Measurement of Glucose in Plasma by a Differential pH Technique


A new automatic apparatus based on the differential measurement of pH between two solutions has been developed. Two 25-μL (internal volume) glass capillary electrodes are used to measure the results of automated (under microcomputer control) chemical reactions that lead to the liberation or the uptake of hydrogen ions. The sensitivity of the differential pH measurements is better than ± 0.0001 pH unit, and the change in H⁺ concentration that can be detected by such an apparatus is 1 μmol/L for plasma and 3 μmol/L for whole blood. The technique has been applied to the measurement of glucose in plasma, giving results in agreement with the specifications of the Food and Drug Administration reference method for quantitative determination of glucose (hexokinase/glucose-6-phosphate dehydrogenase method).

Additional Keyphrases: change in pH as related to enzyme activity 

We have previously shown (1) that differences in pH between two solutions can be measured by using two glass capillary electrodes and we have applied this technique to the determination of glucose in aqueous solutions by measuring the change in pH produced by the hexokinase-catalyzed ATP-glucose reaction (2). The sensitivity of the method was about 5 × 10⁻⁵ pH units, indicating that the ΔpH method might be useful in the measurement of analytes of analytical and clinico-chemical interest. However, our previously described apparatus was not suitable for routine work and we sought to develop a fully automated instrument that could perform a variety of analytical functions, i.e., addition of reagents, filling and standardization of the electrodes, and calculation of results.

Here we describe this new instrument and report the results of its application to the routine determination of glucose in plasma. We have also developed theoretical equations that predict the sensitivity of the method when applied to the determination of analytes in human plasma or whole blood.

Materials and Methods

The Differential pH System

The basic components of the apparatus are shown schematically in Figure 1. Cuvette C is filled by the peristaltic pump P2 with a buffer from a separate reservoir (not shown). By means of the peristaltic pump P3 the content of the cuvette can be brought to constant volume (typically about 800 μL). The contents of cuvette C can be mixed by magnet N, which is covered with inert plastic, magnetically coupled to another magnet, and driven by motor M. E1 and E2 are two capillary electrodes (Ingold AG, Zurich, Switzerland), made specially for this work. The electrodes have an internal volume of about 25 μL and can be filled with the solution contained in cuvette C by the action of peristaltic pumps P4 and P5, respectively. Electrodes E1 and E2 are contained in a stainless-steel block to ensure an equal and constant temperature. They are joined in a Perspex (methyl methacrylate polymer) block, which is connected to cuvette C by a short length of silicone tubing. The liquid junction between the solutions contained in the electrodes is made in an all-liquid T-joint drilled in the Perspex block connecting the two electrodes. The grounding point G is made by a gold wire fixed before or at the liquid junction. The solution after this point is insulated from ground to avoid spurious electrochemical potentials arising from various parts of the system. Cuvette C and removable plug B are both made of Perspex. Peristaltic pump P1 is used to introduce a reagent, such as an enzyme solution, into cuvette C.

The electronic circuitry used to control peristaltic pumps P1 to P5 and motor M, and to monitor the output of electrodes E1 and E2, is based on a Z80A microprocessor (Zilog Inc., Cupertino, CA) run by a program written in machine language. The program takes 8 K bytes of EPROM memory and includes a mathematical package for all calculations. A driver board with solid-state switches activates the pumps and the stirrer. Electrode output is fed into a high-input impedance differential amplifier, as previously described (2). The peak-to-peak noise of the system, when filled with the standard buffer solution used in this work, is less than ±0.0001 pH unit. Data acquisition by the microprocessor is via a 12-bit resolution high-speed analog-to-digital converter (Analog Devices, Norwood, MA 02062). Further technical details will be described elsewhere.

Principles of operation. The apparatus can perform, under microprocessor control, four operations. (a) Wash: in this cycle, pumps P2–P4 are programmed to wash cuvette C, the connecting tubes, and electrodes E1 and E2 with standard buffer, leaving cuvette C filled with a known amount of standard buffer. (b) Calibration: this cycle is started by the operator, who introduces through plug B a known amount, typically 10 μL, of glucose standard. Motor M is automatically activated for 2 s to mix the content of cuvette C, then

