

[54] **AUTOMATED SYSTEM FOR PERFORMING SAMPLE MEASUREMENT, DILUTIONS AND PHOTOMETRIC MEASUREMENTS**

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[58] Field of Search... 73/421 R; 356/36; 23/253, 259

[56] **References Cited**

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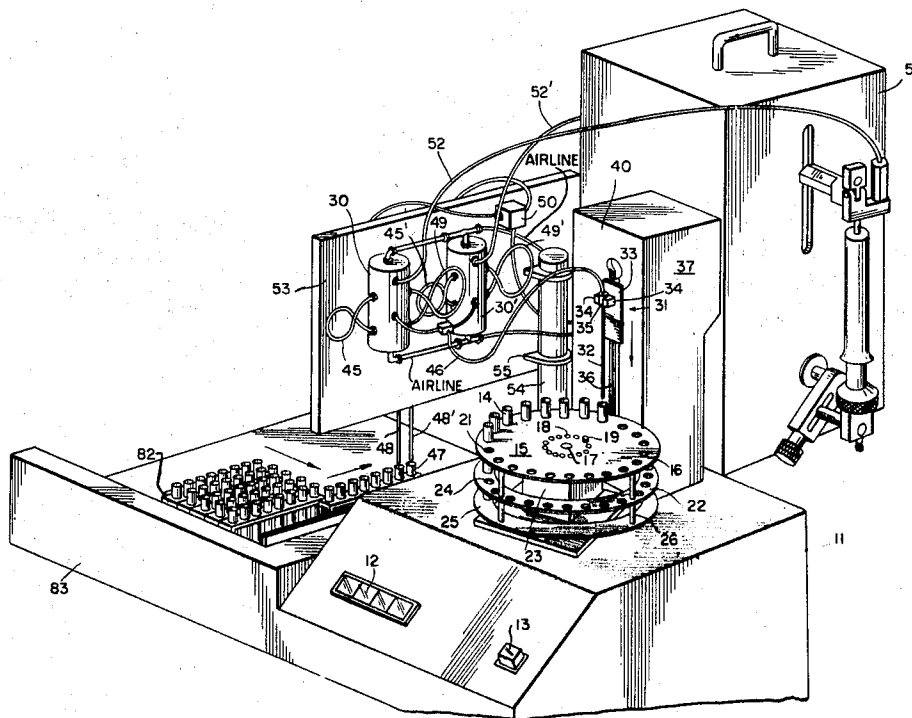
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[57] **ABSTRACT**

An automatic diluter and photometric reader adapted for use in the turbidimetric microbiological assays of antibiotics, vitamins, and the like, as well as in various other analytical assays.

