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(54) ELECTROCHEMICAL TEST STRIP FOR REDUCING THE EFFECT OF DIRECT AND MEDIATED INTERFERENCE CURRENT

(76) Inventors: **Oliver William Hardwicke Davies**, Croy (GB); **Robert Marshall**, Conon Bridge (GB); **Damian Edward Haydon Baskeyfield**, Auldeam (GB); **Lysney Whyte**, Lochardil (GB); **Elaine Leiper**, Inverness (GB)

Correspondence Address:
PHILIP S. JOHNSON
JOHNSON & JOHNSON
ONE JOHNSON & JOHNSON PLAZA
NEW BRUNSWICK, NJ 08933-7003 (US)

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Publication Classification

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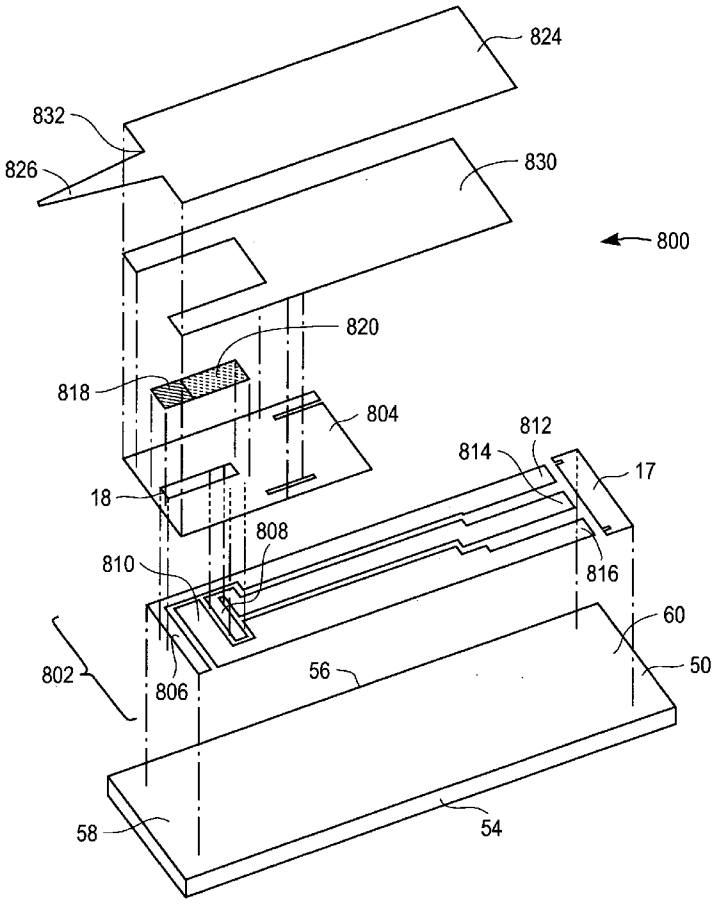
(52) **U.S. Cl.** **204/403.01**

(57) ABSTRACT

The present invention is directed to an electrochemical sensor or electrochemical strip which includes a substrate, a first working electrode disposed on the substrate, a second working electrode disposed on the substrate, a reference electrode, an active reagent layer disposed on the first working electrode, wherein the active reagent layer completely covers the first working electrode and an inactive reagent layer disposed on the second working electrode, wherein the inactive reagent completely covers the second working electrode. The present invention is also directed to an electrochemical sensor an electrochemical sensor including a substrate, a first working electrode disposed on the substrate, a second working electrode disposed on the substrate, a reference electrode, an active reagent layer disposed on the first working electrode, wherein the active reagent layer completely covers the first working electrode, the second working electrode having an active region and an inactive region, the active reagent layer disposed on a active region of the second working electrode and an inactive reagent layer disposed on the inactive region of the second working electrode.



