Enzymic determination of glucose with SMAC: adaption of the dichlorophenol/ aminophenazone chromogen

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Introduction

The currently used SMAC glucose oxidase method is a modification of the procedure of Gochman and Schmitz [1]. In this colorimetirc determination, the specificity of glucose oxidase is combined with a peroxidase indicator coupler, 3-methyl-2 benzothiazolinone hydrazone (MBTH) and dimethylaniline (DMA) to form a coloured indamine dye with a high molar absorption. However, as much as six different reagents have to be prepared by the user, some of them daily. Barham and Trinder [2] described in 1972 a method involving the use of 4-aminophenazone as a colour coupler with sulphonated 2,4-dichlorophenol for determining the hydrogen peroxide produced from glucose with glucose oxidase. This method had a high sensitivity and the additional possibility of using a single reagent stable for several days. This paper presents an adaptation of the former to the SMAC system considerably reducing both the number of reagents involved and the operator-time consumed. The proposed method was compared with the hexokinase manual method and the Gochman-Schmitz method for the SMAC.

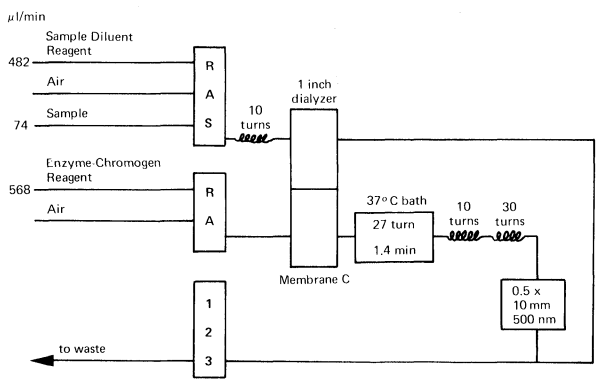

Figure 1. Simplified flow diagram for the glucose channel (DCPS/AP system).

Materials and methods

Reagents

Reagents for SMAC glucose determination (MBTH/DMA)
Reagents were prepared according to the manufacturer [3].
Glucose oxidase working reagent: 1.6 ml of glucose oxidase stock reagent (1000 U/l) were diluted with 163 ml of phosphate buffer 0.1 mol/l, pH 7.0 and 0.16 ml of 300 g/l Brij-35.
MBTH/DMA working reagent: 2.0 ml of 1 g/l MBTH and 3.0 ml of 10 ml/l DMA were diluted with 95.0 ml of 0.5 mol/l acetic acid. DMA and MBTH solutions were prepared in 0.1 mol/l hydrochloric acid.
Peroxidase: 19 U/ml in distilled water.
Glucose sample diluent: 9 g/l saline-0.3 g/l Brij-35.

Reagents proposed for SMAC glucose determination (DCPS/AP)
Enzyme/chromogen mixture: The reagent contains, per litre: 0.8 mmol aminophenazone, 10 mmol sulphonated

