

[54] AUTOMATED SPECTROPHOTOMETER APPARATUS AND COMPUTER SYSTEM FOR SIMULTANEOUS MEASUREMENT OF A PLURALITY OF KINETIC REACTIONS

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[58] Field of Search 356/244, 246, 39, 96, 98, 356/205; 23/259; 233/26, 11

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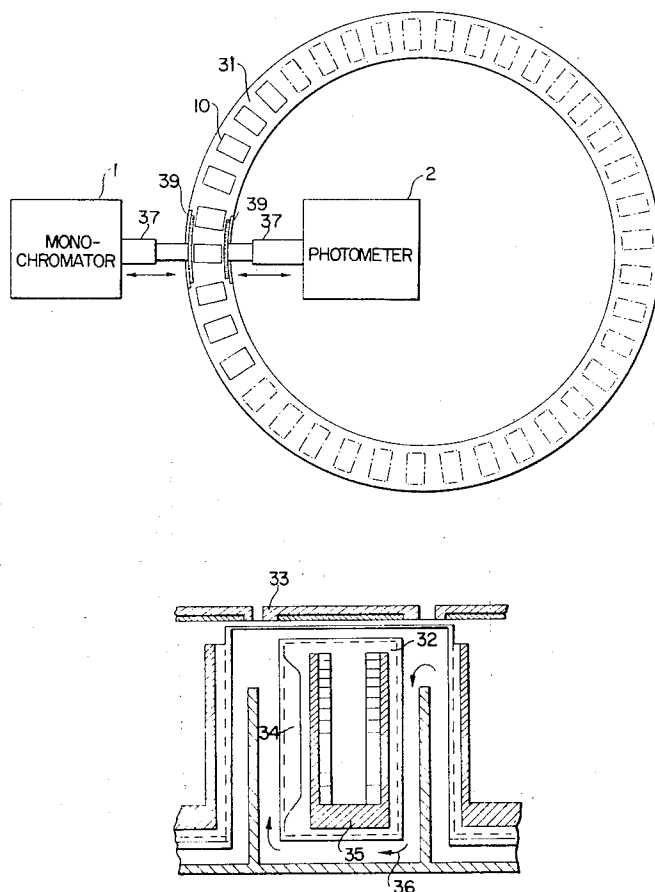
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[57] ABSTRACT

A spectrophotometer apparatus for the automatic positioning of multiple samples and sample blanks for measuring, for example, double differential absorbance, that is, sample absorbance with respect to both blank absorbance and time. The apparatus contains appropriate electronic hardware, for example suitable electronic computer and printout devices to generate a final digital printout of multiple analytical reaction rate results.

