Anal Bioanal Chem (2007) 389:1623–1631
DOI 10.1007/s00216-007-1521-7

ORIGINAL PAPER

Reduction of the interferences of biochemicals and hematocrit ratio on the determination of whole blood glucose using multiple screen-printed carbon electrode test strips

Yueh-Hui Lin · Thomas Y. Shen · C. Allen Chang

Received: 10 April 2007 / Revised: 16 July 2007 / Accepted: 19 July 2007 / Published online: 3 October 2007
© Springer-Verlag 2007

Abstract A practical approach to reduce the interferences of biochemicals and hematocrit ratio (Hct%) in the determination of whole blood glucose using multiple screen-printed carbon electrode (SPCE) test strips is described. SPCE test strips with and without glucose oxidase [i.e., GOD(+)-SPCEs and GOD(-)-SPCEs] were used and the chronoamperometric currents of test glucose solutions with various spiked uric acid concentrations and Hct% were measured. By establishing the interference relationships between glucose concentrations and uric acid concentrations as well as Hct% values and with appropriate corrections, the whole blood glucose determinations could be made to be more accurate and comparable to those determined by the reference YSI method. Specifically, the use of the ΔI value, i.e., the current difference between GOD(+)-SPCE and GOD(-)-SPCE measurements, would reduce most of the uric acid/biochemical interferences. An interpolation method was also established to correct for the glucose determinations with Hct% interferences. The Hct% corrections using the interpolation method are especially important and necessary for those blood samples with glucose concentrations higher than 110 mg dL^{-1} and Hct% values lower than 35%. This approach should also be applicable to other biochemical determinations using similar electrochemical techniques.

Keywords Screen-printed carbon electrodes · Electrochemical biosensor · Glucose · Interference · Hematocrit ratio · Uric acid

Introduction

Chronoamperometric biosensors have high sensitivities and allow the determination of electroactive substances at concentrations as low as 10^{-9} M with a dynamic range of 3–4 orders of magnitude. The concept of an amperometric sensor for glucose was introduced by Clark and Lyons [1] in 1962. In this case, the electrode response of the glucose oxidase (GOD) catalyzed glucose oxidation reaction is dependent on the oxygen concentration in the reaction medium and the product H_2O_2 is detected typically at 500–750 mV vs. the Ag/AgCl reference electrode. However, this method suffers at least two types of interferences for whole blood and serum samples. One is from physiological biochemicals such as uric acid, ascorbic acid and acetaminophen because these interferents are oxidized at the same potential range as H_2O_2 [2, 3]. The other is due to differences of hematocrit ratio (Hct%), i.e., red blood cells ratio [4]. This is because the red blood cell fraction in the sample may affect the diffusion pathways and rates of substrates to the enzymes to produce products and electrochemical signals.

Electronic supplementary material The online version of this article (doi:10.1007/s00216-007-1521-7) contains supplementary material, which is available to authorized users.

Y.-H. Lin
Institute of Biological Engineering,
National Chiao Tung University,
Hsinchu, Taiwan

Y.-H. Lin · T. Y. Shen
APEX Biotechnology Corporation,
Hsinchu Science-Base Industrial Park,
Hsinchu, Taiwan

C. A. Chang (✉)
Department of Biological Science and Technology,
National Chiao Tung University,
Hsinchu, Taiwan
e-mail: changca@cc.nctu.edu.tw

One useful solution to partially reduce the biochemical interferences is to introduce a low-oxidation potential mediator that acts as the electron acceptor of the enzymatic catalyzed oxidation [5–7]. In this approach, a mediator such as ferrocene or a quinine-type compound is added in the reaction vessel to shuttle electrons from the reduced enzyme to the working electrode. Two major advantages for using the mediated sensors are that (1) the electrochemical signals are largely independent of oxygen concentration in the sample and (2) lower oxidation potentials can be used instead of that necessary for H_2O_2. Currently, glucose electrochemical biosensors with screen-printed carbon electrodes (SPCEs) and a mediator are commercially mass-produced for convenient and low-cost homecare applications. Electrochemical biosensors using similar design principles for hemoglobin [8], Hct% [9] and uric acid [10] are also available.

Several other techniques have also been reported to eliminate the unwanted biochemical interferences. These include the preconsumption of the interfering substances by preoxidation employing horseradish peroxidase [11] or ascorbate oxidase [12], and the effective separation of the electrode from the interfering substances using a highly hydrophilic polymeric analyte-regulating membrane [13] or a polycarbonate membrane coated with a negatively charged hydrogel layer [14]. Methods of mediator-free and membrane-free biosensors were also described which provided means to measure the cathodic (i.e., reduction) current of the enzymatically liberated H_2O_2 by metal-dispersed carbon paste electrodes [15, 16]. More recently, a biosensor employing carbon nanotube nanoelectrode ensembles was developed to determine the substrate concentration selectively and avoided the generation of overlapping signals from interferents [17]. A continuously used amperometric biosensor for whole blood sample analysis calibrated with acetaminophen was claimed to achieve good correction of interferences by acetaminophen-specific electrodes [18]. However, the large-scale production of these devices is expected to be more complicated and expensive than that of the SPCEs and may not be readily suitable for homecare applications.

On the other hand, it was usually found that artificially higher responses were obtained for samples with lower Hct% values, and lower responses were obtained for those with higher Hct% values. In the preferred embodiments of a US patent [19], the interferences of Hct% of an electrochemical glucose biosensor were reduced by roughly 50% by the introduction of a dummy electrode without enzyme.

