Immobilization of Glucoamylase on the Plain and on the Spacer Arm-Attached Poly(HEMA-EGDMA) Microspheres

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ABSTRACT: Immobilization glucoamylase onto plain and a six-carbon spacer arm (i.e., hexamethylene diamine, HMDA) attached poly(2-hydroxyethylmethacrylate-ethyleneglycol dimethacrylate) [poly(HEMA-EGDMA] microspheres was studied. The microspheres were prepared by suspension polymerization and the spacer arm was attached covalently by the reaction of carbonyl groups of poly(HEMA-EGDMA). Glucoamylase was then covalently immobilized either on the plain of microspheres via CNBr activation or on the spacer arm-attached microspheres via CNBr activation and/or using carbodiimide (CDI) as a coupling agent. Incorporation of the spacer arm resulted an increase in the apparent activity of the immobilized enzyme with respect to enzyme immobilized on the plain of the microspheres. The activity yield of the immobilized glucoamylase on the spacer arm-attached poly(HEMA-EGDMA) microspheres was 63% for CDI coupling and 82% for CNBr coupling. This was 44% for the enzyme, which was immobilized on the plain of the unmodified poly(HEMA-EGDMA) microspheres via CNBr coupling. The K_m values for the immobilized glucoamylase preparations (on the spacer arm-attached microspheres) via CDI coupling 0.9% dextrin (w/v) and CNBr coupling 0.6% dextrin (w/v) were higher than that of the free enzyme 0.2% dextrin (w/v).The temperature profiles were broader for both immobilized preparations than that of the free enzyme. The operational inactivation rate constants (k_{iop}) of immobilized enzymes were found to be 1.42×10^{-5} min^{-1} for CNBr coupled and 3.23×10^{-5} min^{-1} for CDI coupled glucoamylase. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 2702–2710, 2001

Key words: glucoamylase; enzyme immobilization; covalent bonding; spacer arm; poly(HEMA-EGDMA); microspheres

INTRODUCTION

Enzymes are biological macromolecules that catalyse a variety of chemical reactions.[1–5] In food and fermentation industries, α-amylase is used to break down starch molecules into dextrin, with sufficient hydrolysis occurring to make the products soluble and not susceptible to gelling upon cooling. After this process, the dextrin can be further broken down to syrups with a high glucose content by another saccharifying enzyme, glucoamylase. It attacks α-1,4-linkages at the nonreducing ends of dextrin, and when this reaches an α-1,6-linkage it cleaves that also, but at a slower rate than the α-1,4-linkage.[2–4]

Support materials, which play an important role in the utility of an immobilized enzyme, should be nontoxic, and should provide large sur-

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face area suitable for enzyme reactions. Acrylic copolymers have been used extensively as the support for enzyme immobilization, and they have good chemical and mechanical stability. In addition to these advantages they are resistance to microbial attack.[3,6–11] Acrylic polymeric microspheres have attracted much attention because they can be produced easily in a wide variety of compositions, and can be modified for the immobilization systems by introducing a variety of activation methods.[9–11]

Immobilization of enzymes through covalent attachment has been demonstrated to induce higher resistance to temperature, denaturants, and organic solvents in several cases.[12–14] Covalent immobilization on the external surface of support materials has also been proposed to decrease mass transfer limitations associated with several immobilization techniques, such as entrapment or adsorption in sol-gel. In this method, the main concern is to carry out the covalent attachment on amino acids that are not in the catalytic machinery. This may be difficult to achieve and, usually, enzymes immobilized with this technique lose a part of their initial activity. Although usually the immobilized enzyme shows lower catalytic activity than the free one, it is more stable, reusable, and in consequence, less costly for many applications.[15–20]

In this study, attempts are made to find a suitable procedure to obtain a covalent attachment of glucoamylase to an acrylic copolymer [poly-(HEMA-EGDMA)] microspheres. Two different binding methods employing either carbodiimide or cyanogen bromide as the coupling agent, were used for the covalent immobilization of glucoamylase onto the spacer arm-attached poly(HEMA-EGDMA) microspheres. The effect of immobization processes on the enzyme activity, the kinetic parameters, and storage stabilities of the enzymes were investigated. Finally, the application of immobilized glucoamylase to a continuous flow system is presented.

EXPERIMENTAL

Materials

Glucoamylase (AGD, amyloglucosidase, exo-1,4-α-D-glucosidase, EC 3.2.1.3 from *Aspergillus niger* 51 U(mg^1), 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), carbodiimide (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride), CNBr, *o*-dianisidine dihydrochloride, soluble dextrin, and glucose were all obtained from the Sigma Chemical Company (St. Louis, MO), and used as received.

