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(54) **Apparatus for Continuous Determination of a Plurality of Solution Components**

(57) Apparatus for continuously determining a plurality of components in a solution comprises a plurality of successively arranged enzyme electrodes 1—3 each comprising immobilized enzyme. Constructions of the enzyme electrodes are disclosed.

FIG. 1

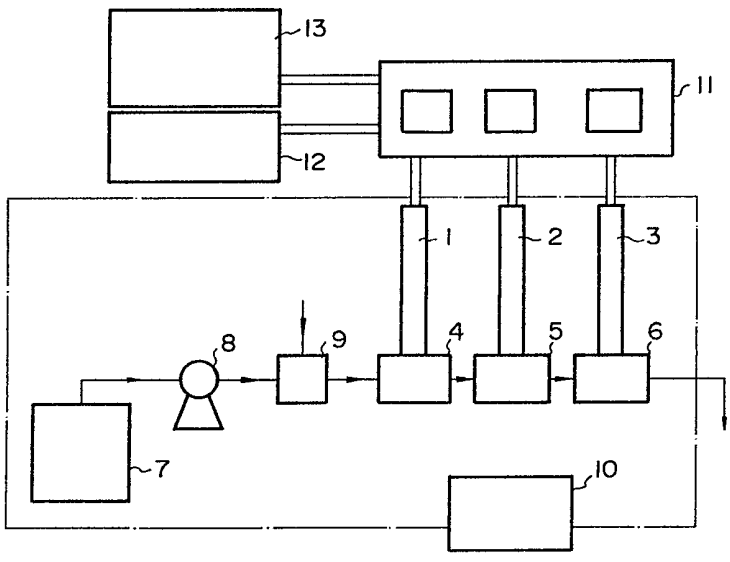

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FIG. 1

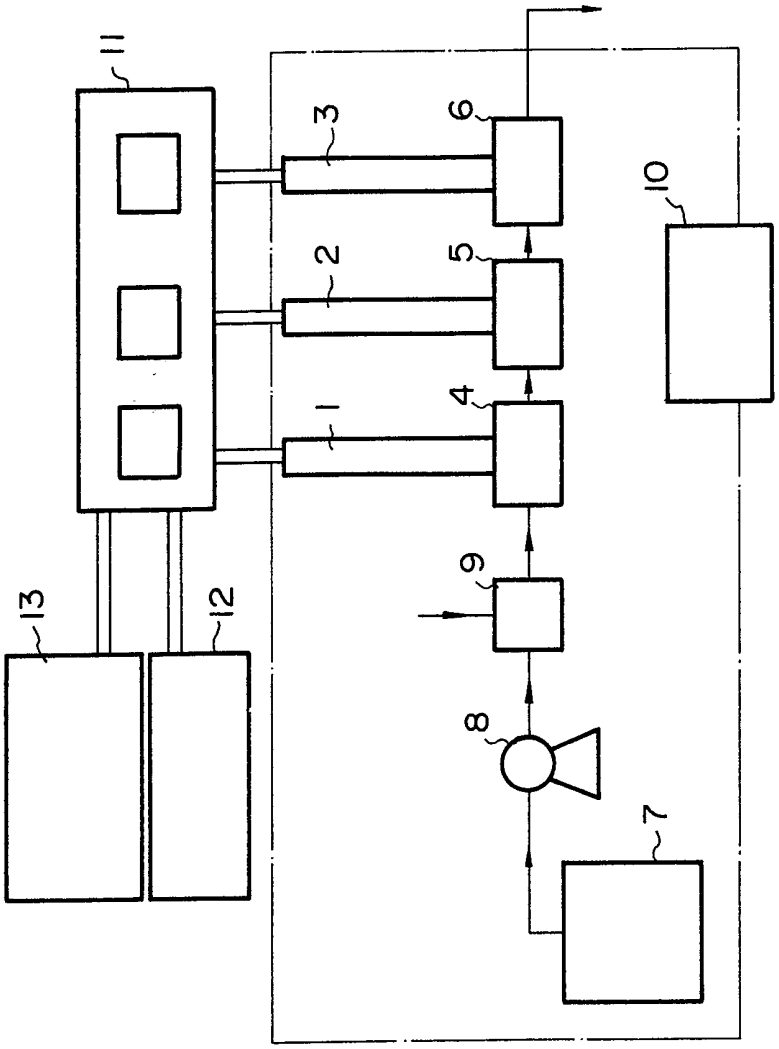


FIG. 2

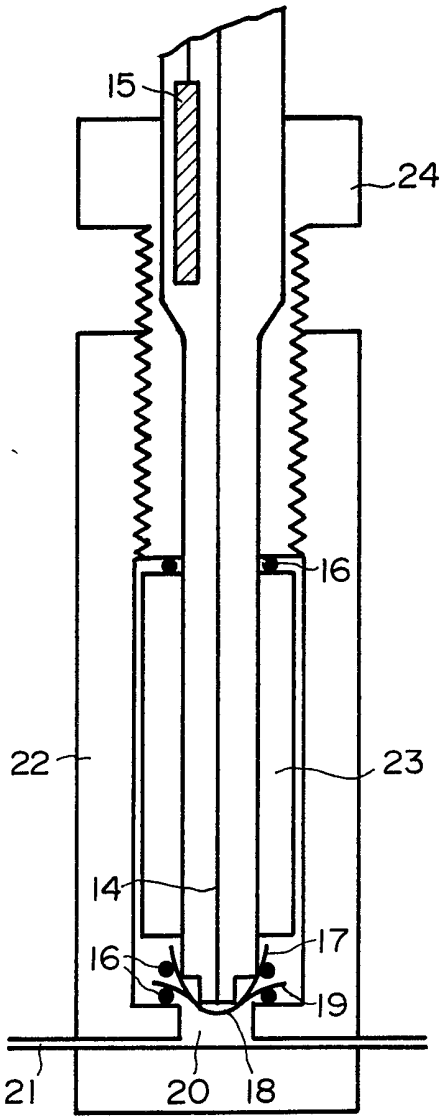
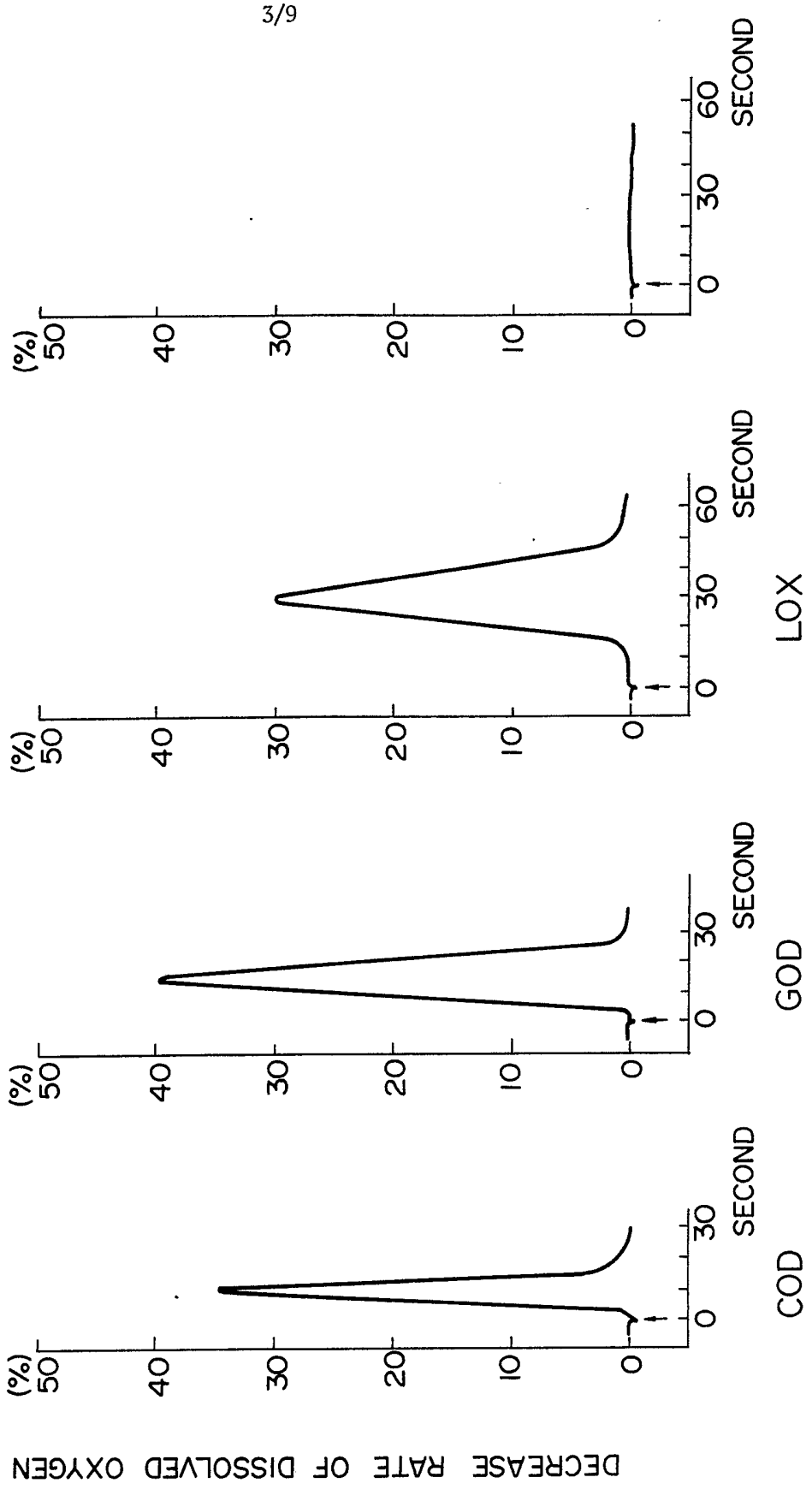


