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(54) **METHOD AND APPARATUS FOR MEASURING SUBSTRATE CONCENTRATION**

VERFAHREN UND VORRICHTUNG ZUR SUBSTRATKONZENTRATIONSMESSUNG

PROCÉDÉ ET APPAREIL DE MESURE DE LA CONCENTRATION D'UN SUBSTRAT

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## Description

TECHNICAL FIELD

**[0001]** The present invention relates to a method, a device and a reagent for carrying out the measurement of a substrate such as glucose.

BACKGROUND ART

**[0002]** There is a conventionally known method called an electrode method, which is a method for measuring a glucose concentration. According to the method, information correlating with a glucose concentration in a specimen is outputted to an electrode in contact with the specimen, and the glucose concentration is calculated based on the output. An enzymatic electrode is conventionally used as the electrode. A conventionally known example of the enzymatic electrode has the structure where an enzyme-immobilized film and a substrate selectively permeable film are multilayered on a surface thereof.

**[0003]** The enzymatic electrode does not have a constant sensitivity. The sensitivity is liable to be degraded depending on environments where it is used, or by a repeated use of the enzymatic electrode. The factors causing the degradation of the sensitivity are, for example, alternation of an activity of the enzyme due to temperature changes and deactivation of the enzyme, deterioration of substrate permeability in the substrate selectively permeable film, and oxidization of the electrode surface. The enzymatic electrode is thus deteriorated as it is more frequently used and needs to be replaced after being used over a definite period, and calibration is a necessary measure to deal with the sensitivity variation before the replacement of the enzymatic electrode.

**[0004]** In an automatic measuring device, for example, the calibration is performed at the startup of the device, and is generally performed at certain time intervals, or per a certain number of measurements in a case where the device is uninterruptedly used over an extended period of time. The sensitivity of the enzymatic electrode is variable owing to the various factors described earlier. In addition to that, the sensitivity may be changeable during the calibration, and it is difficult to predict or grasp what factor causes the sensitivity of the enzymatic electrode to change. Therefore, measured values obtained immediately after the calibration can be very reliable, however, it is not necessarily a case that the reliability of measured values obtained between the calibrations is equally high.

**[0005]** In the case of measuring a substrate concentration using an enzymatic electrode placed in a flow cell, for example, a base current Rb outputted from the enzymatic electrode substantially stays at a constant level as illustrated in Fig. 7A when the sensitivity of the enzymatic electrode remains unchanged, and the output from the enzymatic electrode is increased when a specimen is introduced. On the contrary, the base output is lowered as illustrated in Fig. 7B under such circumstances that the sensitivity of the enzymatic electrode may be degraded.

To calculate the substrate concentration, the output from the enzymatic electrode before the supply of the specimen to the enzymatic electrode (T1) is sampled as a base output Rb, and the output from the enzymatic electrode when a predetermined amount of time passed after the supply of the specimen to the enzymatic electrode (T2) is sampled as an output for measurement Re, and a difference between the output for measurement Re and the base output Rb is obtained, so that the substrate concentration is calculated based on the difference. More specifically describing the calculation, the base output when the output for measurement Re is sampled is used as the base output Rb before the output for measurement Re is sampled on the assumption that the base output (sensitivity of the enzymatic electrode) is constant.

Therefore, as illustrated in Fig. 7B, a calculated value of the substrate concentration is different to a true value in a case where a base output Rb2 obtained by sampling the output for measurement Re is different to a base output Rb1 previously measured.

When the enzymatic electrode deteriorated over time is replaced, the sensitivity of the enzymatic electrode shows a sudden drop immediately after the replacement, and is thereafter stabilized to stay at a certain value. Therefore, it is pointless to perform the calibration before the sensitivity of the enzymatic electrode is stabilized after the replacement of the enzymatic electrode. Thus, the enzyme electrode after the replacement is not ready for measurements until the sensitivity of the enzymatic electrode is stabilized and the calibration is completed.

[Patent Document 1] JP-A-S59-082020
[Patent Document 2] JP-B-3723827

Further, US 2005/089944 A1 discloses an amperometric glucose biosensor comprising a sensing electrode and a reference electrode arranged in a side-by-side parallel configuration on an electrically insulating sheet.

DISCLOSURE OF THE INVENTION

PROBLEM TO BE SOLVED BY THE INVENTION

**[0006]** A main object of the present invention is to improve a measurement accuracy and reliability in the measurement of a concentration of a substrate such as glucose included in a specimen using an enzymatic electrode.

MEANS FOR SOLVING THE PROBLEMS

**[0007]** The problem is solved by a substrate concentration measuring method according to claim 1.

**[0008]** For example, the calculation of the substrate concentration employs a current output for correction for

the specimen to be measured, and a preceding output for correction corresponding to at least one other specimen and measured prior to the current output for correction. In this case, a mean value of the preceding output for correction and the current output for correction may be used to calculate the substrate concentration.

**[0009]** The substrate concentration may be calculated based on a difference between the output for measurement and a base output measured when washing the enzymatic electrode. When the substrate concentration is thus calculated, it is preferable to estimate the base output to be subtracted from the output for measurement to obtain the difference based on an estimation curve drawn so as to correspond to a time-dependent variation of a plurality of base outputs when a change equivalent to at least a given value is detected between the plurality of base outputs.

**[0010]** The enzymatic electrode is placed in, for example, a flow cell. An example of the specimen is whole blood, and an example of the substrate is glucose.

**[0011]** The problem is further solved by a substrate concentration measuring device according to claim 7.

**[0012]** For example, the calculating means is configured to calculate the substrate concentration using a current output for correction for the specimen to be measured and a preceding output for correction corresponding to at least one other specimen and measured prior to the current output for correction. In this case, the calculating means may be configured to calculate the substrate concentration using a mean value of the current output for correction and the preceding output for correction.

**[0013]** The calculating means may be configured to calculate the substrate concentration based on a difference between the output for measurement and a base output measured when washing the enzymatic electrode. When the calculating means thus calculates the substrate concentration, the calculating means is preferably configured to calculate the substrate concentration after estimating the base output to be subtracted from the output for measurement to obtain the difference based on an estimation curve drawn so as to correspond to a time-dependent variation of a plurality of base outputs when a change equivalent to at least a given value is detected between the plurality of base outputs.

**[0014]** The enzymatic electrode is placed in, for example, a flow cell, and an enzymatic electrode which selectively reacts with glucose as a substrate is used as the enzymatic electrode.

**[0015]** The substrate concentration measuring device according to the present invention preferably further includes: a specimen preparing means for preparing the specimen to be reacted with the enzymatic electrode; a reference solution retaining tank for retaining the reference solution for obtaining the output for correction; and a selecting means for selecting one of a state where the specimen is to be supplied and a state where the reference solution is to be supplied for the enzymatic electrode.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0016]**

Fig. 1 is a sectional view schematically illustrating a structure of a concentration measuring device according to the present invention.

Fig. 2 is a sectional view schematically illustrating a structure of an enzyme electrode provided in the concentration measuring device illustrated in Fig. 1.

Fig. 3 is a block diagram of the substrate concentration measuring device illustrated in Fig. 1.

Fig. 4 is a timing chart of outputs from the enzymatic electrode provided to describe an example of an operation in a calculating unit.

Fig. 5 is a timing chart of outputs from the enzymatic electrode provided to describe another example of the operation in the calculating unit.

