United States Patent

[11] **3,555,284**

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[73] Assignee **To the United States of America as represented by the United States Atomic Energy Commission**

[54] **MULTISTATION, SINGLE CHANNEL ANALYTICAL PHOTOMETER AND METHOD OF USE**

10 Claims, 7 Drawing Figs.

[52] **U.S. Cl.** **250/218,** 356/197, 356/246; 250/223; 23/252
[51] **Int. Cl.** **G01n 21/26,** G01n 1/10
[50] **Field of Search** ..... 250/218, 223B, 215, 224; 356/203, 240, 196, 197, 180, 244—246, 181, 183—185; 209/120; 264/310, 311; 23/252, 253

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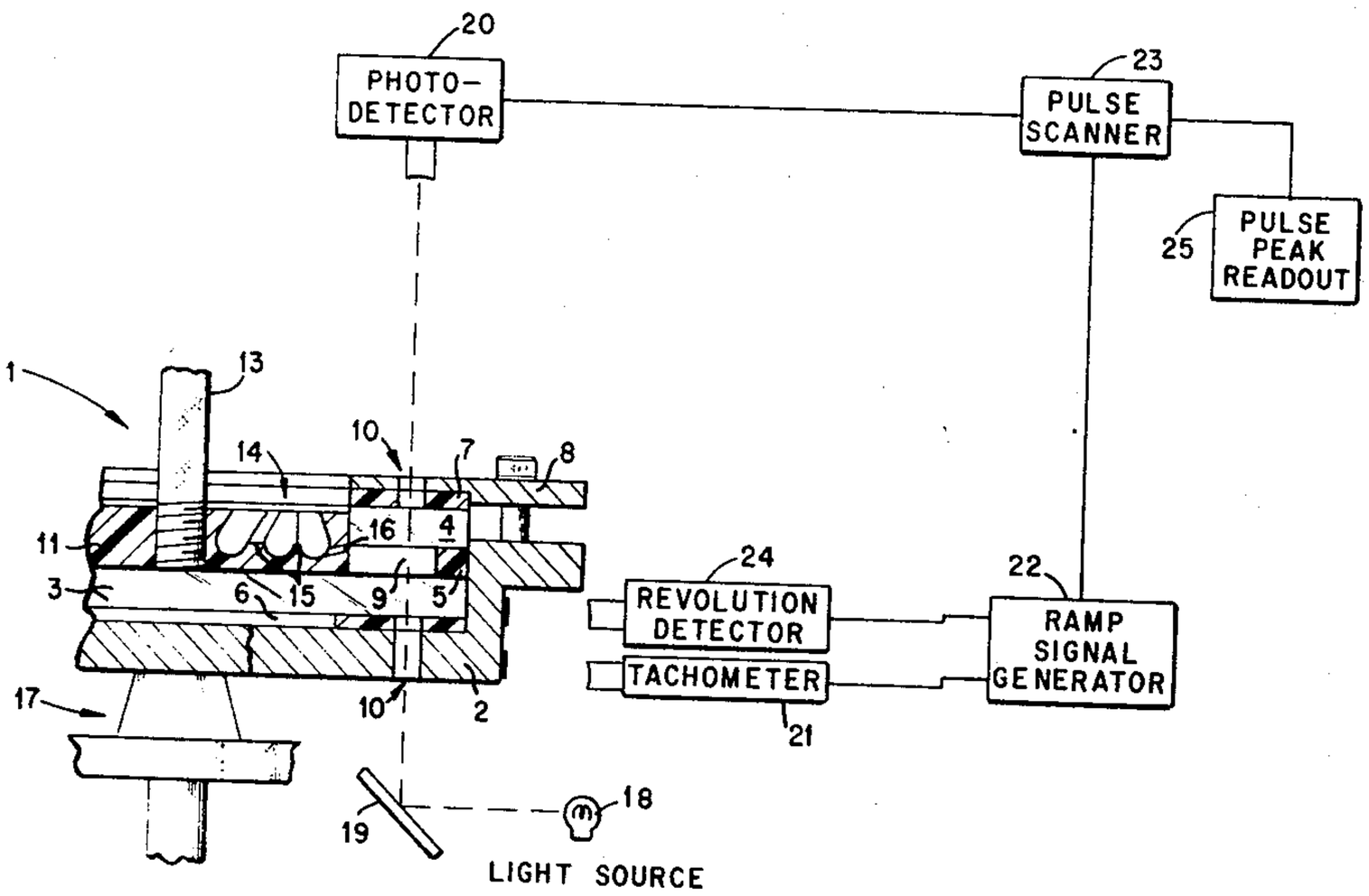
ABSTRACT: An analytical photometer is provided for simultaneously determining the presence of a common substance in a multiplicity of discrete samples. A multiplicity of sample chambers with axially aligned transparent windows are arranged within a centrifuge rotor to provide a rotary cuvette system. Solution handling systems comprising sets of interconnected, solution-accepting chambers are disposed generally in radial alignment with the sample chambers forming the cuvette system. The solution-accepting chambers of the solution handling systems are shaped and sized to retain liquid when the rotor is at rest, and to release the liquid to the cuvettes when the rotor is spinning. A single light source and a photodetecting unit are aligned with the windows to determine chemical species concentrations by light absorbancy in the samples contained in the cuvettes. Means for receiving the output from the photodetecting unit are provided for individually indicating the phototransmittance of the samples within each cuvette.



