of the immobilized amount of invertase by the immobilization pH (invertase immobilization conditions: initial invertase concentration = 2 mg of protein/mL, 1 g of PCMS beads in 25 mL of solution, 4°C, 24 h, 100 cpm).

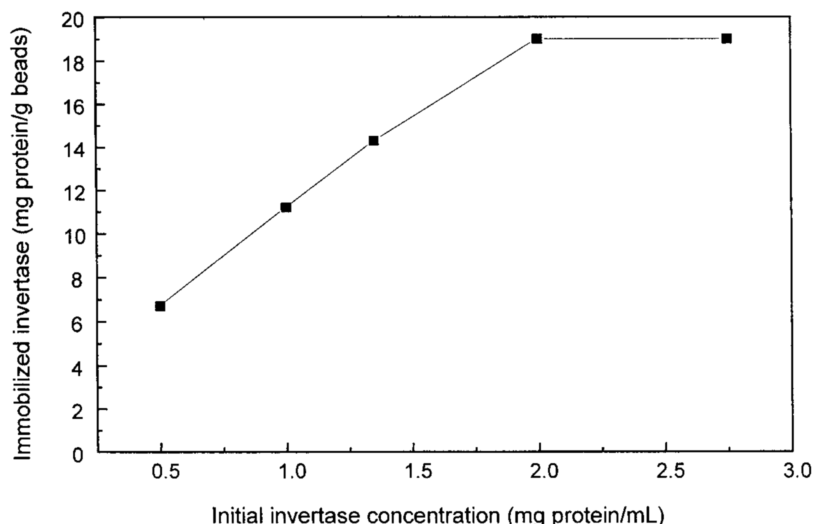

Figure 4 Variation of the immobilized amount of invertase on the PCMS beads by the initial invertase concentration [invertase immobilization conditions: GA concentration = 2.5% (w/v), pH 4.7, 1 g of PCMS beads in 25 mL of solution, 4°C, 24 h, 100 cpm].

lected test conditions (pH 4.7 and 55°C) was evaluated as the optimum value and determined as 4.7, as shown in Figure 3. The determined value was the same with the pH at which the free invertase also showed maximum activity. This should be considered as a factor that reduces the possibility of enzyme denaturation during the immobilization period (i.e., 24 h).

The maximum invertase binding capacity of PCMS beads was determined by the derivation of invertase adsorption isotherm in the existence of GA-activated PCMS beads. In these experiments, we changed the initial enzyme concentration between 0.5 and 2.75 mg/mL (based on total protein content of invertase) by fixing the other immobilization conditions at their appropriate values (i.e., GA concentration = 2.5 wt %, pH 4.7). The variation of the immobilized amount of invertase on the PCMS beads by the initial invertase concentration is given in Figure 4. As seen in this figure, the plateau value of invertase binding capacity was obtained as 19 mg of protein/g of beads with the initial invertase concentrations higher than 2 mg/mL.

Effect of pH on the Activity of the Immobilized Enzyme

The optimum pH of an immobilized enzyme may be different from that of the free one because of the nonuniform distribution of the hydrogen ions between the microenvironment of enzyme and the bulk solution. This effect mostly occurs when the carrier contains ionizable groups.[52,53] Additionally, if the enzyme reaction produces or consumes acid, some special effects may be observed.[54] The activation process may also affect the enzyme structure and the pH–activity profile of the immobilized enzyme. The initial activity of immobilized invertase was determined at different pH values ranging between 3.5 and 6.5 with appropriate buffer solutions. In these experiments, the sucrose concentration and the temperature were fixed at 15 wt % and 55°C, respectively. Identical conditions were also used for the free enzyme. The effects of pH on the relative activities of free and immobilized invertase are shown in Figure 5. As seen here, the maximum activity was obtained at pH 4.7 for both forms. This result indicates that the possible situations regarding pH effect mentioned previously were not significant in our case. Additionally, no significant conformational chance probably occurred in the enzyme by the immobilization. As shown Figure 5, the pH–activity curve was wider than that of free enzyme. This result was attributed to the fact that pH stability of immobilized invertase was better than that of free one.

