Immobilization of Glucoamylase on Magnetic Poly(styrene) Particles

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ABSTRACT: Magnetic poly(styrene) particles including active groups were prepared for enzyme immobilization without any activation process. Glucoamylase, which is widely used in industry, was immobilized onto these particles. The effects of pH, buffer concentration, and temperature on immobilization were investigated; moreover, the effect of immobilization temperature on immobilized glucoamylase activity was determined for the hydrolysis of maltose. The acetate buffer with the concentration of 6 $\times 10^{-4}$ M at pH 4 and 20–30°C was found as the most suitable medium for the immobilization of the glucoamylase. The amount of bound protein is 8 mg/g particle with the immobilization yield of 70%. The maximum activity obtained with immobilized glucoamylase is approximately 70% of the free one. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 72: 69–73, 1999

Key words: magnetic poly(styrene) particles; enzyme immobilization; glucoamylase

INTRODUCTION

Generally, porous small particles are used for the immobilization with the high amount of enzyme. But small particles cause high-pressure drops in packed beds and fluidization, back-mixing, and channeling problems in fluidized beds also. On the other hand, they can cause separation problems in batch reactors with viscous reaction medium after completing reaction. These difficulties coming from the small size may be eliminated with the use of magnetic particles. Magnetic particles can easily and gently be separated by using a magnetic field so that possible enzyme activity loses in the separation by filtration may be reduced by magnetic separation. Back-mixing and channeling problems can be reduced by using magnetic particles and magnetic field in fluidized bed reactors.¹ The void volume of the fluidized

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bed can be adjusted by applying of magnetic field to reduce high-pressure drop.

Enzyme immobilization with covalent coupling to insoluble carriers has been a widely researched and used method. Only negligible amount of enzyme leakage occurs with covalent binding because the strength of binding is very strong. But covalent coupling techniques are generally complex and expensive. The other disadvantage of the covalent binding is the deactivation of enzyme during the immobilization process. Many carriers need an activation process to immobilize enzymes.^{12–17} Activation reagents such as glutaraldehyde, benzoquinone are generally harmful for the enzyme and decrease enzyme activity. Some materials including active groups do not need any activation process, and these materials are very useful for enzyme immobilization and other biochemical applications. The polymers including aldehyde and oxirane groups are the examples of these materials with the high reactivities. $^{2-4}$

Magnetic particles for the enzyme immobilization may be prepared by various methods such as

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silanization of magnetic material,⁵ encapsulation of magnetic particles with a proper polymer for enzyme immobilization, or adsorption of a nonspecific protein followed by cross linking on magnetic particles.⁶

In this study, magnetic poly(styrene) particles including active groups were prepared for the enzyme immobilization by the solvent evaporation method.^{7,11} Glucoamylase was directly immobilized on these particles without using an activation process.

MATERIALS AND METHODS

Chemicals

Glucoamylase E.C. 3.2.1.3. (50 U/mg ART1330) and maltose were purchased from Merck. Poly-(styrene) (MW = 220,000-330,000) and was obtained from the Petkim Co., Turkey. Poly(vinyl alcohol) (MW = 30,000, 88% hydrolyzed) was obtained from the Polisan Co., Turkey. Chloroform was purchased from Birpa, Turkey. Biuret reagent and protein standards were purchased from Sigma. Disperbyk 166 a commercial additive for polymer industry was purchased from BYK Chemie, USA. Magnetite (Bayferrox 318) was purchased from Bayer.

Preparation of Particles

Magnetic poly(styrene) particles, which are microcapsules including magnetic particles, were prepared by the solvent evaporation technique.⁷ Poly(styrene) has no active group to bind enzymes. The additive polymer, Disperbyk 166, including reactive aldehyde groups, was added to the poly(styrene) physically as mentioned below. PVA (1.5 g) as the stabilizer was dissolved in 500 mL distilled water. This aqueous phase was stirred at 700 rpm in a 1000-mL beaker with a mechanical stirrer. Polymer phase was prepared by dissolving 15 g poly(styrene), 0.6 g Disperbyk 166, and by suspending 3 g magnetite in 150 mL chloroform. The polymer phase was suddenly poured into the stirred aqueous phase, and chloroform was evaporated by stirring over 20 h at room temperature. The presence of active groups on manufactured magnetic poly(styrene) beads were determined by the assessment of the FTIR/DRS spectra of poly(styrene), additive polymer, and poly(styrene)-additive polymer particles. Strong peaks

at 1713 cm⁻¹ and medium peaks at 2932–2871, 1418, 1107, and 953 cm⁻¹ indicate that there are aldehyde groups on the particles. These particles were then screened and washed with water three times before use. Particles were stored in water at 4°C for further use.