2-Hydroxyethyl methacrylate (HEMA) was obtained from Fluka AG (Switzerland), distilled under reduced pressure in the presence of hydroquinone and stored at 4°C until use. Ethylene glycol dimethacrylate (EGDMA) was obtained from Fluka AG. Inhibitors were removed by alkaline salt extraction (20% NaCl and 5% NaOH), washed twice with distilled water, dried with $CaCl_2$, and stored at 4°C until use. α-α'-Azoisobutyronitrile (AIBN) and polyvinyl alcohol (PVA) were obtained from Fluka AG and used as received. All other chemicals were of analytical grade, and were purchased from Merck AG (Darmstadt, Germany).

Preparation of Poly(HEMA-EGDMA) Microspheres

The poly(HEMA-EGDMA) microspheres were produced by suspension polymerization; the method was described in detail elsewhere.[11]

The dispersion medium consisted of PVA (0.75 g) in 180 mL phosphate buffer (0.5 M, pH 7.0). The discontinuous phase contained HEMA (30 m:), EGDMA (2 mL), isopropyl alcohol (15 mL), and AIBN (150 mg). The reaction was carried out at 75°C under nitrogen atmosphere for 2.0 h and then for 1.0 h at 90°C. After the reaction, the microspheres were filtered, washed with distilled water and with acetone, and dried in a vacuum oven at 50°C for 48 h. They were sieved to yield a fraction 100–200 μm in diameter.

Spacer Arm Attachment onto Poly(HEMA-EGDMA) Microspheres

The covalent attachment of the 6-carbon spacer arm hexamethylene diamine (HMDA) onto the poly(HEMA-EGDMA) microspheres was carried out by the following method**.** The microspheres (10.0 g) were transferred into tetrahydrofuran (60 mL) containing HMDA (10.0 g) and NaH (3.0 g). The reaction medium was boiled at 67°C in a reactor and refluxed for 20 h. After this period, the medium was cooled down to room temperature and methanol was added dropwise until the end of gas formation. The HMDA derived microspheres were washed and then dried in a vacuum oven. They were stored at 4°C until use.

Immobilization of Glucoamylase onto Poly(HEMA-EGDMA) Microspheres

Two different coupling methods were utilized for the immobilization of glucoamylase on the spacer arm-attached poly(HEMA-EGDMA) microspheres. In the first method, carbodiimide was used as a coupling agent for the immobilization of glucoamylase through carboxylic groups. The microspheres (10 g; diameter 100–200 μm) were equilibrated in phosphate buffer (50 m*M*, pH 7.5) for 2 h. It was then transferred to the same fresh buffer (20 mL) containing glucoamylase (2.0 mg $\cdot$ mL^{-1}) and carbodiimide (10 mg). The reaction was carried out at 4°C for 18 h, while continuously stirring the medium. The enzyme-immobilized microspheres were washed with NaCl solution (20 mL, 0.5 *M*) and then with phosphate buffer (50 m*M*, pH 7.0). In the second method, CNBr was employed for the coupling of the enzyme onto the spacer arm-attached and unmodified poly(HEMA-EGDMA) microspheres. An aqueous solution of CNBr (20 mL, 2.0 mg $\cdot$ mL^{-1}) was prepared in distilled water and the solution pH was adjusted to 11.5 with 0.2 *M* NaOH. The spacer arm-attached or unmodified microspheres (10.0 g) was transferred in this medium and was stirred magnetically at 25°C for 60 min. After the reaction period, the activated microspheres were collected and the remaining CNBr removed by washing first with 0.1 *M* $NaHCO_3$, and then distilled water. The immobilization of glucoamylase onto CNBr activated microspheres was carried out in carbonate buffer (0.1 *M*, pH 9.5, containing 2.0 mg $\cdot$ ml^{-1} glucoamylase) at 4°C for 18 h. After the coupling reaction, the enzyme-microspheres were washed as described above.

Determination of Immobilization Efficiency

The amount of protein in the enzyme solution and in the wash solutions was determined by using Coomassie Brilliant Blue as described by Bradford[21] with BSA as a standard. The amount bound enzyme was calculated to be:

$$q = [(C_i - C_f) \cdot V]/W \quad (1)$$

where q is the amount of bound enzyme onto poly(HEMA-EGDMA) microspheres (mg $\cdot$ g^{-1}), C_i and C_f are the concentrations of the enzyme initial and final in the reaction medium, respectively (mg $\cdot$ mL^{-1}), V is the volume of the reaction medium (mL), and W is the weight of the microspheres (g). All data used in this formula are averages of at least duplicated experiments.