Owing to its convenience and lower costs, the use of electrochemical SPCEs for glucose determination in point-of-care testing (POCT) has continued to increase. Because it is not practical at the POCT setting to perform sample pretreatment to reduce interferences such as in a wet biochemical process, the accuracy and precision of glucose determinations using the SPCE techniques are particularly important. Thus, government regulatory agencies have established the 510K and ISO15197:2003(E) guidelines as performance criteria. In 1987, the American Diabetes Association (ADA) recommended that POCT electrochemical glucose determinations should fall within ±15% of those determined by regular laboratory techniques and the future goal was to reduce this variability to within ±10% for glucose concentrations between 30 and 400 mg dL^{-1}. This goal was later modified by the ADA in 1994–1996 to be within ±5% of those determined by regular laboratory techniques [20–22]. Although the criteria in the government regulatory guidelines have not been changed so far, it is inevitable that the glucose determination specifications are expected to be more rigorous. However, to the best of our knowledge, no report has been published to date concerning attempts to reduce the biochemical and hematocrit interferences and improve the precision and accuracy of the electrochemical glucose determination [23, 24].

In this paper we report the results of our approach to reduce the biochemical and Hct% interferences on the chronoamperometric determination of whole blood glucose using SPCEs. A practical solution could then be developed to improve the accuracy and precision of whole blood glucose determinations to serve the diabetic community better. This simplified approach could also be applied to reduce interferences for chronoamperometric determinations of other analytes in the whole blood sample.

Experimental

Materials and equipment

GOD (EC 1.1.3.4, *Aspergillus niger*, Biozyme Laboratory, code GO3A), and reagent grade L-ascorbic acid, uric acid, bilirubin, carboxymethyl cellulose sodium salt, bovine serum albumin (BSA), lyophilized hemoglobin, deoxycholic acid, acetaminophen and water-soluble cholesterol (Sigma), D-(+)-glucose, 3-acetamidophenol, Triton X-100, potassium hexacyanoferrate(III), $K_2HPO_4.3H_2O$ and KH_2PO_4 (Merck), glucose/HK and UA plus kits (Roche), uric acid standard (Randox) and 0.9% saline (Taiwan Biotech) were used as received. SPCEs and venous and fresh capillary whole blood samples drawn from normal people and diabetic patients by a registered nurse were obtained from APEX Biotechnology Corporation.

A CV50W voltammetric analyzer (Bioanalytical Systems) was used for all electrochemical measurements. For comparison purposes, a YSI 2300 STAT Plus glucose and lactate analyzer (YSI Life Sciences), a HemoCue B-Hemoglobin analyzer, an SP870 spectrophotometer (Meter-

tech) and a KUBOTA 3110 micro hematocrit centrifuge were used for glucose, hemoglobin, uric acid and Hct% determinations, respectively.

Construction of the SPCE test strips with and without GOD

The SPCE test strips were constructed according to a described protocol [25]. Conducting films of silver and carbon ink were screen-printed consecutively on a flat surface of a PVC board to form a two-electrode system and the board was dried by hot air. An about 0.27-mm-thick electrically insulating layer was then applied to the flat board surface to cover most of the electrode area and allowed only the connecting and electrochemical reaction portions to be partially exposed. For SPCE test strips containing GOD [i.e., GOD(+)-SPCEs], a solution with the following composition was added on the surface of the reaction area (% w/w): bovine serum albumin (0.5%), potassium ferricyanide (6.0%), carboxymethyl cellulose (0.5%), Triton X-100 (0.07%), phosphate buffer (pH 5.0, 92.30%) and GOD (0.63%). The test strips were dried at 50 °C for 15 min and cut from the PVC board after a reticular covering mesh had been applied on the reaction layer. For SPCE test strips without GOD [i.e., GOD(-)-SPCEs], a solution with a similar formulation to that of GOD(+)-SPCEs was added to the test strips except that BSA was used instead of GOD. The amount of the formulation solution added onto the 12.6-mm^2 reaction area was approximately 5 μL for both GOD(+)-SPCEs and GOD(-)-SPCEs. All GOD(+)-SPCEs and GOD(-)-SPCEs were freshly prepared for single use.

Initial study of the effects of biochemical and hematocrit interferences

The GOD(+)-SPCEs test strips were used to measure the oxidative cyclic voltamograms for the endogenous and exogenous biochemicals in the potential range from -400 to 400 mV at a scan rate of 100 mV s^{-1} using the CV50W voltammetric analyzer after a 20-s sample incubation period. For a healthy human being, the ADA recommends that the blood sugar level is best kept at 80–120 mg dL^{-1} before meals and at 100–140 mg dL^{-1} at bedtime when using a blood sample drawn from a vein (i.e., a whole blood sample). The numbers increase to 90–130 mg dL^{-1} before meals and 110–150 mg dL^{-1} at bedtime when using a blood sample drawn from a fingertip (i.e., plasma blood sample) [26]. Thus, the our test glucose saline solutions were prepared at concentrations of 71, 145 and 290 mg dL^{-1} to mimic the low-end and high-end glucose levels of a healthy human being and that of a diabetic. The concentrations of the biochemicals (i.e., ascorbic acid, uric acid, bilirubin, acetaminophen, 3-acetamidophenol and hemoglobin) for the interference studies were prepared according to the guidelines set by the National Committee for Clinical Laboratory Standards document *EP7-A: interference testing in clinical chemistry; approved guideline* [27].

