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# Analytical Use of Immobilized Glucose Oxidase

## Kinetic and Operational Studies

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## ABSTRACT

With the aim of immobilizing glucose oxidase (GO) for routine determination of glucose, a covalent bond immobilization method on titanium (IV) chloride activated silica supports was used (*1*). Several parameters were studied in order to optimize the residual activity upon immobilization and during operation. The immobilized enzyme can be reutilized at 25°C for several h a day alternating with storage (4°C) for at least 3,300 h.

**Index Entries:** GO; immobilization; titanium (IV) activation; glucose determination.

## INTRODUCTION

At present, enzymes are being used increasingly as analytical reagents. However, the use of enzymes in solution has been limited by poor enzyme stability, the high cost of enzyme isolation and purification processes and technical difficulties in recovering active enzyme from the reaction mixture (*1*).

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To eliminate some of these drawbacks, many attempts have been made to replace soluble enzymes with immobilized enzymes in clinical and industrial analysis.

A method for the immobilization of (GO) was chosen, based on the transition metal-link method (2) that allowed high operational stability for some enzymes (3).

## MATERIALS AND METHODS

GO (EC 1.1.3.4) was from *Aspergillus niger*, Sigma, type X-S and Peroxidase (EC 1.11.1.7) was from horseradish, Sigma, type I. GO was immobilized by covalent linkage to a carbonyl derivative of porous silica (Spherosil XOB015 -Prolabo- with a particle size between 100 and 200 $\mu$m), using the immobilization method developed by Cabral et al. (2). Technical grade titanium (IV) chloride was from BDH Chemicals Ltd. Protein measurements were taken according to (2). Immobilized GO activity was determined according to (4) with some modifications: glucose (0.5 *M*) was used as substrate; reaction medium samples (1 $cm^{-3}$) were taken each 20″, during 180″. Hydrogen peroxide was quantified using peroxidase, 2,4-dichlorophenol and 4-aminoantipyrine.

## RESULTS AND DISCUSSION

The optimal conditions for GO immobilization on an activated support were selected from the study of the effect of temperature (4–20°C) during immobilization, reaction time (0.5–5 h), pH (7–9) and GO concentration (2–10 mg $cm^{-3}$). The following conclusions were drawn:

1. Temperature is not crucial;
2. Two hours is long enough for the covalent binding of the enzyme;
3. The optimum pH for immobilization was 8.0; and
4. The maximum activity of the immobilized enzyme preparation corresponds to an enzyme solution concentration of 3 mg $cm^{-3}$.

A loss of 30% of protein from the immobilized preparation was observed during the first 50 h. A similar result was observed in previous work on the immobilization of urease, by the same method (3). This may be the result of mechanical effects during stirring and not desorption, as a covalent bond was established between the enzyme and the support (2,3).

Table 1
Characterization of Soluble
and Immobilized Glucose Oxidase

| Parameter | Enzyme form | |
|---|---|---|
| | soluble | immobilized |
| Michaelis constant, $K_m$ (mM) | 33 | 8.3 |
| Maximum velocity, $V_{máx}$ (U/mg) | 168 | 1.83 |
| Optimal temperature (°C) | 45 | 60 |
| Activation energy (kcal $mole^{-1}$) | 2.36 | 4.86 |
| Deactivation energy (kcal $mole^{-1}$) | 5.38 | 9.48 |
| Optimum pH | 6.5 | 6.5 |

Several parameters were studied in order to characterize the soluble and immobilized glucose oxidase during operation (Table 1). The results show that the $K_{mi}^{(app)}$ of the immobilized glucose oxidase is 4 fold lower than the correspondent value for the soluble enzyme. This fact does not have any direct explanation. In fact some authors have observed a similar effect (*1*), but, in most cases, the opposite effect was found (*2,3*), this usually being related to partition or diffusion resistance of mass transfer.

The optimum pH value was not changed by immobilization (*5*). The increase in optimal temperature from 45°C to 60°C observed after immobilization was predictable because of the result of the protective effect already described elsewhere (*1,3,6*). The increased temperature stability of the immobilized enzyme compared to the soluble form, determined by incubation from 25 to 70°C for 30 min, confirms the protective effect of immobilization observed by some authors (*1,6*).

By studying the storage stability of immobilized enzyme in different environments, the optimal storage conditions were established: substrate solution and 4°C (Fig. 1A). According to the operational stability study (Fig. 1B), it was concluded that the immobilized enzyme can be reutilized at operational temperature (25°C) for several hours a day, alternating with storage at 4°C, for at least 3,300 h.

The glucose concentration ($10^{-3}M$) of a glucose test solution was determined using immobilized and soluble enzyme. The results were $7.6 \pm 0.4$(SD) with the immobilized form and $6.9 \pm 0.7$(SD) with the soluble form. The immobilized glucose oxidase was also used for the determination of the glucose concentration in two different individual human blood sera. The results showed good reproductibility with values of $5.4 \pm 0.2$(SD) and $3.4 \pm 0.7$(SD) for the first and second sera, respectively.

The assays with glucose solutions and with human sera indicate that this form of immobilized glucose oxidase could be a useful tool for routine

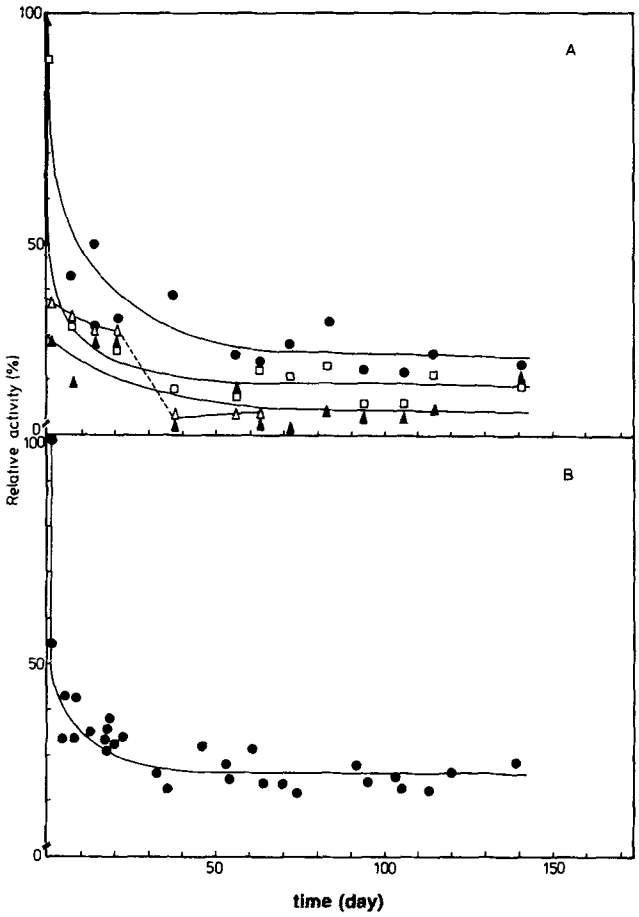


Fig. 1.(A) Storage stability of immobilized enzyme under different environmental conditions: (●) 4°C, glucose, pH 7.0: (□) 4°C, phosphate buffer, pH 7.4; (▲) 25°C, phosphate buffer, pH 7.4; (△) 25°C, glucose, pH 7.0. (B) Operational stability of immobilized enzyme (25°C).

glucose determination. Nevertheless, the appropriate analytical system for immobilized enzyme, such as its use in batch, in nylon tube or in enzyme electrode, would need further improvement.

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