

Sept. 17, 1968

E. W. ANTHON

3,401,591

ANALYTICAL CUVETTE AND SUPPLY SYSTEM WHEREIN THE CUVETTE
INLET AND OUTLET ARE LOCATED ON
THE BOTTOM OF THE CUVETTE

Filed Dec. 9, 1963

2 Sheets-Sheet 1

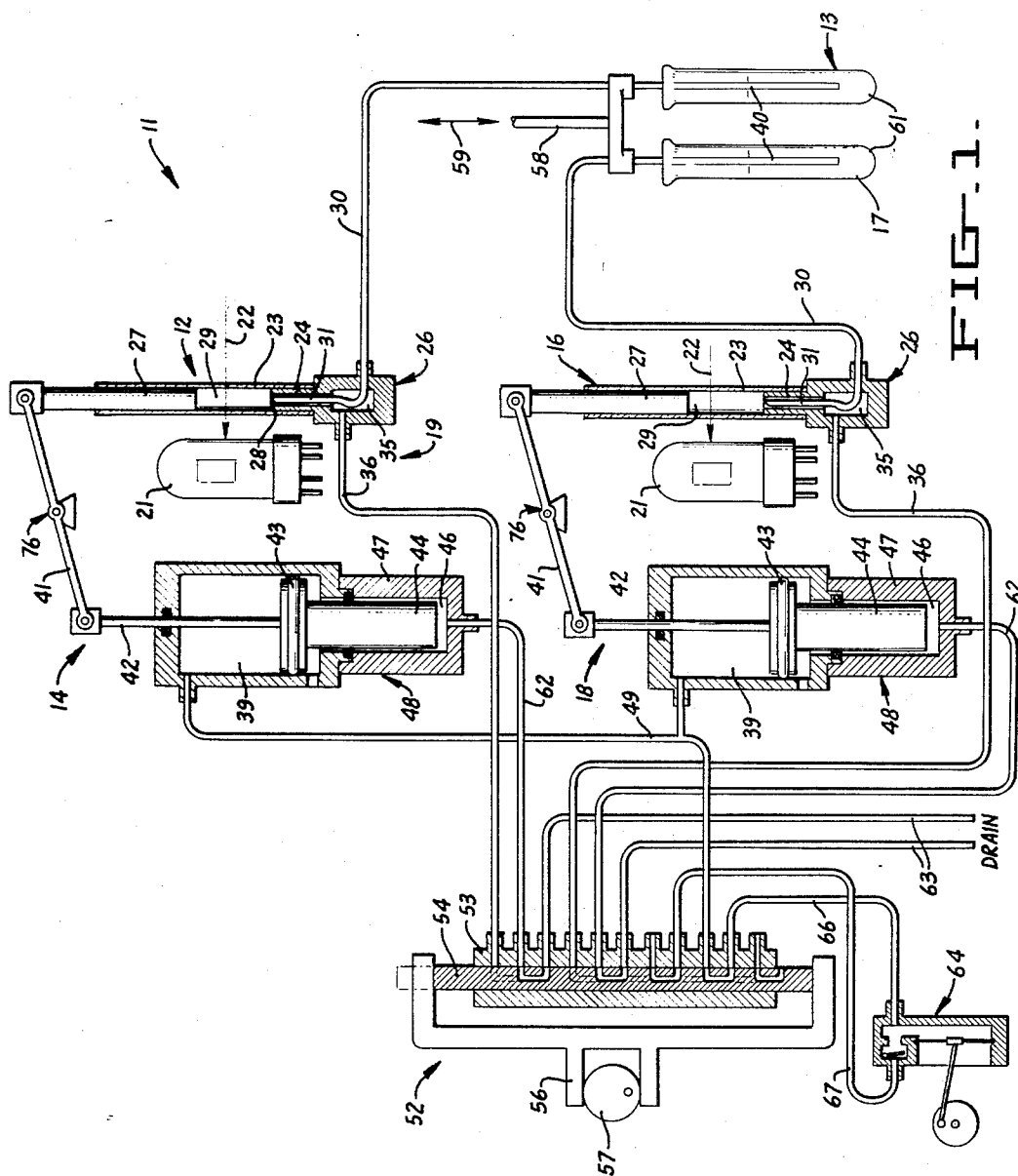


FIG. 1

INVENTOR
ERIK W. ANTHON

