

Continuous-Flow Determination of Reducing Sugars in Serum by Reaction with Periodate Ions, with Use of a Flow-Through Periodate-Sensitive Electrode

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INTRODUCTION

The blood sugar level is raised in diabetes mellitus and in a number of other pathological states. It is depressed after strenuous physical work and after an overdose of insulin in diabetes (6, 9).

Various chemical or enzymatic methods have been described for the determination of glucose in serum. Many chemical methods for the determination of glucose are based on its reducing power; however, these methods are relatively not specific for glucose and measure the total reducing substances in the sample. Many enzymatic methods are based on the oxidation of glucose in the presence of glucose oxidase and coupling of the liberated hydrogen peroxide to a second reaction (6). Enzymatic methods are highly specific for glucose.

Ion-selective electrodes have been successfully adapted as sensors in many clinical analysis schemes because of their low cost, high selectivity, and indifference to optical interference (3, 4).

Periodic acid and its salts are widely used in chemical analysis because they combine a strong oxidative action, under mild conditions, with a high degree of specificity toward certain classes of organic compounds (4, 8). Carbohydrates are a representative class of organic compounds highly reactive toward periodates. The kinetic behavior of certain carbohydrates on their reaction with periodates, in the pH range 4-7, has been the subject of a previous study (5). Considering the fact that relatively simple potentiometric monitoring of periodate activity may be provided with an inexpensive flow-through electrode device, then a totally new automated system for the determination of reducing sugars in serum may be implemented.

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In this paper, a continuous-flow procedure for the determination of reducing sugars in serum is described. The samples are mixed with an excess of periodate solution under controlled and reproducible conditions, with the aid of the Technicon AutoAnalyzer II system. The consumption of periodate is monitored with a periodate-sensitive flow-through electrode. The recorded potential peak heights, through an exponential function, are linearly related to the reducing sugar concentration of the sample expressed as glucose, in the range 50–290 mg/100 ml. No sample pretreatment is required and up to 30 samples per hour may be analyzed, with a sample consumption of 0.160 ml and an average error of about 2%. The relative standard deviation for both aqueous glucose solutions and serum samples was about 1–2%. Comparison studies with an enzymatic method for serum showed an acceptable agreement and correlation.

THEORY OF MEASUREMENTS

When analytical schemes involving relatively slow reactions are implemented with continuous-flow autoanalyzer systems, there are, generally, two choices for the measurements. The first one is to measure the remaining reagent concentration (periodate in the present case) a short time after mixing the sample with an excess of the reagent. In this case, the contribution of the most rapidly reacting components of the sample is expected to be significant. The second choice is to allow the reaction to reach completion before measuring the remaining reagent concentration.

The reaction of periodate with glucose is relatively slow compared with the reaction rate of periodate with other carbohydrates (such as fructose, galactose, and mannose (5)) or ascorbic acid, which are also present in serum but generally in much smaller concentrations.

In order to minimize the contribution to the final result of the other sample components reacting much faster than glucose and take advantage of their smaller concentration relative to that of glucose, it was decided to allow the reaction between reducing substances of the sample and periodate to reach completion.

When glucose (G) is allowed to react with an excess of periodate (P), the overall reaction may be represented by



where n is the number of periodate ions reacting with a single molecule of glucose. The reaction is not expected to be a stoichiometric one and n depends on the actual conditions of the measurement (pH, temperature, and the time delay after mixing of the sample with the periodate solution). Nevertheless, the final periodate concentration, $[P]$, after completion of the reaction, is given by

$$[P] = [P]_0 - n[G]_0 \quad (2)$$

where $[P]_0$ and $[G]_0$ are the initial concentrations of periodate and glucose, respectively, immediately after mixing the sample and the periodate solution.