FIG. 3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0
30
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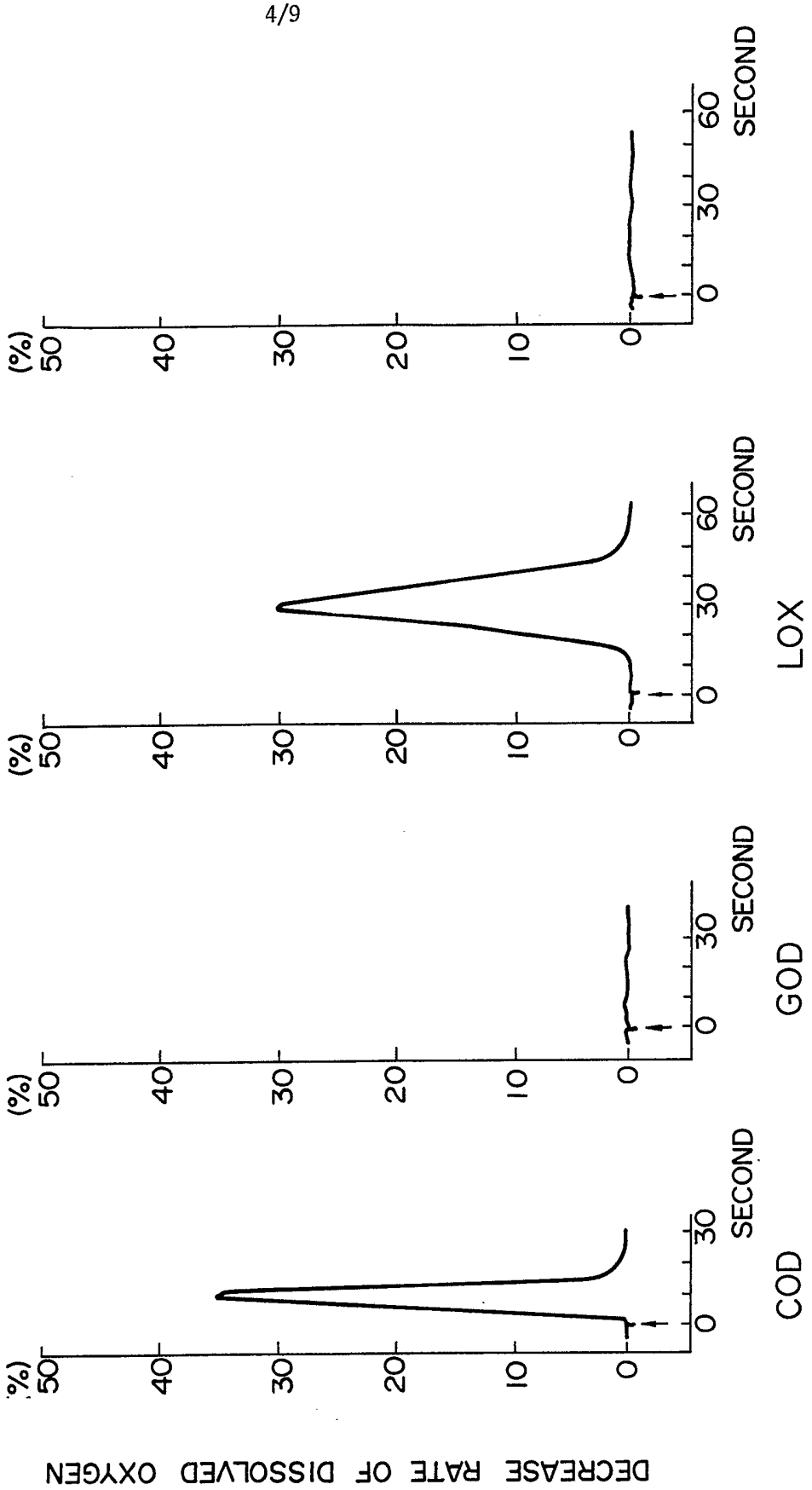
SECOND
COD
GOD
LOX