BY

Schapp & Hatch
ATTORNEYS

Sept. 17, 1968

E. W. ANTHON
ANALYTICAL CUVETTE AND SUPPLY SYSTEM WHEREIN THE CUVETTE
INLET AND OUTLET ARE LOCATED ON
THE BOTTOM OF THE CUVETTE

3,401,591

Filed Dec. 9, 1963

2 Sheets-Sheet 2

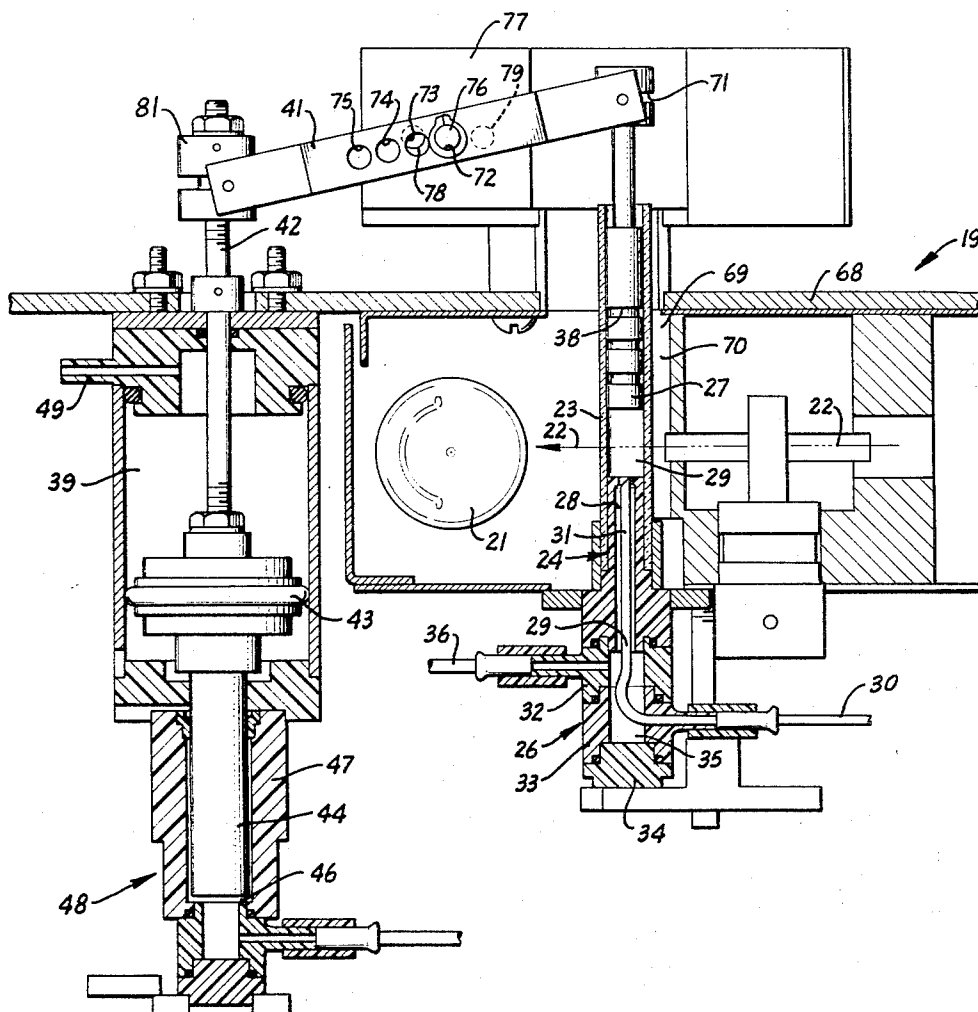


FIG. 2.

