ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Immobilization of Glucose Isomerase in Surface-Modified Chitosan Gel Beads.

Sari Edi Cahyaningrum*, Nuniek Herdyastusi, and Dina Kartika Maharani.

Department of Chemistry, Surabaya State University, Indonesia.

ABSTRACT

In this study, the macroporous chitosan beads was prepared, and could be obtained by chelating Cu^{2+}, Zn^{2+}, Ca^{2+}, and Mg^{2+} ions, respectively on chitosan beads, and Glucose Isomerase (GI) could be adsorbed on the Chitosan beads-metal ions adsorbent through metal–protein interaction forces. Batch adsorption experiments show that adsorption capacity for GI on these chitosan beads-metal ions adsorbent varieties with change of pH. The effect of chelated metal ions species on chitosan beads on the activity and stability of immobilized GI was also evaluated and discussed. Chitosan beads-Zn was the best adsorbent among four kinds of Chitosan beads- metal ions for GI immobilization since it has high enzyme loading, high stability and activity retaining the macroporous metal chelated bead could be pervasively applied in enzyme adsorption or immobilization provided the enzyme had metal chelating ability.

Keywords: immobilization, glucose isomerase, chitosan beads

Corresponding Author

ISSN: 0975-8585

INTRODUCTION

Glucose isomerase (EC 5.3.1.5) catalyzes the isomerisation of glucose into fructose. This is a thermo stable enzyme having wide applications. It is produced by a number of microorganisms to take up available sugar and channelize it into their metabolic pathways. This enzyme also converts xylose into xylulose. Xylose is present in abundance as monomeric units of the polymer xylan in plant residues. Glucose isomerase is one of the popular enzymes of future market. This happens to be in demand due to the increasing requirement of health care products. High Fructose Corn Syrup (HFCS) is formed by converting corn starch into glucose and further isomerising it to fructose. Glucose isomerase (GI) serves as an interesting model for studying structure–function relationships by advanced biochemical and genetic engineering techniques. Besides its academic importance, it has received increased attention by industries for its use in producing high-fructose corn syrup (HFCS) and for its potential application in the production of ethanol from hemicelluloses. The use of GI is expensive because it is an intracellular enzyme, and large quantities are needed to compensate for the high K_m for glucose. Therefore, it is important to immobilize GI for its industrial applications. Recently, several methods for immobilizing GI have been developed [1].

Methods existed for the immobilization of enzymes may be divided into two main categories: physical methods based on molecular interactions between the enzyme and support, and chemical methods based on the formation of covalent bonds. The methods and supports employed for enzyme immobilization are chosen to ensure the highest retention of enzyme activity, stability and durability. Among the immobilization techniques, adsorption may have a higher commercial potential than other methods because it is simpler, less expensive, and retains high catalytic activity [2-4, 7] as well as avoiding use of toxic bisfunctional coupling reagent for enzyme immobilization. However, the physical adsorption of enzyme on most hydrophilic support is not generally strong enough.

In this research, chitosan has been chosen to be a support for glucose isomerase. Chitosan has many significant biological and chemical properties such as biodegradable, biocompatible, bioactive and polycatonic properties [5,6,7]. The primary objective of this work is to produce immobilized glucose isomerase by using chitosan beads as support. Chitosan is an ideal support material for enzyme immobilization because of its hydrophilicity, biocompatibility, biodegradability, and anti-bacterial property. The macromolecule is derived chemically by deacetylation of natural polymer chitin. Furthermore, chitosan exhibits a considerable protein binding capacity and the immobilized enzyme remains considerably active. [8-11].

In this work, the macroporous chitosan beads material was used as a potential glucose isomerase immobilization material by direct absorption. However, protein activity might be changed by metal–protein interaction. In this case, if the active sites of glucose isomerase take part in the metal chelating process, the adsorption of the enzyme on material surface might cause the changing of the enzyme activity. So, the effect of surface bound metal ion species on enzyme activity should be studied. The main purpose of this work was to elucidate the possibility of enzyme immobilization on the novel chitosan beads material and its catalytic behavior change after it was adsorbed through the metal chelating

force. This method has a big potential and may be more versatile since it allows a selection among many chelating metal ions.