FIG. 4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(%)
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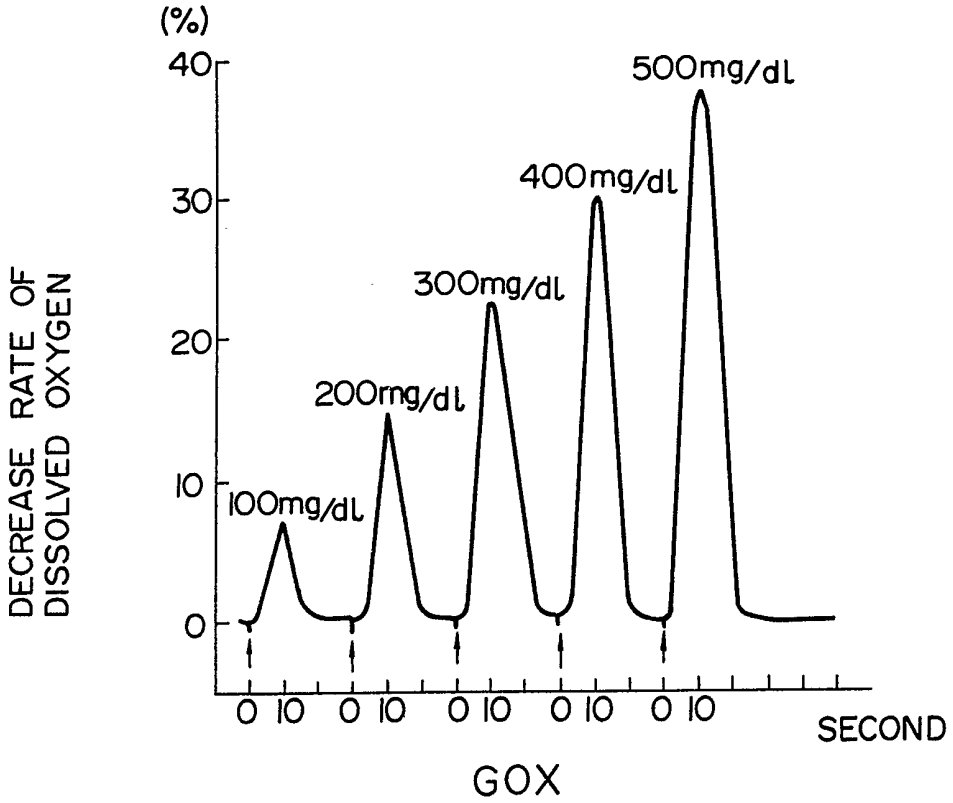
SECOND
COD
GOD
LOX

FIG. 5


(%)
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30
20
10
0
DECREASE RATE OF DISSOLVED OXYGEN
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500mg/dl
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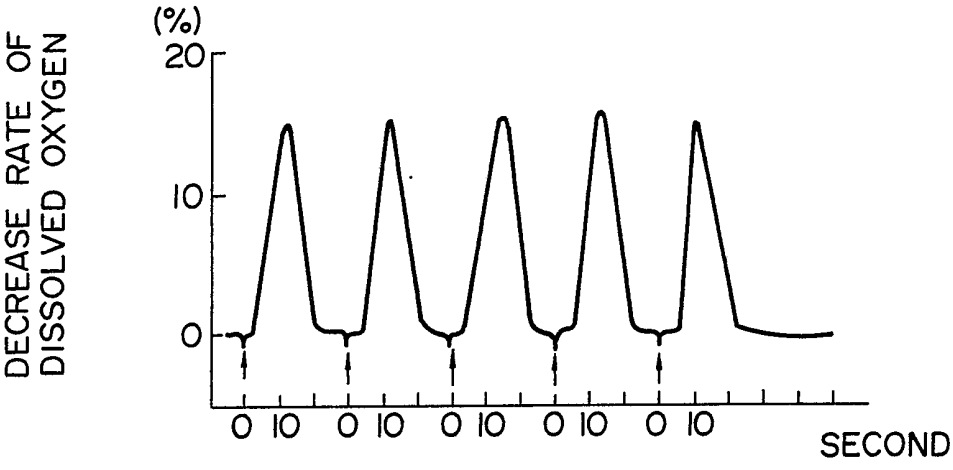
SECOND

GOX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DECREASE RATE OF DISSOLVED OXYGEN
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SECOND

LOX

FIG. 6

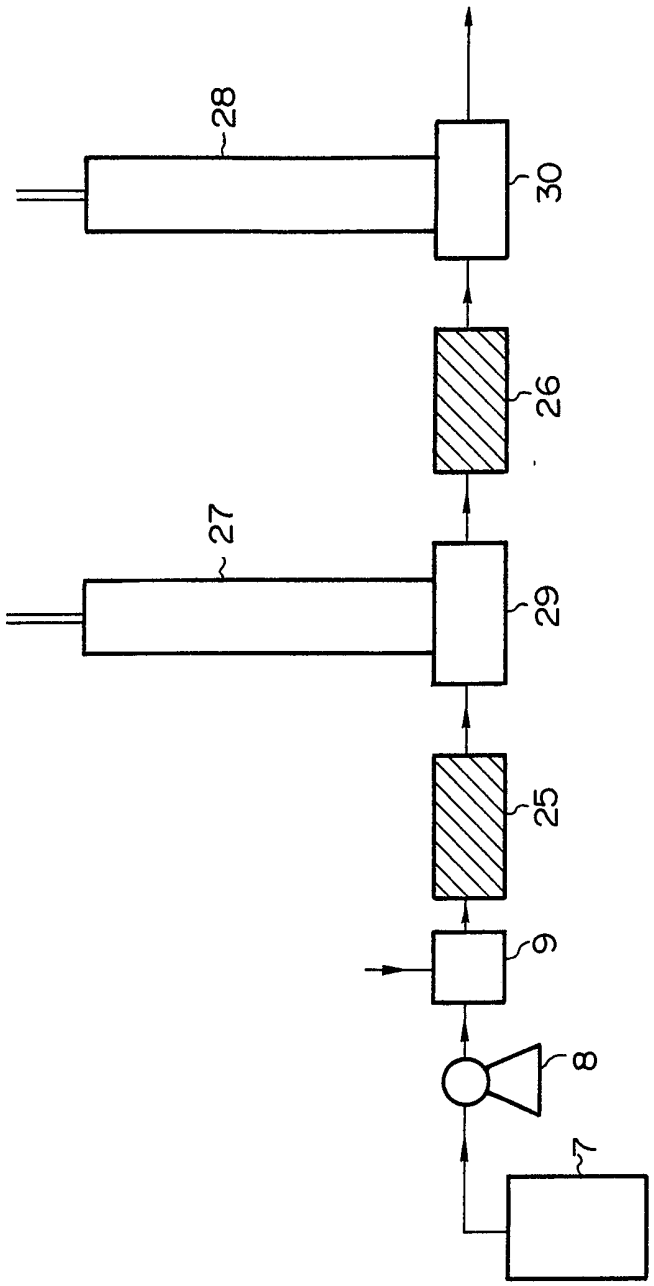
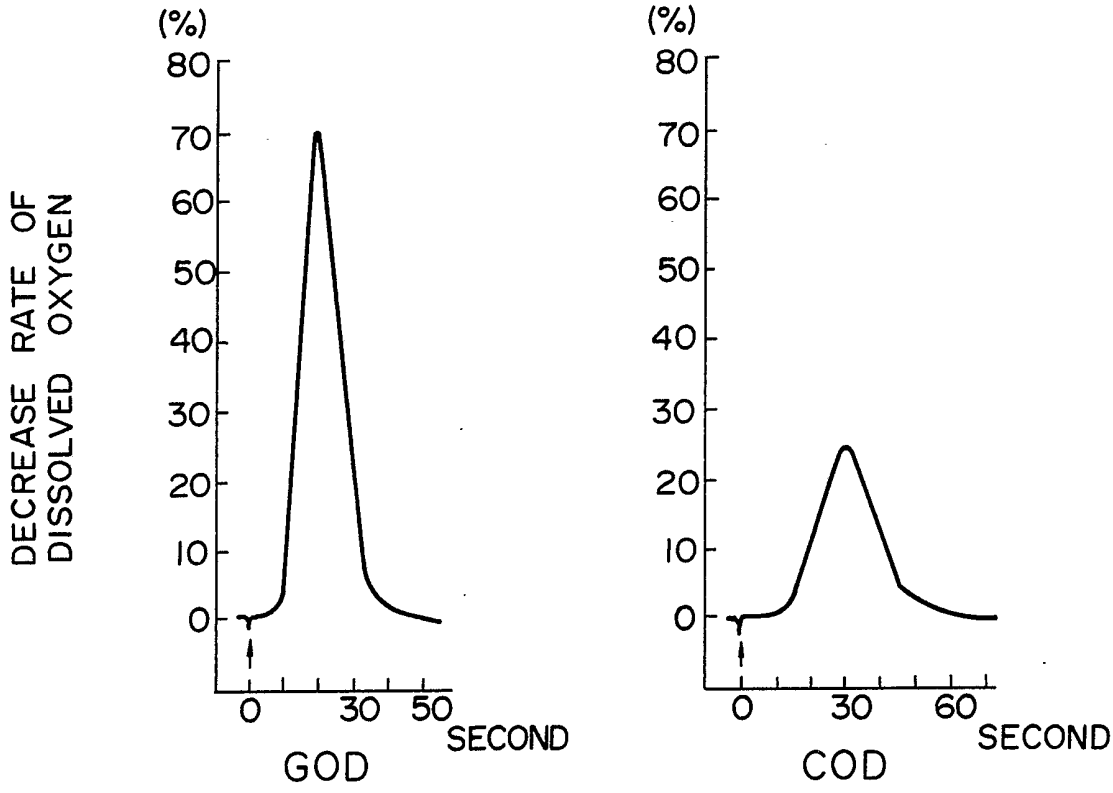