INVENTOR
ERIK W. ANTHON
BY
Schapp & Hatch
ATTORNEYS

1

2

3,401,591

ANALYTICAL CUVETTE AND SUPPLY SYSTEM WHEREIN THE CUVETTE INLET AND OUTLET ARE LOCATED ON THE BOTTOM OF THE CUVETTE

Erik W. Anthon, Kensington, Calif., assignor, by mesne assignments, to Warner-Lambert Pharmaceutical Company, Morris Plains, N.J.

Filed Dec. 9, 1963, Ser. No. 329,201

4 Claims. (Cl. 88—14)

ABSTRACT OF THE DISCLOSURE

A apparatus for transferring samples to a spectrophotometer automatically and in serial fashion including a special cuvette constructed for automatic filling and emptying and capable of self-cleaning from a small quantity of sample, said cuvette being constructed as a cylindrical housing having a bottom and a plunger adapted to reciprocate in said housing so that the plunger and bottom wall fit in matching configuration and the cuvette operates between the substantially zero volume and the filled volume, a filling duct directed vertically through the bottom wall so as to direct incoming sample toward the plunger and a separate and distinct outlet duct extending through the bottom of the chamber for removing fluid therefrom, said filling and exit ducts preferably having their ends terminating at the inner surface of the bottom wall of the chamber and in concentric relation.

The present invention relates to improvements in an analytical cuvette and method of filling same and more particularly to a cuvette adapted to fit in operative position in a sensing device for an automated analytical system and an automatic supply system for moving sample into and out of the cuvette.

In certain types of analytical procedures, large numbers of routine determinations must be carried out. Particularly applicable are those employed in biochemical, physiological and medical laboratories where materials such as blood serum, tissue serum, urine, or other materials are tested and/or analyzed. Such tests are particularly suited for automation because the analytical procedures are repeated a large number of times.

In accordance with many of these procedures, the final measurement is made by a suitable sensing device capable of measuring a physical property which is indicative of the analytical results to be found. For example, many procedures call for the addition of reagents which develop color in an amount depending upon the concentration of a constituent in the sample. When the apparatus is to be fully automated and to be programmed to carry out such a procedure, it is necessary to provide means for holding the sample in operative position in the sensing device and to provide an automatic means for supplying samples to be determined in an orderly and known fashion to said means.

A cuvette and an automatic supply system therefor capable of achieving these results is the subject of the co-pending United States patent application, Ser. No. 228,337 entitled, Cuvette and Supply System Therefor, filed Oct. 4, 1962, now patent No. 3,225,645 by Hans Baruch and Erik W. Anthon, the inventor in the present application, and assigned to the same assignee as the present invention. While this prior application describes and claims an excellent cuvette system, it has been found that improvements can be made on the system, and the present invention is directed to such improvements.

In the device containing the analytical cuvette of the invention, the sensing device may be any type of instru-

ment adapted to measure the physical properties of a substance contained in operative position therein. For example, physical properties such a color, ultraviolet absorption, fluorescence, or any other property utilized for analytical purposes may be measured with a sensing device adapted to measure such properties. The invention is particularly applicable for use with a spectrophotometer or colorimeter which measures color developments or similar optical properties utilized in analytical procedures.

The supply system may be any system capable of moving sample, which has been treated according to the necessary procedure for measurement of the physical property, from a site or location in the apparatus to a cuvette in the sensing device. However, it is important that the supply system be fully automated with its automation properly synchronized into the system, and it is also important to provide a system in which contamination by prior samples handled is completely eliminated.

In order to eliminate or sufficiently reduce this contamination from prior samples, it is necessary to provide a system in which the cuvette and supply ducts are cleaned. The present invention is directed to improvements in the cuvette construction and supply ducts which gives a better cleaning situation and allows for a simpler supply system than that shown in detail in the prior application cited above.

Thus it is the primary object of this invention to provide an improved analytical cuvette and method of filling same of the character described which is capable of moving treated sample into and out of position in a sensing system where a reading which gives the analytical result is made.