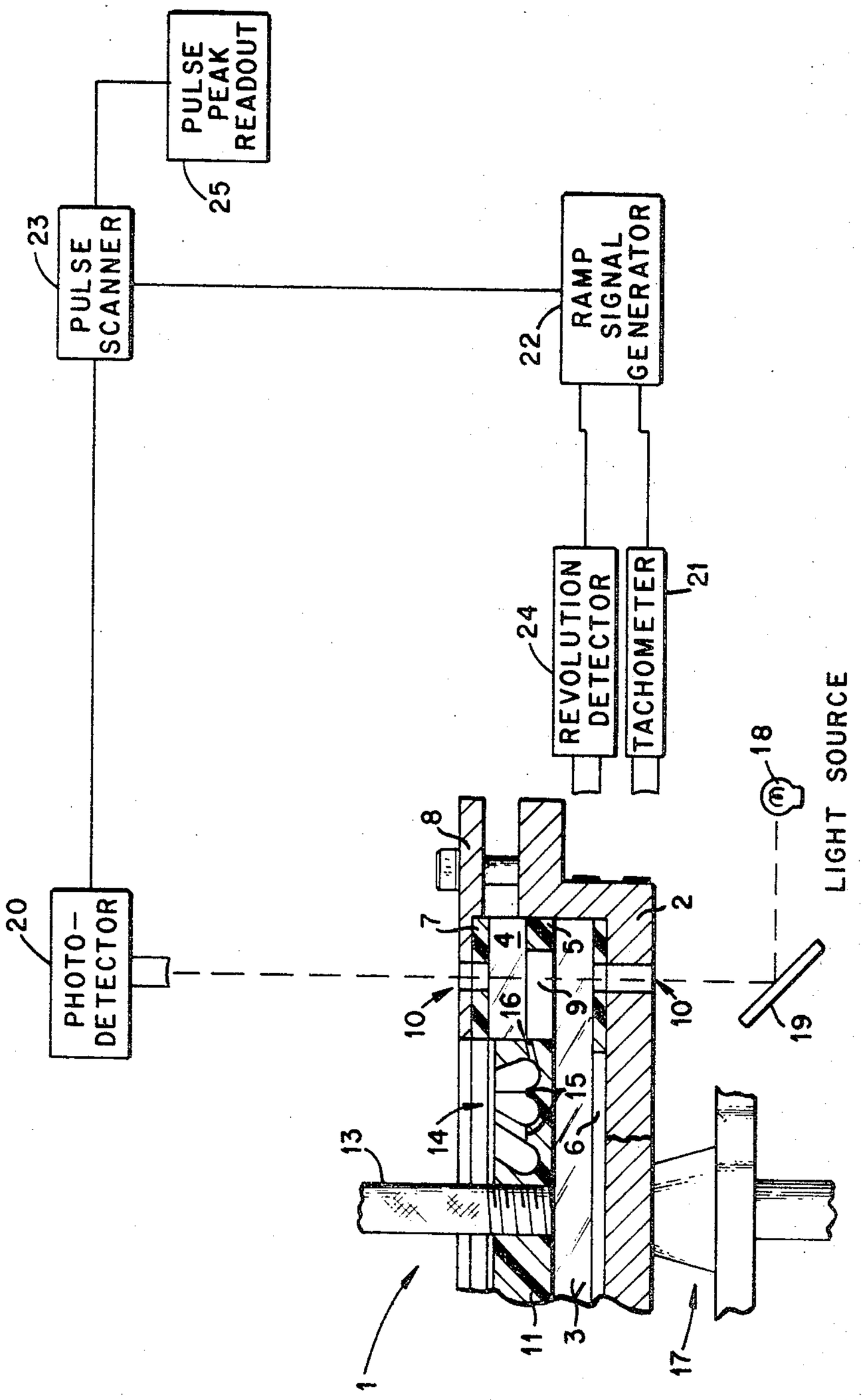


Fig. 1

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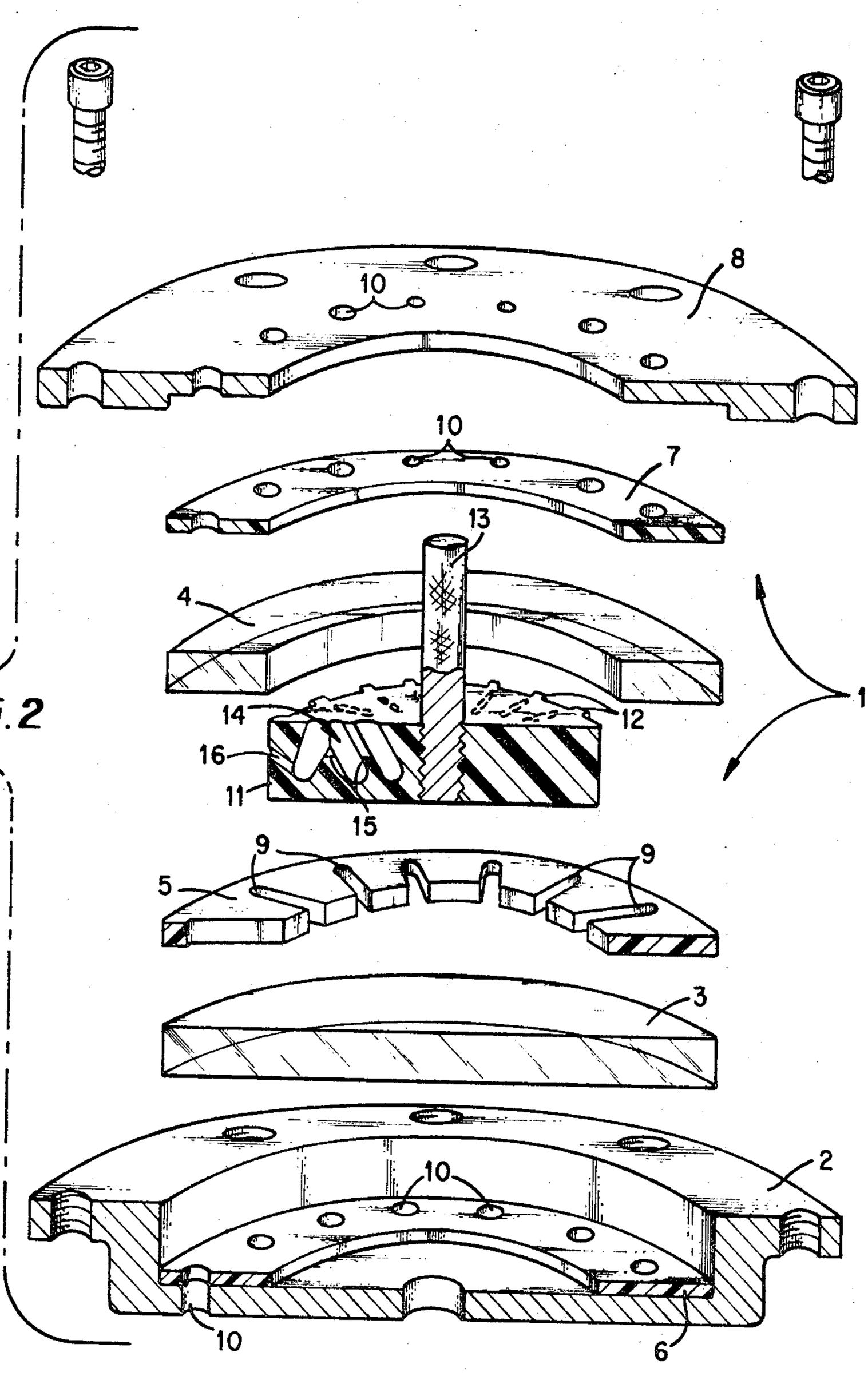