FIG. 7


(%)
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DECREASE RATE OF DISSOLVED OXYGEN
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30
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SECOND
GOD
(%)
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20
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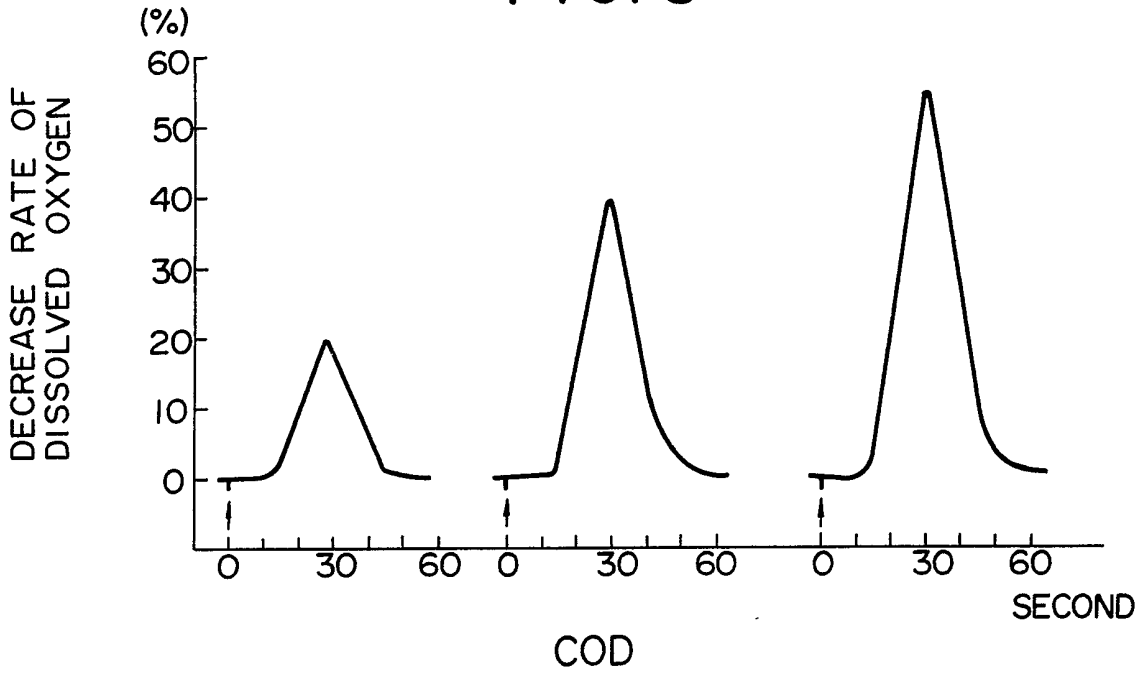
30
60
SECOND
COD

FIG. 8


(%)
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30
20
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30
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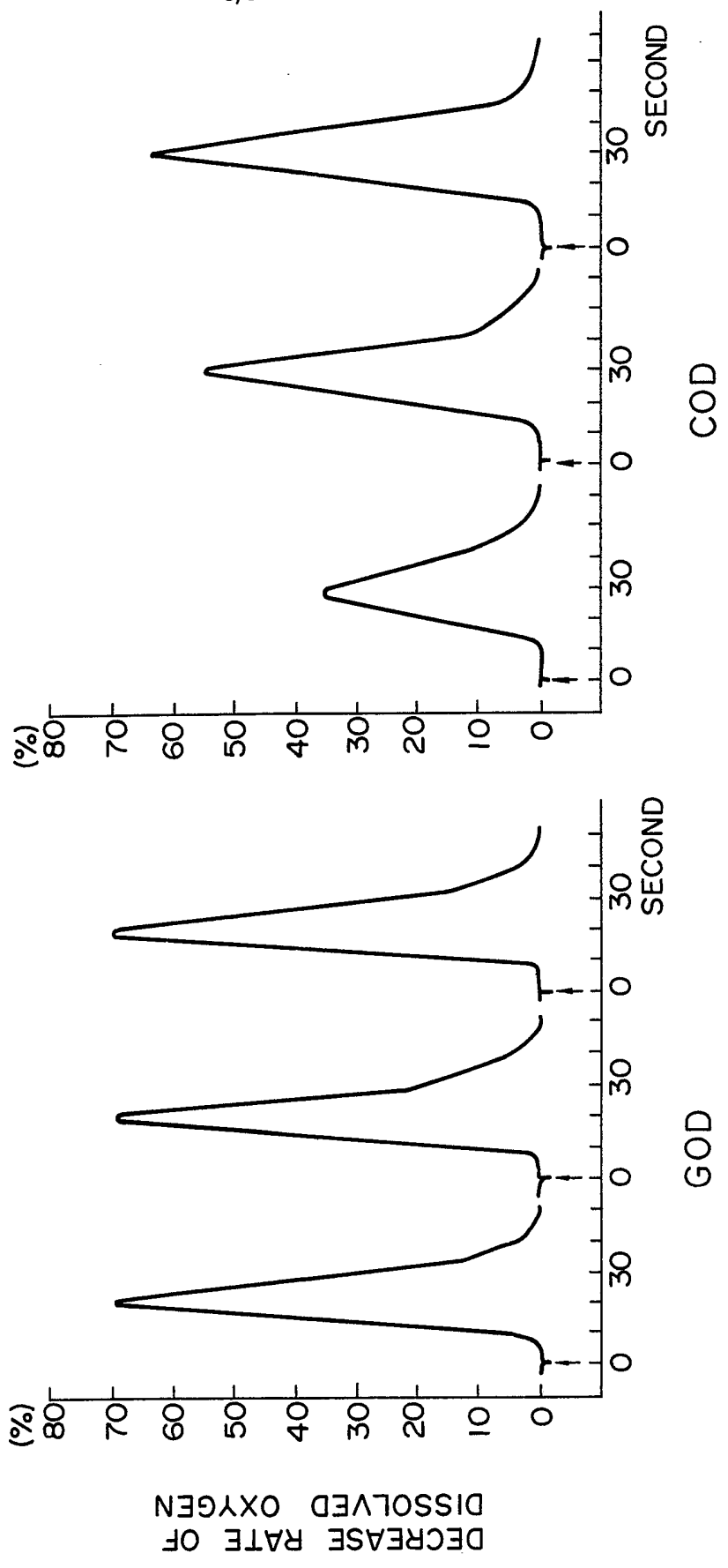
60
0
30
60
SECOND
COD