Another object of the invention is the provision of an improved method and apparatus for filling a cuvette with a series of samples in which the cuvette is washed clean of prior sample by a portion of sample being brought in for measurement, and then the remainder of the sample to be measured is brought into position for accurate determination.

A further object of the invention is to provide an improved cuvette which is capable of continued automatic operation over a long period of time and which is resistant to corrosive chemicals and atmospheres usually found in a laboratory.

A still further object of the invention is to provide an improved analytical cuvette and supply system of the character described which is easily constructed, simple in operation and reliable and accurate in the results obtained.

Further objects and advantages of the invention will be apparent as the specification progresses, and the new and useful features of my analytical cuvette and method of filling the same will be fully defined in the claims attached hereto.

In its broad aspect, the invention provides a cuvette which has a pump associated therewith for moving treated sample into the cuvette chamber and moving treated sample out of the cuvette chamber in a simplified manner while solving the problem of undue cross-contamination. In the process form, the invention operates to utilize the first received sample material to rinse away prior contaminants and then use the last received sample in the container to obtain a reading which is substantially unaffected by prior samples handled.

In its preferred form, the cuvette is constructed with a pump as an integral part thereof, with a cylinder and plunger operating within the cylinder with the cylinder serving as the container for the sample to be measured. In this way, the pump is used to bring in and remove sample from the cuvette. In order to provide the proper flow of liquid, two lines communicate into the cuvette, one of

which brings in the sample while the other takes sample out toward a drain. In order to provide proper flow of liquid, a suitable valving system is used which acts in cooperation with the pump to insure the necessary flow of liquid.

In accordance with the present invention it is possible to carry out the method of the invention with a minimum of apparatus, and it is preferred to utilize a pair of pump units which are driven by air pressure and a valve system which operates all pumps and regulates the associated fluid lines in one operation. In this way, the valve operations are automated by any suitable control systems and the operation of the system follows from the positioning of the valves.

Specifically, the cuvette is designed to have the entrance conduit and the exit conduit for the sample terminate in substantially juxtaposed locations in the cuvette chamber so that efficient washing is effected as sample moves through the cuvette chamber. In its preferred form, the inlet conduit is concentrically disposed within the exit conduit, and the washing or rinsing is accomplished when the cuvette chamber is at minimal volume, the volume of the chamber being varied by a plunger or the like.

The preferred form of the invention is illustrated in the accompanying drawings forming part of this description, in which:

FIGURE 1 is a schematic showing of a complete dual system for loading a cuvette with sample to be determined, and loading another cuvette with a blank sample so that a difference comparison can be made in a spectrophotometer; and

FIGURE 2, an enlarged cross-sectional view of a portion of the apparatus illustrated in FIGURE 1 showing certain of the components in greater detail.

While only the preferred form of the invention is shown in the drawings, it should be understood that various changes or modifications may be made within the scope of the claims attached hereto without departing from the spirit of the invention.

Referring to the drawing in greater detail, there is shown in FIGURE 1 a liquid handling system 11 which comprises a cuvette 12, a sample site 13 and a pump system 14 for moving sample from the sample site into the cuvette and for removing sample from the cuvette. In addition, the system shown also comprises a cuvette 16 adapted to receive blank sample from a blank sample container 17, and a pump system 18 for moving blank sample into and out of the cuvette 16. Thus, a dual system is shown with the cuvette 16 being similar to the cuvette 12, and the pump system 18 being similar to the pump system 14. Accordingly, the description given below for the cuvette 12 and the pump system 14 is also applicable to cuvette 16 and pump system 18, and the similar parts of the two systems are similarly numbered.

In the system shown, the cuvette 12 and the cuvette 16 are both carried in a spectrophotometer 19 which contains a pair of photosensing tubes 21 and means (not shown) for providing light rays as indicated by arrows 22 through the sample and blank simultaneously and to the photosensing tubes 21.