Fig. 6 a timing chart of outputs from the enzymatic electrode provided to describe still another example of the operation in the calculating unit.

Figs. 7A and 7B are timing charts of outputs from an enzymatic electrode provided to describe a conventional substrate concentration calculating method.

## DESCRIPTION OF REFERENCE SYMBOLS

**[0017]**

| | |
|---|---|
| 1 | concentration measuring device |
| 2 | specimen preparation mechanism (specimen preparation means) |
| 21' | reference solution tank (reference solution containing tank) |
| 29 | valve (selecting means) |
| 33 | flow cell |
| 30 | enzyme electrode |
| 5 | calculating unit (calculating means) |

## BEST MODE FOR CARRYING OUT THE INVENTION

**[0018]** The present invention is described below referring to the drawings.

**[0019]** A concentration measuring device 1 illustrated in Fig. 1 is configured to measure a specimen adjusted in a specimen preparation mechanism 2 using a measurement mechanism 3.

**[0020]** The specimen preparation mechanism 2 is a mechanism for preparing a specimen from an analyte, and comprises a nozzle 20, a reagent bottle 21, and a preparation tank 22.

**[0021]** The nozzle 20 is a nozzle for supplying an analyte into the preparation tank 22. Examples of the analyte to be used are biochemical specimens such as blood, urine and saliva, or diluents obtained therefrom. In a case where blood is used as the specimen, any of the whole blood and blood serum or plasma can be used.

**[0022]** The reagent bottle 21 retains therein a reagent

for diluting an analyte or washing the preparation tank 22. The reagent bottle 21 is connected to the preparation tank 22 with a pipe 23 interposed therebetween. A pump 24 is provided at an intermediate position in the pipe 23, and a configuration is given such that a force generated by the pump 24 serves to supply the reagent retained in the reagent bottle 21 into the preparation tank 22.

**[0023]** An example of the the reagent is a buffer solution. The buffer solution is not particularly limited as far as it can adjust the reaction pH of a substrate in a targeted range, and phosphates, for example, can be used. The concentration of the buffer solution in the reagent is set to, for example, 0.0001 to 0.1000 M. The examples of the reagent include, in addition to the buffer solution, conventional hemolytic agents and preservatives such as an azide compound, and the reagent may further include, for example, sodium oxide or potassium oxide.

**[0024]** The preparation tank 22 provides a site where the specimen can be prepared.The preparation tank 22 is configured such that the analyte is supplied by means of the nozzle 20, and the reagent is supplied from the reagent bottle 21. The preparation tank 22 comprises therein an agitator 25, and when the agitator 25 is rotated by a stirrer 26, the analyte and the reagent are mixed with each other. The preparation tank 22 is connected to a drain pipe 27 for discarding the prepared solution of the preparation tank 22 and further connected to an enzymatic electrode 30 of a measurement mechanism 3 by way of a pipe 28. A valve 29 is provided at an intermediate position in the pipe 28, and a reference solution tank 21' is connected to the valve 29. The valve 29 is thereby configured to select one of a state where the specimen prepared in the preparation tank 22, reagent of the reagent bottle 20 or reference solution of the reference solution tank 21' is to be supplied and a state where the above-described specimen, reagent, or reference solution is not to be supplied for the enzymatic electrode 30 of the measurement mechanism 3. As the reference solution can be used a reagent whose substrate concentration is known, for example, diluents obtained from any reference solution which is conventionally used in the calibration (diluted 50 - 200 times).

**[0025]** The measurement mechanism 3 comprises the enzyme electrode 30, a power supply 31 and a current value measuring unit 32.

**[0026]** The enzyme electrode 30 outputs an electrophysical quantity corresponding to the amount of electrons transferred to and received from a substrate in a specimen. The enzyme electrode 30 is arranged in a flow cell 33, and as illustrated in Fig. 2, the enzyme electrode 30 comprises a first selective transmission film 34, an enzyme immobilized film 35, a second selective transmission film 36, and a electrode 37.

**[0027]** The first selective transmission film 34 is a film for selectively supplying a substrate in a specimen to the enzyme immobilized film 35. The first selective transmission film 34 to be used is a various conventionally available film, although being selected depending on the type of a substrate.

**[0028]** The enzyme immobilized film 35 is a film for generating hydrogen peroxide by oxidizing or reducing a substrate, and is configured to include an oxidase. The oxidase used in the present invention is selected depending on the type of a substrate. Examples of the substrate to be measured by the concentration measuring device 1 are glucose and lactate, and glucose oxidase or lactate oxidase can be mentioned as the enzyme.

**[0029]** The second selectively permeable film 36 is provided to selectively supply a reactant such as hydrogen peroxide produced from the substrate by the enzyme to the electrode 37. Usable examples of the second selectively permeable film 36 are acetylcellulose-based and polyarylamine-based films.

**[0030]** The electrode 37 outputs an electrical signal corresponding to the amount of the supplied hydrogen peroxide, in other words, the concentration of the substrate. The electrode 37 to be used is an electrode in which platinum is used as an anode 38, while silver is used as a cathode 39.

**[0031]** The power supply 31 illustrated in Fig. 1 applies a voltage to the enzyme electrode 30 (electrode 37). A direct-current power supply, for example, is used as the power supply 31, and the voltage applied to the enzyme electrode 30 (electrode 37) is set to, for example, 0.1 to 1.0 V

**[0032]** The current value measuring unit 32 is a unit for measuring the current value when a voltage is applied between the anode 38 and the cathode 39. The current value is intermittently measured by the current value measuring unit 32, and a measurement interval is set to, for example, 50 to 200 msec.

**[0033]** As illustrated in Fig. 4, the substrate concentration measuring device 1 further includes a controlling unit 4 and a calculating unit 5.

**[0034]** The controlling unit 4 controls the operations of the respective structural elements. More specifically, the controlling unit 4 controls the operations of the nozzle 20, pump 24, stirrer 26, valve 29 and the like. The controlling unit 4 further controls the operation of the measurement mechanism 3. More specifically, the controlling unit 4 controls the power supply 31 to thereby select one of a state where a voltage is to be applied to the electrode 37 and a state where the voltage is not to be applied thereto, and controls the current value measuring unit 32 to thereby control a timing of measuring a current value. The measuring operation of the current value measuring unit 31 is controlled by the controlling unit 4 so that the current value is repeatedly measured at the intervals of, for example, 50 - 200 μsec.

**[0035]** The calculating unit 5 is provided to calculate a concentration of the substrate, such as glucose, included in the analyte based on a result of the measurement by the current value measuring unit 32. The calculating unit 5 stores therein programs necessary for the calculation, and the operation of the calculating unit is controlled by the controlling unit 4.

**[0036]** Next, an operation of the substrate concentration measuring device 1 is described.

**[0037]** In the substrate concentration measuring device 1, the specimen, prepared solution, reagent and reference solution are repeatedly supplied to the flow cell 33. More specifically, the substrate concentration measuring device 1 repeatedly carries out: washing the enzymatic electrode using the reagent; measuring the substrate using the prepared solution; washing the enzymatic electrode using the reagent; and monitoring the sensitivity of the enzymatic electrode 30 using the reference solution.

**[0038]** When the enzymatic electrode is washed with the reagent, the reagent of the reagent bottle 21 is supplied to the flow cell 33 through the preparation tank 22.