Fig. 1. Schematic diagram of the ΔpH apparatus
P1 to P5, peristaltic pumps; B, a stopper to keep the content of cuvette C anaerobic and constant in volume; N, a magnetic stirrer covered in plastic; M, stirring motor; G, ground; E1 and E2, 25-μL (internal volume) glass capillary electrodes; A, a differential amplifier
about 300 µL of solution is aspirated by pumps P4 and P5 into electrodes E1 and E2. Pump P1 then injects into cuvette C a known quantity of hexokinase solution (typically 5 µL), motor M is activated for another 2 s, and pump P5 aspirates about 300 µL of the enzyme-reacted solution into electrode E2. After a suitable time (about 2–4 s for reaction 2), the difference in pH between E1 and E2 (ΔpH) is calculated from the difference in potential between two electrodes. (c) Measurement: the operation described under the calibration cycle is repeated, except that a known quantity of a solution containing an unknown amount of glucose is added. A second value of the difference in output of the two electrodes (ΔpH2) is then measured. (d) Blank: in this cycle no standard or sample is added, and the effect of the addition of the enzyme to the pH of the solution is determined (ΔpH). 

Determination of Glucose

When glucose is placed in a solution containing ATP in the presence of the enzyme hexokinase, ADP and glucose 6-phosphate (G6P) are formed, and the equilibrium

\[ \text{glucose + ATP} \leftrightarrow \text{G6P + ADP} \]  

(1)
is readily established. Because the pKₐ of ATP differs from those of G6P and ADP, the pH of the solution changes during the reaction course. The actual reaction that occurs in solution at pH = 7.5 can be written symbolically (2) as

\[ \text{glucose + } \Sigma \text{ATP} \leftrightarrow \text{G6P} + \Sigma \text{ADP} + n\text{H}^+ \]  

(2)

where \( \Sigma \text{ATP}, \Sigma \text{ADP}, \) and G6P represent all of the different charged species in the solution for ATP, ADP, and G6P, respectively, and \( n \) is the number of hydrogen ions produced in the reaction. Therefore the liberation of \( n \) protons provides a means of determining the concentration of glucose in the original sample.

Chemicals and solutions. Hexokinase (ATP:p-hexose 6-phosphotransferase, EC 2.7.1.1) was obtained from Sigma Chemical Co., St. Louis, MO 63178, as a lyophilized powder. We generally do not recommend using enzymes suspended in concentrated ammonium sulfate or in strong buffers for this work, because their addition to the solution may considerably change its buffer power. ATP (sodium salt, trihydrate) and NADH (free acid) were obtained from Boehringer, Mannheim, F.R.G. Sterox SE was purchased from Baker Chemicals, Deventer, Holland. Certified National Bureau of Standards (NBS) \( (\pm) \)-glucose and G6P dehydrogenase (\( \pm \)-glucose-6-phosphate:NADP⁺-1-oxidoreductase, EC 1.1.1.49) were a generous gift from Ames Miles, Milano, Italy. Carbon dioxide-free water and reagent water were prepared as described (3). All other reagents were analytical grade.

The buffer medium used in this work to study reaction 2 (hereafter referred to as "standard buffer") has the composition: KCl (0.1 mol/L), MgCl₂ (3 mmol/L), Na₂HPO₄ (0.5 g/L), Sterox SE (1.0 g/L), potassium phosphate (17.7 mmol/L), ATP (1.4 mmol/L), pH 7.50 (at 25°C). The stability of the standard buffer was assessed by measuring the change in pH, ATP concentration, and hexokinase activity after storage in stoppered containers. The pH and ATP concentrations were stable for at least six days in room temperature. The hexokinase, however, lost 17% and 37% of its activity after one and three days, respectively, at room temperature (−0% and 18%, respectively, after one and two days at 5°C). We therefore dissolved the hexokinase in 0.5 mL of standard buffer just before use to give a final concentration of 850 kU/L.

To prepare the working glucose standard we dissolved standard amounts of anhydrous \( (\pm) \)-glucose in 100 mL of 0.1 mol/L KCl containing 0.5 g of NaN₃ per liter. The solutions used for the determination of glucose according to the hexokinase/G6P dehydrogenase method as developed by the FDA (3), referred to in this paper as the "reference method," were prepared according to the instructions in Appendix A of ref. 3. The procedures outlined in the FDA report were strictly followed as regards calibration standards, reagents, instrumentation, data evaluation, and computation.

The spectrophotometer used in determining glucose by the reference method was a Spectracomp 601 (Carlo Erba, Milano, Italy) calibrated against NBS reference material. We also used a Radiometer titration system type TTT61, with an ABU80 autoburette and a PHM84 pH meter (Radiometer, Copenhagen, Denmark), as an alternative system for studying reaction 2 in aqueous solutions.