Quantitation of biochemical and hematocrit interferences

Sample solutions containing the biochemical interferents were prepared by spiking glucose and interfering biochemicals into reconstructed red blood cell saline (RRBC-saline) as well as oxygenated whole blood solutions. RRBC-saline solutions with designated Hct% were prepared by using a fresh venous blood sample, washing four times with three portions of saline solutions and centrifugation to remove unwanted biochemicals, and diluting with appropriate amounts of saline solutions (vide infra). For whole blood oxygenation, 2 mL whole blood in a 7-mL capped container was allowed to equilibrate with the atmosphere on a horizontal rotator for 15 min. The cap was then opened for 30 s, and the container was recapped and the contents were allowed to equilibrate for another 15 min. The sample solutions were added onto the GOD(+)-SPCEs and GOD(-)-SPCEs, and the SPCE strips were connected to the CV50W at 25 °C. A constant potential of 300 mV was applied to the working electrode of the SPCE test strips and the oxidation currents vs. time data were recorded. Freshly prepared SPCE test strips were used for each measurement. The accuracies for the glucose, Hct%, and uric acid determinations were checked by comparing them with those obtained with the YSI 2300 STAT Plus, the KUBOTA 3110 centrifuge, and the Roche UA plus kit/Meterteck SP870 spectrophotometer, respectively.

Results and discussion

Initial study of the effects of biochemical and hematocrit interferences

A total of eight endogenous and exogenous biochemicals were chosen for this study: glucose, ascorbic acid, uric acid, hemoglobin, bilirubin, cholesterol, acetaminophen and acetamidophenol. Figure 1 shows selected cyclic voltamograms of some of these biochemicals at various concentrations using the GOD(+)-SPCEs in the potential range from –400 to 400 mV after 20-s sample incubation. The current readings of the oxidative cyclic voltamograms at various concentrations of these biochemicals at 100, 200, 300 and 400 mV are listed in Table 1. It is noted that the endogenous biochemical concentrations in a normal, healthy person are generally as follows: glucose, below 150 mg dL^{-1}; ascorbic acid, below 5 mg dL^{-1}; uric acid, below 10 mg dL^{-1}; bilirubin, below 5 mg dL^{-1}; cholesterol,

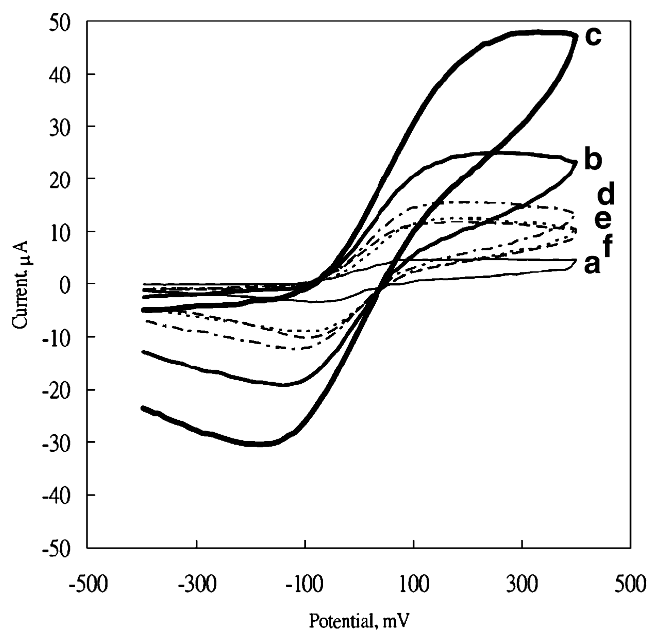

Fig. 1 Voltamograms of glucose and biochemicals in saline solutions on the screen-printed carbon electrode test strips with glucose oxidase [GOD(+)-SPCEs] after 20-s sample incubation (scanning rate, 100 mV/s). *a* Control, glucose, 0 mg dL^{-1}; *b* glucose, 100 mg dL^{-1}; *c* glucose, 200 mg dL^{-1}; *d* ascorbic acid, 20 mg dL^{-1}; *e* uric acid: 20 mg dL^{-1}; *f* hemoglobin, 20 g L^{-1}

below 200 mg dL^{-1}; hemoglobin, below 10 g L^{-1} [28]. Thus, excluding glucose, the higher (than normal) working concentrations chosen for fresh hemoglobin (20 g L^{-1}) and the rest of the biochemicals (300 and 20 mg dL^{-1}, respectively) for this initial study represent quite stressed physiological conditions. From the current readings, it is observed that at 300 mV, most of the potential interfering biochemicals show relatively the greatest interfering signals. It was found that the degree of biochemical interferences for glucose determination is roughly in the order uric acid ~ ascorbic acid > hemoglobin > bilirubin > cholesterol. For the exogenous biochemicals, acetaminophen has a higher degree of interference. However, the effect would be greatly reduced after this material has been excreted from the body.

For subsequent chronoamperometric measurements of various concentrations of glucose and uric acid in saline solutions, a potential of 300 mV was used and the tenth second current readings after 20-s sample incubation were collected, as shown in Fig. 2. Under these conditions, the current signals for glucose measurements in the 50–550 mg dL^{-1} concentration range in saline and whole blood (Hct% of 40%) were linear with good correlation coefficients (Fig. S1). The measured current data for uric acid were also linear with uric acid concentrations (data not shown).

One common complication for diabetic patients is anemia due to hemodialysis [29, 30]. In this case, abnormally low Hct% values were frequently observed for whole blood samples. Thus, it is important to evaluate the determination of blood glucose at low Hct% using SPCEs. The Hct% interference effects on the current measurements using GOD(+)-SPCEs at several glucose concentrations are shown in Fig. 3. As expected, higher current readings were

Table 1 The current readings (μA) at various concentrations of selected biochemicals using the screen-printed carbon electrode test strips with glucose oxidase [GOD(+)-SPCEs] at scanning potentials of 100, 200, 300 and 400 mV