20 Claims, 8 Drawing Figures



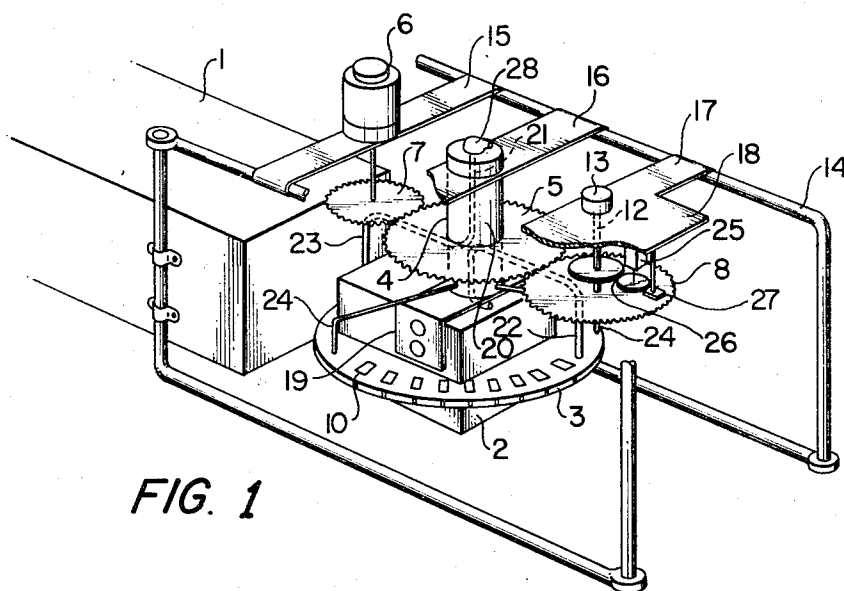


FIG. 1

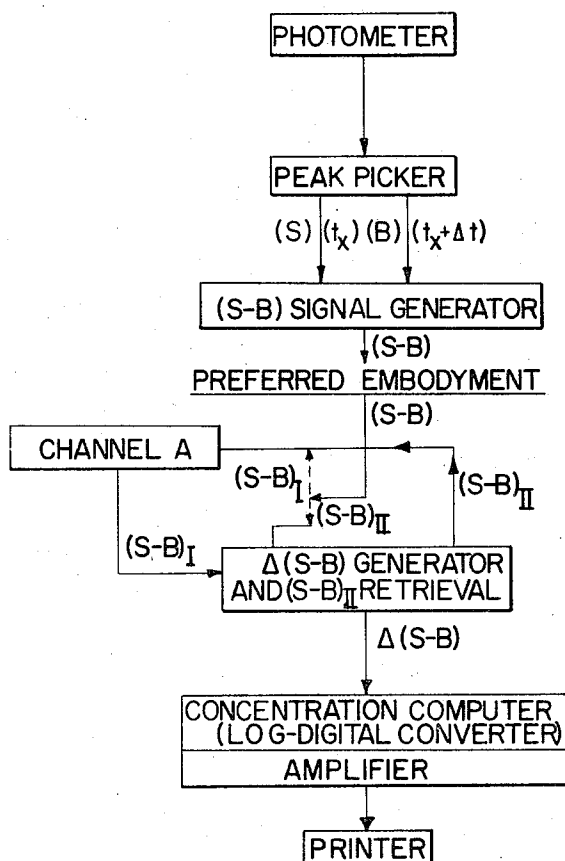
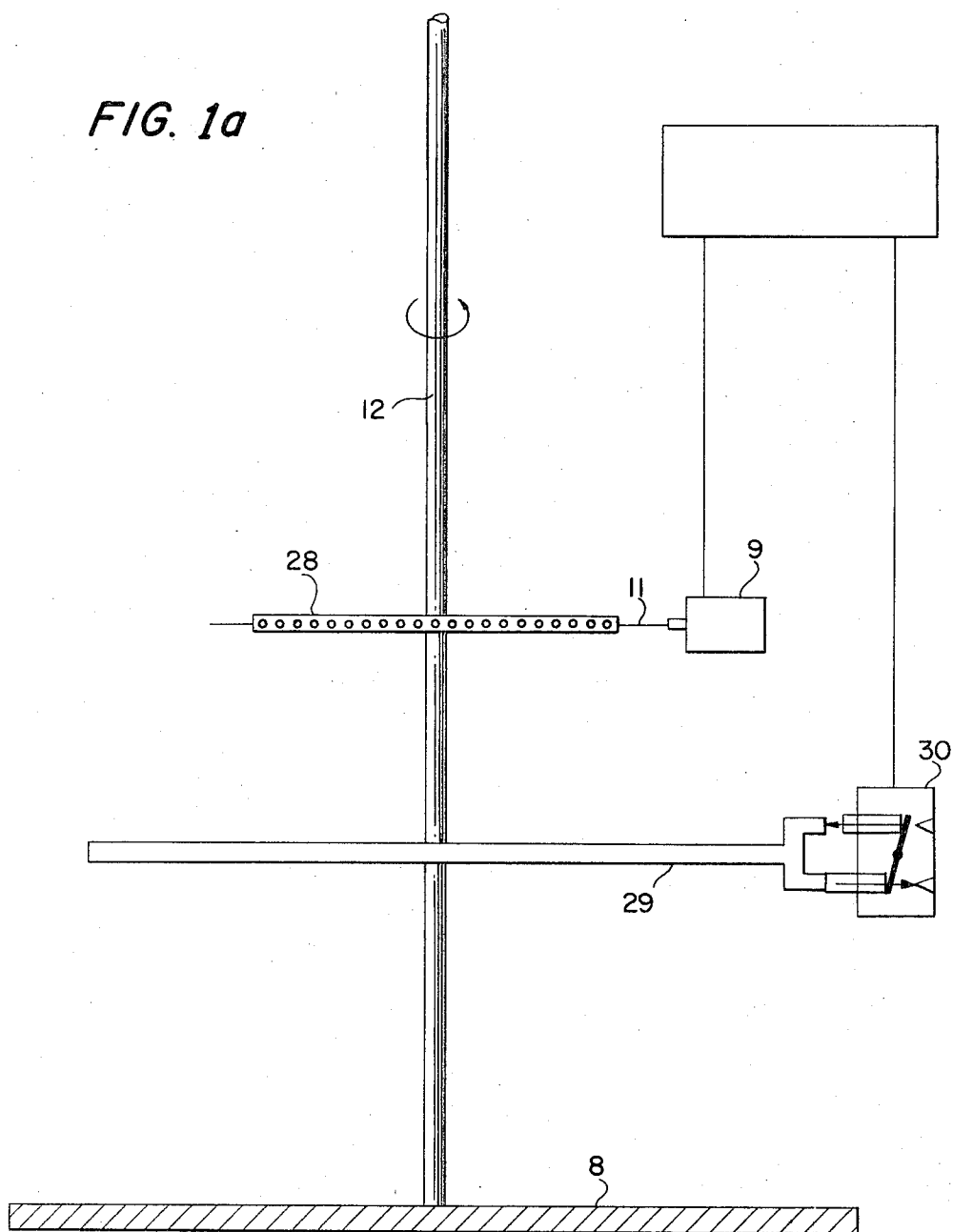
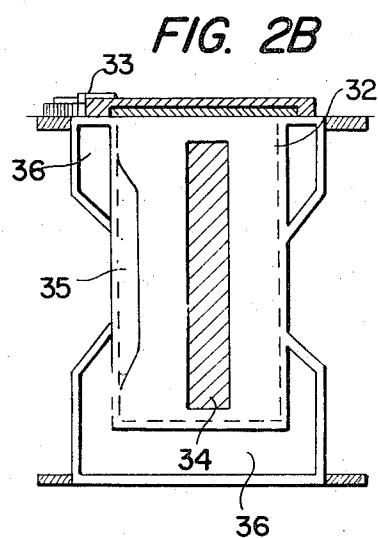
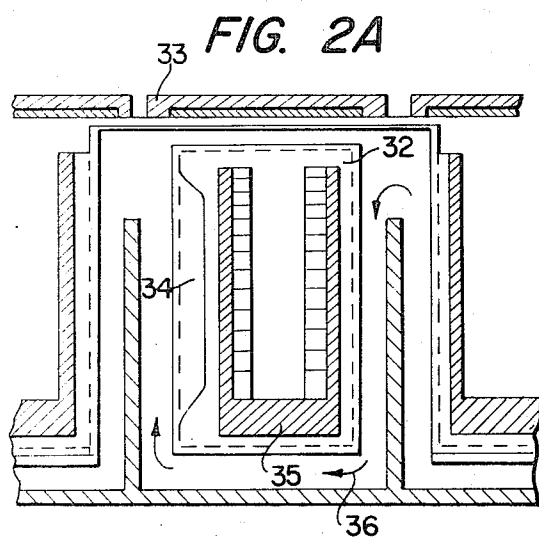
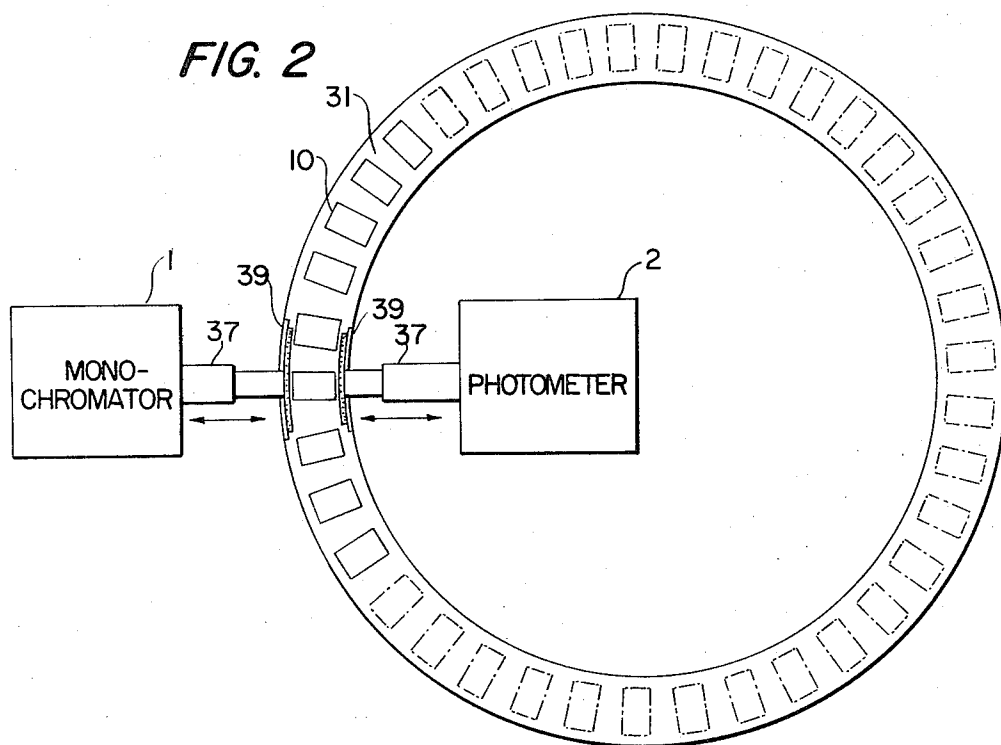