Fig. 2

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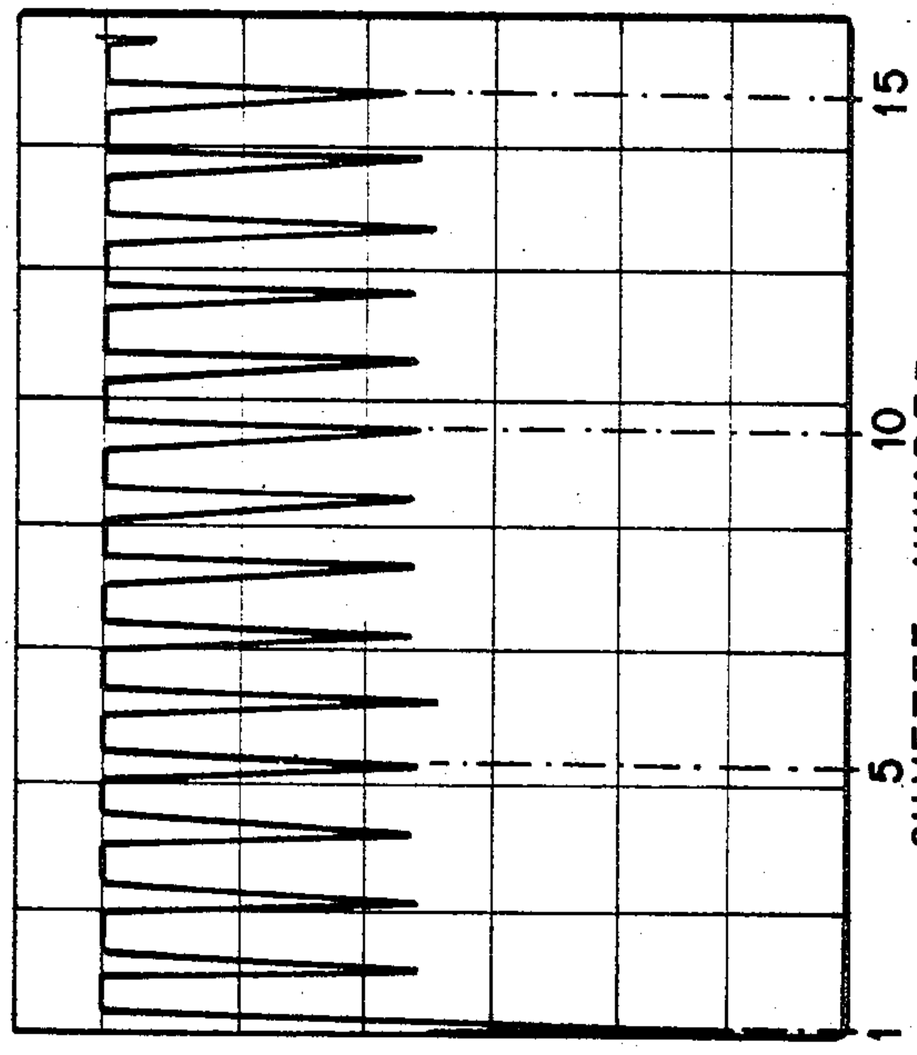