8 Claims, 5 Drawing Figures



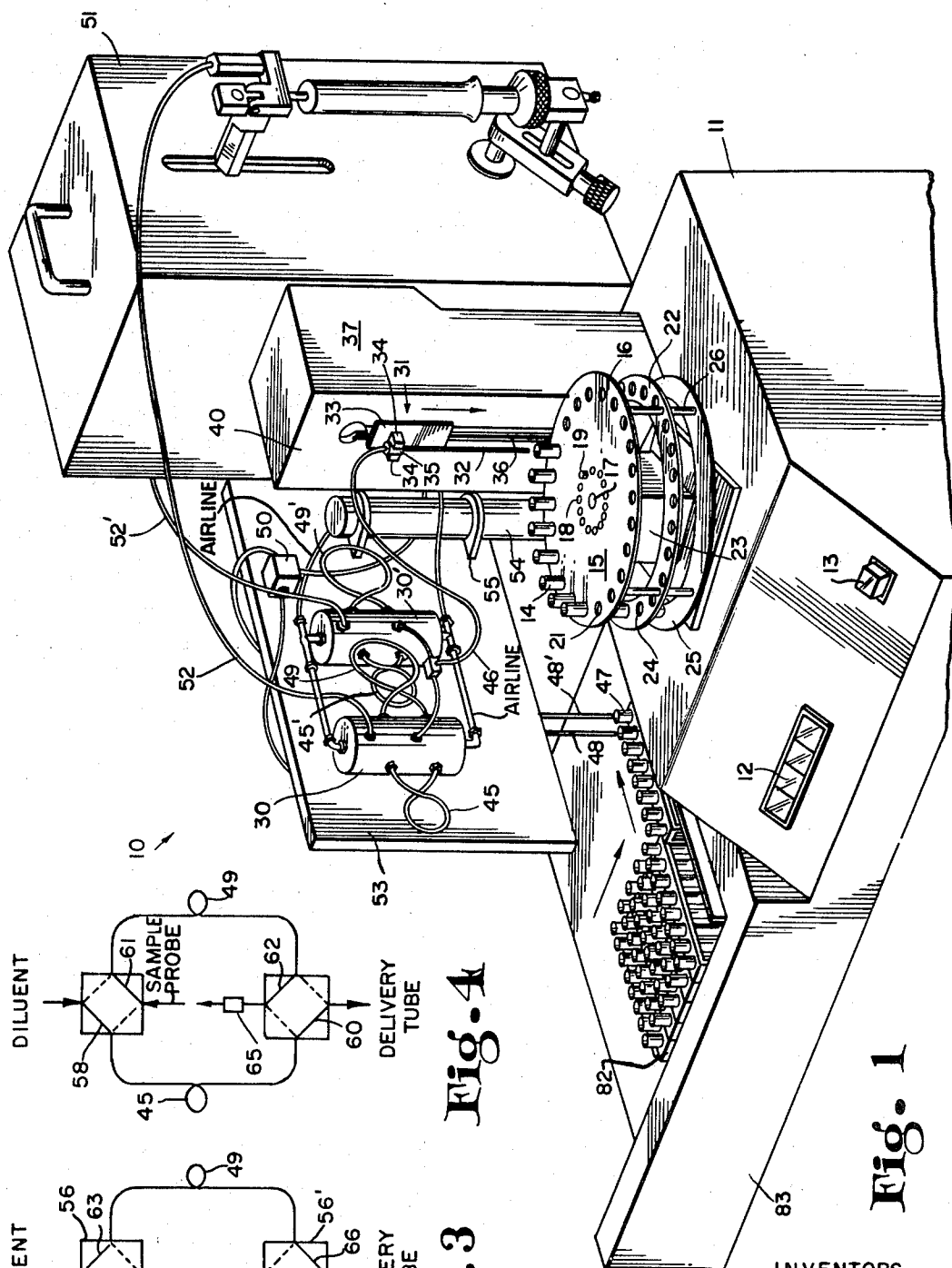


Fig. 1

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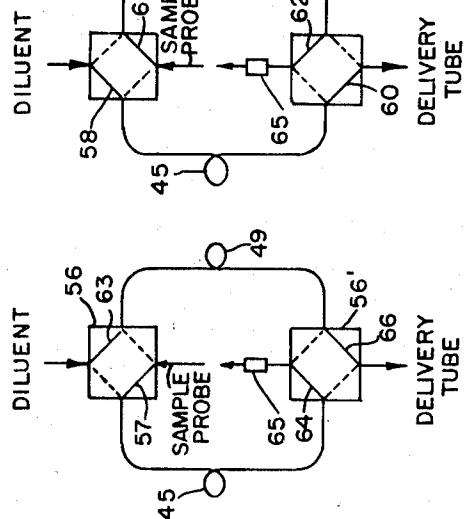