In the absence of glucose in the sample (during the "wash" period in the sampler of the autoanalyzer), the baseline potential recorded for the flow-through periodate-sensitive electrode, E_0 , is given by

$$E_0 = E' - S \log[P]_0 \quad (3)$$

where E' is a constant potential depending among other factors (internal and external reference electrode, junction potentials, etc.) upon the total ionic strength of the measured solution, which is easily maintained constant. S is an experimentally determined constant quantity, ideally equal to the prelogarithmic term $2.303RT/F$, of the Nernst equation.

In the presence of glucose in the sample, the potential recorded for the flow-through periodate-sensitive electrode after the reaction of periodate with glucose is completed, E_1 , is given by

$$E_1 = E' - S \log[P] \quad (4)$$

The measured quantity, in the form of potential peaks, is the difference $\Delta E = E_1 - E_0$ (peak height). Combining equations (2)–(4) we obtain

$$[1 - \text{antilog}(-\Delta E/S)] = n[G]_0/[P]_0 \quad (5)$$

Therefore, a linear relation is expected to hold between the experimentally determined quantity $[1 - \text{antilog}(-\Delta E/S)]$ and the initial concentration of glucose in the sample, $[G]_0$, because $[G]_0$ is linearly related to the actual sample concentration, $[G]_s$, and $[P]_0$ and n are considered constant within a series of measurements.

MATERIALS AND METHODS

Reagents

All solutions were prepared with deionized distilled water from reagent-grade (Merck) materials.

Ionic strength adjustment composite buffer, pH 6.4. Dissolve 322 g of $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ and 70 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in about 700 ml of water, adjust the pH to 6.4 with saturated NaOH solution, and dilute to 1 liter with water.

Sodium periodate working solution, 0.0400 M. Dissolve 8.56 g of NaIO_4 in water and dilute to 1 liter. This solution should be kept in an amber-glass bottle.

Glucose stock solution, 0.100 M. Dissolve 18.0 g of pure anhydrous glucose in water and dilute to 1 liter. Working standards are prepared by

dilution. Glucose standards used in the determination of glucose in serum should contain 5.0% bovine albumin. All glucose solutions should be refrigerated.

Apparatus

A schematic diagram of the automated flow system employed for the analysis is shown in Fig. 1. Sampler II and Proportioning Pump III were from the Technicon AutoAnalyzer II system. A sampling rate of 30 samples/hr and a sample-to-wash ratio of 1:1 were selected. The construction and the general characteristics of the periodate-sensitive flow-through electrode are described in detail elsewhere (1, 7). The periodate flow-through electrode is used in conjunction with an Orion 90-01 single-junction reference electrode (Ag/AgCl). A Corning Model 12 research pH meter, acting as a voltage follower, is inserted between the electrodes and a Sargent-Welch Model XKR potentiometric recorder, to match the high output impedance of the electrodes with the relatively low input impedance of the recorder.

Procedure

(a) *Determination of the prelogarithmic term, S.* Initiate the instrument set under the conditions shown in Fig. 1, but always keep the sampling needle at the "wash" position. Record the electrode signal for several minutes until the baseline potential (E_0) is stabilized to within ± 0.5 mV. Temporarily substitute the periodate working solution with a tenfold diluted one (0.00400 M) and measure the new potential (E_1) as before. The difference $E_1 - E_0$ is the required prelogarithmic term, S . A typical value