Biochemicals	Concentrations		Scanning voltage (mV)			
			100	200	300	400
Saline			4.7	4.8	4.8	4.8
Glucose	71	mg dL^{-1}	20.6	24.7	24.9	23.4
	145		30.6	43.5	47.8	47.2
	290		46.2	67.9	81.1	88.2
Ascorbic acid	10	mg dL^{-1}	8.8	8.9	8.7	7.7
	20		14.6	15.6	15.1	13.5
Uric acid	10	mg dL^{-1}	7.7	8.1	7.9	7.1
	20		11.3	11.8	11.4	10.6
Bilirubin	10	mg dL^{-1}	6.7	6.5	6	5.5
	20		7.9	7.2	6.7	6.2
Cholesterol	150	mg dL^{-1}	5.1	4.9	4.8	4.7
	300		4.2	4.2	4	3.8
Fresh hemoglobin	10	g L^{-1}	7.7	7.8	7.5	6.9
	20		11.5	12.5	11.7	10.7
Lyophilized hemoglobin	10	g L^{-1}	4.9	4.8	4.8	4.8
	20		5.1	5	4.9	4.8
Acetaminophen	10	mg dL^{-1}	5.5	7.7	12.6	12.4
	20		5.3	9.7	18.4	20.9
3-Acetamidophenol	10	mg dL^{-1}	4.9	5	4.8	5
	20		4.7	4.8	4.8	4.8

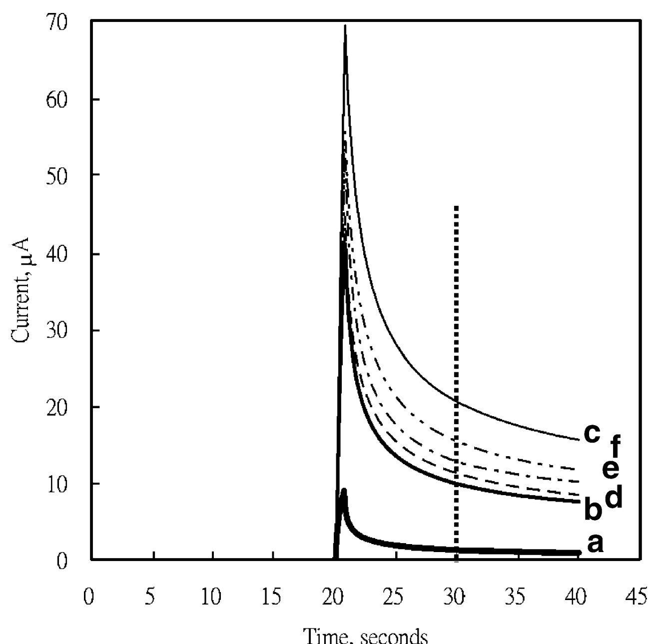

Fig. 2 Chronoamperometric currents of saline solutions containing different glucose and uric acid concentrations vs. time at 300 mV on the GOD(+) SPCEs after 20-s incubation. Glucose: *a* 0, *b* 71 and *c* 145 mg dL^{-1}. Uric acid: *d* 5, *e* 10 and *f* 20 mg dL^{-1}

observed for glucose concentrations with lower Hct% values. An exponential decay relationship, i.e., $I=ke^{-b[\mathrm{Hct\%}]}$, could be established between the current readings and the Hct% values with correlation coefficients $R^2 \geq 0.9088$.

For a given glucose concentration, the current responses at various Hct% values and two uric acid concentrations are shown in Fig. 4 with the use of GOD(-)-SPCEs. A similar result to that of the above-discussed GOD(+)-SPCEs was observed and corroborated well with those reported earlier [19]. The normal adult hematocrit ranges are 39–51% for men and 36–44% for women [31]. Outside this range, the glucose determinations using this technique would give larger errors, particularly for those with lower Hct%.

Initial elimination of interferences of uric acid/biochemicals and Hct%

Three kinds of current signals are defined in this study: (1) $I_{(s+i)}$ is the current reading obtained with the GOD(+)-SPCEs and includes that of glucose with biochemical and hematocrit interferences, (2) I_i is obtained with GOD(-)-SPCEs and is due to biochemicals with minor hematocrit interferences and (3) the calculated corrected current, $\Delta I=I_{(s+i)}-I_i$. Note that the hematocrit interference might not be completely eliminated by just calculating ΔI because the degree of hematocrit interferences in glucose determination is usually greater than that of other biochemicals (vide infra). To minimize the interferences of Hct% completely, additional interpolation was exercised (vide infra).

The tenth second $I_{(s+i)}$ and I_i values were obtained after an initial 20-s sample incubation period. Figure 5 shows the

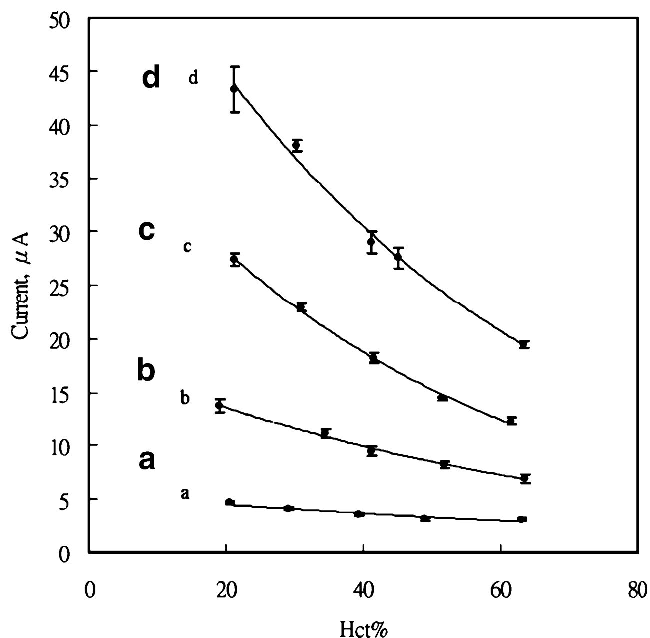

Fig. 3 Whole blood chronoamperometric current readings vs. hematocrit ratio (*Hct%*) at various glucose concentrations using GOD(+)SPCEs. The glucose concentrations were *a* 75.7, *b* 170.4, *c* 318.0 and *d* 462.0 mg dL^{-1}. The uric acid concentration was 6.5 mg dL^{-1}. The best-fitted equations were *a* $Y=5.491e^{-0.0097X}$ (R^2=0.909), *b* $Y=18.599e^{-0.0156X}$ (R^2=0.994), *c* $Y=42.482e^{-0.0204X}$ (R^2=0.997) and *d* $Y=64.524e^{-0.0186X}$ (R^2=0.993)