Activity Measurements

The activities of both the free and the immobilized glucoamylase preparations were determined by measuring the glucose content in the medium according to a method described previously.[3] Assay mixture contained GOD (25 mg), POD (6.0 mg) and *o*-dianisidine (13.2 mg) in phosphate buffer (100 mL, 0.1 *M*, pH 7.0). The assay mixture (2.5 mL) and enzymatically hydrolyzed sample (0.1 mL) were mixed and then incubated at 35°C for 30 min in a water bath. After addition of sulfuric acid (1.5 mL, 30%) absorbance was measured at 525 nm in a UV/Vis spectrophotometer (Shimadzu, Model 1601; Tokyo, Japan). One unit of glucoamylase activity is defined as the amount of enzyme that produces 1.0 mg glucose from dextrin in 3.0 min at 50°C at pH 5.5.

For the determination of immobilized glucoamylase activity, 0.5 g microspheres were introduced to the substrate solutions (5.0 mL) and the glucose content of the solutions were followed as above. The activity of the immobilized enzyme preparations was presented as a percentage of the activity of free enzyme of same quantity.

The kinetic parameters of the free and the immobilized enzyme preparations were determined in a batch system by varying the concentrations of soluble dextrin between [0.25 and 5.0% (w/v)] in acetate buffer (50 m*M*, pH 5.5).

Effect of Temperature and pH on Free and Immobilized Glucoamylase Activity

The effect of temperature on enzyme activity was studied in the range of 20–60°C with the batch operation, and a dextrin concentration 2.0% (w/v) in acetate buffer (50 m*M*, pH 5.5) was used.

The effect of pH on the activity of free and immobilized enzyme was investigated in a batch system at 50°C. The concentration of the dextrin solution was 2.0% (w/v), and was prepared in acetate buffer (50 mM) in the pH range of 4.0–5.5, and in phosphate buffer (50 mM) in the range of pH 6.0–8.0.

Continuous Use of Immobilized Glucoamylases in a Packed Bed Reactor

The reactor (length 10.0 cm, radius 0.5 cm, total volume 7.8 mL), was made from Pyrex® glass. The reactor was charged with immobilized glucoamylase (weight of enzyme-poly(HEMA-EGDMA) microspheres 5.0 g) yielding a void volume of about 3.0 mL. To determine operational stability of im-

mobilized glucoamylase the reactor was operated at 55°C for 60 h with soluble dextrin 2.0% (w/v) in the acetate buffer (50 m*M*, pH 5.5). The dextrin solution was introduced to the reactor at a flow rate of 20 mL·h^{-1} with a peristaltic pump (Cole Parmer, Model 7521-00,USA) through the lower inlet part. The solution leaving the reactor was collected by means of a fraction collector. At the end of each hour the collected sample was assayed for immobilized glucoamylase activity according to the method given in the Activity Assay section.

Thermal and Storage Stability Measurements of Free and Immobilized Enzymes

Thermal stability of the free and immobilized glucoamylase preparations were carried out by measuring the residual activity of the enzyme exposed to two different temperatures (60 and 70°C) in acetate buffer (50 m*M*, pH 5.5) for 4 h. A sample was removed from the medium at each half-hour and assayed for enzymatic activity. The first order inactivation rate constant, k_i was calculated from the equation:

$$\ln A = \ln A_o - k_i t \qquad (2)$$

where A_o and A are the initial activity and the activity after time t (min), respectively.

The activity of free and both immobilized glucoamylase preparations after storage in acetate buffer (50 m*M*, pH 5.5) at 4°C was measured in a batch operation mode, with the experimental conditions as given above.

Characterisation of Poly(HEMA-EGDMA) Microspheres

Scanning Electron Microscopy

Scanning electron micrographs of the poly-(HEMA-EGDMA) microspheres were obtained using a scanning electron microscope (JEOL 5600, Japan) after coating with gold under vacuum.

Elemental Analysis

The amount of attached hexamethylene diamine onto poly(HEMA-EGDMA) microspheres was determined from elemental analysis device (Leco, CHNS-932, USA).

FTIR Spectra

FTIR spectra of the plain and hexamethylene diamine-attached poly(HEMA-EGDMA) microspheres were obtained in KBr tablet form. The microspheres (0.1 g) and KBr (0.1 g) were thoroughly mixed and the mixture was pressed to form a tablet. The spectra was recorded. by using a FTIR spectrophotometer (Model FTIR 8000 Series, Shimadzu, Japan).