Fig. 4

Fig. 3

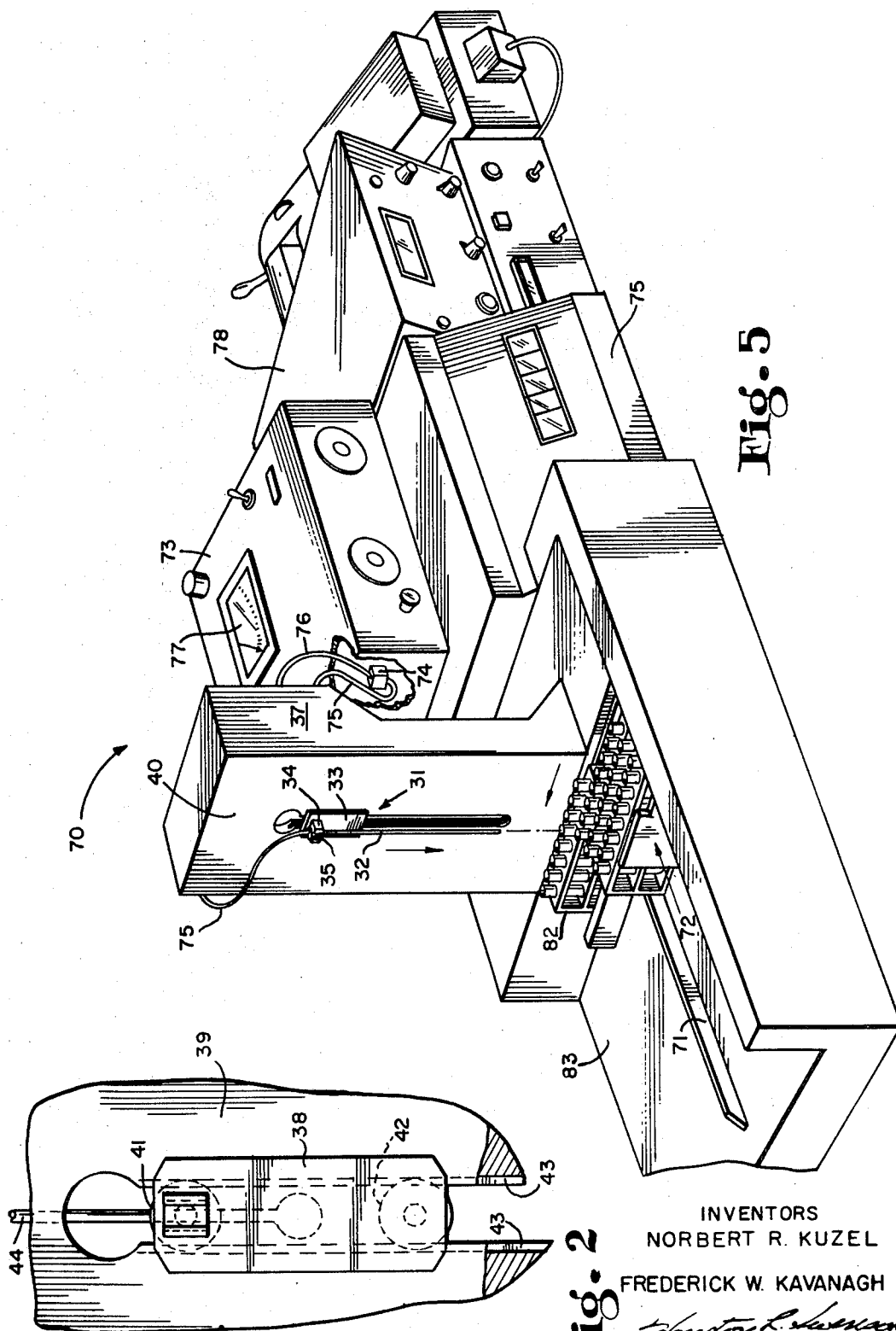


Fig. 2

Fig. 5

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AUTOMATED SYSTEM FOR PERFORMING SAMPLE MEASUREMENT, DILUTIONS AND PHOTOMETRIC MEASUREMENTS

CROSS-REFERENCE TO RELATED APPLICATION

This application is a division of our co-pending U. S. application, Ser. No. 801,369, filed on Feb. 24, 1969 and issued on Sept. 28, 1971, as U.S. Pat. No. 3,609,040.

BACKGROUND OF THE INVENTION

This invention relates to a two-component system for performing turbidimetric microbiological assays on substances such as antibiotics, vitamins, and other bacterial growth promoting or inhibiting substances, as well as various other analytical assays, and the like.

Turbidimetric microbiological assays of antibiotics, vitamins, and the like have long been used as a means for identifying and assaying the potency of such substances. The assay procedures are time consuming and usually involve many manual operations, each subject to some degree of error.

In recent years, there have been attempts to provide automated devices for carrying out such assays. While there are currently several such devices, they are plagued with various problems such as cross-contamination between samples, slow rate of analysis, requirements for large volumes of sample, fixed time of incubation or reaction, lack of versatility, need for method changes to accommodate the equipment, etc. Furthermore, because of the cross-contamination problems of some of the currently existing devices, only one antibiotic, vitamin or the like can be analyzed in a single operation. Before a second substance can be assayed, the equipment must be cleaned.

A further disadvantage of currently available equipment is their lack of reproducibility in assays utilizing rod-shaped organisms such as *Klebsiella*, *Lactobacillus*, *Pseudomonas*, etc., because of the problem of flow birefringence (Kavanagh, F. W., *Analytical Microbiology*, Chapter 4, Academic Press, N.Y., N.Y. 1963).

In addition to the above disadvantages, the cost of many of the currently available devices is prohibitive.

Thus, while attempts have been made to provide automated devices for performing turbidimetric microbiological assays, such attempts have been fraught with problems.