All measurements with the ΔpH apparatus described in Figure 1 were obtained at 22 ± 1°C. However, the difference in temperature between the two glass electrodes was not more than 0.01°C. Under such conditions the effect of temperature on the pH and buffer power of standard buffer can be considered negligible.

Samples and interfering substances. To test the performance of the ΔpH apparatus, by determining the concentration of glucose in plasma, we followed the experimental plan proposed by the FDA, Bureau of Medical Devices (3). Accordingly, we prepared three plasma samples by pooling individual plasma samples with a low glucose concentration (less than 0.70 g/L), dividing this pool into three parts, and enriching two of them by adding \( (\pm) \)-glucose to a concentration of about 1.40 and 2.40 g/L. In addition, we analyzed by the ΔpH method normal and pathological plasma samples containing glucose in the concentration range 0.5 to 4.00 g/L.

To test the specificity of the ΔpH method, we determined the glucose concentration of two pools of plasma (glucose concentration 1.36 g/L) before and after the addition of the following interfering substances (final concentration, g/L): sodium salicylate (0.35), sodium fluoride (2.00), K₂EDTA (1.00), heparin (1.00), sodium citrate (5.00), fructose (0.10), galactose (0.10), xylose (0.10), maltose (0.10), lactose (0.10), creatinine (0.10), bilirubin (0.10), and uric acid (0.10). Whenever the addition caused a change in pH, we added acid or base to bring the specimen back to its initial value. For each interfering substance we compared values for 26 specimens with and without the addition of the interfering substance.

Results

Figure 2a shows a curve for the titration with strong acid of the standard buffer used in this work at 25 and 37°C, and a titration curve of the same buffer with standard glucose according to reaction 2. At pH = 7.5, the titration curves show that the addition of one equivalent of glucose liberates about one equivalent of hydrogen ions, i.e., n \( (\pm) \)-glucose (0.10), xylene (0.10), maltose (0.10), lactose (0.10), creatinine (0.10), bilirubin (0.10), and uric acid (0.10). Whenever the addition caused a change in pH, we added acid or base to bring the specimen back to its initial value. For each interfering substance we compared values for 26 specimens with and without the addition of the interfering substance.

Figure 3a shows how the pH of the standard buffer containing 4 U of hexokinase changes by the addition of various amounts of glucose (line one). Addition of 1.00 g of glucose per liter changes the pH of the standard buffer by 0.01 pH unit; therefore, a system with a sensitivity of at least 0.0001 pH unit is required for the accurate determination of glucose in plasma by the ΔpH method. Line 2 was obtained by connecting point C, which represents the pH change caused by the addition of 2.00 g of glucose per liter,
with the origin. The difference between the two lines, shown in Figure 3b in terms of glucose concentration, is mainly due to the increase in buffer value of the standard buffer and, to a lesser extent, to the decrease in the value of n accompanying the decrease in pH (equation 2). Figure 3b shows that in the glucose concentration range 0 to 4.00 g/L the change in pH can be considered to change linearly with the glucose concentration. In fact, at a glucose concentration of 4.00 g/L the deviation from linearity is less than 2.5%. The deviation increases to about 8% when the glucose concentration is 10.00 g/L, a value 10-fold greater than the normal plasma value.

Table 1 summarizes the precision of this method, as calculated from repeated analysis on three plasma pools by the ΔpH method and by the reference spectrophotometric hexokinase/G6P dehydrogenase method (3). To include the effects of unknown interfering substances present in various amounts in specimens, bias is estimated from data for individual specimens and not from mixtures of a high- and a low-concentration serum pool.

Figure 4 shows a plot of the glucose concentrations obtained by the reference method (x) vs those obtained by the ΔpH method (y). Table 2 reports the effect of various possibly interfering substances on the average value of glucose concentration.
Discussion

Apparatus and Performance

We have previously shown (1) that the sensitivity that can be reached in the measurement of pH by two glass electrodes is about $5 \times 10^{-5}$ pH units. Practical limitations to the application of the differential pH technique have already been discussed, and an improved version of the original differential pH apparatus has been described (2). Here we have shown how by further improvements the differential pH technique can be applied to the automatic determination of analytes in serum, blood, or other biological fluids. Indeed, the technique can be useful in other fields such as analytical, food, and agricultural chemistry; because it relies on sensors specific for the measurement of hydrogen ion activity, it is, to a large extent, independent of the kind of solutions or suspensions being used.