RESULTS AND DISCUSSION

Properties of Poly(HEMA-EGDMA) Microspheres

Poly(HEMA-EGDMA) microspheres were produced by suspension polymerization. Details of production and characterization of these microspheres were discussed in detail elsewhere.[11]

As seen from the optical micrograph [Fig. 1(A)], the microspheres had a regular spherical geometry and a particle size in the 100–200-μm range. Figure 1(B) shows the SEM micrograph of the poly(HEMA-EGDMA) microspheres surface. The SEM micrograph shows that the microspheres have a porous surface and they show irregular pores of varying dimensions. These surface properties of the poly(HEMA-EGDMA) microspheres

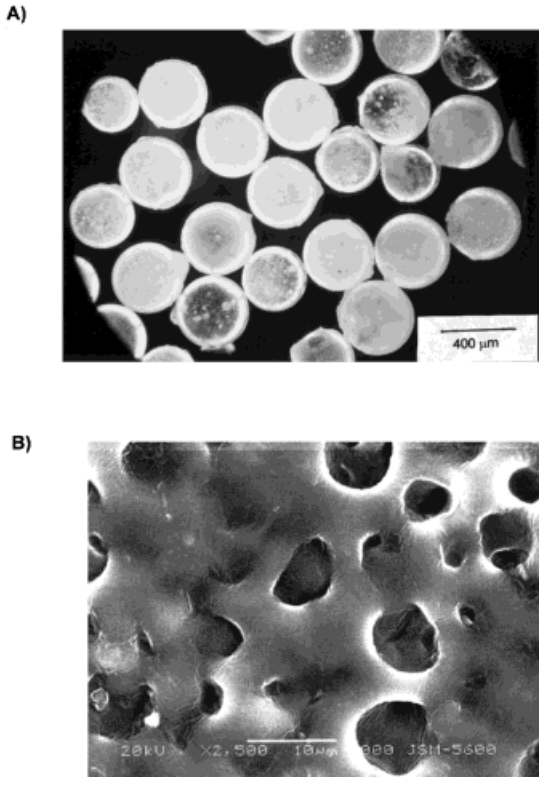

Figure 1 (A) Optical micrograph of poly(HEMA-EGDMA) microspheres. (B) SEM micrograph of poly-(HEMA-EGDMA) microspheres surface.

would favor higher immobilization capacity for the enzyme due to increase in the surface area.

Ester groups in the poly(HEMA-EGDMA) structure were converted to amino groups by reacting with the hexamethylene diamine to form a six-carbon spacer arm. Elemental analyses of the HMDA attached poly(HEMA-EGDMA) microspheres were performed, and the amounts of the incorporated amino group were found to be $236\mu mol \cdot g^1$ poly(HEMA-EGDMA) microspheres from the nitrogen stoichiometry.

FTIR spectra of unmodified and HMDA-attached poly(HEMA-EGDMA) microspheres are presented in Figure 2. The FTIR spectra of HMDA-attached microspheres have some absorption bands different than those of the unmodified microspheres. The most important adsorption bands at 1665 and 1550 cm^1 representing amide I (C=O stretching) and amide II (N—H bending), respectively, are due to HMDA bonded to the poly(HEMA-EGDMA) microspheres.

No enzyme leakage was observed during washing of the freshly prepared microspheres or during batchwise or continuous operation of the enzyme microspheres. All these indicate that the applied immobilization processes were irreversible under the conditions used.

The characterization of the performance of the enzyme immobilized on the poly(HEMA-EGDMA) microspheres with three different immobilization conditions was conducted in the batch and the continuous reactor system. The activity of the free and immobilized glucoamylase preparations were determined by measuring the glucose content of the reaction medium.

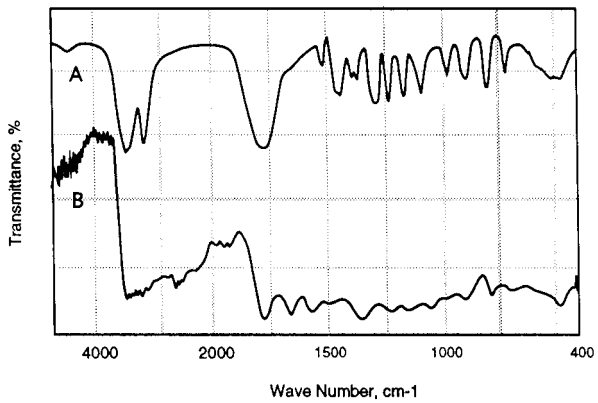

Figure 2 FTIR Spectra of poly(HEMA-EGDMA) microspheres; (A) unmodified microspheres; (B) HMDA-attached microspheres.