Fig. 4

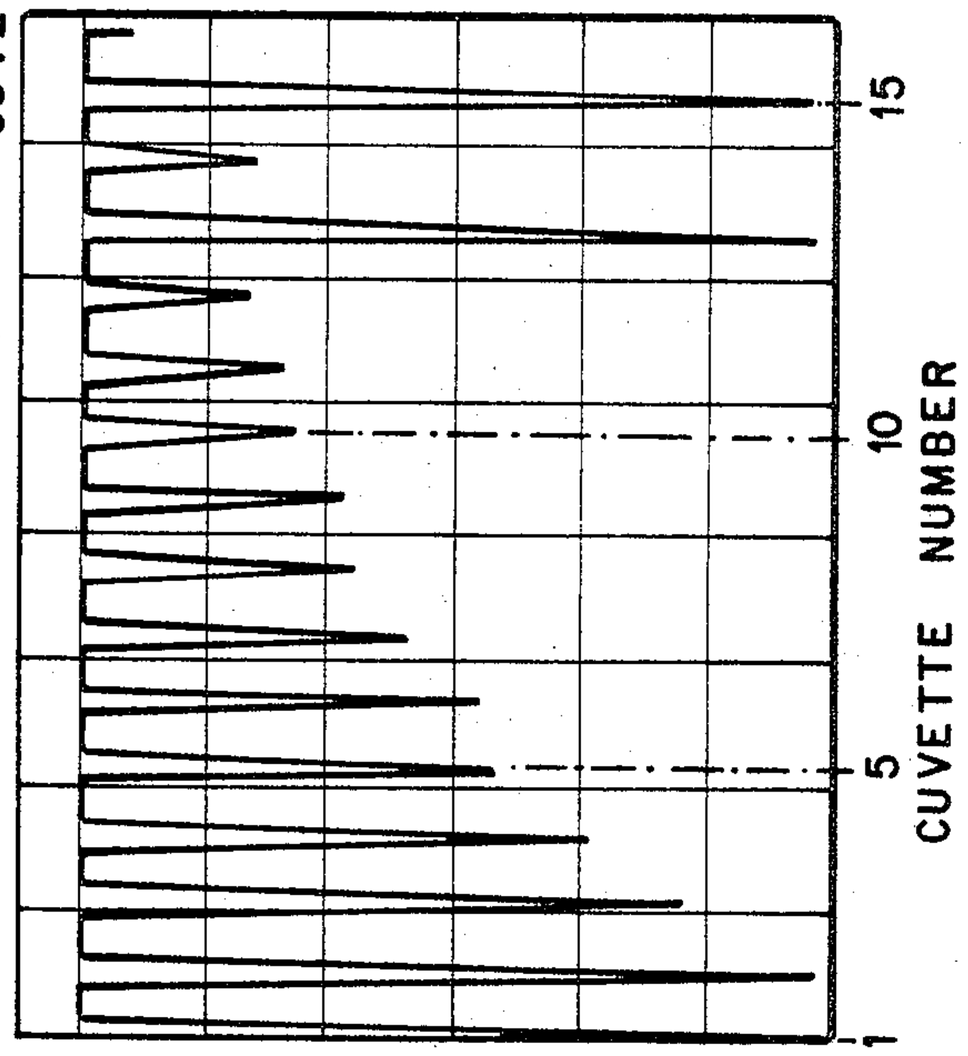

Fig. 5

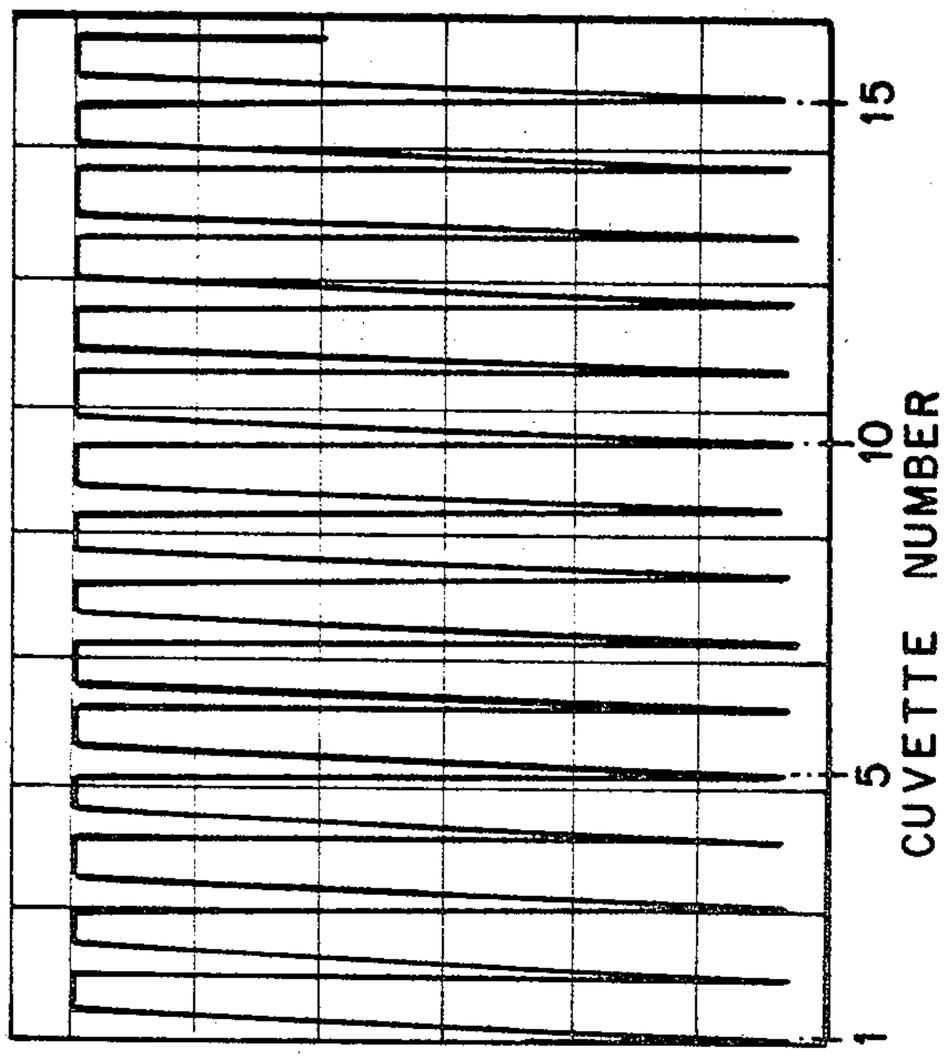

