THE PREPARATION OF IMMOBILIZED GLUCOSE DEHYDROGENASE AND ITS USE IN AUTOMATED ANALYSIS

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Received 6 July 1977
Revised version received 19 July 1977

1. Introduction

Glucose dehydrogenase (EC 1.1.1.47) is one of the enzymes currently used in clinical chemistry laboratories for the specific determination of glucose in body fluids [1,2]. This enzyme, like other dehydrogenases, is particularly interesting because the reduced form of the enzyme which is produced during the reaction is directly accessible for photometric measurement. Hornby et al. [3] have shown that dehydrogenases although less stable than earlier examples of immobilized enzymes can be covalently bound to the surface of nylon 6 with conservation and even enhancement of activity. Glucose dehydrogenase is reasonably stable even in aqueous solution and its immobilization seems promising.

2. Materials and methods

Nylon tube, 3 m × 1.5 mm (Portex, Hythe, Kent) was prepared for the fixation of enzyme according to the method of Hornby et al. [3]. For the attachment of glutaraldehyde, borate buffer at pH 8.5 is used and the coupling buffer consists of 0.12 M phosphate, pH 7.6 containing 8.7 g/l sodium chloride (no EDTA and no dithiodhreithol).

Glucose dehydrogenase, 20 mg (260 U/mg of protein, a gift from E. Merck, Darmstadt) was dissolved in 5 ml of coupling buffer containing 3.3 mg NAD. The tube filled with this solution was incubated for 14 h at 4°C. It was then perfused at 1°C with 100 ml coupling buffer containing 2 mmol sodium borohydride and finally with 100 ml of coupling buffer at a rate of 3.4 ml/min.

The kinetic parameters have been estimated by a method described by Horvath and Salomon [4] using the classic representation of Lineweaver-Burk [5]. The reaction temperature was 25°C and the coenzyme NAD (4.4 mM) was dissolved in coupling buffer, pH 7.6. Figure 1 shows the flow diagram which was used for the automated determination of glucose. All parts were standard equipment from Technicon except the recorder which was from W & W (Münchenstein, Switzerland). NADH concentration was measured at 340 nm and the sampler worked at a rate of 30 samples/h with a 2:1 sample/wash ratio.

3. Results and discussion

Figure 2 shows that the reaction in the nylon tube is under kinetic control and that it can be described by the Michaelis-Menten equation.

The kinetic parameters are derived from the Lineweaver-Burk representation in fig.3 and are summarized in table 1.

When not in use during the night the immobilized glucose dehydrogenase was stored at 4°C in a solution containing potassium phosphate buffer (0.12 M, pH 7.6), sodium chloride (0.15 M) and NADH (0.1 mmol/l). After three weeks the activity decreased about 20%. After six weeks the activity was about 20–30% of the original.

The continuous flow system is very simple as with most immobilized enzyme reactors. Its characteristics

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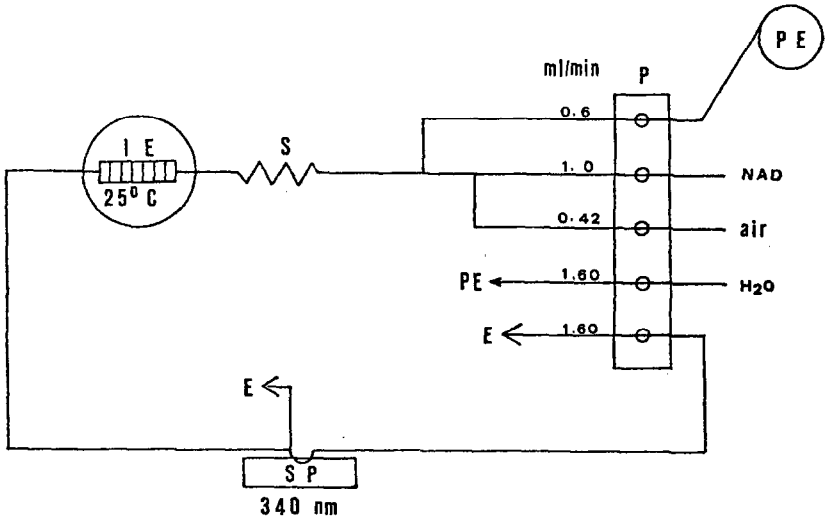

Fig.1. Flow diagram for the automated assay of glucose dehydrogenase–nylon tubes. The immobilized enzyme reactor was maintained at 25°C by immersion in a constant temperature water bath. PE, Sampler. P, Pump. IE, Immobilized enzyme. E, Waste. SP, Photometer. S, Mixing coils.