**[0039]** To measure the substrate, the analyte such as whole blood and the reagent are supplied to the preparation tank 22 and mixed therein, and the specimen thereby prepared is supplied to the flow cell 33 so that the output from the enzymatic electrode 30 is detected. The analyte is supplied to the preparation tank 22 through the nozzle 20, and an amount of the analyte to be supplied is set to 4 to 20 μm in a case where whole blood is used. The reagent is supplied to the preparation tank 22 through the pump 24, an amount of the reagent to be supplied is set to about 100 times as much as the whole blood. The analyte and the reagent are mixed with each other by rotating the agitating member 25 of the stirrer 26.

**[0040]** In the enzymatic electrode 30, the substrate permeates through the first electively permeable film 34 to be supplied to the enzyme-immobilized film 35. In the enzyme-immobilized film 35, the substrate is oxidized or reduced by the enzyme, and a reactant such as hydrogen peroxide is correspondingly produced. The produced reactant permeates through the second selectively permeable film 36 to be supplied to the electrode 37. In the electrode 37, electrons are transferred between the reactant and the anode 38 or the cathode 39 by the voltage applied by the power supply 31. At the time, the transfer of the electrons to and from the anode 38 or the cathode 39 generates a current flow between the anode 38 and the cathode 39, and the current generated then (output for measurement) is measured by the current value measuring unit 32.

**[0041]** To monitor the sensitivity of the enzymatic electrode 30 using the reference solution, the reference solution of the reference solution tank 21' is supplied to the flow cell 33 with the voltage being applied to the electrode 37 by the power supply 31, and a current generated then (output for correction) is measured by the current measuring unit 32.

**[0042]** Fig. 4 illustrates the outputs from the enzymatic electrode 30 when one cycle of processing steps consisting of washing, substrate detection, washing and sensitivity monitoring, is repeatedly performed. In Fig. 4, the outputs for two cycles are illustrated.

**[0043]** The calculating unit 5 calculates the substrate concentration using the output for correction in the enzymatic electrode 30 when the reference solution is supplied to the flow cell in addition to the output for measurement in the enzymatic electrode 30 when the specimen is supplied to the flow cell 33.

**[0044]** More specifically, the calculating unit 5 samples, in the same cycle, a current value (base output Rb) when a predetermined amount of time passed after the wash started (T1), a current value (output for measurement Rs) when a predetermined amount of time passed after the supply of the specimen to the flow cell 33 started (T2), and a current value (output for correction Rm) when a predetermined amount of time passed after the supply of the reference solution. The calculating unit 5 further calculates a monitored reaction value ΔRm by subtracting the base output Rb from the output for correction Rm. The sensitivity of the enzymatic electrode 30 is reflected on the monitored reaction value ΔRm. Moreover, the calculating unit 5 calculates a substrate reaction value ΔRs by subtracting the base output Rb from the output for measurement Rs, and calculates the substrate concentration using the monitored reaction value ΔRm. To calculate the substrate concentration, for example, the substrate reaction value ΔRs is multiplied by a modulus corresponding to the monitored reaction value ΔRm so that a corrected reaction value is calculated, and the corrected reaction value is allocated to a given analytical curve or table. The substrate concentration may be calculated such that the substrate concentration is primarily calculated based on the substrate reaction value ΔRs, and a result of the primary calculation is corrected by the corrected reaction value.

**[0045]** In the substrate concentration measuring device 1, the sensitivity of the enzymatic electrode 30 is monitored (output for correction Rm is measured) by means of the reference solution in order to measure each substrate, and a monitoring result thereby obtained is reflected on the calculation of the substrate concentration. Therefore, the substrate concentration measuring device 1 can suitably calculate the substrate concentration depending on the sensitivity of the enzymatic electrode 30 whatever the factors are that cause the sensitivity of the enzymatic electrode 30 to be variable, which is different to the conventional calibration performed, for example, at the startup of a device, at certain time intervals, or per a certain number of measurements. As a result, the substrate concentration measuring device 1 can improve a measurement accuracy and reliability.

**[0046]** In the substrate concentration measuring device 1 described so far, the sensitivity is monitored once to measure one substrate. The sensitivity may be likewise monitored once for a plurality of substrates in any environment where the base output Rb is stabilized.

**[0047]** Next, another example of the substrate concentration measuring method carried out by the calculating unit 5 is described referring to Fig. 5.

**[0048]** The calculating unit 5 may be configured to calculate the substrate concentration using, in addition to an output for correction Rm1 measured for the substrate

to be measured, outputs for correction Rm2 - Rm5 of the substrates previously measured. More specifically, the calculating unit 5 may be configured to calculate the substrate concentration based on a mean value obtained from the output for correction Rm1 measured for the substrate to be measured and the outputs for correction Rm2 to Rm5 of the substrates previously measured.

**[0049]** Thus configured, any impact of the variability generated in the measurement of the output for correction is controlled by taking the mean value of the current output for correction and the outputs for correction measured in the past. As a result, a measurement reproducibility can be improved.

**[0050]** The number of the outputs for correction measured in the past plus the current output for correction to obtain the mean value is not limited to five as far as at least two outputs for correction can be used.

**[0051]** Next, still another example of the substrate concentration measuring method carried out by the calculating unit 5 is described referring to Fig. 6.

**[0052]** The calculating unit 5 may be configured to compare a base output Rb1 measured for the substrate to be measured to base outputs Rb2 and Rb3 of the substrates previously measured and calculate the substrate concentration based on a comparison result thereby obtained. More specifically, the calculating unit 5 determines that the base output Rb undergoes a large variation, that is, the sensitivity of the enzymatic electrode 30 undergoes a large variation, in a case where the current base output Rb1 is smaller than the past base outputs Rb2 and Rb3 by a given value, and estimates base outputs Rb' and Rb" obtained by sampling the output for measurement Rs and the output for correction Rm in accordance with the time-dependent variation of the base outputs Rb1 to Rb3. Then, the calculating unit 5 calculates the substrate concentration based on the output for measurement Rs, output for correction Rm and estimated base outputs Rb' and Rb".

**[0053]** According to the configuration, the substrate concentration can be measured with a high accuracy even if the sensitivity of the enzymatic electrode 30 deteriorates to a relatively large extent which typically happens immediately after the enzymatic electrode 30 is replaced. When the enzymatic electrode 30 is replaced, it is unnecessary to wait for the sensitivity of the enzymatic electrode 30 to be stabilized before the substrate concentration is measured, which is different to the conventional calibration.

**[0054]** The present invention is not limited to the embodiment described so far, and can be variously modified. For example, the enzyme electrode may be configured to be fixated in the preparation tank, and the substrate concentration may be measured according to the batch system, and further, the preparation tank is omitted, and a configuration may be given such that the reagent of the reagent bottle 21 is continuously supplied to the enzyme electrode 30, and the analyte and specimen are injected from an injection valve.

## Claims

1. A substrate concentration measuring method for measuring a concentration of a substrate included in a specimen based on an output for measurement from an enzymatic electrode (30) when the enzyme electrode (30) and the substrate are reacted with each other, wherein
the substrate concentration is calculated using an output for correction from the enzymatic electrode (30) obtained when a reference solution whose substrate concentration is known and the enzymatic electrode (30) are reacted with each other before or after the enzymatic electrode (30) and the substrate are reacted with each other, **characterized in that** the method comprises the following steps:

   (i) obtaining a substrate reaction value ΔRs by subtracting a base output Rb' from an output for measurement Rs;
   (ii) obtaining a monitored reaction value ΔRm by subtracting a base output Rb " from an output for correction Rm; and
   (iii) calculating a substrate concentration based on the relationship between the substrate reaction value ΔRs and the monitored reaction value ΔRm,

   wherein the output for correction is measured for each specimen.

