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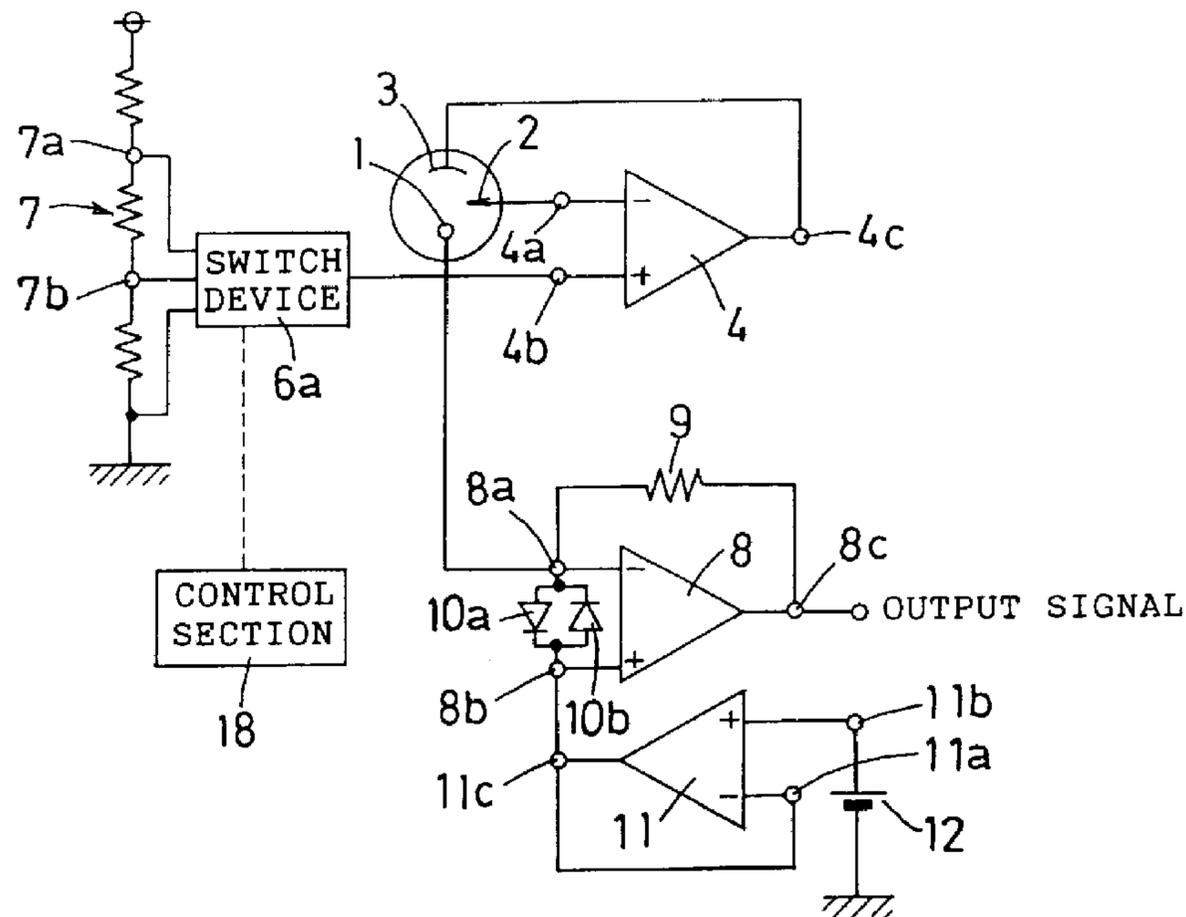
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(54) **METHODE ET DISPOSITIF POUR REMETTRE SOUS TENSION
L'ELECTRODE D'UN BIOSENSEUR**

(54) **METHOD AND APPARATUS FOR REVIVING AN ELECTRODE
OF A BIOSENSOR**



(57) After a reverse bias is applied between a working electrode and a counter electrode or a reference electrode, a forward bias higher than a forward measurement bias is applied between the working electrode and the counter electrode or the reference electrode before the forward measurement bias is applied. This reduces a time period before an actual measuring operation is possible.



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Abstract of the Disclosure

After a reverse bias is applied between a working
electrode and a counter electrode or a reference
5 electrode, a forward bias higher than a forward
measurement bias is applied between the working
electrode and the counter electrode or the reference
electrode before the forward measurement bias is
applied. This reduces a time period before an actual
10 measuring operation is possible.

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Method and Apparatus for Reviving An Electrode of A
Biosensor

Background of the Invention

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The present invention relates to methods and apparatus for reviving an electrode of a biosensor, and more particularly to methods and apparatus for reviving an electrode of a biosensor by supplying a bias having a different polarity from the measurement bias polarity to the electrode before a measuring operation is started.

It is known that a physiologically active substance is capable of selectively detecting a very complicated organic compound, protein or the like with high sensitivity. With attention directed to this characteristic, research has been made in various biosensors.

A typical biosensor having an electrode unit and a physiologically active substance fixed thereon is proposed. The biosensor is used for detecting the existence of an object substance, the relative or active quantity of the object substance and the like based on an electrical signal output from the electrode unit corresponding to the biological reaction of the object

substance, under the condition that a predetermined forward bias is applied to the electrode unit. For example, the electrode unit has a working electrode made of platinum and a counter electrode made of silver. An enzyme-immobilized membrane is fixed onto the electrode unit. When the object substance is to be measured, hydrogen peroxide is generated through an enzyme reaction of the object substance. Then the generated hydrogen peroxide reaches the surface of the electrode unit through a hydrogen peroxide penetration membrane. The electrode unit outputs an electrical signal corresponding to the quantity of hydrogen peroxide that reaches it. The existence of the object substance, the relative or active quantity of the object substance and the like are detected based on the electrical signal. Concretely, the working electrode has a forward bias of 0.6 volts applied with respect to the counter electrode.

In the biosensor described above, an interfering layer such as an oxidized layer and the like which interferes with electrical signals is generated on the working electrode, and the activity of the working electrode is lowered as the object substance measuring operation is continued with the forward bias being applied to the electrode unit. It is proposed that a reverse bias is accordingly applied to the electrode

unit (the working electrode has a reverse bias of about -
0.6 volts applied with respect to the counter electrode,
corresponding to the concrete example) after one or more
measuring is performed, within the time period in which
5 the measurment is not performed, so as to remove the
interfering layer. Then the activity of the working
electrode is revived. The level of the output signal
from the electrode unit is raised to the original level.

(Refer to Japanese Patent Laid Open Sho 60-155959).

10 Lowered measuring sensitivity is recovered by
applying the predetermined reverse bias voltage to the
electrode unit within the time period in which the
measurment is not performed, as is described above, then
measuring with high sensitivity can be performed again.