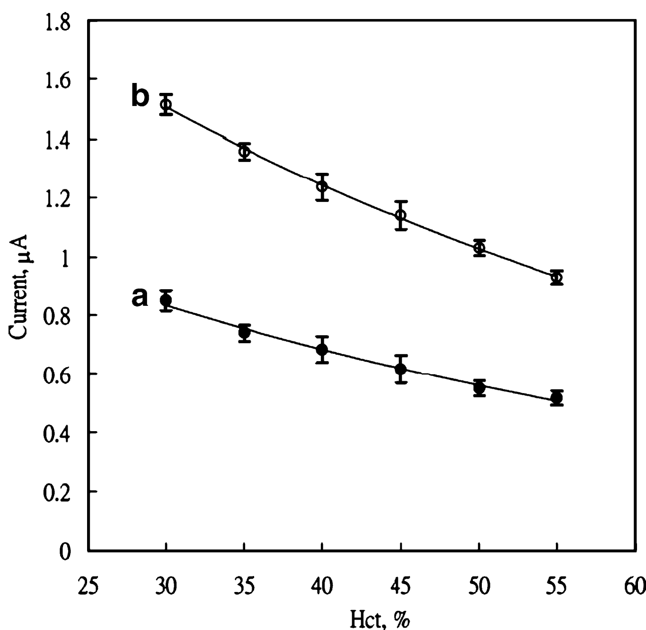

Fig. 4 Whole blood chronoamperometric current readings vs. Hct% at a glucose concentration of 85 mg dL^{-1} using screen-printed carbon electrode test strips without glucose oxidase [GOD(-)SPCEs]. Uric acid concentrations in the whole blood were *a* 4.6 and *b* 16.3 mg dL^{-1}. The best-fitted equations were *a* $Y=1.512e^{-0.0198X}$ (R^2=0.992) and *b* $Y=2.674e^{-0.0191X}$ (R^2=0.992)

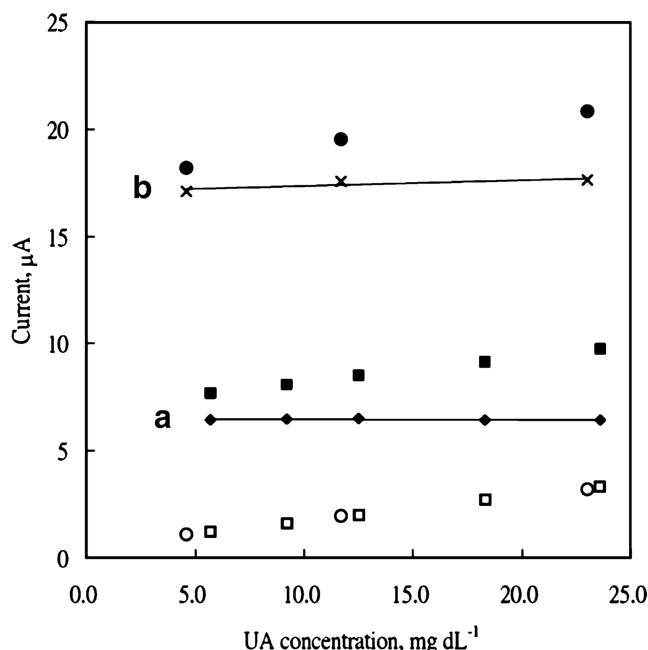

Fig. 5 Effects of spiked uric acid concentrations on the whole blood chronoamperometric current readings. The tenth second $I_{(s+i)}$ and I_i readings were recorded after 20-s sample incubation and ΔI was calculated. *a* Glucose, 93 mg dL^{-1}: $I_{(s+i)}$ (*closed squares*), I_i (*open squares*), ΔI (*diamonds*); best-fitted equation for ΔI, $Y=-0.002X+6.481$. *b* Glucose, 316 mg dL^{-1}: $I_{(s+i)}$ (*closed circles*), I_i (*open circles*), ΔI (*crosses*); best-fitted equation for ΔI, $Y=0.027X+17.089$. The range of all standard deviations is 0.053–0.192. *UA* uric acid

