June 26, 1973 D. G. MITCHELL ET AL 3,741,726

APPARATUS FOR COLLECTING AND DISPENSING LIQUIDS AND FOR PARTICLE COUNTING

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FIG 1

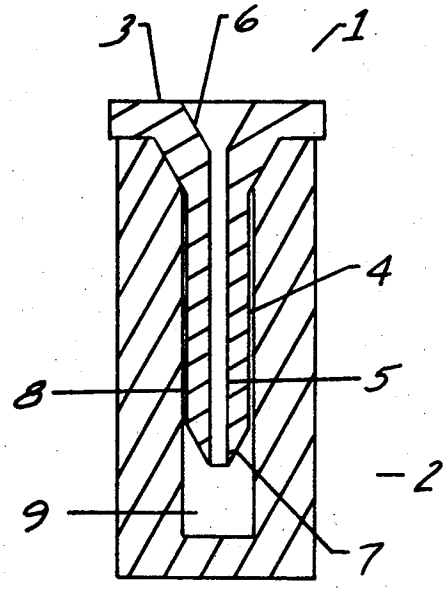
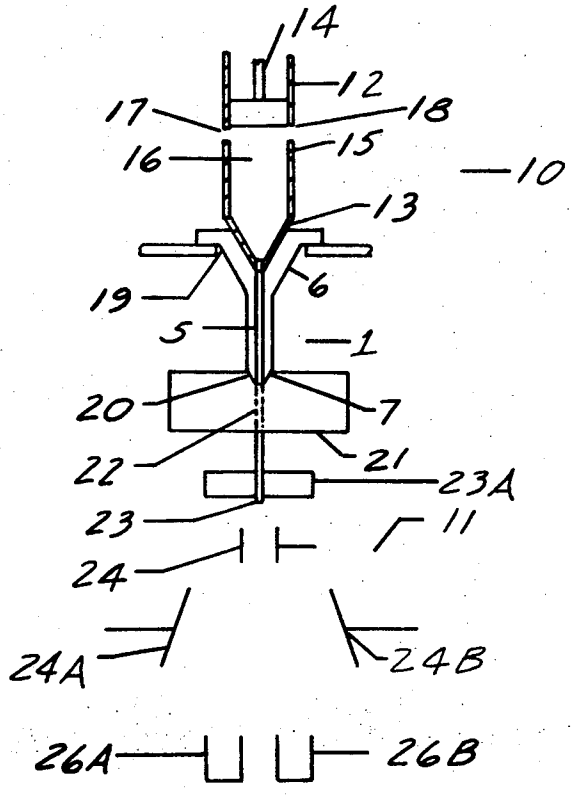


FIG 2



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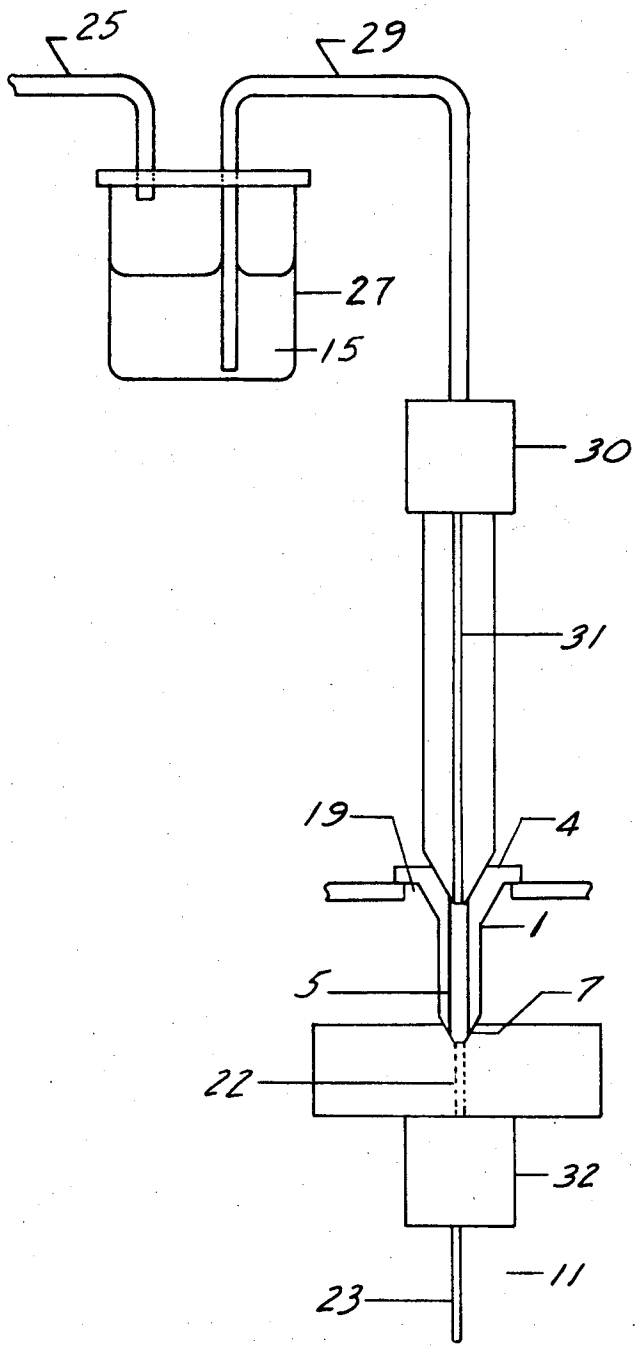


