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(54) Title: SALIVA GLUCOSE MEASUREMENT DEVICES AND METHODS

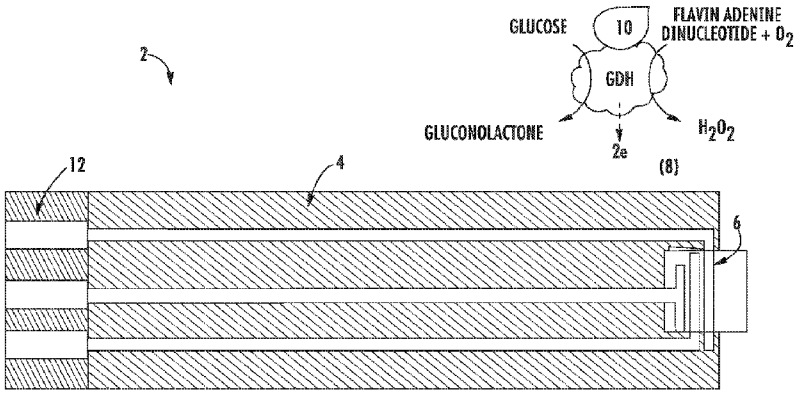


FIG. 12

(57) Abstract: Devices and methods capable of detecting glucose in saliva (FIG. 12). The devices feature a sensor having a substrate containing electrodes and one or more reagents on the electrodes. A detection device is operably coupled with the sensor to detect glucose based on measurement of an electrical parameter when electricity is applied to the electrodes.

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SALIVA GLUCOSE MEASUREMENT DEVICES AND METHODS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/288,747 filed on January 29, 2016.

FIELD OF THE INVENTION

[0002] This disclosure relates to devices and methods of measuring glucose in salvia.

BACKGROUND OF THE INVENTION

[0003] Blood glucose levels commonly are assessed using a blood sample. It is desirable to utilize less- or non-invasively obtained fluid samples for assessing blood glucose levels.

SUMMARY OF THE INVENTION

[0004] Devices and methods are disclosed that are capable of detecting glucose in saliva as a surrogate for blood glucose levels. The devices feature a sensor having a substrate containing electrodes and one or more reagents on the electrodes. A detection device is operably coupled with the sensor to detect glucose based on measurement of an electrical parameter when electricity is applied to the electrodes.

[0005] In the disclosure that follows, the diagnostic relationship between blood glucose and saliva glucose was determined in order to create a non-invasive technology, which can be utilized, for example, by diabetics to control their disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] Fig. 1 depicts a glucose sensor for tear fluid.

[0007] Fig. 2 depicts data for tear fluid measurement of glucose.

[0008] Fig. 3 depicts comparative data for blood versus tear glucose detection.

[0009] Fig. 4A shows multiple measurements taken on a subject before OGTT and after while monitoring blood and saliva glucose simultaneously.

[0010] Fig. 4B depicts a Clarke Error grid of the resulting study (from the data in 4A) showing a correlation between saliva and self-monitored blood glucose in a number of subjects. Most data is found in region A and B (safe regions).

[0011] Figure 5 shows the average lag time between the peaks of SMBG level and saliva glucose current. There are two outliers, subjects E and F. Although subject F has a relatively short lag time, the device failure for subject F makes the time inaccurate. Some device failed while gathering subject E and F's data which skewed the lag time. Subject E and F are thus considered outliers are excluded from the analysis. After avoiding those errors, we notice that the following subjects, H, I and J, have close values of lag time. The average lag time of all 10 subject is 24 minutes. The average lag time without outliers, E and F, is 15 minutes.

[0012] Figure 6. The graphs at Figure 6 show the change in blood-glucose and saliva-glucose over time after three hours of oral glucose challenge test. The graphs suggest that saliva glucose correlates with blood glucose over time. Possible sources of variance include: individual variation in mouth rinsing, Bubbles and viscosity of saliva samples, and manufacturing variance (hand-made devices).

[0013] Figure 7. Clark Error Grid: The Clark Error grid helped to map the data points in reference to an FDA approved reference device (One Touch Ultra SMBG). From this study, 22.1% of results fell in region A, which is the ideal region indicating an error margin under 20%. 76.6% of results fell in region B, which indicates readings with greater than 20% error but still accurate enough to prevent patient harm. The remaining 1.3% of results fell in category C, which indicated a measurement with a chance of causing unnecessary treatment. No results fell into categories D or E, which would indicate a potentially dangerous failure to detect hypoglycemia or hyperglycemia.

[0014] Figure 8. This schematic representation is of a glucose detection mechanism. A) Collection of saliva by naturally salivating. B) Pipette transfer of the sample onto the SG sensor at which (B1) the glucose is catalyzed by GDH-FAD enzyme the resulting electrons are detected by the sensor under an electron mediator, potassium ferricyanide. C) Data processing, where (C1) the electrical current generated after a set amount of time is recorded into the system

and be matched against a (C2) calibration curve, which then calculates the glucose concentration and display the result on D) a monitor.

[0015] Figure 9. The left portion of Fig. 9 is a CV of the GDH modified sensor in saliva with 50 mg/dL of glucose. The red circle indicates current value observed in Amp-it when 0.35V is applied. The right side shows the calibration curve of the linear relationship between glucose concentrations in saliva (mg/dL) and the current (A). The glucose concentrations tested were 0, 0.5, 1, 2, 4, 10, 20, 100, and 200 mg/dL, each with 3 replications and error bars shown. The linear relationship is characterized as Y = -1.05E-07X - 4.93E-07 with R2 value of 0.99, where Y is the electrical current in A and X is the glucose concentration in mg/dL.

[0016] Figure 10. Figure 10 shows how the saliva glucose tracks blood glucose in a non-diabetic subject using disposable SG sensors. The solid squares and lines represent blood glucose measurements and trends. The hollow circles and dashed lines represent saliva glucose measurements and trends. The time stamps are -10, 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes. The oral glucose challenge was given at t = 0 minutes.

[0017] Figure 11. Figure 11 shows the correlation of SG-BG among all subjects. Data points from faulty sensors and mishandling were removed. The slope is characterized by Y = 0.6808X - 16.608 with an R-square value of 0.78.

[0018] Figure 12. This figure schematically depicts a sensor embodiment and system for detection of glucose in saliva.

DETAILED DESCRIPTION OF THE INVENTION

[0019] Embodiments of a device capable of detecting glucose in saliva are disclosed. In one example as shown in Fig. 12, the sensor device 2 includes a substrate 4 containing electrodes 6 and one or more reagents on the electrodes (collectively shown by arrow 8). For example, the reagents may include glucose dehydrogenase (GDH) and an electron mediator, such as ferricyanide or Flavin adenine dinucleotide in 1X PBS buffer. Preferably, the one or more reagents are in dry form and applied to the electrodes prior to contact with a saliva sample 10.

[0020] A detection device 12 is operably coupled with the sensor to detect glucose based on measurement of an electrical parameter when electricity is applied to the electrodes on the

sensor where the saliva sample resides. For example, the detection device 12 collectively may include a power supply, data processor and programing to record the electrical current generated after a set amount of time into the system and match it against a calibration curve, which then calculates the glucose concentration and displays the result on a monitor. In one embodiement such a system is a commercially available electrochemical analyzer (Model 1230A analyzer from CH Instruments, Inc.) and amperometric-current-over-time (Amp i-t) assay. Of course, a tablet or smart phone may additionally or alternatively be used with or as the monitor of the detection system with appropriate wired or wireless components and settings.

