# A NEW METHOD FOR COUPLING GLUCOSE DEHYDROGENASE TO GLASS TUBES ACTIVATED WITH TITANIUM TETRACHLORIDE

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## 1. Introduction

Although a number of papers have been published concerning the immobilization of various enzymes, few or no methods have been reported on the use of glass-tubes as solid support for the coupling of enzymes [1].

However, many studies on enzymes immobilized on controlled-pore glass have appeared in the literature [2–8]. In these methods the controlled-pore glasses were used in particle form of the size of 40–80 mesh with pore diam. 55 nm.

Here, we describe a new approach for coupling of enzymes to glass tubes. The procedure consists in placing chemical coupling agent on the glass surface, involving silanization of glass and activation of silane-coated glass with transition metal salts such as $TiCl_4$.

The glucose dehydrogenase (Glc-DH) bound to glass tube shows a high activity, superior to the one attained by the procedures in [9,10].

## 2. Materials and methods

Borosilicate glass tubes formed to a coil (length, 660 nm; inner diameter, 1 mm; diameter of helix, 8 mm) were used as a carrier for coupling of glucose dehydrogenase. The inner wall of the glass coil was washed with toluene and thoroughly dried overnight at 130°C in vacuum.

The inner surface of the glass tube was silanized by treating the glass with 15% α-aminopropyltriethoxy-silane in toluene for 24 h at 130°C. The coil was allowed to cool down to room temperature, then 50 ml toluene was pumped through the glass tube. The empty coil was then heated at 130°C in vacuum overnight. This silanization process was repeated a second time.

The coated glass tubing was rinsed first with toluene then with *n*-pentane and reacted with $TiCl_4$, 15% in *n*-pentane, at room temperature for 1 h. After drying the tube for 12 h at 50°C in vacuum, it was rinsed with 200 ml 0.5 mol/l NaCl in 0.1 mol/l phosphate buffer (pH 7.6 at 25°C). Glucose dehydrogenase (260 U/mg protein, a gift from E. Merck Darmstadt) was then coupled as in [10].

Glucose concentrations were measured with a dual channel system (Technicon Auto-analyzer II System) wherein the second channel served as a blank channel to correct for initial absorbance at 340 nm (fig.1).

## 3. Results and discussion

Fig.2 shows the effect of pH on the glucose dehydrogenase coupled to the glass tube. The free enzyme shows an optimum at pH 8 [11]. The glass coil coated with Glc-DH shows an optimum at pH 7.7, so the shift in pH optimum is small as compared to the glucose dehydrogenase derivatives in [10].

The classic Hofstee plot yields a straight line. The

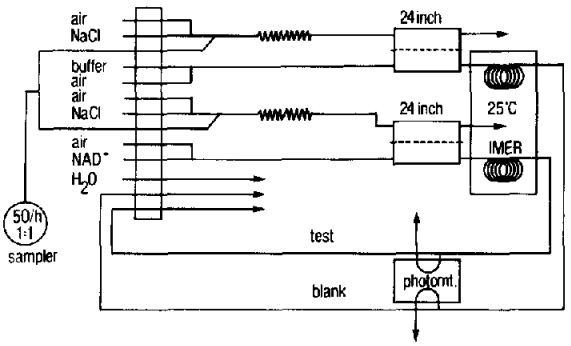


Fig.1. Flow diagram.

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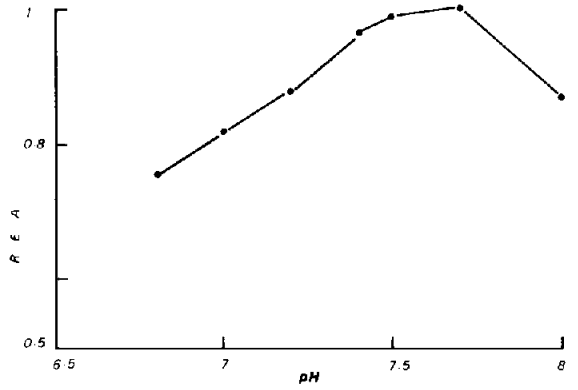



Fig.2. pH Activity profile of glucose dehydrogenase coupled to the glass tube. REA, relative enzyme activity. The reactor was incubated (25 min at 25°C) at different pH values (glucose, 27.8 mmol/l; NAD, 4.4 mmol/l).

$K_m$ is $1.6 \times 10^{-2}$ M and the specific activity was determined to be 9.4 $\mu$mol . $cm^{-1}$ . $min^{-1}$.

The range of linearity for the determination of [glucose] is 0–27.5 mmol glucose/l.

