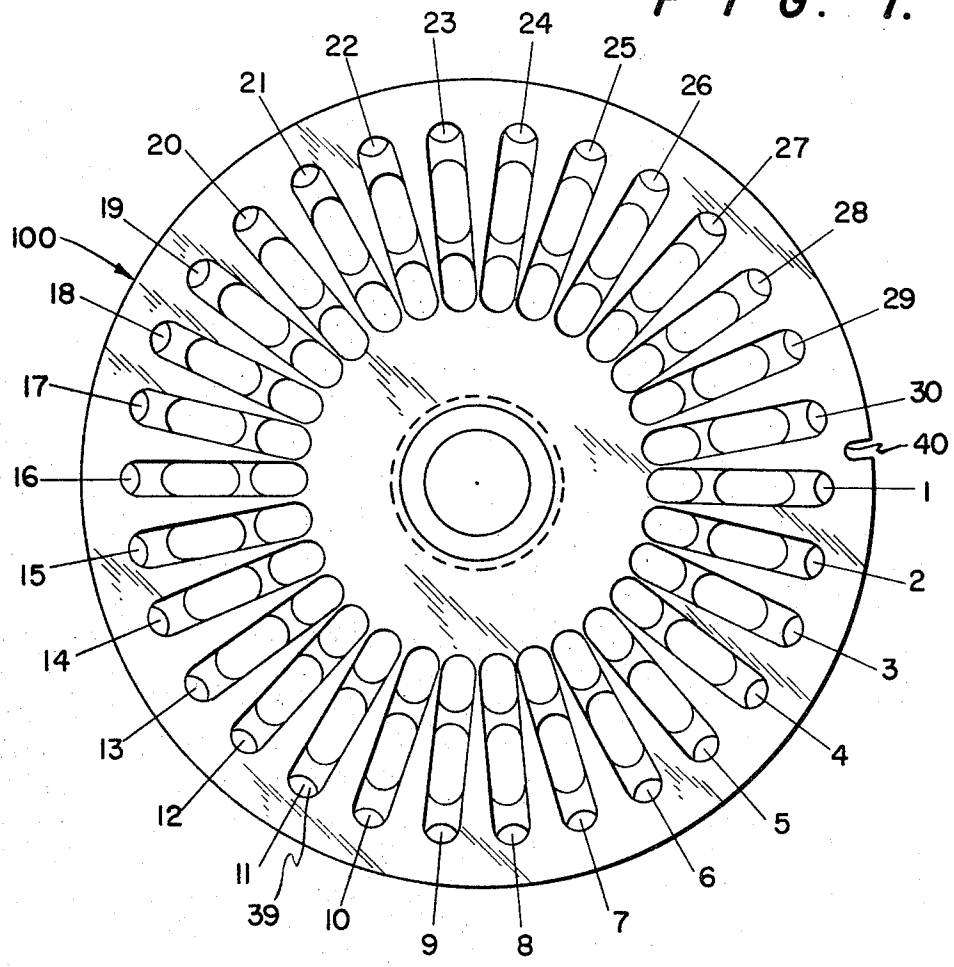
Sept. 18, 1973 H. N. HILL. JR 3,759,666

ANALYTICAL PROCESS

Filed Dec. 9, 1971 2 Sheets-Sheet 1

FIG. 1.