Effect of Temperature on the Activity of the Immobilized Enzyme

To determine the effect of temperature on the initial activity of free and immobilized invertase,

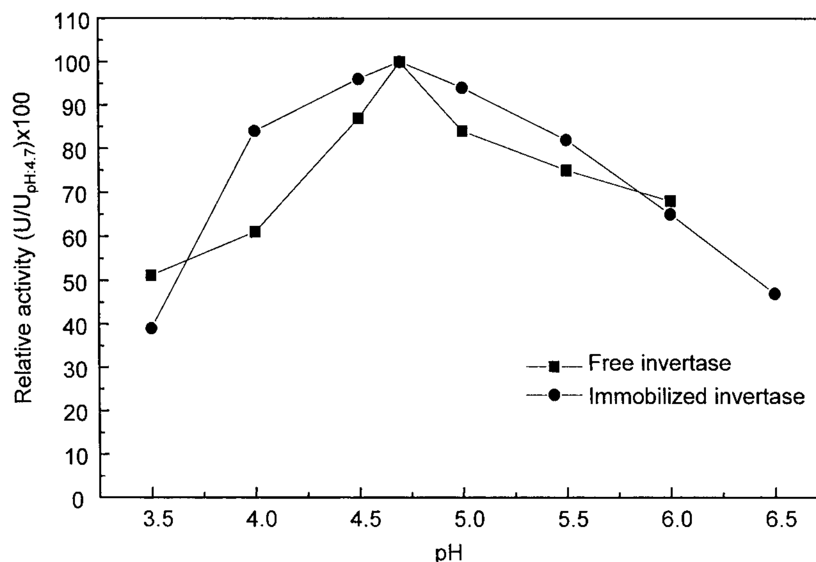

Figure 5 Effects of pH on the relative activities of free and immobilized invertase (amounts of free and immobilized invertase = 0.5 mg based on protein, 30 mL of buffer solution, initial sucrose concentration = 15 wt %, 55°C, 200 rpm).

the activity experiments were performed in batch fashion at different temperatures ranging between 35 and 70°C. In these experiments, the sucrose concentration and pH were fixed at 15 wt % and 4.7, respectively. The effects of temperature on the initial activities of free and immobilized invertase are given in Figure 6. For both free and immobilized enzymes, the relative activity was defined as the ratio of activity at any temperature to the activity obtained at 55°C. For the free enzyme, the relative activity increased with increasing temperature in the range of 35–55°C and exhibited a maximum at 55°C. In this temperature range, the thermal deactivation was probably slow and had no appreciable effect on the rate of the catalyzed reaction. Then, an increase was observed in the relative activity with the increasing temperature. The activity of free enzyme de-

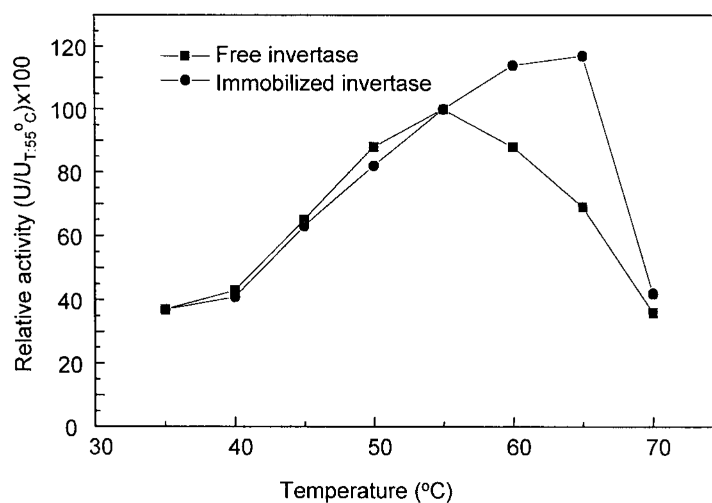

Figure 6 Effects of temperature on the initial activities of free and immobilized invertase invertase (amounts of free and immobilized invertase = 0.5 mg based on protein, 30 mL of buffer solution, initial sucrose concentration = 15 wt %, pH 4.7, 200 rpm).

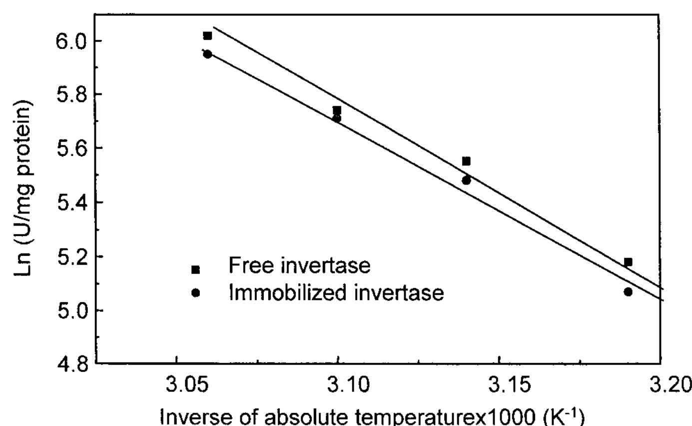

Figure 7 Arrhenius plots sketched for free and immobilized invertase (amounts of free and immobilized invertase = 0.5 mg based on protein, 30 mL of buffer solution, initial sucrose concentration = 15 wt %, pH 4.7, 200 rpm).