FIG. 9


DECREASE RATE OF
DISSOLVED OXYGEN
(%)
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0
30
SECOND
GOD
COD

FIG. 10

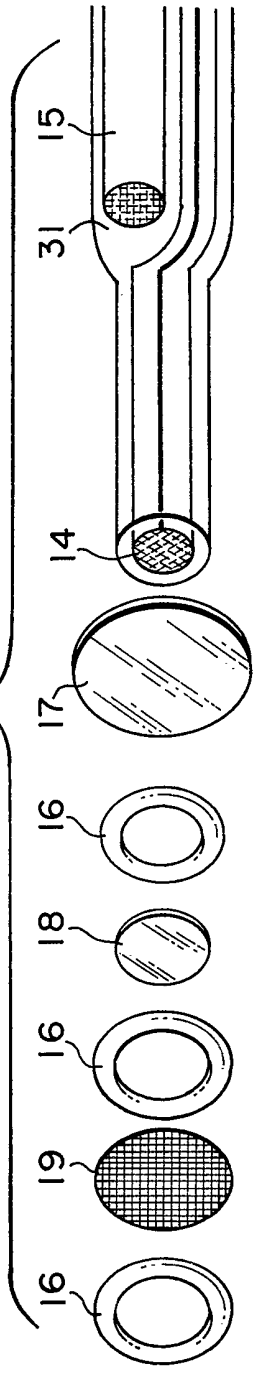


FIG. 11

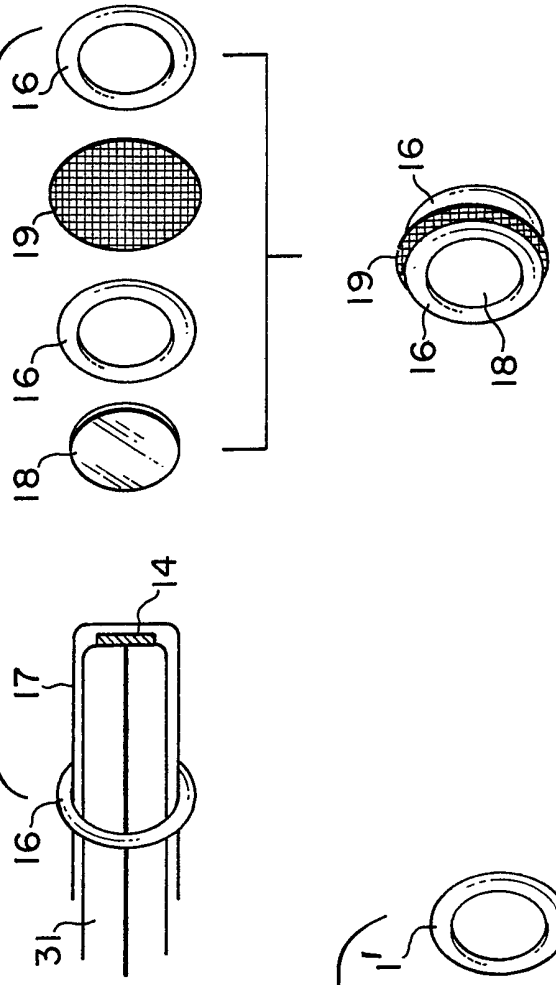
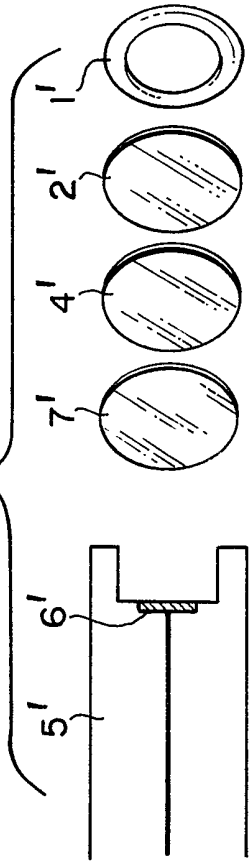


FIG. 12



SPECIFICATION

Multi-item Simultaneously Measuring Apparatus for Continuous Flow System

The present invention relates to a multi-item simultaneously measuring apparatus for a continuous flow system whereby plural components in the sample to be examined are simultaneously measured on multi-items.

In recent years, enzyme analytical methods wherein the quantitative analysis of the components contained in the sample is carried out using enzymes have been developed and widely used. However, this enzyme analytical method has defects such as uneconomical conditions due to large consumption of expensive enzymes and other auxiliary agents, troublesome operations, necessity of long time for measurement and the instability of enzyme.

Accordingly, in order to improve these defects, an immobilized enzyme, particularly, so-called enzyme electrode wherein this immobilized enzyme is combined with an electrochemical measuring method has been drawing attention. The measuring method which uses this enzyme electrode has many advantages such as convenience of the operation, small amount of auxiliary agents such as reagents used, shortened time for measurement and, particularly, remarkable decrese of enzyme consumption, and many enzyme electrodes have been reported.

These enzyme electrodes have been drawing attention particularly in the field of clinical chemistry and, in addition, other fields for component analysis of fermentation liquids such as culture fluid, and raw material, produced material or product of foods.

Further, these enzyme electrodes have been applied to so-called monitoring system of liquid components wherein the components in the sample of dialysis liquid of a living body liquid prepared by ultrafiltration membrane or hollow fiber are continuously measured, for instance, an artificial pancreas monitoring for measuring blood sugar or artificial kidney monitoring (Fermentation and Industry, Vol. 35, No. 11, p. 42—47).

However, in the various measuring methods mentioned above, one test is required for measuring one component because the sample was fitted to the measuring condition when measured; for instance, when the components in blood serum are measured, a considerably large amount of blood serum was required, and a long time was needed because of the many times for measuring multi-items. Such a point was a remarkable defect in the possibility and emergency in sampling particularly in the fields of clinical chemistry and fermentation.

The present inventors have found a method whereby, in the analysis of plural components in the sample, they can be measured using a very small amount of sample, moreover, in a short time and at the same time. Contrary to the conventional methods wherein, for instance, a considerably large amount of blood serum was used because only one component in the blood serum was measured at one test, an analysis of multi-items corresponding to plural components was made possible using a very small amount of blood serum.

At first, the present inventors completed a multi-item simultaneously measuring apparatus and the method therefore which are desirable for a batch process and consist of a plurality of electrodes arranged in the same reaction system (Japanese patent application Nos. 118447/78 and 52717/78) and, in addition, a measuring apparatus for the automation thereof (Japanese patent application No. 140449/79).