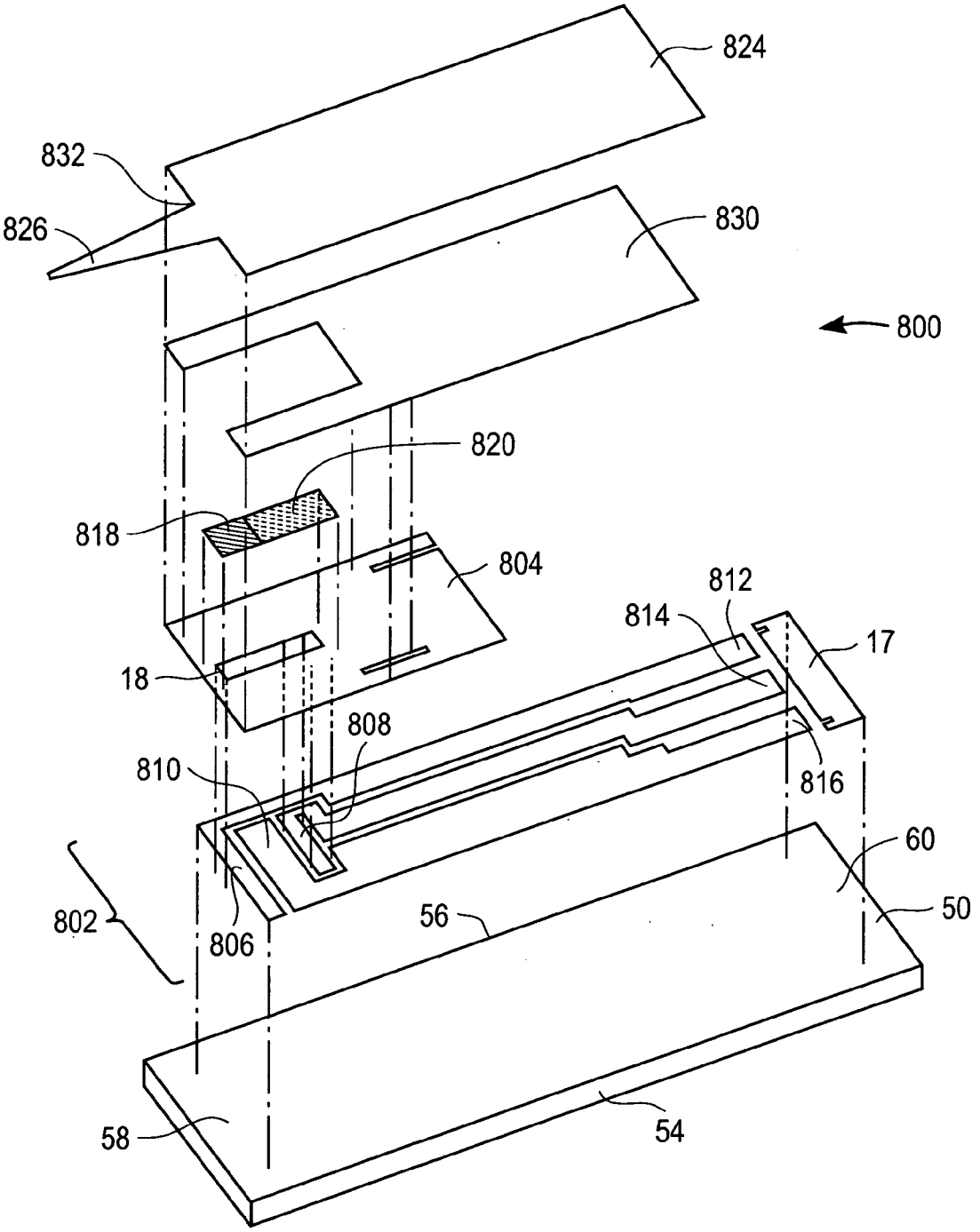

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FIG. 1

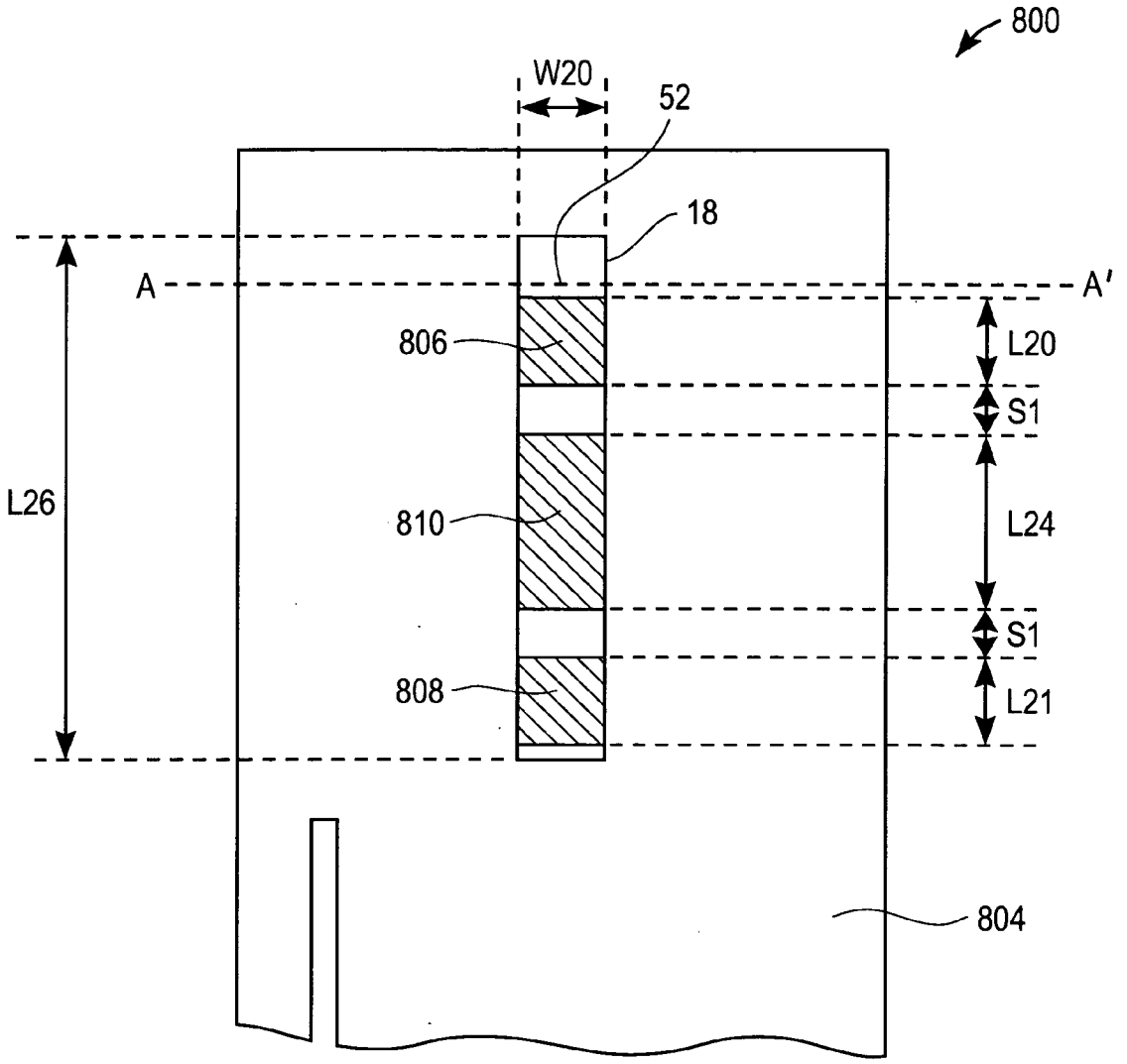


FIG. 2

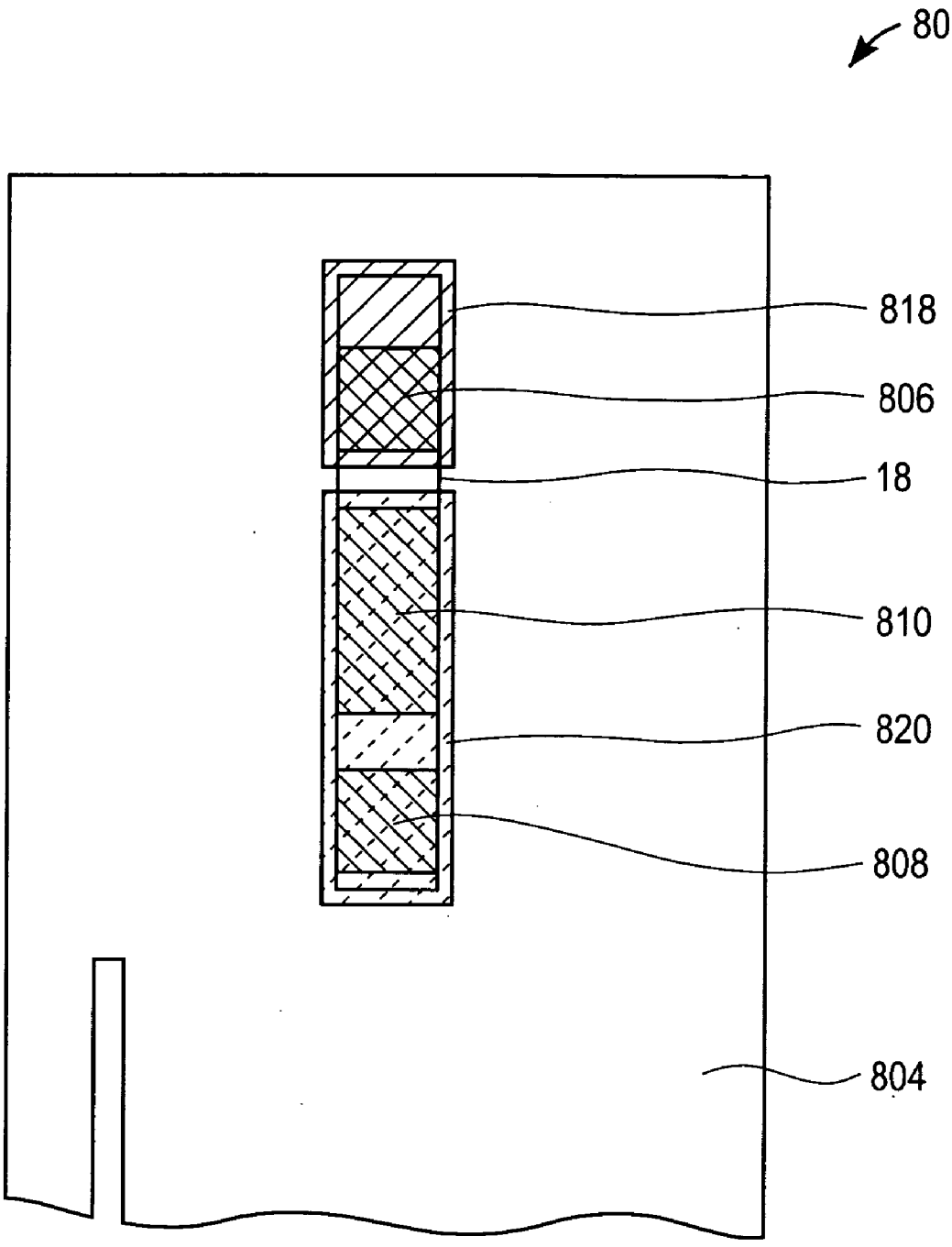


FIG. 3

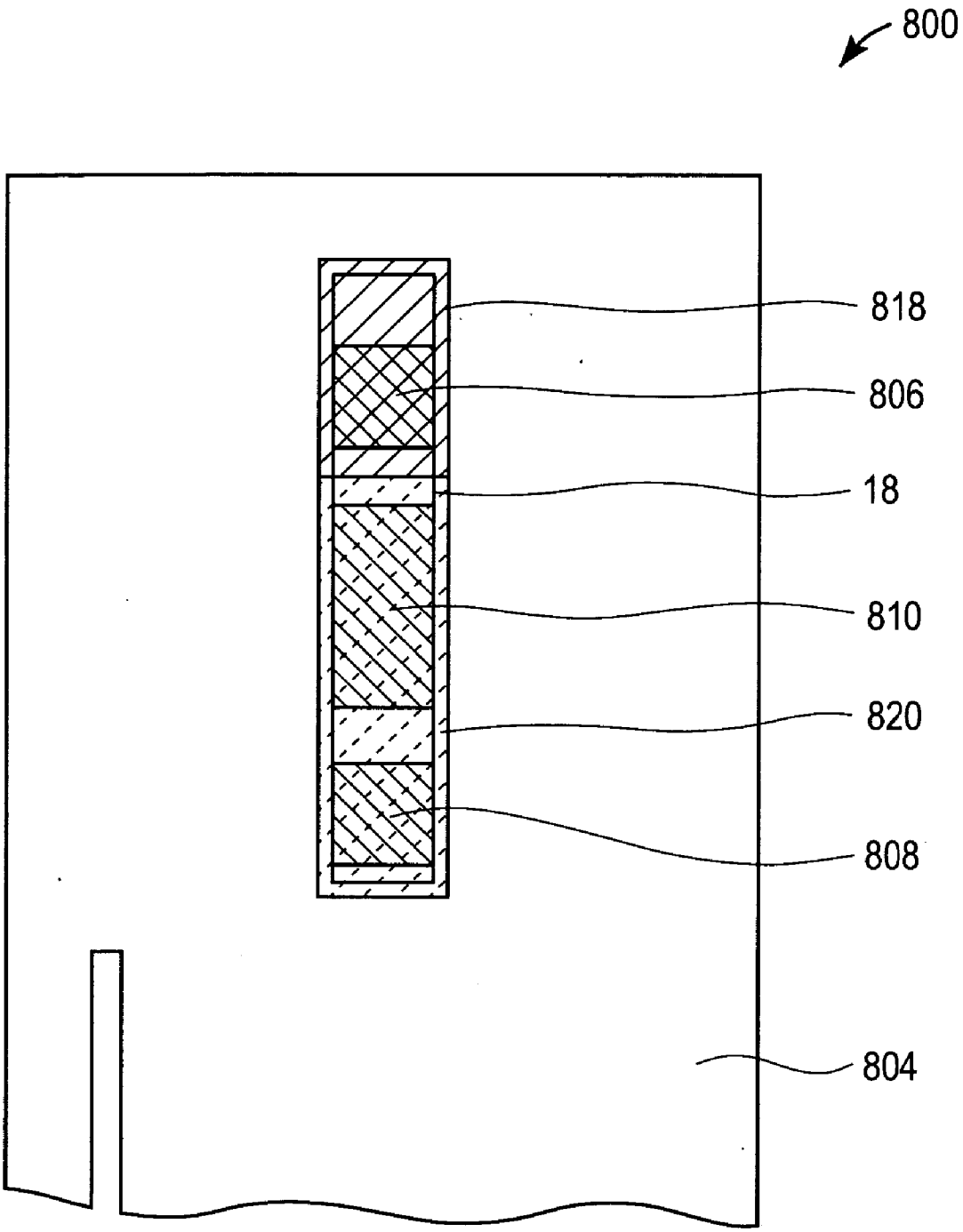


FIG. 4

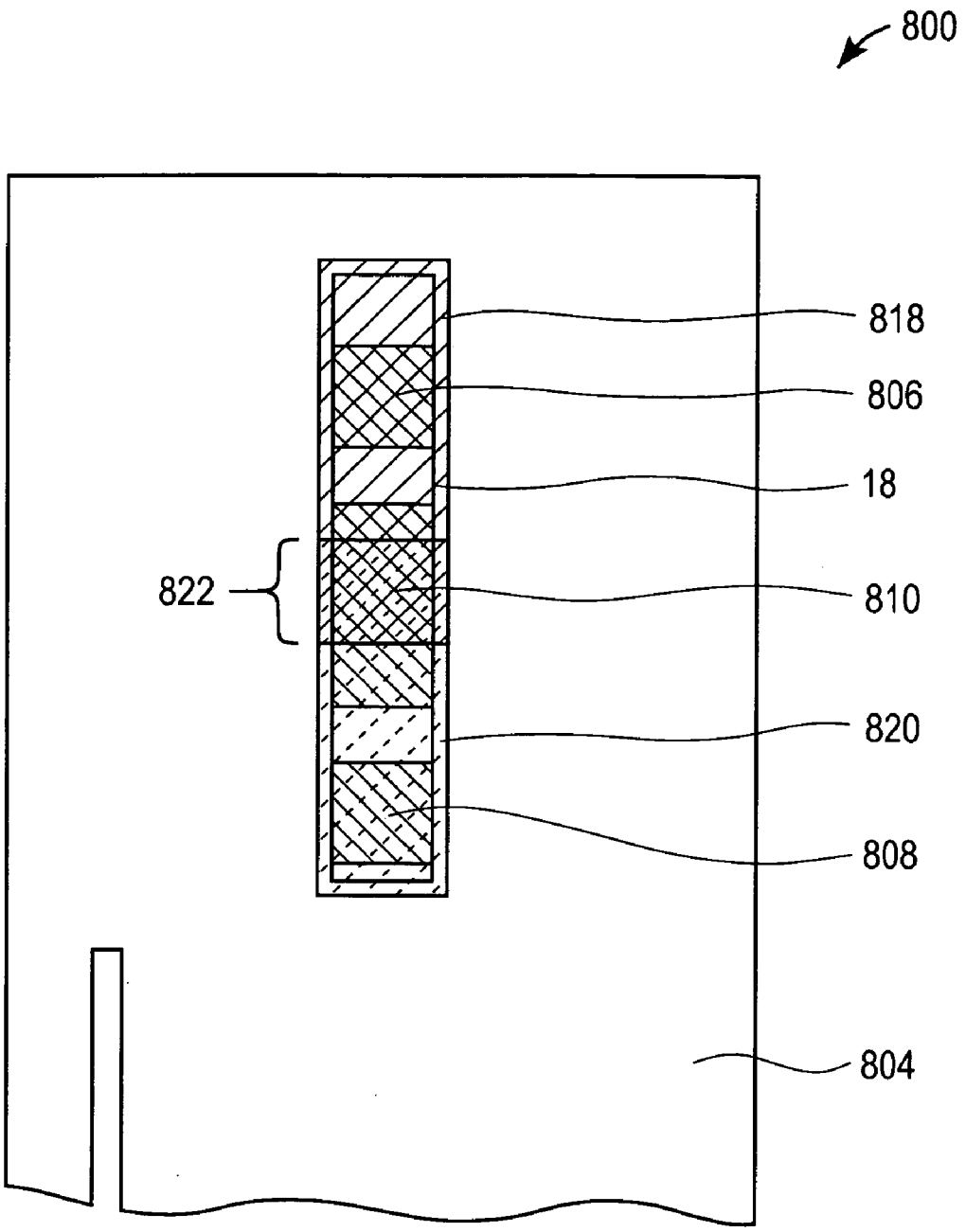


FIG. 5

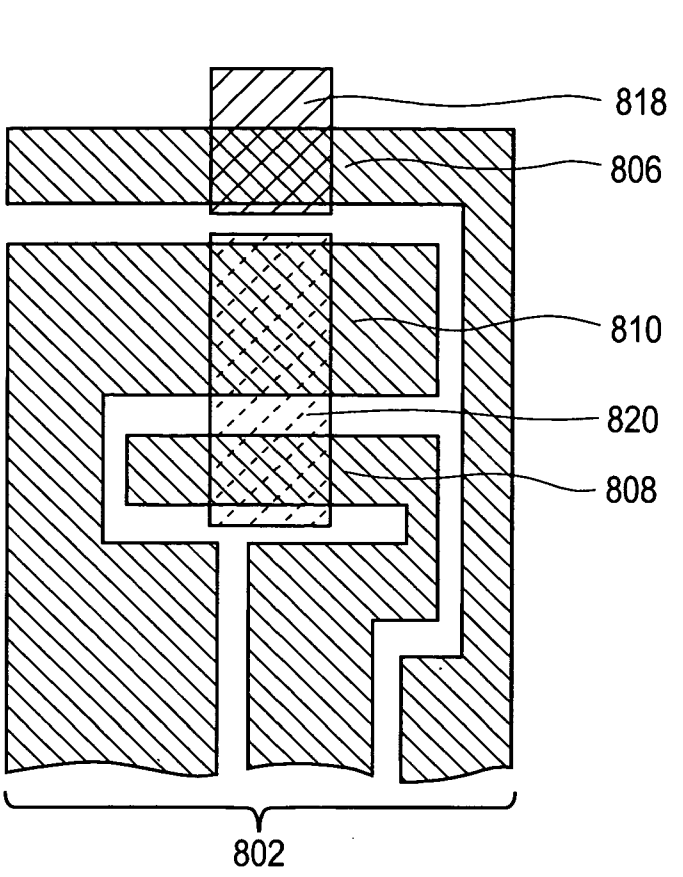


FIG. 6

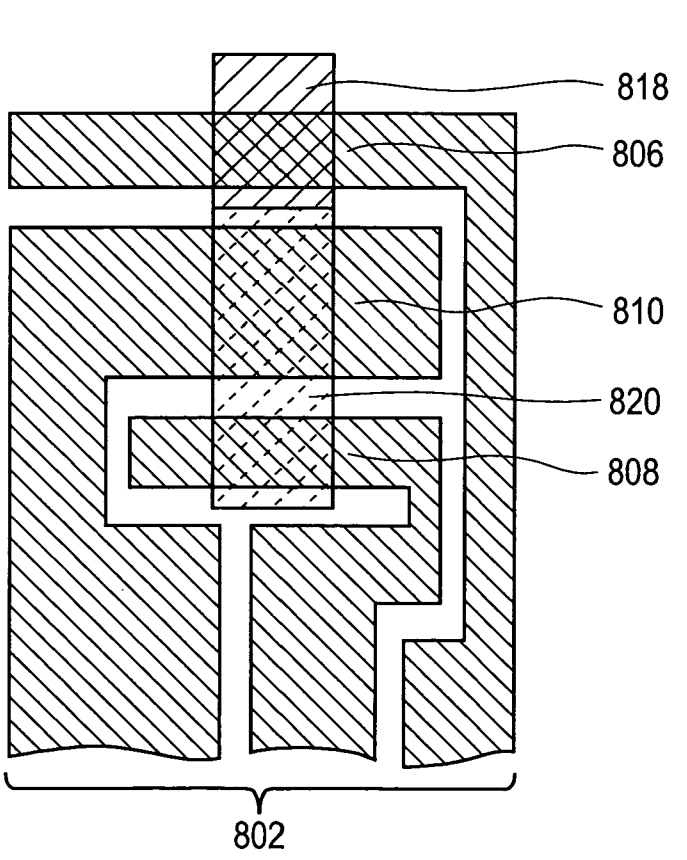


FIG. 7

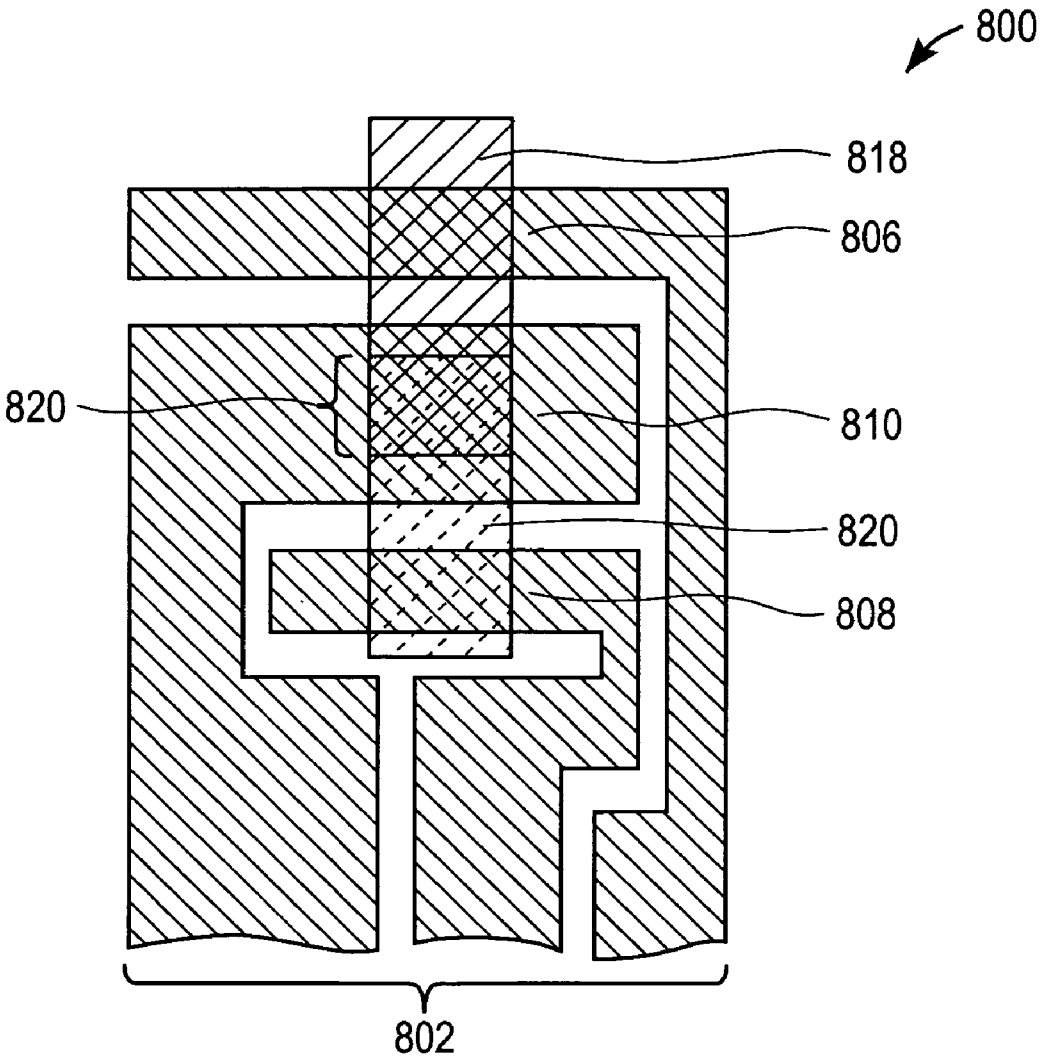


FIG. 8

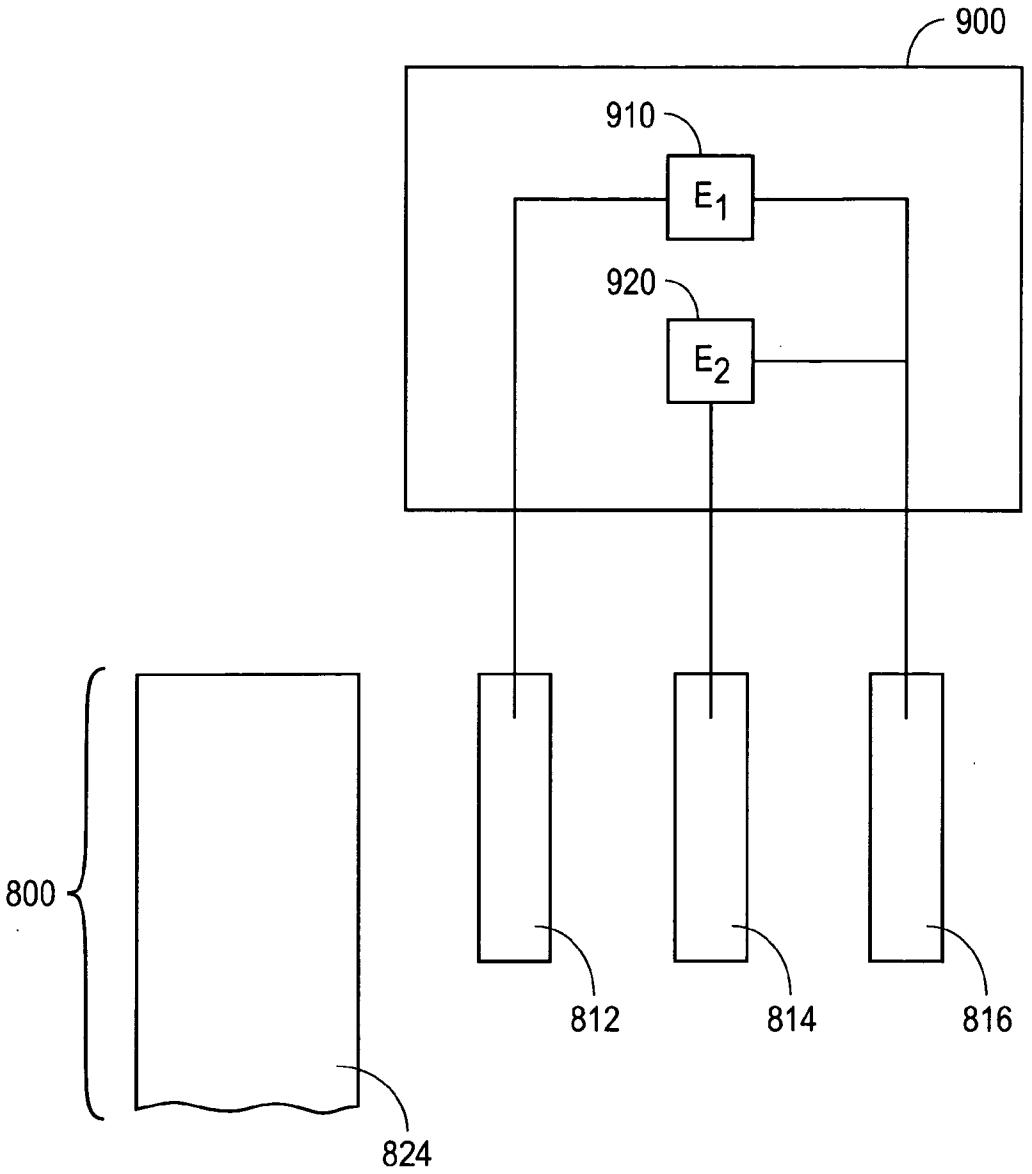


FIG. 9

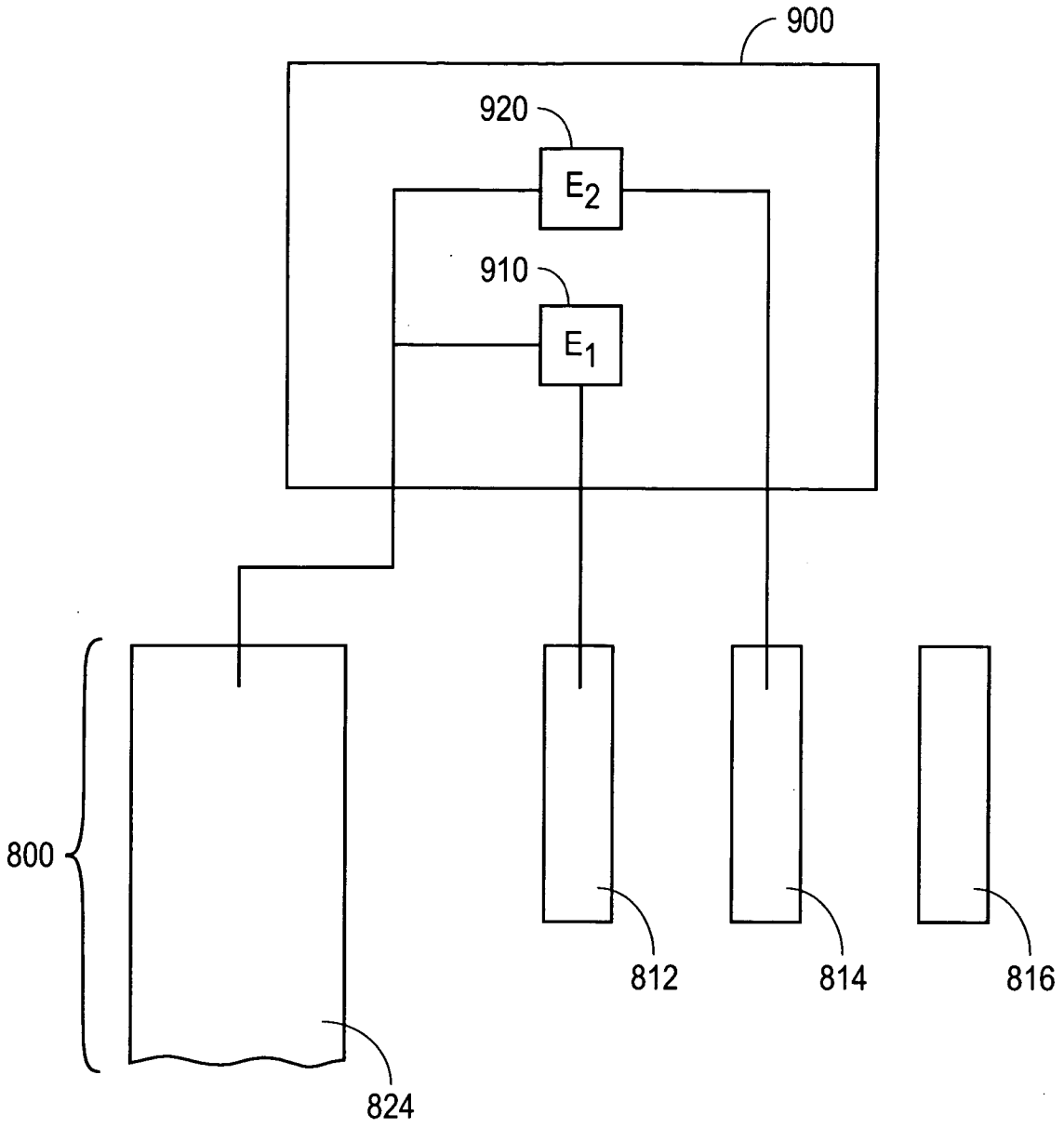


FIG. 10

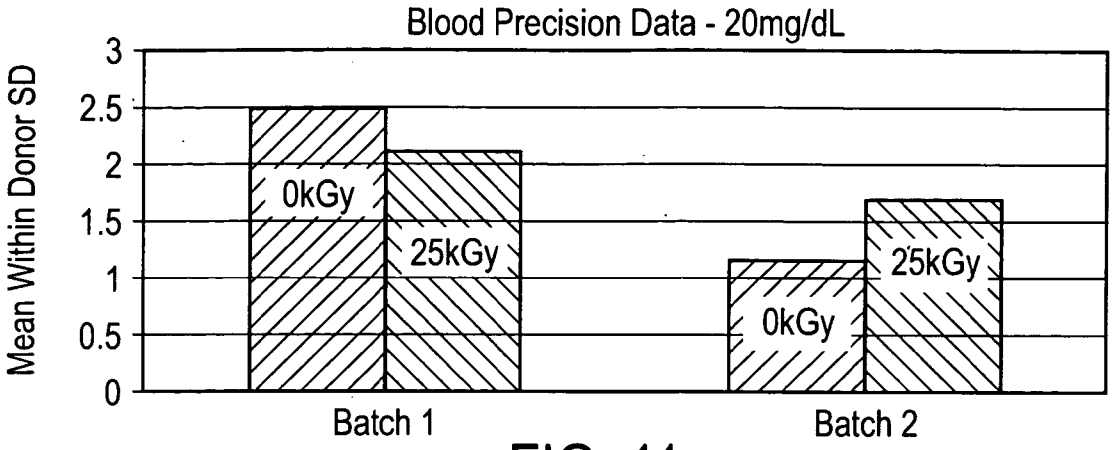


FIG. 11

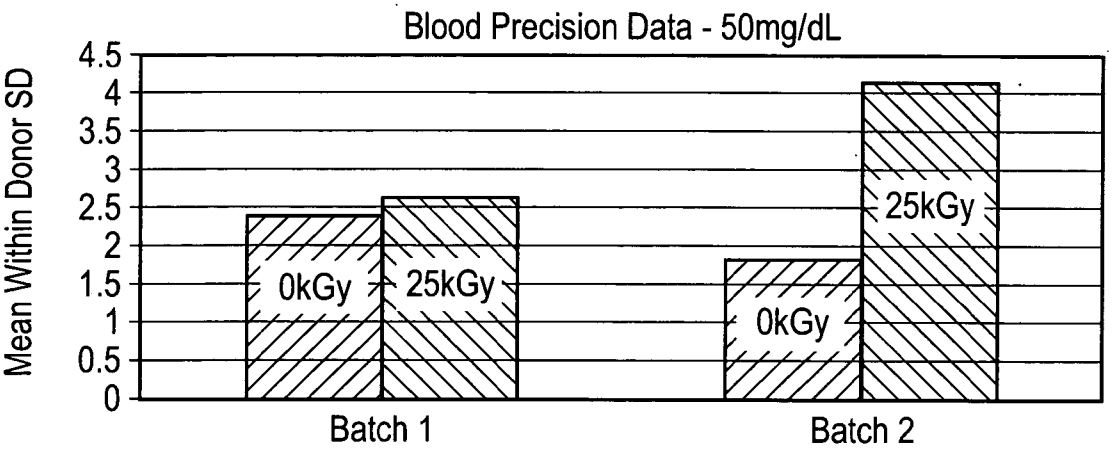


FIG. 12

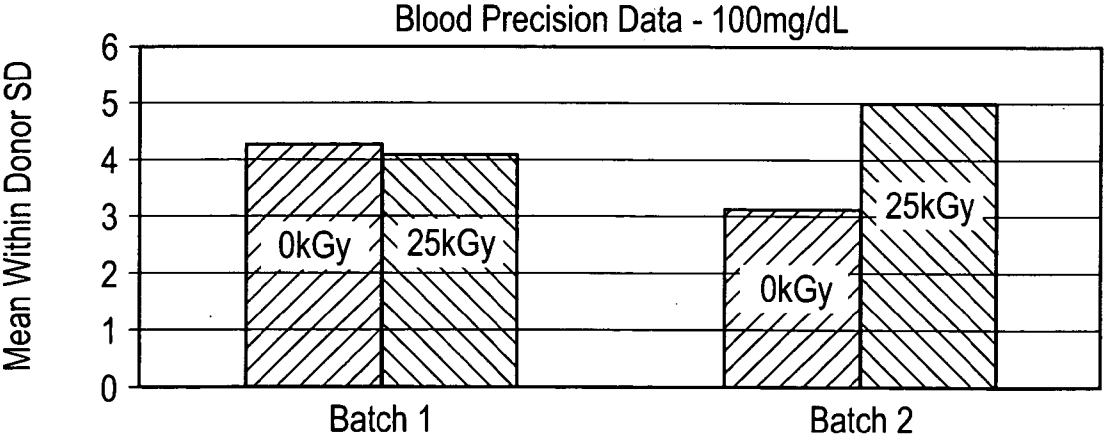


FIG. 13

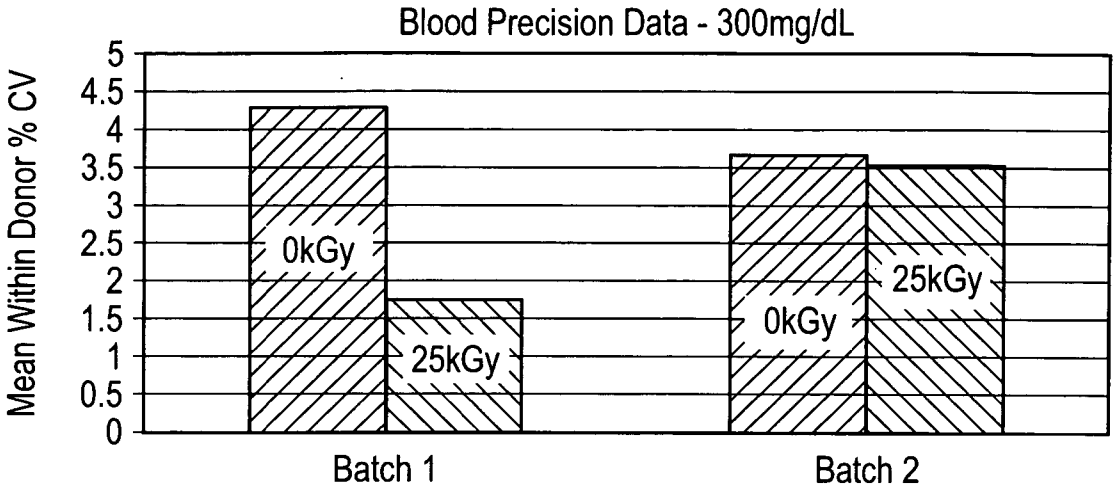