As here shown, the cuvettes 12 and 16 each comprise a cylindrical housing 23 made of precision bore glass tubing, a bottom plug 24 which has a conduit assembly 26 therein, and a plunger 27. The bottom plug is preferably made of Teflon and has a central opening or duct 28 which serves as the outlet conduit for cuvette chamber 29. Inlet tube 31 is preferably concentrically disposed within duct 28 and has its outlet end substantially even with the upper surface of the bottom plug 24 as best seen in FIGURE 2.

The conduit assembly 26 comprises ring shaped fittings 32 and 33 and closure fitting 34 to define a chamber 25 which serves as a part of the exit duct. The exit duct then continues through tube 36 in the fitting 32 as shown. Inlet tube 31 passes through fitting 33 and is carried therein in

tight gripping relation where it is joined with supply line 30. Tube 31 is preferably stiff enough to retain its shape and position during operation. As here shown, tube 31 is also preferably cut at an angle at its end.

The fluid line 30 has a stiff probe section 40 at its end and the line is smooth without joints or irregularities that may cause holdup of liquid and cross-contamination between samples. The use of valves in line 30 is also avoided for the same reason.

The plunger 27 is constructed to slide sealingly within the housing 23, and has an end shaped to match the configuration of the bottom plug 24. In this way, the plunger acts as a pump that draws liquid into and expels liquid out of the chamber 29. The fit is also tight enough that the plunger keeps the walls of the glass tube clean and removes any film that might otherwise collect thereon. Plunger 27 is also preferably constructed with grooves 38 which help provide an air-tight seal between the Teflon plunger and glass housing.

The plunger is moved up and down by an air cylinder 39 through a rocker arm 41 which is connected to a piston arm 42 of a piston 43 carried in the air cylinder. A plunger 44 is also attached to the piston 43, and this plunger fits into a chamber 46 defined by a housing 47. Thus the plunger 44 and chamber 46 serves as a pump means 48. It is seen from FIGURE 1 of the drawing that air cylinder 39 simultaneously operates the pump means 48 and plunger 27. With this operation, the pump means takes in liquid while the cuvette chamber is emptied of its contents, and while the cuvette takes in liquid the pump means expels liquid. This operation is important because pump means prevents the cuvette from emptying its contents back through the probe 40. In order to achieve this result, the pump means should be constructed to draw in a larger amount of liquid than is discharged by the cuvette.

In order to achieve the improved method of loading the cuvette chamber according to the invention it is important that the capacity of the pump means exceeds the added volume of the cuvette chamber and supply line 30. The excess amount corresponds to the volume of sample used to wash the cuvette, and therefore, this excess should be a positive amount of sufficient for the purpose. In a typical example the capacity of the pump means is 2.5 ml., the cuvette chamber 0.5 ml. and the capacity of the supply line 1.5 ml. This provides an excess of sample for washing of 0.5 ml., and such amount is sufficient to achieve sufficient rinsing because the cuvette chamber is reduced to nearly a zero volume during the rinse operation. In this example, the carryover between samples is of the order of 0.2%, and this amount is generally within the required tolerance.

Air pressure or vacuum is supplied to the air cylinders 39 through a line 49 which is controlled by a valve 52. Flow of fluids in the remainder of the system is also effected by the valve 52 as will be more fully described below.

In general, valve 52 may be any valve capable of providing the desired changes in the fluid lines connected thereto, and the size of the valve will vary somewhat on the system utilized. For example, a dual system is shown in FIGURE 1. However, it will be appreciated that a single system could be used, and in such a case certain of the valve positions may be eliminated. As here shown, valve 52 comprises a cylindrical valve unit 53 having passages therein adapted to align with passages in a valve member 54 sliding within the cylindrical valve unit 53.

Valve member 54 is positioned to provide the communication of liquid lines and air lines as shown, or to provide the communication shown in phantom. Adjustment of the position is obtained by reciprocating motion of valve member 54 which in turn is moved by a frame 56. Frame 56 is moved by an eccentric 57 driven by a motor (not shown) at half revolutions. Control for the operation of the valve positioning of the probe in the

samples and readout by the spectrophotometer is preferably obtained by an electric control system such as a simplified form of that shown in the copending application cited above on which the present invention is an improvement.