2. The substrate concentration measuring method as claimed in Claim 1, wherein the output for correction for the specimen to be measured and an output for correction corresponding to at least one other specimen and measured prior to the output for correction are used to calculate the substrate concentration.

3. The substrate concentration measuring method as claimed in Claim 2, wherein a mean value of the output for correction for the specimen to be measured and the output for correction corresponding to at least one other specimen and measured prior to the output for correction is used to calculate the substrate concentration.

4. The substrate concentration measuring method as claimed in Claim 1, wherein the substrate concentration is calculated based on a difference between the output for measurement and a base output measured when washing the enzymatic electrode (30) is completed.

5. The substrate concentration measuring method as claimed in Claim 4, wherein the base output to be subtracted from the output for measurement to obtain the difference is estimated based on an estimation curve drawn so as to correspond to a time-de-

pendent variation of a plurality of base outputs when a change equivalent to at least a given value is detected between the plurality of base outputs.

**6.** The substrate concentration measuring method as claimed in Claim 1, wherein a measurement interval is set to from 50 to 200 msec.

**7.** A substrate concentration measuring device (1) comprising a calculating means (5) for calculating a concentration of a substrate included in a specimen based on an output for measurement from an enzymatic electrode (30) when the enzyme electrode (30) and the substrate are reacted with each other, wherein
the calculating means calculates the substrate concentration using an output for correction from the enzymatic electrode (30) obtained when a reference solution whose substrate concentration is known and the enzymatic electrode (30) are reacted with each other before or after the enzymatic electrode (30) and the substrate are reacted with each other, **characterized in that** the measuring device is arranged to carry out the following steps:

(i) obtaining a substrate reaction value ΔRs by subtracting a base output Rb' from an output for measurement Rs;
(ii) obtaining a monitored reaction value ΔRm by subtracting a base output Rb" from an output for correction Rm; and
(iii) calculating a substrate concentration based on the relationship between the substrate reaction value ΔRs and the monitored reaction value ΔRm,

wherein the calculating means is configured to calculate the substrate concentration using the output for correction measured for each specimen, and further comprising:

a specimen preparing means (2) for preparing the specimen to be reacted with the enzymatic electrode (30);
a reference solution retaining tank for retaining the reference solution for obtaining the output for correction; and
a selecting means (29) for selecting one of a state where the specimen is to be supplied and a state where the reference solution is to be supplied for the enzymatic electrode (30)..

**8.** The substrate concentration measuring device as claimed in Claim 7, wherein the calculating means is configured to calculate the substrate concentration using the output for correction for the specimen to be measured and an output for correction corresponding to at least one other specimen and measured prior to the output for correction.

**9.** The substrate concentration measuring device as claimed in Claim 8, wherein the calculating means is configured to calculate the substrate concentration using a mean value of the current output for correction for the specimen to be measured and the output for correction corresponding to at least one other specimen and measured prior to the current output for correction.

**10.** The substrate concentration measuring device as claimed in Claim 7, wherein the calculating means is configured to calculate the substrate concentration based on a difference between the output for measurement and a base output measured when washing the enzymatic electrode (30).

**11.** The substrate concentration measuring device as claimed in Claim 10, wherein the calculating means is configured to calculate the substrate concentration after estimating the base output to be subtracted from the output for measurement to obtain the difference based on an estimation curve drawn so as to correspond to a time-dependent variation of a plurality of base outputs when a change equivalent to at least a given value is detected between the plurality of base outputs.

**Patentansprüche**

**1.** Substratkonzentrationsmessverfahren zum Messen einer Konzentration eines in einer Probe enthaltenen Substrats basierend auf einer Messausgabe einer enzymatischen Elektrode (30), wenn die enzymatische Elektrode (30) und das Substrat miteinander reagieren, wobei
die Substratkonzentration unter Verwendung einer Korrekturausgabe der enzymatischen Elektrode (30) berechnet wird, die erhalten wird, wenn eine Referenzlösung, deren Substratkonzentration bekannt ist, und die enzymatische Elektrode (30) miteinander zur Reaktion gebracht werden, und zwar bevor die enzymatische Elektrode (30) und das Substrat miteinander zur Reaktion gebracht werden, **dadurch gekennzeichnet, dass** das Verfahren die folgenden Schritte aufweist:

(i) Erhalten eines Substratreaktionswerts ΔRs durch Abziehen einer Basisausgabe Rb' von einer Messausgabe Rs;
(ii) Erhalten eines Reaktionsüberwachungswerts ΔRm durch Subtrahieren einer Basisausgabe Rb " von einer Korrekturausgabe Rm; und
(iii) Berechnen einer Substratkonzentration basierend auf der Beziehung zwischen dem Substratreaktionswert ΔRs und des Reaktionsüber-

wachungswerts ΔRm,

wobei die Korrekturausgabe für jede Probe gemessen wird.

**2.** Substratkonzentrationsmessverfahren nach Anspruch 1, bei dem die Korrekturausgabe für die zu messende Probe und eine Korrekturausgabe, die mit mindestens einer anderen Probe übereinstimmt und vor der Korrekturausgabe gemessen wird, verwendet werden, um die Substratkonzentration zu berechnen.

**3.** Substratkonzentrationsmessverfahren nach Anspruch 2, bei dem ein Mittelwert der Korrekturausgabe für die zu messende Probe und die Korrekturausgabe, die mit mindestens einer anderen Probe übereinstimmt und vor der Korrekturausgabe gemessen wird, verwendet wird, um die Substratkonzentration zu berechnen.

**4.** Substratkonzentrationsmessverfahren nach Anspruch 1, bei dem die Substratkonzentration basierend auf einer Differenz zwischen der Messausgabe und einer Basisausgabe berechnet wird, die gemessen wird, wenn das Waschen der enzymatischen Elektrode (30) abgeschlossen ist.

**5.** Substratkonzentrationsmessverfahren nach Anspruch 4, bei dem, die zur Erhaltung der Differenz von der Messausgabe abzuziehende Basisausgabe auf Grundlage einer Schätzkurve abgeschätzt wird, die so verläuft, um mit einer zeitabhängigen Veränderung einer Vielzahl von Basisausgaben übereinzustimmen, wenn eine Veränderung zwischen der Vielzahl von Basisausgaben erfasst wird, die mindestens einem gegebenen Wert gleicht.

**6.** Substratkonzentrationsmessverfahren nach Anspruch 1, bei dem ein Messintervall zwischen 50 bis 200 ms eingestellt wird.