Fig. 3

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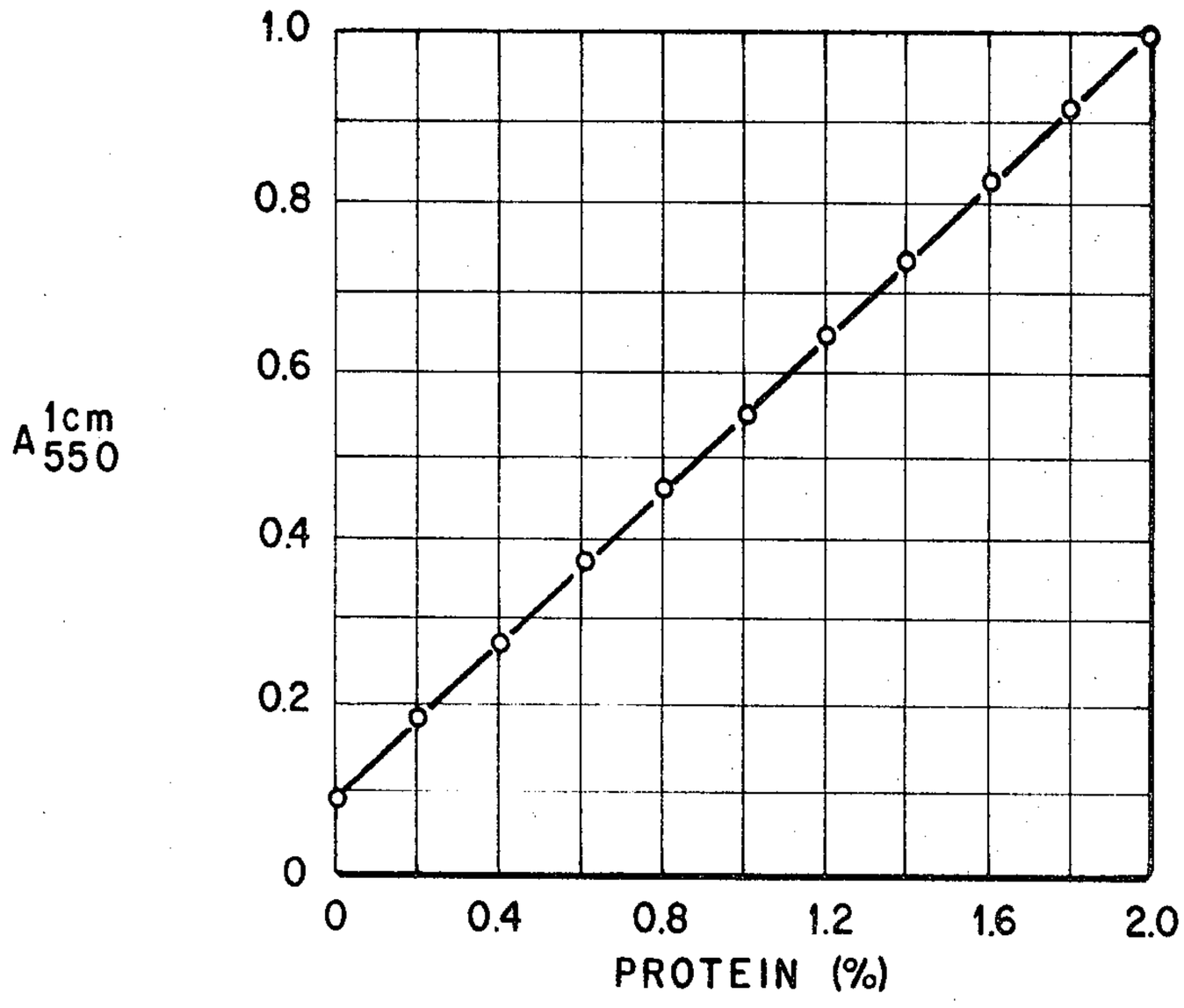