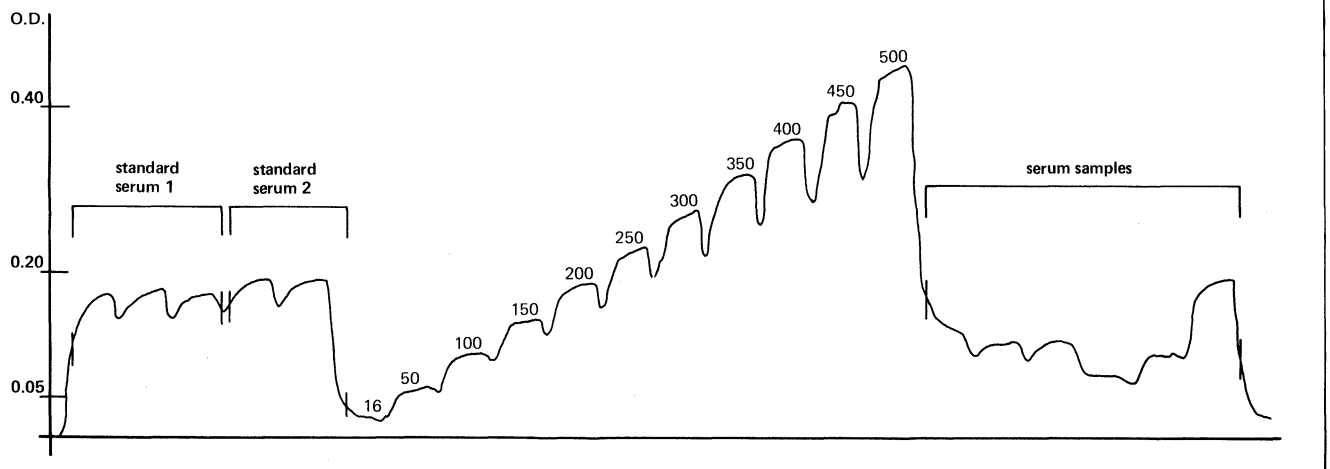

Figure 2. Representative recording of glucose determinations. Number above the peaks indicate the concentration of glucose (mg/100 ml).

2,4-dichlorophenol, 10000 U glucose oxidase and 1100 U peroxidase in phosphate buffer 0.1 mol/l, pH 7.4. It can be prepared in a stable powdered or lyophilised form suitable for up to four days use when appropriately dissolved in water.
Glucose sample diluent: 0.3 g/l Brij-35.

Instrumentation

The instrument used was a SMAC (Technicon Instruments Corp, Tarrytown, NY, 10591). The flow diagram for the DCPS/AP proposed method is shown in Figure 1. Modifications of the original channel are minimal: substitution of the interference filter (500 nm for 600 nm) and disconnection of the MBTH/DMA and peroxidase tubing.

Results and discussion

Linearity

Readings and glucose concentrations were linearly related up to 27.5 mmol/l. A recording of the procedure for continuous sampling of standard sera, an ascending standard curve and results with representative sera are reproduced in Figure 2.

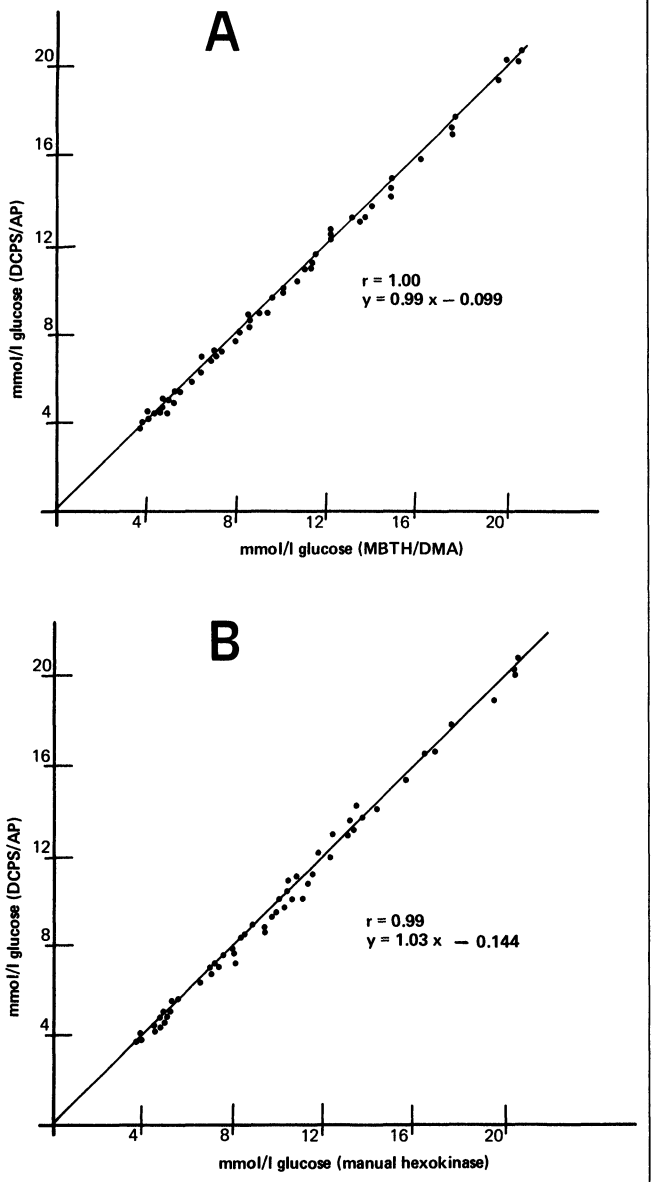

Figure 3. Correlation analysis of serum glucose determined by the DCPS/AP system and the MBTH/DMA system (A) and by the DCPS/AP system and the manual hexokinase procedure (B).