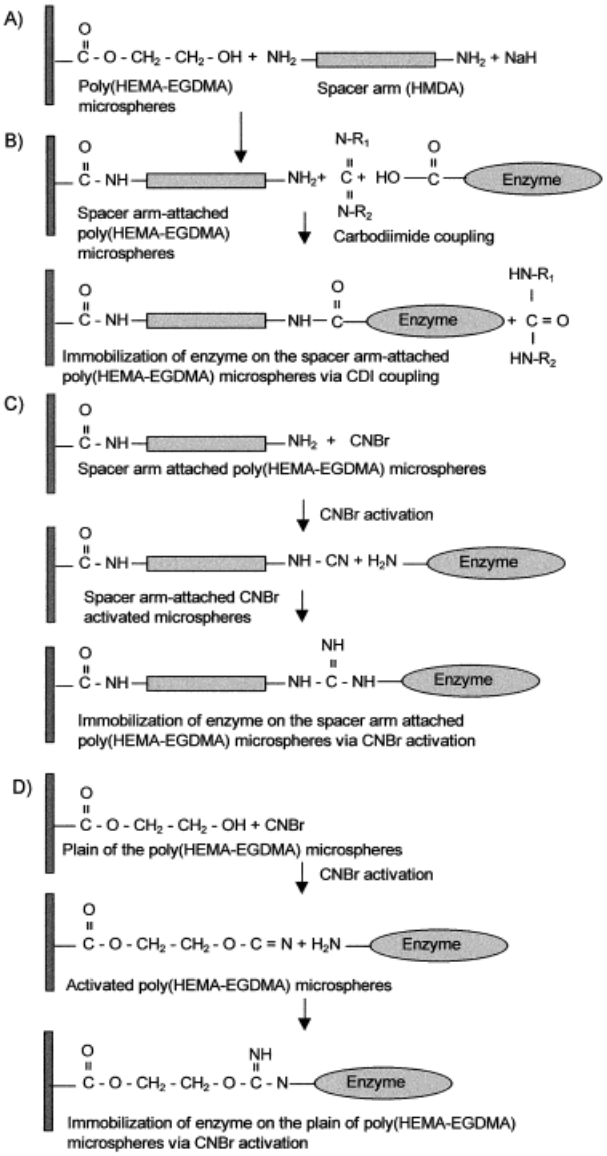

Figure 3 Schematic representation of reaction mechanisms; (A) spacer arm attachment; (B) immobilization of enzyme on the spacer arm-attached microspheres via CDI coupling; (C) immobilization of enzyme on the spacer arm-attached microspheres via CNBr coupling; (D) immobilzation of enzyme on the plain of microspheres via CNBr coupling.

Immobilization of Glucoamylase onto Poly(HEMA-EGDMA) Microspheres

Two different coupling methods were used for the covalent immobilization of glucoamylase on the HMDA-attached poly(HEMA-EGDMA) microspheres (Fig. 3). In the first method, the HMDA- attached poly(HEMA-EGDMA) microspheres and condensing agent (in this case car-

Table I Enzyme Loading and the Kinetic Properties of the Free and the Immobilized Glucoamylases

Glucoamylase	K_m (%, w/v) Dextrin	V_{max} (U · mg^{-1} Enzyme)	Bound Enzyme (mg · g Support^{-1})	U · g^{-1} Support[a]	Relative Activity (%)
Free	0.2	54.7	—	—	100
Immobilized via CNBr coupling (on the plain)	1.1	23.2	3.43	62.4	44
Immobilized via CDI coupling (HMDA-attached)	0.9	33.4	2.87	80.1	63
Immobilized via CNBr coupling (HMDA-attached)	0.6	42.5	4.26	154.7	82

A standard curve was prepared with glucose solutions of different concentration and the slope of the curve was used in the quantification of glucose in the sample.

[a] U · g Support^{-1} = (1.0 mg glucose in ml enzymatically hydrolysed mixture per 3 min)/(g microspheres).

bodiimide) were added simultaneously to the enzyme solution. The amino or hydrazine group-containing supports provide a method of binding enzyme via their carboxyl groups. During the condensation reaction amide bonds are formed between amino groups of the support and carboxyl groups of the enzyme (Fig. 3). In the second method, the activation of amino groups of the HMDA attached and unmodified poly(HEMA-EGDMA) microspheres was achieved by the reaction with cyanogen bromide under alkaline conditions. Glucoamylase was then covalently immobilized via amino group to the activated microspheres. The amino group of the enzyme leading to the formation of guanidino linkage between the enzyme and spacer arm-attached support (Fig. 3).