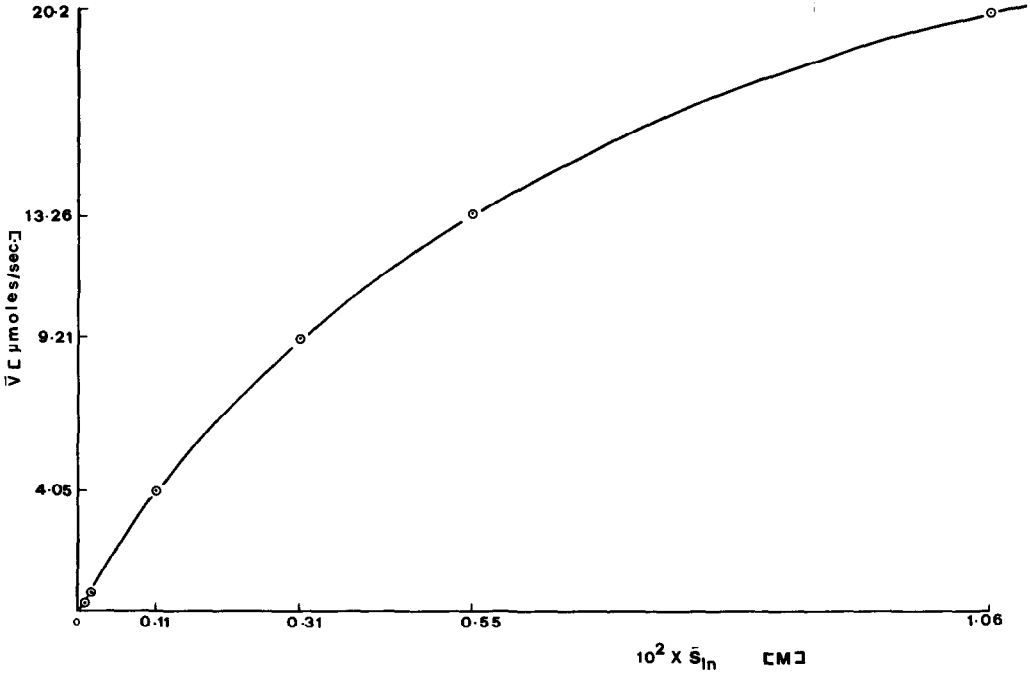

Fig.2. Dependence of overall reaction rate on glucose concentration. Absorbance is in arbitrary units. $\bar{S}_{ln}$ is the logarithmic mean concentration:

$$\bar{S}_{ln} = \frac{[NADH]}{\ln([S]/[S]-[NADH])}$$

(see ref. [4])