As indicated above, any valve capable of achieving the desired result may be used, provided it gives the desired number of fluid line positions, and may be programmed automatically to carry out the pumping steps required. These pumping steps include transfer of sample into the cuvette, removal of the sample from the cuvette, and cleaning of the cuvette. A preferred valve and mechanism for operating the same is described and claimed in the copending United States patent application Ser. No. 183,506, filed Mar. 29, 1962, now Patent No. 3,119,538, entitled Valve by Erik W. Anthon, the inventor in the present application.

The probes 40 are preferably constructed of a material having a hydrophobic surface and therefore are substantially free of sample material after the passage of sample material therethrough. However, if desired, the probes 40 may be moved to a wash site and then to the next sample for pumping sample material into the cuvette.

Any suitable transfer device may be used to effect movement of the probes such as a device moving frame 58, which carries the probes in a reciprocating motion as indicated by arrow 59, while the containers 61 are moved by independent conveying means (not shown). Examples of typical devices capable of effecting the desired transfer of the probe may be found in the copending patent application of Erik W. Anthon, the inventor in the present application, Ser. No. 61,260, entitled, Materials Handling Apparatus, filed Oct. 7, 1960, now Patent No. 3,178,266.

In operation, the cycle ends with the cuvette plunger being up and the pump plunger being down after readout of the previous sample. The valve is positioned as shown with the pump cylinder being connected to the drain through lines 62, valve 52 and lines 63 while the air cylinder 39 is connected to the pressure side of air compressor 64 through lines 49, valve 52 and pressure line 66.

The next sample and reference blank are positioned at the sample site and the probes 40 lowered into position. The valve is then switched to the opposite position as shown in phantom to connect air cylinder 39 to the suction line 67 of the compressor. At the same time, lines 62 of the pump means 48 are connected to exit lines 36 of the cuvettes. With this valve change the air cylinders cause the pump plungers to go up and the cuvette plungers to go down.

Using the volumetric values of a typical example, each pump means draws in a volume of 2.5 ml. while the cuvette plunger goes down and drives out a volume of 0.5 ml. Thus the differential volume of 2.0 ml. is drawn in through the probe and line 30. Since the probe and supply line only holds 1.5 ml., 0.5 ml. of fresh sample passes through the supply line and the cuvette into outlet duct 28.

In the preferred construction shown, the sample is directed into the cuvette in a stream which impinges on the bottom of plunger 27 to give an excellent rinsing action. In this way a small amount of sample is expended in rinsing and an economy of sample is obtained as well as a simplified operation. For example a sample as small as 1.0 ml. could be used since only 0.5 ml. is used for rinsing and 0.5 ml. is used for readout. In this case and any other case where less than 2.0 ml. of sample is available, air is drawn into the probe, but this air remains at the probe end because of the small diameter supply line used.

The probe is then removed from the sample container and the valve is switched back to the position shown in FIGURE 1 which closes tube 36 leading from the cuvette, provides a connection between pump chamber 46 and the drain, and provides pressure in air cylinder 39. This

causes the pump plunger to go down and expel the pump contents to drain while lifting the cuvette plunger and drawing in sample from supply line 30. After this operation, the cuvette chamber is filled and a read-out is made.

After read-out, the cycle is repeated to obtain a determination for the next sample. In a typical unit, cycle time is only 5 seconds plus the time required for the read-out on the spectrophotometer. This rapid operation is achieved because only two strokes are needed for each cycle. It may be observed that in the procedure described, at least 0.5 ml. of air is drawn into the probe. This air slug is valuable in keeping sample slugs separated in the supply line so as to avoid cross-contamination by diffusion between adjacent samples.