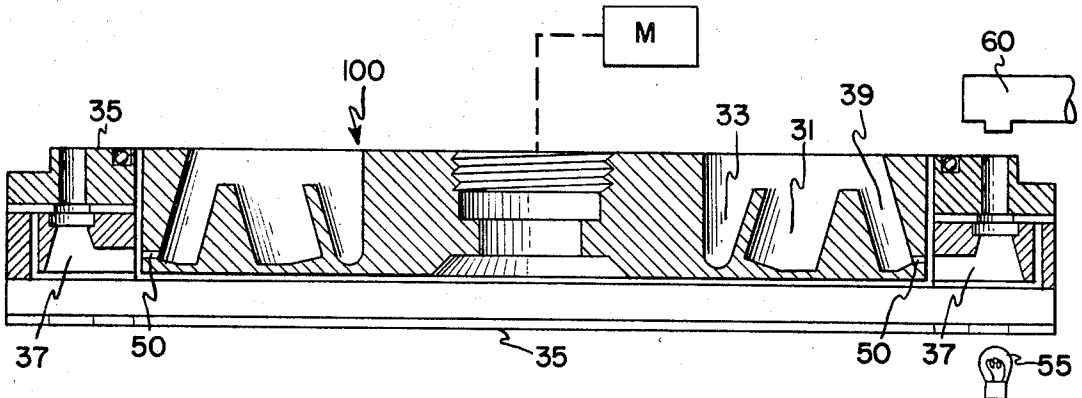


FIG. 2.

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ANALYTICAL PROCESS

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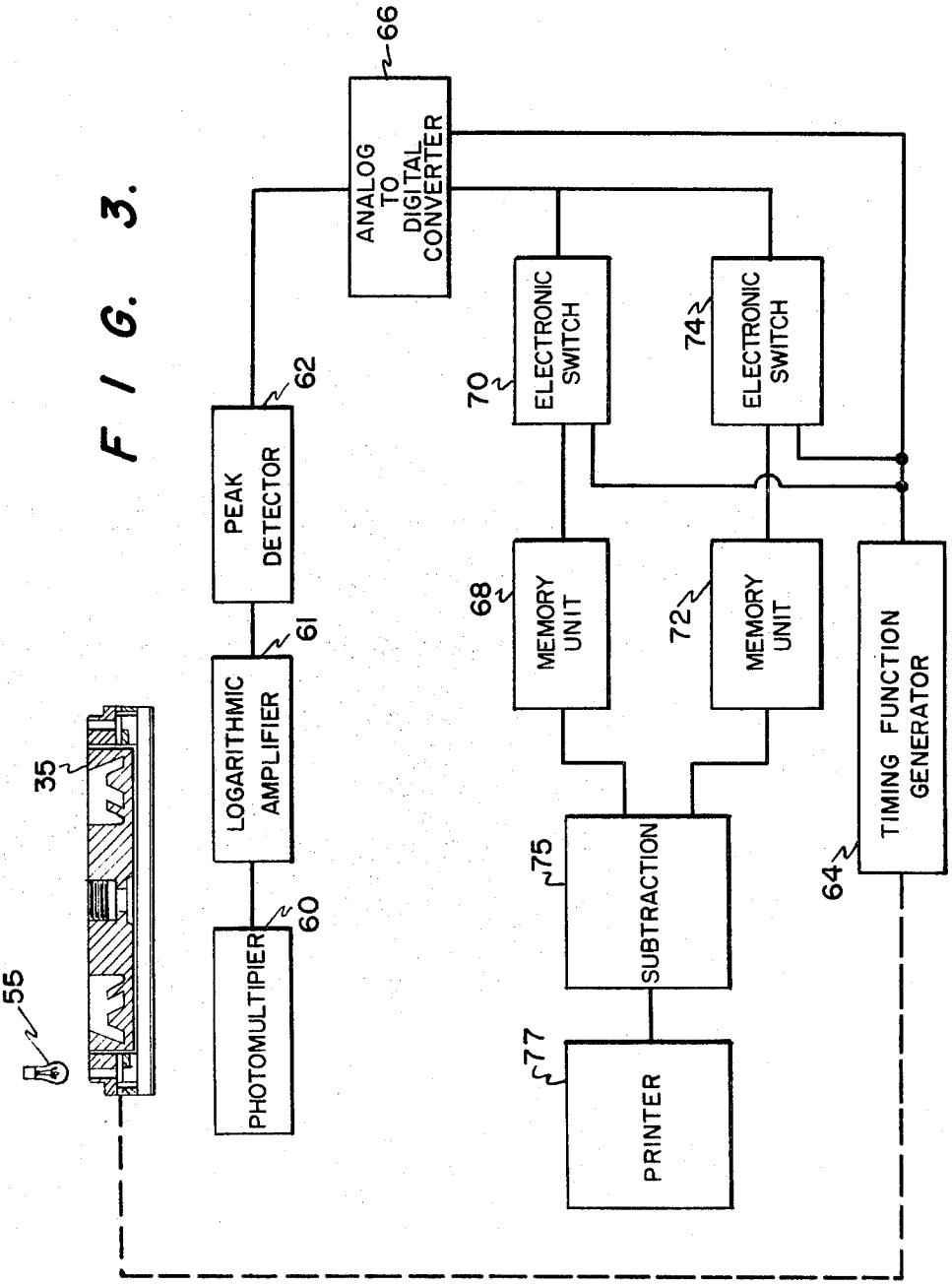
FIG. 3.