[0021] The sensor device may further include a Nafion (i.e., a sulfonated tetrafluoroethylene based fluoropolymer-copolymer) coating or mesoporous carbon coating on the electrodes to reduce nonspecific binding and/or amplify the signal.

[0022] From the above embodiment, it can readily be appreciated that a method of detecting a blood glucose level using saliva also is disclosed. The method in this embodiment includes contacting a sensor containing electrodes and one or more reagents on the electrodes with saliva on the electrodes. Next, electricity is applied to the electrodes such that a measurement of an electrical parameter indicative of an amount of glucose is produced when electricity is applied.

Examples

[0023] A test was conducted of 20 individuals (10 diabetic, 5 Type 1 Diabetes, 5 Type 2 Diabetes) as follows:

Collected SMGB data and tested saliva using the SG device.

Determine correlation between saliva glucose and blood glucose levels.

Evaluate feasibility of saliva capture method.

Determine typical lag time between SG and BG elevation.

Allow saliva to build up in the mouth for approximately thirty seconds.

Deposit saliva on to a curved metal applicator.

Once devices and software are ready, tilt the applicator so that saliva falls onto the electrode of the device.

All devices contain dry reagent applied onto the electrodes earlier.

Reagents: 1.5 mg of GDH : 1 mL of ferricyanide in 1X PBS buffer solution.

Run amperometric- i-t curve for 30 seconds with an initial voltage of 0.35V and a sensitivity of 1E-5.

Take blood glucose measurement using SMBG.

Take 15g glucose solution orally.

Rinse 3x for 3 seconds each.

Wait 10 minutes.

Repeat steps 1-5.

Then repeat steps 1-5 every 15 minutes up until 60 minutes. After 60 minutes, repeat steps 1-5 every 30 minutes until 180 minutes total have elapsed. Stop the experiment with a total of 10 data points.

[0024] Due to manufacturing inconsistencies, a total of 19 devices had to be discarded for failure to read accurately. There are several factors that cause errors, noises and outliers which affect final results.

- Drinking water:
  - Drinking water during the experiment might affect the final result because it could dilute the concentration of glucose in saliva especially when the time between every test is only 15 minutes.
- Bubbles:
  - When a subject bubbly saliva, not clear, the i-t curves contains number of noises and spikes. (See Fig 1, 2, 3 and 4)
- Dry vs wet reagent:
  - Since swirling the Saliva and the dry reagent together before running the software was not a step in the procedure, the reagent might not be well-mixed with the glucose concentration, Saliva. Therefore not accurate result.

[0025] Using the saliva glucose prototype sensor: Significant correlation between BG and SG has been achieved. The saliva glucose sensor has the potential to predict BG. Average lag time is approximately 15 minutes. In accordance with ISO 15197-2013, 98.7% likely to provide a reading which would not harm a patient.

[0026] This disclosure aims to illustrate the design and development of the first disposable SG sensor employing glucose dehydrogenase flavine-adenine dinucleotide (GDH-FAD) capable of quantify SG levels without any sample preparation. The electrochemical approach is outlined in Figure 8. The sensor employed in this work is a commercially available screen printed sensor, Zensor, which can be acquired from CH-Instrument, Texas. It is composed of a carbon working electrode, a carbon counter electrode, and an Ag/AgCl reference electrode.

[0027] The detection reagent is prepared by mixing 1 mL of 100 mM potassium ferricyanide with 1.5 mg of GDH-FAD enzyme. 100 mM potassium ferricyanide is prepared in pH 7.4 1X Phosphate Buffer Saline (PBS). GDH-FAD is highly specific to glucose and is not reactive to other sugars with the exception of xylose. GDH-FAD also employs a signal-to-noise ratio that is 9 times higher than that of glucose oxidase. It has been reported that GDH-FAD has 25 times more enzymatic activity than glucose oxidase, which permits rapid glucose sensing. Dried sensors are prepared by pipetting 27 uL of the reagent onto the sensing well with uniform coverage of all 3 electrodes. The sensors are then placed in a dehydrator at 30℃ for 25 minutes to dry the reagent completely. The completed sensors are carefully examined for visible defects. Sensors with dried reagents outside the sensing well or incomplete coverage of all 3 electrodes were not used for testing. Completed sensors can be stored at room temperature for up to 12 weeks.

[0028] Completed sensors are then tested against various concentrations of glucose (120 μL sample volume) in saliva using an electrochemical analyzer (1230A CH-instrument) and amperometric-current-over-time (Amp i-t) assay for 30 seconds. Cyclic voltammetry (CV) was first conducted in saliva to determine the potential for Amp i-t assay (Figure 9). After evaluating the signal strength and noise of various potentials using Amp-it, a bias potential of 0.35 V was deemed appropriate as it has the lowest noise. This potential was chosen to test all sensors at varying glucose concentrations. A correlation analysis was then conducted to evaluate current readings at several time stamps (t = 10, 20, 30 s.) It was determined that the electrical current signal at t = 10 is a good representative signal for the sample. A calibration curve, at the 10 second mark, has been constructed and is shown in Figure 9. According to the calibration curve, the dynamic range of the sensor is projected to encompass glucose concentrations spanning

from 1.23 mg/dL to 247.07 mg/dL, which covers the clinically relevant SG range of DM subjects reported in literature.

[0029] A preliminary clinical study of 9 non-DM subjects and 3 type 1 DM subjects age 19-25 years (mean age of 23 +/- 1) was conducted. The study was approved by the Arizona State University Institutional Review Board (IRB) under the identification number of STUDY00002778. All procedures and tests were in compliance with IRB requirements. The sample collection steps are as follows.

[0030] Each subject was asked to rinse their mouth with fresh water 3 times for 3 seconds each time. The subject was then asked to accumulate saliva for 30 seconds, and deposit it onto a sterilized metal lab spatula. Ten seconds later, a 120 μL sample of saliva was then transferred, via a pipet tip, to the saliva glucose sensor pre-connected to the electrochemical analyzer. The saliva sample did not undergo any sample preparation or purification prior to testing. Immediately following the deposition of saliva, amperometric i-t technique was performed at a voltage of 0.35 V. The current readings at t = 10 seconds are used as the representative signal for the SG measurement, and have been utilized to determine the appropriate SG concentration. The entire SG measuring process from sampling to obtaining the corresponding current measurement is approximately 1 minute, which is much faster than those ranging from 10 to 20 minutes. Given the short duration of testing, the potential breakdown of glucose by bacteria can be avoided.

[0031] To avoid potential stress induced on diabetic subjects, glucose tolerance testing was performed only on the 9 non-diabetic subjects by administering a 15 gram glucose shot orally (at t= 0 min). After the subject swallowed the glucose shot, he/she was then instructed to immediately rinse their mouth with fresh water 3 times for 3 seconds each time. The SG and BG were then measured every 15 minutes until t = 60 minutes, then were measured once every 30 minutes until t = 180 minutes using the same procedure described above. One representing subject's SG-BG tracking data is shown in Figure 10.

[0032] All SG values from healthy and DM subjects, excluding data points generated by compromised sensors and linked to traceable human error, are plotted against the SMBG values, shown in Figure 11. The average measured SG range for all subject ranges from 34.4 mg/dL to

76.6 mg/dL corresponding to a BG range from 83 mg/dL to 147 mg/dL which contradicts the SG values ranging from 0.19 - 3.82 mg/dL and the SG values ranging from 4 - 13 mg/dL. This disconnect may be attributed to how rapidly the saliva glucose is measured after sample collection and the prevalence of glycolysis.

