

On-chip Hematocrit Correction for Whole Blood Glucose Amperometric Sensing Strip Using a Post-Measurement Potential Step

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Received: 27 February 2017 / Accepted: 19 April 2017 / Published: 12 May 2017

The accuracy of a point-of-care whole blood glucose sensor can be considerably influenced by hematocrit; therefore, an on-chip Hct correction protocol was developed and incorporated to meet the clinical demands. An impulse of DC 0.3 V was first applied for detecting blood glucose amperometrically, followed by an additional 3.2 V DC impulse for Hct estimation. Without Hct correction, the results of blood glucose tests were negatively correlated with Hct levels, and the glucose values were overestimated by 38.8% and underestimated by 43.8% when the Hct levels were 9% and 70%, respectively. On the other hand, all the mean biases of blood glucose tests with 5 designed Hct levels (9%, 25%, 43%, 55% and 70%) were less than $\pm 10\%$ by utilizing the proposed Hct correction method. The method was insensitive to several representative interfering chemicals.

Keywords: Hematocrit compensation, post-measurement potential step, blood glucose sensor, strip

1. INTRODUCTION

According to the World Health Organization and the International Diabetes Federation, the worldwide prevalence of diabetes is projected to double over the next couple of decades, from 342 million people in 2005 to 700 million people in 2030 [1]. To date, although there are practically no methods to cure or prevent diabetes, optimal glycemic control and proper medication can enhance treatment efficiency, mitigate the symptoms, and diminish the complications (e.g. blindness, heart disease, kidney disease, and nerve damage). Therefore, self-monitoring of blood glucose levels is strongly recommended by the American Diabetes Association for diabetes patient [2], a quick and accurate blood glucose monitoring system is highly demanded to respond immediately to blood glucose changes of patients.

Nowadays, point-of-care testing (POCT) blood glucose meters are widely used in homes and hospitals due to its effectiveness, relative inexpensiveness, simplicity and convenience in operation. However, the accuracy of commercially available POCT blood glucose meters are easily interfered by inappropriate blood sample loading volume, oxygen tension, environment temperature and humidity, which will lead to inaccurate readings of glucose values [3, 4]. Besides, it has also been reported that hematocrit (Hct) level is a more significant factor to affect the accuracy of blood glucose measurement [5], the development of an appropriate correction method for the Hct is therefore very important for POCT purpose.

Hct is the volume ratio of red blood cells to whole blood, with a range between 30-50% in healthy reference population [6]. However, the levels in anemic patients, pregnant women, and newborns may differ widely from the normal range [7, 8]. For POCT analytical devices working on whole blood, abnormal Hct levels may severely influence the transparency or the diffusion kinetics of analytes caused by erythrocytes, which all lead to incorrect analytical results [9, 10]. For electrochemical POCT blood glucose sensing strips [11], the most popular POCT devices for diabetes, the sensor responses showed significant negative deviations especially for the samples with higher Hct [12]. To obtain a more accurate data, the blood cells have to be removed prior to the measurement, which is laborious or required a complicated sensor design for the pre-treatment [13-18].

In our previous study, a simple Hct measuring mechanism was developed on a ferricyanide-coated screen-printed carbon electrode. The Hct levels were estimated in only 0.8 s by imposing a 3.0 V DC voltage on the electrode [19]. The current objective of this study is to incorporate the aforementioned mechanism to recalibrate a POCT whole blood glucose sensor for obtaining HCT-insensitive results. The results show no incompatibility between the glucose-measuring and HCT-measuring electrochemical sequences, and the true analytical data can be retrieved in a quick and convenient manner.