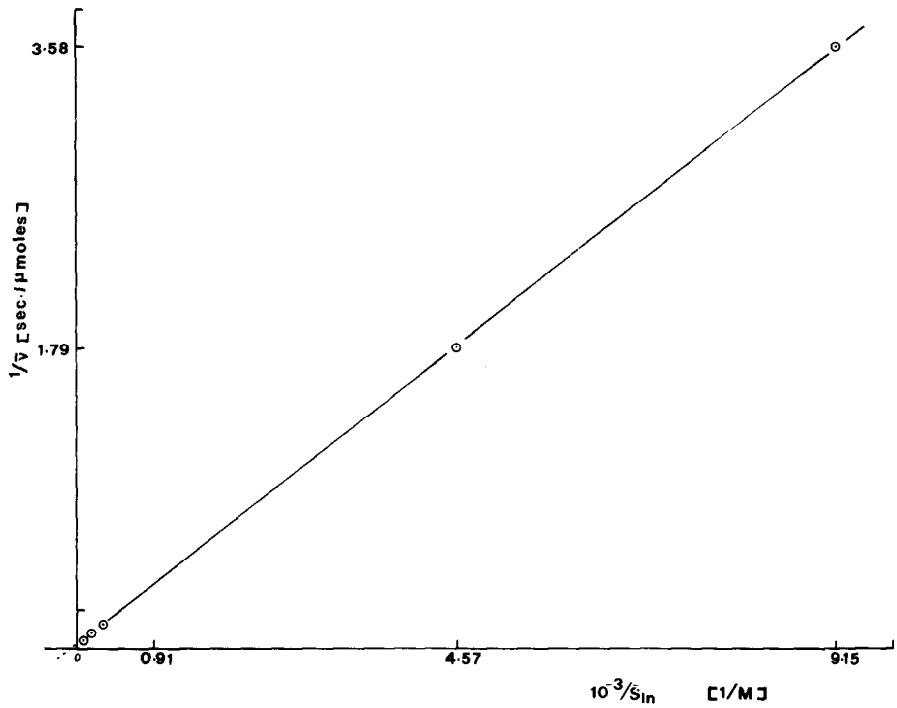

Fig.3. Lineweaver-Burk plot.

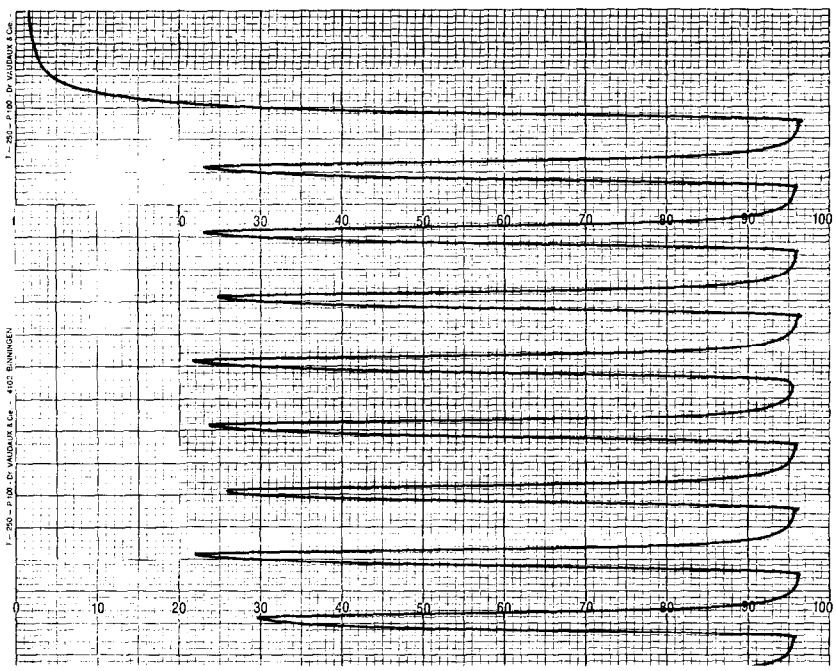

Fig.4. Reproducibility of the automated method for a glucose concentration of 44.4 mmol/l.

Table 1
K_m and $\bar{V}_{max}$ values of immobilized glucose dehydrogenase reactor

Conditions	K_m [M]	$\bar{V}_{max}$ mol/s
0.12 M phosphate pH 7.6 with air segmentation	5.10^{-3}	30
pH 8.0 see ref. [6]	10^{-2}	

are such that the system seems to be suitable for routine analysis. Figure 4 shows that the analytical values are reproducible even at high concentrations (800 mg/100 ml) of glucose. The height of the signals corresponds to 99% of the steady state level and at low concentrations steady state levels are reached much earlier (fig.5).