FIG. 3

FIG. 1a





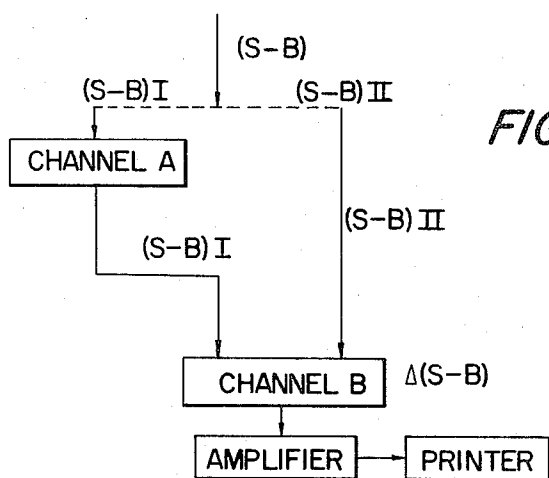


FIG. 3A

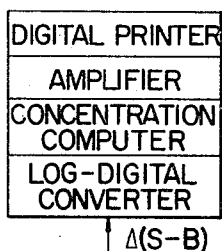
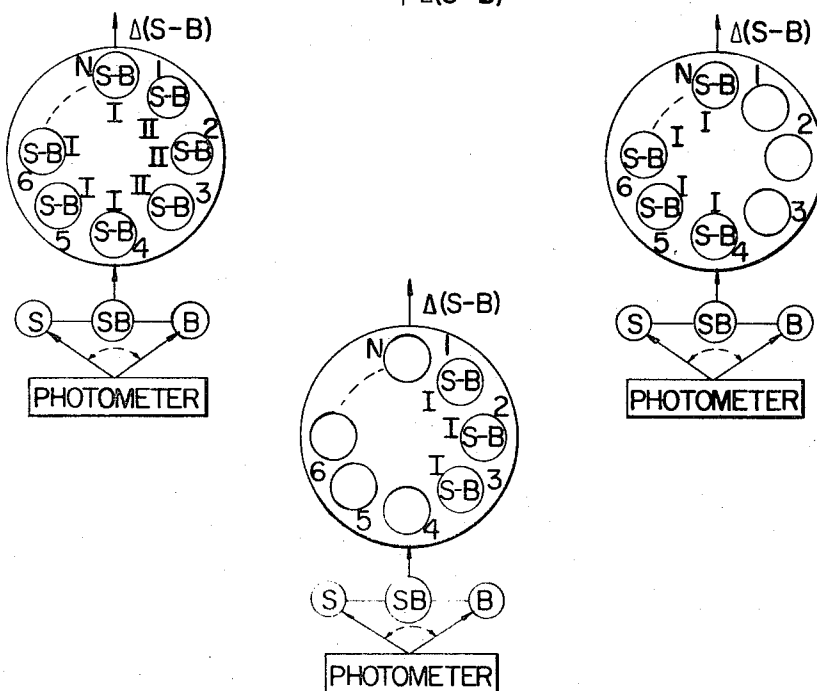


FIG. 4



AUTOMATED SPECTROPHOTOMETER APPARATUS AND COMPUTER SYSTEM FOR SIMULTANEOUS MEASUREMENT OF A PLURALITY OF KINETIC REACTIONS

BACKGROUND OF THE INVENTION

The present invention relates to an improved method and apparatus for determining multiple analytical reaction rate results, particularly for clinical enzyme reactions. More particularly, the present invention is directed to a method and apparatus for the proper positioning of a plurality of samples and sample blanks for simultaneously making a plurality of rate measurements on each sample using a particular arrangement of electronic computer hardware to produce a digital printout of the reaction rate results.

In the area of clinical chemical analysis, much work has been done toward achieving completely automatic analytical capabilities. One of the areas of routine analytical measurement which has not yet obtained a completely automated status with advantageous results is enzyme rate analysis for heterogenous samples, such as for example, blood serum. The instrumentation presently available, such as for example, a centrifuge type analyzer, suffers from at least one of the following limitations. Existing apparatus for measuring clinical enzyme reactions do not perform analysis at a rate (samples per hour) sufficient for routine patient screening needs. Furthermore, the results produced by well known spectrophotometer apparatus are subject to considerable error due to the following factors: (a) not using reactions specific for particular enzyme analysis, that is, many colorimetric procedures are utilized; (b) secondary reactions are frequently present requiring sample blanks for correction; (c) existing systems are not capable of readily checking for linearity of reaction versus time; (d) the spectrophotometer error is related to the extinction coefficient used in making calculations; and (e) no adequate means is provided for checking for inadequate substrates while the tests are being performed.

The elimination of the above sources of error requires the use of a precise spectrophotometer system capable of making accurate measurements in the ultraviolet region of the spectrum, the ability to include a sample blank in all measurements, the ability to make simultaneous measurements on a large number of samples and blanks, the ability to determine the linearity of the reactions and the ability to measure the blank corrected final absorbance and final blank absorbance.

There are three basic systems of positioning a plurality of samples or samples and blanks in the light beam of a spectrophotometer or discrete wavelength photometer. One of the systems is the flow-type system wherein samples flow continuously through a flow-through cuvette fixed in a light path. The flow system can be of the continuous type, such as for example, that represented by the Technicon autoanalyzer flow system or the start-stop type as contained in the Gilford Model 3400 enzyme analyzer. The flow-type system suffers from the following limitations: It has a relatively slow rate of pumping fluids which limits the rate of sampling. Also, a relatively large volume of sample is required together with a micro cuvette. Furthermore, when using such a system it is very difficult to make measurements versus time. The start-stop flow system

also suffers from many limitations including a relatively slow rate of pumping fluids coupled with a start-stop requirement. Thus, the sample rate limiting factors are the flow rate and the length of time required for the stop phase of the cycle.

The second type of system for positioning a plurality of sample or samples and blanks in the light beam of a spectrophotometer is a system requiring a linear back-and-forth motion of discrete samples in individual cuvettes. This system can also be either continuous or of the start-stop type. An example of this type of system is the Gilford Model 244 automatic cuvette positioner. This type of device moves from cuvette number 1 to cuvette number 2 to etc., and then back to cuvette number 1, with a start-stop cycle. The limitations of this system are as follows: The space factor is a problem when considering the total length of a holder which would be required for testing 30 or more samples or a combination of 30 samples and 30 blanks which would require 60 cuvettes. Also, the time needed to move the device from the last position to the first position would introduce a sampling rate limiting factor. In addition, multiple readings for each sample versus time would be required. A further disadvantage is that the mechanism required for changing the direction of the linear motion of the cuvette to give a relatively slow movement in one direction and a very fast reverse movement in the opposite direction, i.e., 30 to 60 times as fast depending upon the number of samples and blanks being tested, to maintain the same measuring sequence, would place severe mechanical strain on the system and complicate the entire apparatus.

The third type of device for positioning a plurality of samples or samples and blanks in the light path of a spectrophotometer is that involving the circular motion of the cuvettes containing the samples and blanks to be analyzed in a wheel assembly. This is the type of motion utilized in the apparatus of the present invention. The features of this type of system are as follows. Optimum single measuring conditions are provided wherein only a minimum sample volume is needed and wherein planar cuvette windows or curved cuvette windows normal to the light at its point of incidence on the cuvette window are used to prevent reflectance and refraction artifacts. The circular motion of cuvettes in a wheel assembly also enables optimum sample-blank differential measurements to be made wherein a single optical system is utilized with only a very small variable in time. Thus only a single monochromator (light source) and a single photometer (detector) is utilized for making sample-blank differential measurements. The circular motion of the cuvette wheel assembly also provides a maximum rate of sequential positioning of the cuvettes in the light path of the spectrophotometer. There is no known linear motion device or flow system which can move cuvettes containing samples to be measured into and out of the light path as fast as the circular motion of the cuvette wheel assembly. Finally, the circular motion of the cuvette wheel assembly produces maximum sample positioning in a minimum amount of space. Thus the circular motion of the cuvettes in the wheel assembly produces optimum physical and theoretical measuring conditions in a minimum amount of laboratory space at a maximum rate of analysis.

SUMMARY OF THE INVENTION

An object of the present invention is to provide an

improved method and apparatus for making automated kinetic measurements for clinical enzyme reactions.

Another object of the present invention is to determine the linearity of the reaction by making a plurality of measurements on each sample.