15 In the reviving apparatus described above, a
constant-voltage for reviving (reverse bias) is
applied between the working electrode and the counter
electrode. The oxydized layer on the working electrode
is reduced, hydrogen, hydrogen ions and the like are
20 accordingly generated. When a constant-voltage for
measurement (forward bias) is applied between the
working electrode and the counter electrode, a diffusion
current flows depending upon the generated hydrogen,
hydrogen ions and the like.

25 As a result, the electrical signal provided for

measuring is affected by lowering, by the diffusion current, so as to remarkably lower measuring accuracy just after the constant-voltage for measurement is applied between the working electrode and the counter electrode. When the measurement is performed after the diffusion current is sufficiently lowered, sufficient measuring accuracy is obtained, but a remarkably long waiting time period (for example, 1-2 minutes) is needed.

10 The same disadvantages as above arises for a biosensor having a reference electrode in addition to the working electrode and the counter electrode.

Summary of the Invention

15 It is an object of the present invention to shorten the time period between performing a reviving operation for an electrode unit to when it is possible to start a measuring operation for an object substance.

20 This present invention first applies a predetermined reverse bias to a working electrode for a predetermined time period, then, applies forward bias higher than a forward measurement bias to the working electrode for a predetermined time period, then, applies

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the forward measurement bias to the working electrode.

In this reviving method, an interfering layer on the working electrode which interferes with the turning on of electricity is reduced and the activity of the working electrode is revived by applying the reverse bias to the working electrode. Substances generated through reduction of the interfering layer are moved to one of the electrodes at high speed by applying the forward bias higher than the forward measurement bias to the working electrode. The substances are rapidly consumed, accordingly, when the forward bias is applied to the working electrode. As a result, a time period between performing a reviving operation for an electrode unit to when it is possible to start a measuring operation for an object substance with a predetermined accuracy maintained, is remarkably shortened.

The time period for applying the higher bias to the working electrode may be nearly equal to the time period for applying the reverse bias to the working electrode.

It is preferred that the predetermined reverse bias is a reverse bias which is gradually increased until its absolute value reaches a predetermined value. Damage of a membrane fixed on the surface of the electrode unit, effected by current is remarkably

reduced.

It is also preferred that the higher forward bias is a forward bias which is gradually increased until its absolute value reaches a higher predetermined value than the forward bias for measuring. Damage of the membrane fixed on the surface of the electrode unit, effected by current is remarkably reduced.

It is further preferred that the predetermined reverse bias is a reverse bias which is gradually increased until its absolute value reaches a predetermined value, and the higher forward bias is a forward bias which is gradually increased until its absolute value reaches a predetermined value higher than the forward measurement bias. Damage of the membrane fixed on the surface of the electrode unit, effected by current is remarkably reduced.

This present invention comprises;

reviving voltage applying means for applying voltage for reviving to a working electrode;

measurement voltage applying means for applying voltage for measuring to the working electrode;

higher voltage applying means for applying a higher voltage than the voltage for measuring to the working electrode;

selection means for sequentially selecting a

condition under which to apply a voltage for reviving to the working electrode from the reviving voltage applying means, a condition under which to apply a higher voltage to the working electrode from the higher voltage

5 applying means, and a condition under which to apply a voltage for measuring to the working electrode from the measurement voltage applying means, when measuring an operation is to be performed.

In this reviving apparatus, the condition of
10 applying voltage for reviving to the working electrode by the reviving voltage applying means and the condition of applying the higher voltage to the working electrode by the higher voltage applying means are selected in this order by the selection means, before an actual
15 measuring operation is started. First, the interfering layer on the surface of the working electrode is removed. After the interfering layer has been removed, substances generated through removal of the interfering layer are moved to one of the electrodes at high speed
20 by applying the higher forward bias to the working electrode. The substances are rapidly consumed when the forward bias is applied to the working electrode. As a result, after a short time period from when a reviving operation for an electrode unit has passed, a measuring
25 operation for an object substance can be started with a

predetermined accuracy maintained.

It is preferred that the reviving apparatus further comprises a first time constant means for applying a predetermined time constant to the reviving
5 voltage. Damage of a membrane fixed on a surface of an electrode unit, effected by current is remarkably reduced.

It is also preferred that the reviving apparatus further comprises a second time constant means for
10 applying a predetermined time constant to the higher voltage. Damage of a membrane fixed on a surface of an electrode unit, effected by current is remarkably reduced.

It is further preferred that the reviving
15 apparatus further comprises a first time constant means for applying a predetermined time constant to the reviving voltage, and a second time constant means for applying a predetermined time constant to the higher
20 voltage. Damage of a membrane fixed on a surface of an electrode unit, effected by current is remarkably reduced.

More specifically, the interfering layer can easily be removed by a reduction operation because the
reviving operation is an operation to remove the
25 interfering layer formed on the working electrode, and

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the interfering layer is generated by oxidization. When
the interfering layer is removed, hydrogen, hydrogen
ions and the like are inevitably generated through
removal of the interfering layer. The generated
5 substances diffuse when the voltage for measuring is
applied to the working electrode. As a result,
concentration measuring of an object substance with high
accuracy is performed only after the diffusion current
is sufficiently lowered. Concretely, when the forward
10 measurement bias is determined to be 0.75 volts and the
reverse bias for reviving is determined to be -1 volt,
the diffusion current is sufficiently lowered only after
1-2 minutes after removal of the interfering layer. The
disadvantage of having to wait a long time period until
15 actual measuring can be started, arises.

On the contrary, in the present invention,
substances generated through removal of the interfering
layer are moved to one of the electrodes at high speed
because the higher voltage is applied to the working
20 electrode before the measurement voltage is applied to
the working electrode. The substances are sufficiently
close to one of the electrodes, accordingly, when the
measurement voltage is applied to the working electrode,
thereby the substances are rapidly consumed when the
25 measurement voltage is applied to the working electrode.

As a result, a time period between removal of the interfering layer to when it is possible to start the concentration measuring of an object substance with high accuracy is remarkably shortened.

5 Figures 4 are diagrams useful in understanding the operation described above. Figs. 4(A1) to fig. 4(A3) correspond to a conventional case, while figs. 4(B1) to fig. 4(B3) correspond to this present invention.