As best seen in FIGURE 2, the cuvette is adapted to be held in operative position in the housing 68 of spectrophotometer 19 or the like, and various sizes of cuvettes may be used if desired. The spectrophotometer components are carried in the same housing as the pumping system of this invention, and the holding means for the cuvette comprises an opening 69 containing a V-shaped wall 70 against which the cuvette is clamped. In general, this clamping may be effected by any suitable means such as that shown in the prior application of Hans Baruch and the present inventor cited above.

The rocker arm 41 which operates between piston arm 42 of the pump means 48 and plunger 27 of the cuvette may be easily removed from the plunger 27 so as to effect a change of cuvettes. This arm is also carried in the end of the cuvette plunger by the cross slot 71 as best seen in FIGURE 2 so that leverage may be effected with various sizes of cuvettes. In addition, rocker arm 41 may be positioned at any one of a number of pivots.

As here shown, rocker arm 41 contains four holes 72, 73, 74 and 75 which serve as pivots for fulcrum means 76. Fulcrum means 76 is in the form of a pin which fits through one of the holes 72 through 75 and into a corresponding hole in support block 77. Thus the position shown illustrates a short stroke with a small cuvette. In order to obtain a longer stroke with the same cuvette, pivot pin 76 is moved over to fit through hole 73 of rocker arm 41 and into hole 78 in support block 77. In order to use a larger cuvette, a larger cuvette is inserted into position and the rocker arm 41 is rotated 180° so that hole 75 of the rocker arm is aligned with hole 79 in the support block. In this way, the pivot may be at 73 with the pin in hole 78, at 74 with the pin located as shown or at 75, or with the pin fitting at 79. Thus the length of the strokes of the plunger 27 may be varied as desired with different sizes of cuvettes and different amounts of sample available.

In the description given above, a filling cycle is described as consisting of two pump strokes. This gives satisfactory results and is the fastest cycle. However, it has often been found more favorable to operate the system with four pump strokes using a lower ratio between the displaced volumes of the pump and cuvette. The operating data for typical four stroke operations are given in the tables below. As used therein, the terms downstroke and upstroke refer to the cuvette plunger movement.

TABLE I

	Pump	Cuvette	Probe
Diameter (I.D.), mm.....	11.1 (7/16)---	6.35 (1/4)---	1.25 (17 GA)
Length or stroke, mm.....	15.9 (5/8)---	12.7 (1/2)---	1,000.
Volume, ml.....	1.5	0.4	1.2.
1. Downstroke, ml.....	1.55 in.....	0.4 out.....	1.15 in.
2. Upstroke, ml.....	1.55 drain---	0.4 in.....	0.4 in.
3. Downstroke, ml.....	1.55 in.....	0.4 out.....	1.15 in.
4. Upstroke, ml.....	1.55 drain---	0.4 in.....	0.4 air.

The operation was tested and it was found that 2.0 ml. of sample were sufficient. Although 3.1 ml. are pumped to drain 0.4 ml. represents air taken in during the last upstroke when sample is moved from the probe to the cuvette, and it is possible to allow up to about 0.7 ml. of air to enter the probe during the second downstroke be-

cause the probe tube holds more liquid than required to fill the cuvette.

Table I above illustrates a procedure for low carryover and Table II below illustrates a procedure where less sample is available. In Table II, the system is designed for 1.9 ml. of sample, but actual tests indicate 1.2 ml. is sufficient. In order to change the ratio, of cuvette volume to pump volume, the pivot pin 76 is moved to holes 73 and 78, and the threaded head 81 on plunger 42 is adjusted. The limits of the strokes are determined by the bottom positions of the pump plunger and cuvette plunger. With the ratio fixed by the rocker arm, the total volumes are adjusted through this nut. After the change, the pump volume is 1.15 ml., and the operative values are given in Table II.

TABLE II

	Pump	Cuvette	Probe
Diameter (I.D.), mm	11.1	6.35	1.25
Length or stroke, mm	11.9 ($\frac{19}{32}$)	12.7	1,000.
Volume, ml.	1.15	0.4	1.2
1. Downstroke, ml.	1.15 in.	0.4 out	0.75 in.
2. Upstroke, ml.	1.15 drain	0.4 in.	0.4 in.
3. Downstroke, ml.	1.15 in.	0.4 out	0.75 in.
4. Upstroke, ml.	1.15 drain	0.4 in.	0.4 air.

