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Paulson et al.

[11] **3,765,841**

[45] **Oct. 16, 1973**

[54] **METHOD AND APPARATUS FOR CHEMICAL ANALYSIS**

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[73] Assignee: **Beckman Instruments, Inc.**, Fullerton, Calif.

[22] Filed: **Aug. 6, 1971**

[21] Appl. No.: **169,687**

[52] **U.S. Cl.** ..... **23/230 R**, 23/253 R, 195/103.5 R, 195/127, 324/30 B

[51] **Int. Cl.**.... **C12k 1/10**, G01n 27/06, G01n 33/16

[58] **Field of Search** ..... 23/230 R, 253 R; 195/103.5, 127; 324/30 B, 30; 204/195

[56] **References Cited**

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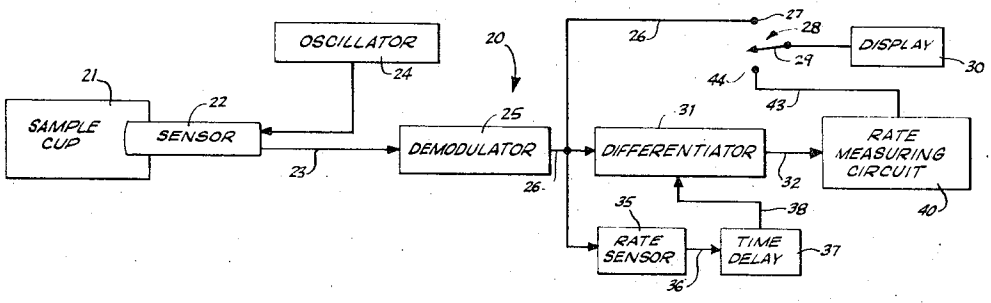
[57] **ABSTRACT**

A method and apparatus for determining the concentration of a component in a sample, i.e. the concentration of urea in biological fluids, such as blood serum, wherein the sample, upon being introduced into solution with a reagent, reacts therewith, causing a continuing change in a characteristic of the solution, and wherein the rate of the reaction is indicative of the concentration of the component in the sample. A sensor is provided for monitoring the characteristic of the solution and for generating a first electrical output signal proportional thereto. Differentiator circuit means are provided for producing a second electrical signal proportional to the time derivative of the first signal, the time derivative signal being indicative of the concentration of the component in the sample.