Still another object of the present invention is to provide an improved spectrophotometer apparatus for the automatic positioning of multiple samples and sample blanks for measuring for example, double differential absorbance, that is, sample absorbance with respect to both blank absorbance and time.

A further object of the present invention is to provide an improved method and apparatus for measuring different types of reaction rates during the same analytical run.

A still further object of the present invention is to provide an improved method and apparatus for automatically substrating a blank measurement during an analytical run which is essential for clinical enzyme analysis if rapid and error-free results are to be realized.

Still another object of the present invention is to provide an improved method and apparatus for making multiple discrete measurements on a plurality of samples and sample blanks to affect simultaneous reaction rate analysis on all of the samples.

Another object of the present invention is to combine with the above spectrophotometer apparatus, appropriate electronic hardware, including electronic computer and printout devices to generate a final digital printout of these simultaneous multiple kinetic measurements for clinical enzyme reactions.

Other objects and further scope of applicability of the present invention will become apparent from the detailed description given hereinafter; it should be understood, however, that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

Pursuant to the present invention it has been found that the above-mentioned disadvantages may be eliminated and a much improved method and apparatus for making kinetic measurements for clinical enzyme reactions may be obtained by utilizing a spectrophotometer taking advantage of the circular motion of cuvettes containing samples and blanks in a wheel assembly and utilizing a single monochromator as a light source, a single photometer as a light sensitive detector and an electronic system for generating a final digital printout of the clinical enzyme reaction rate results. According to the present invention, a cuvette wheel assembly adapted for circular motion is utilized. The purpose of the cuvette wheel assembly is to hold and sequentially position a number of cuvettes containing samples and blanks to be analyzed in the light path of a discrete wavelength or band-pass spectrophotometer. The sequential positioning of the cuvettes in the light path is obtained by rotating the circular cuvette wheel assembly about its axis utilizing appropriate motor and gear means. The positioning of the individual cuvettes in the light path must be controlled to very close tolerance and this sequential positioning must be coordinated with the proper use of said motor-gear drive system as

well as the use of an electronic computer adapted to monitor the electrical output of the photometer.

The general, overall operation of the apparatus of the present invention can be defined as follows. First of all, the cuvette compartments of the cuvette wheel assembly are brought to assay temperature by one of two thermostating systems which will be later defined. The individual cuvettes are then filled with the assay solutions and blank solutions at the assay temperature and loaded into the cuvette compartments of the wheel. The cuvette position number one, which is the first sample, is then moved to a position indicated by a reference line, said position being at a point where the cuvette is ready to pass through the light path of the monochromator. The wheel revolution indicator knob is then turned to position 1. After a preincubation-lag time, which is sometimes necessary, the start button is pushed to begin the measuring procedure. The pushing of the start button simultaneously starts the motor which through a gear mechanism rotates both the cuvette wheel and the idler gear assembly and activates the computer analytical system. Thus the reading and calculating cycle is started. The cuvette wheel first makes one complete rotation with no printout, storing the sample vs blank differential readings, hereinafter noted as (S-B). As the wheel starts its second rotation, the idler gear assembly automatically positions the revolution indicator switch and the computer and printer function to print out the analytical result. On each additional or on each alternate rotation of the cuvette wheel, depending on idler gear assembly and computer data storage capabilities another set of data is printed out. This is continued until, for example, four sets of data (answers) for each of a plurality of samples are generated. In the case where each rotation has been set to take 30 seconds at 2 r.p.m. four sets of answers will be generated in either 2½ minutes or 4 minutes. In the case where the cuvette wheel is designed to hold 30 samples, the corresponding rates of analysis will be either 720 samples per hour or 450 samples per hour. The pushing of the start button also activates a timer which automatically stops the rotation of the cuvette wheel after either the fifth revolution, which corresponds to 2½ minutes of operation or after the eighth revolution, which corresponds to 4 minutes of operation. If linearity of reaction rate has not been achieved for all of the samples, the analysis can be readily repeated by merely pushing the start button.

After an analysis has been completed, a check can be made for "masked" elevated samples by switching from a concentration mode of operation to an absorbance mode of operation and then pushing the start button. This operation generates a set of thirty sample vs blank differential absorbance readings in one rotation of the wheel, which takes 30 seconds. If these readings do not match the corresponding sample concentration readings, then the samples should be repeated after first diluting them. In a similar manner, high levels of NADH (coenzyme) "oxidase" can be checked by removing the sample cuvettes and replacing them with their corresponding cuvettes. Then, with the mode switch set for absorbance measurements, the start button is pushed. The cuvette wheel, by making one revolution, will generate a set of 30 sample-blank absorbance readings. If any of these absorbance readings are well below the majority of the others, indicating a low concentration of NADH during the assay, the amount

of NADH added to these samples is doubled and their analysis is repeated. It is desirable to maintain an optimum NADH concentration during the time of analysis for all enzyme reactions requiring NADH for the analysis.

The above two checks coupled with the routine use of blanks and controls as well as the use of multiple point kinetic method for analysis, as defined by the present invention, substantially eliminates all of the common causes of gross error frequently found in all other presently used systems for clinical enzyme analysis. The system as defined by the present invention not only gives about a 5 to 10 fold increase in the rate of analysis over most systems currently used for enzyme analysis but also is one of the only systems presently available for semi-automated enzyme analysis which produces results which are not subject to gross analytical error.

The rate of rotation of the cuvette wheel is related to several factors and must provide a balance between optical error and variation of sample concentration. As the r.p.m. is decreased, the ratio of optical error to total signal decreases. Any optical error is due mainly to slight misalignment, which decreases as the rate of rotation decreases. As the r.p.m. decreases, the absorbance for a given measurement increases which produces a greater signal. The limiting factors to consider when decreasing the r.p.m. to minimize the measuring error are as follows. In determining the rate of sample analysis, three readings determine a line and four readings allows for one of the readings to be disregarded, for example the first or last reading. At one r.p.m., it takes either one or two minutes per result or four or eight minutes per assay, which is equivalent to four results. Many reactions are linear for only about two to four minutes, therefore all readings after three minutes, for example, are suspect. This supports two r.p.m. rather than one r.p.m., thereby giving a maximum assay time of four minutes and at least 450 samples analyzed per hour which is sufficient because a faster rate of analysis is not presently required.

One of the features of the present invention is to provide the necessary interfacing between the cuvette carrier wheel assembly and the spectrophotometer. This interfacing can be achieved by the use of a "peak-picker" mechanism. As each cuvette rotates into proper alignment in the light path, the peak-picker means closes the circuit from the photometer to the computer. Readings are always taken as pairs, that is, (S-B)₁, (S-B)₂, etc.. Thus, a sample reading is taken and this reading is stored as the cuvette wheel moves and then a blank reading, that is, the second of the pair, is taken. Each pair of readings is used by the computer to generate a set of differential values. The (S-B) differentials for samples 1, 2, 3, etc., noted as (S-B)₁, (S-B)₂, (S-B)₃, etc. are either stored or used to calculate $\Delta(S-B)$ differentials depending on the idler gear assembly function. The idler gear assembly can be used to indicate to the computer which revolution the cuvette wheel is on, i.e., whether the wheel is on a (S-B) storage revolution or on a $\Delta(S-B)$ calculating revolution. The $\Delta(S-B)$ calculating revolution is also the revolution on which a set of results is printed out.