Spacer arm constituted by aliphatic chains of six-carbon atoms has been used to move away-immobilized glucoamylase from the support. The attachment of six-carbon atoms hydrophobic spacer-arm on the poly(HEMA-EGDMA) microspheres surface could prevent undesirable side interaction between large enzyme molecule and support.[7,22] In this way, more areas of the immobilized glucoamylase could become accessible to its large substrate soluble dextrin.

The amount of enzyme, which was bound onto HMDA-attached and unmodified poly(HEMA-EGDMA) microspheres with these immobilization methods and their resultant activities are presented in Table I. The concentration of the soluble dextrin in the activity assay medium was 2.0% (w/v). The conditions were the same for the free and the immobilized preparations as described for the activity assays. As seen in Table I, the attachment of spacer arm significantly increased the immobilized glucoamylase activity. The immobilization of glucoamylase through amino groups via cyanogen bromide coupling onto spacer arm-attached poly(HEMA-EGDMA) microspheres resulted a higher enzyme loading (4.26 mg · g^{-1}) and a higher activity yield (154.7 U · g^{-1} support) than the immobilization of enzyme through carboxyl groups via carbodiimide coupling (2.87 mg · g^{-1}) and (80.1 U · g^{-1} support), respectively. Note that about 3.43 mg glucoamylase was immobilized on the plain of per g microspheres before the spacer arm was attached, the activity yield was about 62.4 U · g^{-1} support. These results indicated that the attachment of the hydrophobic spacer arm on the poly(HEMA-EGDMA) microspheres surface can provide an easy binding process for the high molecular weight substrate dextrin to the active site of the immobilized enzymes. As seen in Table I, the relative activity of the immobilized enzyme on the plain of the microspheres via CNBr coupling was 44%, it was significantly lower than either of the two spacer arm-attached ones (63% for CDI coupling) and (82% for CNBr coupling). The low relative activity obtained the former probably reflect the presence of steric hindrance caused by the immobilization and/or binding difficulty of the large size substrate molecule on the active side of the enzyme.

Kinetic Constants

Kinetic parameters, the Michaelis constant (K_m) and the maximal initial velocity of the reaction (V_{max}) for the free and the immobilized glucoamy-

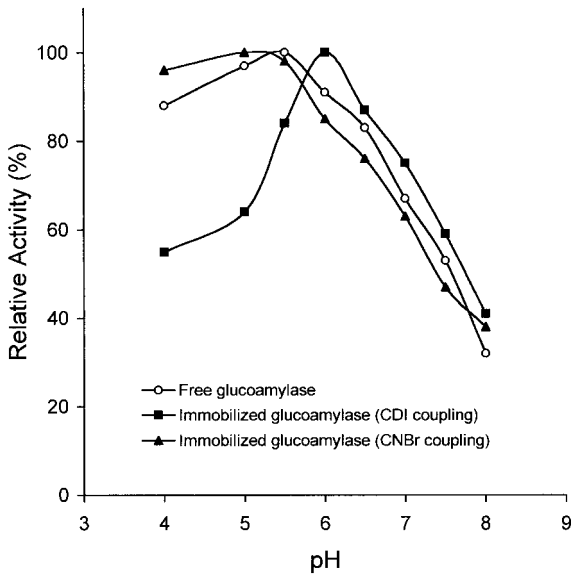

Figure 4 pH profiles of the free and the immobilized glucoamylases.

lase preparations were determined using soluble dextrin as substrate (Table I). For the free enzyme, K_m was found to be 0.2% (w/v), whereas V_{max} was calculated as 54.7 U · mg^{-1} of enzyme). Kinetic constants of the immobilized glucoamylases were also determined in the batch system. K_m values were found to be 0.9% (w/v) for CDI coupled enzyme and 0.6% (w/v) for CNBr coupled enzyme. The larger K_m values of the immobilized enzyme preparations may be explained by diffusional limitations imposed on the substrate. The V_{max} values of the enzyme immobilized onto the spacer arm-attached poly(HEMA-EGDMA) microspheres via CDI and CNBr coupling were estimated from the data as 33.4 and 42.5 U · mg^{-1}, respectively. In our study, as expected, the K_m and V_{max} values were significantly affected after covalent immobilization of glucoamylase onto poly(HEMA-EGDMA) microspheres. These effects were more pronounced when CDI was used as a coupling agent. 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 and by lower accessibility of the large substrate to the active side of the immobilized enzyme. In general, when an enzyme is immobilized, the kinetic parameters K_m and V_{max} undergo changes with respect to corresponding parameters of the free form. These changes are caused by several factor such as protein conformational changes induced by support, steric hindrance, and diffusional effects.[3,23]