2. EXPERIMENTAL

2.1 Materials

Glucose dehydrogenase (GDH, EC 1.1.1.47 from *Pseudomonas* sp.) was provided by DELBio Inc. Ferricyanide, Triton X-100, HPMC (hydroxypropyl methyl cellulose, M.W.10000), PEG (poly ethylene glycol, M.W.10000), acetaminophen, ascorbate, dopamine, gentisic acid, ibuprofen, levo-Dopa, maltose, methyl-Dopa, sodium salicylate, tetracycline, tolazamide, tolbutamide, sodium chloride, uric acid, bilirubin, creatinine, cholesterol, urea and hemoglobin were purchased from Sigma Aldrich (St. Louis, MO, USA). All the chemicals used were of analytical grade. Double distilled deionized water was used to prepare all the solutions. Phosphate buffer solution (PBS) of pH 7.0 was prepared by mixing Na_2HPO_4 (0.1 M) and NaH_2PO_4 (0.1 M) aqueous solutions.

2.2 Blood sample preparation

Venous, heparinized blood was freshly drawn from a single volunteer and was centrifuged (2,500 rpm \times 15 min) to separate plasma and red blood cells. Five representative Hct levels of 9%, 25%, 43%, 55%, and 70% were reconstituted in different tubes by mixing plasma and red blood cells. The Hct values of the “adjusted” blood samples were determined using micro-capillary centrifugation (Model MB, Thermo IEC, Needham Heights, MA, USA) at 12,000 rpm for 5 min. Each Hct level with different target glucose concentrations were prepared by spiking 50000 mg/dL of glucose stock solution into the artificial blood samples. The oxygen level of blood samples were adjusted by treating with pure nitrogen to 70 ± 5 mmHg and confirmed by a blood gas analyzer system (ABL5, Radiometer, Copenhagen, Denmark). Blood samples were analyzed within 15 min after mixing to avoid significant glycolysis. The measurement was performed at 23 ± 5 °C according to ISO 15197 standard. Temperature was maintained within a range of ± 2 °C.

2.3 Preparation of glucose sensing strip

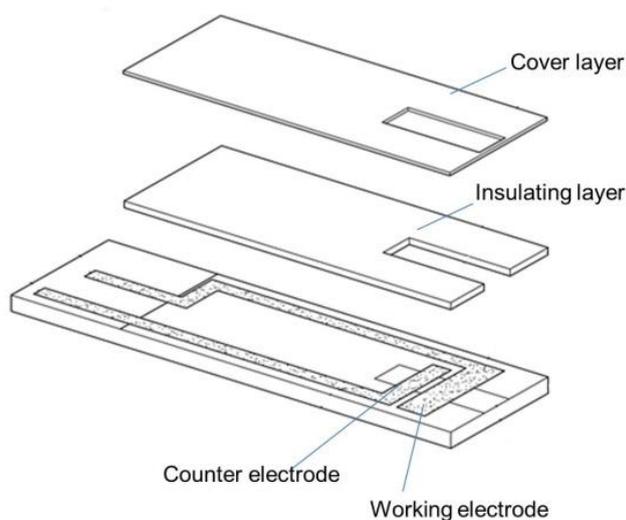


Figure 1. Architecture of the home-made glucose sensing strip.

Screen-printed carbon electrode (SPCE) was used in this study. Figure 1 shows the structure of the test strip with carbon paste (ca. 12 μ m thick) printed on a PET substrate to serve as the working and counter electrode. The reaction area was defined and restricted by the insulating layer. Enzyme solution was prepared by dissolving ferricyanide (0.1M), 1.5% HPMC, 0.15% PEG, 0.05% Triton X-100 and 4U/ μ L of GDH in the PBS solution. The enzyme was immobilized by applying 1.2 μ L of the enzyme solution on the reaction area and then air-dried at 40 °C for 20 mins. After drying, a fixed volume of reaction chamber was formed by adding the cover layer to form a mini-channel for sucking up a defined amount of blood sample by capillary action.