The improvements reported here are (a) the adoption of two capillary pH electrodes (internal volume about 25 µL); (b) the addition to the pH system of a reactor (cuvette C, Figure 1) with a known volume, in which a solution (or a suspension) containing an analyte may be diluted in a proper buffering medium and reacted with a suitable reagent, such as an enzyme; and (c) the adoption of a microcomputer to direct the operation of the system and calculate the concentration of the analyte of interest. Applied to the measurement of analytes in biological fluids, the technique has obvious advantages over standard photometric techniques, because whole blood, plasma, urine, etc., can be analyzed directly without a preliminary deproteinization step. Furthermore, whereas optical techniques often require the use of several consecutive reactions to obtain an absorbance change related to the concentration of the analyte of interest, this often need not be the case for a potentiometric approach.

The main problem, which is inherent to any pH-related analytical technique, is that $\Delta pH$ is the measured variable, whereas $\Delta \text{H}^+$, the amount of hydrogen ions liberated or absorbed in the reaction, is what is required to calculate the stoichiometry of the reaction. The relationship between $\Delta pH$ and $\Delta \text{H}^+$ is given by the well-known Van Slyke buffer equation, which can be applied, for all practical purposes, in the pH interval where the buffer value of water is negligible.

$$d[\text{B}] = -d[\text{H}^+] = \beta \text{dPH}$$

where $\beta$ is the buffer value of the solution. If, at constant pH, a volume $V_b$ of sample with an intrinsic buffer value $\beta_s$ is added to a volume $V_s$ of buffer whose value is $\beta_b$, the total buffer value of the system will be (as a first approximation)

$$\beta' = \frac{V_b \beta_b + V_s \beta_s}{V_b + V_s}$$

Because the sample is also diluted from a volume $V_s$ to $V_f/(V_s + V_b)$, the total change of [B] or [H+] in the undiluted sample will be given by

$$d[\text{B}] = -d[\text{H}^+] = \left[ \frac{V_s + V_b}{V_s} \times \frac{V_b \beta_b + V_s \beta_s}{V_b + V_s} \right] \times \text{dPH}$$

For finite increments, equation 5 becomes

$$\Delta[\text{B}] = -\Delta[H^+] = \left( \frac{V_b \beta_b}{V_s + \beta_s} \right) \Delta \text{PH}$$

The sensitivity (S) may be defined as the ratio of the change in the response ($\Delta \text{PH}$) to the change in the concentration of the quantity that is measured ($\Delta \text{B}$ or $\Delta \text{H}^+$)

$$S = \frac{\Delta \text{PH}}{d[B]} = -\frac{\text{dPH}}{d[H^+]} = \frac{V_s}{V_b \beta_b + V_s \beta_s}$$

Equation 6 shows that the maximum sensitivity is reached when $V_b \beta_b = 0$; under such conditions $S_{\text{max}} = 1/\beta_s$. Thus maximum sensitivity is obtained when measuring pure sample, or sample diluted in pure water, provided that the pH is such that one can assume that $\beta_{bLO} = 0$. For all other conditions, such as when the sample is diluted into a solution of definite buffer value, the sensitivity is correspondingly reduced according to equation 7.

The overall electrochemical performance of the system described in this paper can be assessed in terms of the sensitivity of the differential pH measurement and of its stability. A series of 10 successive readings of $\Delta \text{PH}$ values resulting from 10 calibration cycles involving 10 µL of standard (1.00 g/L) aqueous glucose solutions gave the following typical values: $\Delta \text{PH} = 0.0122$ (SD 0.0001). Ordin-
narily, stability of such values was better than ±0.0002 pH units per hour.

According to equation 3, there are two experimental approaches determining $\Delta (H^+)$ from $\Delta pH$ values: (a) $\beta$ is determined by the addition to the solution (buffer + sample) of a known amount of standard acid or base, or (b) the value of $V_b\beta_b$ (equation 4) is increased to make the contribution of $V_b\beta_b$ negligible ($V_b\beta_b \gg V_p\beta_p$). The latter approach has been followed in this paper, because a much more complex configuration of the differential pH apparatus would be required to make it suitable for the measurement of the buffer value of the solution contained in C.