10 As is illustrated in figs. 4(A1) and 4(B1), the same quantity of generated substances exist between the working electrode and the reference electrode when the interfering layer is removed. Then, in the conventional case, only a fairly small moving force toward the
15 working electrode acts on the generated substances as is illustrated in fig. 4(A2), thereby the time period for consuming a predetermined quantity of the generated substances is lengthened. That is, the generated substances are not so close to the working electrode as
20 is illustrated in fig. 4(A3) after a predetermined time period has passed, thereby a long time period is needed for most of the generated substances to reach the working electrode and be consumed. On the contrary, in
25 this present invention, a fairly large moving force toward the working electrode acts on the generated

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substances as is illustrated in fig. 4(B2), thereby the
generated substances are fairly close to the working
electrode when a time period equal to the above
described time period has passed, as is illustrated in
5 fig. 4(B3). Thereafter, the same moving force as in the
conventional case acts on the generated substances. A
time period for consuming a predetermined quantity of
the generated substances is reduced because the
generated substances are already close to the working
10 electrode. As a result, the time period between removal
of the interfering layer to when it is possible to start
actual measuring is remarkably shortened. A biosensor
having three electrodes is illustrated in figs. 4. A
biosensor having two electrodes, excluding the reference
15 electrode, is similar to the biosensor having three
electrodes.

These and other objectives, features and
advantages of the present invention will be more readily
understood upon consideration of the present invention,
20 taken in conjunction with the accompanying drawings.

Brief Description of the Drawings

Figure 1 is an electronic circuit diagram of an
25 electrode reviving apparatus in accordance with a first

embodiment of the present invention;

Figure 2 is a vertical sectional view of the center portion of an electrode unit;

Figures 3(A) and 3(B) are diagrams showing variations of bias voltage and current when the electrode unit is revived;

Figures 4(A1), 4(A2), 4(A3), 4(B1), 4(B2), and 4(B3) are diagrams showing movement of substances generated through reviving of the electrode unit;

Figure 5 is an electronic circuit diagram of an electrode reviving apparatus in accordance with a second embodiment of the present invention;

Figure 6 is an electronic circuit diagram of an electrode reviving apparatus in accordance with a third embodiment of the present invention;

Figures 7(A) and 7(B) are diagrams showing variations of bias voltage and current when the electrode unit is revived;

Figure 8 is an electronic circuit diagram of an electrode reviving apparatus in accordance with a fourth embodiment of the present invention.

Detailed Description of the Preferred Embodiments

Fig. 1 is an electronic circuit diagram of an

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electrode reviving apparatus in accordance with a first embodiment of the present invention.

The apparatus revives an electrode unit having three electrodes, such as a working electrode 1 made of platinum, a reference electrode 2 and a counter electrode 3, both made of silver.

The reference electrode 2 and the counter electrode 3 are connected to an inverting input terminal 4a and an output terminal 4c of an operational amplifier 4, respectively. A non-inverting input terminal 4b of the operational amplifier 4 is selectively connected to a high voltage tap 7a, a low voltage tap 7b or ground of a voltage divider 7 having resistances by means of a switch device 6a. An inverting input terminal 8a of a current/voltage converting operational amplifier 8 for providing measurement signal is connected to the working electrode 1. A current/voltage converting resistance 9 is connected between an output terminal 8c and the inverting input terminal 8a of the current/voltage converting operational amplifier 8. Diodes 10a and 10b are connected in parallel and in reverse polarity with respect to one another between the inverting input terminal 8a and a non-inverting input terminal 8b of the current/voltage converting operational amplifier 8. A DC power source 12 for measuring is connected to a non-

inverting input terminal 11b of a buffer amplifier 11. An output terminal 11c of the buffer amplifier 11 is connected directly to an inverting input terminal 11a of the buffer amplifier 11. The output terminal 11c of the
5 buffer amplifier 11 is connected to the non-inverting input terminal 8b of the current/voltage converting operational amplifier 8. The DC power source 12 is used for applying a forward bias of 0.75 volts to the working electrode 1. The high voltage tap 7a and the low
10 voltage tap 7b are used for applying biases of -1 volt and 1 volt respectively, to the working electrode 1. A control section 18, provided as a selection means, is used to control the operation of the switch device 6a.

Fig. 2 is a vertical sectional view of the
15 center portion of an electrode unit to which the reviving apparatus is applied.

The working electrode 1 is provided at a predetermined position in an electrode unit body 13. The ring shaped reference electrode 2 and the ring
20 shaped counter electrode 3, both electrodes 2 and 3 surrounding the periphery of the working electrode 1, are provided in the electrode unit body 13 in this order. The electrode unit body 13 has a convex surface on the side on which the working electrode 1, the
25 reference electrode 2 and the counter electrode 3 are

provided. A hydrogen peroxide selective penetration
membrane 14, a glucose oxidase immobilized membrane
(hereinafter referred to as GOD immobilized membrane) 15
and a diffusion-limiting membrane 16 made of polyvinyl
acetate and the like are provided in this order to fit
the convex surface of the electrode unit body 13.

Terminals 17 for supplying output signals are provided
in the electrode unit body 13. The terminals 17 are
connected to the working electrode 1, the reference
electrode 2 and the counter electrode 3, respectively.

The operation of the sensor for measuring
glucose concentrations having the arrangement described
above is as follows.

When measuring of glucose concentrations is
carried out, the non-inverting input terminal 4b of the
operational amplifier 4 is connected to the high voltage
tap 7a of the voltage divider 7 by operating the switch
device 6a. Then, a constant voltage for reviving (for
example the constant voltage of -1 volt) is applied
between the working electrode 1 and the reference
electrode 2 (Refer to a region R1 in figs. 3(A) and
3(B)).

In this condition, current flows to reduce
interfering substances which are formed on a surface of
the working electrode 1. The interfering substances are

completely removed and activity of the working electrode 1 is revived by continuing to apply the constant voltage for reviving between the electrodes 1 and 2 for a fairly short time period (for example a time period of about 4
5 seconds). When the interfering substances are completely removed, hydrogen, hydrogen ions and the like are disposed between the working electrode 1 and the reference electrode 2.

Thereafter, the non-inverting input terminal 4b
10 of the operational amplifier 4 is connected to the low voltage tap 7b of the voltage divider 7 by operating the switch device 6a. Then, a higher voltage (for example the higher voltage of 1 volt) higher than a measurement voltage is applied between the working electrode 1 and
15 the reference electrode 2 (Refer to a region R2 in figs. 3(A) and 3(B)).

In this condition, a large moving force acts on the floating hydrogen, hydrogen ions and the like to move them toward the working electrode 1. Most
20 hydrogen, hydrogen ions and the like are moved close to the working electrode 1 by applying a high voltage between the working electrode 1 and the reference electrode 2 for a fairly short time period (for example, a time period of about 5 seconds).

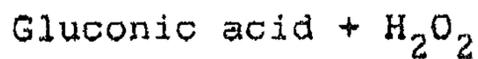
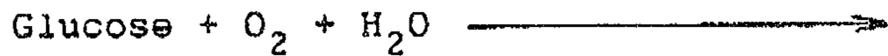
25 Thereafter, the non-inverting input terminal 4b

of the operational amplifier 4 is connected to ground of the voltage divider 7 by operating the switch device 6a. Then, a predetermined voltage (for example a voltage of 0.75 volts) is applied as a forward bias to the working electrode 1 on the basis of the reference electrode 2. The hydrogen, hydrogen ions and the like are consumed within a short time period, then glucose concentrations may be measured. The time period between removal of interfering substances to when measuring is permitted 1-2 minutes in conventional reviving apparatus, while the time period is reduced to about 30-45 seconds in this embodiment.