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ANALYTICAL PROCESS

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U.S. Cl. 23—230 B 1 Claim

ABSTRACT OF THE DISCLOSURE

Known rotating spectrophotometers have a series of cuvets arranged concentrically around a horizontally rotatable disc so that when the disc is rotated, centrifugal force mixes and transfers reagents and samples to the cuvets. As each cuvet passes a light source, the absorbance of each individual sample is detected and measured photometrically and an electrical signal provided which is indicative of the extent of reaction between reagent and sample. To obtain an accurate measurement of the extent of reaction a reference electrical signal is required. This is provided in the present invention by rotating the cuvet containing disc at a speed such that an initial electrical signal is obtained before there is any substantial reaction between reagent and sample in the cuvets.

This invention relates to a method for rapidly operating a spectrophotometer adapted to analyze the presence of a substance in a plurality of samples which are rotating in a centrifugal field.

In recent years, the need for rapid, automatic analytical devices has increased markedly, owing to the numerous microanalytical studies in biochemical research, routine clinical testing for physicians and hospitals, enzymatic studies, and the like. However, few devices are available which can analyze sufficiently rapidly and accurately to handle the increasing number of varied tests desired by clinicians and research workers.

Recently, multistation analytical photometers which utilize a centrifugal field have become available for the rapid microanalysis of a wide variety of liquids such as blood serum and other body fluids, food products, and the like. Since numerous analyses can be performed rapidly and simultaneously, these devices are of particular interest wherein a large number of samples is involved or a variety of tests on one sample is desired. Moreover, since these devices allow the use of relatively small volumes of reagents, the use of expensive reagents can be minimized.

One such device which utilizes a centrifugal field in microanalytical studies is described in "Analytical Biochemistry," 28, 545–562 (1969). This device employs the principle of double-beam spectrophotometry wherein absorbancies of a liquid sample and a reference solution are intercompared. The system is basically a series of cuvets arranged around the periphery of a rotor so that when it is spun, centrifugal force simultaneously mixes and transfers reagents and samples to the cuvets where an analysis is made spectrophotometrically. A sample loading disc is provided which contains rows of cavities arranged concentrically. In the afore-mentioned device serum samples to be analyzed are placed in the inner cavities, and the reagents are placed in cavities at a greater radial distance than those containing the serum samples. The sample loading disc is then indexed and positioned in the rotor and as the rotor is accelerated, centrifugal force moves the sample to the cavity containing the reagent, where they are mixed and the mixture of reagent and sample is then moved through a communicating passage into the cuvet. The filled cuvets rapidly spin past a fixed light beam, and the transmission of light through the cuvets, i.e., through the samples, is measured.

In the past, when a variety of tests was to be performed,

a reagent "blank," was provided for each sample-serum mixture to establish a zero absorbancy value for comparison with color changes during serum-reagent reaction whereby the extent of reaction between serum and reagent was established. This required that serum samples be placed in alternate cavities only, thus limiting the number of tests that could be performed at one time and the speed with which such tests could be made.

