**FLUORESCENCE APPLICATIONS**

**DETERMINATION OF GLUCOSE USING THE MODEL LS-50**

**INTRODUCTION**

The measurement of serum or plasma glucose is important in diabetes and in the detection of low serum glucose in premature infants. The measurement of serum glucose is also necessary in various tolerance tests. Both hypo and hyperglycaemia occur in disease. A rise in blood sugar occurs in sepsis and a number of infectious diseases e.g. common cold; also a number of intracranial diseases such as meningitis give rise to hyperglycaemia.

The determination of blood glucose is based upon the use of glucose oxidase which converts glucose to gluconic acid and hydrogen peroxide. Under the action of peroxidase, hydrogen peroxide oxidizes homovanillic acid to a highly fluorescent product (1,2).

**MATERIALS**

Disodium hydrogen phosphate (AnalaR, 10248) was obtained from BDH, Poole, UK.

Homovanillic acid (H-1252), horseradish peroxidase (D-8125), glucose oxidase (G-6125) sodium phosphate (S-0751) and benzoic acid (B-3250) were obtained from SIGMA Chemical Co.

Deionized water was used throughout the analysis.

Analysis was performed using the PerkinElmer Model LS-50 Luminescence Spectrometer fitted with a thermostatted stirred cell changer (Part No. L225-0134).

**METHOD**

A solution of 13.9 g NaH₂PO₄ was prepared in 1 litre of water. Also, a solution of 26.8 g of Na₂HPO₄.12H₂O was prepared in 1 litre of water. From this solution a phosphate buffer of 0.1 M and pH 7.0 was prepared by mixing in the ratio 2:3 and adjusting as necessary.

Working reagent was prepared by dissolving 50 mg of homovanillic acid, 3000 units of horseradish peroxidase and 200 units of glucose oxidase in 35 mL of the phosphate buffer. This was then diluted to 50 mL with the phosphate buffer.

Saturated benzoic acid solution was made by shaking 1 g of benzoic acid in 800 mL of water and making up to 1 litre.

A glucose stock standard of 1 g of glucose dissolved in 75 mL saturated benzoic acid and made up to 100 mL was prepared. This was then diluted 10-fold to produce a working standard.

2.5 mL of the working reagent were pipetted into a cuvette held in the thermostatted stirred cell changer, the stirrer was set at low and the temperature thermostatted to 30 °C by pumping water through the cell holder from an external water-bath. A calibration curve was obtained by adding different amounts of stock glucose solution to fresh amounts of the working solution. The reaction was allowed to proceed for 30 minutes and then a reading was taken.

The spectrometer was set at excitation 310 nm, emission 445 nm and slits 5/5 nm.
RESULTS

Figure 1 is the calibration curve obtained from the above analysis. Linearity was seen for the given range. A limit of detection was obtained for the above analysis and was found to be $2 \times 10^{-7}$ moles per litre.

CONCLUSION

The PerkinElmer Model LS-50 provides a simple and sensitive technique for the analysis of plasma and/or serum glucose. The limit of detection ($2 \times 10^{-7}$ M) is at least 20,000 times more sensitive than the normal level of glucose in blood ($4 \times 10^{-3}$ M). Severe cases of Addison's and Simmond's diseases can lead to levels down to $1 \times 10^{-5}$ M and this can obviously be measured by the Model LS-50.

REFERENCES