**7.** Substratkonzentrationsmessgerät (1) mit einem Berechnungsmittel (5) zum Berechnen einer in einer Probe enthaltenen Substratkonzentration basierend auf einer Messausgabe einer enzymatischen Elektrode (30), wenn die enzymatische Elektrode (30) und das Substrat miteinander zur Reaktion gebracht werden, wobei
das Berechnungsmittel die Substratkonzentration unter Verwendung einer Korrekturausgabe der enzymatischen Elektrode (30) berechnet, die erhalten wird, wenn eine Referenzlösung, deren Substratkonzentration bekannt ist, und die enzymatische Elektrode (30) miteinander zur Reaktion gebracht werden, und zwar vor oder nachdem die enzymatische Elektrode (30) und das Substrat miteinander zur Reaktion gebracht werden, **dadurch gekennzeichnet, dass** das Messgerät eingerichtet ist, um die folgenden Schritte auszuführen:

(i) Erhalten eines Substratreaktionswerts ΔRs durch Abziehen einer Basisausgabe Rb' von einer Messausgabe Rs;
(ii) Erhalten eines Reaktionsüberwachungswerts ΔRm durch Subtrahieren einer Basisausgabe Rb" von einer Korrekturausgabe Rm; und
(iii) Berechnen einer Substratkonzentration basierend auf der Beziehung zwischen dem Substratreaktionswert ΔRs und dem Reaktionsüberwachungswert ΔRm,

wobei das Berechnungsmittel eingerichtet ist, die Substratkonzentration unter Verwendung der Korrekturausgabe zu berechnen, die für jede Probe gemessen wird, und
des Weiteren mit:

einem Probenvorbereitungsmittel (2) zum Vorbereiten der mit der enzymatischen Elektrode (30) zur Reaktion zu bringenden Probe;
einem Referenzlösungsaufnahmetank zur Aufnahme der Referenzlösung zum Erhalten der Korrekturausgabe; und
einem Auswahlmittel (29) zum Auswählen eines Zustands, in dem die Probe zuzuführen ist, und eines Zustands, in dem die Referenzlösung der enzymatischen Elektrode (30) zuzuführen ist.

**8.** Substratkonzentrationsmessgerät nach Anspruch 7, bei dem das Berechnungsmittel eingerichtet ist, die Substratkonzentration unter Verwendung der Korrekturausgabe für die zu messende Probe und einer Korrekturausgabe, die mit mindestens einer anderen Probe übereinstimmt und vor der Korrekturausgabe gemessen wird, zu berechnen.

**9.** Substratkonzentrationsmessgerät nach Anspruch 8, bei dem das Berechnungsmittel eingerichtet ist, die Substratkonzentration unter Verwendung eines Mittelwerts der aktuellen Korrekturausgabe für die zu messende Probe und der Korrekturausgabe, die mit mindestens einer anderen Probe übereinstimmt und vor der aktuellen Korrekturausgabe gemessen wird ist, zu berechnen.

**10.** Substratkonzentrationsmessgerät nach Anspruch 7, bei dem das Berechnungsmittel eingerichtet ist, die Substratkonzentration basierend auf einer Differenz zwischen der Messausgabe und einer Basisausgabe zu berechnen, die beim Waschen der enzymatischen Elektrode (30) bestimmt wird.

**11.** Substratkonzentrationsmessgerät nach Anspruch 10, bei dem das Berechnungsmittel eingerichtet ist, die Substratkonzentration zu berechnen, und zwar

nach einem Schätzen der von der Messausgabe abzuziehenden Basisausgabe, um die Differenz basierend auf einer abgeleiteten Schätzkurve zu erhalten, die so verläuft, um mit einer zeitabhängigen Veränderung einer Vielzahl von Basisausgaben übereinzustimmen, wenn eine Veränderung zwischen der Vielzahl von Basisausgaben detektiert wird, die mindestens einem gegebenen Wert gleicht.

**Revendications**

**1.** Procédé de mesure de la concentration d'un substrat destiné à mesurer une concentration d'un substrat inclus dans un échantillon basée sur une sortie pour mesure provenant d'une électrode enzymatique (30) quand l'électrode enzymatique (30) et le substrat sont mis à réagir l'un avec l'autre, dans lequel :

la concentration du substrat est calculée à l'aide d'une sortie pour correction provenant de l'électrode enzymatique (30) obtenue quand une solution de référence dont la concentration du substrat est connue et l'électrode enzymatique (30) sont mises à réagir l'une avec l'autre avant ou après que l'électrode enzymatique (30) et le substrat soient mis à réagir l'un avec l'autre, **caractérisé en ce que** le procédé comprend les étapes suivantes consistant à :

(i) obtenir une valeur de réaction du substrat ΔRs en soustrayant une sortie de base Rb' d'une sortie pour mesure Rs ;
(ii) obtenir une valeur de réaction surveillée ΔRm en soustrayant une sortie de base Rb" d'une sortie pour correction Rm ; et
(iii) calculer une concentration du substrat en se basant sur la relation entre la valeur de réaction du substrat ΔRs et la valeur de réaction surveillée ΔRm,

dans lequel la sortie pour correction est mesurée pour chaque échantillon.

**2.** Procédé de mesure de la concentration d'un substrat selon la revendication 1, dans lequel la sortie pour correction pour l'échantillon à mesurer et une sortie pour correction correspondant au moins à un autre échantillon et mesurée avant la sortie pour correction sont utilisées pour calculer la concentration du substrat.

**3.** Procédé de mesure de la concentration d'un substrat selon la revendication 2, dans lequel une valeur moyenne de la sortie pour correction pour l'échantillon à mesurer et de la sortie pour correction correspondant au moins à un autre échantillon et mesurée avant la sortie pour correction est utilisée pour calculer la concentration du substrat.

**4.** Procédé de mesure de la concentration d'un substrat selon la revendication 1, dans lequel la concentration du substrat est calculée sur la base d'une différence entre la sortie pour mesure et une sortie de base mesurée quand le lavage de l'électrode enzymatique (30) est terminé.

**5.** Procédé de mesure de la concentration d'un substrat selon la revendication 4, dans lequel la sortie de base à soustraire de la sortie pour mesure afin d'obtenir la différence est estimée sur la base d'une courbe d'estimation tracée de manière à correspondre à une variation dépendant du temps d'une pluralité de sorties de base quand un changement équivalent au moins à une valeur donnée est détecté parmi la pluralité de sorties de base.

**6.** Procédé de mesure de la concentration d'un substrat selon la revendication 1, dans lequel un intervalle de mesure est défini à 50 à 200 msec.

**7.** Appareil de mesure de la concentration d'un substrat (1) comprenant un moyen de calcul (5) pour calculer une concentration d'un substrat inclus dans un échantillon en sur la base d'une sortie pour mesure provenant d'une électrode enzymatique (30) quand l'électrode enzymatique (30) et le substrat sont mis à réagir l'un avec l'autre, dans lequel :

le moyen de calcul calcule la concentration du substrat à l'aide d'une sortie pour correction provenant de l'électrode enzymatique (30) obtenue quand une solution de référence dont la concentration du substrat est connue et l'électrode enzymatique (30) sont mises à réagir l'une avec l'autre avant ou après que l'électrode enzymatique (30) et le substrat soient mis à réagir l'un avec l'autre, **caractérisé en ce que** le procédé de mesure est arrangé pour mettre en oeuvre les étapes suivantes consistant à :

(i) obtenir une valeur de réaction du substrat ΔRs en soustrayant une sortie de base Rb' d'une sortie pour mesure Rs ;
(ii) obtenir une valeur de réaction surveillée ΔRm en soustrayant une sortie de base Rb" d'une sortie pour correction Rm ; et
(iii) calculer une concentration du substrat sur la base de la relation entre la valeur de réaction du substrat ΔRs et la valeur de réaction surveillée ΔRm,

dans lequel le moyen de calcul est configuré pour calculer la concentration du substrat à l'aide de la sortie pour correction mesurée pour chaque échantillon, et comprenant en outre :

un moyen de préparation de l'échantillon (2) pour préparer l'échantillon à mettre à réagir avec l'électrode enzymatique (30) ;
une cuve de retenue de la solution de référence pour contenir la solution de référence afin d'obtenir la sortie pour correction ; et
un moyen de sélection (29) pour sélectionner un des états où l'échantillon doit être fourni et un état où la solution de référence doit être fournie pour l'électrode enzymatique (30).