Further, the present inventors completed a multi-item simultaneously measuring method as a desirable method for the measurement in a continuous flow system consisting of, as a reactor type process, a plurality of flow cell wherein immobilized enzyme columns and measuring electrodes are arranged (Japanese patent application No. 90976/79). However, in case of this reactor type method, the concentrations of oxygen, hydrogen peroxide, etc. remarkably vary in the system when a system comprising an oxidase type enzyme is used. This variation gives a large effect on the variation of the concentration of oxygen in the system or that of hydrogen peroxide and the like. A multi-item simultaneous measurement in a continuous flow system was made possible by various means such as a method to introduce a measuring means for obtaining the effective difference between the variations of oxygen, hydrogen peroxide, etc. in the preceding flow cell and those in the following flow cell, a method to make the concentration of dissolved oxygen recover to the extent where the variation in the preceding flow cell does not affect that in the following flow cell and a method to dilute the hydrogen peroxide. However, these methods are not satisfactory yet as the accurate quantitative measuring method.

The present inventors further eagerly studied the multi-item simultaneous measurement in a continuous flow system and have found that, by arranging a plurality of reaction cell part in a continuous flow system which consists of enzyme electrodes to which immobilized enzymes comprising various immobilized oxidase type enzymes are attached by coating or other means similar to it, the variation in concentrations of each component which relates to an oxygen reaction such as the component consumed by an enzyme reaction, e.g., oxygen or the component produced, e.g., hydrogen peroxide, etc. does not result in defects such as a remarkable variation in the system as in case of said reactor type process and an effect thereof on the following system; and, in the relationship between enzyme and dissolved oxygen, the dissolved oxygen diffused in the immobilized enzyme participates in the enzyme reaction; the dissolved oxygen is directly reduced at the surface of functional electrodes, for example, those of platinum, silver, rhodium, etc, to which enzymes are applied; the decrease

of oxygen is very small being limited to only in the neighborhood of the immobilized enzyme causing no variation in the concentration of oxygen in the system and, in addition, even such a very small variation can be measured by translating this value to an electrical variation. Thus, the present inventors have succeeded in the preparation of a measuring apparatus which enables multi-item simultaneous measurement effectively in a continuous flow system by arranging a plurality of this reaction cell part in a continuous flow system. Also it has been found that, in case of a component produced by an enzyme reaction which occurs simultaneously with the decrease of oxygen, e.g., hydrogen peroxide, very little variation occurs only in the neighborhood of the immobilized enzyme; this variation is a measurable one; further, this variation does not affect the measurement carried out at the following reaction cell part, and it has been found that a multi-item simultaneous measurement can be carried out on components produced such as this hydrogen peroxide.

The present invention has been completed based upon the finding mentioned above, and it relates to a multi-item simultaneously measuring apparatus for a continuous flow system comprising, in a multi-item simultaneously measuring apparatus, a plurality of reaction cell part successively arranged consisting of at least more than one enzyme electrode which is prepared by applying immobilized enzymes to the functional electrode.

In the accompanying drawings,

Fig. 1 shows the multi-item simultaneously measuring apparatus of the present invention; Fig. 2 shows the reaction cell part thereof; Fig. 3, Fig. 4 and Fig. 5 show the results of measurement wherein the apparatus of the present invention is used; Fig. 6 shows an apparatus based upon the reactor type process wherein immobilized enzyme columns are used; Fig. 7, Fig. 8 and Fig. 9 show the results of measurement wherein the apparatus based upon the reactor type process is used; Fig. 10 and Fig. 11 show the structure for enzyme electrode and the enzyme electrode consisting of fundamental electrode part and Fig. 12 shows the enzyme electrode on the market.

At first, as the material to be measured with the multi-item simultaneously measuring apparatus of the present invention, the material such as, for instance, a living body liquid including blood such as blood serum or blood plasma and urine, a fermentation liquid obtained by culture and food products can be illustrated. Also as the components in the sample to be measured, components in a living body liquid related to the clinical diagnosis, components of nutritive source products and the like for microorganisms in culture and raw material components of food products are illustrated; they are, for example, glucose, succharose, lactose, lactic acid, cholesterol, cholesterol esters, triglyceride, phospholipid, starch, protein, grycerol, pyruvic acid, creatinine, cretine, amino acids, monoamines, diamines, etc.

Further, as the enzyme used to measure the components mentioned above, oxidase type enzymes consisting of the components to be measured or the reaction products thereof as the substrate are used; for instance, glucose oxidase may be used in case of glucose; lactic acid oxidase may be used in case of lactic acid; cholesterol oxidase may be used in case of cholesterol; cholesterol esterase and cholesterol oxidase may be used in case of cholesterol esters; lipase or lipoprotein lipase and glycerin oxidase, or glycerokinase, ATP, L-α-glycerophosphoric acid oxidase may be used in case of triglyceride; cholesterol oxidase may be used in case of choline; phospholipase D or phospholipase C, alkali phosphatase and choline oxidase may be used in case of phospholipids; glycerin oxidase or glycerokinase, ATP, L-α-glycerophosphoric acid oxidase may be used in case of glycerol; uricase may be used in case of uric acid; pyruvic acid oxidase may be used in case of pyruvic acid; creatininase, creatinase and sarcosine oxidase may be used in case of creatine; amino acid oxidase may be used in case of amino acids and amine oxidase may be used in case of amines. Thus, various enzymes are selected, combined and used corresponding to the components to be measured.

Further, as the immobilized enzymes used in the present invention, one kind of or the combination of enzyme mentioned above is used. The immobilized enzymes may be obtained by various immobilizing methods such as

(1) inclusion and immobilization with acrylamide,

(2) immobilization by blending with insoluble protein and cross-linking the mixture,

(3) immobilization by inclusion with or covalently coupling to collagen, etc.,

(4) immobilization by adsorption to or covalently coupling to a porous polymer material, and

(5) immobilization by using a photosetting resin.

Usually they are used in a film-like form or a fibrous form and processed in accordance with the form of electrode used for measurement.

As the electrode, when the component to be measured is the substrate, any material which can be used for measuring a component consumed or produced by enzyme reaction may be used. For instance, as the oxygen electrode to capture oxygen, a Clark type one or a Galvani type one is conveniently used and a hydrogen peroxide electrode, an ammonia electrode and the like can be illustrated in order to capture hydrogen peroxide. The functional electrode means an electrode which shows an electrical variation by perceiving a variation of components.

Furthermore, there is no particular limitation on the enzyme electrode; however, an enzyme electrode wherein an immobilized enzyme is applied to the electrode for measurement prepared by the procedure mentioned below is

conveniently and suitably used. For instance, an enzyme electrode may be used which is obtained by allowing said electrode to be the fundamental electrode and allowing a thin membrane of immobilized enzyme to attach to the detecting part which is the functional electrode, or by forming the thin membrane at the detecting part, or by enveloping the detecting part to which said thin membrane is attached with a nylon net membrane or a dialysis membrane and fixing it with an O-ring; or, in order to obtain it more conveniently, a one-touch type enzyme electrode may be used which is obtained by previously bonding a net-like membrane to the O-ring used for fixing it to the detecting part of the fundamental electrode, preparing a structure wherein an immobilized enzyme is placed in the O-ring to which the net-like membrane is bonded and inserting this structure into the detecting part of the fundamental electrode when used.

As the O-ring used for fixing the enzyme electrode to the functional electrode of the fundamental electrode, a ring-shaped elastic O-ring such as one made of natural or synthetic rubber or silicone rubber is generally used. There is no particular limitation on the diameter of this O-ring as far as it is suitable for the fixing to the functional electrode. Usually an O-ring having a diameter slightly smaller than that of the functional electrode is preferably used for fixing.