Immobilization Method

Enzyme immobilization can be done by treating the enzyme solution with the particles directly, because the particles themselves contain active groups to bind enzyme. Effects of pH and buffer concentration on glucoamylase immobilization were examined for different pH values and concentrations of acetate and phosphate buffers. Particles (1.4 g), having average diameter of 75 μ m, were added to the 10 mL enzyme solution containing 2 mg/mL glucoamylase in buffer at 30°C. The bounded enzyme was determined by taking samples with time and measuring the remaining concentration of protein in the sample by Biuret Method. After immobilization, particles were washed three times with pH 4.5, $6 \times 10^{-2} M$ NaAc buffer for 15 min in the shaker and kept at 4°C before use, and a released enzyme in the washing steps was also determined. Prepared particles were kept at 4°C in acetate buffer ready for use.

The amount of bound glucoamylase was found when the immobilization was carried out in NaAc buffer solution at pH 4 and the different temperatures in the range of 20 and 50°C to determine the effect of temperature on immobilization yield. The effect of immobilization temperature on the bound enzyme activity was also investigated.

Determination of Enzyme Activity

Glucoamylase activity may be given as the amount of the produced glucose for unit time. Experiments were performed in a standard stirred batch reactor (Mettler DL 21) at a desired pH, temperature, and substrate concentration. Activities were calculated by measuring the produced glucose amount with time. Glucose concentrations were determined with a glucose analyzer (YSI Model 27, USA). In the experiments, 0.65 mg of immobilized or free glucoamylase was added to the reactor containing 50 mL of maltose solution (10% w/v), and the produced glucose was measured with time to calculate activities.

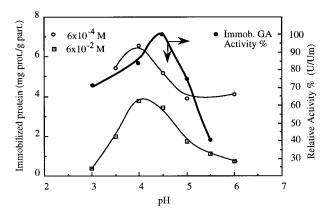


Figure 1 The effects of pH and buffer concentration on immobilized amount of glucoamylase ($T = 30^{\circ}$ C), and the effect of pH on immobilized glucoamylase activity ($T = 50^{\circ}$ C, 10% w/v Maltose as substrate).

RESULTS AND DISCUSSION

Effect of pH and Buffer Concentration On Glucoamylase Immobilization

pH and the ionic strength are the important factors for the enzyme immobilization. Reactivities of the groups on the enzyme molecule and the particle may change with pH and ion concentration.^{8,13,14} Effects of pH and buffer concentration were determined at 30°C for different pH values and buffer concentrations, and the results are given in Figure 1.

The maximum enzyme immobilization was observed at pH 4 for both buffer concentrations. At pH 4 glucoamylase is very active and stable, so that this value is proper for the further immobilization process.⁹ As shown in Figure 1, higher glucoamylase amount was bound with dilute buffer solution with the concentration of $6 imes 10^{-4}$ *M*. This showed that the conformation of the glucoamylase and/or reactivities of the groups on glucoamylase and/or particle at high ion concentration was not proper for this immobilization process. An optimum pH value of 4 is very close to the glucoamylase's isoelectrical point of pH 4.2, so there should be no ionic immobilization at this pH. $6 \times 10^{-4} M$ NaAc buffer at pH 4 was determined as the optimum immobilization medium. The effect of pH on the activity of glucoamylase that was immobilized at these optimum conditions was also determined and given in Figure 1. The activity of immobilized glycoamylase was found as quite high, in the range of pH 4 and 5; therefore, pH 4 is very suitable for this immobilization.

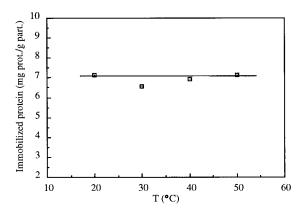


Figure 2 Effect of temperature on immobilized amount of glucoamylase (pH = 4, immobilization time: 120 min).

Effect of Temperature On Glucoamylase Immobilization

Glucoamylase immobilization was carried out at different temperatures, between 20 and 50°C, to determine optimum immobilization temperature.

Figure 2 shows that temperature has no effect on the amount of total bounded protein if the immobilization time is long enough (120 min). In these experiments, approximately 7 mg protein was immobilized on 1 g of particles.