[0033] Given the sensor can measure glucose in approximately 25 seconds (15 seconds for sample collection and a 10 second duration before the utilized current readings are collected), it is possible to obtain higher saliva glucose measurements when compared to the 5-20 minute processing times of other techniques. Nevertheless, The SG seems to track BG well with a lag time ranging from -15 minutes to 15 minutes, which is consistent with literature. The lag time can be attributed to individual dietary patterns, lifestyles, and race. A positive correlation between SG and BG is also identified, which is consistent with literature.

[0034] In summary, an easy-to-use, rapid, and disposable SG sensor featuring GDH-FAD and no sample preparation is underway.

[0035] The following claims are not intended to be limited by the embodiments and examples herein.

What is claimed is:

1. A device configured to detect glucose in saliva, comprising:

a sensor having a substrate containing electrodes and one or more glucose-detection reagents on said electrodes; and

a detection device operably coupled with said sensor to detect said glucose based on measurement of an electrical parameter when electricity is applied to said electrodes.

2. The sensor device of claim 1, wherein said one or more reagents comprise glucose dehydrogenase (GDH) and ferricyanide in 1X PBS buffer.

3. The sensor device of claim 1, further including a sulfonated tetrafluoroethylene-based fluoropolymer-copolymer coating or mesoporous carbon coating on one or more of the electrodes.

4. The sensor device of claim 2, wherein said reagents are in dry form.

5. The sensor of claim 1, wherein said one or more reagents comprise glucose dehydrogenase flavine-adenine dinucleotide (GDH-FAD) and ferricyanide in 1X PBS buffer.

6. A method of detecting a blood glucose level using saliva, the method comprising:

contacting a sensor containing electrodes and one or more glucose-detection reagents on said electrodes with saliva; and

energizing said electrodes such that a measurement of an electrical parameter indicative of an amount of glucose in said saliva is produced and measured.

7. The method of claim 6, wherein said one or more reagents comprise glucose dehydrogenase (GDH) and ferricyanide in 1X PBS buffer.

8. The method of claim 6, wherein said one or more reagents comprise glucose dehydrogenase flavine-adenine dinucleotide (GDH-FAD) and ferricyanide in 1X PBS buffer.

9. A method of monitoring a glucose level against a desired range in a patient, comprising:

contacting a sensor containing electrodes and one or more glucose-detection reagents on said electrodes with saliva; and

energizing said electrodes such that a measurement of an electrical parameter indicative of an amount of glucose in said saliva is produced and measured against said desired range.

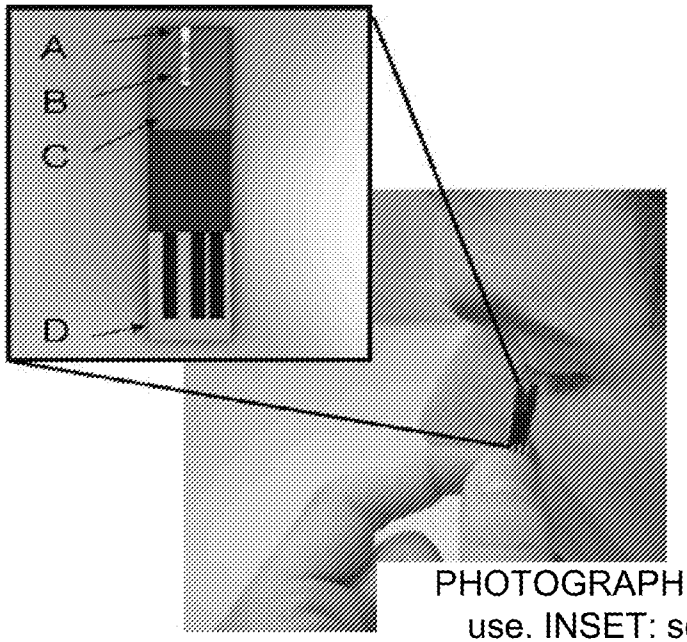
A little background...

Tear Glucose

User touches device to conjunctiva, collecting tear fluid.

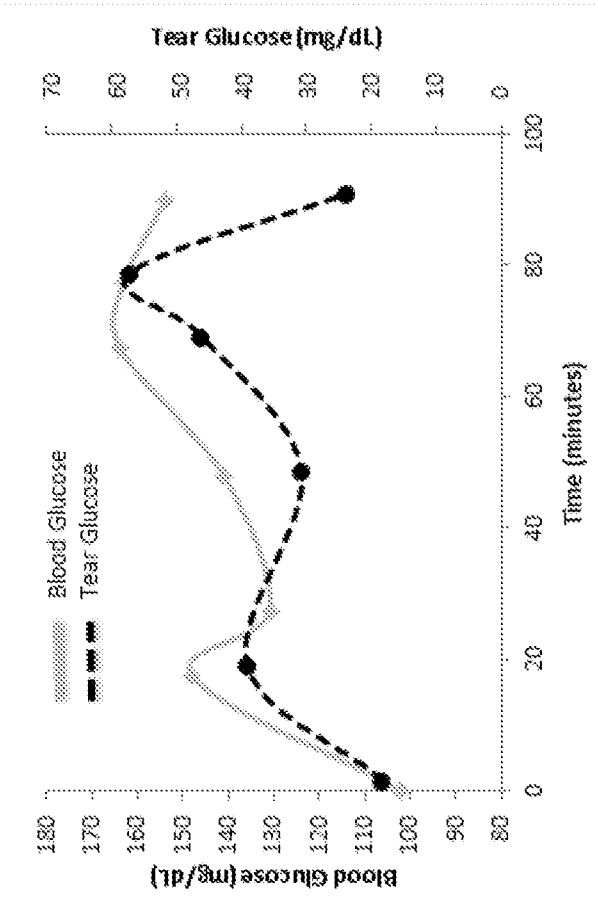
In 9 seconds, glucose levels are displayed.

The relationship between blood glucose and tear glucose has been successfully identified.



PHOTOGRAPH: device use. INSET: sensor depicting integrated (A) absorbent capture material, (B) microfluidic channel, (C) sensor-assay area and (D), sensor strip.

Fig. 1



Lag time between tear glucose and blood glucose (less than 10 seconds).

Linear correlated relationship between blood glucose and tear glucose.