Effect of pH and Temperature on the Free and Immobilized Enzyme Activity

The effect of pH on the activity of free and immobilized glucoamylase in dextrin hydrolysis was carried out in the pH range of 4.0–8.0, and the results are presented in Figure 4. The maximum activity for the free enzyme was observed at pH 5.5. The optimum pH value of the free enzyme shifted to a 0.5 unit to more acidic and less acidic region after immobilization of glucoamylase via CNBr and CDI coupling on the spacer arm-attached poly(HEMA-EGDMA) microspheres, respectively. The shift depends on the method of immobilization as well as on the structure and charge of the matrix.

The maximum activity for free and immobilized preparations was observed at 50 and 55°C, respectively (Fig. 5). The increase in optimum temperature was caused by changing the physical and chemical properties of the enzyme. The covalent bond formation between the enzyme and support might also reduce the conformational flexibility, and may result in a higher activation energy for the molecule to reorganize to the proper conformation for the binding to substrate. In general, the effect of changes in temperature on the rates of enzyme-catalyzed reactions does not provide much information on the mechanism of biocatalysts. However, these effects can be important in indicating structural changes in enzyme.[3,7,22,23]

Thermal Stability

Knowledge of thermal stability of immobilized enzyme is very useful in the investigation of poten-

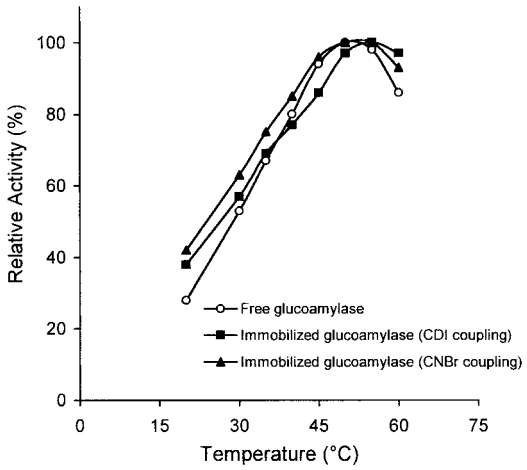

Figure 5 Temperature profiles of the free and the immobilized glucoamylases.

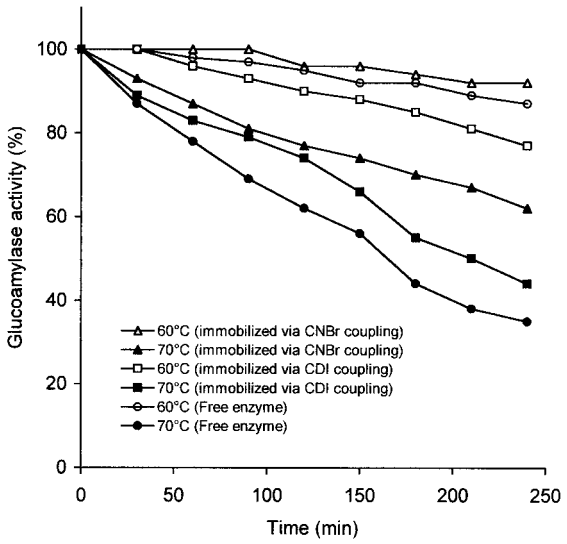

Figure 6 Effect of temperature on the stability of the free and the immobilized glucoamylases.