Fig. 6

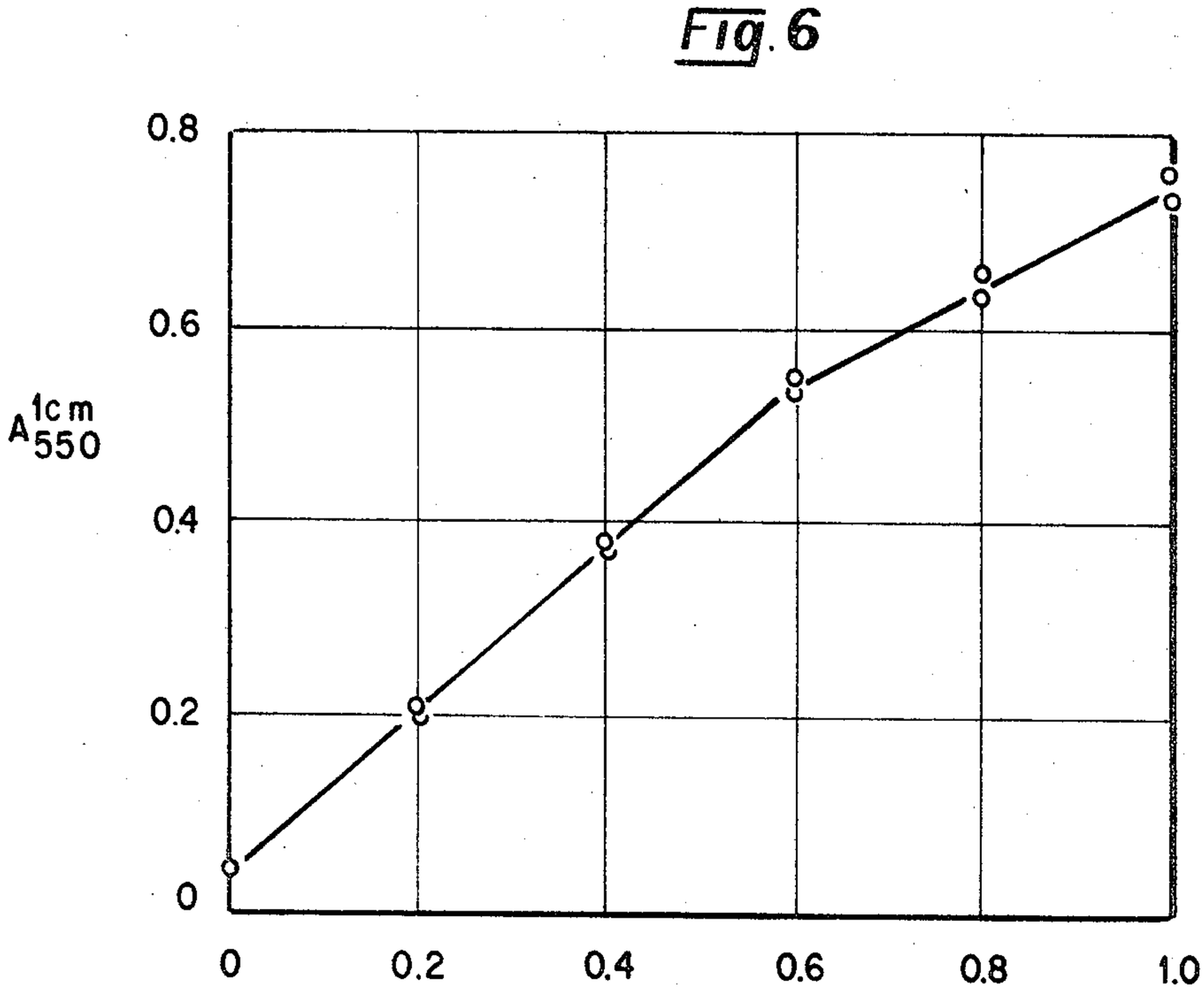

Fig. 7

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MULTISTATION, SINGLE CHANNEL ANALYTICAL PHOTOMETER AND METHOD OF USE

BACKGROUND OF THE INVENTION

The invention described herein relates generally to photometers and more particularly to a photometer for simultaneously determining the presence of a common substance in a multiplicity of discrete samples. It was made in the course of, or under, a contract with the U. S. Atomic Energy Commission.

The term "photometric" as used herein should not be considered in a restrictive sense as it is intended to be generic to the terms "colorimetric", "fluorometric" and "spectrometric". Consistent with such usage, the term "photometer" is also used in a broad sense to include those devices sometimes referred to in the art as "colorimeters", "fluorometers" and "-spectrometers". The term "light" as used herein includes radiant energy in both the visible and invisible spectrums as well as radiant energy restricted to specific wave lengths. Thus the invention should be understood to encompass systems which utilize different types of radiation to accomplish the measurement desired. The need for a photometric system capable of performing analyses on a large number of discrete samples simultaneously has long existed in clinical and analytical laboratories. Qualitative and quantitative measurements of metabolites, hormones, vitamins, enzymes, minerals, body waste products, bile constituents and gastric contents are made daily in great numbers in such laboratories in the diagnosis of disease as well as for research purposes. A system which can perform measurements of this type rapidly, accurately and cheaply will effect large manpower and cost savings while providing improved results. Most prior art instruments are capable of performing analyses only in sequence, rather than simultaneously. Not only does sequential analysis limit the analytical production, but in the case of analyzing very small samples, the analytical results are usually unreliable.

Another deficiency common in prior art, discrete-sample analyzers is the requirement that samples for photometric analysis be prepared in many time consuming steps in several entirely separate machines. Such an arrangement further limits analytical production by causing it to be even more time consuming and expensive.

Still another deficiency in many prior art photometric instruments is that volumes of samples, enzymes and other expensive reagents larger than desirable are required. This deficiency is in some cases the result of continuous flow monitoring systems which are inefficient when small numbers of samples are analyzed. A further deficiency is the undesirability of handling many small, discrete volumes of samples and reagents individually and mixing them at timed intervals.

It is, accordingly, a general object of the invention to provide a photometric system capable of performing analyses on a large number of discrete samples simultaneously.

Another object of the invention is to provide a photometric system wherein the steps of volumetric measurement, liquid transfer, solution mixing, reaction, photometric measurement, and data reduction may be performed within a single system.

Other objects of the invention will be apparent from an examination of the following description of the invention and the appended drawings.

SUMMARY OF THE INVENTION

In accordance with the invention, a photometer for simultaneously determining the presence of a common substance in a multiplicity of discrete samples is provided. A multiplicity of sample chambers with axially aligned transparent windows are arranged within a centrifuge rotor to provide a rotary cuvette system. Solution handling systems comprising sets of interconnected, solution-accepting chambers are disposed generally in radial alignment with the sample chambers forming the cuvette system. The solution-accepting chambers of the solution handling systems are shaped and sized to retain liquid when the rotor is at rest, and to release the liquid to the cuvettes when the rotor is spinning. A single light source and photodetecting unit are aligned with the windows to determine chemical species concentrations by light absorbency in the samples contained in the cuvettes. Means receiving the output from the photodetecting unit are provided for indicating the phototransmittance of the samples within each cuvette. Thus a system is provided wherein a multiplicity of samples may be tested simultaneously and wherein volume measurement, mixing, liquid transfer and data reduction are performed by a single system.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 schematically illustrates a photometric system designed in accordance with the present invention.

FIG. 2 is an exploded perspective view, in section of the rotor used in the system illustrated in FIG. 1.

FIG. 3 is an oscillogram obtained with the system of FIG. 1 using a 660 μ filter and distilled water in all cuvettes.

FIG. 4 is an oscillogram obtained with the system of FIG. 1 using a 660 μ filter wherein a uniform solution containing water, bovine serum albumen and bromphenol blue was introduced into cuvettes numbered 2—15 during rotation.

FIG. 5 is an oscillogram obtained with the system of FIG. 1 using a 550 μ filter and a series of incremented standards in cuvettes numbered 3—12.

FIG. 6 is a plot of the data obtained from FIG. 5.

FIG. 7 is a plot of absorbencies against protein concentration obtained using the system of FIG. 1 in performing the experiment described in Example II.