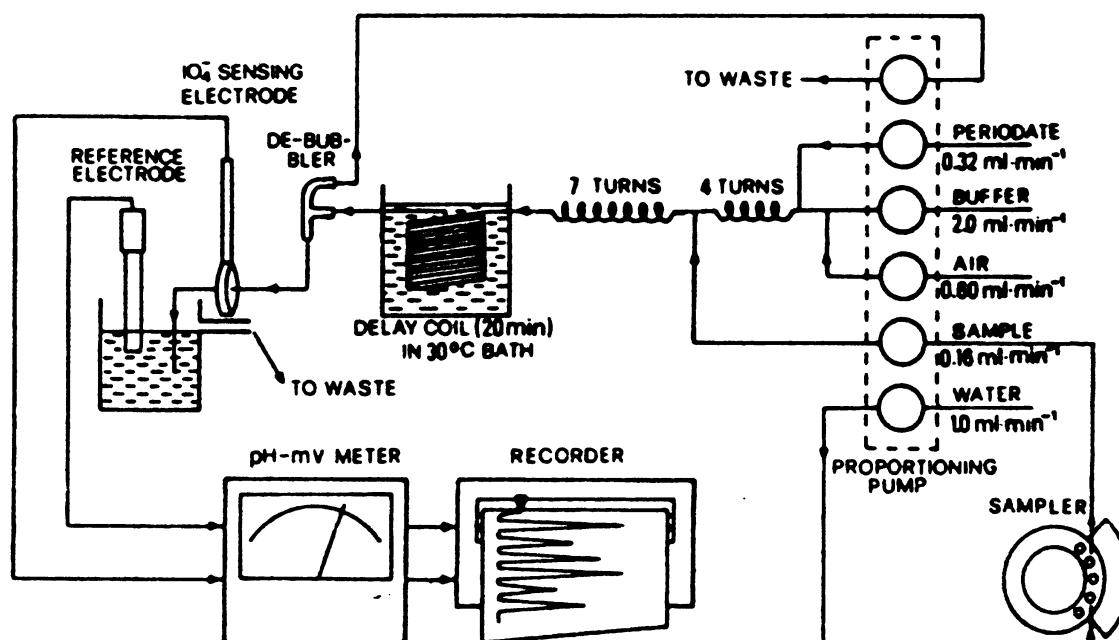


FIG. 1. Schematic diagram of the automated analysis system.

of S is 50 mV. This determination must be carried out just before or soon after a series of measurements.

(b) *Measurements.* Initiate the instrument set under the conditions shown in Fig. 1. The sample consumption is 0.160 ml per measurement. Measure the peak height, $\Delta E = E_{\text{peak}} - E_0$, corresponding to each sample. Include three standard solutions of glucose, 0.00300, 0.0100, and 0.0160 M, which are usually sufficient to establish the working curve, $1 - \text{antilog}(-\Delta E/S)$ vs [glucose]. The working curve should be a straight line passing through the origin.

RESULTS AND DISCUSSION

In Fig. 2 recorded tracings of a typical calibration sequence are shown along with tracings for a series of serum samples. In the same figure the corresponding working curve is also shown.

Aqueous glucose samples in the range 108–288 mg/100 ml were analyzed by the proposed method. The results and their precision are