32 Claims, 11 Drawing Figures



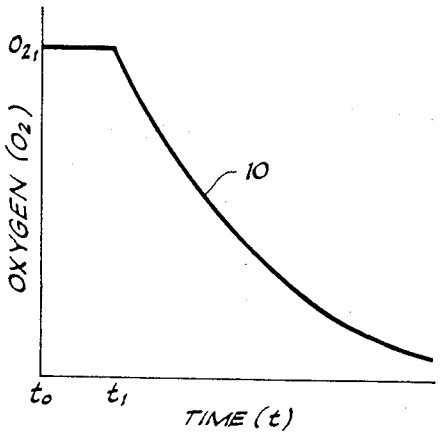


Fig. 1

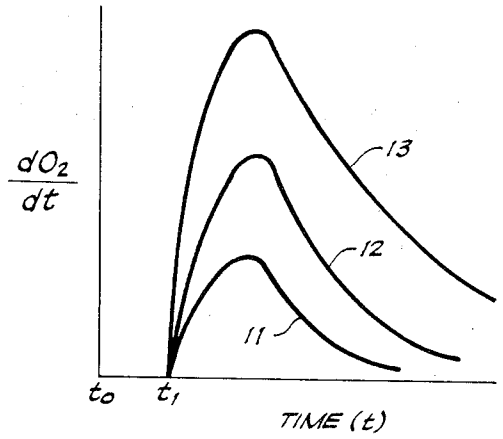


Fig. 2

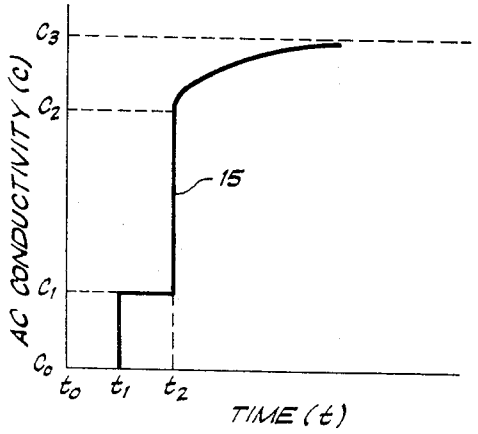


Fig. 3

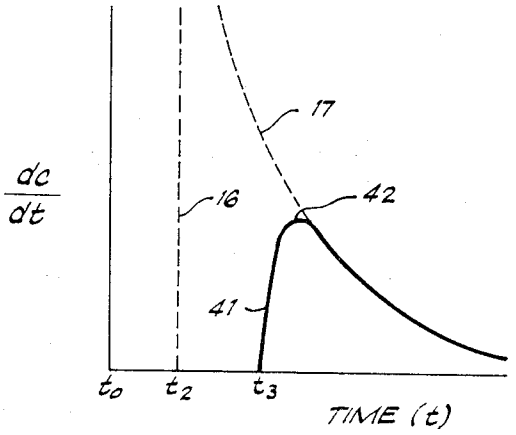


Fig. 4

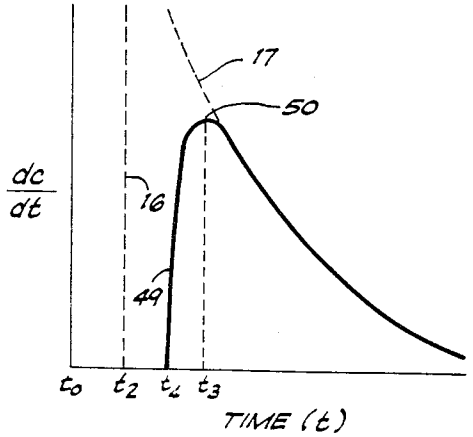


Fig. 5

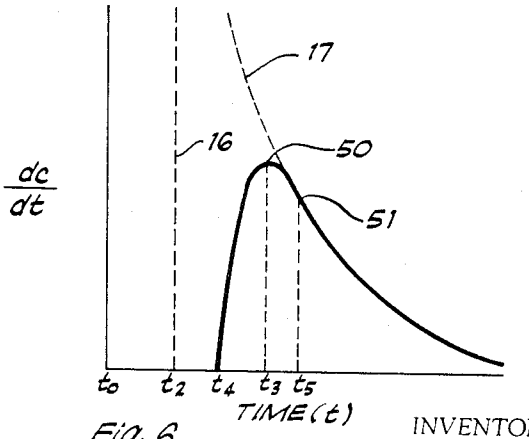


Fig. 6

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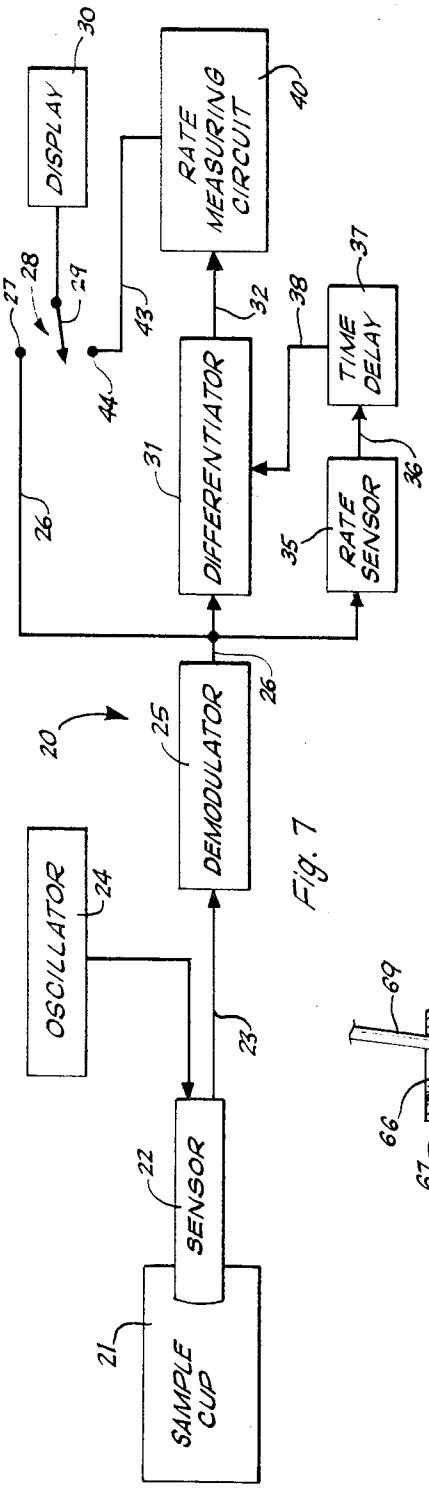

Fig. 7

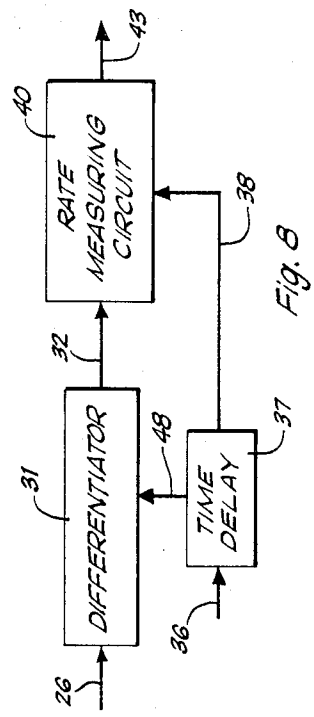

Fig. 8

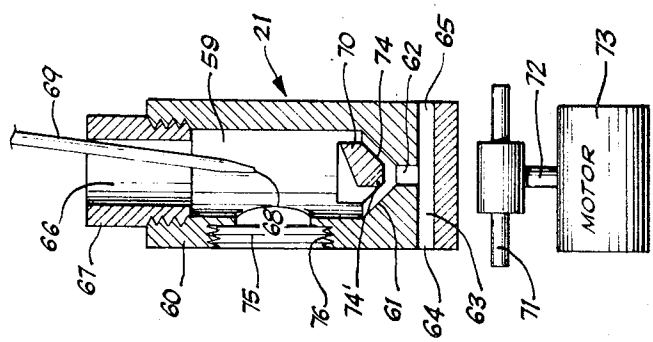

Fig. 9

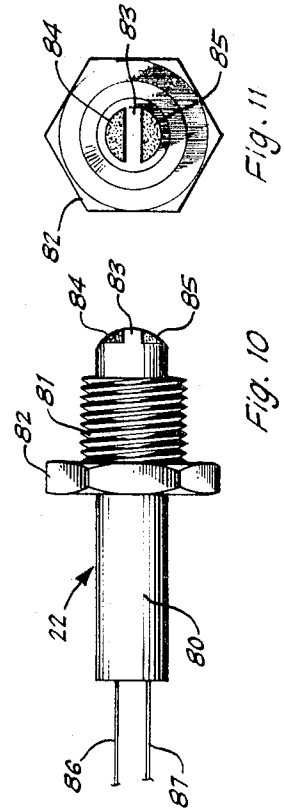

Fig. 10

Fig. 11

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METHOD AND APPARATUS FOR CHEMICAL ANALYSIS

According to the present invention, a large, instantaneous change in the characteristic of the solution being measured also takes place when the sample is added. Therefore, means are provided for measuring the value of the time derivative signal after a predetermined, fixed time interval from introduction of the sample into the reagent so as to eliminate the effect of the instantaneous change in the characteristic of the solution and to permit thorough mixing of the sample with the reagent.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a method and apparatus for chemical analysis and, more particularly, to a method and apparatus for the quantitative determination of the concentration of substances which are reactive with enzymes.

Description of the Prior Art

One general area within the field of this invention is the chemical analysis of biological substances to determine the chemical composition thereof. For example, a common procedure is to determine the concentration of glucose in blood or urine since the concentration of glucose in these body fluids is indicative of the operation of various fundamental body functions. Another common procedure is to determine the concentration of urea in blood serum since the concentration of urea in this body fluid is indicative of the operation of the kidneys.

Most available analyzing systems for determining the chemical composition of biological substances rely on colorimetric analysis. For example, one known technique for the enzymatic assay of glucose in blood and urine relies on the oxidation of the glucose in blood with the enzyme glucose-oxidase to produce hydrogen peroxide and gluconic acid. A presently available chemical analyzer relies on the spectrophotometric response of the color reaction between hydrogen peroxide, peroxidase and a chromogen. Another example would be in the determination of urea in blood by the reaction of urea with the enzyme urease to produce ammonium carbonate and using colorimetric techniques for determining the intensity of the product of the reaction.