DESCRIPTION OF THE PREFERRED EMBODIMENT

FIG. 1 schematically illustrates an analyzer made in accordance with the invention. A pancake-shaped rotor assembly 1, illustrated in greater detail in the exploded view of FIG. 2 where like numerals are used to denote like parts, comprises a bolt flanged steel rotor body 2, glass rings 3 and 4, a slotted polytetrafluorethylene cuvette ring 5, polytetrafluorethylene retaining rings 6 and 7, and a steel bolted flange ring 8. Rings 3, 4, 5, 6 and 7 are compressed between rotor body 2 and flange ring 8 to form a multiplicity of radially oriented cuvettes 9 in slotted cuvette ring 5. Spaced holes 10, axially aligned with cuvettes 9, are provided in rotor body 2, retaining rings 6 and 7, and flange ring 8 so as to provide axially extending passageways permitting passage of a light beam through the cuvettes. A centrally positioned removable transfer disc 11 is provided with small radial projections 12 which are spaced about its periphery to mate with cuvettes in ring 5. Handle 13 is provided to facilitate removal of transfer disc 11 from the rotor assembly. Transfer disc 11 is provided with a set of chambers 14 corresponding to each cuvette 9 for receiving sample liquids and reactants while the rotor is at rest. Chambers 14 comprise a plurality of sloping cylindrical cavities which are interconnected at their upper ends and separated by partitions 15 at their lower ends. Partitions 15 prevent mixing of the sample and reactant liquids when the rotor is at rest while permitting such liquids to pass to cuvettes 9 when the rotor is spinning. A passageway 16 extends from the radially outermost cavity of each chamber 14 to the periphery of a corresponding radial projection 12 to permit passage of liquid from each chamber to a corresponding cuvette when the rotor is rotated. A drive motor 17 supports rotor assembly 1 as well as rotating it.

A photometric light source and projecting means is provided to project a light beam of constant intensity intersecting rotor assembly 1 at a point corresponding to the radial positions of cuvettes 9 and spaced holes 10. The light beam is aligned in such a manner so as to be transmitted through each hole 10 and cuvette 9 as they pass through the beam. The photometric light source comprises an incandescent lamp 18 with a reflecting mirror 19 disposed below the rotor assembly and oriented to reflect the light beam upward, substantially normal to the plane of rotation.

Electronic photodetecting means **20** is disposed above rotor assembly **1** and aligned to receive light transmitted through the cuvettes during rotation. Photodetecting means **20** is designed to respond electronically with an output which is proportional to the intensity of the light transmitted from light source **18** through the cuvettes. Photodetector **20** comprises a photomultiplier tube disposed directly above the cuvette circle to receive all light transmitted upwardly through the axially aligned openings.

The remaining electronic components illustrated schematically in FIG. **1** include a proportional tachometer **21** which supplies a voltage signal proportional to the rotor speed to a ramp signal generator **22** which, in turn, provides a signal to a pulse scanner **23**. A revolution detector **24** synchronizes the ramp signal frequency with the rotor speed. Pulse scanning means **23**, synchronizable by the ramp signal generator frequency, responds proportionately to pulses originating in photodetecting means **20** and sorts the pulses therefrom as to origin. Pulse peak readout means **25** continuously and simultaneously indicates phototransmittance of the liquid contents in each cuvette. Electronic components **21** through **25** are described in greater detail in corresponding U.S. Pat. No. 3,514,613 issued May 26, 1970, to common assignee.

In operation, samples and reagents are initially inserted in chamber **14** while the rotor assembly **1** is at rest and then moved centrifugally into corresponding cuvettes **9** by spinning the rotor. Since the transfer into cuvettes **9** occurs during a relatively short period of time as the rotor accelerates. All reactions in the cuvettes start essentially simultaneously and may be followed continuously on an oscilloscope or other readout means **25**. By providing three cavities within each chamber **14** in the transfer disc **11**, a sample and two reagents may be loaded without mixing while the rotor is at rest and then, by spinning the rotor, caused to drain centrifugally into a corresponding cuvette where they are mixed. Connections to the cuvettes are by small passageways through projections **12** as illustrated in FIGS. **1** and **2**. The transfer disc **11** may be adapted to hold transfer tubes as described in copending application S. N. 756,265 of common assignee, or small, commercially available, disposable microliter pipettes. Such devices allow single or multiple addition reactions to be used or reactions in which a reaction time occurs between two additions. In reactions that produce precipitates, the suspended solids can be moved out of the optical path by centrifugal force, allowing the absorbencies of a clear supernatant to be measured.

In the rotor described, the radial orientation of the cuvettes causes a difference in tangential velocity to exist between the radially innermost end of each cuvette and its radially outermost end. Rapid acceleration and deceleration of the rotor during transfer of liquid into the cuvettes cause circular flow of the liquid therein and enhance mixing. Such mixing is considered desirable as it aids the reaction between sample and reagent and provides more uniform results. In practice the rotor is accelerated rapidly to transfer fluid to the cuvettes, decelerated rapidly to facilitate mixing, and then reaccelerated to the speed desired for testing.

EXAMPLE I

To determine whether reproducible curves could be obtained with standard solutions using apparatus as described above, a solution containing 1.5 g. of crystalline bovine serum albumen (BSA) and 15 mg. of bromphenol blue (BPB) in 100 ml. of water was diluted with distilled water to give a series of solutions containing 10 percent increments of stock solution. FIG. **4** is an oscillogram showing the pattern observed using a 660 μ filter and distilled water in all cuvettes. The oscillogram was obtained from an oscilloscope which provided the pulse peak readout means **25** described in an earlier reference to FIG. **1**. The oscillogram of FIG. **5** was attained by introducing a solution containing water and the BSA-BPB solution in a 1:1 volume ratio into the cuvettes numbered **2** through **15** during rotation. The differences in peak height, though small, agreed with those observed by direct measurement. The oscillogram of FIG. **6** was obtained by providing a complete series of incremented standards in cuvettes numbered **3** through **12**, with a duplication of the solution used in the cuvettes numbered **12** also being used in the cuvette numbered **14**. The four remaining cuvettes contained distilled water only. Measurements were made from photographic enlargements of the patterns observed on the oscilloscope, and all peaks converted to 1/percent T by dividing the first blank by each subsequent reading in turn. The log of 1/T is the absorbence which, after blank subtraction, was then multiplied by the cuvette factor to give absorbency for a one cm. path length. The data obtained in this manner from the oscillogram of FIG. **5** is plotted in FIG. **6**.