There are at least three types of peak-picker means which can be used for proper interfacing, i.e., for closing the circuit from the photometer to the computer when a cuvette rotates into proper alignment in the

light path of the monochromator. One possible way of using a peak-picker means for closing the circuit from the photometer to the computer is through the use of an interval timing device to close the circuit at constant time intervals, ΔT . This type of device is not shown in the drawings. It would function as part of the computer means. A second peak-picker means is to use the idler gear means to indicate when a cuvette is in the proper position for taking transmittance readings. For example, the computer takes readings from the photometers continuous output of electrical signals only when a micro-switch is closed and it is closed only when the cuvette is in proper alignment with the optical axis as indicated by a trip-pin on the idler gear assembly. A third peak-picker means would be an electronic switch peak picker. In operation, the light generated from the monochromator is continuous and the electrical output from the photometer is also continuous with the change in signal from the photometer being determined by variations in the amount of light reaching the photometer. The amount of light reaching the photometer is determined by the position of the cuvette carrier wheel relative to the optical axis (light path). When each cuvette is positioned with its optical face perpendicular to this imaginary line (optical axis) the amount of light reaching the photometer will be at a maximum. As the cuvette moves through this position, the light reaching the photometer increases to this maximum and then decreases. The decrease on either side of this maximum is the result of light lost by reflection, defraction and increase effective cuvette path length. This maximum signal can be used to signal the computer to take a reading at this instant of time.

In a preferred embodiment of the present invention, an electronic switch contained in the computer closes the circuit each time the detector signal reaches a maximum. In this embodiment after the first revolution, a trip-pin on the idler gear turns the revolution indicator to its alternate position. This opens the (S-B)_I (I corresponds to the first revolution) circuit to channel A (see FIG. 3) and closes the (S-B)_{II} (II corresponds to the second revolution) circuit to the $\Delta(S-B)$ generator. This activates the sequential release of previously stored (S-B)_I signals from channel A to be used with each corresponding successive (S-B)_{II} signal to generate the corresponding $\Delta(S-B)$ signal. The trip-pin also activates a timer which turns the motor off after a time interval sufficient for four revolutions of the cuvette wheel. Each (S-B)_{II} signal is stored in channel A and the corresponding $\Delta(S-B)$ signal is converted to a digital concentration printout (answer). This operation continues for three additional revolutions, after which the motor is automatically shut off thereby stopping the analysis. Thus, four "equivalent" answers have been generated for each sample being analyzed. Thus, in the preferred embodiment, the second, (S-B)_{II} reading is used with the first (S-B)_I reading (which has been stored) to determine a $\Delta(S-B)$ and then the second (S-B)_{II} reading is substituted for the first (S-B)_I reading in the storage circuit. The difference between the above two signals or readings, $\Delta(S-B)$ is used by the computer to calculate a digital answer which is automatically printed out.

In an alternative embodiment of the electronic system utilized in the present invention, the wheel is rotated until an indicator light, not shown in the drawing, corresponding to the first of two coupled revolution

lights and the reference lines are matched. This is an indication that the double trip-pin on the idler gear has positioned the revolution indicator switch to close the (S-B)_I circuit to channel A. The motor-on switch is then pushed which starts the rotation of the cuvette wheel thereby initiating the assaying of the samples. This step also starts an interval timer which automatically turns the motor off after a time interval sufficient for eight revolutions of the wheel. As each cuvette rotates into proper alignment in the light path of the monochromator, a peak-picker means momentarily closes the circuit from the photometer to the computer. After the first revolution, the double trip-pin on the idler gear positions the revolution indicating switch to open the (S-B)_I circuit and close the (S-B)_{II} circuit to channel B (see FIG. 3a). At the same time, the sequential release of previously stored (S-B)_I signals from channel A is also activated to be used with each corresponding successive (S-B)_{II} signal to generate the corresponding Δ (S-B) signal. The Δ (S-B) signal is converted to a digital concentration printout (answer). After the second revolution, the double trip-pin on the idler gear assembly positions the revolution indicator switch to open the (S-B)_{II} circuit and close the (S-B)_I circuit. The stored (S-B)_I signals are then used with the (S-B)_{II} signals as they are generated to determine a Δ (S-B) signal. In this operation the storage circuit for each sample is cleared and the entire operation is repeated to obtain a second set of answers. The entire operation is repeated and the cycle continues for eight revolutions at which time the motor is automatically shut off, thereby stopping the analysis. Four "equivalent" answers have thus been generated for each sample being analyzed. As will be readily recognized, the only difference between the preferred and the alternative embodiments of the electronic system, as described above, is a factor of two in the rate of sample analysis.

In all probability, a combination of mechanical and electronic devices discussed above will be necessary to realize the optimum interfacing between the cuvette holder wheel and the monochromator-photometer system.

If it is technically difficult to obtain the proper combination using cuvettes with planar windows, cuvettes with curved windows having a center of curvature which is coincident with the axis of the cuvette carrier wheel can be used. In this case, a slight uncertainty in the position of the wheel at the time of measurement would not significantly affect the measurement. This would allow a simple timing device in the computer coupled with a synchronous motor-driven simple gear system to keep the wheel and spectrophotometer in phase. There are two technical problems with this type of system. First of all, it is technically more difficult to produce optically accurate curved optical glass cuvette windows than it is to produce planar cuvette windows. Furthermore, most spectrophotometers would not have the light focused properly to avoid reflectance and refraction losses at the curved surfaces of the cuvettes. A lens system could be utilized to eliminate this problem.

In positioning the cuvettes in the light path of the spectrophotometer, it is essential that each cuvette, at the time of measurement, is positioned such that all light striking the optical surface (window) is normal to said surface at the point of incidence. Two different

means must be used depending upon whether planar or curved surfaces (windows) are utilized. In the case of cuvettes with planar windows, a horizontal plane bisecting the wheel contains the optical axis which is perpendicular to the axis of rotation of the wheel. Each rectangular cuvette chamber is thus positioned in the wheel so that one of two adjacent and mutually perpendicular sides of each chamber is parallel to a plane containing a radius of the wheel and the axis of rotation of the wheel. The cuvette can be automatically positioned in this chamber through the use of springs which force the cuvette against said two mutually perpendicular sides of the chamber. The light incident on the cuvette optical surface (window) is collimated (focused at infinity) and therefore is normal to the cuvette surface at the point of incidence. In the case of cuvettes with curved windows, a horizontal plane bisecting the wheel also contains the optical axis and is also perpendicular to the axis of rotation of the wheel. The curved optical surfaces of each cuvette are coincident with two series of vertically displaced circles with fixed, but different radii for each series with all circles being centered around the axis of rotation of the wheel. The light incident on the cuvette optical surface is focused on the axis of rotation of the wheel and therefore is normal to this surface at the point of incidence. The absolute rotational position of the wheel is not critical as long as some portion of the optical surface is in the light path at the time measurements are taken.

The fact that "double differential" measurements, that is, the (S-B) differential and the differences in (S-B) differentials are being made, reduce considerably the optical requirements for matching the cuvette with the spectrophotometer. The accuracy of any single transmittance reading is of only secondary importance because it is only the differential readings that are used in the analysis as defined by the present invention and any systematic errors, for example, optical errors, will cancel themselves out and thus not contribute in an adverse way to the final result. Because of this feature, plastic cuvettes could be readily produced through molding, rather than the more difficult to produce optical glass cuvettes, thus eliminating the latter problem. Thus, either the planar cuvette window or the curved cuvette window system could be used equally well as far as the final results are concerned. From the above discussion it is apparent that the three systems discussed above, that is, the motor-gear drive system, the planar or curved cuvette system and the electronic computer system have some flexibility with the final choice of systems being based upon technical considerations.