creased at temperatures higher than 55°C, probably due to thermal deactivation. However, the activity of immobilized invertase continuously increased with increasing temperature in the range of 35–65°C. Therefore, we came to the conclusion that the immobilization caused a significant improvement in the thermal stability of invertase, especially in the range of 55–65°C.

Activation Energy

The SEM results obtained from our previous studies indicated that although PCMS beads had a rough and nonporous surface, they possessed a highly macroporous interior.[43] The electron micrograph given in Figure 1 also showed the nonporous character of the particle surface. However, these electron micrographs were obtained with dried beads,[43] and it was difficult to ensure that these photographs exactly reflect the real surface structure in the aqueous medium. In other words, the surface morphology in the aqueous medium may be different than that observed in the dry state because some morphological changes occur in the particles during the drying process. For instance, the bead surface may be porous in the aqueous medium, and surface porosity may disappear in the drying process. In the case of a porous surface in the enzymatic reaction media, the immobilized enzyme system involves a diffusion reaction process, whereas only a surface reaction is possible with the beads with nonporous surfaces.

Activation energy is an important parameter for immobilized enzyme systems because it may indicate diffusion limitations. Miyamoto et al. detected the contribution of intraparticle diffusion of the substrate to the overall reaction rate.[55] Arrhenius plots were sketched for the hydrolysis of maltose by both free and immobilized glucoamylase. They observed that the apparent activation energies decreased as a result of the influence of the intraparticle diffusion with increasing particle size.[55] Sharp et al. reported that β-galactosidase immobilized onto porous cellulase sheets had a smaller activation energy than that of the free enzyme, and their reaction system was slightly limited by the internal diffusion.[56] Whalley showed that as the effectiveness factor fell below unity, the measured activation energy also fell, tending toward the arithmetic mean of the activation energies for the diffusion process and the chemical reaction.[57]

Arrhenius plots were sketched for free and immobilized invertase for the determination of apparent activation energies. Here, the activity data collected in the temperature range of 35–55°C (where no significant denaturation of enzyme was observed for both forms) were used. The results are given in Figure 7 and in Table I. As seen here, the apparent activation energies of both forms of invertase were nearly the same. In this case, no intraparticle diffusion resistance was possible for the immobilized enzyme. This result was also consistent with the nonporous view of the bead surface (Fig. 1).

For the proposed carrier, two specific morphological properties probably played an important role in the kinetic behavior of the immobilized enzyme. The rough character of the bead surface provided a reasonably higher surface area (14.1

Table I Kinetic Parameters of Free and Immobilized Invertase

Parameter	Free Invertase	Immobilized Invertase
K_M (wt %)	2.0	3.5
V_M (U/mg of protein)	495	410
Activation energy (cal/mol)	2829	2818
Kd (min^{-1})	0.00075	0.000143
Activity half-life (min)	924	4842

pH = 4.7, 55°C.

m^2/g) for the interaction of the immobilized invertase and the substrate (Fig. 1). The nonporous bead surface probably prevented the diffusion of the substrate into the particle interior. Hence, the possible limiting effect of intraparticle diffusion resistance on the overall substrate consumption rate was not observed. The enzyme–substrate interaction only occurred on the nonporous bead surface. In the case of a relatively high particle surface area used for the enzyme–substrate interaction, an immobilized enzyme system working based on a surface reaction (i.e., without inclusion of an intraparticle diffusion process) should produce substrate consumption rates comparable to those of free enzyme. To test this idea, the kinetic behavior of the immobilized enzyme was determined and compared to that of the free enzyme.

Kinetic Parameters K_M and V_M

To determine the kinetic parameters of immobilized invertase, the variation of initial activity with the initial sucrose concentration was investigated. In these group of experiments, the initial sucrose concentration was varied between 0.5 and 25.0 wt %. pH and temperature were fixed at 4.7 and 55°C, respectively. The activity experiments were also conducted with free invertase under identical conditions. The variation of activity with the initial sucrose concentration is given in Figure 8. Here, the specific enzyme activity is defined as the U based on per milligram of protein. For both forms of invertase, the plateau value of activity was observed after the initial sucrose concentration of 10 wt %. The relation between the activity and the initial sucrose concentration could be adequately described by the Michaelis–Menten model. The apparent kinetic parameters determined based on this model (i.e. K_M and V_M) are presented in Table I.