After reviving is finished, a signal corresponding to the concentration of glucose is output as follows by dropping an object solution onto the electrode unit.

The dropped object solution is guided to the GOD immobilized membrane 15 with limited penetration by the glucose to some degree, by the diffusion-limiting membrane 16. Then, the following reaction takes place:

GOD



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Hydrogen peroxide, the quantity of which corresponds to concentration of existing glucose, is accordingly generated. The generated hydrogen peroxide is guided to the surface of the working electrode 1 which is revived to have sufficient activity, through the hydrogen peroxide selective penetration membrane 14. The forward bias is kept applied to the working electrode 1. An oxydation reaction is carried out on the surface of the working electrode 1 and current corresponding to the amount of hydrogen peroxide flows in through the working electrode 1. The current is applied to the inverting input terminal 8a of the current/voltage converting operational amplifier 8, then a voltage signal is output from the output terminal 8c of the current/voltage converting operational amplifier 8. The voltage signal is generated by adding an offset voltage caused by the forward bias and a voltage signal being proportional to the current.

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Thereafter, only the voltage signal proportional to the current is extracted, then the extracted voltage

signal is differentiated to obtain a first-order differential value, then a peak value of the first-order differential value is detected. Finally, a glucose concentration detection signal with high accuracy is
5 obtained by performing the necessary operations.

Second Embodiment

Fig. 5 is an electronic circuit diagram of an
10 electrode reviving apparatus in accordance with a second embodiment of the present invention.

Different points from the first embodiment are as follows,

(1) an electrode unit having two electrodes,
15 one is the working electrode 1 made of platinum while another, the counter electrode 3 made of silver, is used for being revived, and

(2) the counter electrode 3 is directly
20 connected to the switch device 6a by omitting the operational amplifier 4.

In this embodiment, reviving for an electrode unit having two electrodes can be performed. Also, the time period between removal of an interfering substances to when it is possible to start actual measuring can be
25 shortened.

Third Embodiment

Fig. 6 is an electronic circuit diagram of an
5 electrode reviving apparatus in accordance with a third
embodiment of the present invention.

Different points from the first embodiment are
as follows,

(1) the non-inverting input terminal 4b of the
10 operational amplifier 4 is connected to ground through a
condenser 5,

(2) the non-inverting input terminal 4b is
selectively connected to the high voltage tap 7a and the
low voltage tap 7b of the voltage divider 7 through a
15 resistance 5a and the switch device 6a.

In this embodiment, bias voltages are increased
in absolute value (refer to fig. 7(A)) based on a time
constant (for example, a time constant of 0.5 seconds).
The time constant is determined based on the resistance
20 5a and the condenser 5. As a result, current
undershoots and overshoots are remarkably suppressed as
is illustrated in fig. 7(B). That is, an excessive
current is securely prevented from flowing. Damage to a
membrane or membranes fixed on the surface of the
25 electrode unit, effected by current is remarkably

reduced, accordingly. As a result, the life of a membrane or membranes is extended up to about one year while the life of a membrane or membranes applied in the conventional reviving apparatus is about one month. The
5 life is a time period when the penetration ratio of the hydrogen peroxide selective penetration membrane increases by 10 %.

Fourth Embodiment

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Fig. 8 is an electronic circuit diagram of an electrode reviving apparatus in accordance with a fourth embodiment of the present invention.

Different points from the third embodiment are
15 as follows,

(1) an electrode unit having two electrodes, one is the working electrode 1 made of platinum while another, the counter electrode 3 made of silver, is used for being revived, and

20 (2) the counter electrode 3 is directly connected to the switch device 6a by omitting the operational amplifier 4.

In this embodiment, reviving for an electrode unit having two electrodes can be performed. Also, the
25 time period between removal of interfering substances to

when it is possible to start actual measuring can be shortened. Furthermore, damage to a membrane or membranes fixed on the surface of the electrode unit, effected by current is remarkably reduced.

5 This invention is not limited to the foregoing
embodiments. Preferably, the voltage for reviving and
higher voltage are changed. Preferably, the time period
for applying voltage is changed corresponding to the
voltage value. Preferably, the reviving method and
10 apparatus are applicable to apparatus for measuring
concentrations of substances other than glucose.
Preferably, a programmable power source which varies the
output voltage under control by a microcomputer, is
employed instead of the time constant circuit comprising
15 the resistance 5a and the condenser 5.

 The terms and expressions which have been
employed here are used as terms of description and not
of limitations, and there is no intention, in the use of
such terms and expressions of excluding equivalents of
20 the features shown and described, or portions thereof,
it being recognized that various modifications are
possible within the scope of the invention as claimed.

CLAIMS

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1. A method for reviving an electrode unit of a biosensor which includes a working electrode and a counter electrode, and which measures a concentration of an object substance based upon an electrical signal generated between the working electrode and the counter electrode when a predetermined forward measurement bias is applied to the working electrode on which a physiologically active substance is placed, the electrical signal being generated based upon a biological reaction of the object substance, said method comprising the steps of;

(1) applying a predetermined reverse bias to the working electrode for a time period which is in seconds order;

and

(2) applying a higher forward bias than the forward measurement bias to the working electrode for a time period which is in seconds order before the forward measurement bias is applied to the function electrode.

2. A method as set forth in claim 1, wherein the predetermined reverse bias is a reverse bias which is exponentially increased until its absolute value reaches a predetermined value.

3. A method as set forth in claim 1, wherein the higher forward bias is a forward bias which is exponentially increased until its absolute value reaches a predetermined value which is higher than the forward measurement bias.

B

4. A method as set forth in claim 1, wherein the predetermined reverse bias is a reverse bias which is exponentially increased until its absolute value reaches a predetermined value, and the higher forward bias is a forward bias which is exponentially increased until its absolute value reaches a predetermined value which is higher than the forward measurement bias.

5. An apparatus for reviving an electrode unit of a biosensor which includes a working electrode and a counter electrode, and which measures a concentration of an object substance based upon an electrical signal generated between said working electrode and said counter electrode when a predetermined forward measurement bias is applied to said working electrode on which a physiologically active substance is placed, said electrical signal being generated based upon a biological reaction of said object substance, said apparatus comprising;

reviving voltage applying means for applying a reviving voltage to said working electrode;

measurement voltage applying means for applying a measurement voltage to said working electrode;

higher voltage applying means for applying a voltage higher than the measurement voltage to said working electrode; and

selection means for sequentially selecting a condition under which to apply a reviving voltage to said working electrode from said reviving voltage applying means, a condition under which to apply a higher voltage to said working electrode from said

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higher voltage applying means, and a condition under which to apply a measurement voltage to said working electrode from said measurement voltage applying means, when a measuring operation is to be performed.

