World Applied Sciences Journal 14 (Special Issue of Food and Environment): 15-19, 2011
ISSN 1818-4952

The Survey of Kinetic Behavior of Immobilized Glucose Oxidase on Gum Tragacanth Carrier

[1]*Maryam Otadi and* [2]*Shirin Mobayen*

[1]Department of Chemical Engineering, Central Tehran Branch of Islamic Azad University, Tehran, Iran
[2]Biochemistry Department, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract: In this study, glucose oxidase (GOD) was encapsulated within tragacanth gel capsules. Gum tragacanth is a viscous, odorless, tasteless, water-soluble mixture of polysaccharides obtained from sap which is drained from the root of the plant and dried. The effects of pH, temperature, oxygen, concentrations of substrate (glucose) and enzyme were studied and the optimal conditions for immobilized GOD obtained. The kinetic parameters, V_{max} (maximum reaction rate) and K_m (substrate affinity), of both free and immobilized enzymes were determined. Oxidation of glucose to gluconic acid followed Michaelis-Menten kinetics.

Key words: Glucose · Glucose oxidase · Tragacanth gum · Encapsulation · Optimum conditions · Kinetics

INTRODUCTION

Glucose oxidase (β-D-glucose: oxygen-oxidoreductase, EC 1.1.3.4) from *A. niger*, is a flavoprotein which catalyses the oxidation of β-D-glucose to D-glucono-δ-lactone and hydrogen peroxide, using molecular oxygen as the electron acceptor. Apart from being an analytical tool in biosensors for medical applications and environmental monitoring glucose oxidase (GOD) finds application in food and fermentation industry [1-3]. This protein is a dimer of two identical subunits with a molecular weight of 160 kDa. The dimer contains two disulfide bonds, two free sulfhydryl groups and two FAD molecules (tightly bound) to the enzyme which are responsible for the oxidation-reduction properties of the enzyme [4,5]. Under denaturing conditions, the subunits of GOD dissociate accompanied and the loss of cofactor FAD occurs [6-8]. Many studies were performed involving the immobilization of GOD in different matrices to enhance properties such as reusability, recovery, stability, thermostability and shelf life [9,10]. Tragacanth is one of the biopolymer that can be employed in the formation of the semipermeable membrane in the capsules. In this context, the main objective of this work was to evaluate the use of tragacanth gel capsules as a matrix for enzyme immobilization and GOD was chosen as a model in this study. The optimum conditions for immobilized enzyme were determined and compared with the free enzyme. Furthermore, apparent kinetic parameters of the encapsulated GOD were compared with the intrinsic kinetic parameters of the soluble enzyme.

MATERIALS AND METHODS

Materials: Glucose oxidase (β-D-glucose: oxygen-l-oxidoreductase, EC 1.1.3.4.) from *A.niger*,200 U/mg protein was purchased from Fluka BioChemika, β-D-(+)-glucose from Sigma Chemical Co. and aluminum chloride, and iron (III) chloride were from MERCK Chemical Co.,Iranian gum tragacanth (*A. gossypinus*) was used in this study, which was purchased from the local market in Tehran. All the solutions were prepared with distilled water. (Enzyme and glucose were dissolved in 0.1M phosphate buffer)

GOD Encapsulation Procedure: In order to immobilize GOD within tragacanth gel capsules, tragacanth solution (1% w/v) was prepared. The tragacanth solution was maintained under constant stirring (700rpm), using a magnetic stirrer at the bottom of the vessel, and the temperature was held constant at 50°C. The tragacanth solution formed a gel after 2 hrs. After gelation, the enzyme solution (dissolved in 0.1 mol/l phosphate buffer at the desired pH) and iron chloride (10% w/v in distilled water) were added to the tragacanth solution and mixed until the solution was homogenized. Droplets of the

Corresponding Author: Maryam Otadi, Department of Chemical Engineering,
Central Tehran Branch of Islamic Azad University, Tehran, Iran.
Tel: +98 912 3088841.

solution ($FeCl_3$/Tragacanth solution/GOD) were dropped through a syringe into 50 ml aluminum chloride solution (1% w/v). The aluminum chloride solution was maintained under constant stirring (300 rpm), using a magnetic stirrer situated at the bottom of the vessel, in order to prevent the droplets from sticking together and to minimize the external mass transfer resistance. Stirring the solution was carried on for 60 min. A dropping height of 10 cm was used to ensure that spherical droplets were formed. Once the $FeCl_{3/}$ tragacanth solution/GOD solution had been dropped into the aluminum chloride solution, a capsular membrane formed instantaneously around each droplet.