Fig. 2

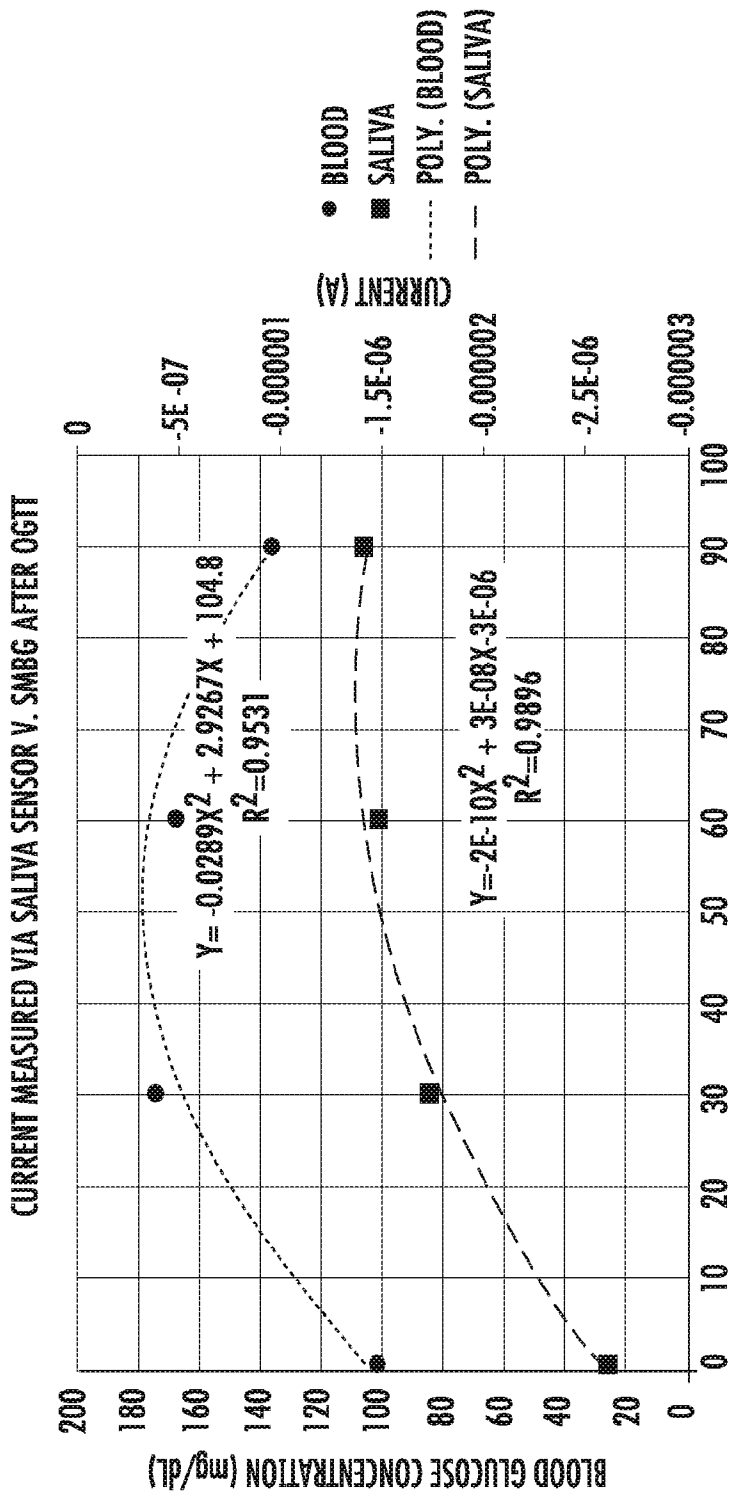
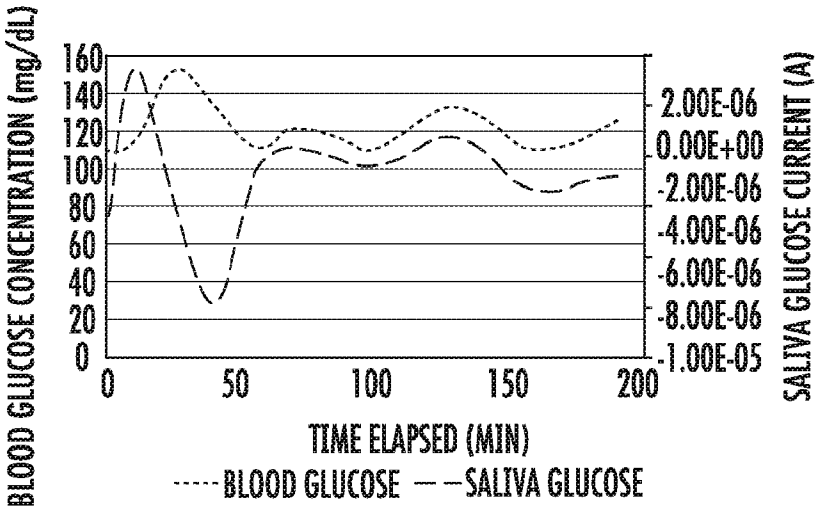
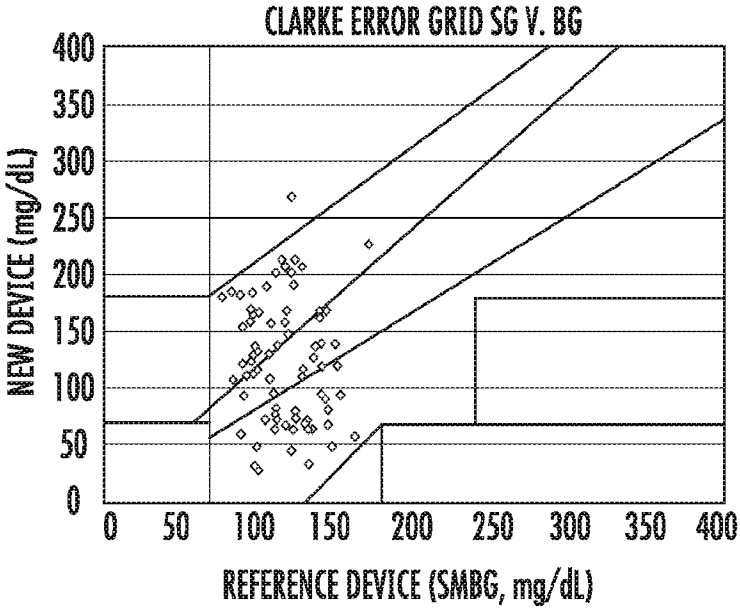

CURRENT MEASURED VIA SALIVA SENSOR V. SMBG AFTER OGTT
BLOOD GLUCOSE CONCENTRATION (mg/dL)
TIME SINCE GLUCOSE ADMINISTERED (M)
CURRENT (A)
BLOOD
SALIVA
POLY. (BLOOD)
POLY. (SALIVA)
Y= -0.0289X2 + 2.9267X + 104.8
R2=0.9531
Y=-2E-10X2 + 3E-08X-3E-06
R2=0.9896
0
20
40
60
80
100
120
140
160
180
200
0
10
20
30
40
50
60
70
80
90
100
0
-5E-07
-0.000001
-1.5E-06
-0.000002
-2.5E-06
-0.000003

FIG. 3



LAG TIME BETWEEN SALIVA GLUCOSE AND BLOOD GLUCOSE (LESS THAN 20 SECONDS)

FIG. 4A



CLARKE ERROR GRID RELATING BLOOD GLUCOSE VALUES AS MEASURE USING SMBG AND THE SALIVA GLUCOSE TECHNOLOGY.