6. An apparatus as set forth in claim 6, further comprising a first time constant means for controlling a reviving voltage supplied by said reviving voltage applying means according to a predetermined time constant.

7. An apparatus as set forth in claim 6, further comprising a second time constant means for controlling a higher voltage supplied by said higher voltage applying means according to a predetermined time constant.

8. An apparatus as set forth in claim 6, further comprising a first time constant means for controlling a reviving voltage applied by said reviving voltage applying means according to a predetermined time constant, and a second time constant means for controlling a higher voltage applied by said higher voltage applying means according to a predetermined time constant.

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Fig. 1

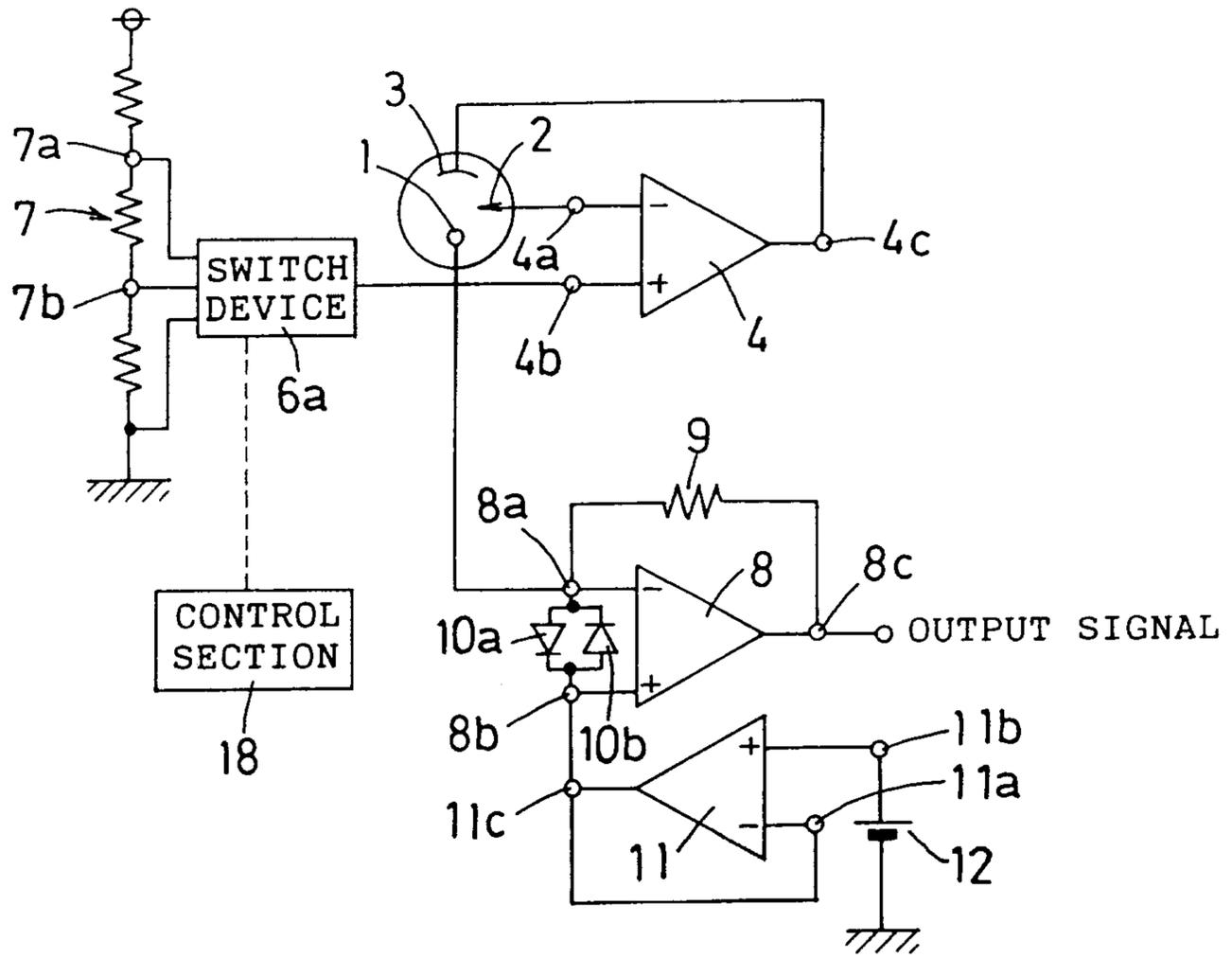
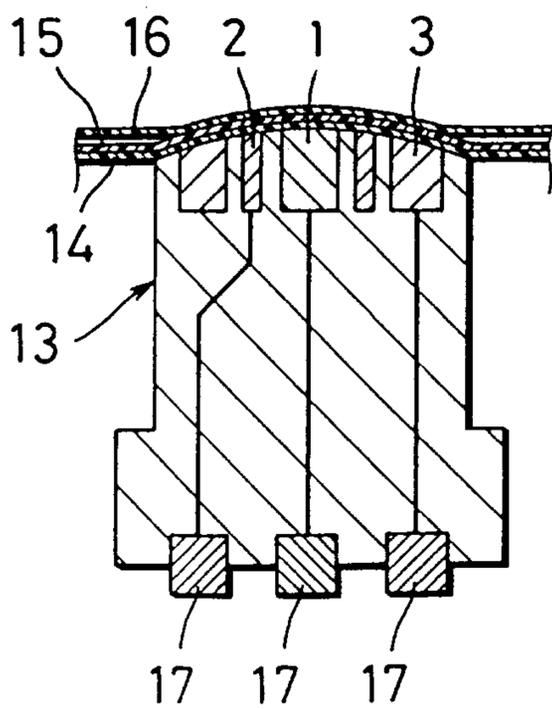