As is well known in the spectrophotometry field of technology, it is imperative to maintain a constant environmental temperature for the cuvettes throughout the entire period of the reaction rate analysis. In one of the features of the present invention, the cuvette wheel is provided with a hollow axle-bearing system for the introduction and removal of a thermostating liquid into and out of the cuvette wheel. Thus, the thermostatic liquid having a constant temperature controlled outside of the spectrophotometer apparatus is introduced into and removed from the cuvette compartment through the axis of the cuvette wheel. Incidentally, because of the manner in which the thermostating liquid is circulated in the cuvette compartment around the cuvette, no thermostatic liquid is disposed between the mono-

chromator and the sample cuvette as well as between the photometer and the sample cuvette.

In an alternative means for thermostating the cuvette carrier wheel, a thermoelectric heating-cooling means could be used. For example, the wheel could be made of metal with thermoelectric heating-cooling elements provided therein. Electrical contacts could then be made with an exterior power supply through the hollow axle and bearing of the cuvette wheel. In such an arrangement, spring loaded carbon tip contacts could be mounted in the bearing with corresponding copper contact strips mounted in the axle bearing seat.

To provide a substantially light-tight system from the monochromator to the cuvette wheel and from the cuvette wheel to the photometer a telescopic light pipe device has been found to be particularly effective. This feature provides a light-tight mechanical coupling of the stationary parts of the system, that is, the monochromator and the photometer, with the constantly moving or rotating part of the system, that is, the cuvette wheel. The light pipe device of the present invention can be telescopically adjusted between the monochromator and the photometer to provide a substantially light-tight environment between these elements and the rotating cuvette wheel. Alternatively, a large black box can be utilized to enclose the entire system. One of the advantages of using the box is that it could serve the dual function of not only providing a light-tight system when the wheel is in use but could function as a storage box for the wheel when it is not in use. The box can be disposed upside down over the wheel assembly and fastened permanently to the wheel support rack. When the wheel is not in use the box and wheel assembly could be removed and the box turned right side up. The lid to the box, with a handle attached thereto, could then be attached thus making a convenient carrying and storage case for the wheel assembly.

It should be pointed out that the rate of revolution of the cuvette wheel is somewhat flexible as are the number of sample cuvettes in the wheel and the number of revolutions of the wheel required for a complete analysis of all of the samples. The conditions specified above are essentially optimum for minimizing analytical error, including a convenient number of samples for a single analysis and a rate of analysis which is sufficient to meet the needs of a primary user, that is, a clinical chemistry laboratory. Because the reagent mixing step in the analytical procedure is not part of the automated operation of the present invention, different types of reaction rate measurements can be made during the same analytical run. This is a particularly advantageous feature of the present invention. The automated kinetic measuring spectrophotometer system of the present invention is the only such known device which can automatically subtract a sample blank during an analytical run. This is an essential step for clinical enzyme analysis if both rapid and error free results are to be realized.

DESCRIPTION OF THE DRAWINGS

The present invention will become more fully understood from the detailed description given hereinbelow and the accompanying drawings which are given by way of illustration only and thus are not limitative of the present invention and wherein,

FIG. 1 shows a perspective view of the spectrophotometer apparatus of the present invention including a monochromator, a photometer, a cuvette wheel assembly, and an idler gear means;

FIG. 1a shows another embodiment of the idler gear means of the present invention;

FIG. 2 shows a plan view of the cuvette wheel in conjunction with a telescopic light pipe device, a monochromator and a photometer;

FIG. 2a shows a front view section of the individual cuvette compartments in the cuvette wheel;

FIG. 2b shows a side view section of the individual cuvette compartments in the cuvette wheel;

FIG. 3 shows a schematic illustration of the computer elements starting from the point where the signals are taken from the photometer;

FIG. 3a shows a schematic illustration of another embodiment of the computer elements utilized in the present invention; and

FIG. 4 shows a schematic illustration of both a preferred embodiment and an alternative embodiment of the computer function of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The spectrophotometer apparatus of the present invention according to FIGS. 1 and 1a comprises a monochromator 1 (light source) a photometer 2 (detector) and a cuvette wheel 3 provided with a motor-gear-axle assembly. The cuvette wheel is mounted for rotation about an axle assembly 4 between the monochromator and the photometer. The axle assembly 4 is provided at its upper portion with an axle drive gear 5. A synchronous motor 6, which is located in the vicinity of the upper portion of the axle assembly is provided with a motor drive gear 7 which is adapted to engage the axle drive gear 5 for rotating the cuvette wheel. The axle drive gear is also associated with an idler gear 8 of an idler gear synchronizing mechanism. Because the idler gear engages the axle drive gear, the idler gear remains in synchronous phase with the cuvette wheel at all times and can be used together with other associated elements to trip switches indicating to the electronic components the relative position of the wheel and what electronic function should be performed. The idler gear mechanism could perform the following functions: It is adapted to indicate when a cuvette is in the proper position for taking "transmittance readings." For example, readings are taken from the photometer's continuous output of electrical signals only when a given micro-switch 9 is closed and it is closed only when the cuvette 10 is in proper alignment with the optical axis, as indicated by a trip pin 11 disposed on the idler gear shaft 12 of the idler gear 8. Measurements are always taken as pairs, that is, $(S-B)_1$, $(S-B)_2$, etc. The $(S-B)$ difference readings are then transformed into $\Delta (S-B)$ readings using the computer methods and devices discussed above and hereinafter. One electronic means capable of handling the above pairs of readings is embodied in the Gilford Model 2430 oscillating cell $(S-B)$ system. This device takes a sample reading, stores this reading, moves the cell holder linearly and takes a blank reading. Both readings are taken as "voltage" which is directly proportional to absorbance. The difference between these measurements, i.e., $(S-B)$ is determined and in this case recorded on a chart recorder. The $(S-B)$ difference readings are then transformed

into Δ (S-B) readings as stated above, using the computer methods and devices discussed above and hereinafter.

The idler gear mechanism is provided at its upper end, that is, the end opposite the idler gear, with a bearing sleeve 13 which is adapted to receive the idler gear shaft 12. The idler gear is constructed to be identical in size with the axle drive gear of the axle assembly so that the rotation of the idler gear is synchronous with the rotation of the cuvette wheel. The synchronous motor, the axle for the cuvette wheel and the idler gear mechanism is supported above the photometer by a support rack 14. The upper portion of the support rack is provided with a motor support 15 for supporting the synchronous motor, a top-axle bearing support 16 for supporting the top portion of the axle for the cuvette wheel, an idler gear shaft bearing support 17 for supporting the idler gear mechanism and a revolution indicator support 18 for supporting the revolution indicator. The lower portion of the axle for the cuvette wheel is supported by a bottom axle bearing support 19 which is adapted to be secured to the upper portion of the photometer. As one of the possible arrangements of the support rack, right angle elements of the support rack are provided at their opposite ends with male and female end portions which provide for the ready assembly and disassembly of the support rack about the photometer.

In one of the advantageous features of the present invention, a hollow axle 20 for the cuvette wheel is utilized, said hollow axle being provided with upper and lower axle bearing means provided with channels 21 which communicate with conduit means 22 and 23 for introducing a thermostating liquid into and removing it from the cuvette compartments of the cuvette wheel from a source outside of the spectrophotometer. Thus a thermostating liquid which is controlled to a desired temperature, depending upon the particular samples being analyzed is introduced through the hollow axle of the cuvette wheel via conduit means 22 to the cuvette compartment which contains the individual cuvettes. The thermostating liquid is removed from the cuvette compartment via conduit means 23 through the hollow axle for the cuvette wheel and returned to the point where the liquid is being controlled at a predetermined temperature outside of the spectrophotometer. The cuvette wheel and the cuvette axle are provided with additional support brackets 24 which further stabilize the cuvette wheel axle with respect to the cuvette wheel. The individual cuvette compartments which contain the cuvettes are provided with a hinged cover to exclude extraneous light from the cuvette compartments.