While such colorimetric chemical analyzing systems are capable of producing accurate indications of the concentration of a component in a sample, there are several problems associated therewith. In the first instance, most colorimetric techniques are subject to large disturbances and interferences which may provide grossly inaccurate indications. For example, in the enzymatic assay of glucose in blood and urine by the oxidation of glucose with glucose-oxidase, the strong oxidizing agent, hydrogen peroxide, can react with other reducible substances and other impurities interfere with the peroxide-peroxidase reaction causing a loss in specificity and accuracy. In addition, availabe colorimetric and analyzing systems require measurement of the intensity of the color of the product at the completion of the reaction. Accordingly, the analysis is time consuming. In addition, the assay often cannot be conducted without deproteinization of the blood samples or prepurification of urine samples.

With respect to some enzymatic reactions, it has been proposed to use conductivity measurements in order to determine the concentration of a component in a sample. More specifically, in certain enzymatic reactions, a change occurs from a non-ionic to an ionic species or from an ionic to a non-ionic species. In such cases, the AC conductance of the medium serves as a direct measure of the extent of the reaction and the rate of change of AC conductance measures the rate of the reaction. Since the rate of reaction is directly proportional to the concentrations of certain reactants, such as the enzyme and the substrate, the concentrations of these species can be monitored by measuring the rate of change of AC conductance.

An example of this type of reaction is the reaction that occurs when blood, containing urea, is mixed with the enzyme urease. The non-ionic urea in the serum reacts with the enzyme urease to form ionic ammonium carbonate. The rate at which ammonium carbonate is formed is proportional to the quantity of urea in the serum sample. Since ammonium carbonate is ionic, the AC conductivity of the solution will change at a rate proportional to the quantity of urea present.

U. S. Pat. No. 3,421,982 to F. C. Schultz et al for Enzymatic Analysis proposes a system for measuring this change in AC conductivity. The system of Schultz et al employs conventional conductance electrodes and heretofore conventional levels of urease to provide a constant rate of change of concentration with time. Schultz et al indicate that the conversion of the substrate proceeds for several minutes but that the rate of change of conductivity in the first minute is essentially linear. These conditions are required so that a two-point kinetic method can meaningfully be used, with an adequate approximation to the rate of reaction being determined by measuring the finite change in conductance occurring over a fixed time interval within the linear portion of the reaction. Mathematically, this is a measurement of $66\ C/\Delta t$ where ΔC is the change in conductance during the fixed time interval, Δt, which is approximately one minute. As a result, the system of Schultz et al is cumbersome, time consuming and subject to inaccuracies.

One system for solving not only the problems inherent in colorimetric analysis systems but also in the conductimetric system of Schultz et al is disclosed in copending application Ser. No. 618,859, filed Feb. 27, 1967 in the name of James C. Sternberg for Rate Sensing Batch Analyzer and assigned to Beckman Instruments, Inc., the assignee of the present application. The analyzer disclosed therein provides a convenient method for rapidly determining quantitative information concerning a series of chemical, and especially biological samples. That analyzer determines the concentration of substances reactive with enzymes rapidly and accurately and uses small sample sizes. The analyzer of that application relies on the measurement of true instantaneous rate of reaction at very early stages of the reaction, before much reactant is consumed. The recorded rate signal results in a sharply defined peak corresponding to apparent maximum rate which is directly proportional to initial concentration. The apparent maximum rate is obtained in a relatively short time interval, thus saving analysis time and permitting more samples to be run in the same time interval. As applied to the direct monitoring of oxygen consumed in a glucose oxidase-glucose reaction, the invention does not

require preliminary purification or deproteinization of blood or urine samples, gives highly accurate results on an absolute basis and is insensitive to many impurities known to interfere with many other analytical procedures.

While such Rate Sensing Batch Analyzer solves the problems of the prior art discussed hereinbefore, it has been found that such analyzer is not ideally suited for many enzyme reactions. For example, the Sternberg analyzer determines the quantity of glucose in blood or urine by using an oxygen sensor to measure the rate of oxidation of glucose with glucose-oxidase to produce hydrogen peroxide and gluconic acid. The reaction may be controlled so that there is no initial change in oxygen level when the sample is introduced into solution with the reagent. On the other hand, when determining the concentration of urea in blood serum by reacting the serum with urease to form ammonium carbonate, obviously an oxygen sensor cannot be utilized to monitor the reaction. If a conductivity sensor is utilized for measuring change in conductance of the solution, problems are encountered in measuring the maximum value of the time derivative of the output of the sensor, as taught and claimed in said copending application. This is because blood serum itself is conductive and there is a large conductivity change in the solution when the serum is added to the reagent. This instantaneous jump in solution conductivity generates an apparent maximum value of time rate of change of conductivity which approaches infinity such that a meaningful output cannot be obtained.

SUMMARY OF THE INVENTION

According to the present invention, there is provided a method and apparatus for chemical analysis which not only solves the problems of the prior art solved by the before-mentioned Rate Sensing Batch Analyzer, but is applicable to a wider variety of enzyme reactions. The present method and apparatus is capable of rapidly determining the concentration of a component in a sample, such as biological fluids. The present apparatus is rapidly set up and put into operation, makes the determinations rapidly and accurately, uses a small sample size, and measures true concentration.

The present method and apparatus relies on the measurement of true instantaneous rate at a very early stage of a reaction before the reactant is consumed and, even with gaseous reactants, the reactions can be open to the atmosphere since the indicative data is collected before back diffusion of gas into the solution can influence the results. The present method and apparatus recognizes that introduction of the sample into solution with the reagent may cause an instantaneous change in the characteristic of the solution which is being measured. Accordingly, the present system inhibits measurement of the rate of change signal during a predetermined, fixed time interval starting with introduction of the sample into the reagent, which time interval is sufficient to eliminate the effect of the instantaneous change in the solution as well as to permit thorough mixing of the sample with the reagent. Immediately after the termination of the fixed time interval, the present system measures the value of the rate of change of the reaction.

Briefly, the present invention contemplates a method and apparatus for determining the concentration of a component in a sample, i.e. the concentration of urea in biological fluids, such as blood serum, wherein the sample, upon being introduced into solution with a reagent, reacts therewith, causing a continuing change in a characteristic of the solution, and wherein the rate of the reaction is indicative of the concentration of the component in the sample. A sensor is provided for monitoring the characteristic of the solution and for generating a first electrical output signal proportional thereto. Differentiator circuit means are provided for producing a second electrical signal proportional to the time derivative of the first signal, the time derivative signal being indicative of the concentration of the component in the sample.

According to the present invention, a large, instantaneous change in the characteristic of the solution being measured also takes place when the sample is added. Therefore, means are provided for measuring the value of the time derivative signal after a predetermined, fixed time interval from introduction of the sample into the reagent so as to eliminate the effect of the instantaneous change in the characteristic of the solution and to permit thorough mixing of the sample with the reagent. Thus, the present invention is capable of handling a large instantaneous change in the solution which is of little or no interest followed by a smaller and slower change which is indicative of the concentration of the component of interest.