Fig. 2



by Rogers, Bereskin & Parr

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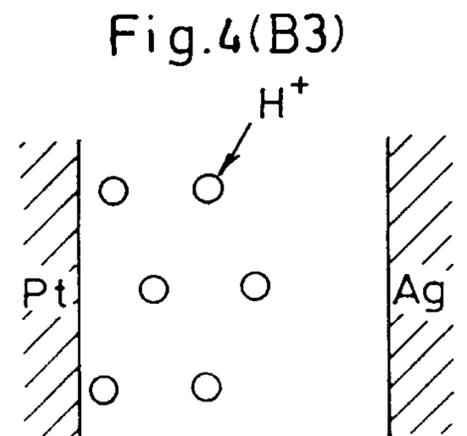
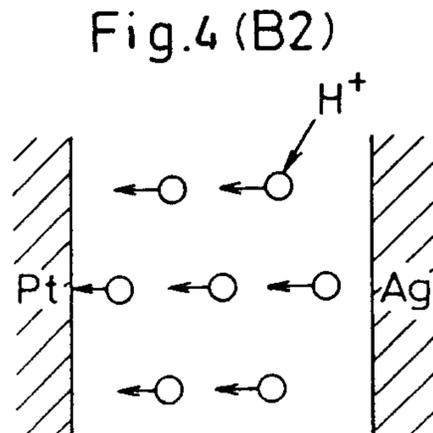
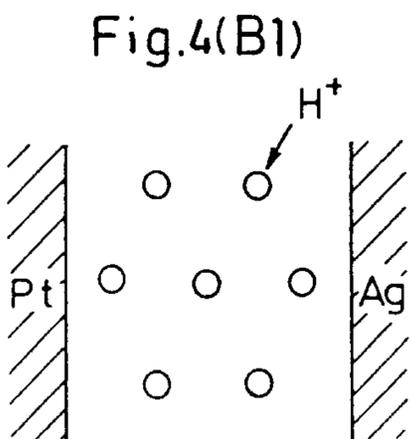
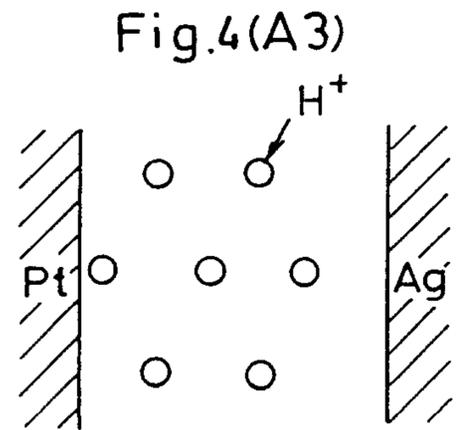
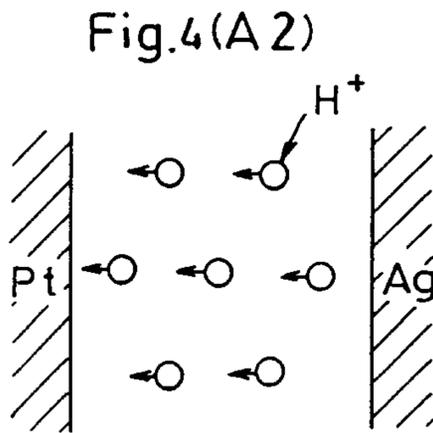
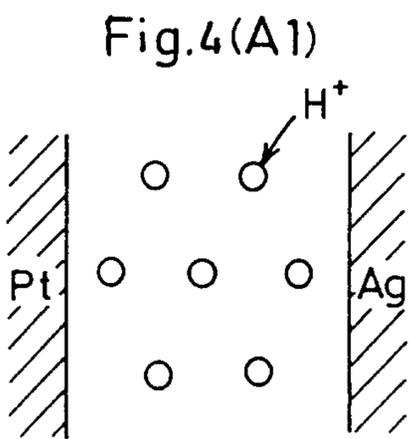
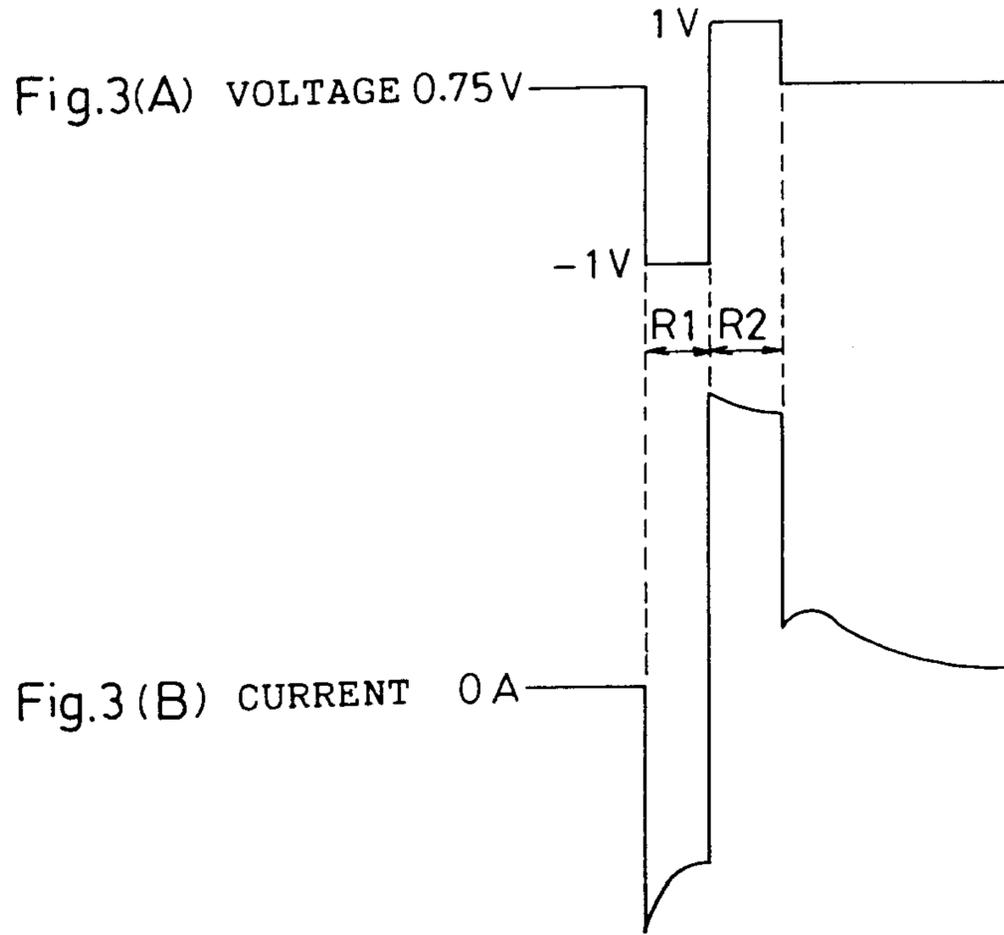