tial applications for enzymes. Figure 6 shows the thermal stabilities of the free and the enzyme immobilized onto the spacer arm-attached microspheres. Thermal stability experiments were carried out with free and immobilized enzymes, which were incubated in the absence of substrate at various temperatures. The thermal stability of all the glucoamylase preparations decreased with an increase in temperature in the tested temperature range. The free glucoamylase retained about 87 and 35% of its initial activity at 60 and 70°C after a 240-min incubation period, respectively. After a 240-min treatment at 60°C, immobilized glucoamylase via CNBr and CDI coupling retained about 92 and 77% of their initial activities. At 70°C, these were 61 and 44%, respectively. The half lives at 70°C were determined as 185 min for free, 400 min for CNBr, and 214 min for CDI coupled enzymes. The thermal inactivation rate constants (k_i) were calculated as 4.36×10^{-3} min^{-1} for the free enzyme, 1.99×10^{-3} min^{-1} for CNBr coupled and 3.42×10^{-3} min^{-1} for CDI-coupled enzymes. These results suggest that the thermostability of immobilized glucoamylase increased considerably as a result of covalent immobilization onto spacer arm-attached poly(HEMA-EGDMA) microspheres. Similar results have been previously reported for various immobilized enzymes.[3,7,22,23] Ulbrich et al.[23] report that the activity of the immobilized preparation, especially in a covalently bound system is more resistant than that of the soluble form against heat and denaturing agents.

Operational Stability

The operational stabilities of immobilized glucoamylase preparations were studied in a continuous flow system for 60 h. CNBr-coupled and CDI-coupled glucoamylase preparations lost about 5 and 11% of their initial activities after 60 h of the continuous operation, respectively (Fig. 7). The operational inactivation rate constants (k_{iop}) of immobilized enzymes at 50°C with 2.0% (w/v) dextrin in acetate buffer (50 m*M*, pH 5.5) were calculated as 1.42×10^{-5} min^{-1} for CNBr coupled and 3.23×10^{-5} min^{-1} for CDI-coupled glucoamylase. Thus, the high operational stabilities obtained with immobilized glucoamylase preparations indicate that these immobilized enzyme preparations can successfully be used for continuous production of glucose from soluble dextrin.

Complete Hydrolysis of Dextrin with Immobilized Glucoamylase

The complete hydrolysis of dextrin with immobilized glucoamylase preparations (reactor loaded with 5 g enzyme-microspheres CNBr or CDI coupled) was achieved by recycling the dextrin solution (5%, 100 mL) through the reactor. Hydrolysis results were calculated from the amount of glucose produced after enzymatic degradation of dextrin (MW of glucose was taken as 162.14 g). Although complete hydrolysis was expected in 22 min for CNBr coupled enzyme (reactor loaded

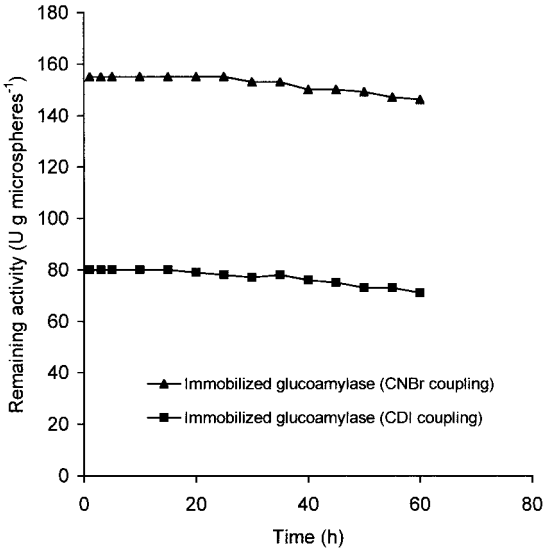

Figure 7 Operational stability of the immobilized glucoamylase preparation in continuous-flow system.

about 773 U enzyme-microspheres) and 42 min for CDI coupled enzyme (reactor loaded about 400 U enzyme-microspheres), it took about 105 and 180 min, respectively. The increase in hydrolysis time may be resulted from reversion product formation. Glucose is not the sole final product; however, in the presence of glucoamylase, one molecule of β-glucose and a second molecule, either α or β-glucose quickly form a lower concentration of maltose and more slowly produce a higher concentration of nigerose and isomaltose.[24,25] This series of linked reactions can cause diffusion limitation of the product species from the pore spaces of the enzyme microspheres, and this phenomena could affect the overall rate of hydrolysis. Lee et al.[26] reported that when the glucose concentration were very high, a higher rate of reversion was observed with the products being isomaltose and nigorose.

CONCLUSION

The porous poly(HEMA-EGDMA) microspheres were prepared by suspension polymerization. Immobilization of a glucoamylase on the plain and spacer arm-attached microspheres was performed. The attachment of spacer arm on the support surface resulted a significant increase in the immobilized enzyme activity. It was observed that the enzyme immobilized on the spacer arm-attached microspheres, especially the CNBr coupling method yielded a high residual activity, and a high operational and thermal stability than that of the CDI coupling method. A high operational stability obtained with these preparations indicates that these immobilized enzymes can successfully be used for continuous production of glucose from soluble dextrin.

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