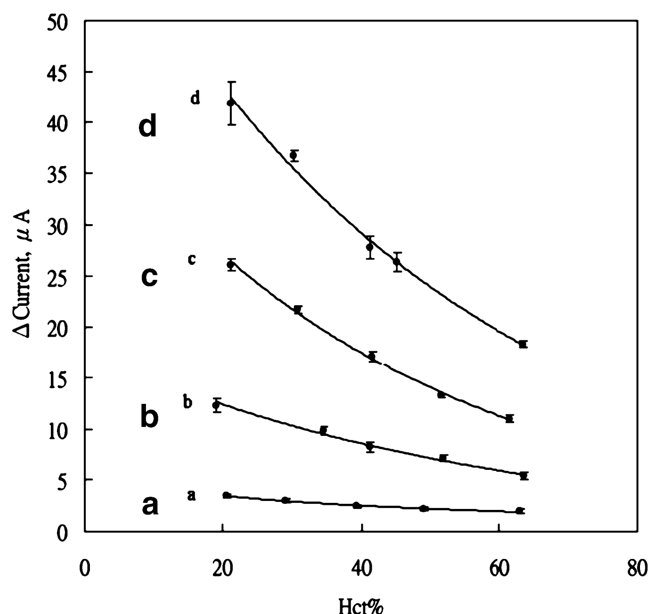

Fig. 6 Whole blood chronoamperometric ΔI values vs. Hct% at various glucose concentrations. The tenth second $I_{(s+i)}$ and I_i readings were recorded after 20-s sample incubation and ΔI was calculated. Glucose concentrations *a* 75.7, *b* 170.4, *c* 318.0 and *d* 462.0. Best-fitted equations *a* $Y=4.419e^{-0.0133X}$ ($R^2=0.909$), *b* $Y=17.958e^{-0.0184X}$ ($R^2=0.990$), *c* $Y=42.023e^{-0.0219X}$ ($R^2=0.998$) and *d* $Y=64.696e^{-0.0199X}$ ($R^2=0.995$)

current (i.e., $I_{(s+i)}$ and I_i) plots against spiked uric acid concentrations in two sets of blood glucose solutions, i.e., 93 mg dL^{-1} (Hct% 46%) and 316 mg dL^{-1} (Hct% 42%). The ΔI values are also plotted in Fig. 5. It is observed that the slopes of both $I_{(s+i)}$ and I_i plots against uric acid concentrations are similar and the slopes of both ΔI plots against uric acid concentrations are close to zero. This implies that after uric acid concentration corrections, the glucose oxidation currents are independent of uric acid concentration, if Hct% values are not very different.

In an attempt to eliminate Hct% interferences, a series of glucose solutions were prepared with various concentrations and Hct% values. The solutions were spiked with various amounts of uric acid. The $I_{(s+i)}$ and I_i readings were recorded in the same way as those of Fig. 5, and corresponding ΔI values were calculated. Figure 6 shows plots of ΔI values against Hct% values at four blood glucose concentrations. From these plots, it is observed that for a glucose concentration of 75.7 mg dL^{-1}, the current readings are similar in the Hct% range tested. However, for glucose concentrations of 170.4, 318.0 and 462.0 mg dL^{-1}, the current readings are increasingly higher at lower Hct% values, as expected (vide supra). Additional efforts to eliminate Hct% interferences are described later.

Practical applications

Previously a correlation for the determination of whole blood glucose between the more convenient YSI method and that of the more accurate photometric hexokinase method [32] was established in our laboratory using 96 plasma samples. The correlation equation was $[\text{glucose}]_{\text{YSI}}=1.064[\text{glucose}]_{\text{hexokinase}}-12.63$, with $R^2=0.9781$. For convenience, the YSI method was used for the practical applications discussed in this section.

The above-discussed methods to eliminate biochemical and Hct% interferences were applied to 37 preclinical human samples (16 from men and 21 from women). The experimentally obtained $I_{(s+i)}$ and I_i values as well as calculated ΔI values are listed in Table 2. The data in column 2 (i.e., $[\text{glucose}]_{\text{YSI}}$), column 3 (i.e., $[\text{UA}]_{\text{optic}}$) and column 4 (i.e., $[\text{Hct}]_{\text{KUBOTA}}$) of Table 2 are the glucose and uric acid concentrations and the Hct% values determined directly using the methods of the YSI 2300 STAT Plus, the Roche UA plus kit/Meterteck SP870 spectrophotometer and the KUBOTA 3110 centrifuge, respectively. The uncorrected glucose concentrations in column 6 (i.e., $[\text{glucose}]_{\text{uncorr}}$) were obtained using the $I_{(s+i)}$ values (column 5) and a calibration curve was established between the data in columns 2 and 5 [33–35], i.e., $I_{(s+i)}=0.0613[\text{glucose}]_{\text{uncorr}}+0.0168$. For each sample, a percentage bias value is calculated using the corresponding values in columns 2 and 6 and the sum of the absolute percentage bias values for all samples is 204.2. Similarly, the

Table 2 Comparisons of the $I_{(s+i)}$ and ΔI readings as well as uncorrected and corrected glucose concentrations for 37 preclinical human samples