Blood Precision Data - 300mg/dL
Mean Within Donor % CV
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0kGy
25kGy
0kGy
25kGy
Batch 1
Batch 2

FIG. 14

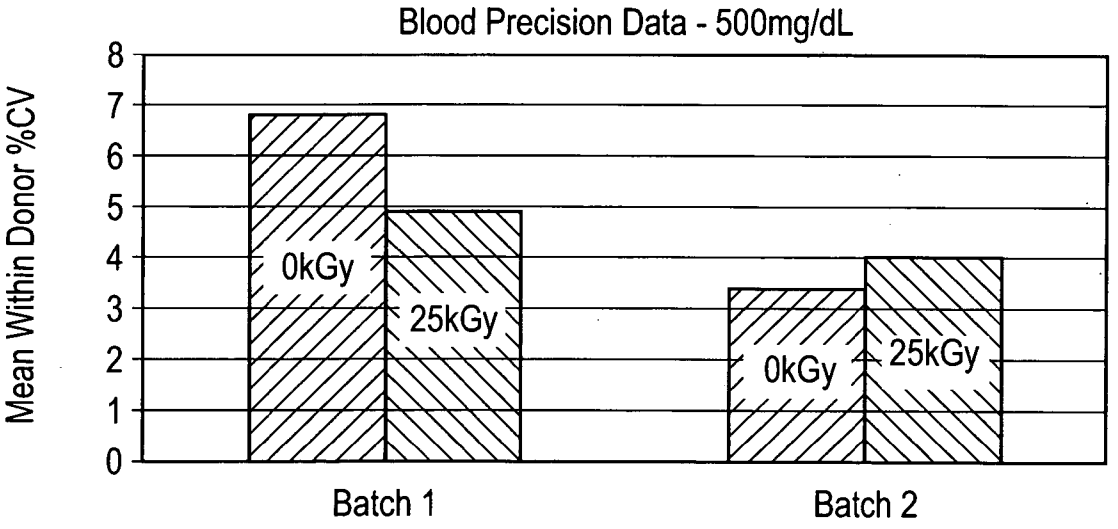

Blood Precision Data - 500mg/dL
Mean Within Donor %CV
8
7
6
5
4
3
2
1
0
0kGy
25kGy
0kGy
25kGy
Batch 1
Batch 2

FIG. 15

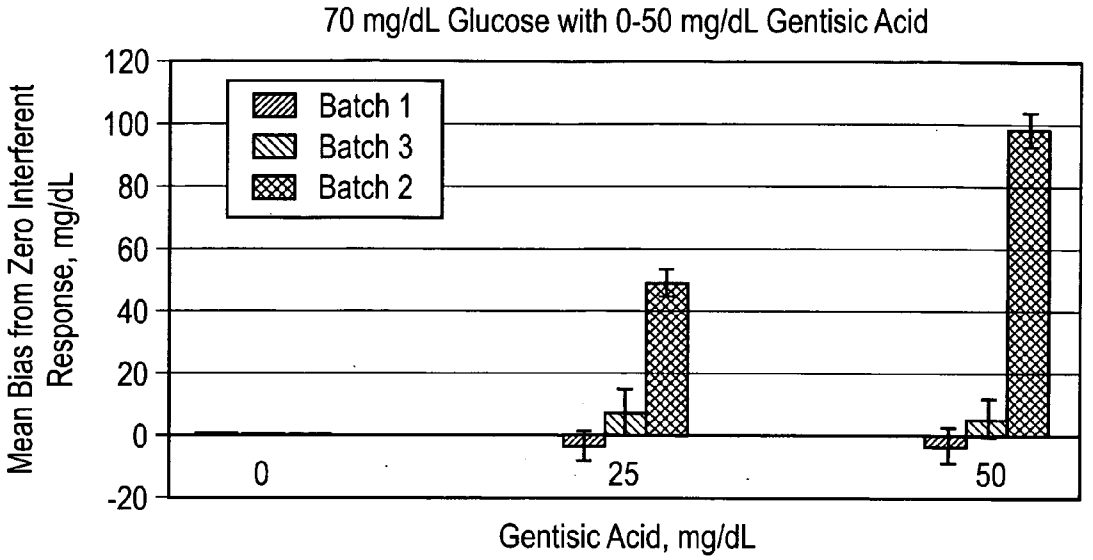

70 mg/dL Glucose with 0-50 mg/dL Gentisic Acid
Mean Bias from Zero Interferent Response, mg/dL
120
100
80
60
40
20
0
-20
Batch 1
Batch 3
Batch 2
0
25
50
Gentisic Acid, mg/dL

FIG. 16

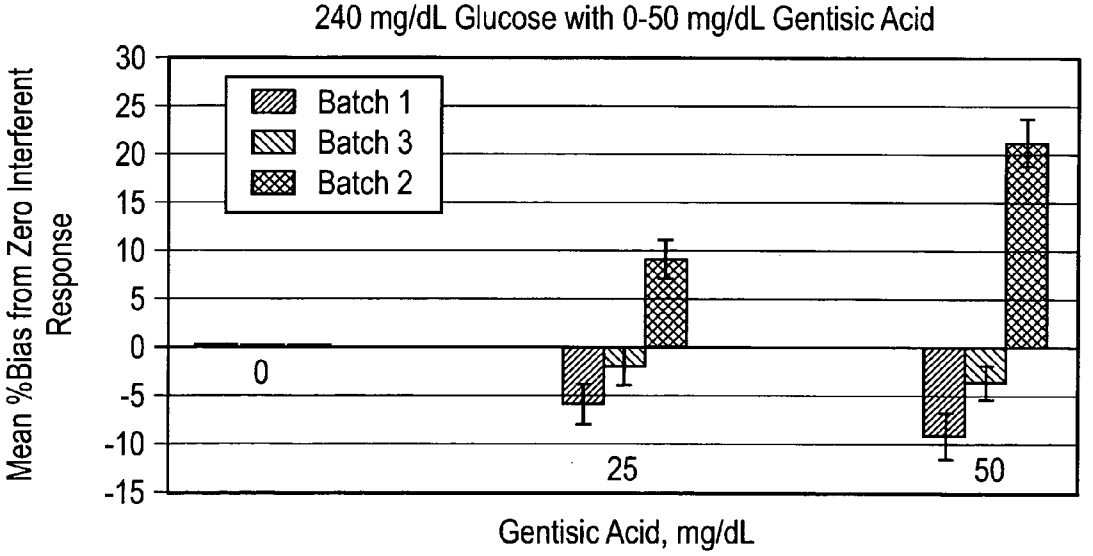

240 mg/dL Glucose with 0-50 mg/dL Gentisic Acid
Mean %Bias from Zero Interferent Response
30
25
20
15
10
5
0
-5
-10
-15
Batch 1
Batch 3
Batch 2
0
25
50
Gentisic Acid, mg/dL

