THE USE OF IMMOBILISED DERIVATIVES OF UREASE AND URATE OXIDASE IN AUTOMATED ANALYSIS

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1. Introduction

The use of immobilised enzymes in automated analysis has already been described for the assay of glucose with glucose oxidase chemically attached to the inside surface of polystyrene tubes [1]; however, the usefulness of these tubes as a support for enzymes may be limited by the extreme hydrophobic nature of this material [2]. Nylon, on the other hand, while possessing many of the mechanical properties of polystyrene is more hydrophilic and consequently may be a more suitable structure for enzymes in general. This paper describes the preparation of nylon tube-supported urease and nylon powder supported urate oxidase, together with the use of these preparations for the automated determination of urea and uric acid.

2. Experimental

Jack bean urease (Type IV, Sigma Chemical Co.) was coupled to the partially hydrolysed inside surface of a nylon tube (Type 66 nylon, 1 mm internal diameter, John Tullis and Co., Tullibody, Alloa, Scotland) by the method of Sundaram and Hornby [3]. A freshly prepared glutaraldehyde-nylon tube (2 m long) was perfused in a closed loop with a solution of the enzyme (7.5 mg/ml in 0.05 M phosphate buffer, pH 7.5, containing 1 mM EDTA and 2 mM mercaptoethanol) at 0° for 75 min at a flow rate of 1 ml/min. Finally the tube was washed through with 0.05 M phosphate buffer, pH 7.0, 1 mM in EDTA and 0.25 M in NaCl until the effluent contained no trace of urease activity.

Type 66 low molecular weight nylon powder, 120–150 mesh, (a gift from Dr. M.D. Lilly of University College London) was prepared for attachment of the enzyme by first coupling the free amino groups with glutaraldehyde. 100 mg of the powder were sus-

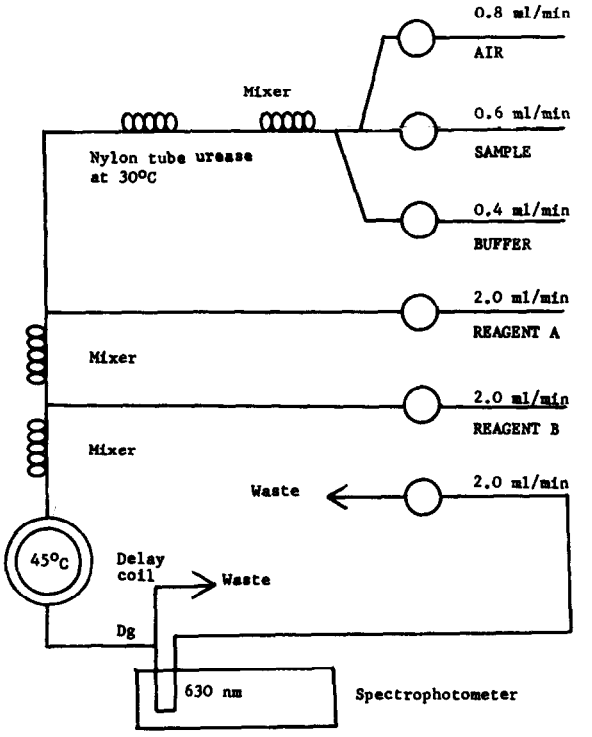

Fig. 1. Autoanalyser flow diagram for the automated determination of urea using nylon tube-supported urease. Reagent A: 0.006% (w/v) sodium nitroprusside in 4.7% (w/v) aqueous phenol. Reagent B: sodium hypochlorite in 0.5 M NaOH, containing 0.10 – 0.15% available chlorine. (Dg. : degasser).

pended in a mixture of 5 ml 20% (w/v) glutaraldehyde and 1.0 ml 0.1 M phosphate buffer, pH 8.0, and stirred at 0° for 30 min. The powder was then washed with 0.1 M phosphate buffer, pH 6.0, and immediately suspended in a solution of 1.62 mg urate oxidase (Sigma Chemical Co., Type IV from *Candida utilis*) in 5.0 ml 0.1 M phosphate buffer, pH 6.0. The suspension was stirred at 0° for 1 hr, after which 2 ml of 0.1 M phosphate buffer, pH 8.0, were added and the stirring continued for a further 15 min. The resulting nylon powder-supported urate oxidase was packed into a column (2.0 X 0.2 cm internal diameter) and washed thoroughly by perfusion with 1 M NaCl in 0.02 M borate buffer, pH 8.5, until the effluent contained no trace of urate oxidase activity.

Both immobilised enzyme preparations were incorporated into Technicon Auto Analyser circuits