Prior to the removal of capsules, the capsules were rinsed more than two times by adding the appropriate amount of deionized water and phosphate buffer. This rinsing removes excess $AlCl_{3.}$ Capsules were recovered by Whatman paper and transferred to phosphate buffer solution before they were used.

Glucose Oxidase Assay: Activity of GOD is usually estimated by measuring the amount of H_2O_2 or gluconic acid produced in the oxidation of β-D-glucose:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{GOD}} \text{D-gluconic acid} + H_2O_2$$

The enzyme produces gluconic acid, so the pH of the reaction solution should decrease without an addition of a base. The activity of GOD was estimated by measuring the amount of base needed to neutralize the gluconic acid and maintain the pH at constant value. The kinetics of β-D-glucose oxidation by immobilized GOD was studied in batch operation mode. A glass reactor with a capacity of 250 ml and a working volume of 200 ml was used. The reactor was equipped with an oxygen pump generating an oxygen rate of 1 lit/min and the temperature was kept constant at the desired temperature using a cooling/heating bath, with an accuracy range of ±0.1°C. At different reaction conditions the optimum assay conditions for immobilized GOD could thus be determined.

One unit of GOD activity (U) was defined as the amount of enzyme that liberates a titratable amount of D-gluconic acid equivalent to 1 µmol of NaOH per minute at 36°C and pH 6 and uses the first 60 min of titration curves to calculate enzymatic activities of the GOD [14].

Kinetics Studies of Free and Immobilized GOD: The Michaelis constant (K_m) and (V_{max}) for free and immobilized GOD were determined by using Lineweaver-Burk plot [14]. The substrate was glucose, with concentrations of 20, 30, 50, 100, 150 and 200 mmol/l. At 36°C, the reaction rates were determined in 0.1 M phosphate buffer (pH 6) according to the method mentioned above in section 2.3. Based on the obtained Lineweaver-Burk plot, the Michaelis constant (K_m) and (V_{max}) were calculated.

RESULT AND DISCUSSION

The Effect of pH on Glucose Oxidation: The effect of pH value on the activity of GOD for free and immobilized GOD was examined at pH 4.5-6.5 at 36 °C. For determination of optimum pH, glucose solution (0.10M) were prepared with the tested pH values, encapsulated GOD or free enzyme was added to the solution.

The results are shown in Fig. 1. Although immobilization usually alters the optimum pH value for enzymes, this was not observed in the current case. The optimum pH value was 6.0 for both free and immobilized GOD, which indicates that the catalytic function of the GOD does not seem to be affected by the immobilization process proposed in this work.

The Effect of Temperature on Oxidation of Glucose: The effect of temperature on the free and immobilized GOD activity was studied at pH 6. The temperature range of 35 to 39 °C was chosen. The results are shown in Fig. 2. The optimum temperature value was 36 °C for both free and immobilized GOD. The probably reason why the temperature optimum of encapsulated GOD did not increase in comparison with the free enzyme is the possibility, that possibility, that higher temperature may lead to higher diffusion limitations.

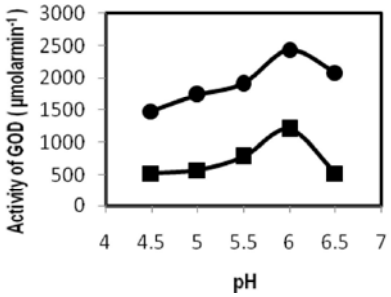

Fig. 1: Activity of free (●) and immobilized (■) GOD at different pH values. The reaction of GOD catalysis was performed for 60 min at 36°C; using glucose concentration of 0.10M; and 0.001g free enzyme or GOD-immobilized in tragacanth gel capsules. The enzyme activity was estimated by measuring the amount of base needed to neutralize the gluconic acid.

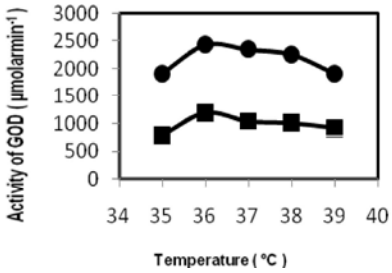

Fig. 2: Activity of free (●) and immobilized (■) GOD at different temperatures. The reaction of GOD catalysis was performed for 60 min at 36 °C; using glucose concentration of 0.10M and 0.001g free enzyme or GOD-immobilized in tragacanth gel capsules. The enzyme activity was estimated by measuring the amount of base needed to neutralize the gluconic acid.