FIG. 17

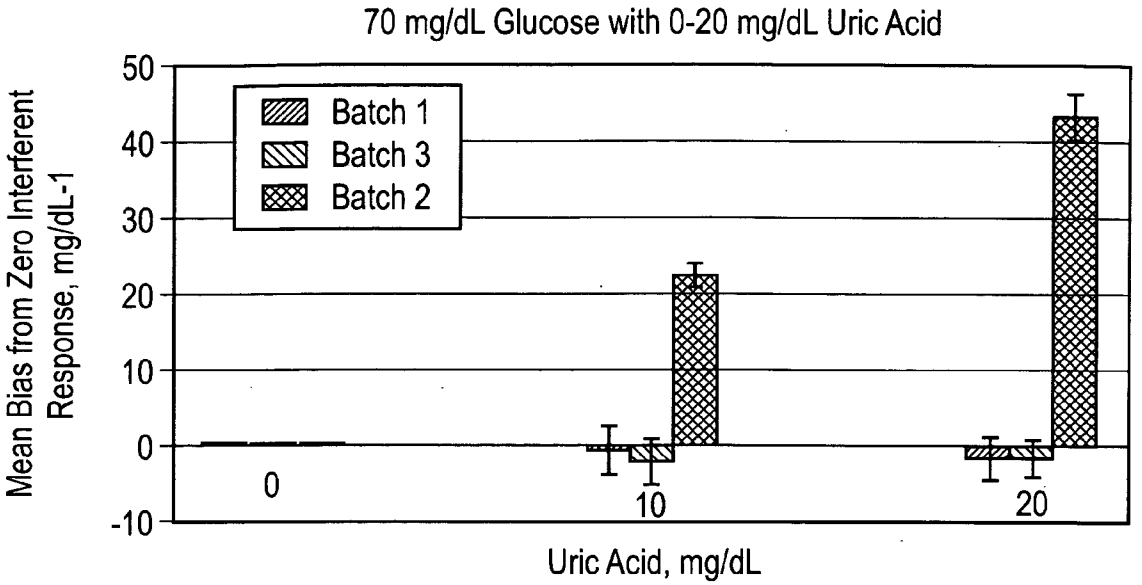

70 mg/dL Glucose with 0-20 mg/dL Uric Acid
Mean Bias from Zero Interferent Response, mg/dL-1
50
40
30
20
10
0
-10
Batch 1
Batch 3
Batch 2
0
10
20
Uric Acid, mg/dL

FIG. 18

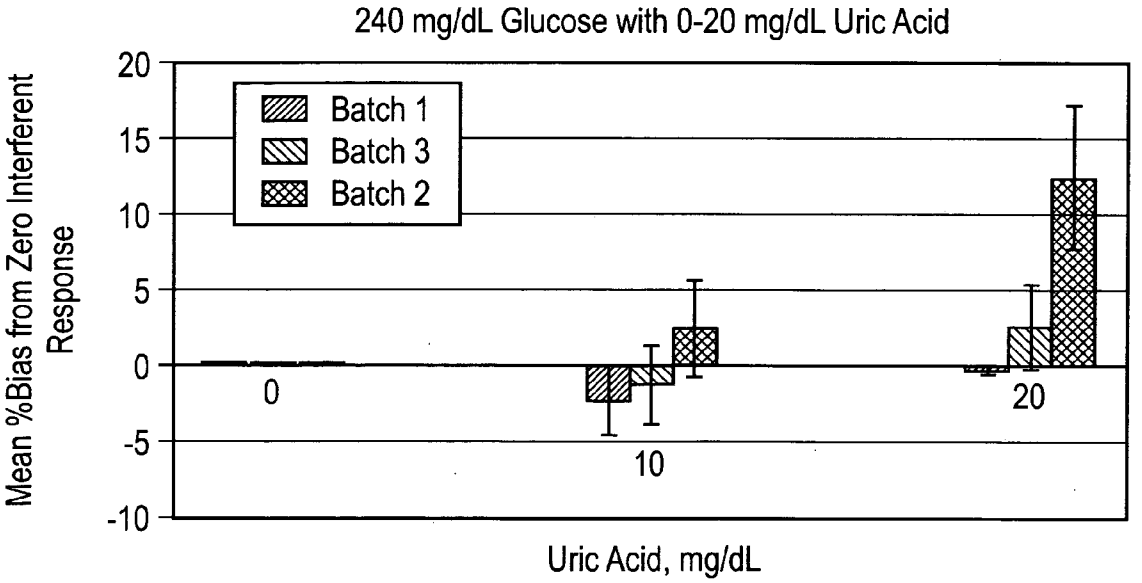

240 mg/dL Glucose with 0-20 mg/dL Uric Acid
Mean %Bias from Zero Interferent Response
20
15
10
5
0
-5
-10
Batch 1
Batch 3
Batch 2
0
10
20
Uric Acid, mg/dL

FIG. 19

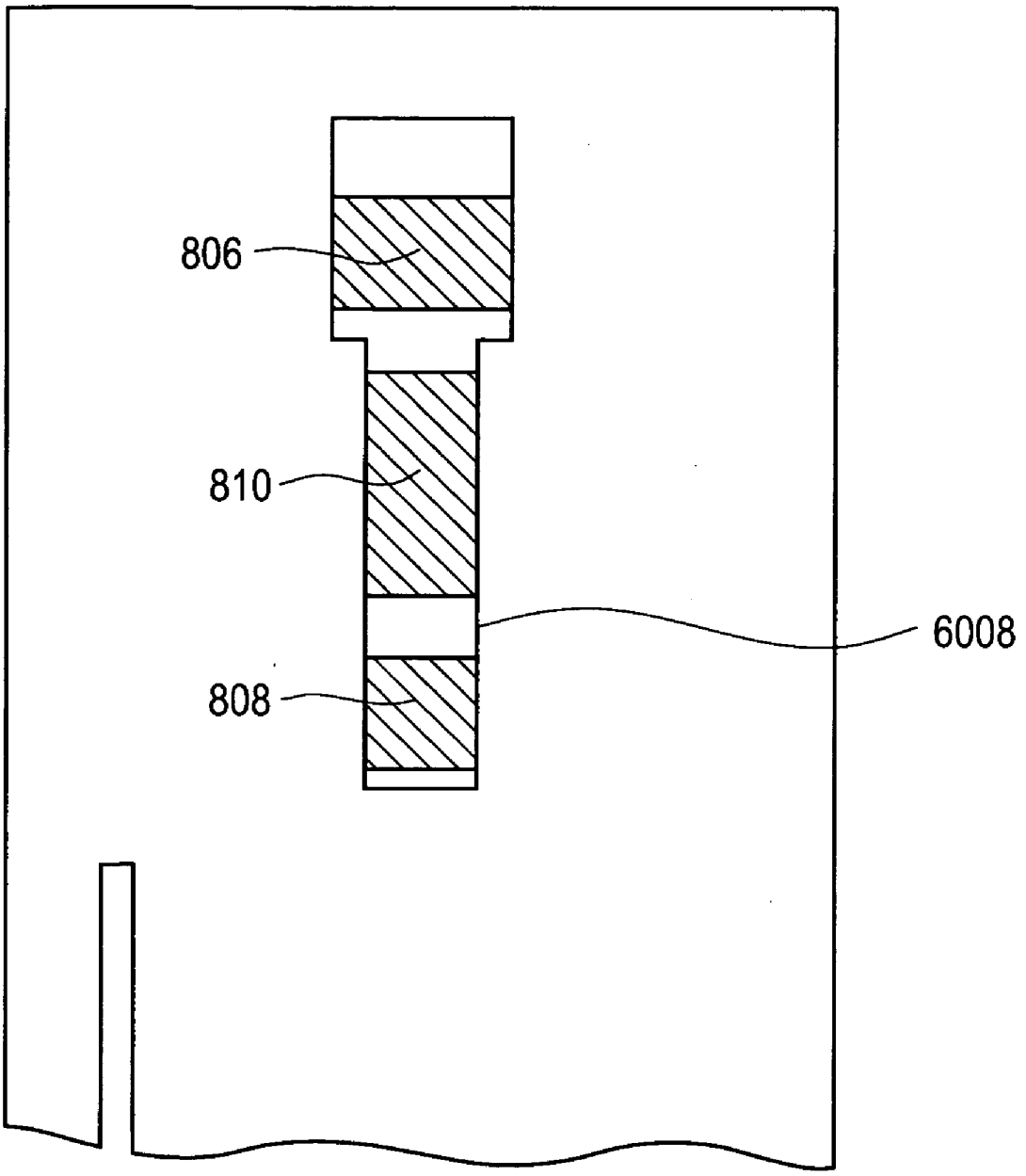
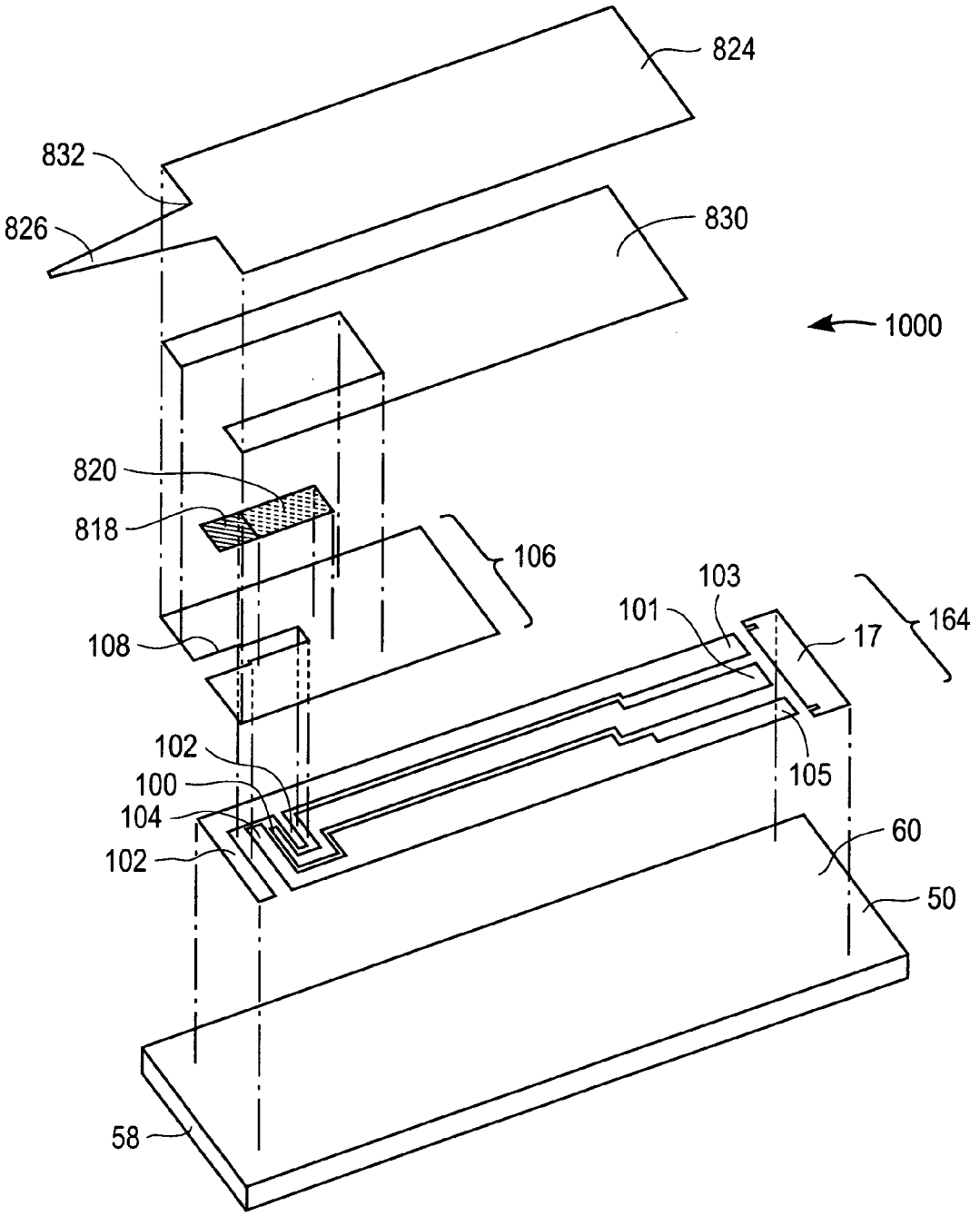


FIG. 20


824
832
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102
60
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58

FIG. 21

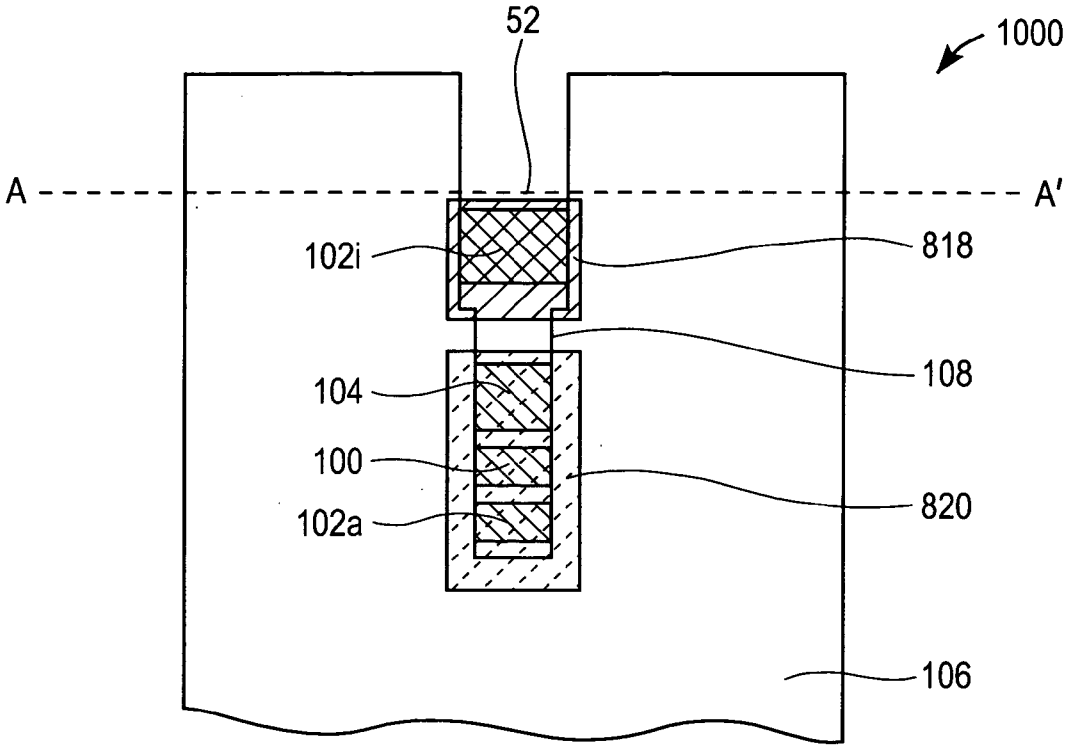
52
1000
A
A'
102i
818
108
104
100
820
102a
106

FIG. 22

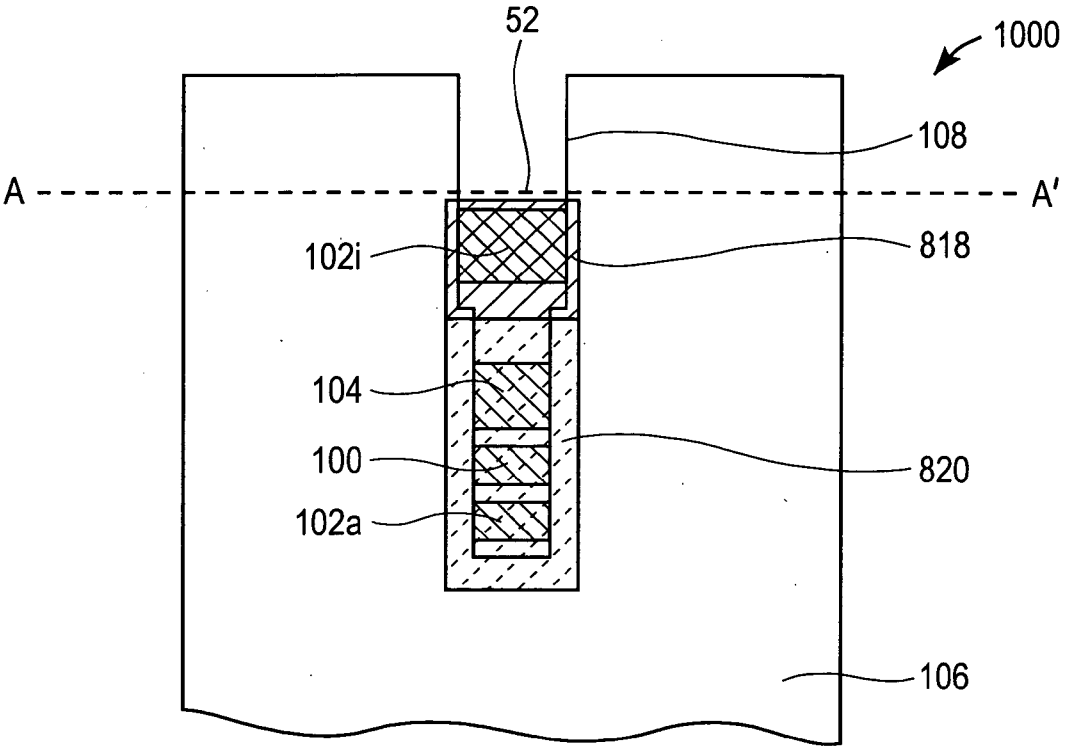
52
1000
108
A
A'
102i
818
104
100
820
102a
106