It is therefore an object of the present invention to provide a novel method and apparatus for chemical analysis.

It is a further object of the present invention to provide a method and apparatus for the quantitative determination of the concentration of substances which are reactive with enzymes.

It is a still further object of the present invention to determine the concentration of a substance reactive with an enzyme by measuring the value of the rate of the reaction after a predetermined, fixed time interval from introduction of the substance into the enzyme.

It is another object of the present invention to provide a method and apparatus for measuring the concentration of urea in blood serum.

It is still another object of the present invention to measure enzymatic reactions rapidly and accurately with a minimum sample size.

Another object of the present invention is the provision of a novel electrode for measuring AC conductance.

Still other objects, features and attendant advantages of the present invention will become apparent to those skilled in the art from a reading of the following detailed description of the preferred embodiments constructed in accordance therewith, taken in conjunction with the accompanying drawings, wherein like numerals designate like parts in the several figures and wherein:

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph illustrating oxygen (O_2) concentration as a function of time (t) in a glucose oxidase-glucose reaction;

FIG. 2 is a graph illustrating the time derivative of oxygen concentration (dO_2/dt) for reaction of FIG. 1;

FIG. 3 is a graph illustrating AC conductivity (C) as a function of time (t) for certain enzyme reactions, such as a urea-urease reaction;

FIGS. 4–6 are graphs illustrating the rate of change of AC conductivity (dC/dt) versus time (t) for the graph of FIG. 3 and showing three alternative methods for measuring the value of the rate of change signal after a predetermined, fixed time interval from introduction of the sample into the reagent;

FIG. 7 is a simplified block diagram showing a preferred embodiment of apparatus constructed in accordance with the teachings of the present invention;

FIG. 8 is a partial block diagram showing a possible modification to the apparatus of FIG. 7;

FIG. 9 is a view, partly in section, showing a preferred embodiment of sample cup for use in the apparatus of FIG. 7;

FIG. 10 is a side elevation view of a preferred embodiment of sensor for use in the apparatus of FIG. 7; and

FIG. 11 is an end elevation view of the sensor of FIG. 10.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The beforementioned copending application of James C. Sternberg discloses a convenient method for rapidly determining the concentration of substances reactive with enzymes by measuring true instantaneous rate of reaction at very early stages of the reaction. As applied to the determination of the quantity of glucose in blood or urine, such analyzer contemplates measurement of the rate of oxidation of glucose with glucose-oxidase to produce hydrogen peroxide and gluconic acid. Measurement is made by positioning an oxygen sensor within a sample cup and measuring the rate of change of oxygen concentration.

Referring now to the drawings and, more particularly, to FIG. 1 thereof, at a time t_0, prior to introduction of the glucose-containing sample into the oxygenated glucose oxidase, the level of oxygen O_2 may have a value O_{2_1}. Upon introduction of the sample, at time t_1, the level of oxygen follows a curve 10 which decreases asymptotically. If the output of the oxygen sensor is applied to a differentiating circuit, an electrical signal may be derived which is the time derivative of the oxygen concentration signal and thus proportional to the time rate of change of concentration of oxygen. With reference to FIG. 2, curves 11, 12 and 13 represent three possible outputs of such differentiating circuit. More specifically, upon differentiating the output of the oxygen sensor, the time derivative increases to a maximum value and then decreases as the rate of reaction decreases. The maximum value of the output time rate of change signal is directly proportional to the initial concentration of glucose and provides a convenient, rapid and accurate output signal.

Many other enzymatic reactions are such that introduction of the sample into solution with the reagent cuases no instantaneous change in the characteristic of the solution being measured such that the teachings of the Sternberg application are applicable. On the other hand, in some enzymatic reactions, introduction of the sample into solution with the reagent causes an instantaneous change in a characteristic of the solution which it is desired to measure. For example, it is possible to determine the concentration of urea in blood serum by reacting the serum with the enzyme urease to form ammonium carbonate. The rate at which ammonium carbonate is formed is proportional to the quantity of urea in the serum sample. Since the serum is initially nonionic and since ammonium carbonate is ionic, the AC conductivity of the solution will change at a rate proportional to the quantity of urea present. However, problems are encountered in measuring the maximum value of the output of an AC conductance sensor since blood serum itself is conductive and there is a large conductivity change in the solution when the serum is added to the reagent. Referring now to FIG. 3, curve 15 shows the change in AC conductance with time measured by a conductance sensor positioned within a sample cup. At time t_0, when the cup is empty, the AC conductance has a value C_0=0. At time t_1, when the sample cup is filled with the enzyme urease, the AC conductivity jumps to a value C_1 becuase of the conductivity of the reagent. At time t_2, when the serum is introduced into the sample cup, there is an immediate jump in conductivity to a value C_2 because of the conductivity of the blood serum. Thereafter, the conductivity continues to increase asymptotically to a maximum value C_3, the change in conductivity from C_2 to C_3 being caused by the formation of ammonium carbonate.

Referring now to FIG. 4, becuase of the instantaneous jump in solution conductivity at time t_2, the rate of change of AC conductivity dC/dt initially jumps to infinity, as shown by the dashed curve 16. After time t_2, dC/dt decreases asymptotically along dashed curve 17. However, it will be appreciated by those skilled in the art that this instantaneous jump in solution conductivity, at t_2, generates an apparent maximum value of dC/dt which approaches infinity such that a system which measures the maximum value of time rate of change of the sensor output is incapable of providing a useful output signal.

Referring now to FIG. 7, a simplified block diagram of the present method and apparatus for chemical analysis, generally designated 20, includes a sample cup 21 in which the enzyme reaction occurs. Sample cup 21 may have any one of many known configurations and includes means for permitting introduction of the reagent and the sample as well as means for insuring thorough mixing of the solution. A preferred embodiment of sample cup 21 will be described hereinafter with reference to FIG. 9.

Extending into sample cup 21 is a sensor 22 for monitoring a characteristic of the solution or a component or a product of the reaction and for producing a first electrical output signal on a line 23 proportional to such characteristic. Accordingly, sensor 22 may be any type of known sensor such as the oxygen sensor of the before-mentioned copending application of Sternberg, such as a spectrophotometric sensor or the like. According to the preferred embodiment of the present invention, sensor 22 is a conductivity sensor, of a type to be described more fully hereinafter with regard to FIGS. 10 and 11, including first and second spaced electrodes for sensing the AC conductance of the solution within sample cup 21.