FIG. 4B

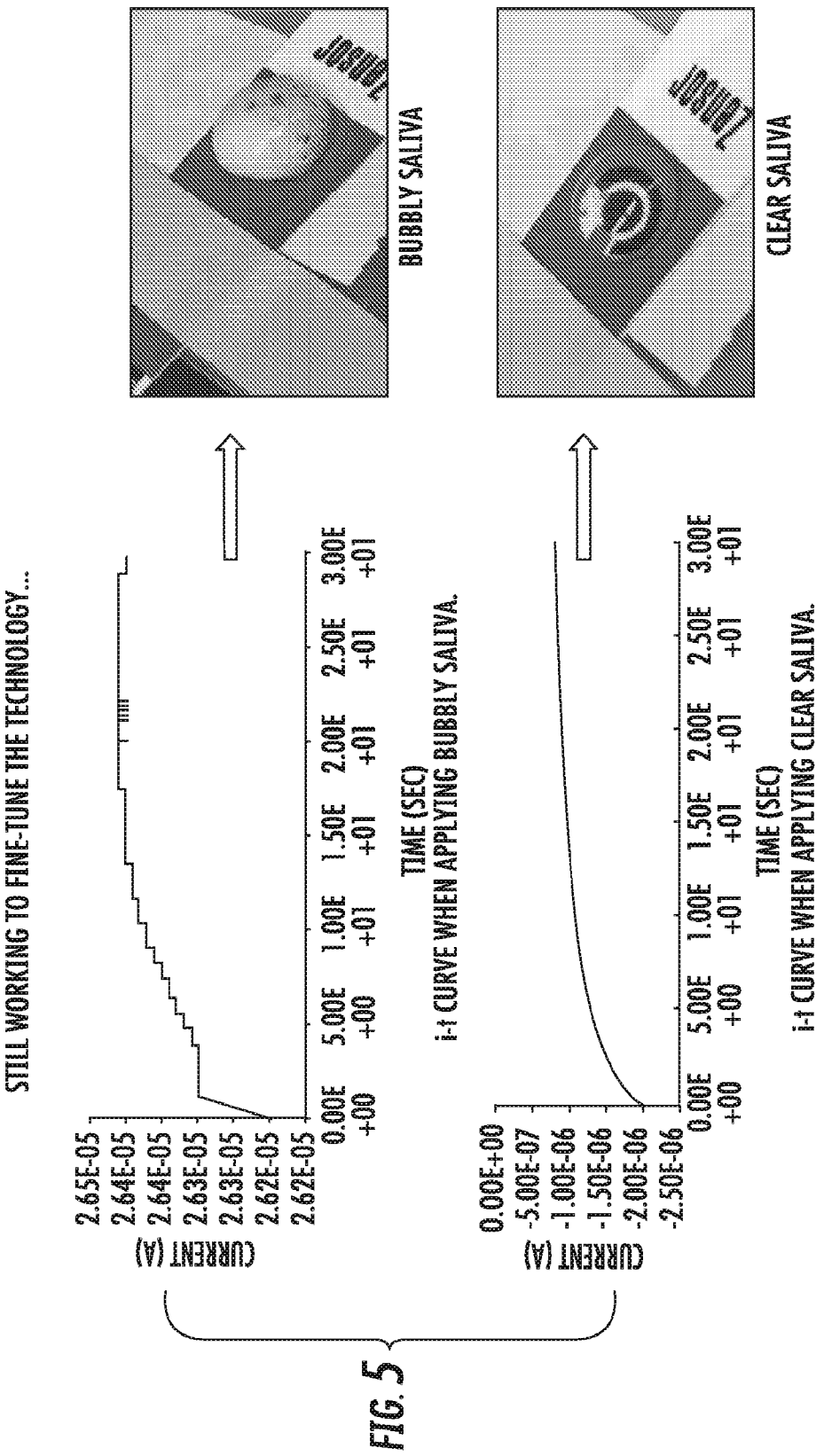

STILL WORKING TO FINE-TUNE THE TECHNOLOGY...
CURRENT (A)
2.65E-05
2.64E-05
2.64E-05
2.63E-05
2.63E-05
2.62E-05
2.62E-05
0.00E +00
5.00E +00
1.00E +01
1.50E +01
2.00E +01
2.50E +01
3.00E +01
TIME (SEC)
i-t CURVE WHEN APPLYING BUBBLY SALIVA.
BUBBLY SALIVA
CURRENT (A)
0.00E+00
-5.00E-07
-1.00E-06
-1.50E-06
-2.00E-06
-2.50E-06
0.00E +00
5.00E +00
1.00E +01
1.50E +01
2.00E +01
2.50E +01
3.00E +01
TIME (SEC)
i-t CURVE WHEN APPLYING CLEAR SALIVA.
CLEAR SALIVA

FIG. 5

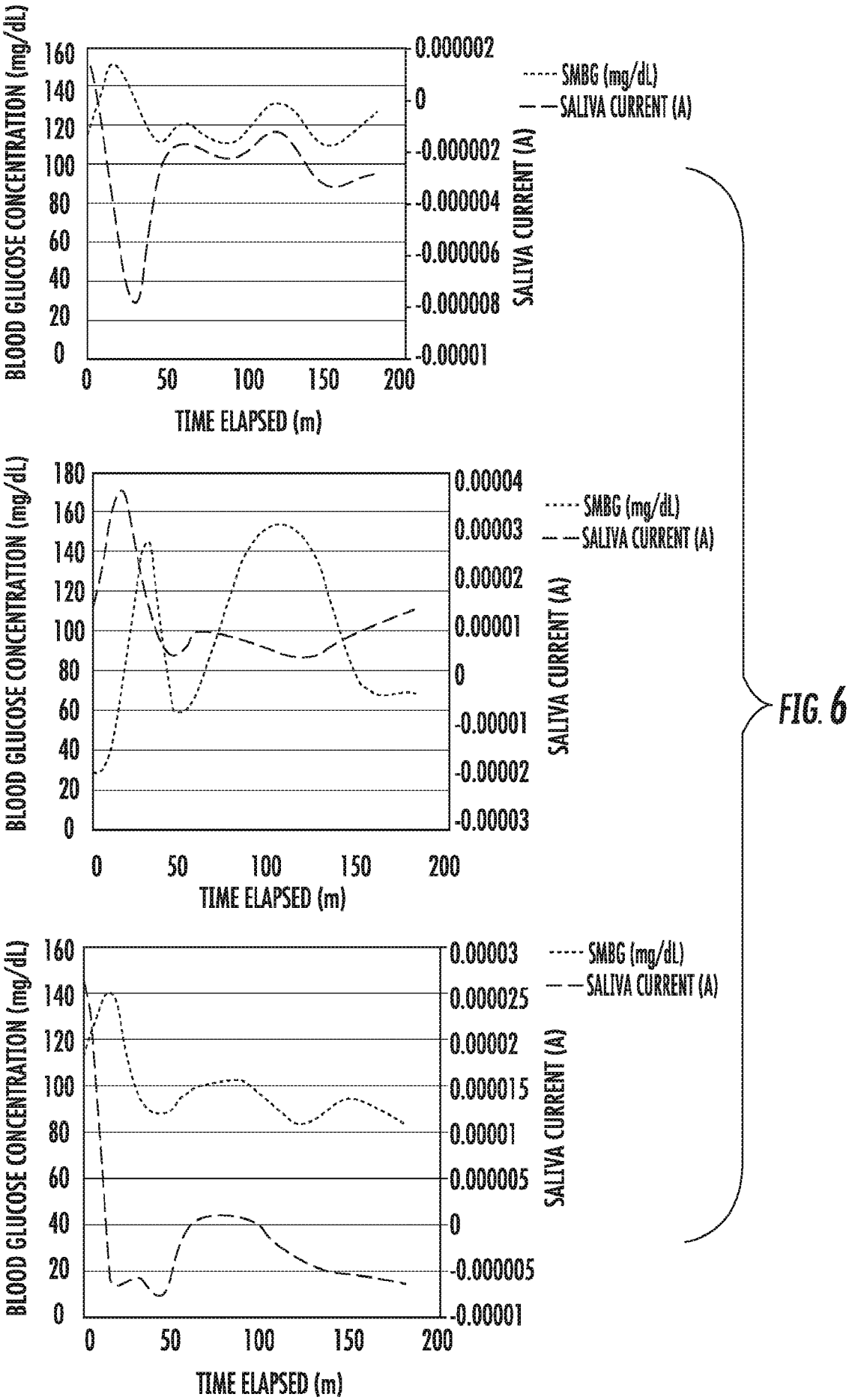

BLOOD GLUCOSE CONCENTRATION (mg/dL)
SALIVA CURRENT (A)
TIME ELAPSED (m)
SMBG (mg/dL)
SALIVA CURRENT (A)
0
50
100
150
200
0.000002
0
-0.000002
-0.000004
-0.000006
-0.000008
-0.00001
0.00004
0.00003
0.00002
0.00001
-0.00001
-0.00002
-0.00003
0.000025
0.000015
0.000005
-0.000005