There is very low background noise and carry over is low even at a glucose concentration of 44.4 mmol/l (800 mg/100 ml). The recorder peak height is linear

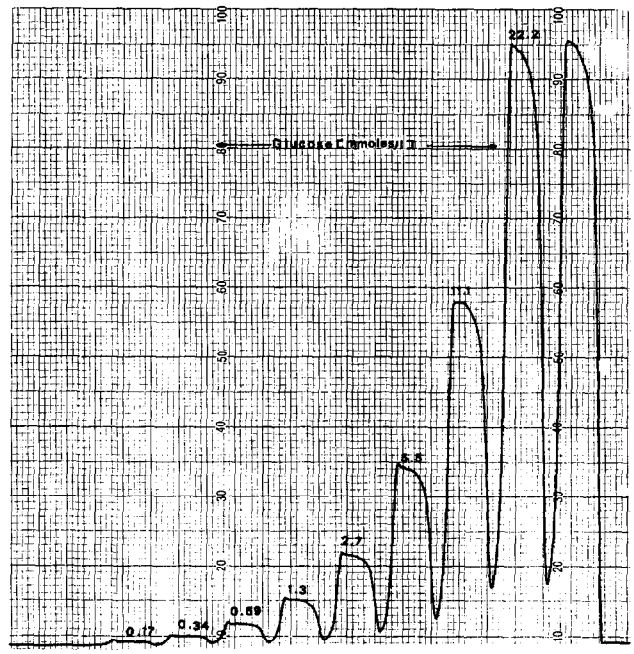

Fig.5. Dependence of peak heights and curve characteristics on glucose concentration.

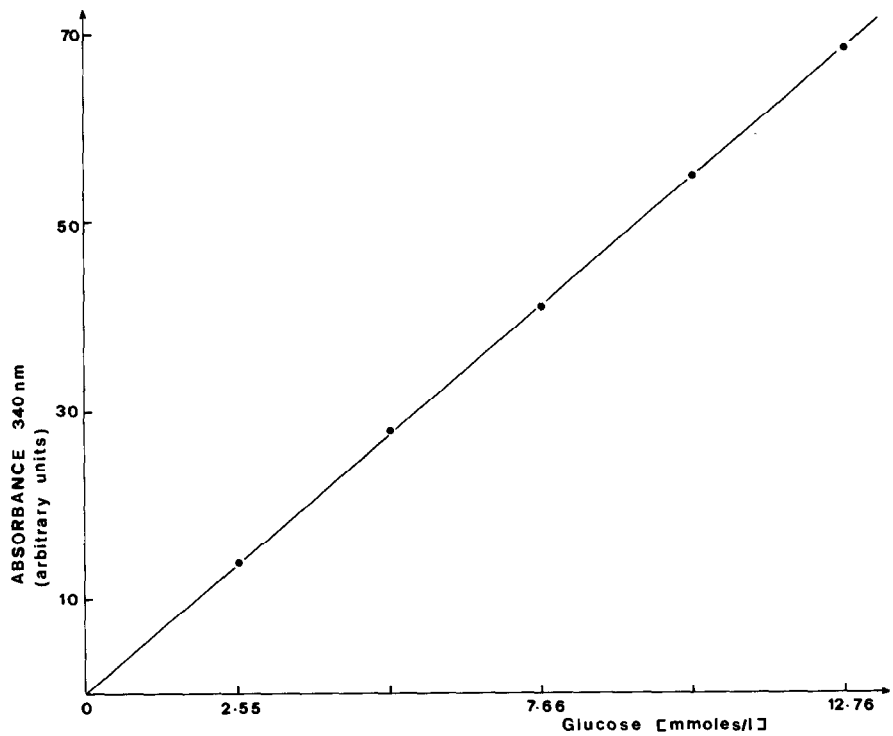

Fig.6. Linearity of the automated method.

with glucose concentration up to a limit of 13 mmol/l (fig.6).

The photometric detection is very sensitive. It is therefore possible to dialize biological material although the yield of the dialysing procedure might be as low as 20%. First results of analysis of blood serum are very encouraging. A longer period of evaluation will be necessary however in order to prove the practicability of this new immobilized enzyme reactor in the daily routine for clinical chemistry.

Acknowledgement

We wish to thank the Swiss National Foundation for Scientific Research for their support.

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