A constant amplitude AC voltage is applied to one electrode of sensor 22 from an oscillator 24. The output of oscillator 24 may be a symetrical wave of any shape, i.e. sinusoidal, square, triangular, etc., having any desired frequency, depending on circuit parameters, as will be explained more fully hereinafter. The change in AC conductivity of the solution produces a change in the current on line 23 connected to the other

electrode of sensor **22**, which current is reflected as an amplitude modulation of the basic frequency signal from oscillator **24**.

The amplitude modulated signal from sensor **22** is applied to a demodulator **25** which produces a DC voltage on a line **26** proportional to the AC conductivity of the solution in cup **21**. Line **26** is connected to one fixed terminal **27** of a switch **28** which includes a moveable arm **29**. Arm **29** is connected to a suitable display device **30** such as a digital voltmeter. Accordingly, by positioning arm **29** of switch **28** in contact with terminal **27**, the DC voltage proportional to the AC conductivity of the solution may be directly read out on display **30**. This signal should appear as curve **15** in FIG. **3**. This feature permits monitoring of the actual AC conductance of the solution in sample cup **21** to determine the values of C_0, C_1, C_2 and C_3.

The DC voltage proportional to AC conductivity on line **26** is also applied to a differentiator circuit **31** which is operative to produce, on a line **32**, a second electrical output signal proportional to the time derivative of the AC conductivity signal on line **26**. Thus, the electrical signal on line **32** is proportional to the time rate of change of AC conductance of the solution in sample cup **21** and is directly proportional to the concentration of the reactants in sample cup **21**.

It can therefore be seen that apparatus **20** is useful in monitoring a large class of enzymatic reactions, such as those where a change occurs from a non-ionic to an ionic species, or vice-versa, as described more fully in the before-mentioned copending application of Sternberg. Such a reaction occurs when blood serum containing urea is reacted with the enzyme urease to form ammonium carbonate. As explained previously, since the urea is initially non-ionic and since ammonium carbonate is ionic, the AC conductivity of the solution will change, and at a rate proportional to the initial concentration of urea.

Referring again to FIGS. **3** and **4**, curve **15** shows the output of demodulator **25** on line **26** as a function of time. At time t_0, with sample cup **21** empty, the conductance has a value $C_0=0$. A measured volume of reagent, containing the enzyme urease, is injected into sample cup **21** at time t_1, completely immersing sensor **22**. When this occurs, the AC conductivity on line **26** jumps to a value C_1 because of the conductivity of the reagent. A more elaborate discussion of the reagent will be provided hereinafter. A very small volume of sample serum is then introduced into sample cup **21** at time t_2 and mixed with the reagent. Accordingly, and as shown in FIG. **3**, at time t_2 there is an immediate jump in conductivity to a value C_2 because of the conductivity of the blood serum. In addition, the non-ionic urea reacts with the urease to form ammonium carbonate at a rate which is proportional to the quantity of urea in the sample. Accordingly, the conductivity continues to increase until a maximum value C_3 is reached.

Differentiator **31** provides an output voltage proportional to the rate of change of AC conductivity. Because of the instantaneous jump in solution conductivity at time t_2, the rate of change of conductivity initially jumps toward infinity (dotted curve **16**), preventing the measurement of maximum value of time rate of change. However, according to the present invention, the output on line **26** from demodulator **25** is applied to a rate sensing circuit **35** which senses the jump in conductivity when the serum sample is injected and which generates an electrical signal on a line **36** indicative of such jump. Alternatively, the output on line **26** from demodulator **25** may be applied to a conductivity level sensing circuit (not shown) which would sense the jump in conductivity when the sample is injected and which would also generate an electrical signal indicative of such jump. In any event, the signal on line **36** is applied to a time delay circuit **37** which generates, on a line **38**, a suitable electrical control signal, a characteristic of which changes after a predetermined, fixed time interval. The length of this fixed time interval is chosen based upon several considerations. In the first instance, the time interval is selected to be long enough to permit the transient from the jump in conductivity to disappear sufficiently to make an accurate measurement of rate of change of conductivity. The time interval is also selected to permit elimination of other transients, such as temperature upset and the like. Finally, the fixed time interval is selected to be long enough to permit thorough mixing of the sample serum with the reagent. In a preferred embodiment, as described hereinafter, the change in characteristic of the output of time delay **37** occurs approximately 12 seconds after sample introduction.

In any event, according to a first embodiment of the present invention, the output of time delay **37**, on line **38**, is applied to differentiator **31** for inhibiting the operation thereof until the end of the time interval, at time t_3. At time t_3, after the termination of the time interval, and as shown in FIG. **4**, the output **41** of differentiator **31**, on line **32**, rises to the actual signal level (dotted curve **17**) and then falls with the reaction rate. When this occurs, a signal peak **42** is obtained which is proportional to the value of the rate of change signal after a predetermined, fixed time interval from introduction of the sample into the reagent and is, therefore, proportional to the urea concentration in the sample. This output from differentiator **31**, on line **32**, is applied to a rate measuring circuit **40** which, in this embodiment, senses and holds peak value **42** and applies this value as an output signal over a line **43** to a second fixed terminal **44** of switch **28**. Accordingly, by moving arm **29** of switch **28** into contact with terminal **44**, the peak signal from differentiator **31** may be read out on display **30**.

It will be appreciated by those skilled in the art that the operation of time delay **37**, differentiator **31** and rate measuring circuit **40** just described is only one specific manner of affectuating the broader teaching of the present invention, namely measuring the value of the output of differentiator **31** after a predetermined, fixed time interval from introduction of sample into the reagent. In the embodiment shown in FIGS. **4** and **7**, the control signal from time delay **37**, on line **38**, is used to inhibit the operation of differentiator circuit **31** until time t_3 whereupon rate measuring circuit **40** measures the maximum value of the signal on line **32** immediately thereafter. Other techniques are obviously possible. For example, with reference to FIGS. **5** and **8**, rate measuring circuit **40** may be in the nature of a sample and hold circuit and the control signal from time delay **37**, on line **38**, may be applied to rate measuring circuit **40** to select the time or times for sampling the output of differentiator **31**. More specifically, time delay **37** may be operative to generate on a line **48**, a second electrical control signal, a characteristic of which changes at a time t_4 occurring after time t_2 but prior to

time t_3. As shown in FIG. 5, this second control signal on line 48 inhibits differentiator 31 from time t_2 to time t_4, to prevent the disturbance of differentiator 31 in the presence of the large jump in conductivity at time t_2. When such transient has been eliminated, the second control signal on line 48 permits differentiator circuit 31 to begin operation so that the output thereof rises along curve 49 until reaching the actual signal level (dotted curve 17) and then falls with the reaction rate. However, even though differentiator 31 is permitted to start operation at time t_4, it is still desirable to wait until time t_3 to measure the output of differentiator 31 so as to provide a sufficient amount of time to eliminate the effects discussed previously. Accordingly, the output of time delay 37 on line 38 is applied to rate measuring circuit 40 which is activated at time t_3. Rate measuring circuit 40 measures the instantaneous value 50 of the output of differentiator circuit 31, at time t_3, and applies such value as an output signal to display 30 via switch 28. According to another embodiment of the present invention, and as shown in FIG. 6, rate measuring circuit 40 samples the value 51 of the signal on line 32 at a time t_5 so as to derive the value of the signal on line 32 at a predetermined time t_5, which need not necessarily coincide with the apparent rate peak 50.