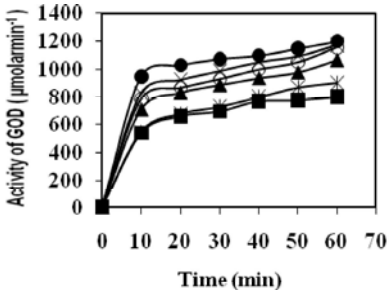

Fig. 3: Effect of glucose concentration on glucose oxidation (pH = 6, temperature = 36 °C, 0.001 g GOD-immobilized tragacanth gel capsules and O_2 flow rate = 1 L/min). Symbols: (■) C = 0.02M; (*) C = 0.03M; (▲) C = 0.05M; (●) C = 0.10; (×) C = 0.15M; (○) C = 0.20M

Similar temperature and pH optima of immobilized GOD have been reported also by other authors [10,15].

The Effect of Glucose Concentration on Oxidation of Glucose: The influence of glucose concentration on the activity of GOD, is represented in Fig. 3. For this experiment, concentration of glucose solution varied in the concentration range of 0.02-0.2 M and the reaction was carried out at 36°C and pH 6.As shown in Fig. 4, GOD activity at 0.1M was higher than that at 0.02, 0.05, 0.15 and 0.2M. Therefore, the optimum glucose concentration is 0.1M at 36°C and pH 6 in this experiment.

The Effect of Enzyme Concentration on GOD Activity: The range of 50-250 units immobilized enzyme was chosen in this study and the reaction was carried out at the optimum condition. As shown in Fig. 4,

Table 3: The effect of glucose concentration on oxidation of glucose

Glucose concentration (mM)	20	30	50	100	150	200
Activity of immobilized GOD (U)	800	904	1067	1200	1175	1160

Table 4: The effect of enzyme concentration on GOD activity

Enzyme concentration (mg/ml)	0.25	0.50	75	1	1.5
Activity of immobilized GOD (U)	1200	1325	1419	1514	1619

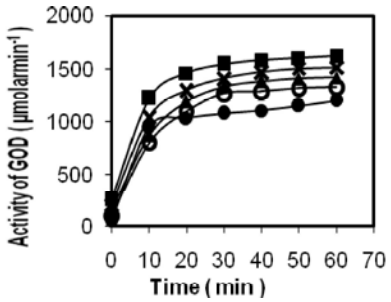

Fig. 4: Effect of enzyme concentration on glucose oxidation (pH =6, T= 36 °C, glucose concentration = 0.1M and O_2 flow rate = 1 L/min). Symbols: (●) 50 units of glucose oxidase; (○) 100 units of glucose oxidase; (▲) 150 units of glucose oxidase; (×) 200 units of glucose oxidase; (■) 250 units of glucose oxidase.

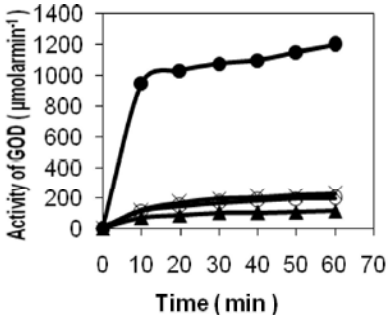

Fig. 5: Glucose oxidation with different aeration rates (pH = 6, temperature = 36 °C, 0.143 g GOD-immobilized tragacanth gel capsules). Symbols: (▲) Air flow rate= 0.5 Lit/min; (○) Air flow rate = 1.0 Lit/min; (×) Air flow rate = 1.5 Lit/min; (●) O_2 flow rate = 1.0 Lit/min.

GOD activity increased when the enzyme concentration increased from 0.25 to 1.25 mg/ml. The maximum activity of immobilized GOD reached 1619 U at 1.5 mg/ml GOD.

Different Aeration Rates: Oxygen as well as glucose is the substrate for GOD. Different aeration rates were employed to study the effect of oxygen. As shown in Fig. 5, oxygen flow rate is 1 lit/min, while the aeration range of 0.5 to 1.5 lit/min was examined. The enzyme activity was high when the oxygen flow rate was used.

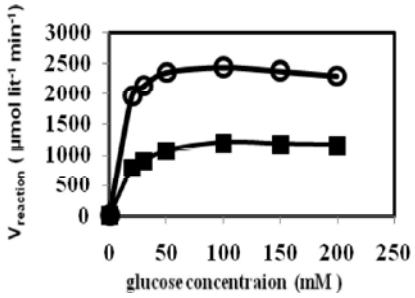

Fig. 6: Reaction rates of free (■) and encapsulated GOD (○) at different glucose concentrations. pH 6; temperature 36°C; 0.001 g free enzyme or 0.001 g GOD-immobilized tragacanth gel capsules.

Table 5: Kinetic parameters for free and encapsulated GOD

GOD	V_{max} (mM min^{-1})	K_m (mM)	r^2
Free	2.5	9.00	0.97
Encapsulated	1.25	12.375	0.99

It can be concluded when the air flow rate was employed the amount of oxygen in solution was less than the time employed by the pure oxygen.