FIG. 6

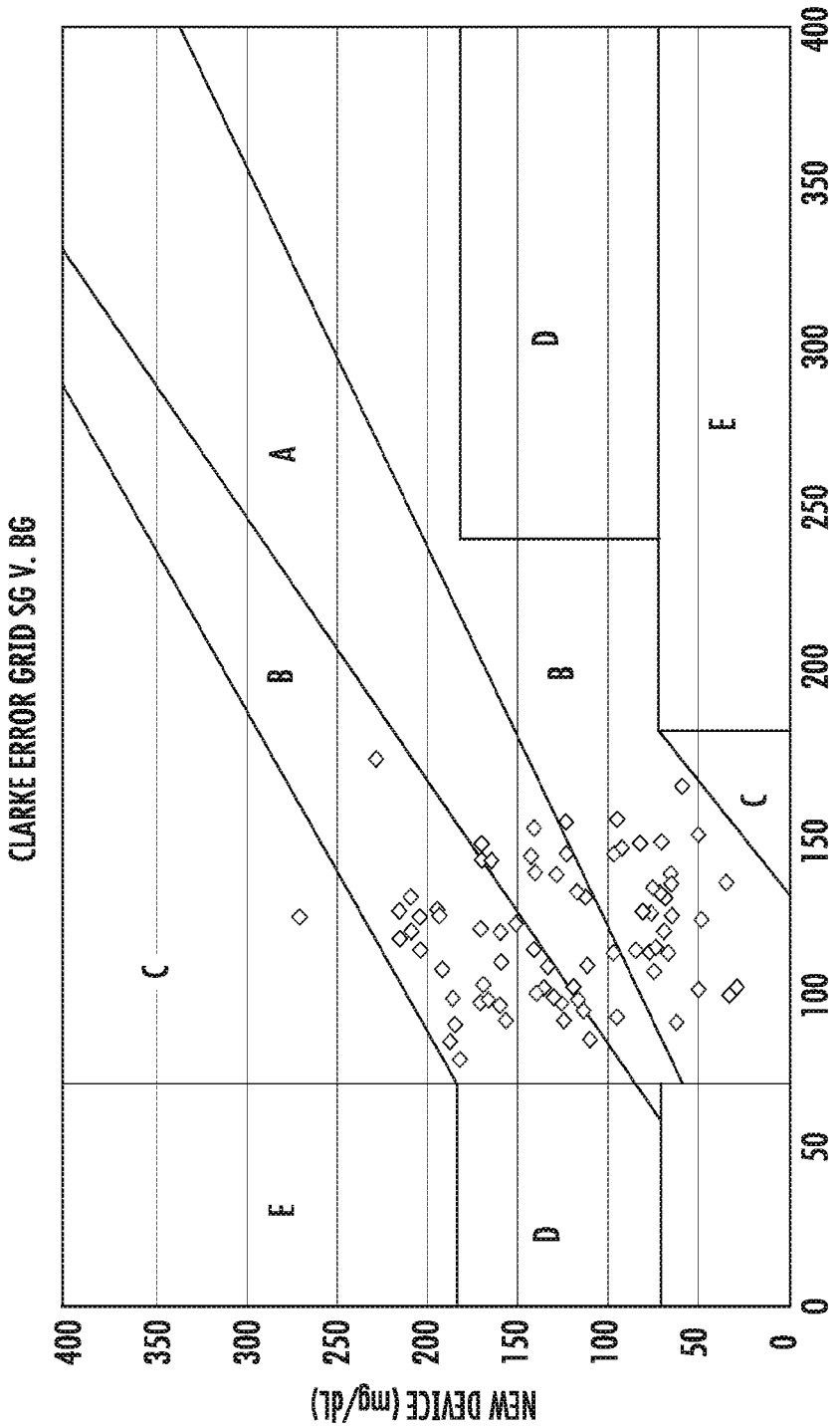

CLARKE ERROR GRID SG V. BG
NEW DEVICE (mg/dL)
REFERENCE DEVICE (mg/dL)
0
50
100
150
200
250
300
350
400
A
B
C
D
E

FIG. 7

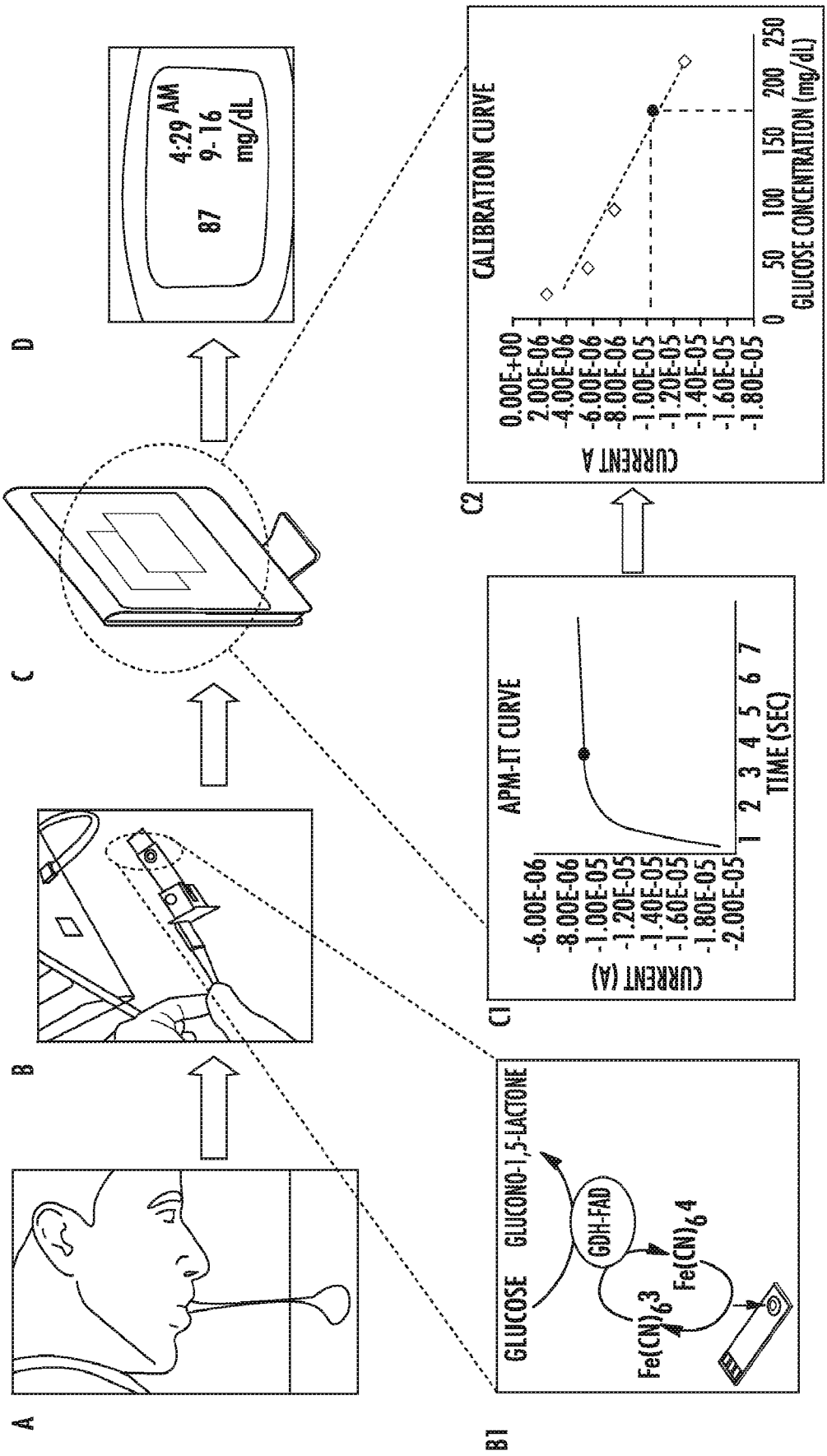

A
B
C
D
4:29 AM
87 9-16 mg/dL
B1
GLUCOSE
GLUCONO-1,5-LACTONE
GDH-FAD
Fe(CN)6 3
Fe(CN)6 4
C1
APM-IT CURVE
CURRENT (A)
-6.00E-06
-8.00E-06
-1.00E-05
-1.20E-05
-1.40E-05
-1.60E-05
-1.80E-05
-2.00E-05
1 2 3 4 5 6 7
TIME (SEC)
C2
CALIBRATION CURVE
CURRENT A
0.00E+00
-2.00E-06
-4.00E-06
-6.00E-06
-8.00E-06
-1.00E-05
-1.20E-05
-1.40E-05
-1.60E-05
-1.80E-05