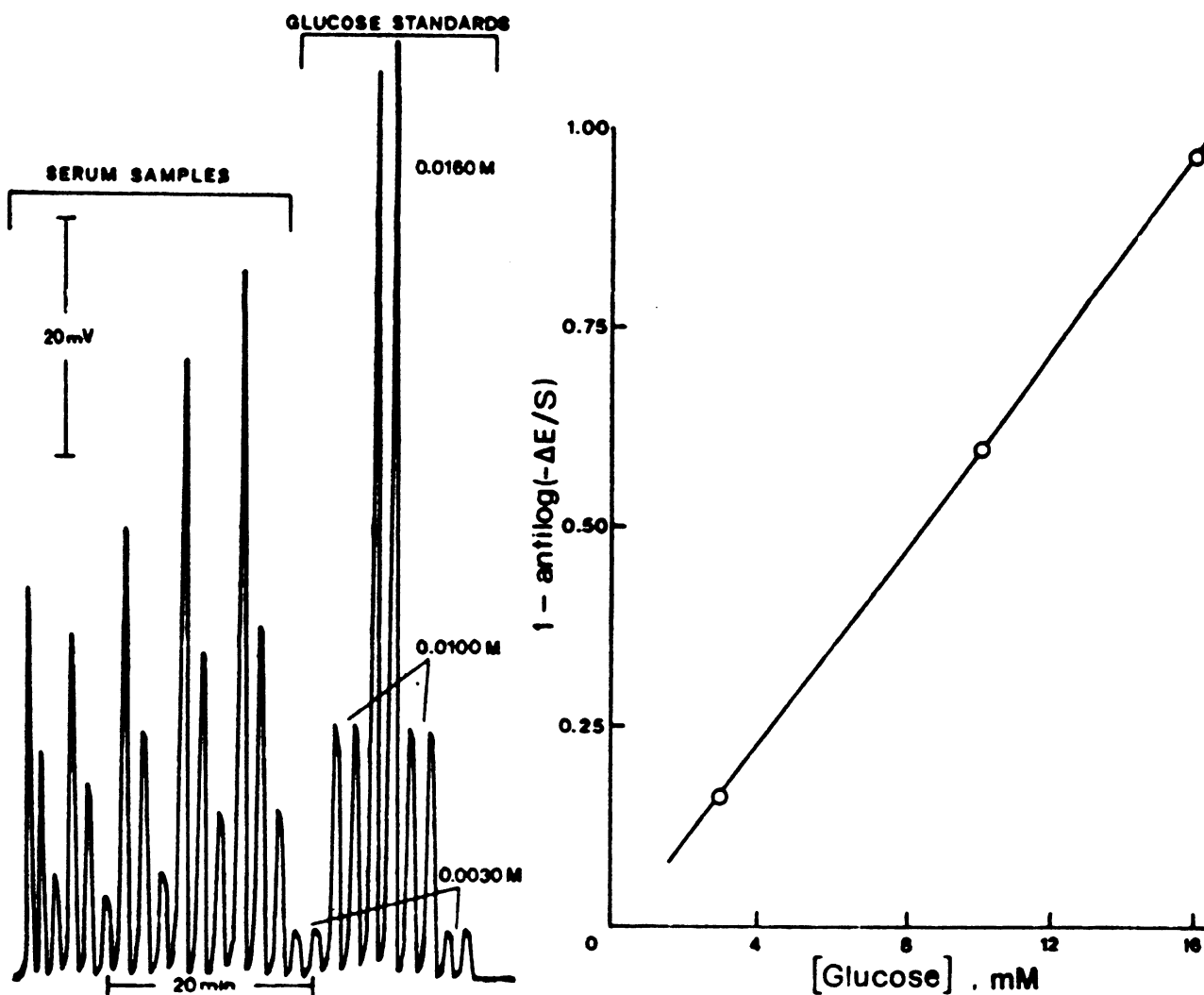


FIG. 2. Recorded tracings for glucose standards and serum samples and the corresponding working curve.

TABLE 1
ACCURACY AND PRECISION OF THE METHOD FOR AQUEOUS GLUCOSE SOLUTIONS

Glucose, (mg/100 ml)		Error (%)	RSD (%)
Taken ^a	Found ^b		
108.0	105.5	-2.3	—
144.0	145.8	+1.2	0.86 (<i>N</i> = 14) ^c
180.0	183.6	+2.0	0.55 (<i>N</i> = 10)
216.0	226.8	+5.0	—
252.0	—	—	0.93 (<i>N</i> = 10)
288.0	284.4	-1.2	—
		Av 2.3	

^a Initial sample concentration. Dilution factor 0.0645.

^b Average of two measurements.

^c *N* = number of determinations.

TABLE 2
PRECISION OF THE METHOD FOR SERUM SAMPLES

Serum sample	Glucose (mg/100 ml) ^a	RSD (%)	<i>N</i> ^b
I	123.4	1.94	10
II	134.3	0.94	9
III	291.7	1.84	10

^a Concentration found by the proposed method.

^b Number of determinations.