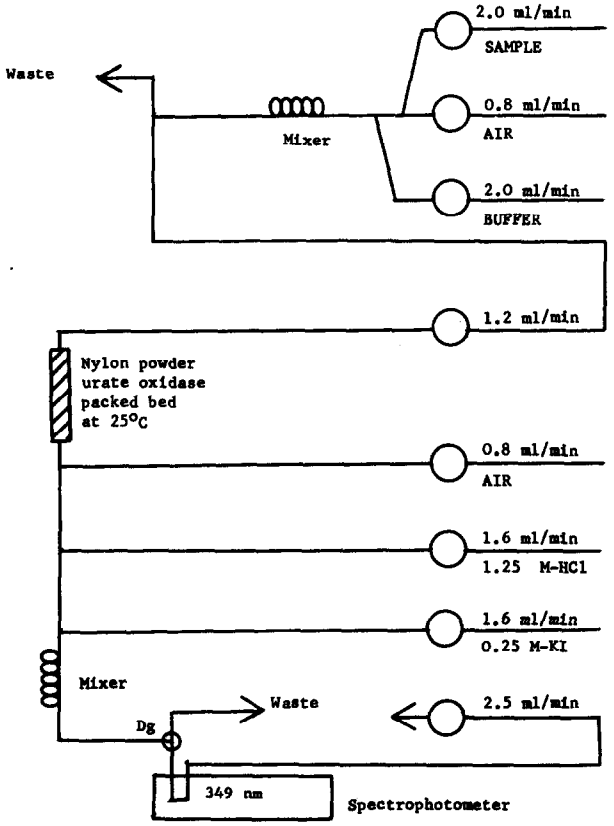

Fig. 2. Autoanalyser flow diagram for the automated determination of uric acid using a small packed bed of nylon powder-supported urate oxidase. (Dg.: degasser).

for the continuous estimation of their respective substrates. The method used for the determination of urea with nylon tube-supported urease is shown as a flow diagram in fig. 1. In this procedure the urea is measured by the determination of the ammonia by the method of Chaney and Marbach [4]. The automated method used for the determination of uric acid with a small packed bed of nylon powder-supported urate oxidase is shown as a flow diagram in fig. 2. In this method the uric acid is measured by the determination of one of its reaction products, hydrogen peroxide, with acid potassium iodide.

The lyophilised standard reference sera were obtained from Hyland Div., Trevanol Labs. Inc., Costa Mesa, California, USA. These materials were reconstituted with water immediately before they were required.

3. Results and discussion

Fig. 3(A) shows the results obtained when standard solutions of urea were assayed using nylon tube-supported urease. These data show that this system can be used for the estimation of urea in the concentration range 0.3–3.0 mM. This procedure was also used to measure the concentration of urea in 2 samples of reference sera. Urea contents corresponding to 12.6 and 48.6 mg urea nitrogen per 100 ml were found for the Hyland Normal and Hyland Abnormal sera, respectively. These figures are within 5% of the stated values of 12.0 and 50.0 mg urea nitrogen per 100 ml for these reference sera.

The results obtained when standard solutions of uric acid were assayed using a small packed bed of nylon powder-supported urate oxidase are shown in fig. 3(B). The data show that this system can be used for the estimation of uric acid in the concentration range 0.01–0.10 mM.

Both immobilised enzyme preparations showed enhanced stability relative to their water-soluble counterparts. For instance, the nylon tube-supported urease was used over a period of 4 mon and during this time over 400 analyses were performed.

Both the enhanced stability of immobilised enzymes and their new physical characteristics make it possible to use the same preparation for repetitive substrate estimations in a continuous process. Fur-

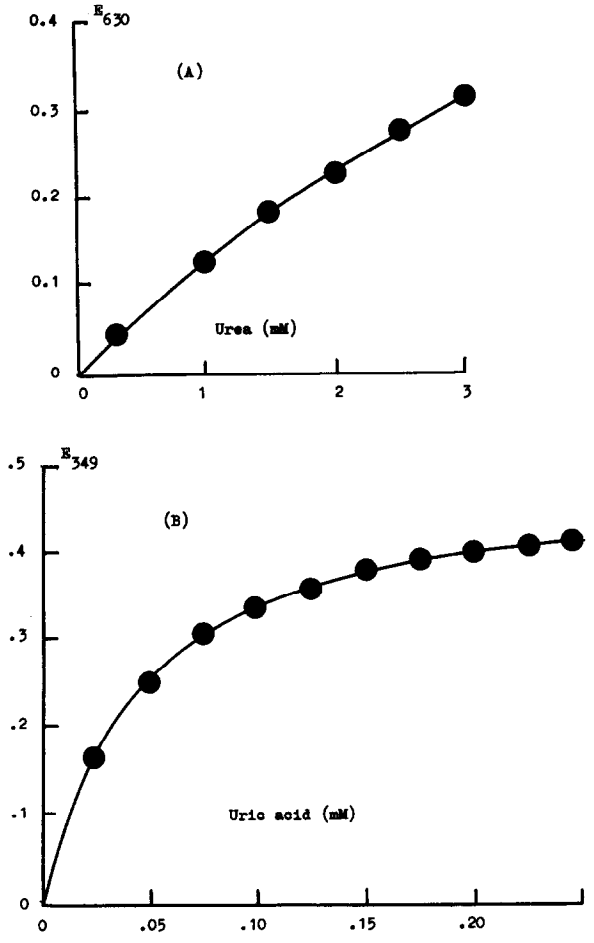

Fig. 3. (A) Standard curve for the automated determination of urea using nylon tube-supported urease. Samples were assayed at the rate of 30/hr. (B) Standard curve for the automated determination of uric acid using a small packed bed of nylon powder-supported urate oxidase. Samples were assayed at the rate of 30/hr.

thermore, immobilised enzymes may also afford a more stable standard than free enzymes in the calibration of analytical techniques applied to the measurement of enzymic activity. In both applications the use of enzymes in the immobilised form should make for considerable economies in terms of the amount of enzyme needed per analysis.

Acknowledgement

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