FIG. 23

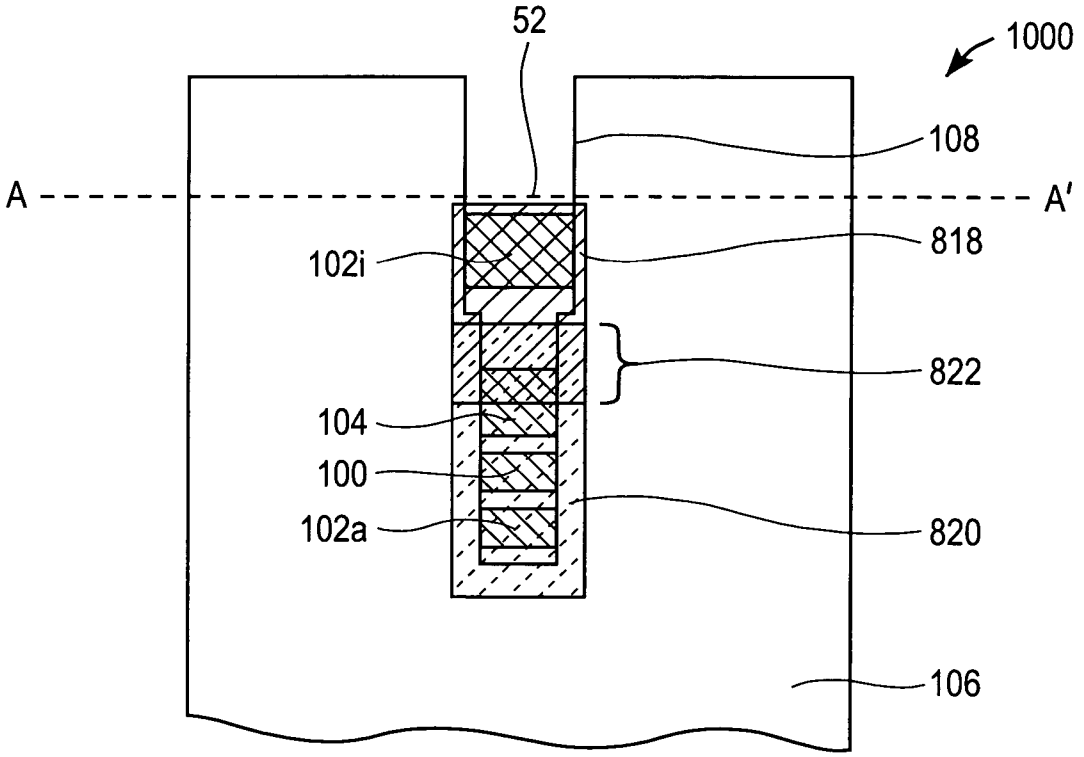

FIG. 24

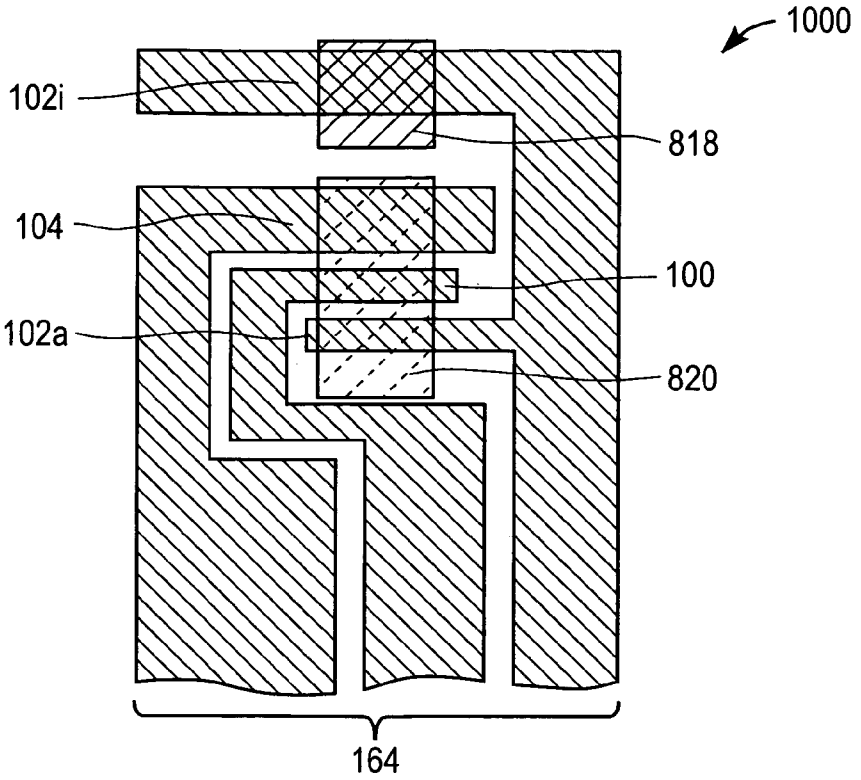
1000
102i
818
104
100
102a
820
164

FIG. 25

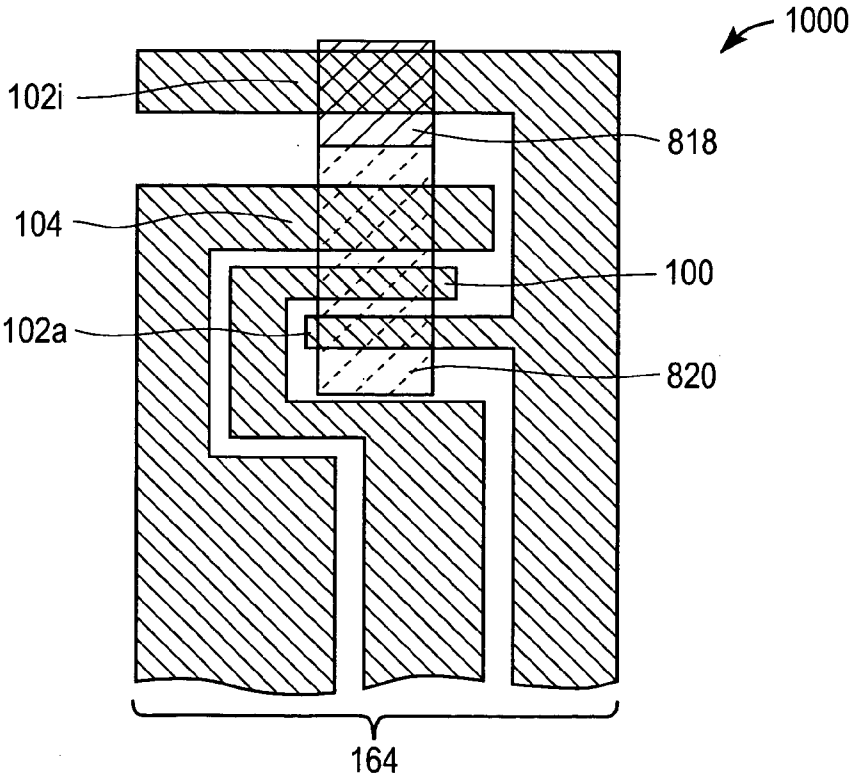
1000
102i
818
104
100
102a
820
164

FIG. 26

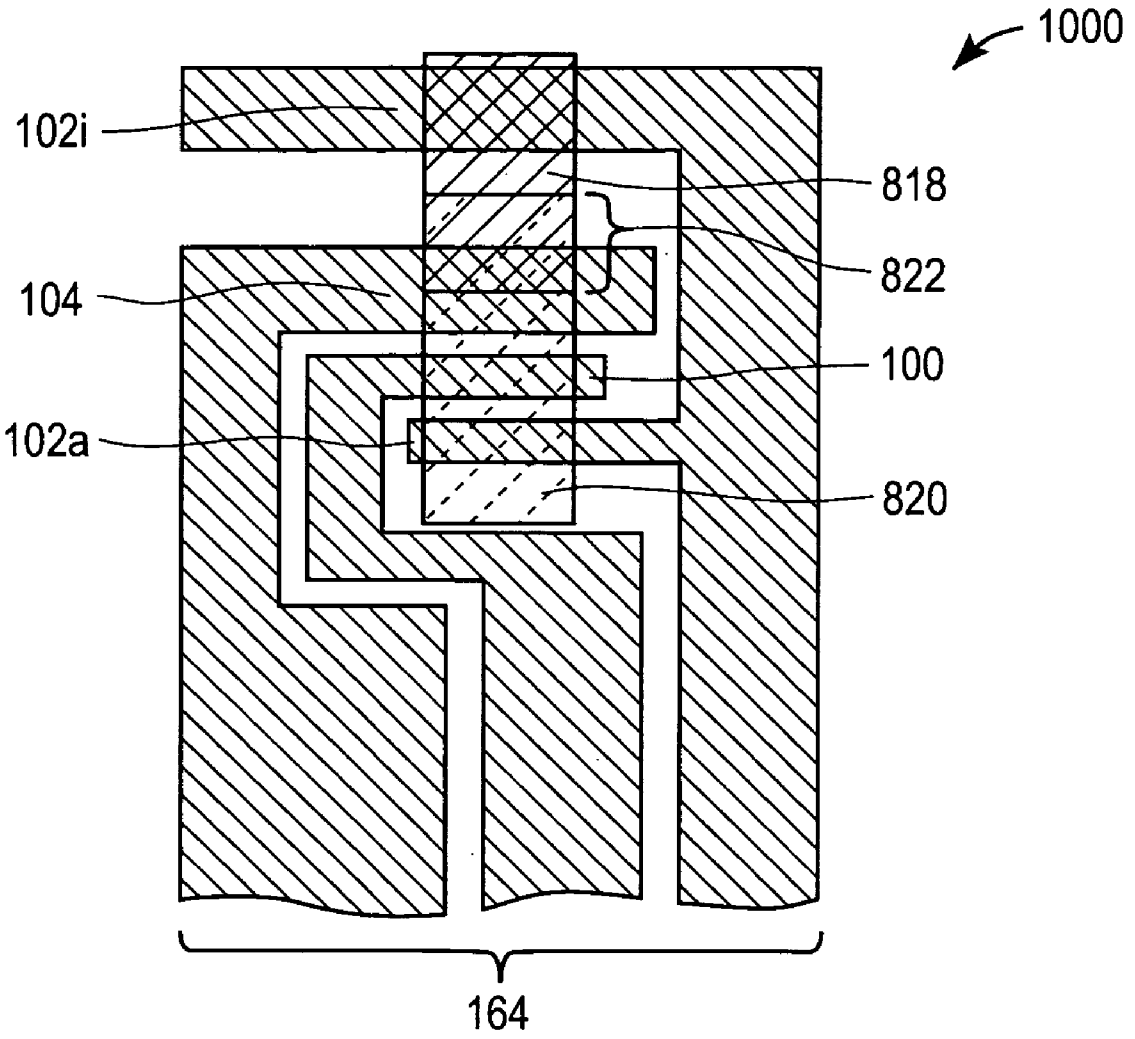

FIG. 27

ELECTROCHEMICAL TEST STRIP FOR REDUCING THE EFFECT OF DIRECT AND MEDIATED INTERFERENCE CURRENT

PRIORITY

[0001] The present invention claims priority to the following US Provisional Applications: U.S. Provisional Application Ser. No. 60/516,252 filed Oct. 31, 2003; U.S. Provisional Application Ser. No. 60/558,424 filed Mar. 31, 2004; and U.S. Provisional Application Ser. No. 60/558,728 filed Mar. 31, 2004, which applications are hereby incorporated herein by reference.

RELATED APPLICATIONS

[0002] The present invention is related to the following co-pending US Applications:

[0003] U.S. patent application Ser. No. _____ [Attorney Docket Number DDI-5027], filed on Oct. 29, 2004; U.S. patent application Ser. No. _____ [Attorney Docket Number DDI-5042], filed on Oct. 29, 2004; U.S. patent application Ser. No. _____ [Attorney Docket Number DDI-5064], filed on Oct. 29, 2004; U.S. patent application Ser. No. _____ [Attorney Docket Number DDI-5065], filed on Oct. 29, 2004; and U.S. patent application Ser. No. _____ [Attorney Docket Number DDI-5067], filed on Oct. 29, 2004.

FIELD OF THE INVENTION

[0004] The present invention is related, in general to electrochemical strips and systems which are designed to reduce the effect of interfering compounds on measurements taken by such analyte measurement systems and, more particularly, to an improved electrochemical strip for reducing the effects of direct interference currents and mediated interference currents in a glucose monitoring system wherein the electrochemical strip has electrodes with regions coated by active reagent and regions coated with inactive reagent.

BACKGROUND OF INVENTION

[0005] In many cases, an electrochemical glucose measuring system may have an elevated oxidation current due to the oxidation of interfering compounds commonly found in physiological fluids such as, for example, acetaminophen, ascorbic acid, bilirubin, dopamine, gentisic acid, glutathione, levodopa, methyldopa, tolazimide, tolbutamide, and uric acid. The accuracy of glucose meters may, therefore, be improved by reducing or eliminating the portion of the oxidation current generated by interfering compounds. Ideally, there should be no oxidation current generated from any of the interfering compounds so that the entire oxidation current would depend only on the glucose concentration.

[0006] It is, therefore, desirable to improve the accuracy of electrochemical sensors in the presence of potentially interfering compounds such as, for example, ascorbate, urate, and, acetaminophen, commonly found in physiological fluids. Examples of analytes for such electrochemical sensors may include glucose, lactate, and fructosamine. Although glucose will be the main analyte discussed, it will be obvious to one skilled in the art that the invention set forth herein may also be used with other analytes.

[0007] Oxidation current may be generated in several ways. In particular, desirable oxidation current results from the interaction of the mediator with the analyte of interest (e.g., glucose) while undesirable oxidation current is generally comprised of interfering compounds being oxidized at the electrode surface and by interaction with the mediator. For example, some interfering compounds (e.g., acetominophen) are oxidized at the electrode surface. Other interfering compounds (e.g., ascorbic acid) are oxidized by chemical reaction with the mediator. This oxidation of the interfering compound in a glucose measuring system causes the measured oxidation current to be dependent on the concentration of both the glucose and any interfering compound. Therefore, in the situation where the concentration of interfering compound oxidizes as efficiently as glucose and the interferent concentration is high relative to the glucose concentration, the measurement of the glucose concentration would be improved by reducing or eliminating the contribution of the interfering compounds to the total oxidation current.

[0008] One known strategy that can be used to decrease the effects of interfering compounds is to use a negatively charged membrane to cover the working electrode. As an example, a sulfonated fluoropolymer such as NAFION™ may be used to repel all negatively charged chemicals. In general, most interfering compounds such as ascorbate and urate have a negative charge, thus, the negatively charged membrane prevents the negatively charged interfering compounds from reaching the electrode surface and being oxidized at that surface. However, this technique is not always successful since some interfering compounds such as acetaminophen do not have a net negative charge, and thus, can pass through a negatively charged membrane. Nor would this technique reduce the oxidation current resulting from the interaction of interfering compounds with some mediators. The use of a negatively charged membrane on the working electrode could also prevent some commonly used mediators, such as ferricyanide, from passing through the negatively charged membrane to exchange electrons with the electrode.

[0009] Another known strategy that can be used to decrease the effects of interfering compounds is to use a size selective membrane on top of the working electrode. As an example, a 100 Dalton exclusion membrane such as cellulose acetate may be used to cover the working electrode to exclude all chemicals with a molecular weight greater than 100 Daltons. In general, most interfering compounds have a molecular weight greater than 100 Daltons, and thus, are excluded from being oxidized at the electrode surface. However, such selective membranes typically make the test strip more complicated to manufacture and increase the test time because the oxidized glucose must diffuse through the selective membrane to get to the electrode.