FIG. 1 shows a preferred embodiment of the idler gear mechanism of the present invention wherein an automatic switch reset element is associated with the idler gear shaft for resetting the switch after the first revolution of the cuvette wheel. For this purpose revolution indicating switch 25 is associated with a reset mechanism 26. Manual lever 27 is also associated with the reset mechanism for setting the switch in its proper first revolution position. The revolution indicating switch 25 is supported on support rack 14 by support frame 18.

FIG. 1a shows an alternative embodiment of the idler gear mechanism of the present invention wherein the sample-blank cuvette position indicator (trip pin) 28 is

provided with a number of pins 11 corresponding to the number of sample-blank cuvettes disposed in the cuvette wheel. Because the sample-blank cuvette position indicator is attached to the idler gear shaft, it rotates with the rotation of the cuvette wheel. As the sample disposed in the cuvette wheel is rotated into light communication with the monochromator and photometer, the indicator 11 makes contact with a mechanical switch peak picker (micro-switch) 9 which enables the signal of the sample to be recorded at a time T_1 . The peak picker indicates to the computer that the signal of the next cuvette which contains a blank be recorded at time T_2 . Through the use of appropriate electronic components the difference (S-B) can be readily computed and stored. The idler gear shaft is also provided with a double trip pin element 29 which is associated with an alternate revolution indicator switch 30.

FIG. 2 shows a plan view of the cuvette wheel of the present invention showing the top of each of the cuvettes 10 disposed in the cuvette compartments 31 of the cuvette wheel. FIG. 2a and 2b show a front sectional view of the cuvette wheel at a plane parallel to the wheel axis and a side sectional view of the cuvettes disposed in the cuvette compartments, respectively. The individual cuvette compartments 32 contain a hinged cover 33. The individual cuvettes are held in position against the cuvette compartment walls by spring means 34 and 35. As can be readily seen, the thermostating liquid 36 is free to travel through the cuvette compartments around the individual cuvettes. It should be noted that the cuvette compartments are provided with a front and back slit which is adapted to transmit the light from the monochromator to the photometer. As can be readily seen, no thermostating liquid is disposed between the monochromator and the sample or between the photometer and the sample. This arrangement, of course, eliminates any error which might be introduced by a thermostating liquid interposed between the sample and these elements.

FIG. 2 also shows a plan view of the monochromator-photometer-cuvette wheel wherein a telescopic light pipe 37 is disposed between the monochromator and the cuvette wheel as well as between the photometer and the cuvette wheel as a stray light trap. The telescopic light pipe prevents the light from the monochromator from being lost to the environment and furthermore it performs the same function with respect to the light received by the photometer. The telescopic light pipe also prevents light present in the environment from effecting sample measurements. Because of the telescopic feature, the monochromator and the photometer can be moved toward or away from the cuvette wheel. FIG. 2 further shows the use of felt pads 38 and a metal flange 39 to prevent extraneous light from entering into the system. As can be readily seen, the cuvette compartments are provided with slits on the inside and the outside of the cuvette wheel adjacent to each cuvette sample. Advantageously, the slit should be smaller than the diameter of the light pipe.

FIG. 3 shows a schematic illustration of the computer elements which can be utilized to produce a final answer from the appropriate signal generated by the photometer. The continuous electrical signal from the photometer is fed into the peak-picker means of the computer. The peak-picker means picks the maximum signal from the photometer for each of the samples being analyzed at time T_x and then the maximum signal of the

blank being analyzed at time T_x plus ΔT . The signals of the sample S and the blank B are then fed into a signal generator which produces the differential reading (S-B). From this point the obtaining of a printout of the answer can follow one of two courses. In the preferred embodiment of the present invention, during the first revolution, the revolution indicator switch 25 is manually set to its data (S-B) storage mode, i.e., the circuit to channel A is closed and all (S-B) readings are stored in channel A. After the first revolution a trip-pin on the idler gear automatically turns the revolution indicator switch 25 to its alternate position. This opens the (S-B)_I circuit to channel A, and at the same time, the (S-B)_{II} circuit to the Δ (S-B) generator is closed. The differential reading (S-B)_{II} represents that reading taken on the second revolution of the cuvette wheel. As the differential readings (S-B)_{II} are introduced into the Δ (S-B) generator, the sequential release of the previously stored (S-B)_I signals are activated from channel A to be introduced into the Δ (S-B) generator to be used with each corresponding successive (S-B)_{II} signal to generate the corresponding Δ (S-B) signal. At the same time, a timer which turns the motor off after a time interval sufficient for four revolutions of the wheel is activated. The (S-B)_{II} signal which was originally introduced into the Δ (S-B) generator is now stored in channel A to be used in subsequent calculations and the Δ (S-B) signal is converted to a digital concentration printout (answer). This operation continues for three additional revolutions, at which time the motor is automatically shut off stopping the analysis. Four equivalent answers have thus been generated for each sample being analyzed.

FIG. 3a represents a schematic of the computer elements utilized in an alternative embodiment for obtaining a digital printout of the answer. In this embodiment, the (S-B) differential reading is stored in channel A and noted as (S-B)_I differential readings. The differential readings stored in channel A is the direct result of the closing of the (S-B)_I circuit to said channel as a result of the operation of the double trip pin on the idler gear. After the first revolution, the double trip pin on the idler gear positions the switch to open the (S-B)_I circuit and close the (S-B)_{II} circuit to channel B. At the same time it also activates the sequential release of previously stored (S-B)_I signals from channel A to be used with each corresponding successive (S-B)_{II} signal to generate a corresponding Δ (S-B) signal. The Δ (S-B) signal is then converted to a digital concentration printout (answer). The difference between the operation of the electronic elements of FIG. 3a when compared to FIG. 3 is that the differential reading (S-B)_{II} which is used to calculate a Δ (S-B) signal is not restored in channel A so that it can be used to calculate a new Δ (S-B) with further readings. Rather, after the printout of the Δ (S-B) answer, the (S-B)_{II} signal is removed from the computer system so that in order to calculate a new Δ (S-B) signal for subsequent measurements, the (S-B)_{III} signals must be stored in channel A. Thus, eight revolutions of the cuvette wheel are necessary to generate four equivalent answers for each sample being analyzed. This is to be compared with the preferred embodiment as shown in FIG. 3 wherein only five revolutions of the cuvette wheel are required to generate four equivalent answers for each sample being analyzed.

FIG. 4 is a schematic drawing illustrating the computer function described with respect to FIGS. 3 and

3a. The signals received from the photometer in the first revolution are fed into a computer to determine a set of (S-B) differentials which are stored. The second set of (S-B) differentials generated on the second revolution of the wheel are used along with the said previously stored (S-B) differentials to generate a Δ (S-B) differential. In the preferred computer storage system the second set of (S-B) differentials, i.e., (S-B)_{II}, remains stored to be used on the third revolution to generate a second set of Δ (S-B) differentials. This is the storage system illustrated schematically in FIG. 3. In the alternate storage system the second set of (S-B) differentials is not stored and the entire process must be repeated. This is the storage system illustrated schematically in FIG. 3a. Independent of which computer storage system is utilized, the Δ (S-B) signal is subsequently introduced into a log-digital converter, a concentration computer, and an amplifier and finally a digital printout of the answer is obtained.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be apparent to one skilled in the art are intended to be included within the scope of the following claims.