**8.** Appareil de mesure de la concentration d'un substrat selon la revendication 7, dans lequel le moyen de calcul est configuré pour calculer la concentration du substrat à l'aide de la sortie pour correction pour l'échantillon à mesurer et une sortie pour correction correspondant au moins à un autre échantillon et mesurée avant la sortie pour correction.

**9.** Appareil de mesure de la concentration d'un substrat selon la revendication 8, dans lequel le moyen de calcul est configuré pour calculer la concentration du substrat à l'aide d'une valeur moyenne de la sortie de courant pour la correction pour l'échantillon à mesurer et de la sortie pour correction correspondant au moins à un autre échantillon et mesurée avant la sortie de courant pour la correction.

**10.** Appareil de mesure de la concentration d'un substrat selon la revendication 7, dans lequel le moyen de calcul est configuré pour calculer la concentration du substrat sur la base d'une différence entre la sortie pour mesure et une sortie de base mesurée quand le lavage de l'électrode enzymatique (30) est terminé.

**11.** Appareil de mesure de la concentration d'un substrat selon la revendication 10, dans lequel le moyen de calcul est configuré pour calculer la concentration du substrat après estimation de la sortie de base à soustraire de la sortie pour mesure afin d'obtenir la différence sur la base d'une courbe d'estimation tracée de manière à correspondre à une variation dépendant du temps d'une pluralité de sorties de base quand un changement équivalent au moins à une valeur donnée est détecté parmi la pluralité de sorties de base.