EXAMPLE II

A further experiment was performed to demonstrate that the system can be used to follow reactions occurring in the cuvettes. The biuret reaction for protein is a single one-reagent analysis which is of general interest and is suitable for evaluating the efficiency of the transfer discs, of mixing, and of the ability of the system to read absorbencies early in the course of the reactions. The Weichselbaum biuret reagent may be used with protein solutions in a range of ratios varying from 0 to 50 percent reagent in the final mixture, providing that identical solutions are used to obtain the standard curve.

An experiment was run using 200 microliters of reagent and duplicate protein solutions containing 200 microliters of protein solutions containing 0.2, 0.4, 0.6, 0.8, and 1.0 percent protein. These solutions were placed in appropriate chambers in the center disc and transferred to the cuvettes by starting the rotor. Thirty seconds later an oscillogram was obtained in the same manner as in the experiments of Example I and the results plotted in FIG. **7**, using water as the reference standard.

The experimental embodiment of the apparatus used in the examples described above permitted **15** reactions to be initiated simultaneously and the absorbencies of the samples to be observed and measured within very short intervals after the reactions were initiated. A larger number of reactions could be run by using a larger rotor with a correspondingly larger number of cuvettes, or a smaller number by simply using only a portion of the available cuvettes.

Unlike sequential analyzers, no carry over was observed between the samples and the oscilloscope tracing returned to 0 percent transmission between each sample reading. By providing one or more water blanks in each series, readings for the samples, 0, and 100 percent transmission were made during each revolution. At a rotational speed of 1200 r.p.m., 20 revolutions per second occur permitting 20 sets of measurements to be made. Where an exposure time of one second is used, the result represents the average of 20 readings. The time between peaks is ample to allow computer averaging of digitalized peak height.

If small fluid volumes are added to the rotor initially, the rotor may be brought to a complete stop and the sample-reagent disc replaced. In this manner reactions depending upon sequential timed additions may be performed. The centrifugal capabilities of the rotor may also be employed, where desired, to sediment particulate matter or to ensure that the solutions are not turbid when their absorbencies are measured.

The above description of one embodiment of the invention is offered for illustrative purposes only and should not be interpreted in a limiting sense. For example, rotor assemblies **1** may be fabricated with more or less cuvettes than shown or with different materials such as transparent plastics. The centrally positioned transfer disc may also be provided with more or less chambers for receiving sample and reactant liquids and such chambers may vary from the particular shape illustrated. It is intended rather that the invention be limited only by the scope of the appended claims.

I claim:

1. A photometric solution analyzer for the simultaneous determination of a common substance in a multiplicity of discrete samples comprising:

a. a power-driven rotor assembly defining;

1. a multiplicity of sample analysis chambers for accepting liquid samples to be analyzed, said rotor assembly having transparent walls adjacent said sample analysis chambers for permitting the passage of light therethrough, and

2. a multiplicity of chambers adapted to retain liquid samples and reactants when said rotor assembly is at rest, and to release said liquid samples and reactants to said sample analysis chambers when said rotor is rotated;

b. a light source for providing a beam of light incident on said rotor assembly at a point corresponding to the radial position of said sample analysis chambers;

c. means for detecting light from said light source after it has passed through said sample analysis chambers, said means for detecting light generating an output signal proportional to the intensity of light detected; and

d. means receiving the output from said light detecting means for continuously and simultaneously indicating the presence of said common substance within each of said sample analysis chambers.

2. The photometric analyzer of claim 1 wherein said sample analysis chambers comprise a multiplicity of radially oriented elongated cavities disposed in a circular array about the center of rotation of said rotor assembly.

3. The photometric analyzer of claim 2 wherein said sample analysis chambers are fabricated by sandwiching a slotted ring between layers of transparent material.

4. The photometric analyzer of claim 3 wherein said slotted ring is fabricated of polytetrafluorethylene and said layers of transparent material are fabricated of glass.

5. The photometric analyzer of claim 1 wherein said chambers adapted to retain liquid samples and reactants when said rotor assembly is at rest are disposed in a circular array in radial alignment with and spaced radially inward from said sample analysis chambers with respect to the center of rotation of said rotor assembly.

6. The photometric analyzer of claim 1 wherein said means for detecting light comprises a photomultiplier tube.

7. The photometric analyzer of claim 1 wherein said means for continuously and simultaneously indicating the presence of said common substance within each of said sample analysis chambers comprises an oscilloscope.

8. A method for photometrically analyzing a multiplicity of discrete samples to simultaneously determine the presence of a single substance therein, comprising:

a. introducing preselected volumes of liquids necessary to produce photometrically measurable solutions into a first series of chambers within a rotor assembly while said rotor assembly is at rest;

b. rotating said rotor assembly at a speed wherein centrifugal force causes said volumes of liquids to be transferred to a second series of chambers located radially from the center of rotation of said rotor system a greater distance than said first series of chambers; and

c. continuously and simultaneously scanning the phototransmittance of the contents of said second series of chambers while said rotor is rotating to determine the concentration of a preselected substance contained therein.

9. The method of claim 8 wherein following the introduction of liquids into said first series of chambers, the rotor is accelerated, decelerated, and then reaccelerated to facilitate mixing within said second series of chambers.

10. The method of claim 8 wherein following the rotation of said rotor assembly at a speed causing transfer of said volumes of liquid from said first series of chambers to said second series of chambers, said rotor assembly is brought to rest and said first series of chambers replaced with a third series of chambers containing further preselected volumes of liquids, and wherein said rotor assembly is again rotated to effect the transfer of liquids from said third series of chambers to said second series of chambers.