0 50 100 150 200 250
GLUCOSE CONCENTRATION (mg/dL)

FIG. 8

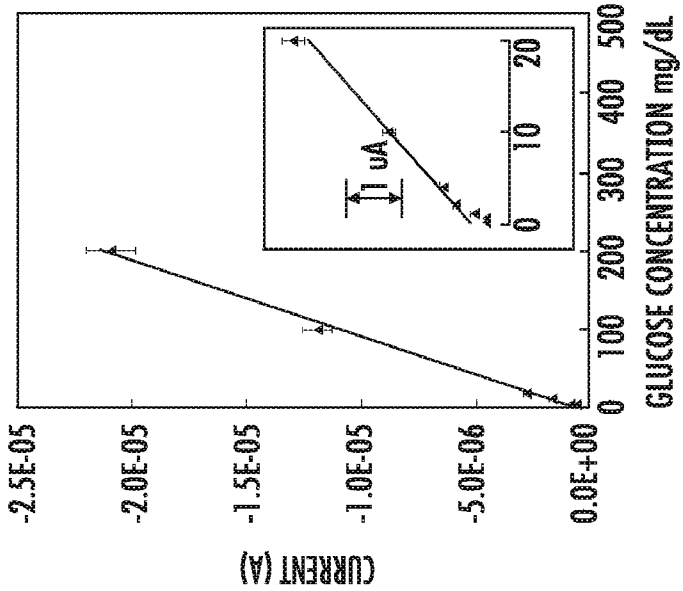

CURRENT (A)
-2.5E-05
-2.0E-05
-1.5E-05
-1.0E-05
-5.0E-06
0.0E+00
0
100
200
300
400
500
GLUCOSE CONCENTRATION mg/dL
1 uA
0
10
20

FIG. 9B

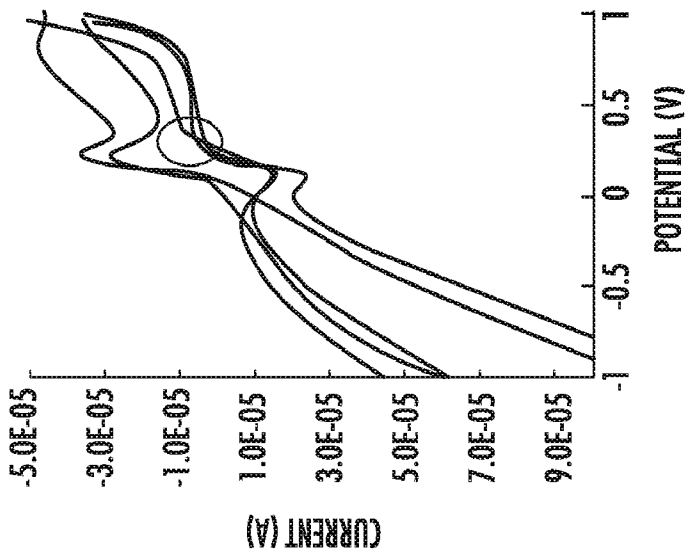

CURRENT (A)
-5.0E-05
-3.0E-05
-1.0E-05
1.0E-05
3.0E-05
5.0E-05
7.0E-05
9.0E-05
-1
-0.5
0
0.5
1
POTENTIAL (V)

FIG. 9A

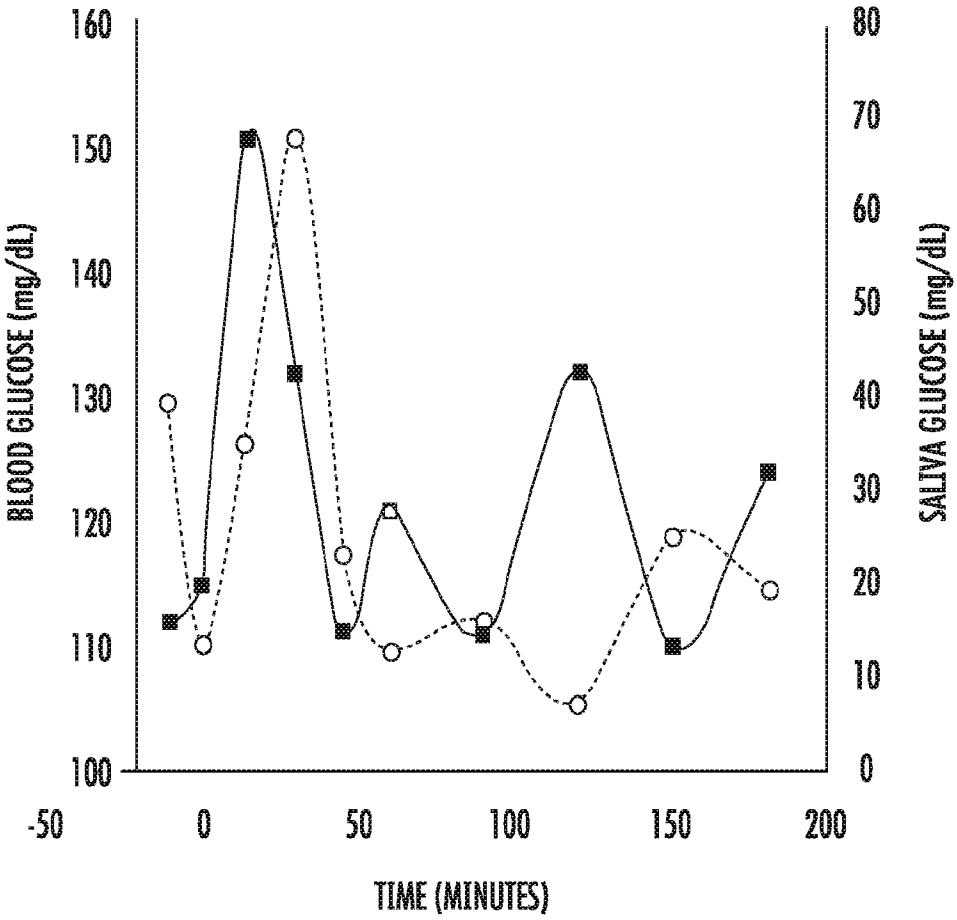

160
150
140
130
120
110
100
BLOOD GLUCOSE (mg/dL)
80
70
60
50
40
30
20
10
0
SALIVA GLUCOSE (mg/dL)
-50
0
50
100
150
200
TIME (MINUTES)