Referring now to FIG. 9, a preferred embodiment of sample cup includes a cylindrical, hollow body 60, forming a chamber 59, the bottom of which is tapered at 61. The apex of tapered section 61 is connected to a vertical passageway 62 which is connected to a horizontal passageway 63 extending entirely through body 60, adjacent the bottom thereof. One end 64 of passageway 63 provides an inlet for conducting reagent from a suitable source through passageways 63 and 62 into chamber 59. The other end 65 of passageway 63 provides a convenient location for emptying the solution in cup 21. It will be apparent that exit 65 is blocked during filling of cup 21 whereas inlet 64 is blocked during draining of cup 21.

Body 60 is open at the upper end thereof, at 66, and may include a suitable collar 67 if desired. The tip 68 of a pipette 69 is adapted to be extended through the open upper end 66 of body 60 to introduce a very small volume of sample, such as serum, into the reagent in chamber 59. In order to insure thorough mixing of the sample with the reagent in sample cup 21, sample cup 21 includes a stirrer 70. Stirrer 70 should have a shape similar to that shown in FIG. 9. To prevent the problem of coupling a drive element to stirrer 70, stirrer 70 may be magnetized and may be driven by the rotating magnetic force generated by a rotating drive magnet 71 connected by a shaft 72 to a motor 73. In order to support stirrer 70 for rotation within body 60 of cup 21, the lower end of stirrer 70 may be tapered, as at 74, at approximately the same angle as tapered section 61 of chamber 59. By making stirrer 70 of some suitable material, such as teflon, the tapered surfaces 74 and 61 provide adequate bearing surfaces for stirrer 70. A drainage passage from cup 21 is provided by slots 74' in the bottom of stirrer 70. A suitable type of stirrer is disclosed in U. S. Pat. No. 3,591,309 issued to Robert A. Ray et al and assigned to Beckman Instruments, Inc.

In operation, motor 73 is activated to rotate magnet 71 at any desired speed whereby stirrer 70 follows such speed of rotation. A measured amount of reagent is introduced into chamber 59 of cup 21 via inlet 64 and passageways 63 and 62. Thereafter, a small volume of sample, such as serum, is introduced into sample cup 21 via pipette 69 where it is mixed with the reagent due to the action of stirrer 70.

As shown in FIG. 9, body 60 of sample cup 21 may include an opening 75 in the side thereof, which is partially threaded, at 76, for receipt of sensor 22. Any suitable sensor having a pair of electrodes may be used for measuring AC conductivity. For example, the beforementioned U. S. Pat. No. 3,421,982 to Shultz et al teaches the use of a pair of parallel electrodes in a conductimetric system. However, in accordance with the teachings of the present invention, such electrodes should have a specific construction in order to eliminate many problems that occur in conductance measuring systems. However, before discussing in detail the preferred embodiment of electrode constructed in accordance with the present invention, the following discussion of the problems involved is provided.

Classical conductance is defined as the reciprocal of the electrical DC resistance and is represented by the equation:

$$C = 1/R$$

where C equals conductance and R equals DC resistance. However, the polarization effects of DC systems have required that most instruments use an AC voltage to measure this so-called conductance. In fact, an AC system measures the reciprocal impedance Z in accordance with the equation:

$$Z = (X_c^2 + R^2)$$

where X_c is the capacitive reactance due to ions in the solution. Normally the capacitive reactance term X_c remains quite large until frequencies on the order of several megacycles are reached. Since frequencies this high are not practical, most conventional conductimeters have a capacitance balancing circuit built in to accomodate this problem.

In accordance with the teachings of the present invention, it has been determined that the reason for this large capacitive reactance term is the use of conventional parallel-plate sensors. Referring now to FIGS. 10 and 11, there is shown a preferred embodiment of sensor 22 which substantially solves this problem. Sensor 22 includes an elongated cylindrical body 80 having a diameter equal to the diameter of the opening 75 in body 60 of sample cup 21. The forward end of body 80 may be threaded at 81 to engage with threads 76 in opening 75. Body 80 may also include a retaining nut 82 which is adapted to be tightened against the outer surface of body 60 of cup 21. Extending into sample cup 21 is a surface 83. Positioned on surface 83 are first and second electrodes 84 and 85 which are connected to leads 86 and 87, respectively, extending through body 80 of sensor 22. Leads 86 and 87 may be connected to oscillator 24 and demodulator 25, respectively.

According to the teachings of the present invention, the capacitive reactance term may be minimized and, in effect, eliminated, by making electrodes 84 and 85 planar and by positioning them coplanar. By so positioning electrodes 84 and 85, the DC resistance is uneffected but the capacitance is substantially minimized thereby permitting the capacitive reactance term X_c to become very small at a much lower frequency.

Surface 83 may be a flat surface and electrodes 84 and 85 may be conductive areas deposited thereon. As

a practical matter, making surface **83** flat and planar is not compatible with the cylindrical configuration of the wall of chamber **59** and would prevent rapid and thorough mixing of the solution therein and efficient drainage thereof. Accordingly, and as shown in FIGS. **10** and **11**, surface **83** is generally curved, having the shape of a segment of a sphere. With such a configuration, electrodes **84** and **85** may be in the shape of half circles positioned with the straight sides parallel and spaced apart. With such a configuration, it has been found that the capacitive reactance term X_c in the reciprocal impedence Z is effectively reduced to zero by increasing the frequency of oscillator **24** to 10 kHz. Of course, the frequency at which the reciprocal impedence plateaus is quite dependent upon the electrode configuration and the **10** kHz value only corresponds to the electrode configuration shown in FIGS. **9–11.** In any event, with such an electrode configuration, the AC impedance becomes a very close approximation to the DC resistance and no capacitance balancing circuit is required. The output of oscillator **24** may be connected directly to one of leads **86** or **87** whereupon the other lead provides the electrical signal on line **23** for direct connection to demodulator **25.**

As explained previously, one of the advantages common to the present invention and the Rate Sensing Batch Analyzer of James C. Sternberg is the provision of convenient methods for rapidly determining quantitative information concerning biological samples. The present analyzer, as well as that of Sternberg, relies on the measurement of true instantaneous rate of reaction at a very early stage of the reaction, before much reactant is consumed. By obtaining this rate in a relatively short time interval, analysis time is saved permitting more samples to be run in the same time interval. Accordingly, as applied to the present invention, the quantity of enzyme reagent used is large compared to usual methods whereby the reaction proceeds at a relatively rapid rate. The useful reaction is an approximately exponential change of conductance, as shown in both FIGS. **1** and **3,** having a typical time constant of 20 seconds. This is diametrically opposed to the teachings of Schultz et al who use such a small amount of reagent that the reaction proceeds very slowly and can be stated to be approximately linear.