MATERIALS AND METHODS

Glucose Isomerase (5.3.1.5) were produced from a selected strain of *Steptomyces murinus* and Glucose were obtained from Sigma Chem. Co. (st. Louis, USA). Chitosan was obtained from shell of shrimp with Meyer Methods (1989). All other chemicals were of analytical grade. Glucose isomerase activity was determined by reaction of 1 ml of glucose isomerase in a mixture of 0.8 M glucose, 0.05 M buffer pH 7.0, 0.01 M Mg_2 at 60°C for 1 hour. The reaction was stopped by addition of 4.5 ml hyper chloride solution. The amount of fructose converted from glucose was then assayed for the determination of glucose isomerase activity. Glucose Isomerase Unite is 1 micromole glucose converted to fructose in one minute at pH 7, temperature 60°C, 300 g / l glucose concentration.

Preparation of Chitosan Beads

To prepare highly swollen beads, an amount of chitosan flakes (1g) was completely dissolved in 0.1l of 1-mol/l acetic acid. The resulting solution was sprayed into 125 of ml deionized water containing 15 g NaOH and 25 ml of 95% ethanol through a nozzle (1.2 mm diameter). The chitosan beads were swelled and washed with deionized water until the solution became neutral. The diameter of wet beads approximately 2.3 mm. The BET surface area of swollen beads was not measured because the drying was difficult.

Determination of Immobilization GI

The protein content of the chitosan-GI conjugate was calculated by subtracting the amount of protein determined in the centrifuged and washings following immobilization from the amount of papain used for immobilization. The GI in the solutions was determined by the Bradford method [13].

Adsorption isotherm of GI

Adsorption isotherm of GI was determined by equilibrating different concentration of GI with 0.5 g of Chitosan-metal ion gel beads in 15mMPBS (pH 7.0) for 100 min at room temperature. After equilibration, the amount GI bound was analyzed using Langmuir isotherm models..

The pH profiles of free and immobilized GI

Effect of pH value on the activities of free and immobilized GI were carried out at pH range 3–8.0 (by using appropriate buffer solutions). Activity of pH profiles was determined at indicated pH values using a 10 g L Glucose solution at 60^{o}C.

ISSN: 0975-8585

The Temperature Profiles and Thermal Stability of Free and Immobilized GI

Activity of temperature profiles was determined at indicated temperatures (20–70^{o}C) using glucose solution. The thermal stability of free and immobilized GI was ascertained by measuring the activity of the residual enzyme exposed at various temperatures (20–70^{o}C) solution for 5 h. Activities of samples were performed at optimum conditions.

The Operational Stability of Free and Immobilized GI

The retention of the immobilized enzyme activity was tested as described in the activity assays of GI. After each reaction run, the enzyme immobilized chitosan beads were removed and washed with 0.05 M HCl solution to remove any residual substrate within the chitosan beads. They were then reintroduced into fresh reaction medium and enzyme activities were detected at optimum conditions.

Kinetic Study

The effect of substrate concentration on the activity was tested by using increasing concentrations of glucose, Vmax and Km values of immobilized and free GI were determined.

RESULTS AND DISCUSSION

Adsorption isotherm

In this experiment, adsorption isotherm of GI on the four kinds of Chitosan gel beads was similar to the Langmuir adsorptive isotherm. The four kinds of chitosan gel beads adsorbent have different affinity for GI at pH 7.0. Data shown in Table 1 were fitted to the linear form of the Langmuir equation:

$$\frac{[Ce]}{[Q]} = \frac{1}{k[Q]\max} + \frac{[Ce]}{[Q]\max} \qquad (1)$$

where [*C*e] and [*Q*] represent the equilibrium concentration of trypsin and the adsorbed activity on per gram weight of immobilized chitosan, respectively; [*Q*]max is the maximum GI activity adsorbed on per gram weight of chitosan gel beads-metal ion; *k* is the adsorption–desorption equilibrium constant related to the binding energy.

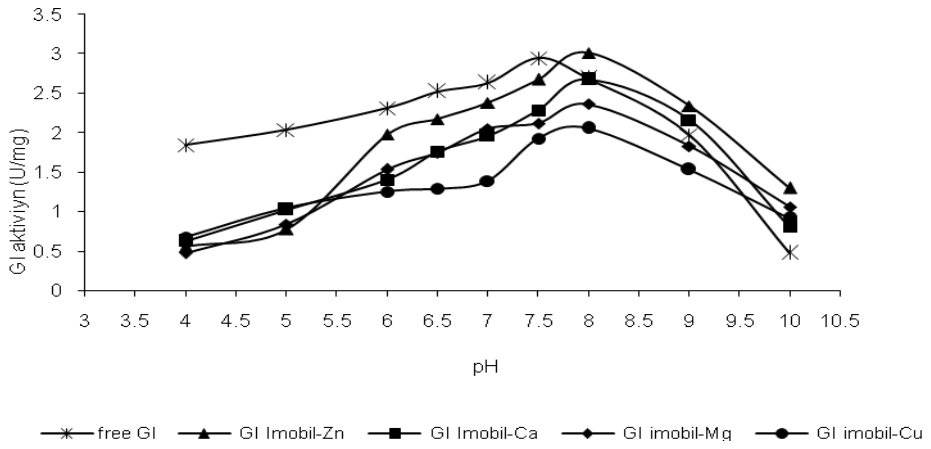
Table 1. Immobilization Capacity of Glucose Isomerase