TABLE 3
RECOVERY OF GLUCOSE ADDED TO SERUM SAMPLES

Initially present ^a	Glucose (mg/100 ml)			Recovery (%)
	Added	Total	Found ^a	
103.6	90.0	193.6	185.5	91.0
	150.0	253.6	233.2	86.4
98.8	90.0	188.8	185.0	95.8
	150.0	248.8	243.9	96.7
85.4	90.0	175.4	184.5	110.1
	150.0	235.4	237.4	101.3
40.6	90.0	130.6	126.6	95.6
	150.0	190.6	187.1	97.7
73.1	90.0	163.1	166.3	103.6
	150.0	223.1	221.4	98.9
				Av. 97.7

^a Average of two determinations.

summarized in Table 1. The precision of the method was also tested in three pooled serum samples. Results are shown in Table 2.

Recovery experiments were carried out by adding aqueous glucose standard solutions to serum samples. The results are presented in Table 3. Recovery ranged from 86.4 to 110.1% with an average of 97.7%.

Analytical results with serum samples were compared with those obtained by a well-established enzymatic method (Glu-cinet, GOD-POD colorimetric method, Sclavo Diagnostics, Italy). The correlation plot is shown in Fig. 3. From Fig. 3 it becomes obvious that the correlation of the proposed method with the enzymatic method for serum is satisfactory. The positive blank occurring with the periodate method may be attributed to the reaction of periodate with other reducing substances normally occurring in serum, such as other carbohydrates and small amounts of ascorbic acid.

CONCLUSIONS

In conclusion, the proposed method is satisfactory for the determination of reducing sugars in blood serum and provides results which compare and correlate well with those of an enzymatic procedure. The method has also been applied to the determination of reducing substances in urine.

SUMMARY

A continuous-flow method for the determination of reducing sugars in serum is described. The sample reacts with an excess of periodate in a flow system and the decrease in periodate

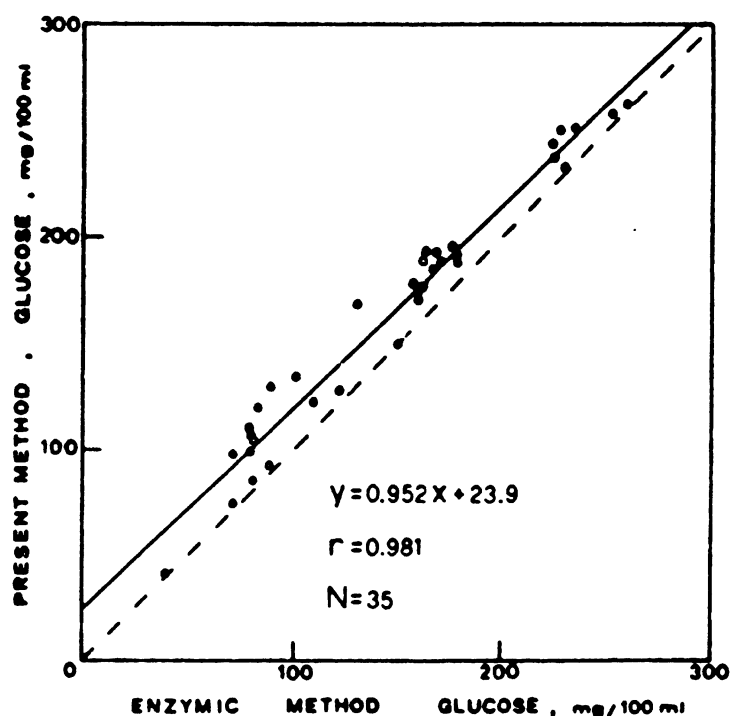


Fig. 3. Correlation plot of the proposed automated method with the enzymatic method.

activity is monitored with a periodate-sensitive flow-through electrode. The recorded potential peak heights are indirectly linearly related to the reducing sugar concentration expressed as glucose, in the range 50–290 mg/100 ml. The analysis is completely automated and requires no sample pretreatment, and samples can be analyzed at the rate of 30 per hour with average errors and relative standard deviation of about 1–2%. Comparison with an enzymatic method for serum gave satisfactory results.

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