Kinetic Analysis: In order to study effect of encapsulation on the kinetic parameters of the reaction, the initial rates of glucose oxidation reaction by free and encapsulated GOD were measured at various glucose concentrations. Free enzyme (0.001 g) or 0.001 g GOD-immobilized tragacanth gel capsules were thus emplyed. Fig. 6 shows the Michaelis-Menten plots for free and immobilized GOD.

The calculated values of V_{max} and K_m for free and immobilized GOD are summarized in Table 5. As indicated in the table, the immobilized GOD showed a 1.375 fold increase in the value of K_m and a 2 fold decrease in the value of V_{max} as compared with the free GOD. The increase in the value of K_m could be attributed to the lower accessibility of the active sites of immobilized GOD to the glucose molecules. The decrease in *V*max value as a result of immobilization is considered to be associated with the increase in *K*m value, since the lower the *K*m value, the greater the affinity of the enzyme for the substrate.

CONCLUSION

Tragacanth gel capsules have been used as an enzyme immobilization matrix in this study. The optimum conditions selected for the effective encapsulation of glucose oxidase were 1% w/v tragacanth gel, 10% w/v $FeCl_3$, 1% w/v $AlCl_3$, 2 hrs gelation time and at 50°C. The optimum conditions for enzyme activity were studied. The kinetics parameters of both free and immobilized GOD were calculated and values of 9.00 and 12.375 mmol were obtained for the K_m and 2.50 and 1.25 mM min^{-1} were observed for the V_{max} of free and immobilized GOD, respectively.

REFERENCES

1. Hecht, H.J., H.M. Kalisz, J. Hendle, R.D. Schmid and D. Schomburg, 1993. Crystal structure of glucose oxidase from *Aspergillus niger* refined at 2.3 Å resolution. J. Mol. Biol., 229: 153-172.
2. Gerharrtz, W., 1990. Industrial uses of enzymes. In: W. Gerharrtz, editor. Enzymes in industry. Production and applications. New York: VCH Publishers, pp: 77-91.
3. Gibson, Q.H., B.E.P. Swoboda and V. Massey, 1964. Kinetics and mechanism of action of glucose oxidase. J. Biol. Chem., 239: 3927-3934.
4. Müller, F., 1991. In: F. Müller, editor. Chemistry and biochemistry of flavoenzymes. CRC Press: Boca Raton, pp: 1-71.
5. Meyer, M., G. Wohlfahrt, J. Knäblein and D. Schomburg, 1998. Aspects of the mechanism of catalysis of glucose oxidase: A docking, molecular mechanics and quantum chemical study. J. Comput Aided. Mol. Des., 12: 425-440.
6. Ahmed, A., M.S. Akhtar and V. Bhakuni, 2001. Monovalent cation-induced conformational changes in glucose oxidase leading to stabilization of the enzyme. Biochem., 40: 1945-1955.
7. Jones, M.N., P. Manley and A. Wilkinson, 1982. The dissociation of glucose oxidase by sodium n-dodecyl sulphate. Biochem. J., 203: 285-291.
8. O'Malley, J.J. and J.L. Weaver, 1972. Subunit structure of glucose oxidase from *Aspergillus niger*. Biochem., 11: 3527-3532.
9. Blandino, A., M. Macı´as and D. Cantero, 2001. Immobilization of glucose oxidase within calcium alginate gel capsules. Process Biochem., 36: 601-606.
10. Vikartovská, A., M. Bučko, D. Mislovičová, V. Pätoprsty, I. Lacík and P. Gemeiner, 2007. Improvement of the stability of glucose oxidase via encapsulation in sodium alginate-cellulose sulfate-poly (methylene-*co*-guanidine) capsules. Enzyme Microb. Technol., 41: 748-755.
11. Nigam, S.C., I.F. Tsao, A. Sakoda and H.Y. Wang, 1988. Techniques for preparing hydrogel membrane capsules. Biotechnol. Tech., 2: 271-6.

12. Washington, F.D.A., 1974. Fed. Register, 39(185): 34207.
13. Weiping, W. and A. Branwell, 2000. Tragacanth, Karaya. In: G.O. Phillips, P.A. Williams, editors. Handbook of Hydrocolloids, Cambridge: Wood head publishing Ltd., pp: 231-246.
14. Lineweaver, H. and D. Burk, 1934. J. Am. Chem. Soc., 56: 658.
15. Yang, Y.M., J.W. Wang and R.X. Tan, 2004. Immobilization of glucose oxidase on chitosan-SiO2 gel. Enzyme Microb. Technol., 34: 126-131.