The stability of immobilized enzyme in continuous operation is the major problem in analytical systems with bound enzymes. The durability of glass coils coated with Glc-DH is good. The decrease in activity on continuous operation for 4 weeks is illustrated in fig.5. During this period the reactor was connected to the SMAC (Technicon, Tarritown NY) (high speed continuous-flow analyzer) system and ~400 samples were analyzed every day. The activity was reduced to 50% after 24 days only. The half-life

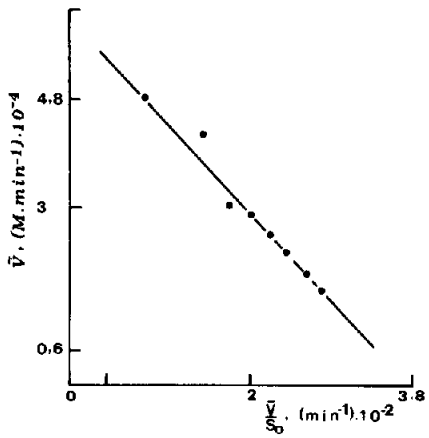



Fig.3. Classic Hofstee plot of $\bar{v}$ versus $\bar{v}/S_0$ ($\bar{v}$ is the mean rate of the reaction). Glucose concentration range 2.4–57 mmol/l.

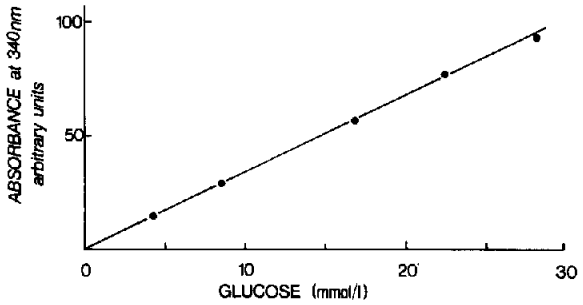



Fig.4. Linearity of the method over 0–30 mmol glucose/l.

determined from linear regression of a representation of the logarithm of activity versus time was found to be 24 days. In order to gain information on how the silanized glass is activated by $TiCl_4$, we investigated some factors affecting the yield in activity of Glc–DH bound to glass tubes. Compared to the above procedure, the yield in activity was 76% and 55% when tetraethoxysilane and diphenylsilanediol were used, respectively, in place of α-aminotriethoxysilane. It was 6% when the coupling of enzyme was carried out with a silanized glass without preactivation with $TiCl_4$. These results permit one to conclude that:

1. $TiCl_4$ condenses with unreacted hydrolyzable groups (ester or hydroxyl) on the surface of the silanized glass.

2. The coupling of enzyme occurs through the Ti(IV)-ion.

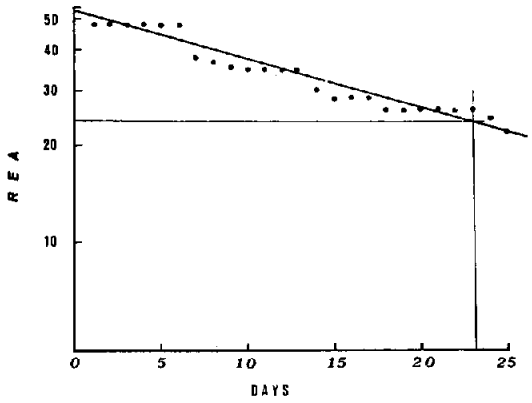
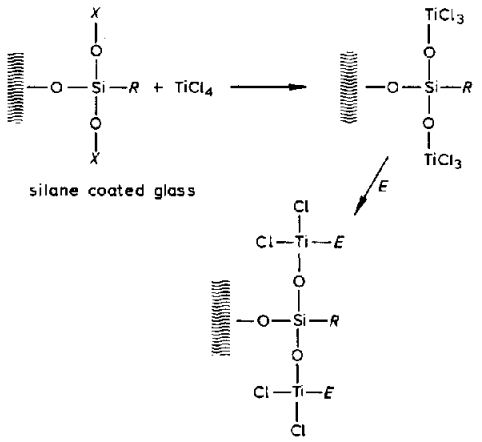



Fig.5. Decline of activity of Glc-DH glass-coils during continuous operation (4 weeks): (•) activity of reactor as function of time; (■) linear regression of log activity versus time for half-life determination. Half-life = 24 days. REA, relative enzyme activity.

We therefore propose the schematic representation of the coupling reaction as follows:




X = hydrolyzable groups

R = organic functional or alkyl groups

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