FIG. 10

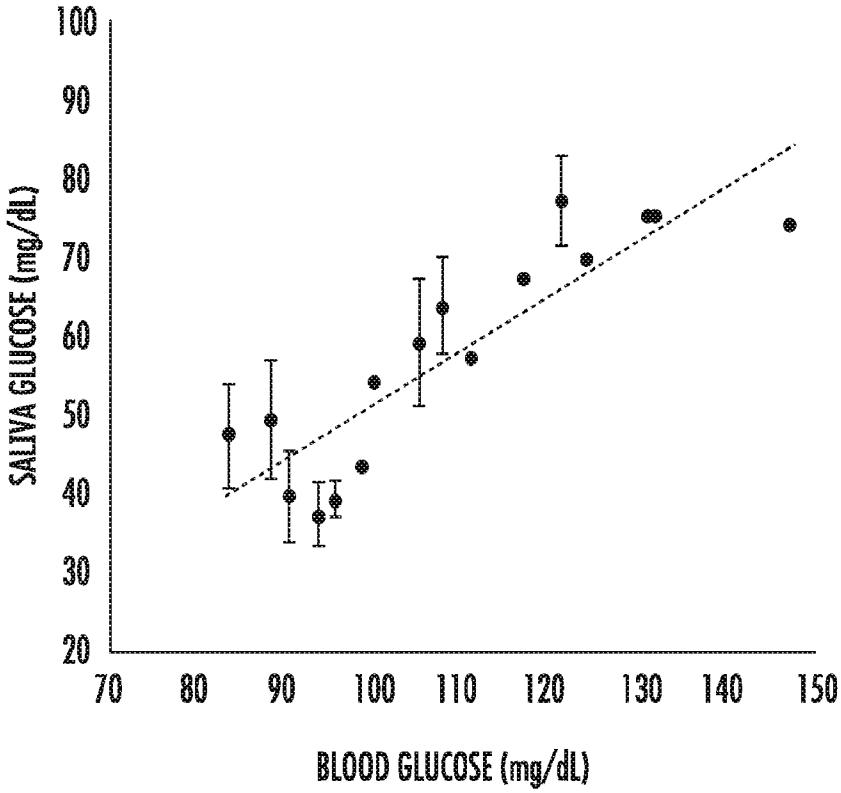

100
90
80
70
60
50
40
30
20
SALIVA GLUCOSE (mg/dL)
70
80
90
100
110
120
130
140
150
BLOOD GLUCOSE (mg/dL)

FIG. 11

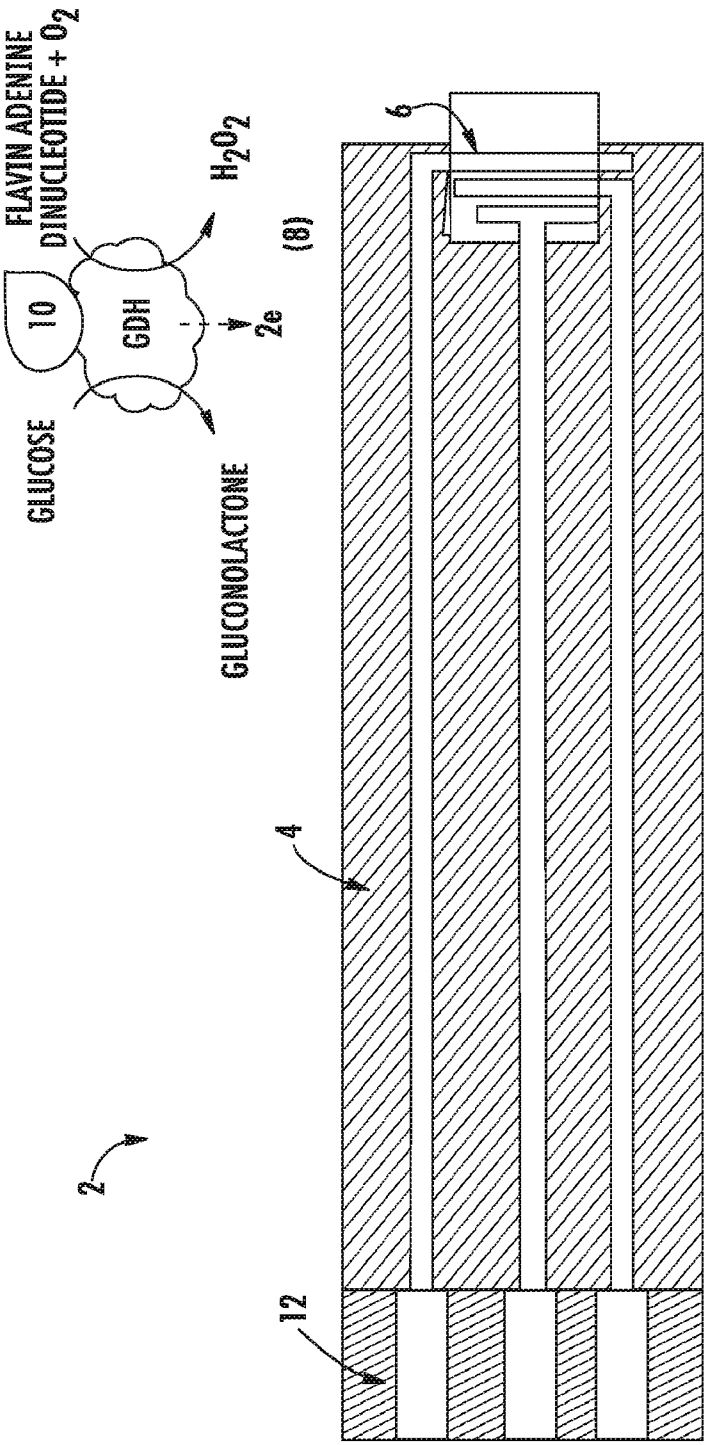

FLAVIN ADENINE DINUCLEOTIDE + O_2
H_2O_2
(8)
10
GDH
2e
GLUCOSE
GLUCONOLACTONE
6
4
12
2

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/15434

A. CLASSIFICATION OF SUBJECT MATTER
IPC - G01N 27/31, 27/403, 27/70; A61B 5/1477; C12Q 1/00; H01L 51/00 (2017.01)
CPC - G01N 27/31, 27/403, 27/70; A61B 5/1477; C12Q 1/00; H01L 51/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	US 2013/0026050 A1 (Harding, I et al.) January 31, 2013; abstract; figure 4; paragraphs [0022], [0025], [0045], [0047]-[0049], [0051], [0099], [0130]; table 1, line 2	1-2, 4 - 3, 5, 6-9
Y	US 2015/0037827 A1 (NEWCASTLE INNOVATION LIMITED) February 5, 2015; abstract; figure 1; paragraph [0103]	3
Y	US 2013/0075276 A1 (ARKRAY, INC.) March 28, 2013; abstract	5, 8
Y	US 2001/0023324 A1 (PRONOVOST, A et al.) September 20, 2001; abstract; figures 13, 14; paragraphs [0089], [0094]	6-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 March 2017 (08.03.2017)	05 APR 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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