adsorbent	Langmuir Parameter			
	[Q]max (10^{-6} mol/g)	K $(mol/L)^{-1}$	E (kJ/mol)	R^2
Chitosan beads-Mg	26,61	297132,96	31,43	0,9933
Chitosan beads- Ca	47,98	579133,85	33,10	0,9920
Chitosan beads- Zn	59,84	711647,73	33,61	0,9904
Chitosan beads- Cu	73,27	630647,06	33,31	0,9951

The maximal adsorption capacity could be found in the Cu^{2+}-chitosan gel beads with the value of of 73.27. 10^{-6} mol/g the adsorbent, while the maximal adsorption capacity for GI on Zn^{2+}, Ca^{2+} and Mg^{2+} loaded support was $59.84.10^{-6}$ mol/g, $47.98.10^{-6}$ mol/g and $26.61.10^{-6}$ mol/g, respectively. It seems that the binding strength of the enzyme with the metal ions follow the order of Cu^{2+} >Zn^{2+} > Ca^{2+}>Mg^{2+}. The result also indicated that the adsorption of GI should be mainly through the coordination force between the metal chelating amino acid of the enzyme and metal ions chelated on the support. The Langmuir isotherm is designed for monolayer adsorption of a species on a homogeneous surface with adsorption energy is the same for all active site regardless of the degree of coverage. The Langmuir's adsorption capacity is representation of the capacity of nitrogen on the N acetyl group in adsorbing metal ions. The Langmuir's adsorption capacity may indicate the adsorption capacity of this $–NH_2$.

Effect of pH on the GI Activity

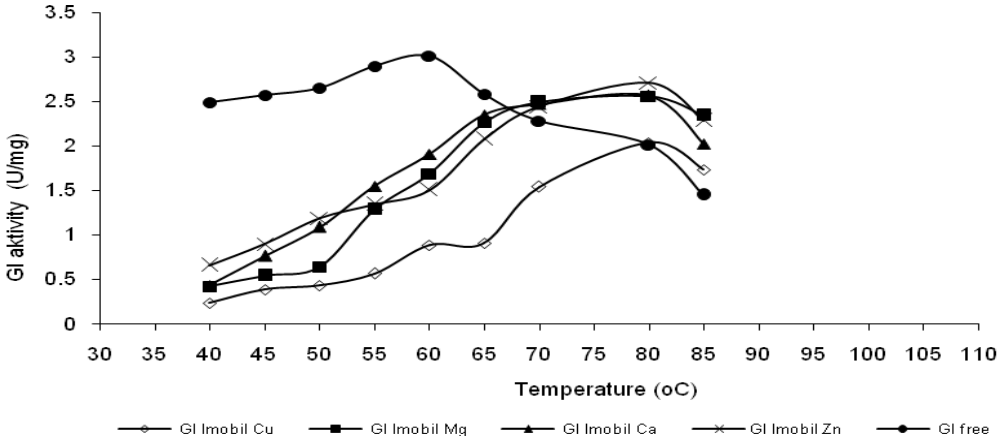
The pH activity profiles of free and immobilized GI were compared. The results are given in Fig. 1. The optimum pH values for free and immobilized GI were the don't same. The optimum pH range was obtained as 7.5 for free and 8.0 for immobilized GI. The stability of various immobilized enzymes can frequently be improved when inorganic supports are used instead of organic polymers because of greater dimensional stabilities of inorganic supports. Shifts in pH optimum with immobilization have been found for many enzymes. Anionic supports tend to shift the pH optimum toward the alkaline. The immobilization of enzymes to charged supports often leads to displacements in the immobilized enzyme and the bulk phase due the electrostatic interactions with the matrix.