[0010] Another strategy that can be used to decrease the effects of interfering compounds is to use a mediator with a low redox potential, for example, between about −300 mV and +100 mV (when measured with respect to a saturated calomel electrode). Because the mediator has a low redox potential, the voltage applied to the working electrode may also be relatively low which, in turn, decreases the rate at which interfering compounds are oxidized by the working electrode. Examples of mediators having a relatively low redox potential include osmium bipyridyl complexes, fer-

rocene derivatives, and quinone derivatives. A disadvantage of this strategy is that mediators having a relatively low potential are often difficult to synthesize, unstable and have a low water solubility.

[0011] Another known strategy that can be used to decrease the effects of interfering compounds is to use a dummy electrode which is coated with a mediator. In some instances the dummy electrode may also be coated with an inert protein or deactivated redox enzyme. The purpose of the dummy electrode is to oxidize the interfering compound at the electrode surface and/or to oxidize the mediator reduced by the interfering compound. In this strategy, the current measured at the dummy electrode is subtracted from the total oxidizing current measured at the working electrode to remove the interference effect. A disadvantage of this strategy is that it requires that the test strip include an additional electrode and electrical connection (i.e., the dummy electrode) which cannot be used to measure glucose. The inclusion of dummy electrode is an inefficient use of an electrode in a glucose measuring system.

SUMMARY OF INVENTION

[0012] The present invention is directed to an electrochemical sensor or electrochemical strip which includes a substrate, a first working electrode disposed on the substrate, a second working electrode disposed on the substrate, a reference electrode, an active reagent layer disposed on the first working electrode, wherein the active reagent layer completely covers the first working electrode and an inactive reagent layer disposed on the second working electrode, wherein the inactive reagent completely covers the second working electrode. The present invention further includes an electrochemical sensor wherein the first working electrode, the second working electrode and the reference electrode are positioned in a sample receiving chamber, the sample receiving chamber has a proximal and a distal end, the distal end including a first opening which is adapted to receive bodily fluids and the second working electrode being positioned adjacent the first opening. The present invention further includes an electrochemical sensor wherein the first working electrode and the reference electrode are positioned proximal to the second working electrode.

[0013] The present invention is also directed to an electrochemical sensor an electrochemical sensor including a substrate, a first working electrode disposed on the substrate, a second working electrode disposed on the substrate, a reference electrode, an active reagent layer disposed on the first working electrode, wherein the active reagent layer completely covers the first working electrode, the second working electrode having an active region and an inactive region, the active reagent layer disposed on an active region of the second working electrode and an inactive reagent layer disposed on the inactive region of the second working electrode. The present invention further includes an electrochemical sensor wherein the first working electrode, the second working electrode and the reference electrode are positioned in a sample receiving chamber, the sample receiving chamber having a proximal and a distal end, the distal end including a first opening which is adapted to receive bodily fluids and the inactive region of the second working electrode being positioned adjacent the first opening. The present invention further includes an electrochemical sensor wherein the active region of the second working electrode and the first working electrode are positioned proximal to the inactive region of the second working electrode.

BRIEF DESCRIPTION OF DRAWINGS

[0014] A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings, of which:

[0015] **FIG. 1** is an exploded perspective view of a test strip according to an exemplary embodiment of the present invention;

[0016] **FIG. 2** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 1** including a conductive layer and an insulation layer;

[0017] **FIG. 3** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 1**, wherein the position of an active and an inactive reagent layer do not touch each other and are illustrated with the insulation and conductive layer;

[0018] **FIG. 4** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 1**, wherein the position of the active and the inactive reagent layer are immediately adjacent to each other and are illustrated with the insulation and conductive layer;

[0019] **FIG. 5** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 1**, wherein the position of the active and the inactive reagent layer that overlap with each other and are illustrated with the insulation and conductive layer;

[0020] **FIG. 6** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 1**, wherein the active and the inactive reagent layer do not touch each other and are illustrated with the conductive layer;

[0021] **FIG. 7** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 1**, wherein the active and the inactive reagent layer are immediately adjacent to each other and are illustrated with the conductive layer;

[0022] **FIG. 8** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 1**, wherein the active and the inactive reagent layer overlap with each other and are illustrated with the conductive layer;

[0023] **FIG. 9** is a simplified schematic showing a meter interfacing with a test strip that has a first contact, second contact, and reference contact disposed on a substrate;

[0024] **FIG. 10** is a simplified schematic showing a meter interfacing with a test strip that has a first contact and a second contact disposed on a substrate and a reference contact which is orientated in a facing orientation with the first contact and second contact;

[0025] **FIG. 11** is a graph showing the effects of gamma radiation on precision for test strips tested at a 20 mg/dL glucose concentration;

[0026] **FIG. 12** is a graph showing the effects of gamma radiation on precision for test strips tested at a 50 mg/dL glucose concentration;

[0027] **FIG. 13** is a graph showing the effects of gamma radiation on precision for test strips tested at a 100 mg/dL glucose concentration;

[0028] **FIG. 14** is a graph showing the effects of gamma radiation on precision for test strips tested at a 300 mg/dL glucose concentration;

[0029] **FIG. 15** is a graph showing the effects of gamma radiation on precision for test strips tested at a 500 mg/dL glucose concentration;

[0030] **FIG. 16** is a graph showing the effects of gentisic acid on accuracy for test strips tested at a 70 mg/dL glucose concentration;

[0031] **FIG. 17** is a graph showing the effects of gentisic acid on accuracy for test strips tested at a 240 mg/dL glucose concentration;

[0032] **FIG. 18** is a graph showing the effects of uric acid on accuracy for test strips tested at a 70 mg/dL glucose concentration;

[0033] **FIG. 19** is a graph showing the effects of uric acid on accuracy for test strips tested at a 240 mg/dL glucose concentration;

[0034] **FIG. 20 a** simplified plane view of a distal portion of a test showing a modified cutout that allows the area of a second working electrode to be increased;

[0035] **FIG. 21** is an exploded perspective view of a test strip according to another exemplary embodiment of the present invention;

[0036] **FIG. 22** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 21**, wherein the position of an active and an inactive reagent layer do not touch each other and are illustrated with the insulation and conductive layer;

[0037] **FIG. 23** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 21**, wherein the position of the active and the inactive reagent layer are immediately adjacent to each other and are illustrated with the insulation and conductive layer;

[0038] **FIG. 24** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 21**, wherein the position of the active and the inactive reagent layer that overlap with each other and are illustrated with the insulation and conductive layer;

[0039] **FIG. 25** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 21**, wherein the active and the inactive reagent layer do not touch each other and are illustrated with the conductive layer;

[0040] **FIG. 26** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 21**, wherein the active and the inactive reagent layer are immediately adjacent to each other and are illustrated with the conductive layer; and

[0041] **FIG. 27** is a simplified plane view of a distal portion of a test strip according to the embodiment of the present invention illustrated in **FIG. 21**, wherein the active and the inactive reagent layer overlap with each other and are illustrated with the conductive layer;

DETAILED DESCRIPTION OF THE INVENTION

[0042] The invention described herein includes a test strip to improve the accuracy of a glucose measurement in the presence of interfering compounds. Under certain circumstances, a type of interfering compound may develop in the test strip itself before bodily fluid such as, for example, blood is added. An example this type of interfering compound may be a reduced mediator (e.g. ferrocyanide) which develops from the conversion of an oxidized mediator (e.g. ferricyanide). This causes the background signal to increase which, in turn, decreases the accuracy of the test strip measurement. It should be noted that in this circumstance the interfering compound develops in the test strip itself as opposed to being provided to the test strip in the form of a bodily fluid.

[0043] Typically, an oxidized mediator is disposed on a working electrode with the intent that the oxidized mediator will be stable and not transition over to the reduced redox state. The generation of reduced mediator causes the background signal to increase for electrochemical sensors which use an oxidation current to correlate with the glucose concentration. In general, ferricyanide (e.g. oxidized mediator) tends to become reduced over time to the reduced redox state. Ferricyanide generally transitions to the reduced redox state more rapidly when exposed to environmental conditions which include but are not limited to, basic pH, elevated temperature, elevated humidity, bright light conditions, electron beam radiation, and gamma radiation.

[0044] Recently, a lance and a test strip have been integrated into a single medical device. These integrated medical devices can be employed, along with an associated meter, to monitor various analytes, including glucose. Depending on the situation, test strips can be designed to monitor analytes in an episodic single-use format, semi-continuous format, or continuous format. The integration of the lance and the test strip simplifies a monitoring procedure by eliminating the need for a user to coordinate the extraction of a bodily fluid from a sample site with the subsequent transfer of that bodily fluid to the test strip. In such a case, the lance and test strip must be sterilized together so as to mitigate the risk of infection.

[0045] Ionizing radiation may be used to sterilize test strips with a lance. Possible sources of ionizing radiation are electron beam, gamma, and x-ray. However, one of the challenges in sterilizing a test strip is to provide a sufficiently high intensity of radiation such that a sufficiently high proportion of microorganisms are neutralized for an entire package of test strips, while at the same time not adversely affecting the reagent layer. Typically, a batch or package of test strips are exposed to an ionizing radiation dose ranging

from about 10 KGy to about 50 KGy. For the case using e-beam sterilization, the energy of the incident e-beam source can range from about 3 MeV to about 12 MeV. The impingent ionizing radiation may often have some non-uniformities in its intensity causing a particular portion of the package to receive more ionizing radiation than another portion of the package. Experiments have shown that both gamma radiation and electron beam radiation cause the background signal of the electrochemical sensors to increase. Furthermore, the relatively non-uniform nature of the radiation causes the background signal to increase in a non-uniform nature for a sterilized batch of test strips. This causes the precision to decrease when testing a particular batch of sterilized glucose test strips. In addition, the decrease in precision is exacerbated at the low glucose concentration range (e.g. about 20 mg/dL to about 100 mg/dL) because the proportion of reduced mediator is relatively high with respect to the low glucose concentration range.

[0046] **FIG. 1** shows an exploded perspective view of a test strip **800** that is designed to compensate for the variations in increased background potentially caused by the conversion of oxidized mediator to reduced mediator. In the embodiment of the present invention illustrated in **FIG. 1**, an electrochemical test strip **800**, which may be used for measuring glucose concentration in bodily fluids such as blood or interstitial fluid, includes a first working electrode **808**, a second working electrode **806**, and a reference electrode **810**. An active reagent layer **820** is disposed on first working electrode **808** and reference electrode **810** where active reagent layer **820** completely covers first working electrode **808** and at least partially covers reference electrode **810**. An inactive reagent layer **818** is disposed on second working electrode **806**.

[0047] In an embodiment of this invention, active reagent layer **820** may include, for example, glucose oxidase and a mediator such as, for example, ferricyanide. Inactive reagent layer **818** may include a mediator, but no active enzymes which are specific for the analyte of interest. Because ferricyanide has a redox potential of approximately 400 mV (when measured with respect to a saturated calomel electrode) at a carbon electrode, the introduction of a bodily fluid e.g., blood may generate a significant and undesirable oxidation of interferents by the mediator and/or the working electrode. Therefore, the oxidation current measured at first working electrode **808** will be a superposition of oxidation current sources: a first, desirable, oxidation current generated by the oxidation of glucose; a second, undesirable, direct oxidation of interferents at the electrode (direct interference current); and a third, undesirable, indirect oxidation of interferents via a mediator (mediated interference current). The oxidation current measured at second working electrode **806** will also be a superposition of oxidation current sources similar to first working electrode **808**, but the first, desirable, oxidation current should not occur because there is no enzyme present on second working electrode **806**. Because the oxidation current measured at second working electrode **806** depends only on interferents, and the oxidation current measured at first working electrode **808** depends on glucose and interferents, it is possible to calculate a corrected glucose current which is independent to the effects of interfering compounds oxidized at first working electrode **808** and second working electrode **806**. In such a case, the current density of first working electrode **808** is subtracted from the current density of the second working electrode **806** to calculate a corrected glucose current density G where

$$G=W_E-WE_2 \quad \text{(Eq 8)}$$

[0048] where WE_1 is the current density at first working electrode **808** and WE_2 is the current density at second working electrode **806**.

[0049] In an alternative embodiment to this invention, the interferent oxidation current density at second working electrode **806** may be slightly different than the interferent oxidation current density at first working electrode **808** because there is no enzyme on second working electrode **806**. In such a case, a constant K can be used to correct for such non-idealities in the correction method. Equation 9 shows how constant K would modify the previously described Equation 8.

$$G=WE_1-(K\times WE_2) \quad \text{(Eq 9)}$$

[0050] where K can range from about 0.5 to about 1.5.

[0051] Test strip **800** includes a substrate **50**, a conductive layer **802**, an insulation layer **804**, inactive reagent layer **818**, active reagent layer **820**, an adhesive layer **830**, and a top layer **824**. Test strip **800** may be manufactured by sequentially printing five layers which are conductive layer **802**, insulation layer **804**, inactive reagent layer **818**, active reagent layer **820**, and adhesive layer **830** onto substrate **50**. Top layer **824** may be assembled by a lamination process. Test strip **800** further includes a first side **54**, a second side **56**, a distal portion **58**, and a proximal portion **60**.

[0052] In one embodiment of the present invention, substrate **50** is an electrically insulating material such as plastic, glass, ceramic, and the like. In an embodiment of this invention, substrate **50** may be a plastic such as, for example, nylon, polycarbonate, polyimide, polyvinylchloride, polyethylene, polypropylene, PETG, or polyester. More particularly the polyester may be, for example Melinex® ST328 which is manufactured by DuPont Teijin Films. Substrate **50** may also include an acrylic coating which is applied to one or both sides to improve ink adhesion.

[0053] The first layer deposited on substrate **50** is conductive layer **802** which includes first working electrode **808**, second working electrode **806**, reference electrode **810**, and strip detection bar **17**. In accordance with the present invention, a screen mesh with an emulsion pattern may be used to deposit a material such as, for example, a conductive carbon ink in a defined geometry as illustrated in **FIG. 1**. Conductive layer **802** may be disposed on substrate **50** by using screen printing, rotogravure printing, sputtering, evaporation, electroless plating, ink jetting, sublimation, chemical vapor deposition, and the like. Suitable materials which may be used for conductive layer **802** are Au, Pd, Ir, Pt, Rh, stainless steel, doped tin oxide, carbon, and the like. In an embodiment of this invention, the carbon ink layer may have a height between 1 and 100 microns, more particularly between 5 and 25 microns, and yet even more particularly at approximately 13 microns. The height of conductive layer **802** can vary depending on the desired resistance and conductivity of the printed material.

[0054] A first contact **814**, a second contact **812**, and a reference contact **816** may be used to electrically interface with a meter. This allows the meter to electrically commu-

nicate to first working electrode **808**, second working electrode **806**, and reference electrode **810** via, respective, first contact **814**, second contact **812**, and reference contact **816**.

[**0055**] The second layer deposited on substrate **50** is insulation layer **804**. Insulation layer **804** is disposed on at least a portion of conductive layer **802** as shown in **FIGS. 1 and 2**. **FIG. 2** is a simplified plane view of distal portion **58** of test strip **800** which highlights the position of first working electrode **808**, second working electrode **806**, and reference electrode **810** with respect to insulation layer **804**. Insulation layer **804** further includes a cutout **18** which may have a rectangular shaped structure as shown in **FIG. 1** and **2**. Cutout **18** exposes a portions of first working electrode **808**, second working electrode **806**, and reference electrode **810** which can be wetted with liquid. Cutout **18** includes a cutout width **W20** and a cutout length **L26**. Cutout width **W20** corresponds to a width of second working electrode **806**, reference electrode **810**, and first working electrode **808** as illustrated in **FIG. 2**. In an embodiment of this invention, cutout width **W20** may range from about 0.7 mm to about 1.4 mm, and cutout length **L26** may range from about 0.4 mm and about 3.4 mm.

[**0056**] In one embodiment of the present invention, second working electrode **806** and first working electrode **808** have a respective length of **L20** and **L21** which may be the same and range from about 0.1 mm to about 0.8 mm. Reference electrode **810** may have a length **L24** which may range from about 0.2 mm to about 1.6 mm. In accordance with the present invention, electrode spacing **S1** is a distance between second working electrode **806** and reference electrode **810**; and between reference electrode **810** and first working electrode **808** which may range from about 0.2 mm to about 0.6 mm.

[**0057**] In an alternative embodiment of the present invention, an area of first working electrode **808** may be different than an area of second working electrode **806**. A ratio of first working electrode **808** area:second working electrode **806** area may range from about 1:1 to about 1:3. Under certain situations, the reduction in background can be improved by increasing the relative area of second working electrode **806**. The area of second working electrode **806** may be increased by modifying the geometry of a cutout **6008** as shown in **FIG. 20**.

[**0058**] **FIG. 2** shows that strip **800** may be cut along incision line A-A' after it is fully laminated as illustrated in **FIG. 1**. In the process of cutting test strip **800** along incision line A-A' as illustrated in **FIG. 1**, a sample inlet **52** is created in which a liquid sample can be applied for dosing test strip **800**.