Sample	Reference methods			GOD(+)-SPCEs only			GOD(+)-SPCEs and GOD(−)- SPCEs		
	$[\text{Glucose}]_{YSI}$	$[\text{UA}]_{optic}$	$[\text{Hct}]_{KUBOTA}$	$I_{(s+i)}=0.0613\times[\text{glucose}]_{uncorr}+0.0168$			$\Delta I=0.0601\times[\text{glucose}]_{uacorr}-0.4421$		
	(mg dL^{-1})	(mg dL^{-1})	(%)	$I_{(s+i)}$	$[\text{Glucose}]_{uncorr}$	|Bias%|	I	$[\text{Glucose}]_{uacorr}$	|Bias%|
1	352.0	7.1	39.0	21.2	345.4	1.9	20.5	348.7	0.9
2	330.0	9.1	44.0	21.3	347.5	5.3	20.1	341.1	3.4
3	329.0	2.5	43.0	20.5	333.9	1.5	20.0	340.0	3.4
4	298.0	4.0	43.0	18.0	292.6	1.8	17.0	290.1	2.6
5	270.0	2.4	45.0	14.1	230.1	14.8	13.7	234.7	13.1
6	258.0	4.4	33.0	18.0	292.6	13.4	17.0	290.1	12.5
7	257.0	6.7	44.0	15.0	243.7	5.2	14.3	244.7	4.8
8	248.0	5.4	38.0	15.4	250.2	0.9	14.7	252.7	1.9
9	248.0	8.4	43.0	15.5	251.9	1.6	14.7	251.3	1.3
10	232.0	5.0	43.5	13.1	213.3	8.1	12.5	214.7	7.5
11	200.0	4.0	31.0	15.0	244.8	22.4	13.7	235.8	17.9
12	175.0	3.6	45.5	9.5	154.0	12.0	8.8	154.0	12.0
13	175.0	4.6	37.0	11.6	188.8	7.9	10.7	185.9	6.2
14	134.0	4.0	39.0	6.5	106.2	20.8	6.0	106.6	20.4
Subsum						117.4			107.9
15	112.0	4.8	43.0	6.5	106.5	5.0	6.2	109.8	2.0
17	102.0	4.4	45.0	6.4	104.4	2.3	5.8	103.6	1.6
18	101.9	3.8	42.0	6.2	101.2	0.7	5.7	102.4	0.5
19	98.7	5.3	41.0	6.5	105.6	7.0	6.0	106.4	7.8
20	98.5	3.7	38.0	6.4	104.2	5.8	5.5	99.5	1.0
21	95.6	4.3	38.0	6.2	101.4	6.1	5.7	102.3	7.0
22	92.5	5.6	44.0	6.0	98.0	6.0	5.5	98.5	6.4
23	89.9	5.2	40.0	5.4	87.6	2.5	5.0	90.5	0.7
24	89.6	7.1	44.0	5.7	92.3	3.0	5.1	91.9	2.6
25	89.4	6.4	51.0	5.0	81.8	8.5	4.8	87.0	2.6
26	87.0	3.9	41.0	5.4	88.1	1.3	5.0	90.2	3.7
27	86.5	3.4	36.0	5.4	87.7	1.4	4.9	89.6	3.6
28	85.0	4.1	41.0	5.1	82.8	2.6	4.5	81.7	3.9
29	84.8	5.4	45.0	5.3	86.9	2.5	4.7	85.4	0.7
30	82.2	9.0	47.0	5.3	86.2	4.9	4.6	83.8	2.0
31	81.0	6.6	41.0	5.1	83.3	2.9	4.6	83.9	3.5
32	80.2	4.7	42.0	4.7	76.4	4.7	4.1	75.8	5.4
33	78.0	3.7	42.0	4.5	73.0	6.4	4.0	74.4	4.6
34	72.3	3.2	42.0	4.5	73.8	2.0	4.0	74.0	2.4
35	70.5	6.2	51.0	4.5	72.4	2.6	3.9	72.0	2.1
36	69.0	2.7	33.0	4.4	71.4	3.5	3.8	70.6	2.3
37	67.0	4.0	39.0	4.1	66.5	0.8	3.5	65.0	3.0
Subsum						86.8			73.8
Sum						204.2			181.7

GOD(−)-SPCEs screen-printed carbon electrode test strips without glucose oxidase, *$[\text{glucose}]_{YSI}$* glucose concentration determined using the YSI 2300 STAT Plus, *$[\text{UA}]_{optic}$* uric acid concentration determined using the Roche UA plus kit/Meterteck SP870, *$[\text{Hct}]_{KUBOTA}$* hematocrit ratio determined using the KUBOTA 3110 centrifuge, *$[\text{glucose}]_{uncorr}$* uncorrected glucose concentration, *$[\text{glucose}]_{uacorr}$* uric acid corrected glucose concentration

uric acid corrected glucose concentrations in column 9 (i.e., $[\text{glucose}]_{uacorr}$) were obtained using the ΔI values (column 8) and a calibration curve was established between the data in columns 2 and 8, i.e., $\Delta I=0.0601[\text{glucose}]_{uacorr}-0.4421$. The sum of the absolute percentage bias values for $[\text{glucose}]_{uacorr}$ is reduced to 181.7, indicating some improvement in accuracy. If the 37 samples are divided into a high-glucose group (the first 14 samples; more than 120 mg dL^{-1} glucose) and a normal-glucose group (the last 23 samples; less than 120 mg dL^{-1} glucose), it is found that the percentage bias is largely contributed by the samples from the high-glucose group, i.e., samples from the higher-risk patients.

Compared with Hct% interferences, uric acid/biochemical interferences and others such as sample/strip back-

ground interferences are relatively lower and the use of ΔI values would eliminate most of these minor interferences. However, glucose measurements using SPCE test strips are also interfered with by Hct%, particularly for samples with high glucose concentration and low Hct% (Figs. 3, 6). For example, although both samples 27 and 36 have abnormally low uric acid concentrations (i.e., outside the 3.5– 8.5 mg dL^{-1} range) and Hct% values (i.e., outside the 36–51% range), the percentage bias values are relatively low because they were in the low glucose concentration group. On the other hand, samples 6 (Hct% 33%) and 11 (Hct% 31%) with normal uric acid concentrations but abnormally low Hct% values tend to have much greater percentage bias values.

To further improve the accuracy of the glucose measurements, we propose the following method to reduce the Hct% interferences, using sample 11 as one example. The initially determined glucose concentration of sample 11 (i.e., 244.8 mg dL^{-1}) fell between 170.4 and 318.0 mg dL^{-1}; therefore, the exponential decay equations represented by curves b and c in Fig. 3 are applicable to calculate the two current values, i.e., 11.5 μA (using the equation represented by curve b) and 22.6 μA (using equation represented by c) at Hct%=31%. Using the determined $I_{(s+i)}$ value of 15.0 μA in Table 2 (column 5, sample 11) and by interpolation, we calculated $[glucose]_{Hct\text{-}corr}$ of sample 11 to be 217.71 mg dL^{-1} for the $I_{(s+i)}$ system. The percentage bias is reduced to 8.8 and is lower than the corresponding value, 22.4 (Table 2, column 7, sample 11), without any corrections. If the ΔI value of 13.7 μA for sample 11 and the equations represented by curves b and c in Fig. 6 are used, the value of $[glucose]_{Hct\text{-}corr.}$ in the ΔI system is 217.69 mg dL^{-1}. The percentage bias is also reduced to 8.8 and is again lower than the corresponding value, 17.9 (Table 2, column 10, sample 11), with only uric acid/biochemical correction (cf. Table S1 for sample calculations). Similarly, the respective percentage bias values of sample 6 after Hct% corrections are 3.1 and 4.9, and are lower than the respective values, 13.4 (Table 2, column 7, sample 6) and 12.5 (Table 2, column 10, sample 6). The differences of the percentage bias values of Hct% corrected values and that of Hct% uncorrected values for other samples with normal Hct% are not as large as for samples 6 and 11. Thus, *hematocrit corrections are especially important for samples with abnormal hematocrit values*.