When sufficient sample is available a longer light path through the sample is obtained by using a larger cuvette and reversing the rocker arm as explained above. A typical operation using a larger cuvette is given in Table III below. In this run, 2.7 ml. of sample represents the minimum requirement while 3.1 ml. is the designed take-up.

TABLE III

	Pump	Cuvette	Probe
Diameter (I.D.), mm	11.1	12.7 ($\frac{1}{2}$)	155 (15 GA.)
Length or stroke, mm	23.8	11.9	1,000.
Volume, ml.	2.3	1.5	1.9.
1. Downstroke, ml.	2.3 in.	1.5 out	0.8 in.
2. Upstroke, ml.	2.3 drain	1.5 in.	1.5 in.
3. Downstroke, ml.	2.3 in.	1.5 out	0.8 in.
4. Upstroke, ml.	2.3 drain	1.5 in.	1.5 air.

The apparatus may be constructed of any suitable materials which are chemically resistant, but I prefer to use precision bore glass tubes for the cuvettes, and to use a Teflon plunger and plug therein. Other materials which are preferably used include polypropylene tubing and Viton rubber O-rings on the pump pistons. These materials are eminently satisfactory in providing long wear and resistance to chemical attack.

From the foregoing description, it is seen that I have provided an improved self-cleaning, fully automated system for moving samples into reading position in a cuvette, and for removing said samples from the cuvette and cleaning same for the next operation. It is also seen that I have provided an improved form of cuvette, which is simple in operation and capable of rapid accurate operation with a minimum of sample.

I claim:

1. An apparatus for transferring individual samples to

a sensing device from a sample site where samples are provided in one-at-a-time fashion, comprising a cuvette in the sensing device for receiving a sample, said cuvette having radiation permeable walls and a plunger for drawing in and expelling fluid from the cuvette, a pump for collecting liquids removed from the cuvette, a first duct in communication with the cuvette positionable to receive sample from the sample site, a second duct in communication with the cuvette chamber and the pump, said first duct and said second duct each having a separate and distinct end thereof terminating at the inner surface of the bottom wall of the cuvette chamber, and valve means for controlling the operation of the cuvette plunger, pump and the opening in the second duct, said first duct being oriented to direct its fluid toward the plunger, and said pump being formed to remove fluid from the cuvette at a rate greater than the displacement of the plunger in the cuvette whereby a new sample is directed toward the plunger as a prior sample is removed to wash away old sample and make the cuvette more receptive to receipt of the new sample.

2. The apparatus for transferring samples to a sensing device defined in claim 1, in which the end portion of the first duct terminating in the cuvette chamber is located inside the passage at the end portion of the second duct.

3. The cuvette defined in claim 1, in which the ends of the first and second duct are in concentric alignment.

4. A cuvette adapted to receive and hold a liquid sample in automated analytical equipment in position for further operation thereon, comprising a housing having side walls and a bottom defining a chamber for holding liquids, said walls being formed for accommodating said further operation, a plunger adapted to reciprocate in said housing, said plunger having side walls and a bottom wall in matching configuration with said housing, a first duct having a passage in communication with said chamber through said bottom and extending substantially to the inner surface of said bottom, and a second duct in communication with said chamber through said bottom, said second duct having a passage separate and distinct from the passage of said first duct and extending substantially to the inner surface of said bottom, and said first duct having its end thereof entirely inside of the passage of the second duct at the bottom of the cuvette, with the passage of the first duct being directed toward the plunger whereby sample moving through the first duct is adapted to be directed against the plunger during at least a part of the operation thereof to wash the plunger.

References Cited

UNITED STATES PATENTS

3,225,645 12/1965 Baruch et al.
2,878,388 3/1959 Bergson.

JEWELL H. PEDERSEN, *Primary Examiner*.
O. B. CHEW, *Assistant Examiner*.