SUMMARY OF THE INVENTION

The two-component assay system of this invention comprises an automatic diluter module and an automatic photometric reader module. The modules perform the same basic operations involved in traditional manual analytical or microbiological turbidimetric methods such as pipetting, diluting, and the like, with far greater accuracy, precision, and efficiency. The modules, for example, can utilize the same organisms, the same media, and can measure the same bacteriological response employed in manual turbidimetric microbiological assays. The system is able to accommodate similar assay designs and standardization procedures as have been used heretofore. It provides for unlimited incubation or reaction times.

Generally speaking, the assay system of this invention consists of two modules, a diluter module, and a photometric reader module.

The diluter module includes a programmed control means, and a sampler tray which is adapted to advance at a predetermined time, making each sample tube available to a first sample probe means. A portion of the sample is withdrawn by a first sample probe means into a first metering means having a zero dead-volume. The combined operations of the probe and metering means performs the pipetting operation. After the sample has been measured, a predetermined amount of diluent is directed through the metering means by a first diluent delivery means, thereby washing out and diluting the sample into a diluted sample receiving means.

The operations are repeated until the desired number of samples have been measured and diluted. So long as the same diluent is used, different substances can be run by different methods without cleaning the equipment between substances. The diluter module is self-cleaning. That is, by carefully choosing the materials of construction, and by providing a sufficiently great dilution factor, the diluent serves to completely flush the sample from the metering means, thereby eliminating cross-contamination without necessitating an intermediate cleaning operation between samples.

A variety of dilutions can be obtained by either changing the volume of the diluent delivered or by altering the volume of sample measured by the metering means.

The diluter module can additionally include an automatic means for conveying the diluted sample receiving means to a delivery tube which is cooperatively associated with the metering means.

The diluted samples can be transferred in suitable carriers, after allowing for sufficient incubation or reaction time, to a photometric reader, preferably to the reader module of this invention.

The reader module of this invention includes a photometric reader such as a photometer having a quartz flow cell as the cuvette. The reader module has an automatic sample conveying means, such as a conventional linear fraction collector or the like, cooperatively associated therewith for conveying each sample to be read to a means for transferring the sample into the flow cell. The sample conveying means can advantageously be a probe means, or the like, which is adapted to withdraw a sample and convey it to the flow cell. Before each sample reaches the flow cell, the flow is momentarily interrupted to provide an "air hammer" effect which rids the sample flow of air bubbles. Once the sample has been measured, it is drawn through egress means associated with the flow cell to waste. The reading for each sample can be taken from the photometric reader, or the reader module can additionally include a suitable readout means or can be keyed directly into a computer. As to the case with the diluter module, the functions of the reader module are controlled by a programmed control means.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective view of the diluter module.

FIG. 2 is a rear plan view of the probe assembly with the housing therefor broken away.

FIG. 3 is a flow diagram of a metering cell in its first position.

FIG. 4 is a flow diagram of a metering cell in the second position.

FIG. 5 is a perspective view of the reader module with the position of the flow cell designated by dotted lines.

DESCRIPTION OF PREFERRED EMBODIMENTS

Referring to the drawing, one embodiment of the diluter module is shown generally at 10 in FIG. 1. The diluter module performs the functions of pipetting and delivering a precise, predetermined amount of sample into a sample holder and of adding a precisely measured amount of diluent or reagent thereto.

The diluter module includes a housing 11 for a programmed control unit and a drive mechanism therefor (not shown). The control unit and drive mechanism are actuated by control switches 12, and the main power switch 13. The samples and standards are poured into sample tubes 14 which are then placed into the sample holder 15. While any suitable sample holder can be utilized, the preferred sample holder 15 includes three circular, spaced-apart plates. The top plate 16 has a centrally located aperture adapted to have a drive shaft 17 removably journaled therein, a plurality of spaced-apart locating apertures 18 forming a concentric circle about said centrally located aperture, said apertures being adapted to receive a positioning pin 19 which is coupled to a mounting and supporting plate (not shown) and which limits the rotational motion of the sample holder so that each sample tube will be aligned with the sampler probe 32, and a plurality of spaced-apart apertures 21 for receiving sample tubes 14, the apertures being conveniently spaced apart adjacent the outer periphery of the plate. The center plate 22 has a central aperture of sufficient size to fit over the housing 23 for the driving motor which controls the indexing of the sample holder. The driving means in turn is controlled by the control unit. Apertures 24 in plate 22 are aligned with apertures 21 of the top plate 16. The bottom plate 25 similarly has a central aperture of sufficient size to fit over housing 23. The bottom plate serves as a base for the bottom surface of the sample tubes. The three plates are maintained in their spaced-apart positions by spacing posts 26.