There are several reasons why a different kinetic behavior is observed with an enzyme immobilized onto a solid support relative to the free enzyme. First, the immobilization may cause some conformational changes in the enzyme molecules. Second, the immobilized enzyme is located in an environment different from that when it is in the free solution, and this can have a significant effect on the kinetics.[54] Third, there is a partitioning of substrate between the solution

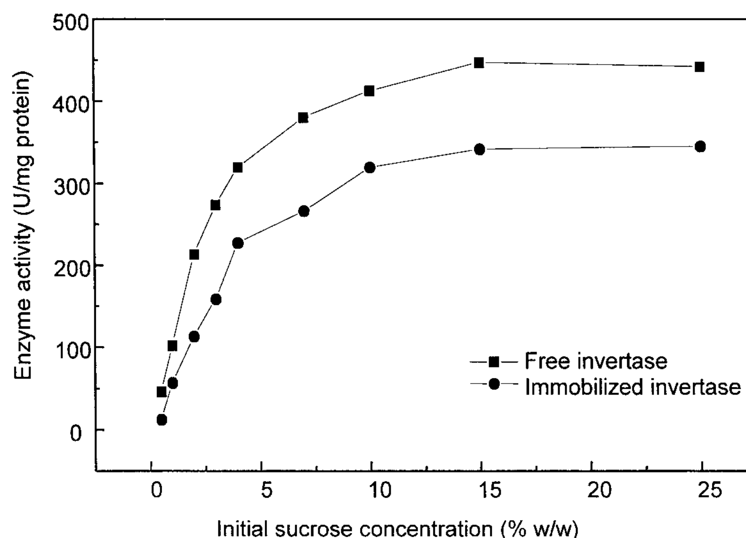

Figure 8 Variation of activity with the initial sucrose concentration (amounts of free and immobilized invertase = 0.5 mg based on protein, 30 mL of buffer solution, 55°C, pH 4.7, 200 rpm).

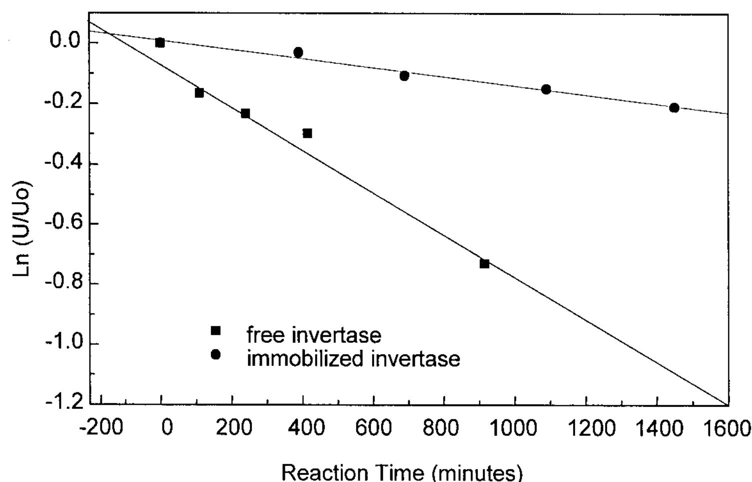

Figure 9 Deactivation of both forms of invertase (amounts of free and immobilized invertase = 0.5 mg based on protein, initial sucrose concentration = 15 wt %, 30 mL of buffer solution, pH 4.7, 55°C, pH 4.7, 200 rpm).

and support; hence, the substrate concentration in the neighborhood of the enzyme may be significantly different from that in the bulk solution.[54] The results show that the apparent K_M value of the immobilized invertase (3.5 wt %) was approximately 1.75 times higher than that of the free invertase, (2 wt %). The small increase in the apparent K_M value of the immobilized invertase was probably related to the conformational changes. V_M of the immobilized invertase [410.0 U/mg (μmol of sucrose hydrolized min^{-1} mg of $protein^{-1}$)] was 83% of the corresponding value of the free invertase (495 U/mg). The comparison of kinetic parameters for the free and immobilized invertases indicated that conformational changes or the deactivation that originated from the selected immobilization method were not too significant.