Fig. 5

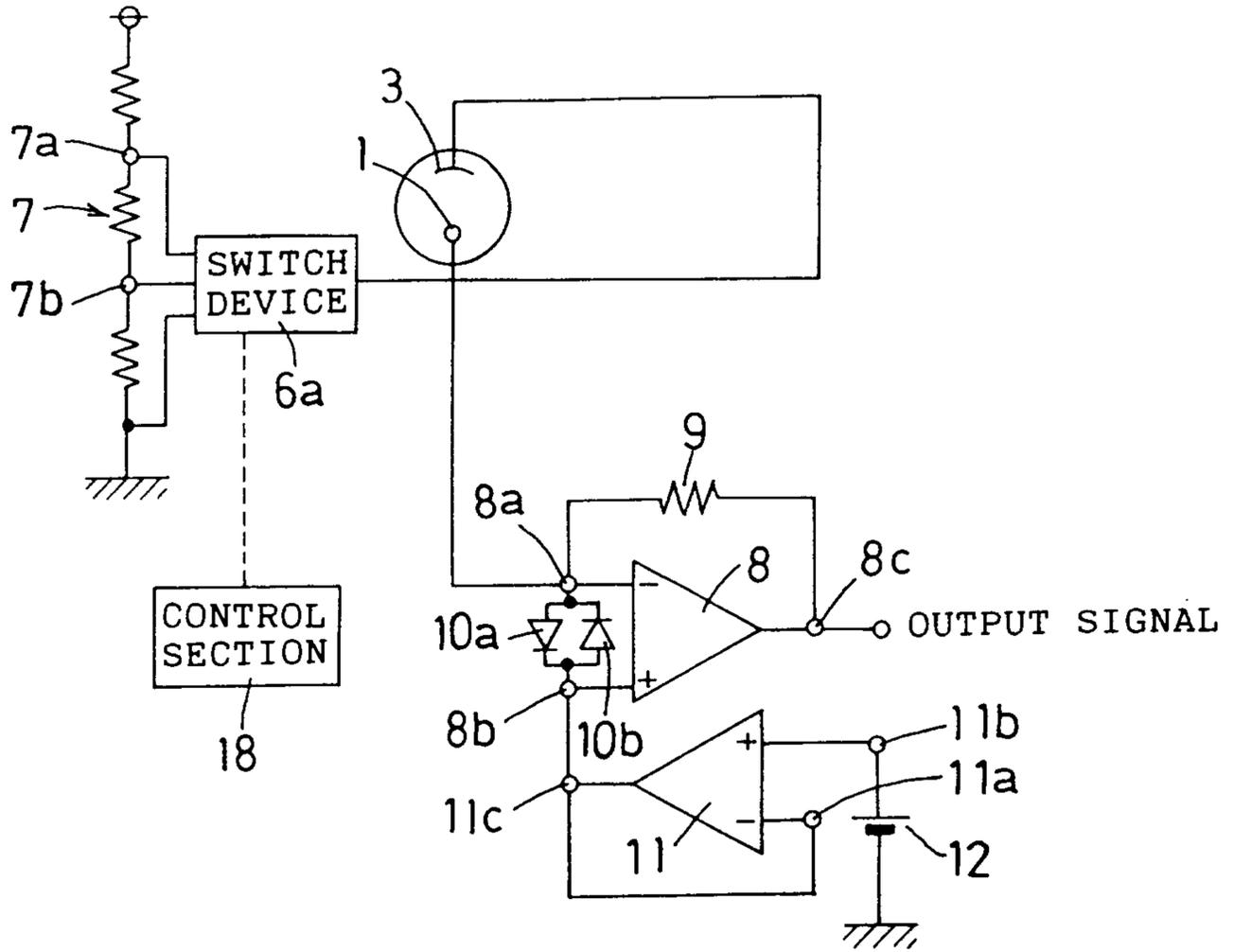
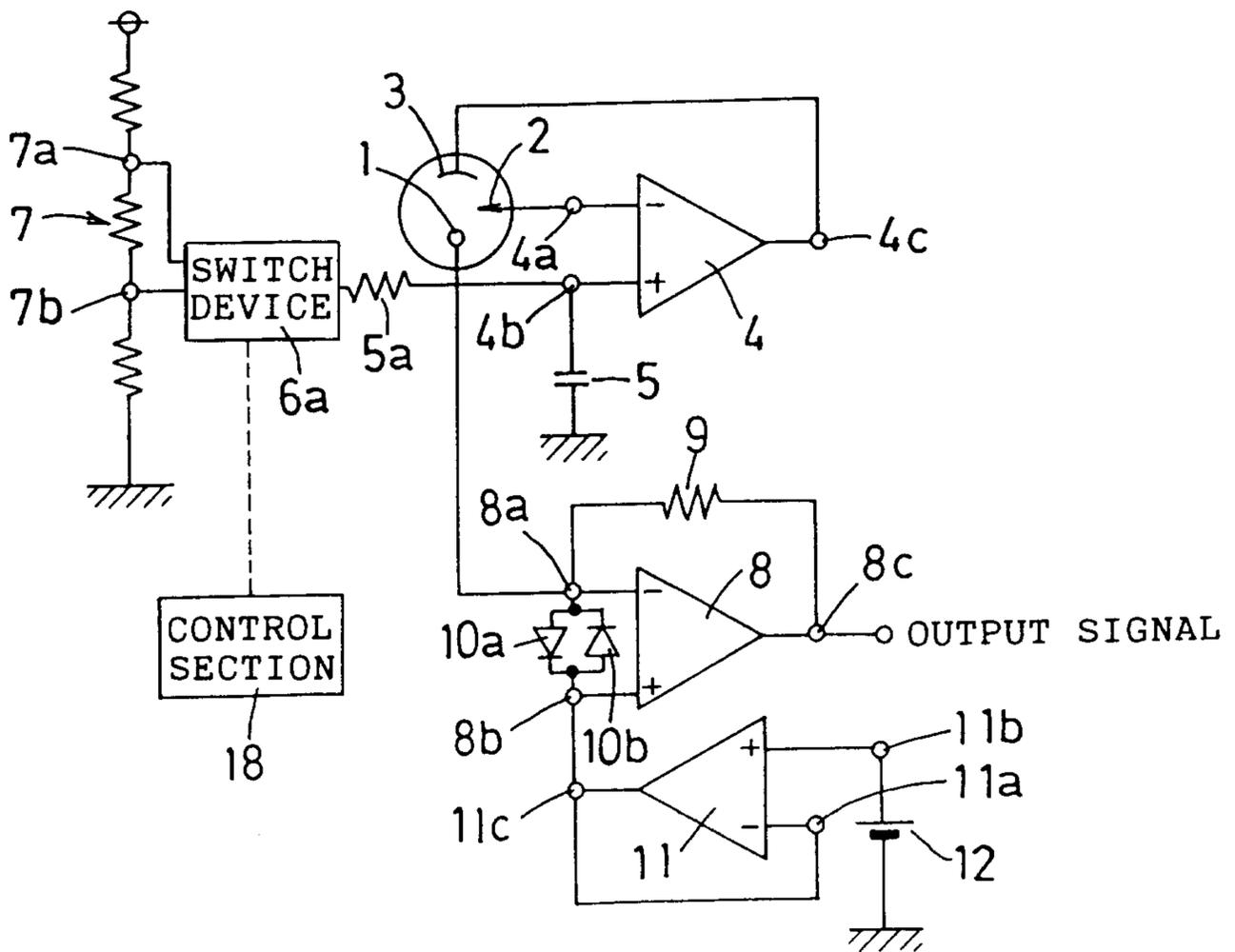