2.4 Measurements

All electrochemical measurements were performed at $23 \pm 2 \text{ }^\circ\text{C}$, and the experimental solutions were all purged with pure nitrogen to avoid interference from oxygen. The amperometric measurement was performed with CHI 6141D potentiostat (CH Instruments, USA). Parts of the samples were carefully centrifuged to obtain plasma and the plasma glucose concentrations were determined on the YSI 2300 reference device (Yellow Springs Instruments Inc., Yellow Springs, OH, USA).

3. RESULTS AND DISCUSSION

3.1 Effect of Hct variation on blood glucose measurement

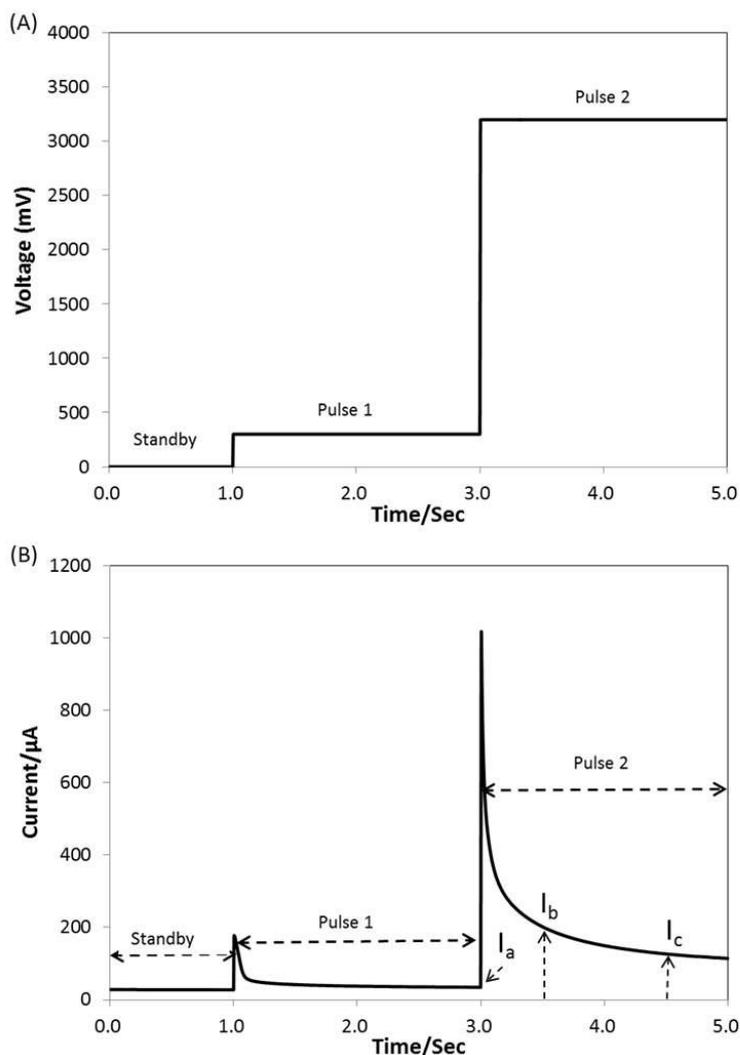


Figure 2. (A) Voltage waveform applied to the blood glucose sensing strip during the 5 s test time. (B) An example of a current/time output following the application of the voltage waveform of (A).

To incorporate the previous electrochemical Hct assay using ferricyanide, the biosensing approach immobilized glucose dehydrogenase (GDH) that can also use ferricyanide as the redox mediator [20]. The immobilized GDH catalyzed the electron transfer between the redox couple of glucose / ferricyanide, and the reduced ferrocyanide was then oxidized to generate an amperometric current proportional to glucose concentration in a pseudo-first order manner [21].