Figure 3 shows the effect of the time and temperature on immobilization process. According to the data in Figure 3, the temperature affects only the immobilization rate. At a high temperature of 50°C, the immobilization rate is high, but the plateau value of immobilized amount of glucoamylase is the same at low temperature of 20°C for the long-time immobilization of 120 min. Although 47% of the immobilization was completed

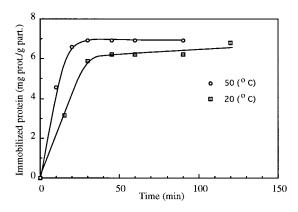


Figure 3 Change of immobilized amount of glucoamylase by time (pH = 4).

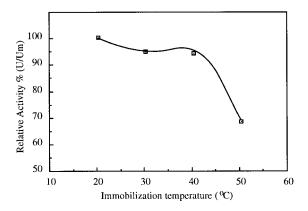


Figure 4 Effect of immobilization temperature on activity ($T = 50^{\circ}$ C, pH = 4.5).

in the first 15 min at 20°C, 66% was completed at 50°C in 10 min.

Effect of Immobilization Temperature on Immobilized Glucoamylase Activity

Immobilization temperature effect on bounded glucoamylase activity was studied. Activities for different immobilization temperatures between 20 and 50°C were determined at 50°C and pH 4.5 with 10% (w/v) maltose¹⁰ as substrate, and the results are given in Figure 4. In the range of the immobilization temperatures of 20 and 40°C, activities of bound glucoamylase does not change too much, but the activity decreases at higher temperatures because of the thermal deactivation of the enzyme during the immobilization. Therefore, the optimum immobilization temperature range for glucoamylase to these particles was determined as 20-30°C, and the required time for immobilization was found to be 60-90 min at these temperatures.

When the maximum activity of immobilized glucoamylase is compared with that of the free enzyme, it was seen that free and immobilized glucoamylase activities are 31.64 U and 22.21 U,

respectively. The retained activity after immobilization is 70% of the free glucoamylase, and this is a reasonable value for the enzyme immobilization.

Immobilization Yield

Immobilized amounts of glucoamylase per gram particle for different initial protein concentrations were studied to determine the optimum immobilization yields. Results are given in Table I.

High initial protein concentrations do not affect the immobilized protein amount too much, and the maximum immobilization yield was found to be 70%. This immobilization yield is quite high compared to the enzyme immobilization studies generally.^{10,15,16}

CONCLUSION

One of the important points of this study is the preparation of the magnetic particles having active groups for the enzyme immobilization without any particle activation process. The particles prepared in this study present some advantages. First, preparation of particles is very easy and cheap. Second, enzyme immobilization on these particles is very simple, and there is no need to any activation of particles. Activation processes of particles are generally difficult and expensive. Activation agents such as glutaraldehyde, benzoquinone, or CNBr can affect the enzyme negatively and cause the activity loss because these agents are generally harmful. In this study, glucoamylase immobilization yield is 70%. This value is quite high for an enzyme immobilization process, and enzyme loss is very small. The retained activity after immobilization is 70% of the free glucoamylase activity at the initial stage, and this is, again a very reasonable value for enzyme studies. On the other hand, the magnetic particles provide

V _o (mL)	$[E]_o (mg/mL) $ (Initial)	$[E]_{f} (mg/mL)$ (Final)	Immobilized Protein (mg/g Part.)	Immob. Yield %
10	1.59	0.47	8.0	70
10	2.17	0.98	8.5	55
10	2.88	1.76	8.0	39
15	1.77	1.12	7.0	37

some advantages, especially in the column studies, such as the low pressure drop, the prevention of channeling, moreover, easy separation in a batch reactor operation by applying a magnetic field.

NOMENCLATURE

FTIR/DRS	Fourier transform infrared spectros-
	copy/diffuse reflectance spectros-
	copy
M_w	Molecular weight
PVA	Poly(vinyl alcohol)
U	Glucoamylase activity unit (μ mol
	glucose produced per minute)
U_m	Maximum glucoamylase activity unit (µmol glucose produced per
	minute)
V_o	Initial volume of enzyme solution (mL)
$[\mathbf{E}]_o$	Initial concentration of glucoamy- lase (mg/mL)
$[\mathbf{E}]_f$	Final concentration of glucoamylase (mg/mL)

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