Table 1. Day-to-day precision of automated glucose method (10 daily analysis).

	Serum A		Serum B	
	MBTH/DMA system	DCPS/AP system	MBTH/DMA system	DCPS/AP system
Mean (mmol/l)	4.17	4.21	13.37	13.57
SD	0.15	0.12	0.33	0.28
CV(%)	3.60	2.85	2.47	2.06
n	25	25	25	25

Table 2. Interferences study. Solutions of the examined compounds were added to a serum pool and the glucose concentration was tested and compared with the same pool diluted with saline. Glucose found is the mean of triplicate results.

Added Substance	Concentration tested (mg/ml)	Normal concentration (mg/ml)*	Glucose found (mmol/l)
Saline	–	–	6.39
Heparine	150,000 U/l	–	6.39
Citrate	200	–	6.33
EDTA	60	–	6.50
NaF	300	–	6.39
Oxalate	30	–	6.44
Glutathione	30	3.1	5.83
	7	3.1	6.44
Ascorbic acid	1	0.3	6.33
	5	0.3	4.67
Uric acid	1	0.5	6.39
Bilirubin	2	0.1	6.44
Cysteine	7.5	0.001	6.33
Fructose	30	–	6.39
Mannose	30	–	6.39
Xylose	30	–	6.33
Maltose	30	–	23.33
Galactose	30	–	6.39
Saccharose	30	–	6.44
Dextran	30	–	6.36
Creatinine	2	0.1	6.39
Salicylic acid	2	–	6.39
Acetylsalicylic acid	10	–	6.33
Gentisic acid	0.2	0.04	6.39
L-DOPA	0.1	0.03-0.003	6.39
Haemoglobin	50	–	6.44
Haemolysate	50	–	4.72

**Approximate normal values of the compound in serum (physiological or therapeutical).*

Precision

The precision of the method was determined by daily analysis of 2 lyophilised control sera with low and high glucose values. The results are shown in Table 1. Data shows satisfactory precision for consecutive runs involving change of reagents, standards, etc.

Interferences

Various anticoagulants, drugs and metabolites were tested for potential interferences with the method (Table 2). A number of saccharides did not interfere reflecting the specificity of the method, maltose being an exception. The interference from maltose was due to the common contamination of glucose oxidase with maltase. None of the anticoagulants tested interfered. Glutathione, uric acid, ascorbic acid and bilirubin did not interfere at concentrations

Table 3. Recovery of glucose added to dialysed serum. Found glucose is average of triplicate determinations.

Added glucose (mmol/l)	Found glucose (mmol/l)	Recovery (%)
2.78	2.83	102
5.56	5.50	99
13.89	14.17	102
22.22	22.44	101

higher than those found in serum. The serum drug concentrations studied here were higher than would be expected after therapeutic doses and no interference was observed. Haemoglobin did not interfere up to 50 mg/ml, but a haemolysate with the same concentration of haemoglobin produced a decrease in the observed glucose value.

Accuracy and comparison of methods

Accuracy of the method was investigated by recovery studies and by comparison with the spectrophotometric hexokinase method. To investigate recovery a pool of human serum was prepared by dialysing it overnight against saline. Various amounts of glucose solution (555 mmol/l) and water were added to the dialysed serum and analysed. The results are shown in Table 3. Recovery was excellent at low and high glucose concentration. We compared our results for this method to those obtained by the Boehringer-Mannheim hexokinase manual procedure and by the MBTH/DMA SMAC method for 85 freshly collected patients' sera. The data were correlated (Figure 3) and showed excellent agreement over a wide range of plasma glucose values.

In conclusion, the proposed system is simpler and more economical than the MBTH/DMA method, performing similarly in linearity, sensitivity, resistance to interfering substances, accuracy and precision.

Recently obtained data have shown a similar performance of the proposed method in the SMA II system modified in the glucose channel as described for the SMAC (results not shown).

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