Further, as the net-like membrane to be bonded to the O-ring, any one which does not prevent the enzyme reaction of the immobilized enzyme, can maintain the immobilized enzyme and, in addition, has an elasticity which enables the tight adhesion of the immobilized enzyme to the perceiving part may be used. Usually a nylon net membrane (having a space of 0.1—0.2 mm and about 1 mm when stretched) is used.

Said enzyme electrode is as shown in Fig. 10 and Fig. 11. At first, in Fig. 10 and Fig. 11, 16 is O-ring and 19 is nylon net membrane. An epoxy type adhesive is applied to the circumference of O-ring and this is put on the nylon net membrane for bonding. Then, the surplus part of the membrane may be cut, or a nylon net membrane previously cut for fitting to the diameter of the O-ring may be used. Further, in order to reinforce the bonding of the net membrane and the O-ring, another O-ring 16 is bonded to the net membrane side of this nylon net membrane 19 to which said O-ring 16 is bonded by the procedure similar to that mentioned above so that the nylon net membrane is put for bonding between the double layers of O-rings. Then, an immobilized enzyme 18 may be placed to this. When the immobilized enzyme 18 is a membrane-like material, it can be easily placed into the O-ring 16 by forming and using it as a membrane having a diameter smaller than that of the O-ring 16. Thus, a structure for the enzyme electrode can be obtained. Structures having various sizes of inside diameter fitted to the diameter of O-ring used can be obtained and a suitable one having a diameter in accordance with the functional electrode 14 of the fundamental electrode 31 may be selected and used. In such a case, an enzyme electrode can be obtained by fixing the structure for the enzyme electrode to the functional electrode of the fundamental electrode such as an oxygen electrode which is covered by an oxygen-permeable teflon membrane or a hydrogen peroxide-permeable polycarbonate membrane 17 with O-ring 16.

For comparison, an enzyme electrode already known in shown in Fig. 12.

Fig. 12 is a rough sketch of an enzyme electrode on the market (Sugar Analyzer, manufactured by YSI Inc.), in which 1′ is O-ring; 2′, polycarbonate membrane; 4′, immobilized membrane; 5′, fundamental electrode; 6′, functional electrode and 7′, cellulose acetate membrane. The structure thereof is as follows: the fundamental electrode 5′ has a cavity at its end; a cellulose acetate membrane 7′, an immobilized enzyme membrane 4′ and a polycarbonate membrane 2′ are placed in this order at the detecting part 6′ in said cavity and they are fixed by an O-ring 1′ inserted into the cavity.

As the structure of these enzyme electrodes, an electrode prepared by suitable processing may be adopted and there is no limitation on the structure as far as it can be used for measurement.

Then such an enzyme electrode is placed at the reaction cell part consisting of flow cells and having a reactor capacity of, for instance, 0.05—0.5 ml; thus, a reaction cell part consisting of at least more than one of the functional electrodes to which the immobilized enzyme is applied is formed. An enzyme electrode wherein immobilized enzymes are suitably applied to the functional electrode responding to the capacity of reaction cell and the items of components to be measured may by used. Further, a plurality of the reaction cell part thus obtained can be successively arranged. In this case, usually the space between each reaction cell part may be preferably larger than 10 mm. Since this space causes, in the electrical variation detected, the time difference of more than about several seconds between each reaction cell part, there is a possibility that it can be effectively used in an electrical measurement; also satisfactory measurements can be carried out at each reaction cell part by arranging 2—20 reaction cell parts.

The quantitative analysis based upon the enzyme reaction can be carried out by measuring the electrical variations at enzyme electrodes; any one of the known methods up to the present such as, for instance, a current variation value method, an endpoint method, an initial velocity method and an acceleration method can be used. When an electrode which shows a slow response is used, a differentiation method such as initial velocity method is desirable to obtain a quick response; on the contrary, when an electrode which shows a very quick response is used, a differential output type method which detects an

electrical variation after it reaches a stable state in the analysis is preferable because, if a differentiation type method is used, there is a fear to cause error due to the effect of agitation condition and the like in the analysis.

Next, in connection with the drawings, an example of the present invention is mentioned below.

An apparatus suitable to carry out the present invention is as shown in Fig. 1. That is, a continuous flow system is prepared by placing enzyme electrodes 1—3 into the flow cells 4—6, respectively, and successively combining plural reaction cell parts having enzyme electrodes. In this case, a buffer solution tank 7, a positive displacement pump 8, an injector 9 and a thermostat 10 are prepared together with a converter 11 for electrical variations from each of the enzyme electrodes; further, a recorder 12 and a printer 13 are equipped thereto.

As the reaction cell part, one consisting of a combination of an enzyme electrode having a structure as shown in Fig. 2 and a flow cell is illustrated. In Fig. 2, an enzyme electrode consisting of a functional electrode (cathode) 14 to detect the variation in an enzyme reaction, a counter electrode (anode) 15, an O-ring 16, a Teflon (Registered Trade Mark) membrane 17 as an oxygen-permeable membrane to detect the consumption of oxygen in an enzyme reaction, an immobilized enzyme 18 and a nylon net 19 to support the immobilized enzyme are combined with a flow cell 22 having a reaction vessel 20 through an electrode supporter I 23 and an electrode supporter II 24. Also a flow path 21 to send the sample and the buffer solution into the reaction vessel or to drain them from the reaction vessel is prepared in this flow cell.

These reaction cell parts are arranged so that no mutual buffer action occurs; the number thereof is affected to some extent by the conditions such as the diffused state of the sample in the reaction system; it is, however, usually 2—20 preferably about 2—10. They may be connected with said flow paths leaving a space of more than about 10 mm between the center positions of each reaction cell. The diameter of the flow path is usually 0.1—1 mm.

Further, in case mentioned above, when the oxygen as a component consumed in the enzyme reaction is detected, a polypropylene membrane, other than said teflon membrane, may be used as the oxygen-permeable membrane; when the hydrogen peroxide as a component produced is detected, no membrane or, in some cases, a hydrogen peroxide-permeable membrane such as a cellulose triacetate membrane or a polycarbonate membrane is used.

A multi-item simultaneously measuring apparatus for a continuous flow system is prepared as mentioned above; a sample being injected thereinto through the injector, the sample being flowed at a constant velocity, the component to be measured being detected at each reaction cell part, the result measured being converted to the variation at each enzyme electrode which can be read by the recorder and the printer through the converter. These operations may be carried out manually or automatically by the electrical regulation wherein the electrical variation is held by a microcomputer and, in this case, sending in and out of the buffer solution, the positive displacement pump for that purpose and the signals held are detected and recorded with a zero adjusted circuit, a differential output circuit or a differentiation circuit.