FIG.1

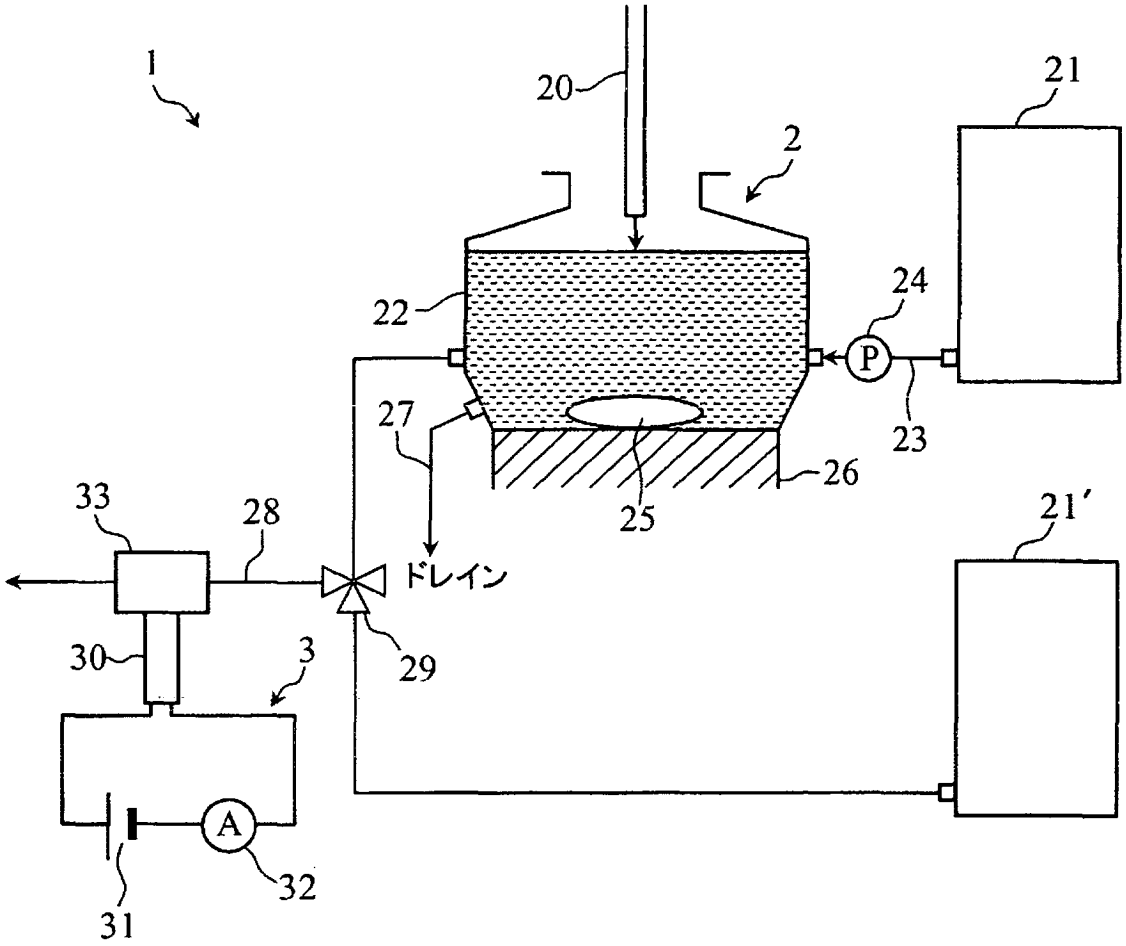


FIG.2

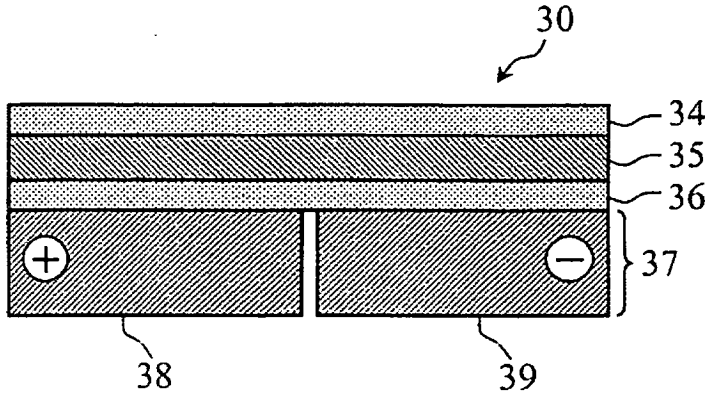

30
34
35
36
37
38
39

FIG.3

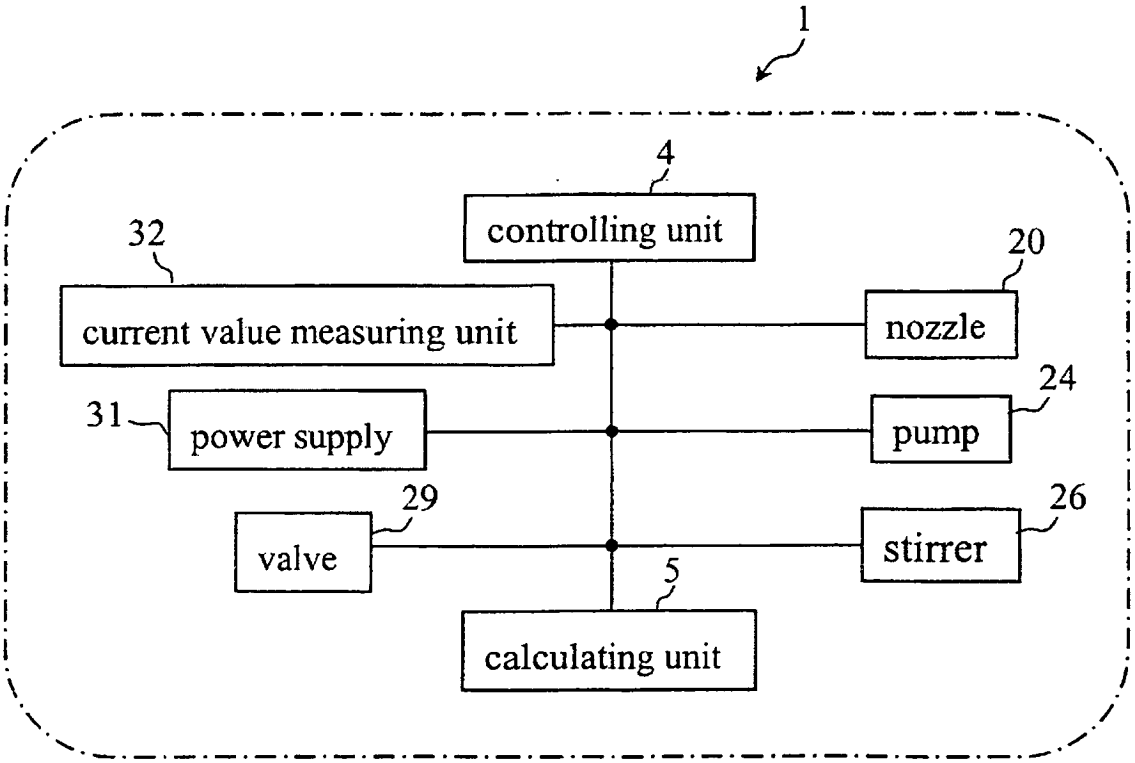

1
4
controlling unit
32
current value measuring unit
20
nozzle
31
power supply
24
pump
29
valve
26
stirrer
5
calculating unit

FIG.4

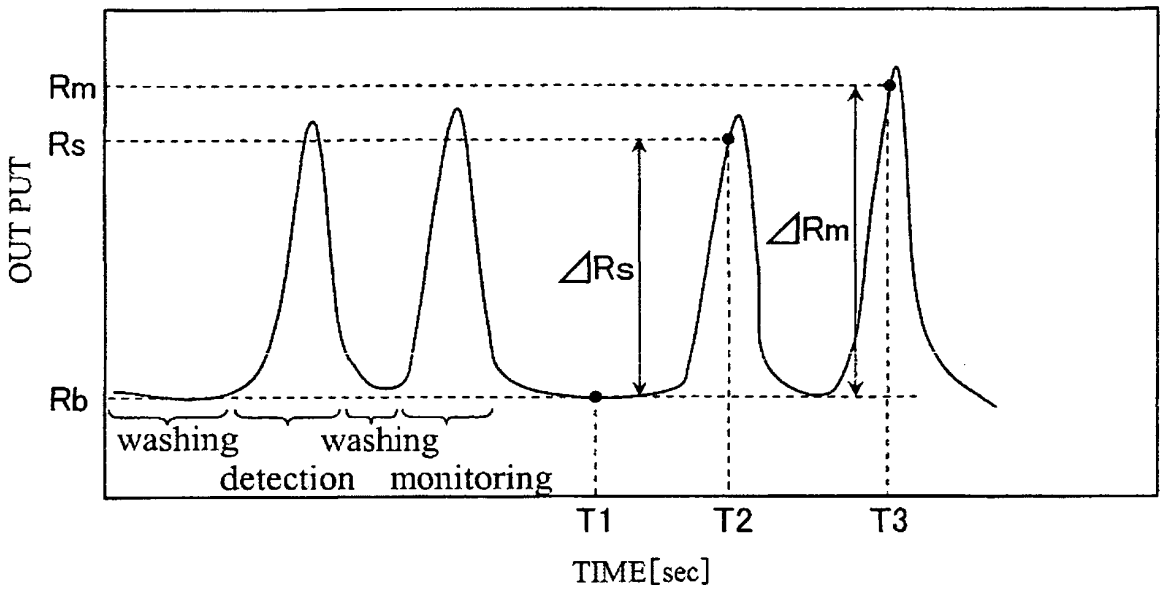

Rm
Rs
Rb
OUT PUT
⊿Rs
⊿Rm
washing
washing
detection
monitoring
T1
T2
T3
TIME[sec]

FIG.5

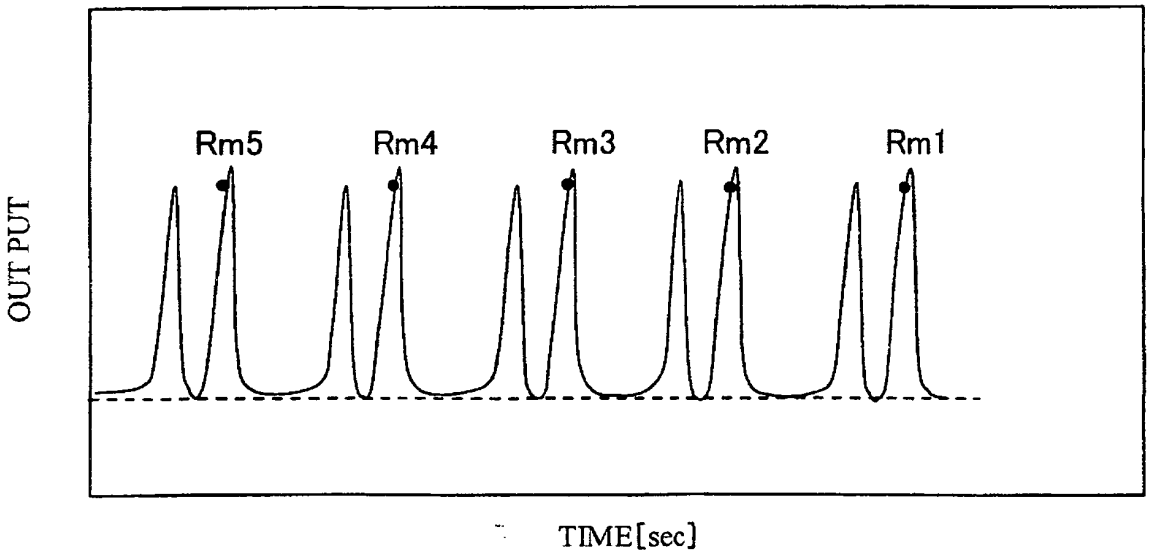

Rm5
Rm4
Rm3
Rm2
Rm1
OUT PUT
TIME[sec]