It is therefore an object of the present invention to provide a method for increasing the testing capability of rotating spectrophotometers employed for analytical testing.

Other objects will be apparent from the following description and claim taken in conjunction with the drawing wherein

FIG. 1 shows a plan view of the rotatable disc of a spectrophotometer used in analytical testing

FIG. 2 is an elevation view of the device of FIG. 1 also showing a light source, photodetection means, cuvet assembly and motor drive used in connection with the device of FIG. 1, and FIG. 3 shows schematically an electrical arrangement used in conjunction with the device of FIGS. 1 and 2.

The present invention will be more fully understood by reference to the drawing. With reference to FIGS. 1 and 2 there is shown a rotatable loading disc **100** of a spectrophotometer of the type previously described which comprises thirty rows of cavities numbered **1** to **30** with each row having a serum cavity **33** and a reagent cavity **31**. A slot to enable indexing of the loading disc is shown at **40**. In the past, standard operating procedure was to place reagent in the cavities **31** in alternate positions e.g. **1, 3, 5** etc. through **29** with the adjacent radially aligned serum cavities **33** being left empty. Both serum and reagent were placed in their respective cavities **33** and **31** in positions **2, 4, 6, 8** etc. through **30**. Thus a reagent "blank" was provided for each analysis to be performed in positions **1, 3, 5** etc. through **20**. With the sample-reagent containing disc indexed and positioned in rotor assembly **35** each row of cavities was aligned with a respective cuvette **37** and as the rotor assembly **35** was driven, centrifugal force moved the contents of the serum and reagents to the outermost cavities **39**. Serum and reagents from positions **2, 4, 6, 8** etc. were mixed and transferred from cavity **39** through channels **50** to their respective cuvettes **37**. The cuvettes communicating with the cavities of positions **1, 3, 5** etc. were filled only with reagent while the cuvettes **37** communicating with the cavities of positions **2, 4, 6,** etc. were filled with a mixture of serum and reagent. The filled cuvettes **37** rotated between light source **55** and photomultiplier **60**. The cuvettes **37** for positions **1, 3, 5** etc. contained only reagent, and photomultiplier **60** therefore provided a value which could be designated as zero absorbance i.e. a "blank." The signals provided by the photomultiplier for the other positions **2, 4, 6** etc. could be compared to its corresponding "blank" thereby indicating any color changes due to reaction between reagent and serum.

Based on the observation that serum-reagent reactions require a finite period of time to exhibit a significant color change, i.e. a change in absorbance, a method is provided in the present invention whereby all positions **1** through **30** are provided with both serum and reagent in the respectve cavities **33** and **31**. The disc **100** is then rotated very rapidly e.g. 1000 r.p.m. whereby all cuvettes are passed between light source **55** and photomultiplier **60** in a very short period of time e.g. less than $\frac{1}{10}$ of a second. Thus, the signals provided by photomultiplier **60** during the initial rotation correspond to a "blank" since no significant color change due to serum-reagent reaction occurs in this interval. The electrical current signals from photomultiplier **60**, with reference to FIG. 3, are applied to a