The samples are drawn into a first metering valve 30 via probe assembly shown generally at 31. Probe assembly 31 includes a probe 32 which is removably mounted at its upper end to a first mounting plate 33 via bracket means 34. A retaining means 35 can conveniently be fitted over the top end of the probe to insure a secure fit into the bracket means. The probe is preferably of stainless steel or a like material. The probe is adapted to move vertically along channel 36 in probe assembly housing 37. As can be seen in FIG. 2, a second plate 38 is cooperatively associated with plate 33 so that plate 38 is disposed adjacent the interior surface 39 of the front wall 40 of the probe assembly housing. Bearings 41 and 42 are rotatably retained between plates 33 and 38. As the probe assembly moves along channel 32, bearings 41 and 42 ride along bearing runners 43. Drive shaft 44 is coupled to and driven by, for example, a bell crank and motor (not shown).

When the probe assembly is actuated, the probe moves downwardly and into the sample contained in the particular tube which is presently aligned with the probe. A vacuum system (not shown) is then actuated and sample is drawn through the probe and into a first

loop 45 of a first metering valve means 30, via sample inlet tube 46. The vacuum system is then shut off whereupon the loop remains full. The size of the sample which is ultimately delivered into sample receiving means 47 via delivery tube 48 is determined by the volume of the loop 45.

Once the sample is drawn into a first loop 45, and the vacuum is shut off via vacuum solenoid (not shown), the sample is delivered via delivery tube 48, into sample receiving means 47, which can be, for example, a test tube, as a predetermined amount of diluent is passed into the valve and through loop 45, through the valve, and into the sample retaining means via delivery tube 48. The presently preferred ratio of diluent to sample is at least 10:1 to insure complete flushing of the valve, thereby eliminating cross-contamination. However, it is only necessary that sufficient diluent be passed through the valve to flush out sample. Thus, for example, when large volumes of diluent are received, part of the diluent can be passed directly into the sample receiving means. On the next cycle, the same sample is passed through and measured by the second loop 49, which can differ in volume from the first loop 45 and is again washed out with diluent via delivery tube 48 and into another sample receiving means.

Any unit capable of adding a specified amount of diluent can be utilized in the practice of this invention, for example, the filling unit manufactured by National Instruments Co. Diluent is pumped from the filling unit 51 into the valve, via diluent inlet tube 52.

In the preferred embodiment of this invention, two metering valves 30 and 30' are utilized in order to obtain, for example, two 0.15 ml. samples and two 0.1 ml. samples. The valves are conveniently mounted on a first surface of mounting board 53, which is, in turn, conveniently coupled to housing 11 via support member 54 and mounting brackets 55.

The sample injection valve manufactured by Chromatix Inc. is particularly suitable for providing the sample metering means of the present invention when modified to operate in accord with the above discussion. However, any other suitable metering means can be utilized.

The operation of the valve 30 can be more fully understood by the diagram of FIG. 3. The sample is drawn into a first section 56 of valve 30 via sample probe 32. The sample follows flow passage 57 to loop 45, and is drawn into a second section 56' of the valves, through passage 64, through vacuum valve 65 and to waste. When the vacuum valve is shut off, loop 45 remains filled until sections 56 and 56' are switched to their alternate positions as shown in FIG. 4. The diluent then passes through passage 58, into loop 45, thereby washing out the sample via passage 60 and through the delivery tube to the awaiting sample receiving means.

Concurrently with the diluent delivery through loop 45, loop 49 is connected to the sample probe via passage 61 and 62, thereby filling loop 49 with the same sample. Return of 56 and 56' to the first position now connects loop 49 with the diluent via passage 63 and 66.

The valves are preferably of Teflon and Kel-F, although other suitable materials can be employed. Similarly, the loops are preferably Teflon.

The sample receiving means 47 are conveniently held in racks 82. The racks are then conveniently advanced automatically into the proper position by an automatic conveyor means 83, cooperatively associated with housing 11 and mounting board 53 so that for each delivery cycle, a sample receiving means is aligned beneath a delivery tube.