FIG.6

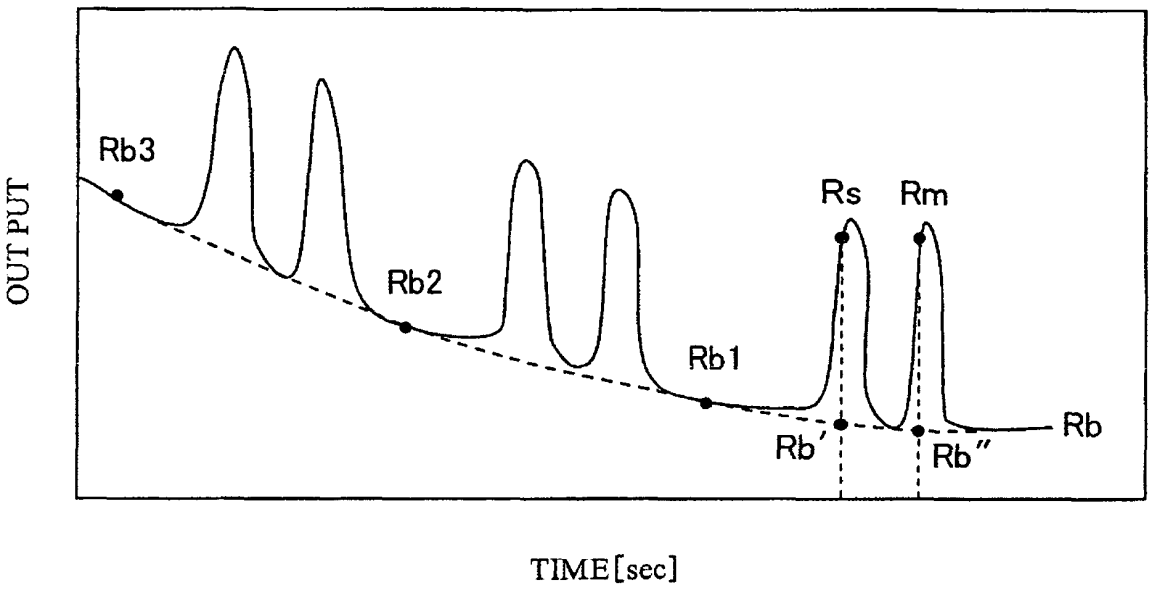


FIG.7A

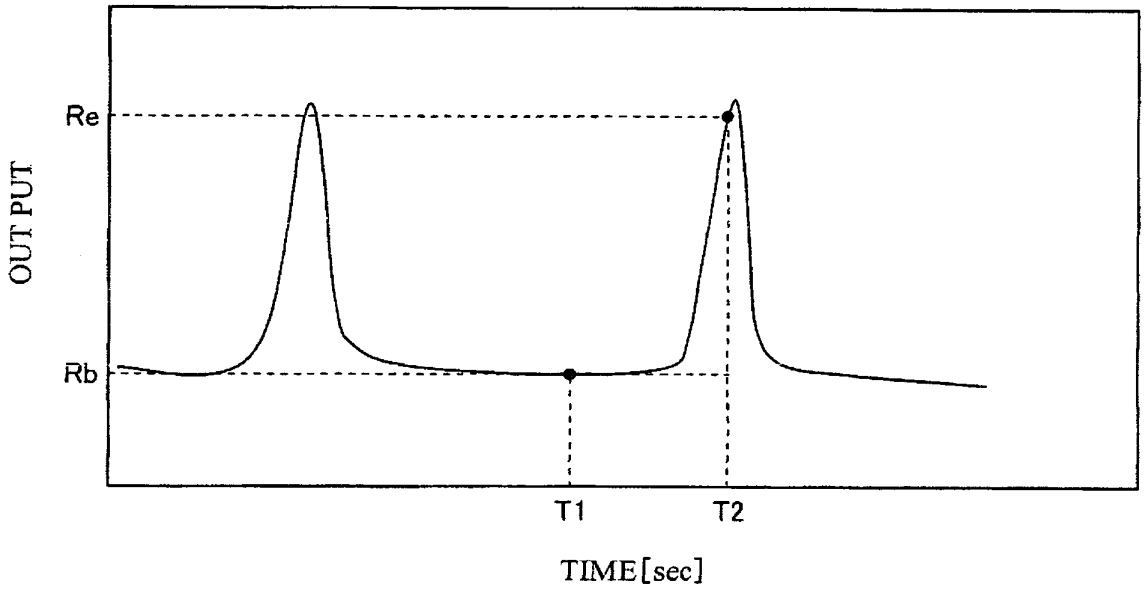
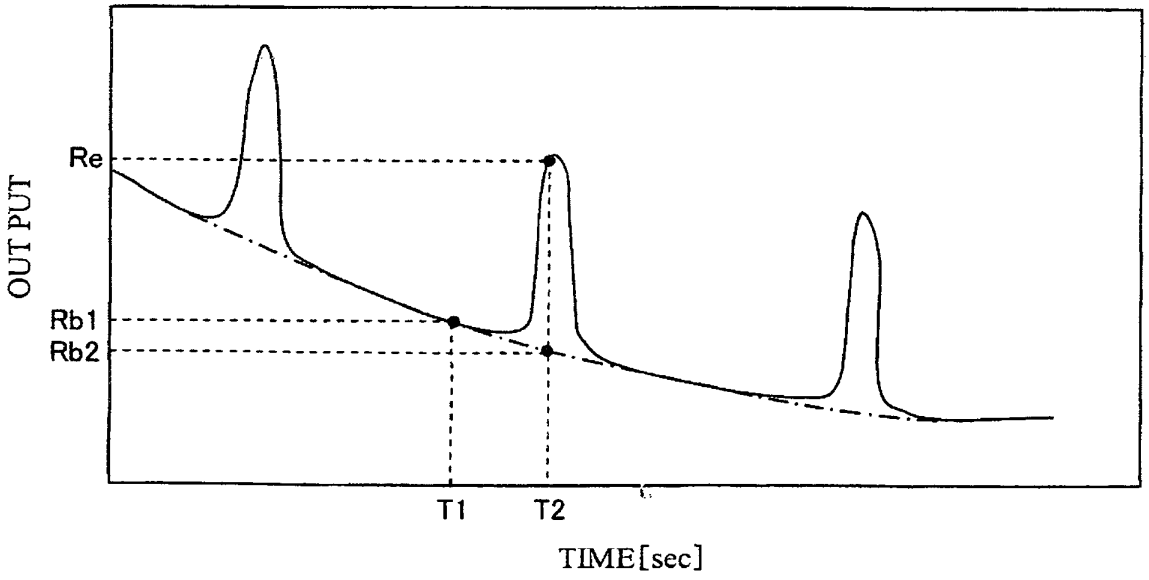



FIG.7B



## REFERENCES CITED IN THE DESCRIPTION

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|---|---|---|---|
| 专利名称(译) | 用于测量底物浓度的方法和设备 | | |
| 公开(公告)号 | EP2154521B1 | 公开(公告)日 | 2012-08-22 |
| 申请号 | EP2008740452 | 申请日 | 2008-04-15 |
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| [标]发明人 | KAMATA TATSUO<br>TAKAGI TAKESHI<br>ITO YUKI | | |
| 发明人 | KAMATA, TATSUO<br>TAKAGI, TAKESHI<br>ITO, YUKI | | |
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| CPC分类号 | C12Q1/001 A61B5/14532 A61B5/1486 A61B5/1495 A61B2560/0223 | | |
| 优先权 | 2007108630 2007-04-17 JP | | |
| 其他公开文献 | EP2154521A4<br>EP2154521A1 | | |
| 外部链接 | Espacenet | | |


摘要(译)

本发明提供一种基板浓度测量方法，用于当酶电极和基板相互反应时，基于来自酶电极的测量输出测量样品中包含的基板的浓度，使用输出计算基板浓度用于在酶电极和底物相互反应之前或之后，当底物浓度已知的参比溶液和酶电极彼此反应时从酶电极校正。例如，校正输出由每个样本测量。在该方法中，可以使用用于校正待测样本的输出和对应于至少一个其他样本的校正输出来计算基板浓度，并在校正输出之前测量。


FIG.1