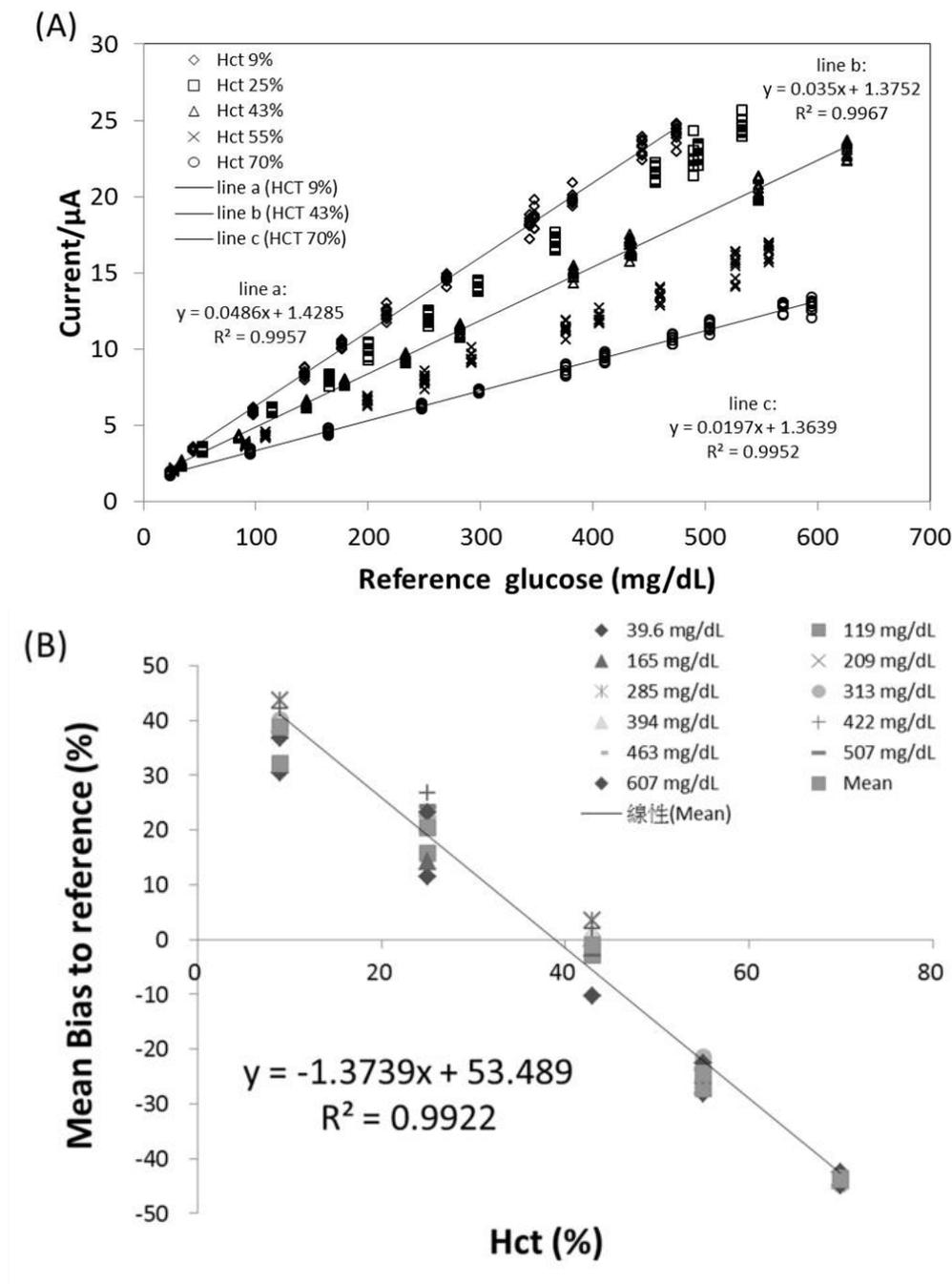


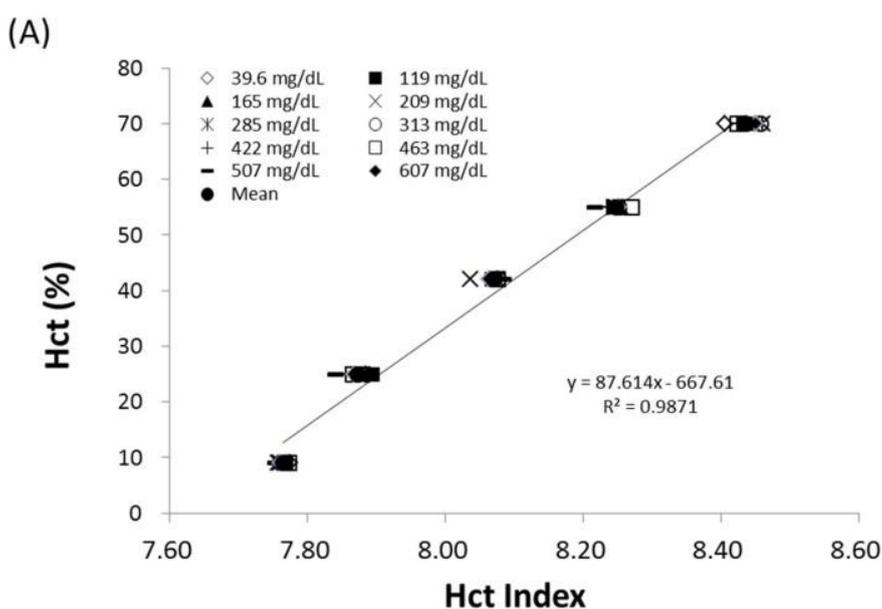
Figure 3. (A) The relationship between the monitored amperometric current response and the glucose concentration with different Hct level (9%, 25%, 43%, 55% and 70%) by simple chronoamperometry. (B) The mean bias of glucose detection to referenced value with different Hct levels by simple chronoamperometry.

As in Figure 2A, 2s of 0.3V pulse (Pulse 1) was imposed to oxidize the ferrocyanide after the standby period, the oxidation current after the pulse (I_a) was utilized for glucose detection (Figure 2B). For Hct correction, the second higher pulse (Pulse 2 in Figure 2A, 3.2V×2s) was applied to break down the red blood cell membrane and estimate the Hct using both I_b (current at 3.5th second) and I_c (current at 4.5th second) in Figure 2B. I_b was correlated with Hct levels and I_c can serve as the background level of each strip for data normalization. As a consequence, the logarithmic value of I_b/I_c value can be used to estimate Hct value [19].

The results of blood glucose detection with 5 designed Hct levels (9%, 25%, 43%, 55% and 70%) were shown in Fig. 3A, the correlation coefficient was calculated to be only 0.6742 if the influence of Hct was not considered. On the other hand, with fixed Hct levels (e.g. 9%, 43%, 70%), the current responses were highly correlated with blood glucose concentrations, which means that accurate blood glucose measurement could be achieved only after knowing the sample Hct value. In this case, the sensitivity of blood glucose detection (the slope of the linear regression on Figure 3A) gradually decreased when Hct level was elevated due to the mass transfer hindrance by erythrocytes [22].

Figure 3B indicates the mean bias of glucose detection with Hct levels between 9% - 70% (the reference calibration curve for glucose detection was constructed with Hct level of 43%), an obvious negative correlation was observed. The glucose values were overestimated by 20.4% and 38.8% when the Hct levels were set as 25% and 9%, respectively. Contrarily, the glucose values were underestimated by 24.4% and 43.8% when the Hct levels were set as 55% and 70%, respectively. These phenomena not only implied that the conventional chronoamperometry for blood glucose measurements were highly Hct dependent ($R^2 = 0.9922$) but also suggested an alternative way to remove the effect of Hct variation on glucose measurement.