I claim:

1. An apparatus for making automated kinetic measurements comprising a standard light source, a standard light sensitive detector and a cuvette wheel assembly disposed between said light source and light sensitive detector, and mounted for rotation about an axle assembly, said cuvette wheel assembly containing a plurality of cuvettes containing the samples to be analyzed, means for rotating the axle assembly, thereby providing the sequential positioning of the samples in the light path of the light source, an idler gear assembly associated with the axle assembly and in synchronous phase with the cuvette wheel assembly, a "peak-picker" means associated with the detector indicating when a sample is in the proper position for recording transmittance readings received by the detector and an electronic computer system associated with the peak-picker means and the idler gear assembly for generating a final digital printout of multiple analytical reaction rate results.

2. The apparatus of claim 1, wherein the axle assembly is provided with a standard axle drive gear and the idler gear assembly is provided with a standard idler gear, said axle drive gear and said idle gear being substantially the same size and being in engaging relationship with each other.

3. The apparatus of claim 1, wherein the peak-picker means is an interval timing device which forms part of the electronic computer system, said interval timing device functioning to close the circuit between the detector and the computer-system at constant time intervals, ΔT for recording signals received from the detector.

4. The apparatus of claim 2 wherein the peak-picker means comprises a plurality of trip-pins associated for rotation with the idler gear assembly, each of said trip-pins corresponding to a sample or blank cuvette and being adapted to sequentially engage a micro-switch which momentarily closes the circuit between the detector and the computer system for recording signals received from the detector.

5. The apparatus of claim 1, wherein the peak-picker means is an electronic switch which forms part of the electronic computer system, said electronic switch functioning to close the circuit between the detector and the computer system when a maximum signal is received from the detector.

6. The apparatus of claim 1, wherein the computer system comprises a signal generator which generates differential readings for each revolution of the cuvette wheel, a storage channel for storing said differential readings for the first of said revolutions, a double differential generator for receiving the differential readings from said storage channel and said signal generator corresponding to the second revolution of the cuvette wheel, means for simultaneously releasing said stored differential readings, means for converting the double differential readings to a final digital printout of multiple analytical reaction rate results and means for using the differential readings corresponding to the second revolution of the cuvette wheel with subsequent differential readings corresponding to a third revolution of the cuvette wheel to generate another digital printout, and means for continuing this process until the desired number of analytical measurements have been made.

7. The apparatus of claim 6, wherein revolution indicator means are associated with the idler gear assembly for indicating to the computer system which differential readings are to be stored and which differential readings are to be sent directly to the double differential generator.

8. The apparatus of claim 2, wherein revolution indicator means are associated with the idler gear assembly for indicating to the computer system which differential readings are to be stored and which differential readings are to be sent directly to the double differential generator.

9. The apparatus of claim 7, wherein said revolution indicator means comprises a trip pin on the idler gear assembly operatively associated with a revolution indicator switch.

10. The apparatus of claim 1, wherein the computer system comprises a signal generator which generates differential readings for each revolution of the cuvette wheel, a storage channel for storing said differential readings for the first of said revolutions, a double differential generator for receiving the differential readings from said storage channel and said signal generator corresponding to the second revolution of the cuvette wheel, means for simultaneously releasing said stored differential readings from said storage channel to said generator thereby generating double differential readings, means for converting the double differential readings to a final digital printout of multiple analytical reaction rate results and means for clearing the storage circuit.

11. The apparatus of claim 10, wherein the means for clearing the storage circuit comprises an alternate revolution indicator means associated with the idler gear assembly for indicating to the computer system which differential readings are to be stored and which differential readings are to be sent directly to the double differential generator.

12. The apparatus of claim 1, wherein the cuvette wheel is provided with a hollow axle-bearing-conduit system for the introduction and removal of said thermostating liquid into and out of a cuvette wheel.

13. The apparatus of claim 12, wherein thermostating fluid from a standard recirculating liquid thermostating means enters the hollow axle-bearing-conduit system through a first conduit means axially communicating with the bottom end of the hollow axle-bearing system for introducing a thermostating liquid thereto, second conduit means provide communication between said hollow axle-bearing and the hollow cuvette wheel assembly for introducing said thermostating liquid thereto, third conduit means provide communication between said hollow cuvette wheel and said hollow axle-bearing system for removing said thermostating liquid from said cuvette wheel and fourth conduit means axially communicating with the top end of the hollow axle-bearing system for removing said thermostating liquid therefrom back to the said standard recirculating liquid thermostating means.

14. The apparatus of claim 1, wherein said apparatus is utilized for standard clinical enzyme reactions.

15. A method for making automated kinetic measurements comprising placing a plurality of samples (S) and blanks (B) to be analyzed in a cuvette wheel, passing said samples and blanks in a circular path sequentially through the light path of a spectrophotometer, measuring the transmittance readings for each S vs B as they pass through said light path and generating a plurality of (S-B) differential readings for the first revolution of the cuvette wheel, storing the (S-B) differential readings, generating a plurality of (S-B) differential readings for the second revolution of the cuvette wheel and generating double differential readings $\Delta(S-B)$ through the sequential release of said previously stored (S-B) differential readings from said first revolution, converting the $\Delta(S-B)$ differential readings to a digital concentration printout of multiple analytical reaction rate results, using the (S-B) differential readings from the second revolution of the cuvette wheel with the (S-B) differential readings from the third revolution of the cuvette wheel to generate new double differential readings and subsequent digital concentration printouts, and continuing this procedure until the desired number of analytical measurements have been made.

16. The method of claim 15, wherein said method is utilized for standard clinical enzyme reactions.

17. The method of claim 15 wherein the signals for each of the samples and blanks are recorded when the cuvette is rotated into proper alignment with the optical axis of the spectrophotometer as indicated by the momentary completion of an electrical circuit for each of said samples and blanks in synchronism with the alignment of said samples and blanks with the optical axis.

18. The method of claim 15, wherein the signals received from the detector for each of the samples and blanks are recorded at constant time intervals, ΔT .

19. The method of claim 15, wherein the signals for each of the samples and blanks are recorded when the cuvette is rotated into proper alignment with the optical axis of the spectrophotometer as indicated by a maximum signal received from the detector.

20. A method for making automated kinetic measurements comprising placing a plurality of samples (S) and blanks (B) to be analyzed in a cuvette wheel, passing said samples and blanks in a circular path sequentially through the light path of a spectrophotometer, measuring the transmittance readings for each S vs B as they pass through said light path and generating a

plurality of (S-B) differential readings for the first revolution of the cuvette wheel, storing the (S-B) differential readings, generating a plurality of (S-B) differential readings for the second revolution of the cuvette wheel and generating double differential readings $\Delta(S-B)$ 5 through the sequential release of said previously stored (S-B) differential readings from said first revolution, converting the $\Delta(S-B)$ differential readings to a digital concentration printout of multiple analytical reaction

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rate results, using the (S-B) differential readings from the third revolution of the cuvette wheel with the (S-B) differential readings from the fourth revolution of the cuvette wheel to generate a second set of double differential readings and subsequent digital concentration printouts, and continuing this procedure until the desired number of analytical measurements have been made.

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