To calculate the value of $\beta_b$ for which (at any given $V_b$ and $V_p$) $V_b\beta_b \gg V_p\beta_p$, one must first know the value of $\beta_p$, $\beta_b$ values for human plasma and for human whole blood can be calculated from the data reported by Siggaard-Andersen (4), and turn out to be $9.4 \times 10^{-3}$ and $30 \times 10^{-3}$, respectively. Given a sensitivity of the $\Delta pH$ measurement of about 1 $\times 10^{-4}$ pH units, the change in [B] or [H$^+$] concentration that can be detected in pure plasma or whole blood with the apparatus shown in Figure 1 is about 1 $\mu$mol/L for plasma and −3 $\mu$mol/L for whole blood. If $V_b = 0.8$ mL, $\beta_b = 5.8 \times 10^{-3}$ (standard buffer), and $V_p = 0.01$ mL, then $\beta_p$ (equation 4) $= 5.84 \times 10^{-3}$ (plasma) and $6.1 \times 10^{-3}$ (whole blood). The minimum change in [H$^+$] that can be detected decreases, according to equation 5, to [$(81 \times 5.84) + (8.41) \times 10^{-3}$] = 48 $\mu$mol/L for plasma and 51 $\mu$mol/L for blood.

Therefore, under the conditions of our experiments, the contribution of the buffer value of plasma and of whole blood to the total buffer value of standard buffer is very small, i.e., 0.04/5.84 = 0.7% (plasma) and 0.30/6.10 = 4.9% (whole blood).

**Determination of Glucose in Plasma**

In a blank cycle the effect of addition of enzyme solution to standard buffer is first determined. From equations 3 and 4:

$$[\text{glucose}]_b = \frac{\beta_b V_b' + \beta_p V_p}{V_b' + V_p} \Delta pH_b' = \Delta \Delta pH_b'$$

(8)

$\beta_b$ is the buffer value of the standard buffer at pH = 7.50 (Figure 2b); $V_b'$, the volume of the buffer left in cuvette C after the first electrode has been filled with standard buffer, is typically 500 $\mu$L; $V_p$, the volume of enzyme solution, is about 5 $\mu$L; and $\beta_e = \beta_p$ because we used a lyophilized enzyme preparation dissolved in standard buffer.

In a calibration cycle, 10 $\mu$L of a standard glucose solution (2.00 g/L) is added to the standard buffer and a value of $\Delta pH$ is determined. If we assume a linear relationship between the concentration of glucose and $\Delta pH$, as is found in the glucose concentration range 0 to 4.00 g/L, then:

$$[\text{glucose}] = f \left( \frac{\beta_b V_b' + \beta_p V_p}{V_b' + V_p} \Delta pH_1 \right)$$

(9)

whence

$$[\text{glucose}] = f \times B \times (\Delta pH_1 - \Delta pH_2)$$

(10)

where $f$ is the dilution coefficient = ($V_b + V_p + V_e)/V_p$.

A measuring cycle is then performed by the addition of a known volume (10 $\mu$L) of plasma with an unknown glucose concentration. Again assuming a linear relationship between glucose concentration and $\Delta pH$, then:

$$[\text{glucose}]_p = f \left( \frac{\beta_b V_b' + \beta_p V_p}{V_b' + V_p} \Delta pH_2 \right)$$

$$- \left( \frac{\beta_b V_b' + \beta_p V_p}{V_b' + V_p} \Delta pH_1 \right)$$

whence

$$[\text{glucose}]_p = (\text{fC}(\Delta pH_2 - \Delta pH_1))$$

(12)

The buffer power of standard buffer $\beta_p$ should be changed from $\beta_b$ to $\beta_p$ to take into account the effect of the addition of plasma on the pH and hence on the $\beta_p$ of standard buffer. In fact, the effect is very small. If $\beta_p = 9.4 \times 10^{-3}$ and the pH variation in individual plasma samples does not exceed ±0.5 pH unit, the total amount of base or acid added to the standard would be at most $\pm 9.4 \times 0.5 \times 10^{-5}$/81 = ±58 $\mu$mol/L, causing a maximum change of $(\pm 5.8 \times 10^{-5}$/58 $\times 10^{-5}) = \pm 0.01$ pH unit in the standard buffer. This corresponds to a total maximum change of 1% in $\beta_p$ (Figure 2b). Thus the effect of analyzing plasma samples with extreme pH values (i.e., 6.9–7.9) has only a negligible effect on the determination of their glucose content by this method.