[**0059**] FIGS. **3** to **5** are a simplified plane view of distal portion **58** of test strip **800** according to the embodiment of the present invention illustrated in **FIG. 1**, which show various positions of active reagent layer **820** and inactive reagent layer **818** with respect to each other. FIGS. **6** to **8**, which correspond to FIGS. **3** to **5** respectively, do not show insulation layer **804** to help demonstrate more clearly the relationship between the conductive layer **802**, active reagent layer **820**, and inactive reagent layer **818**.

[**0060**] Test strip **800** may have inactive reagent layer **818** disposed on second working electrode **806** such that it completely covers second working electrode **806** as is illustrated in FIGS. **3** to **5**. In one embodiment of this invention, inactive reagent layer **818** completely covers second working electrode **806**, but does not touch reference electrode **810** as is illustrated in **FIGS. 3 and 4**. In another embodiment of this invention, inactive reagent layer **818** completely covers second working electrode **806** and at least partially covers reference electrode **810** as is illustrated in **FIG. 5**.

[**0061**] In an embodiment of this invention, inactive reagent layer **818** includes at least an oxidized mediator, such as ferricyanide, and may optionally include an inert protein or inactivated enzyme. Inactive reagent layer **818** may further include a citrate buffer at pH 6, a polyvinyl alcohol, a polyvinyl pyrrolidone-vinyl acetate, a Dow Corning DC1500 antifoam, a hydroxyethyl cellulose (Natrosol 250G, Hercules), and a surface modified silica (Cab-o-sil TS 610, Cabot) having both hydrophilic and hydrophobic domains. Examples of oxidized mediators may be ferricyanide, ferricinium complexes, quinone complexes, and osmium complexes. Examples of inert protein may be crotein or albumin (e.g. bovine or human). Examples of inactivated enzyme may be the apo form of PQQ-glucose dehydrogenase (where PQQ is an acronym for pyrroloquinoline-quinone) or apo glucose oxidase (e.g. enzyme with no active site). Enzyme may also be deactivated or sufficiently attenuated by heat treatment or by treatment with denaturing agents such as urea. Because inactive reagent layer **818** does not include an active enzyme, the oxidation current measured at second working electrode **806** is not proportional to the glucose concentration. For this reason, one skilled in the art may refer to second working electrode **806** as a dummy electrode.

[**0062**] In an embodiment of this invention, the inert protein or deactivated enzyme in inactive reagent layer **818** may act as a stabilizer for the mediator. The inert protein or deactivated enzyme may shield the mediator during the drying process at elevated temperature. In addition, the inert protein or deactivated enzyme may act as a desiccant which helps protect the mediator from moisture that may potentially destabilize the mediator.

[**0063**] Test strip **800** has active reagent layer **820** disposed on first working electrode **808** as illustrated in FIGS. **3** to **5**. In another embodiment of this invention, active reagent layer **820** completely covers first working electrode **808**, but does not touch reference electrode **810**. In another embodiment of this invention, active reagent layer **820** completely covers first working electrode **808** and at least partially covers reference electrode **810** as illustrated in FIGS. **3** to **5**.

[**0064**] In an embodiment of this invention, active reagent layer **820** includes at least an oxidized mediator, and an enzyme. Active reagent layer **820** may further include a citrate buffer at pH 6, a polyvinyl alcohol, a polyvinyl pyrrolidone-vinyl acetate, a Dow Corning DC1500 antifoam, a hydroxyethyl cellulose (Natrosol 250G, Hercules), and a surface modified silica (Cab-o-sil TS 610, Cabot) having both hydrophilic and hydrophobic domains. Examples of oxidized mediators may be ferricyanide, ferricinium complexes quinone complexes, and osmium complexes. Examples of the enzyme may be glucose oxidase, glucose dehydrogenase using a PQQ co-factor, and glucose dehydrogenase using a nicotinamide adenine dinucleotide co-factor. Because active reagent layer **820** does include the

enzyme, the oxidation current measured at first working electrode **808** is proportional to the glucose concentration.

[**0065**] It should be noted that if screen printing were used for depositing both inactive reagent layer **818** and active reagent layer **820**, then two separate screen printing steps would be required to deposit the respective reagent layers onto the appropriate electrode(s). It should be noted that screen printing is not well-suited for printing two discrete reagents on the same screen. The squeegee motion during printing may cause the two respective reagents to mix during the screen printing process. **FIG. 3** shows an embodiment of this invention which has inactive reagent layer **818** disposed on second working electrode **806**, and active reagent layer **820** disposed on first working electrode **808** and reference electrode **810**. In this embodiment, inactive reagent layer **818** does not touch or overlap with active reagent layer **820**. Because the area of second working electrode **806**, first working electrode **808** and reference electrode **810** is relatively small, it can be difficult to sequentially align and coat inactive reagent layer **818** and active reagent layer **820**, respectively, with the desired yield. It should also be noted that relatively small electrode areas (e.g. about 0.6 mm^2) are preferred because this allows the volume of liquid sample required for a test strip to be small.

[**0066**] In an embodiment of this invention, inactive reagent layer **818** is printed first and then dried at an elevated temperature. Active reagent layer **820** is then subsequently printed followed by another drying step at an elevated temperature as described in International Application serial number PCT/GB/03004708 which is hereby incorporated by reference herein. Because active reagent layer **820** is deposited second, it is exposed to only one drying step as opposed to the two drying steps for inactive reagent layer **818**. This helps stabilize both mediator and enzyme within active reagent layer **820** because under certain conditions enzymes can degrade with continued exposure to elevated temperatures.

[**0067**] In an embodiment of this invention, **FIG. 4** shows inactive reagent layer **818** disposed on second working electrode **806**, and active reagent layer **820** disposed on first working electrode **808** and reference electrode **810**. In this embodiment inactive reagent layer **818** and active reagent layer **820** are immediately adjacent to each other. In such a case, the inactive reagent layer **818** and active reagent layer **820** would touch, but typically not overlap with each other to any significant extent. Although the printing process targets the alignment such that inactive reagent layer **818** and active reagent layer **820** are immediately adjacent to each other, normal manufacturing variation will cause some overlap to occur with a certain frequency between inactive reagent layer **818** and active reagent layer **820**. Likewise, such variation will also cause inactive reagent layer **818** to sometimes not touch active reagent layer **820**. Because inactive reagent layer **818** was allowed to touch or not touch active reagent layer **820** and the operation of the method of the invention still works to reduce the variation in the background in either circumstance, the yield of acceptable test strips was improved.

[**0068**] It should be noted that the overlap of inactive reagent layer **818** with active reagent layer **820** does not affect the glucose measurement as long as the enzyme from active reagent layer **820** cannot diffuse, to any significant extent in the time allowed for the measurement (i.e. about 5 seconds or less), to second working electrode **806**. If enzyme were to diffuse to second working electrode **806**, then first working electrode **808** would measure a glucose current in addition to the non-enzyme specific currents. This would prevent test strip **800** from effectively reducing the background signal.

[**0069**] It should also be noted that if the overlap of inactive reagent layer **818** with active reagent layer **820** were to occur on reference electrode **810** that this would not affect the glucose measurement. In such a case, the amount of enzyme and/or oxidized mediator on reference electrode **810** will increase, but should not affect the glucose measurement or the background correction algorithm.

[**0070**] Yet another embodiment of this invention which improves upon the method of coating inactive reagent layer **818** and active reagent layer **820** is shown in **FIG. 5** Inactive reagent layer **818** may be coated such that it completely covers second working electrode **806** and a portion of reference electrode **810**. Similarly, active reagent layer **820** may be coated such that it completely covers first working electrode **808** and at least a portion of reference electrode **810**. In an embodiment of this invention, the printing process can target the alignment such that inactive reagent layer **818** and active reagent layer **820** substantially overlap with each other on reference electrode **810** at an overlap zone **822**. In such a case, inactive reagent layer **818** and active reagent layer **820** may mix with each other at overlap zone **822**. Because the length of both inactive reagent layer **818** and active reagent layer **820** was further increased compared to the embodiment described in **FIG. 4**, the alignment and coating of active reagent layer **820** and inactive reagent layer **818** to first working electrode **808** and second working electrode **806** was yet further improved.

[**0071**] It should be noted that second working electrode **806** (e.g. dummy electrode) is located on distal portion **58** of test strip **800** as illustrated in FIGS. **1** to **5**. This causes the physiological fluid to sequentially wet in the following order—second working electrode **806**, reference electrode **810**, and then first working electrode **808**. Test strip **800** was purposefully designed to have inactive reagent layer **818** (which contains no enzyme) upstream of active reagent layer **820** (which does contain enzyme). This reduces the possibility of enzyme being present at both second working electrode **806** and first working electrode **808**. If active reagent layer **820**, which contains enzyme, was coated over second working electrode **806**, and no enzyme were present over first working electrode **808** then it would be possible that some enzyme could be swept to first working electrode **808** from second working electrode **806**. The presence of a significant amount of enzyme on first working electrode **808** would prevent the background signal from being reduced through the use of the dummy electrode format.

[**0072**] In an embodiment of this invention, top layer **824** may be in the form of an integrated lance **826** as shown in **FIG. 1**. In such an embodiment, top layer **824** may include a lance **826** which is located at distal portion **58**. Lance **826**, which may also be referred to as a penetration member, may be adapted to pierce a user's skin and draw blood into test strip **800** such that second working electrode **806**, first working electrode **808**, and reference electrode **810** are wetted. Top layer **824** is adhered to test strip **800** by adhesive

layer **830**. This adhesive layer **830** can be a heat seal or a pressure sensitive adhesive. Lance **826** includes a lancet base **832** that terminates at distal portion **58** of assembled test strip **800**. Lance **826** may be made with either an insulating material such as plastic, glass, and silicon, or a conducting material such as stainless steel and gold. For the case in which top layer **824** is conductive, top layer **824** may also be used as a reference electrode **810** which is orientated with a facing relationship to second working electrode **806** and first working electrode **808**. Further descriptions of integrated medical devices that use an integrated lance can be found in International Application No. PCT/GB01/05634 and U.S. patent application Ser. No. 10/143,399 which are hereby fully incorporated by reference herein. In addition, lance **826** can be fabricated, for example, by a progressive die-stamping technique, as disclosed in the aforementioned International Application No. PCT/GB01/05634 and U.S. patent application Ser. No. 10/143,399 which are hereby fully incorporated by reference herein.

[**0073**] In an embodiment of the present invention, adhesive layer **830** has a height of about 70 to 110 microns. Adhesive layer **830** may include a double sided pressure sensitive adhesive, a UV cured adhesive, heat activated adhesive, thermosetting plastic, or other adhesive known to those skilled in the art. As a non-limiting example, adhesive layer **830** may be formed by screen printing a pressure sensitive adhesive such as, for example, a water based acrylic copolymer pressure sensitive adhesive which is commercially available from Tape Specialties LTD in Tring, Herts, United Kingdom (part#A6435).

[**0074**] In a method of this invention, the background variations are reduced by subtracting a first current from first working electrode **808** from a second current from second working electrode **806**. To initiate a test, a sample is applied to sample inlet **52** which allows a current to be measured at second working electrode **806** and first working electrode **808**. Because second working electrode **806** does not have a glucose oxidizing enzyme disposed thereon, a magnitude of an oxidation current at second working electrode **806** is proportional to an amount of interfering compounds present on test strip **800** and also an amount of interfering compounds originating from the sample. This allows a corrected current value to be calculated using a difference between first working electrode **808** and second working electrode **806** to reduce the effects of interfering compounds present in the sample and also for interfering compounds that may be present on test strip **800**.

[**0075**] **FIG. 9** is a simplified schematic showing a meter **900** interfacing with test strip **800**. Meter **900** has at least three electrical contacts that form an electrical connection to second working electrode **806**, first working electrode **808**, and reference electrode **810**. In particular second contact **812** and reference contact **816** connect to first voltage source **910**; first contact **814** and reference contact **816** connect to second voltage source **920**. When performing a test, first voltage source **910** applies a first potential E1 between second working electrode **806** and reference electrode **810**; and second voltage source **920** applies a second potential E2 between first working electrode **808** and reference electrode **810**.

[**0076**] In one embodiment of this invention, first potential E1 and second potential E2 may be the same such as for example about +0.4 V. In another embodiment of this invention, first potential E1 and second potential E2 may be different. A sample of blood is applied such that second working electrode **806**, first working electrode **808**, and reference electrode **810** are covered with blood. This allows second working electrode **806** and first working electrode **808** to measure a current which is proportional to glucose and/or non-enzyme specific sources. After about 5 seconds from the sample application, meter **900** measures an oxidation current for both second working electrode **806** and first working electrode **808**.

[**0077**] **FIG. 10** is a simplified schematic showing a meter **900** interfacing with test strip **800**. In contrast to **FIG. 9**, top layer **824** is conductive and used as a reference electrode instead of reference electrode **810** which is disposed on substrate **50**. More particularly, **FIG. 10** shows that top layer **824**, in the form of a reference electrode, has a facing relationship with first working electrode **808** and second working electrode **806**. In this case, meter **900** forms an electrical contact to top layer **824** instead of at reference contact **816** as is shown in **FIG. 1**.

[**0078**] **FIG. 21** is an exploded perspective view of a test strip **1000** according to another embodiment of the present invention. The oxidation current measured at a first working electrode **100** will be a superposition of oxidation current sources: a first, desirable, oxidation current generated by the oxidation of glucose and a second, undesirable, oxidation current generated by the interferents. The oxidation of interferents may occur directly at first working electrode **100** and indirectly through a mediated mechanism via a mediator.

[**0079**] Second working electrode **102** has a geometric trace that has an active portion **102***a* which is coated with active reagent **820** and an inactive portion **102***i* which is coated with inactive reagent **818** as illustrated in FIGS. **22** to **27**. The oxidation current sources measured at active portion **102***a* will be similar to first working electrode **100**. Inactive portion **102***i* of second working electrode **102** will oxidize interferents and not oxidize glucose because there is no enzyme present. Further, inactive portion **102***i* will oxidize interferents directly at second working electrode **102** and indirectly through a mediated mechanism via a mediator. Because the oxidation current measured at inactive portion **102***i* does not depend on glucose and the area of inactive portion **102***i* is known, it is possible to calculate its contribution to the interferent oxidation current measured at second working electrode **102**. In turn, using the interferent oxidation current calculated for inactive portion **102***i* and knowing the area of first working electrode **100** and the area of active portion **102***a*, it is possible to calculate a corrected glucose current which accounts for the effects of interfering compounds oxidized at the electrode. It should be noted that in the present invention, inactive portion **102***i* helps correct the glucose current for direct and mediated interference oxidation. It should also be noted that inactive portion **102***i* and active portion **102***a* may sometimes by referred to as an inactive region and an active region, respectively.

[**0080**] An algorithm may, therefore be used to calculate a corrected glucose current that is independent of interferences. After dosing a sample onto test strip **1000**, a constant potential is applied to first working electrode **100** and second working electrode **102** and a current is measured for both

electrodes. At first working electrode **100** where active reagent layer **820** covers the entire electrode area, the following equation can be used to describe the components contributing to the oxidation current,

$$WE_1=G+I_{1a} \quad \text{(Eq 1)}$$

[**0081**] where WE_1 is a current density at the first working electrode, G is a current density due to glucose which is independent of interferences, and I_{1a} is a current density due to interferences oxidized at first working electrode **100** which is covered with active reagent **820**.

[**0082**] At second working electrode **102** which is partially covered with active reagent **820** and inactive reagent **818**, the following equation can be used to describe the components contributing to the oxidation current,

$$WE_2=G+I_{2a}+I_{2i} \quad \text{(Eq 2)}$$

[**0083**] where WE_2 is a current density at the second working electrode, I_{2a} is a current density due to interferences at the active portion **102***a,* and I^{2i} is a current density due to interferences at inactive portion **102***i.*

[**0084**] To reduce the effects of interferences, an equation is formulated which describes the relationship between the interferent current at active portion **102***a* and inactive portion **102***i.* It is approximated that the interferent oxidation current density measured at active portion **102***a* is the same as the current density measured at the inactive portion **102***i.* This relationship is further described by the following equation,

$$I_{2a} = \frac{A_{2a}}{A_{2i}} \times I_{2i} \quad \text{(Eq 3a)}$$

[**0085**] where A_{2a} is an area of second working electrode covered with active reagent layer **820** and A_{2i} is an area of second working electrode covered with inactive reagent layer **818**.

[**0086**] Inactive portion **102***i* can oxidize interferents, but not glucose because it is not coated with enzyme. Active portion **102***a* can oxidize glucose and interferents. Because it was experimentally found that inactive portion **102***i* oxidizes interferents in a manner proportional to the area of active portion **102***a,* it is possible to predict the proportion of interferent current measured overall at second working electrode **102**. This allows the overall current measured at second working electrode **102** (i.e. WE_2) to be corrected by subtracting the contribution of the interferent current. In an embodiment of the present invention the ratio of A_{2i}:A_{2a} may be between about 0.5:1 to 5:1, and is preferably about 3:1. More details describing this mathematical algorithm for current correction will be described in the subsequent sections.