Stability of the Immobilized Invertase

Thermal stability of the immobilized invertase was tested by two methods. First, the rate of activity decrease was determined for the free and immobilized invertases at 55°C. These experiments were performed in a batch reactor with 150 mL of reaction medium. Sucrose concentration and pH were 15 wt % and 4.7, respectively. The activity half-lives of free and immobilized invertase were calculated according to first-order deactivation kinetics.[58] The deactivation kinetics of both forms of invertase are shown in Figure 9. As seen here, the experimental results obtained for both forms could be adequately described by the first-order deactivation model. The activity half-lives of free and immobilized invertases were found as 15.4 and 80.7 h, respectively (Table I). Under optimum conditions, a fivefold longer activity half-life could be achieved by the immobilized enzyme.

Second, we tested the reusability of the immobilized invertase by performing 26 batch experiments with the same sample of particles carrying immobilized invertase. Each experiment continued for 1 h, and we completed 26 batch experiments in a time period of 13 days by performing two batch experiments per day. Here, the relative activity of immobilized enzyme was defined as the ratio of initial activity at any run to the initial activity observed in the first run (U/U_1). The variation of relative activity of the immobilized invertase with the run number is given in Figure 10. As seen here, immobilized enzyme exhibited quite stable behavior. The activity of the immobilized invertase decreased about 20% after 25 batch experiments.

CONCLUSIONS

In this study, crosslinked PCMS beads produced by a suspension polymerization process were investigated as an alternative carrier for the covalent immobilization of the glycoenzyme invertase. For this purpose, a chemical derivatization route including amine and aldehyde functionalization steps (i.e. PEI attachment and GA activation) was

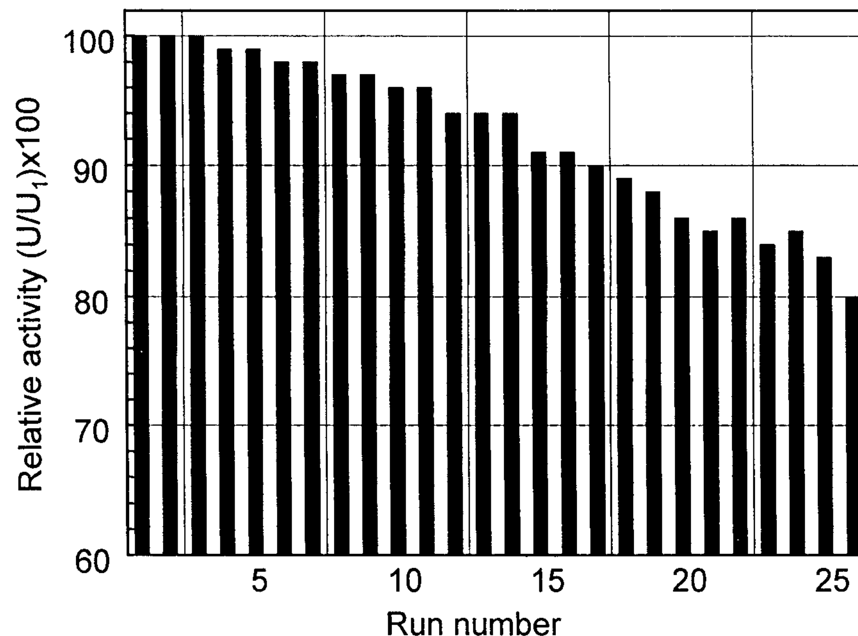

Figure 10 Variation of relative activity of the immobilized invertase with the run number (amount of immobilized invertase = 0.5 mg based on protein, initial sucrose concentration = 15 wt %, 30 mL of buffer solution, pH 4.7, 55°C, 200 rpm).

applied onto the PCMS beads. Invertase was then covalently attached onto the beads with free aldehyde groups. The kinetic parameters (K_M and V_M), the apparent activation energy, and the thermal stability of immobilized enzyme were determined and compared to those of the free one. As shown by the electron microscopic study, PCMS beads had a nonporous but reasonably rough surface. The rough character of the bead surface provided a reasonably higher surface area (14.1 m^2/g) for the interaction of immobilized invertase and substrate. The nonporous character prevented the diffusion of substrate into the particle interior. Hence, no significant intraparticle diffusion resistance was detected for this system. This result was obtained by the comparison of activation energies determined both for free and immobilized enzymes. The interaction between the immobilized enzyme and substrate probably occurred on the bead surface. So, high apparent substrate consumption rates could be achieved due to the large external surface area and the absence of intraparticle diffusion resistance. The substrate consumption rate of immobilized enzyme exhibited only a 20% decrease relative to that of the free one under optimum conditions.

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