3.2 Effect of Hct compensation on blood glucose measurement



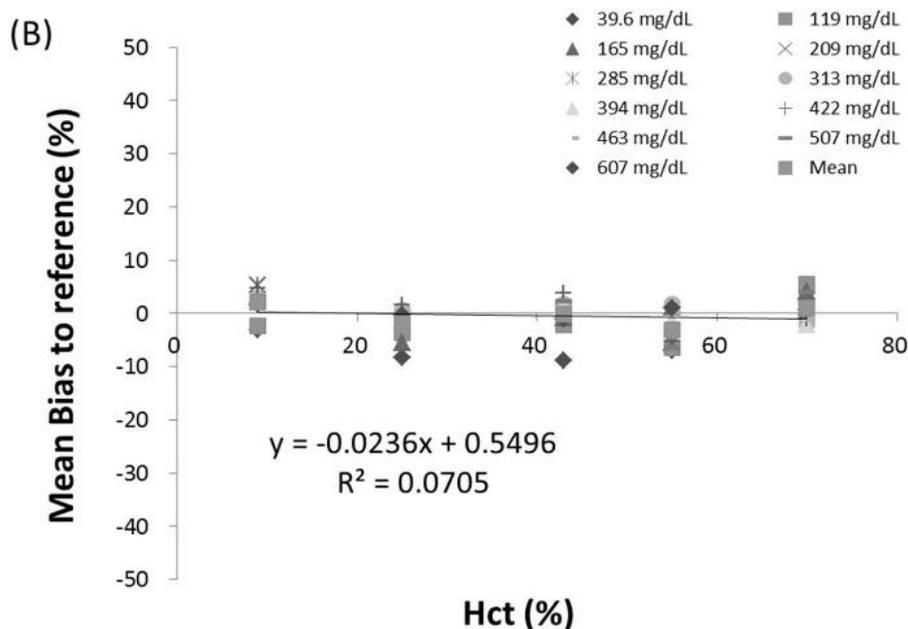


Figure 4. (A) The relationship between the calculated Hct index from I_b and I_c and the designated Hct level (9%, 25%, 43%, 55% and 70%). (B) The mean bias of glucose detection to referenced value with different Hct levels by Hct correction method.

The accuracy of Hct index calculated from the amperometric current responses of I_b and I_c (refer to Fig. 2B) on the home-made glucose sensing strip was shown in Fig. 4A, the analytical results for 5 designed Hct levels (9%, 25%, 43%, 55% and 70%) were glucose independent and correlated well as expected. Based on the relationship between Hct level and the mean bias of glucose detection (i.e. the regressive equation displayed in Fig. 3B), the effect of Hct variation on blood glucose measurement could be easily compensated with Hct levels. With Hct compensation, the mean bias of blood glucose detection showed no dependency to Hct level (Fig. 4B).

According to the ISO 15197: 2013 [23], the mean bias of blood glucose tests have to be in the range of $\pm 10\%$ for POCT blood glucose meters. In the present study, all the mean biases of blood glucose measurements with 5 designed Hct levels were less than $\pm 10\%$ by utilizing the proposed Hct correction method.

3.3 Interferences Evaluation

Because of the proposed additional potential step for hematocrit measurement, some other co-existing electrochemically active interferences in whole blood may alter the precision of the glucose measurement.

In Table 1, 19 potential therapeutic or physiological interferences were selected and investigated according to both ISO 15197: 2013 and CLSI EP7-A2 statements [24]. As shown in Fig. 5, no substance caused significant interference (all the mean biases are less than $\pm 10\%$) either under normal blood glucose level or hyperglycemia.

Table 1. Representative interferences in glucose measurement: therapeutic or physiological levels and test concentrations.