conventional logarithmic amplifier **61**. These signals are in the form of pulses of electrical current due to the "chopping" effect of the rotor assembly **35**. A logarithmic amplifier is desirable due to the inherent logarithmic character of the absorbance phenomenon of serum-reagent reactions. In amplifier **61** the pulses of electrical current generated by photo-multiplier **60** are converted to pulses of voltage having a logarithmic relationship to the current pulses. The voltage pulses from logarithmic amplifier **60** are applied to a conventional peak detector **62** which can be a Peak Detector Module 4084/25 available from the Burr-Brown Research Corporation. The peak detector **62** observes the peak value of each pulse applied and holds this value until re-set by timing function generator **64** which is synchronized with and coupled to rotor assembly **35**. Thus, during the initial rotation of rotor assembly **35**, peak signals are applied to peak detector **62** for each of the cuvet positions **1** through **30**, with the timing function generator **64** re-setting the peak detector **62** after each pulse is received. Each peak signal received by peak detector **62** is applied to analog-to-digital converter **66** which generates a group of pulses, using the conventional binary number system, having a value corresponding to the peak observed by the peak detector **62**. The analog-to-digital converter can be a Teledyne Philbrick Nexus model 4103. The pulse groups produced by analog-to-digital converter **66** during the initial rotation of rotor assembly **35** are stored and held in memory unit **68** via electronic switch **70** which is actuated at the appropriate time by timing function generator **64**. These pulse groups represent "blanks" since no significant reaction occurs between serum and reagent during the initial revolution of rotor assembly **35**. The pulse groups produced by analog-to-digital converter **66** for subsequent revolutions of rotor assembly **35**, at pre-selected times, are applied to memory unit **72** via electronic switch **74** which is actuated at the appropriate time by timing function generator **64**.

When pulse groups are stored in memory unit **72**, the contents of memory units **68** and **72** are subtracted in subtraction unit **75** and the resulting data is indicated at print-out unit **77**, for example, a Moduprint Mode A available from Practical Automation Inc. This data is a measure of the color change or absorbance change in the cuvettes **37** of rotor assembly **35** and hence the extent of reaction between serum and reagent in the cuvettes.

By way of further illustration, a frequently performed analytical test is the determination of glucose in blood serum. In this analysis, 5 microliters of serum is placed in serum cavities **33** and 350 microliters of glucose reagent is placed in the reagent cavities **31** of sample disc **100**. The glucose reagent is a 0.3 molar triethanolamine buffer of pH 7.5 containing 0.0004 mol/liter NADP, 0.0005 mol/liter ATP, 70 mg./liter hexokinase, 140 mg./liter glucose-6-phosphate dehydrogenase and 0.004 mol/liter $MgSO_4$. The combined action of ATP (adenosine triphosphate) and NADP (nicotineamide adenine dinucleotide phosphate) in the presence of the enzymes hexokinase and glucose 6 phosphate dehydrogenase leads to the reduction of NADP which is followed spectrophotometrically by detecting changes in absorbance at a wavelength of 340 nm. An initial "blank" reading for this situation can be taken as much as two seconds after initiating the revolution of the rotor assembly **35** and stored in memory unit **68** to provide a "blank." After a period of ten minutes a final reading is taken and stored in memory unit **72**. The "blank" is subtracted from the value in memory unit **72** and the measured change is proportional to the change in absorbance due to the extent of oxidation of glucose which is concurrent with the reduction of NADP.

What is claimed is:

1. A method for determining the progress of a chemical reaction between serum samples and reagent which comprises

(1) providing (a) a light source (b) a photodetector means spaced from and arranged in juxtaposition therewith, said photodetector means providing an electrical signal proportional to the optical density of the media by which it is separated from said light source and (c) a rotatably movable rotor assembly arranged to rotate between said light source and said photodetector means having (i) a plurality of light transmitting sample analysis chambers located at a common radial position in said rotor assembly and (ii) a reagent containing chamber and a serum containing chamber radially aligned with each of said sample analysis chambers whereby upon rotation of the rotor assembly the contents of the reagent and serum chambers are caused to mix and pass into the radially aligned sample analysis chambers

(2) rotating said rotor assembly at a speed such that mixtures of serum and reagent enter the sample analysis chamber before any substantial reaction occurs therebetween

(3) applying the electrical signal provided by said photodetector means at a time before any substantial reaction between reagent and serum occurs to a device adapted to store said electrical signal

(4) applying an electrical signal provided by said photodetector means at a time after substantial reaction has occurred between reagent and serum to a device adapted to store said electrical signal

(5) providing an electrical signal proportional to the difference between the two aforesaid electrical signals as a measure of the extent of reaction between serum and reagent.

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23—230 R, 253 R, 259; 233—26; 250—218; 356—39