Still another factor must be considered in making a conductivity measurement as described herein. More specifically, in enzyme reactions generally, the reagent is usually buffered with salts which are highly conductive so that the pH of the solution remains relatively constant as the reaction continues. This technique permits the reaction rate to proceed at its maximum potential value. With the present invention, it is apparent that this would be objectionable since it is herein desired to allow the conductivity of the solution to change, which change and the rate thereof is measured to determine the concentration of one of the components of the reaction. Accordingly, the present invention contemplates starting with essentially pure urease, dissolved in water, a low salt preparation having a relatively low initial conductivity. Typically, before the sample is injected, the conductance C_1, as in FIG. **3,** is about 20–25 percent of the final conductance C_3. Again, the concentration of enzyme reagent relative to the usual concentration of enzyme is relatively high. When the sample is injected at time t_2, the conductance jumps to C_2 which will have a value in the vicinity of 80 percent of the final conductance C_3. Of course, the initial conductance C_1 of the reagent may have a wide range of values since there still will be a change of conductance during formation of ammonium carbonate. However, because of the inherent difficulty of measuring a small change in a large signal, it is desirable to keep the initial concentration as small as possible.

It can therefore be seen that in accordance with the present invention, there is provided a method and apparatus for chemical anaylsis which not only solves the problems of the prior art solved by the beforementioned Rate Sensing Batch Analyzer, but is applicable to a wider variety of enzyme reactions. The present method and apparatus is capable of rapidly determining the concentration of a component in a sample, such as the concentration of components in biological fluids. The method and apparatus represented by block diagram **20** may be rapidly set up and put into operation to make quantitative determinations of true concentration rapidly and accurately, using small sample sizes.

The present method and apparatus relies on the measurement of true instantaneous rate at a very early stage of a reaction before the reactant is consumed and during a non-linear portion of the reaction. On the other hand, the present invention reconizes that introduction of a sample into solution with a reagent may cause an instantaneous change in the characteristic of the solution which is being measured. Accordingly, the present system inhibits measurement of the rate of change signal during a predetermined, fixed time interval starting with introduction of the sample into the reagent, which time interval is sufficient to eliminate the effect of the instantaneous change in the solution as well as to permit thorough mixing of the sample with the reagent. Immediately after the termination of the fixed time interval, the present invention contemplates measuring a value of the rate of change of the reaction. Several specific embodiments have been described for accomplishing this result. In addition, the present invention contemplates a novel conductivity sensor for eliminating capacitive influences in a conductance measurement.

While the invention has been described with respect to several physical embodiments constructed in accordance therewith, it will be apparent to those skilled in the art that various modifications and improvements may be made without departing from the scope and spirit of the invention. Accordingly, it is to be understood that the invention is not to be limited by the specific illustrative embodiments, but only by the scope of the appended claims.

We claim:

1. A method for determining the concentration of a component in a sample, wherein the sample, upon being introduced into solution with a reagent, reacts therewith, the rate of the reaction being indicative of said concentration, comprising:

monitoring a characteristic of said solution or a component or product of said reaction which is proportional to said concentration;

generating an output signal proportional to the time rate of change of said characteristic;

measuring the value of said output signal; and

inhibiting the measurement of the value of said output signal for a predetermined, fixed time interval from introduction of said sample into said reagent,

said time interval being sufficient to permit thorough mixing of said sample with said reagent.

2. A method according to claim 1 wherein said fixed time interval is long enough to permit thorough mixing of said sample with said reagent.

3. In a chemical analyzing system for determining the concentration of a component in a sample, wherein said sample, upon being introduced into solution with a reagent, reacts therewith, the rate of the reaction being indicative of said concentration, such system comprising sensor means for monitoring a characteristic of said solution or a component or a product of said reaction and for producing a first electrical output signal porportional thereto, and differentiator circuit means for producing a second electrical signal proportional to the time derivative of said first signal, said time derivative signal being indicative of said concentration of said component in said sample, the improvement comprising:

means for inhibiting operation of said differentiator circuit means for a predetermined, fixed time interval from introduction of said sample into said reagent, said time interval being sufficient to permit thorough mixing of said sample with said reagent.

4. In a chemical anaylzing system for determining the concentration of a component in a sample, wherein said sample, upon being introduced into solution with a reagent, reacts therewith, the rate of the reaction being indicative of said concentration, such system comprising sensor means for monitoring a characteristic of said solution or a component or a product of said reaction and for producing a first electrical output signal proportional thereto, differentiator circuit means for producing a second electrical signal proportional to the time derivative of said first signal, said time derivative signal being indicative of said concentration of said component in said sample, and means responsive to said second signal for generating an output signal indicative of the value thereof, the improvement comprising:

means for inhibiting the generation of said output signal for a predetermined, fixed time interval intitiated automatically upon introduction of said sample into said reagent, said time interval being sufficient to eliminate the effect of transients occurring upon introduction of said sample into said reagent.

5. In a chemical analyzing system according to claim 4, the improvement wherein said inhibiting means inhibits the operation of said differentiator circuit means during said fixed time interval.

6. In a chemical analyzing system according to claim 4, the improvement wherein said inhibiting means inhibits the operation of said generating means during said fixed time interval.

7. In a chemical analyzing system for determining the concentration of a component in a sample, wherein said sample, upon being introduced into solution with a reagent, reacts therewith, the rate of the reaction being indicative of said concentration, such system comprising sensor means for monitoring a characteristic of said solution or a component or a product of said reaction and for producing a first electrical output signal proportional thereto, differentiator circuit means for producing a second electrical signal proportional to the time derivative of said first signal, said time derivative signal being indicative of said concentration of said component in said sample, and means for measuring the value of said time derivative signal, the improvement comprising:

means for inhibiting the measurement of said time derivative signal for a predetermined, fixed time interval from introduction of said sample into said reagent, said time interval being sufficient to eliminate the effect of transients occurring upon introduction of said sample into said reagent.