The detection and the measurement of multi-item components were carried out using the multi-item simultaneously measuring apparatus thus obtained. When this measurement was carried out, a 0.1 N phosphoric acid buffer solution (pH 7.5) was used, the solution being transported at a slow rate of 2 ml/min with a positive displacement pump, an enzyme electrode consisting of a choline oxidase immobilized enzyme, a glucose immobilized enzyme or a lactic acid oxidase immobilized enzyme being fixed to each flow cell, respectively (in Fig. 1, the enzyme electrode consisting of choline oxidase is 1, the enzyme electrode consisting of glucose oxidase is 2 and the enzyme electrode consisting of lactic acid oxidase is 3), these electrodes being successively connected leaving a space of 10 mm, further, an enzyme electrode being, for comparison, connected at the end thereof in order to measure the variation of dissolved oxygen in the reaction system. Each immobilized enzyme was prepared according to the method of British Patent No. 215001 wherein the enzyme electrode is fixed to a polyacrylonitrile membrane having amino groups. The enzyme electrode was prepared by cutting this membrane to pieces having a size of about 5×5 mm (about 0.7—0.8 mg). Each of them was an immobilized enzyme having a choline oxidase activity of 35 U/g, a glucose oxidase activity of 23 U/g and a lactic acid oxidase activity of 32 U/g, respectively. They were fixed to the detecting part of the enzyme electrode with an O-ring in the order of teflon membrane, immobilized enzyme and nylon net. Then the system was maintained at 37°C and 5 μl of a mixture solution containing choline chloride (100 mg/dl), glucose (500 mg/dl) and DL-lactic acid (20 mg/dl) was injected as a sample.

As the result, as shown in Fig. 3, after the injection of the sample, at first choline (COD in Fig. 3), then glucose (GOD in Fig. 3), further, lactic acid (LOX in Fig. 3) were measured at each reaction cell part; thus it was known that satisfactory reactions occurred at each reaction cell part. Also a peak value of decreasing rate of dissolved oxygen was seen after 10 sec. in the first cell part and after 30 sec. in the third cell part, respectively. In the oxygen electrode prepared for comparison, almost no decrease rate of dissolved oxygen was shown. Accordingly, it was made clear that the decrease of dissolved oxygen in the measurement at each reaction cell part did not give any effect in the system; thus the

components to be measured in multi-item was simultaneously measured satisfactorily in a continuous flow system.

Using the same apparatus as that mentioned above, 5 μl of a mixture solution containing choline chloride (100 mg/dl) and DL-lactic acid (20 mg/dl) was injected thereinto as a sample and the effect of dissolved oxygen on the enzyme electrode consisting of glucose oxidase and the oxygen electrode for comparison in addition to the enzyme electrode consisting of choline oxidase and the enzyme electrode consisting of lactic acid oxidase was investigated.

As the result, as shown in Fig. 4, after the injection of the sample, at first choline (COD in Fig. 4), then perfectly no glucose (GOD in Fig. 4), further, lactic acid (LOX in Fig. 4) were measured at each reaction cell part and, in addition, it was detected that, according to the oxygen electrode, there is no variation of dissolved oxygen in the system. The decrease of dissolved oxygen due to choline oxidase did not give any effect on the enzyme electrode consisting of glucose oxidase and also the decrease of dissolved oxygen due to choline oxidase and lactic acid oxidase did not give any effect in the system; thus the measurement was carried out satisfactorily.

From the phenomena mentioned above, it is recognized that, in the measurement, a very small amount of dissolved oxygen was decreased in the neighborhood of the immobilized enzyme on the enzyme electrode having immobilized enzyme and such a very small amount gave almost no effect on the decrease of dissolved oxygen in the system.

According to the procedure similar to that mentioned above, a two electrode type apparatus was prepared by connecting an enzyme electrode consisting of glucose oxidase and an enzyme electrode consisting of lactic acid oxidase. Five microliter of the mixture solution of glucose having different concentrations (100—500 mg/dl) and DL-lactic acid having a constant concentration (10 mg/dl) was injected into this apparatus and the variation of lactic acid measured was compared with the variation in concentration of glucose.

As the result, as shown in Fig. 5, glucose (GOD in Fig. 5) was measured corresponding to each concentration and, in addition, lactic acid (LOX in Fig. 5) was satisfactorily measured at the detection time nearly the same as that of glucose.

Compared with this, the measurement according to a reactor type process as the measurement in a continuous flow system using immobilized enzyme columns is mentioned as follows.

At first, as an apparatus according to the reactor type process one which is shown in Fig. 6 was prepared In Fig. 6, immobilized enzyme columns (2.8ϕ×20 mm, respectively) 25 and 26 (25 is a glucose oxidase immobilized enzyme column and 26 is a choline oxidase immobilized enzyme column) are connected to flow cells 29 and 30, respectively; an enzyme electrode 27 or 28 is attached to each flow cell and they are connected to a buffer solution tank 7, a positive displacement pump 8 and injector 9.

Into this apparatus was injected 10 μl of a sample containing only glucose (100 mg/dl). Glucose caused reaction in the glucose oxidase immobilized enzyme column resulting in the decrease of dissolved oxygen to be measured; glucose was measured by detecting it with oxygen electrode (GOD in Fig. 7); however, the variation of this dissolved oxygen affected the oxygen electrode of choline oxidase immobilized enzyme column and the detected result was as if a choline component were present (COD in Fig. 7).

Next, into this apparatus based on reactor type process was injected choline chloride (20 mg/dl, 40 mg/dl, 80 mg/dl). In this case, no enzyme reaction occurred in the glucose oxidase immobilized enzyme column; consequently, as shown in Fig. 8, choline component was satisfactorily detected.

On the other hand, when 10 μl of a mixture solution of glucose (100 mg/dl) and choline chloride (20 mg/dl, 40 mg/dl, 80 mg/dl) was injected into said apparatus, glucose was satisfactorily detected as shown in Fig. 9 (GOD in Fig. 9); however, the measured value of choline component (COD in Fig. 9) was higher than that shown in Fig. 8 due to the effect of glucose on variation of dissolved oxygen.

From this phenomenon, it is obvious that, when a sample which contains a plurality of components is measured by a reactor type process, this process is not suitable for a measurement system because the preparation of a calibration curve and the measurement are impossible.

While the reactor type process wherein immobilized enzyme columns are used is, as mentioned above, not suitable for a multi-item simultaneously measuring apparatus in a continuous flow system, the apparatus of the present invention advantageously enables multi-item simultaneous measurement of a plurality of components contained in one sample in a continuous flow system.

Claims

1. A multi-item simultaneously measuring apparatus for a continuous flow system which comprises a plurality of the reaction cell part successively arranged consisting of enzyme electrodes wherein immobilized enzymes are applied to at least more than two functional electrodes.

2. A multi-item simultaneously measuring apparatus for a continuous flow system of claim 1 wherein the space more than 10 mm between each reaction cell part is left in successive arrangement thereof.

3. A multi-item simultaneously measuring apparatus for a continuous flow system of claim 1 or 2 wherein the plurality is 2—20.

4. A multi-item simultaneously measuring

apparatus for a continuous flow system of claim 1 wherein a net-like membrane is bonded to an O-ring which is used to insert an enzyme electrode into the functional electrode of the fundamental electrode and an immobilized enzyme is placed in this O-ring.

5. A multi-item simultaneously measuring apparatus for a continuous flow system of Claim 4 wherein two O-rings form a double-layer and a net-like membrane is bonded to them so that said membrane is held between said layers.

6. A multi-item simultaneously measuring apparatus for a continuous flow system of claim 4 or 5 wherein the immobilized enzyme is a membrane-like material of immobilized enzyme or a fibrous material of immobilized enzyme.

7. A multi-item simultaneously measuring apparatus for a continuous flow system of claim 6 wherein the membrane-like material of immobilized enzyme has a diameter smaller than the inside diameter of the O-ring.

8. A multi-item simultaneously measuring apparatus for a continuous flow system substantially as herein described with reference to, and as illustrated in the accompanying drawings.

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