[**0087**] In an alternative embodiment of the present invention, I_{2a} may be different than I_{2i}. This may be ascribed to a more efficient or less efficient oxidation of interferents at the active portion **102***a* because of the presence of enzyme. For not well described reasons, it is possible that the presence of enzyme may affect the electrode's ability to oxidize mediator and/or interferents. This behavior may be phenomenologically modeled by re-writing Equation 3a to the following form,

$$I_{2a}=f\times I_{2i} \quad \text{(Eq 3b)}$$

[**0088**] where f is a correction factor which incorporates the effects of the interferent oxidation efficiency of the active portion **102***a* to inactive portion **120***i.*

[**0089**] In an embodiment of the present invention, Equation 1, 2, and 3a may be manipulated to derive an equation that outputs a corrected glucose current density independent of interferences. It should be noted that the three equations (Equation 1, 2, and 3a) collectively have **4** unknowns which are G, I_{2i}, I_{2a}, and I_{1a}. However, I_{1a} and I_{2a} can be conservatively assumed to be equal because they are measured at the same conductive material and coated with the same active reagent layer **820**. Equation 1 can be rearranged to the following form.

$$G=WE_1-I_{1a}=WE_1-I_{2a} \quad \text{(Eq 4)}$$

[**0090**] Next, I_{2a} from Equation 3a can be substituted into Equation 4 to yield Equation 5.

$$G = WE_l - \left[\frac{A_{2a}}{A_{2i}} \times I_{2i}\right] \quad \text{(Eq 5)}$$

[**0091**] Next, Equation 1 and Equation 2 can be combined to yield Equation 6.

$$I_{2i}=WE_2-WE_1 \quad \text{(Eq 6)}$$

[**0092**] Next, I_{2i} from Equation 6 can be substituted into Equation 5 to yield Equation 7a.

$$G = WE_1 - \left\{\left(\frac{A_{2a}}{A_{2i}}\right)X(WE_2 - WE_1)\right\} \quad \text{(Eq 7a)}$$

[**0093**] Equation 7a outputs a corrected glucose current density G which removes the effects of interferences requiring only the measured current density from first working electrode **100** and second working electrode **102** (i.e. WE_1 and WE_2), and a proportion of an area of the second working electrode covered with active reagent to an area of the second working electrode covered with inactive reagent

$$\left(\text{i.e } \frac{A_{2a}}{A_{2i}}\right).$$

[**0094**] In one embodiment of the present invention the proportion

$$\frac{A_{2a}}{A_{2i}}$$

[**0095**] may be programmed into a glucose meter, in, for example, a read only memory. In another embodiment of the present invention, the proportion

$$\frac{A_{2a}}{A_{2i}}$$

[0096] may be transferred to the meter via a calibration code chip which would may account for manufacturing variations in A_{2a} or A_{2i}.

[0097] In an alternative embodiment to the present invention Equation 1, 2, and 3b may be used when the interferent oxidation current density for active portion **102***a* is different from the interferent oxidation current density of inactive portion **102***i*. In such a case, an alternative correction Equation 7b is derived as shown below.

$$G=WE_1\{f\times(WE_2-WE_1)\} \quad \text{(Eq 7b)}$$

[0098] In another embodiment of the present invention, the corrected glucose current Equation 7a or 7b may be used by the meter only when a certain threshold is exceeded. For example, if WE_2 is about 10% or greater than WE_1, then the meter would use Equation 7a or 7b to correct for the current output. However, if WE_2 is about 10% or less than WE_1, the meter would simple take an average current value between WE_1 and WE_2 to improve the accuracy and precision of the measurement. The strategy of using Equation 7a or 7b only under certain situations where it is likely that a significant level of interferences are in the sample mitigates the risk of overcorrecting the measured glucose current. It should be noted that when WE_2 is sufficiently greater than WE_1 (e.g. about 20% or more), this is an indicator of having a sufficiently high concentration of interferents. In such a case, it may be desirable to output an error message instead of a glucose value because a very high level of interferents may cause a breakdown in the accuracy of Equation 7a or 7b.

[0099] **FIG. 21** shows an exploded perspective view of a test strip embodiment that is designed to compensate for variations in increased background caused by the conversion of oxidized mediator to reduced mediator. Test strip **1000** includes a substrate **50**, a conductive layer **164**, an insulation layer **106**, an inactive reagent layer **818**, an active reagent layer **820**, an adhesive layer **830**, and a top layer **824**. Test strip **1000** further includes a distal end **58** and a proximal end **60**. It should be noted that test strip **1000** is a modification of test strip **800** so that an active reagent coating **820** covers a portion of both a first working electrode **100** and a second working electrode **102**. This allows for two glucose measurements to be made while at the same time allows for the correction of interferents which develop within test strip **1000** or are dosed into test strip **1000**. Test strip **1000** would employ either Equation 7a or 7b for reducing the effect of interfering compounds or increased background. In contrast to test strip **800**, test strip **1000** has a modification to conductive layer **164** and insulation layer **106**. Substrate **50**, inactive reagent layer **818**, active reagent layer **820**, adhesive layer **830** and top layer **824** are similar in both shape and material for both test strip **1000** and test strip **800**.

[0100] FIGS. **22** to **24** are a simplified plane view of distal portion **58** of test strip **1000**, according to the embodiment of the present invention illustrated in **FIG. 21**, which show various positions of active reagent layer **820** and inactive reagent layer **818** with respect to each other. FIGS. **25** to **27**, which correspond to FIGS. **22** to **24** respectively, do not show insulation layer **804** to help demonstrate more clearly the relationship between the conductive layer **164**, active reagent layer **820**, and inactive reagent layer **818**.

[0101] In test strip **1000**, conductive layer **164** is disposed on substrate **50**. Conductive layer **164** includes a first working electrode **100**, a second working electrode **102**, a reference electrode **104**, a first contact **101**, a second contact **103**, a reference contact **105**, a strip detection bar **17**, as shown in **FIG. 21**. In contrast to test strip **800**, second working electrode **806** and first working electrode **102** has a C-shape.

[0102] **FIG. 22** is a simplified plane view of first working electrode **100**, second working electrode **102**, and reference electrode **104**, insulation layer **106**, inactive reagent layer **818**, and active reagent layer **820**. Insulation layer **106** includes a cutout **108** which defines the area of second working electrode **102** to have an inactive portion **102***i* and an active portion **102***a*. In this embodiment, inactive reagent layer **818** was disposed on inactive portion **102***i* and active reagent layer **820** was disposed on active portion **102***a*, first working electrode **100**, and reference electrode **104**. **FIG. 22** shows that inactive reagent layer **818** does not touch or overlap with active reagent layer **820**.

[0103] Test strip **1000** differs from test strip **800** in that both inactive reagent layer **818** and active reagent layer **820** both coat a portion of second working electrode **102**. This allows two glucose measurements to be performed while at the same time reduce the effects of background and/or interferences. One of the challenges with making test strip **1000** as shown in **FIG. 22** is that it can be difficult to sequentially align and coat the respective inactive reagent layer **818** and active reagent layer **820** so that they do not touch each other with the desired yield because the area of first working electrode **100**, second working electrode **102** and reference electrode **104** is relatively small.

[0104] In an embodiment of this invention, **FIG. 23** shows inactive reagent layer **818** disposed on inactive portion **102***i*, and active reagent layer **820** disposed on active portion **102***a*, first working electrode **100**, and reference electrode **104**. In this embodiment inactive reagent layer **818** and active reagent layer **820** are immediately adjacent to each other. In such an ideal case the inactive reagent layer **818** and active reagent layer **820** would touch, but not substantially overlap with each other. Although the printing process targets the alignment such that inactive reagent layer **818** and active reagent layer **820** are immediately adjacent to each other, normal manufacturing variation will cause some overlap to occur with a certain frequency between inactive reagent layer **818** and active reagent layer **820**. Likewise, such variation will also cause inactive reagent layer **818** to not touch active reagent layer **820** at a certain frequency. Because inactive reagent layer **818** was allowed to touch or not touch active reagent layer **820**, the yield of acceptable test strips was improved.

[0105] Yet another embodiment of this invention which improves upon the method of coating inactive reagent layer **818** and active reagent layer **820** is shown in **FIG. 24**. Inactive reagent layer **818** may be coated such that it completely covers inactive portion **102***i* and a portion of reference electrode **104**. Similarly, active reagent layer **820** may be coated such that it completely covers active portion **102***a*, first working electrode **100** and at least a portion of reference electrode **104**. In an embodiment of this invention, the printing process can target the alignment such that inactive reagent layer **818** and active reagent layer **820** substantially overlap with each other on reference electrode **810** at an overlap zone **822**. In such a case, inactive reagent

layer **818** and active reagent layer **820** may mix with each other at overlap zone **822**. Because the length of both inactive reagent layer **818** and active reagent layer **820** was further increased compared to the embodiment described in **FIG. 23**, the alignment and coating of active reagent layer **820** and inactive reagent layer **818** was yet further improved in terms of manufacturing yield.

[**0106**] It is an advantage of this invention in that two reagent layers are used which helps reduce the effects of increased background. The ability to sufficiently compensate for varying levels of reduced mediator such as ferrocyanide in the test strip itself enables a high level of accuracy and precision to be achieved. There are several factors that may influence the conversion of oxidized mediator to the reduced form during the manufacturing, testing, and storage process. Therefore, this allows for corrections to be made which account for manufacturing variations such as reagent layer height (within batch and batch-to-batch), heat seal adhesive manufacturing conditions, high temperature drying, packaging, and sterilization conditions. Because the correction accounts for these variation, a more robust process can be envisaged in which rigorous process controls are not needed to monitor and control such manufacturing variations. The measurement of background currents may also improve the stability of test strip to withstand adverse storage conditions such as high temperature and humidity. This may allow simpler cartridges to be designed for storing test strips which may not need a rigorous seal to withhold moisture

EXAMPLE 1

[**0107**] Test strips **800** were prepared as illustrated in FIGS. **1** to **3***a*. Test strips **800** were tested in blood which were exposed to varying levels of sterilizing radiation. To test strips **800**, they were electrically connected to a potentiostat which has the means to apply a constant potential of +0.4 volts between first working electrode **808** and reference electrode **810**; and second working electrode **806** and the reference electrode **810**. A sample of blood is applied to sample inlet **52** allowing the blood to wick into the sample receiving chamber and to wet first working electrode **808**, reference electrode **810**, and second working electrode **806**. Active layer **820** becomes hydrated with blood and then generates ferrocyanide which may be proportional to the amount of glucose and/or interferent concentration present in the sample. In contrast, inactive layer **818** becomes hydrated with blood and does not generate additional ferrocyanide that was not present within inactive layer **818** before hydration. After about 5 seconds from the sample application to test strip **800**, an oxidation of ferrocyanide and/or interferences are measured as a current for both first working electrode **808** and second working electrode **806**.

EXAMPLE 2

[**0108**] Two batches of test strips were prepared to show that the use of inactive reagent layer **818** and active reagent layer **820** improved the overall precision for test strips sterilized by gamma radiation. Both batches of test strips were tested in a similar manner as described in Example 1. The first test strip batch is test strip **800** and is referred to as Batch **1**. The second test strip batch, which is referred to as Batch **2**, is also similar to test strip **800**, but does not include inactive reagent layer **818** and also has a modified active reagent layer which covers both first working electrode **808**, second working electrode **806**, and reference electrode **810**. When testing Batch **1**, the difference in current from first working electrode **808** and second working electrode **806** was used to calculate a corrected signal current which was then converted to a glucose concentration. When testing Batch **2**, the current from second working electrode **806** and first working electrode **808** were summed together to determine a value which was then used to calculate an uncorrected glucose concentration. Before testing with blood, both Batch **1** and Batch **2** test strips were treated with 0 kGy and 25 kGy of gamma radiation. Next, the four test cases, which are Batch **1**—0 kGy, Batch **1**—25 kGy, Batch **2**—0 kGy, and Batch **2**—25 kGy, were evaluated for precision by testing 24 test strips with blood for each test case at 5 glucose concentrations which was 20, 50, 100, 300, and 500 mg/dL.

[**0109**] FIGS. **11** to **15** show that Batch **1** test strips did not suffer from a degradation in precision after being sterilized with 25 kGy of gamma radiation. For all five glucose concentrations, the precision was substantially similar or better after sterilization for Batch **1** test strips. This shows that the use of active reagent layer **820** and inactive reagent layer **818** helps compensate for background levels of ferrocyanide produced during the sterilization process.

[**0110**] FIGS. **11** to **13** show that Batch **2** test strips did suffer from a degradation in precision after being sterilized with 25 kGy of gamma radiation. This control experiment verifies that there is a degradation in precision when not using the background reduction method of the present invention. Because Batch **2** test strip did not have inactive reagent layer **818**, the background reduction method could not be implemented. Batch **2** test strips, did not suffer from a degradation in precision after being sterilized because relatively high glucose concentrations were tested (300 and 500 mg/dL) in which the effect of sterilization on precision is not as significant. In this case, the amount of ferrocyanide generated by glucose oxidase is significantly higher than ferrocyanide generated (e.g. by sterilization processes) before hydrating the test strip.

EXAMPLE

[**0111**] Another batch of test strips, which is referred to as Batch **3**, was prepared in a manner similar to test strip **800** except that second working electrode **806** was not coated with either active reagent layer **820** or inactive reagent layer **818**. In this example, Batches **1** to **3** were tested to evaluate the overall accuracy in the presence of interfering compounds such as uric acid and gentisic acid.

[**0112**] Batch **1**, Batch **2**, and Batch **3** test strips were tested in blood at three concentrations of gentisic acid which were 0, 25, and 50 mg/dL. For each gentisic acid concentration, two glucose concentrations were tested which were 70 and 240 mg/dL. **FIGS. 16 and 17** show that Batch **1** and Batch **3** test strips had an insignificant change (<10 mg/dL or 10%) in bias when testing them at 25 and 50 mg/dL gentisic acid concentration. In contrast, Batch **2** test strips had a significant change (>10 mg/dL or 10%) in bias when testing them at a 25 and a 50 mg/dL gentisic acid concentration. This shows that the use of second working electrode **806** not coated with enzyme allows for an effective correction of the glucose signal in the presence of high concentrations of gentisic acid.

[0113] Batch **1**, Batch **2**, and Batch **3** test strips were tested in blood at three concentrations of uric acid which were 0, 10, and 20 mg/dL. For each uric acid concentration, two glucose concentrations were tested which were 70 and 240 mg/dL. **FIGS. 18 and 19** show that Batch **1** and Batch **3** test strips had an insignificant change (<10 mg/dL or 10%) in bias when testing them at 10 and 20 mg/dL uric acid concentration. In contrast, Batch **2** test strips had a significant change (>10 mg/dL or 10%) in bias when testing them at a 10 and a 20 mg/dL uric acid concentration. This shows that the use of second working electrode **806** not coated with enzyme allows for an effective correction of the glucose signal in the presence of high concentrations of uric acid.

[0114] It will be recognized that equivalent structures may be substituted for the structures illustrated and described herein and that the described embodiment of the invention is not the only structure which may be employed to implement the claimed invention. In addition, it should be understood that every structure described above has a function and such structure can be referred to as a means for performing that function. While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to hose skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

What is claimed is:

1. An electrochemical sensor comprising:

a substrate;

a first working electrode disposed on said substrate;

a second working electrode disposed on said substrate;

a reference electrode;

an active reagent layer disposed on said first working electrode, wherein said active reagent layer completely covers said first working electrode; and

an inactive reagent layer disposed on said second working electrode, wherein said inactive reagent completely covers said second working electrode.

2. An electrochemical sensor according to claim 1 wherein:

said first working electrode, said second working electrode and said reference electrode are positioned in a sample receiving chamber;

said sample receiving chamber having a proximal and a distal end, said distal end including a first opening which is adapted to receive bodily fluids; and

said second working electrode being positioned adjacent said first opening.

3. An electrochemical sensor according to claim 2 wherein said first working electrode and said reference electrode are positioned proximal to said second working electrode.

4. An electrochemical sensor comprising:

a substrate;

a first working electrode disposed on said substrate;

a second working electrode disposed on said substrate;

a reference electrode;

an active reagent layer disposed on said first working electrode, wherein said active reagent layer completely covers said first working electrode;

said second working electrode having an active region and an inactive region, said active reagent layer disposed on a active region of said second working electrode and an inactive reagent layer disposed on said inactive region of said second working electrode.

5. An electrochemical sensor according to claim 4 wherein:

said first working electrode, said second working electrode and said reference electrode are positioned in a sample receiving chamber;

said sample receiving chamber having a proximal and a distal end, said distal end including a first opening which is adapted to receive bodily fluids; and

said inactive region of said second working electrode being positioned adjacent said first opening.

6. An electrochemical sensor according to claim 5 wherein said active region of said second working electrode and said first working electrode are positioned proximal to said inactive region of said second working electrode.

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