Picture 1. Effect of pH on Glucose Isomerase activity

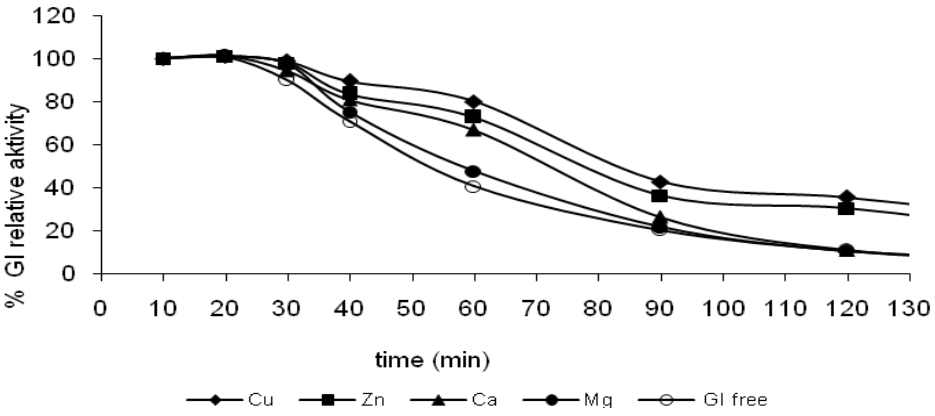
The temperature dependence of the activities of the free and immobilized GI studied in 15 mM PBS at temperature range 40-100 ^{o}C and temperature profiles of free and immobilized GI shown in Fig.2. The optimum temperature range for free and immobilized GI was found to be about 60 and 80^{o}C, respectively. The conformational flexibility of the GI was affected by immobilization. The immobilization of GI on chitosan beads with metal ions bifunctional agent caused an increase in GI rigidity which is commonly reflected by increase in stability towards denaturation by raising the temperature [8, 13]. The binding strength of the enzyme with the metal ions follow the order of Cu^{2+} >Zn^{2+} > Ca^{2+}>Mg^{2+}, but the highest

activities of GI immobilized is GI immobile –Zn because Cu^{2+} is one of inhibitor metal for GI. [8,13].

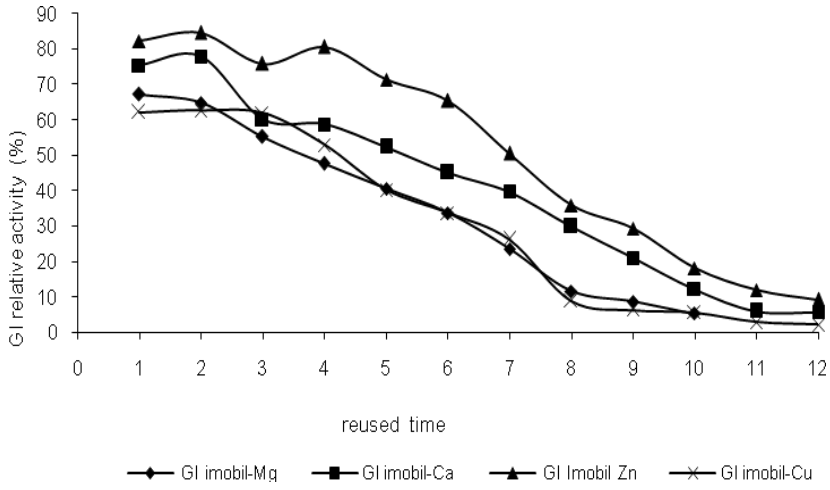


Picture 2. Effect of temperature on GI activity

The thermal stability of immobilized GI was markedly increased relative to that of the native enzyme. The thermal stability chitosan beads-metal ions GI at 60°C was improved dramatically, follow the order of Cu^{2+} >Zn^{2+} > Ca^{2+}>Mg^{2+}.



Picture 3. Thermal stability of free GI and GI Immobile



Picture. 4. Reusability of GI Immobile

To investigate the reusability, the enzyme-immobilized chitosan beads metal was washed with deionized water after one catalysis run and reintroduced into a casein solution for another hydrolysis. Fig 4. shows the effect of repeated use on the activity of the immobilized GI. It can be seen that the activity of the immobilized GI decay with recycled. The residual activity of GI immobilized on chitosan beads-Zn was about 40%, Chitosan beads-Ca, Chitosan beads-Mg and Chitosan beads Cu were 30%, 15% and 10%, respectively after 8 cycles of bath operation. Although the binding strength of the enzyme with the metal ions follow the order of Cu^{2+} >Zn^{2+} > Ca^{2+}>Mg^{2+}, because Cu^{2+} is inhibitor for GI, the activities was minimal. The activity loss could be related to the inactivation of the enzyme caused by the denaturation of the protein and the leakage of protein and metal ions from the support's surface.