In “Initial elimination of interferences of uric acid/biochemicals and Hct%,” only glucose solutions with spiked uric acid and adjusted Hct% were examined to illustrate the interference effects, i.e., the sample background complexity of blood glucose determination was somewhat simplified. However, the results of the simplified exponential decay models were still quite useful in practical applications, because by only determining the uric acid concentrations and Hct% values for the samples in Table 2, we could make significant correction improvements for the glucose determinations. From the results discussed in this section, Hct% corrections have been found to be more important for low Hct% samples. Corrections with uric acid/biochemicals interferences, although evident, may not be as drastic as those with Hct% corrections. The present approach should also be applicable to other analytes that can be determined by SPCE electrochemical techniques.

Acknowledgements The authors wish to thank the National Science Council of the Republic of China (Taiwan) for financial support (grant number NSC-95-2113-M-009-025) of this work. Thanks are also due APEX Biotechnology Corporation for donating SPCE test strips and blood samples.

References

1. Clark LC, Lyons C (1962) Ann N Y Acad Sci 102:29–45
2. Chen T, Friedman K, Lei I, Heller A (2002) Anal Chem 72:3757–3763
3. Brahim S, Narinesingh D, Guiseppi-Elie A (2002) Biosens Bioelectron 17:973–981
4. Fogh-Andersen N, D'Orazio P (1998) Clin Chem 44:655–659
5. Turner APF, Chen B, Piletsky SA (1999) Clin Chem 45:1596–1601
6. Quan X, Wightman M (1997) Anal Chim Acta 341:43–51
7. Cass AEG, Davis G, Francis GD, Hill HAO, Aston WJ (1984) Anal Chem 56:667–671
8. Green MJ, Lias RJ (1989) US Patent 4,876,205
9. Kuhn LS, Ochs ML, Morris GC (1995) US Patent 5,385,864
10. Kuo CS, Hwu CM, Lin YH, Huang YH, Kao WY, Weih MJ, Hsiao LC, Kwok CF, Ho LT (2002) J Clin Lab Anal 16:109–114
11. Shin MC, Yoon HC, Kim HS (1996) Anal Chim Acta 329:223–230
12. Maidan R, Heller A (1991) J Am Chem Soc 113:9003–9004
13. Xie H, Tan XL, Gao Z (2005) Front Biosci 10:1797–1801
14. Vaidya R, Wilkins E (1994) Med Eng Phys 16:416–421
15. Wang J, Liu J, Chen L, Lu F (1994) Anal Chem 66:3600–3603
16. Wang J, Lu F. Angnes L, Liu J, Sakslund H, Chen Q, Pedrero M, Chen L (1995) Anal Chim Acta 305:3–7
17. Lin Y, Lu F, Tu Y, Ren Z (2004) Nano Lett 4:191–195
18. Schaffar BPH, Kontschieder H, Ritter C, Berger H (1999) Clin Chem 45:1678–1679
19. Carter NF, Chambers GR, Hughes GJ, Scott S, Sanghera GS, Watkin JL (1997) US Patent 5,628,890
20. American Diabetes Association (1987) Diabetes Care 10:95–9
21. American Diabetes Association (1994) Diabetes Care 17:81–6
22. American Diabetes Association (1996) Diabetes Care 19:S62–66
23. Chance JJ, Li DJ (1999) Am J Clin Pathol 111:547–56
24. Yuoh C, Elghetany MT, Petersen JR, Mohammad A, Okorodudu AO (2001) Clin Chim Acta 307:119–123
25. Huang YC, Shen TYS (2005) US Patent 6,923,894
26. American Diabetes Association (2006) Diabetes Care 29:S3–S42
27. National Committee for Clinical Laboratory Standards (2002) EP7-A: interference testing in clinical chemistry; approved guideline. National Committee for Clinical Laboratory Standards, Wayne
28. Burtis CA, Ashwood ER (1999) Tietz textbook of clinical chemistry, 3rd edn. Saunders, Philidelphia

29. Ma JZ, Ebben J, Xia H, Collins AJ (1999) J Am Soc Nephrol 10:610–619
30. Anatole Besarab A, Bolton WK, Browne JK, Egrie JC, Nissenson AR, Okamoto DM, Schwab SJ, Goodkin DA (1998) N Engl J Med 339:2023–2024
31. Minetti L (1997) J Nephrol 10:117–119
32. Schmidt FH (1961) Klin Wochenschr 39:1244–1247
33. Collison ME, Stout PJ, Glushko TS, Pokela KN, Mullins-Hirte DJ, Racchini JR, Walter MA, Mecca SP, Rundquist J, Allen JJ, Hilgers ME, Hoegh TB (1999) Clin Chem 45:1665–1673
34. Tieszen KL, New JP (2003) Diabet Med 20:325–328
35. Kovatchev BP, Clarke WL, Breton M, Brayman K, McCall A (2005) Diabetes Technol Ther 7:849–862