Because, under the experimental conditions chosen, $\Delta pH_b = \Delta pH_0 = \Delta pH_2$, equations 10 and 11 can be combined, giving:

$$[\text{glucose}]_p = [\text{glucose}]_b \times \frac{B}{C} \times \frac{\Delta pH_2 - \Delta pH_0}{\Delta pH_1 - \Delta pH_0}$$

(13)

The determination of glucose in plasma by use of equation 13 allows greater analytical precision than does the use of the absolute values of the parameters of equation 11, in that the dilution factor (f), the initial pH, the composition of the standard buffer, and $V_E$, $\beta_E$, and $V_p$ do not need to be known on an absolute basis.

Equation 13 has been developed on the assumption that both $[\text{glucose}]_b$ and $[\text{glucose}]_p$ are linearly related to $\Delta pH_1$ and $\Delta pH_2$, respectively. Figure 2b shows that for all practical purposes this is true in the range of glucose concentration 0 to 4.00 g/L. At greater concentrations there is a marked deviation from linearity. If $[\text{glucose}] = 2.00$ g/L, there is an 8% deviation from linearity when $[\text{glucose}] = 10.00$ g/L, for reasons discussed earlier. If linearity at greater concentrations is required, a two-point calibration overlapping the concentration range of interest can be used. Alternatively, a composite buffer with a constant buffer value in the working pH range can be used. Because the main object of this paper was to verify the performance of the $\Delta pH$ apparatus and of the simplified chemical system used for glucose determination with reference to the FDA method, we limited our work to the glucose concentration range 0 to 4.00 g/L. Equation 13 shows how the glucose concentration in plasma can be determined relative to the glucose concentration of a standard aqueous solution.

Table 1 and Figure 4 compare both the precision and the results obtained by the $\Delta pH$ method and by the FDA reference method (3). To make such a comparison meaningful at the level of precision required, all data obtained by the $\Delta pH$ method and by the use of equation 13 (with reference to $[\text{glucose}]_b = 2.00$ g/L) have been corrected as follows:

- for the residual nonlinearity between glucose concentration and $\Delta pH$ (Figure 2b); the size of this correction is, at most, 2.5% of the value of glucose concentration at the highest limit of glucose concentration studied (4.00 g/L).
- for the small (less than 0.5%) effect of changing the buffer value of the standard buffer after the addition of plasma.
for the volume effect due to plasma proteins, a correction made according to the following equation (5): $\Delta c = c_1[(100/\ (100 - Vc_2)] - 1$, where $c_1$ = concentration of glucose in plasma, $c_2$ = concentration of glucose in the protein-free supernate, $\Delta c = c_2 - c_1$, $c_p$ = concentration of protein in g/ dL, and $V$ = mean partial specific volume of the proteins. $\Delta c$ calculated from the above formula is, for human plasma of normal protein content, less than 5% of the value of the glucose concentration. The correction is based on the assumption that in the FDA reference method there is no coprecipitated or adsorbed glucose in the precipitate.

Table 2 shows the effect on the mean glucose concentration of various interfering substances. Clearly, only fructose interferes significantly. This is to be expected because hexokinase is known to catalyze the phosphorylation of both glucose and fructose. However, $K_m$ for glucose is $1.0 \times 10^{-4}$ mol/L as compared with $7.0 \times 10^{-4}$ mol/L for fructose (6), which probably explains why fructose interference is equivalent to less than half of its total concentration. Other carbohydrates (e.g., galactose and xylose) or disaccharides (maltose and lactose) do not produce an appreciable effect.

We were interested at this point to see whether the simplified method of enzymatic analysis of glucose concentration based on reaction 2 and $\Delta p$H measurement would qualify as an FDA in vitro diagnostic device for the quantitative measurement of glucose in plasma or serum. With regard to bias, which according to FDA specification (3) is the limiting mean difference between the reference method value and the $\Delta p$H method value obtained with specimens representative of the population to which the test is applied (measured as a percentage of the reference method value), statistical analysis of all available data showed that the $\Delta p$H method passed the requirements set by the FDA report for qualification as a diagnostic device for the quantitative measurement of glucose in plasma. No value of glucose concentration by the $\Delta p$H method was found to differ by more than 15% of the value obtained by the reference FDA method. Analysis of data on the specificity of the $\Delta p$H method (Table 2) showed that the effect of all substances tested, including fructose, was within the interference limit set by the FDA specifications.

The small discrepancy (averaging about 0.04 g/L) between glucose concentrations determined by the $\Delta p$H method with a single enzyme and by the FDA reference method was probably due to the simultaneous phosphorylation of fructose as well as of glucose when only hexokinase was used. However, the fructose concentration in plasma of normal fasting individuals is very small (7), probably 0.02–0.04 g/L, and would not introduce clinically relevant errors.

References