Item	Interferent	Levels [a]	Test [b]
Exogenous substance			
1	Acetaminophen	1~3 mg/dL	30 µg/ml
2	Ascorbic Acid	0.4~2 mg/dL	6.0 mg/dL
3	Dopamine	0.03 mg/dL	0.09 mg/dL
4	Gentisic Acid	0.2~0.6 mg/dL	1.8 mg/dL
5	Ibuprofen	1~7 mg/dL	50.0 mg/dL
6	Levo-Dopa	0.3 mg/dL	3.0 mg/dL
7	Maltose	99~120 mg/dL	1000 mg/dL
8	Methyl-DOPA	0.1~0.75 mg/dL	1.5 mg/dL
9	Sodium Salicylate	10~30 mg/dL	70 mg/dL
10	Tetracycline	0.2~0.5 mg/dL	1.5 mg/dL
11	Tolazamide	1.86~2.49 mg/dL	6.0 mg/mL
12	Tolbutamide	5.4~10.8 mg/dL	64 mg/dL
13	Sodium chloride	135~145 mmol/L	1050 mg/dL
Endogenous substance			
14	Uric acid	2.5~8 mg/dL	23.5 mg/dL
15	Bilirubin	0.3~1.2 mg/dL	40 mg/dL
16	Creatinine	0.6~1.3 mg/dL	5.0 mg/dL
17	Cholesterol	114~201 mg/dL	500 mg/dL
18	Urea	6.6~85.8 mg/dL	300 mg/dL
19	Hemoglobin	100~200 mg/dL	800 mg/dL

[a] therapeutic or physiological. [b] Test concentrations

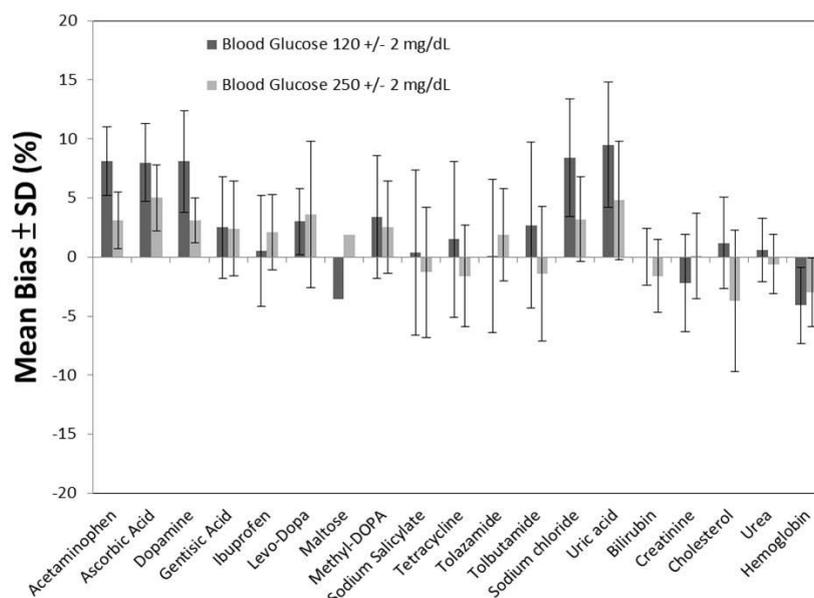


Figure 5. The interference tests of 19 chemicals on blood glucose measurements (mean ± SD, n =10) under normal blood glucose level (120 ± 2 mg/dL) or hyperglycemia (250 ± 2 mg/dL).

4. CONCLUSION

The previous rapid on-chip electrochemical Hct assay was successfully incorporated into an amperometric glucose sensing strip for post-measurement Hct correction, and the accuracy for blood glucose measurement was extensively improved especially for the samples with abnormally high or low Hct levels. With the proposed Hct compensation mechanism, the mean bias of blood glucose measurements was less than $\pm 10\%$ to meet the requirement of ISO 15197: 2013. Moreover, all the potential interferences in blood glucose tests showed insignificant interference in the present study. These results revealed the versatility and compatibility of the Hct measuring mechanism in terms of clinical and POCT applications.

ACKNOWLEDGMENT

The support from DELBio Inc. of Taiwan is gratefully acknowledged.

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