It can be seen that the diluter module provides a convenient, accurate means for automatically pipetting a given amount of sample, delivering the sample to a sample holder such as a test tube or the like, and adding thereto, a predetermined amount of medium, diluent, reagent, or the like.

Referring now to FIG. 5, the reader module of this invention is shown generally at 70. The diluted samples obtained from the diluter module, or any other suitable means, are, after proper incubation or reaction time, transferred in the racks 82 to an automatic conveyor means 83, or other suitable advancing means. It can be seen that the racks are guided by guide means 71, and that a retaining plate 72 is positioned behind the last rack. The samples are drawn into a suitable photometer 73 by probe assembly 31. The samples are passed through flow cell 74 via inlet tube 75 and through outlet tube 76 to a waste receiving means (not shown). The flow cell is preferably quartz or a like material, and replaces the cuvette of conventional readers. A solenoid valve (not shown) in the control unit 79 controls the sample flow. The control circuit is adapted to momentarily drop out the solenoid and interrupt the sample flow before it reaches the flow cell. This produces an "air hammer" effect which takes care of any air bubbles present in the flow stream. It is presently preferred, when determining the turbidity of rod-shaped organisms, that the flow cell permit a rate of flow of not less than 0.8 ml./sec., and preferably a rate of 1 ml./sec. or higher in order to obtain highly accurate, reproducible results. For other assays, the flow rate is not critical. The readings can be taken from the scale 77 of the photometer 73, or, as in the preferred embodiment, a suitable "readout" device 78 can be cooperatively associated with the photometer.

The readout is compatible with a variety of data acquisition devices such as printed tape, punched paper tape, punched cards, or can be interfaced directly to a computer.

The reader module of this invention provides a great advance in the art by providing a means for overcoming the problems of measuring the turbidity of rod-shaped organisms by avoiding the flow birefringence problem normally encountered with rod-shaped particles. By producing a sufficiently rapid flow through the flow cell, rod-shaped particles, which would otherwise orient themselves with the slow currents in a static cell, leading to inconsistent readings, are read with the same precision as are spherical particles. Thus the present reader module now makes it possible to assay for substances which affect various rod-shaped organisms such as *Klebsiella*, *Lactobacillus*, *Pseudomonas*, and the like, and to determine them with a precision equivalent to spherical organisms, all other factors being equal.

While the present invention is particularly suited for turbidimetric microbiological assays, it will be apparent to those skilled in the art that the diluter module and the reader module can be used jointly or separately in any number of analytical procedures, medical laboratory tests, and the like. Therefore, while the following discussion is directed toward turbidimetric microbiological assays, the modules of this invention are applicable to any suitable analytical procedures.

We claim:

1. In an automatic diluter module the improvement comprising: a valve means having first and second sections, said first section having a diluent supply inlet connected to a metered diluent supply and a sample supply inlet connected to a sample supply, a pair of loops of predetermined volume having their first ends connected to a pair of respective outlets in said valve means first section in alternately selective communication with said diluent supply and said sample supply, said loops having their second ends in alternately selective communication with a pair of respective discharge outlets in said second section of said valve means, said pair of discharge outlets in said second section of said valve means being in communication with a waste receptacle and a delivery receptacle, and control means associated with said first and second sections alternately effecting coupling of each of said loops between (a) said diluent supply inlet and said delivery receptacle; and (b) said sample supply inlet and said waste receptacle.

2. In an automatic diluter module the improvement in accordance with claim 1 in which said loops are interchangeably mounted.

3. In an automatic diluter module the improvement in accordance with claim 2 in which the coupling of one loop between said diluent supply inlet and said delivery receptacle is concurrent with the coupling of the other loop between said sample supply inlet and said waste receptacle.

4. In an automatic diluter module the improvement in accordance with claim 3 in which the communication between said sample supply inlet and said sample supply includes a probe extending into said sample supply.

5. In an automatic diluter module the improvement in accordance with claim 4 in which said sample supply is contained in a plurality of open-mouth receptacles.

6. In an automatic diluter module the improvement in accordance with claim 5 in which said probe is reciprocally mounted and associated with a drive means for imparting vertical movement thereto.

7. In an automatic diluter module the improvement in accordance with claim 6 in which said open-mouth receptacles containing said sample supply are automatically driven in succession under said probe and in synchronization with the vertical movement of said probe.

8. In an automatic diluter module the improvement in accordance with claim 7 in which a plurality of delivery receptacles are automatically driven in successive synchronized communication with the discharge of diluted samples from the one of said discharge outlets.

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