Fig. 6



by ROGERS, Bereskin & Parr

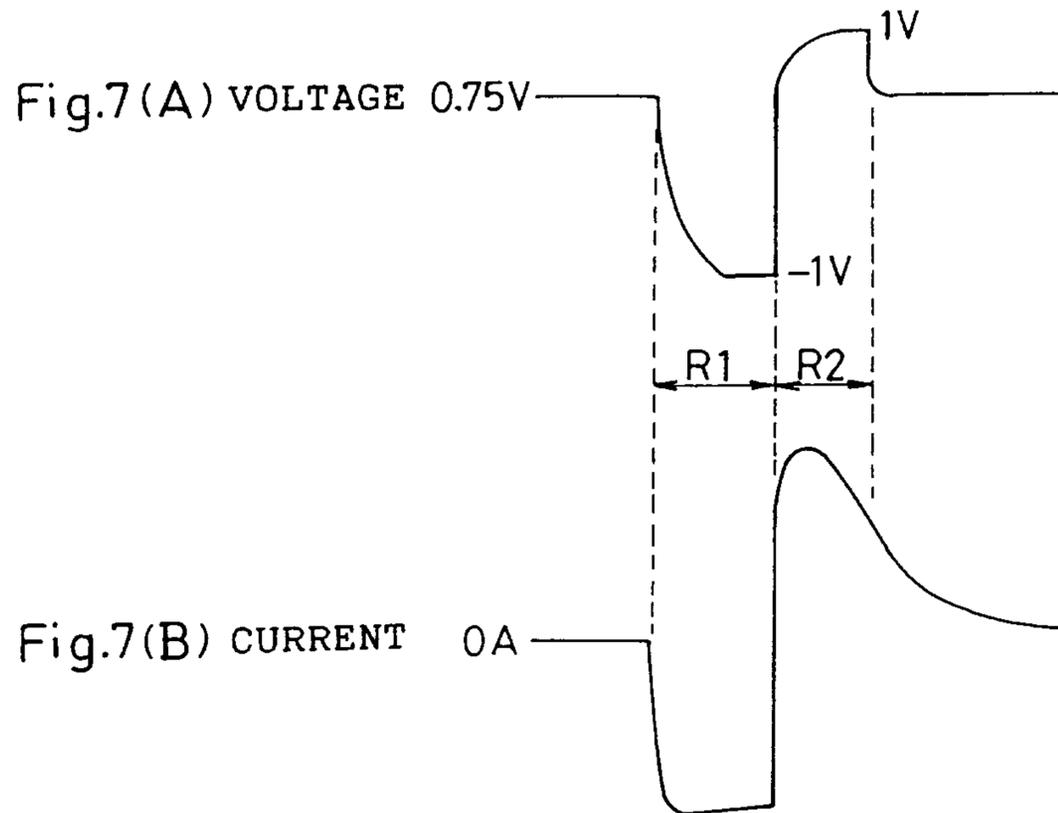
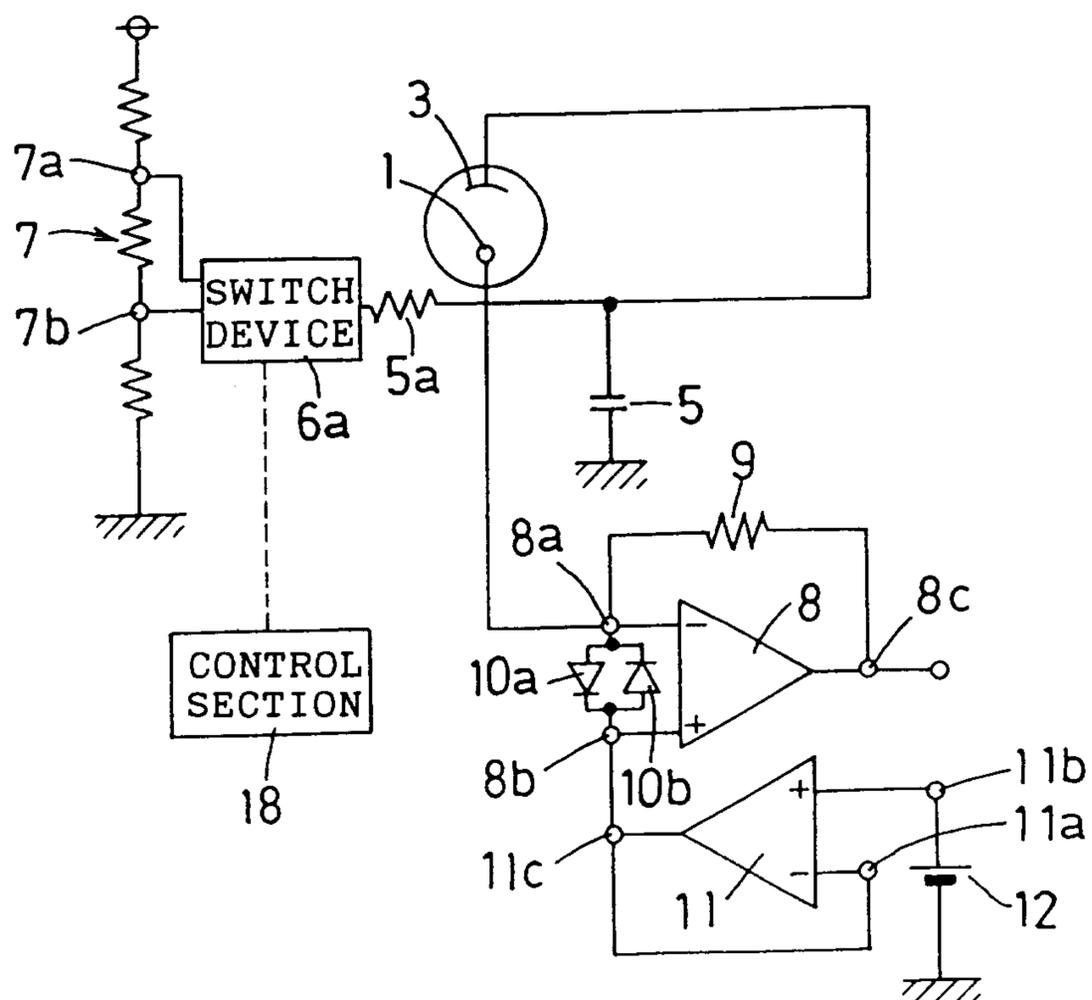


Fig.8



by ROGERS, BERESKIN & PAIR