Kinetics of the activity of free and immobilized GI was investigated at various concentrations of denatured glucose as a substrate. These data were plotted according to the method of Lineweaver–Burk and kinetic parameters, apparent Km and Vmax were calculated from the graphs [13]. The apparent Km value of free GI was found to be lower than that of immobilized GI. This increase in apparent Km value might be either due to structural changes in the enzyme induced by the applied immobilization procedure or due to the lower accessibility of the substrate to the active site of the immobilized enzyme [14,15]. A similar result involving change in Km and Vmax values of enzyme after immobilization has been reported in the literature [13-15]. Our results are also in accordance with the literature report. The Km and Vmax for free and immobilized GI was evaluated, as shown in Table 2.

Table.2 Km and Vmax free and immobilized GI

Enzyme	KM(mg/mL)	Vmax(U/mg)
Free GI	$2.143.10^{-2}$	$3.193.10^{-2}$
GI immobile-Zn	$3.082.10^{-2}$	$3.1187.10^{-2}$
GI immobile-Ca	$3.234.10^{-2}$	$3.1105.10^{-2}$
GI immobile-Mg	$3.849.10^{-2}$	$2.863.10^{-2}$
GI immobile-Cu	$3.9697.10^{-2}$	$2.839.10^{-2}$

The activation energy of the immobilized GI was lower than that of the free pepsin, which indicates a lower sensitivity to temperature as well as significantly higher affinity for the active site of the chitosan support. GI that immobilized on alginate gel beads have condition the optimum pH and temperature of free and immobilized glucose isomerase were found to be the same values as 7.5 and 60^{o}C, respectively. For free and immobilized enzymes, kinetic parameters were calculated as 1.79×10^{-2} and 8.27×10^{-3} mol/L for K_m, and 2.39×10^{-3} and 6.03×10^{-3} mol/L min for V_{max}, respectively. After 42 days of storage at 4C, free enzyme retained 56% of its initial activity, while for the immobilized enzyme, this value was observed as 86%. The immobilized samples were used repeatedly 22 times by retaining more than 85% of their initial activity [1].

ISSN: 0975-8585

CONCLUSIONS

The macroporous chitosan gel beads could effectively chelate with Cu^{2+}, Zn^{2+}, Ca^{2+} and Mg^{2} metal ions. GI could be directly adsorbed on the prepared chitosan beads. The metal ion species had significant effects on the adsorptive capacity and the activity retaining of GI. Chitosan beads-Zn was the best adsorbent among four kinds of Chitosan beads- metal ions for GI immobilization since it has high enzyme loading, high stability and activity retaining The macroporous metal chelated bead could be pervasively applied in enzyme adsorption or immobilization provided the enzyme had metal chelating ability.

REFERENCES

[1] Hayrettin Tumturk, Gokhan Demirel, Hay dar Altinok, Serpil Aksoy, Nesrin Hasirci. J Food Chem 2008;23(2): 234-246.
[2] Nadir Dizge, Coskun Aydiner, Derya Y. Imer, Mahmut Bayramoglu, Aziz Tanriseven and Bülent Keskinler. Biores Technol 2007. 100: 1983-1991.
[3] Vaidya BK, GC Ingavle, S. Ponrathnam, BD Kulkarni and SN Nene. Biores Technol 2008;99:3623-3629.
[4] Li S, Hu J, Liu B. Biosystems 2004;77:25–32.
[5] Cetinus SA, & Oztop HN. Enz Microb Technol 2003;32: 889–894.
[6] Ignatova M, Starbova K, Markova N, Manolova N, Rashkov I. Carbohyd Res 2006;341:2098–107.
[7] M Gavrilescu and Y. Chisti. Biotechnol Adv 2005;23(7-8): 471-499.
[8] S Çetinus, E Sahin and D Saraydin. Food Chem 2009;114(3):962- 69.
[9] MM Elnashar and AM Yassin. J App Poly Sci 2009;114(1):17-24.
[10] EN Danial, MM Elnashar and GE Awad. Chem Eng Res 2010;49(7):3120- 3125.
[11] Chiou SH and WT Wu. Biomater 2004. 25: 197-204.
[12] Tang ZX, JQ Qian and LE Shi. Mater Lett 2007.61:37-40.
[13] Gamze Durgun Altun, Senay Akkus Cetinus. Food Chem 2007.(100):967-971.
[14] Kennedy JF, Melo EHM. Chem Eng Prog 1990. 81–89.
[15] Cetinus SA, Oztop HN. Enz Microbial Technol 2003;32: 889–894.