8. In a chemical analyzing system according to claim 7, the improvement wherein said fixed time interval is long enough to permit thorough mixing of said sample with said reagent.

9. In a chemical analyzing system according to claim 7, the improvement wherein the rate of said reaction is not necessarily linear.

10. In a chemical analyzing system according to claim 7, the improvement wherein said inhibiting means inhibits the operation of said differentiator circuit means during said fixed time interval.

11. In a chemical analyzing system according to claim 7, the improvement wherein said inhibiting means inhibits the operation of said measuring means during said fixed time interval.

12. A chemical anaylzer comprising:

means for receiving a sample and a reagent;

sensor means operatively associated with said receiving means for monitoring the concentration of a component or product of the reaction between said sample and said reagent and for producing a first output signal proportional to said concentration;

differentiator circuit means coupled to said sensor means and responsive to said first output signal for producing a second output signal proportional to the time derivative of said first output signal and thus proportional to the time rate of change of concentration of said conponent or product;

means coupled to said differentiator circuit means for measuring the value of said second signal; and

means coupled to said sensor means for inhibiting the measurement of the value of said second signal for a predetermined, fixed time interval from introduction of said sample and said reagent into said receiving means, said time interval being sufficient to eliminate the effect of transients occurring upon introduction of said sample and said reagent into said receiving means.

13. A chemical analyzer according to claim 12 wherein said inhibiting means comprises:

timing means responsive to an abrupt change in said first output signal for producing a control signal, a characteristic of which changes after said predetermined, fixed time interval; and wherein said measuring means comprises:

means responsive to said second output signal and operative upon the occurrence of said change in said characteristic of said control signal for determining the value of said second output signal.

14. A chemical anaylzer according to claim 13 wherein said control signal is applied to said differentiator circuit means for inhibiting the operation thereof during said fixed time interval and wherein said measuring means measures the maximum value of said second output signal.

15. A chemical anaylzer according to claim 13 wherein said timing means produces a second control signal, a characteristic of which changes before the ter-

mination of said fixed time interval, wherein said second control signal is applied to said differentiator circuit means for inhibiting the operation thereof during a first portion of said fixed time interval, and wherein said first-mentioned control signal is applied to said measuring means for inhibiting the operation thereof during said fixed time interval.

16. A chemical analyzer according to claim **12** wherein said sensor means comprises:

first and second electrodes extending into said receiving means for monitoring the conductance of the solution therein, wherein:

said second output signal is proportional to the rate of change of conductance of said solution, and wherein:

said measuring means measures the value of said rate of change of conductance after said predetermined, fixed time interval.

17. A chemical anaylzer according to claim **16** further comprising:

oscillator means operatively coupled to one of said electrodes of said sensor means, said oscillator means generating an AC output signal; and

demodulator means operatively coupled to the other of said electrodes of said sensor means, said demodulator means receiving an amplitude modulated signal and producing a DC signal which comprises said second output signal.

18. A chemical analyzer according to claim **16** wherein said sensor means includes a surface which is exposed to said solution, and wherein said electrodes comprise first and second conductive areas positioned on said surface, said conductive areas being spaced apart.

19. A chemical analyzer according to claim **16** wherein said sensor means includes a surface which is exposed to said solution, said surface conforming to a segment of a sphere, and wherein said electrodes comprise first and second conductive areas positioned on said surface.

20. A chemical analyzer according to claim **19** wherein said conductive areas have the shape of half circles and are positioned with their straight sides parallel and spaced apart.

21. In a chemical analyzing system for determing the concentration of a component in a sample, wherein said sample, upon being introduced into solution with a reagent, reacts therewith causing an instantaneous change in a characteristic of said solution or a component or a product of said reaction, the rate of the reaction being indicative of said concentration, such system comprising sensor means for monitoring said characteristic, component or product and for producing a first electrical output signal proportional thereto, said change being sensed by said sensor means producing an abrupt change in said first output signal, and differentiator circuit means for producing a second electrical signal proportional to the time derivative of said first signal, said time derivative signal being indicative of said concentration of said component in said sample, the improvement comprising:

timing means responsive to an abrupt change in said first signal for producing a control signal, a characteristic of which changes after a predetermined, fixed time interval after said abrupt change in said first signal; and

means responsive to said second output signal and operative upon the occurrence of said change in said characteristic of said control signal for determining the value of said second signal.

22. In a chemical analyzing system according to claim **21,** the improvement wherein said control signal is applied to said differentiator circuit means for inhibiting the operation thereof during said fixed time interval and wherein said determining means measures the maximum value of said second signal.

23. In a chemical anaylzing system according to claim **21,** the improvement wherein said determining means measures the instantaneous value of said second signal upon termination of said fixed time interval.

24. In a chemical analyzing system according to claim **21,** the improvement wherein said determining means measures the peak value of said second signal after termination of said fixed time interval.

25. In a chemical analyzing system according to claim **21,** the improvement further comprising:

means for displaying the determined value of said second signal.

26. In a chemical anaylzing system according to claim **21,** the improvement wherein said timing means produces a second control signal, a characteristic of which changes before the termination of said predetermined fixed time interval, wherein said second control signal is applied to said differentiator circuit means for inhibiting the operation thereof during a first portion of said fixed time interval, and wherein said first-mentioned control signal is applied to said determining means for inhibiting the operation thereof during said fixed time interval.

27. In a chemical analyzing system according to claim **26,** the improvement wherein said determining means measures the instantaneous value of said second signal upon termination of said fixed time interval.

28. In a chemical analyzing system according to claim **26,** the improvement wherein said determining means measures the value of said second signal at a known time after termination of said fixed time interval.

29. In a chemical analyzing system according to claim **21,** the improvement wherein said sensor means comprises first and second conductive elements for monitoring the conductance of said solution, wherein said second electrical signal is proportional to the rate of change of conductance of said solution, and wherein said determining means measures the value of said rate of change of conductance after said predetermined, fixed time interval from introduction of said sample into said reagent.

30. In a chemical analyzing system according to claim **29,** the improvement wherein said conductive elements are substantially planar and are positioned in said sample coplanar to minimize capacitance effects on the measurement of conductance.

31. In a chemical analyzing system according to claim **29,** the improvement wherein said sensor means includes a surface which is exposed to said solution, said surface conforming to a segment of a sphere, and wherein said conductive elements comprise first and second conductive areas positioned on said surface.

32. In a chemical analyzing system according to claim **31,** the improvement wherein said conductive areas have the shape of half circles which are positioned with their straight sides parallel and spaced apart.

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