FIG 3

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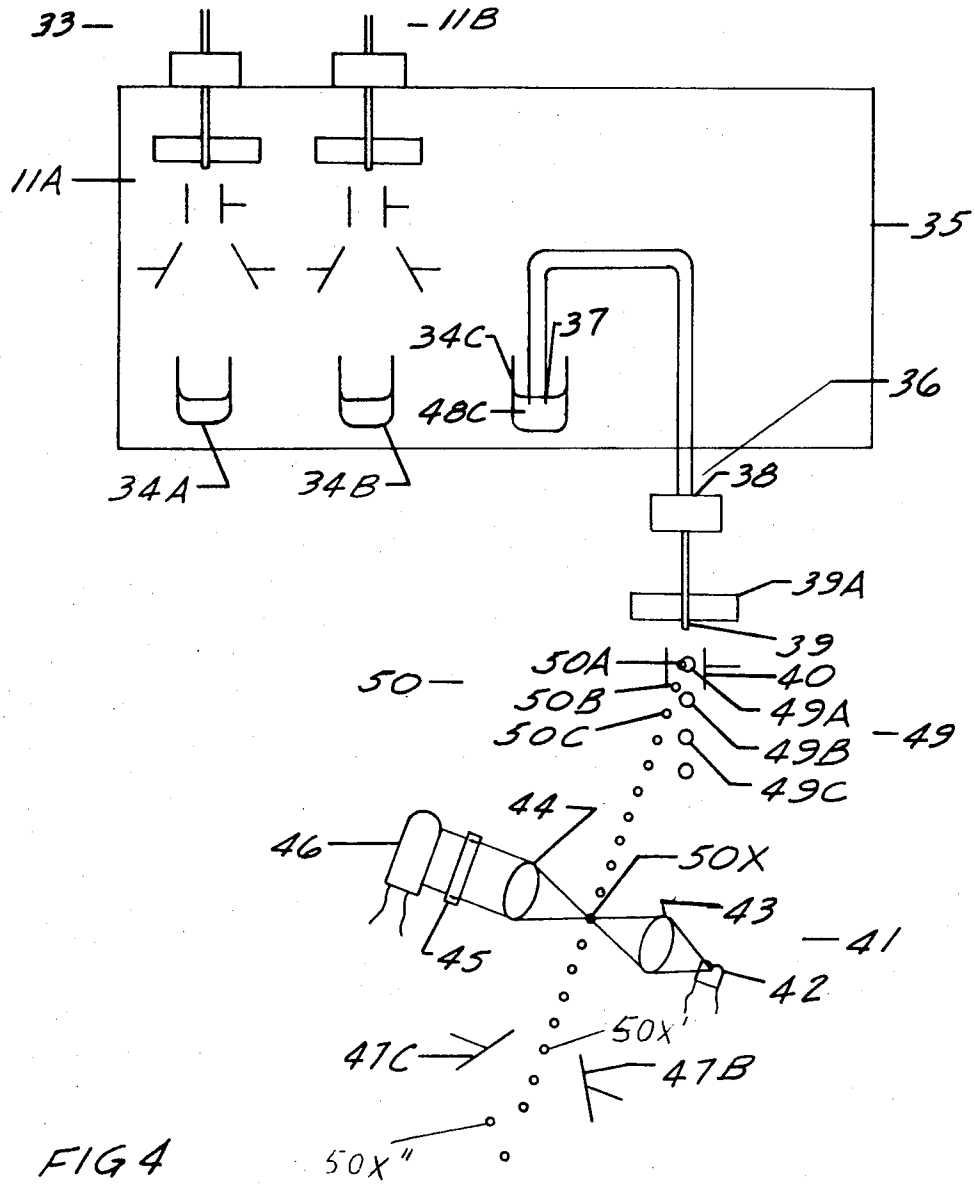


FIG 4

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APPARATUS FOR COLLECTING AND DISPENSING LIQUIDS AND FOR PARTICLE COUNTING

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ABSTRACT OF THE DISCLOSURE

Method and apparatus for collecting analytical samples, particularly blood samples, for centrifuging these samples and separating them into two or more fractions and for dispensing the sample or sample fraction into two or more aliquots comprising a sample collector in the form of a body means having generally central capillary tubing which is generally separable into two or more parts, one of which can be manually or automatically fitted as an integral part of tubing which feeds liquid including sample or sample fraction to a drop generator means wherein sample or sample fraction is dispensed into one or more aliquots. Means are also provided for incorporating this sample dispensing system in apparatus for chemical analysis by molecular absorption spectroscopy, molecular fluorescence spectroscopy, and other analytical techniques. Means are also provided to enable a sample to be diluted or reacted as necessary with diluent or reagent preferably using drop generator means and dispensed as a stream of drops preferably of much smaller diameter than the diameter of the drop generator means and passed through particle counting and separating means for accurate particle counting and separating.

This invention relates to new and improved method and apparatus for collecting, separating and dispensing analytical samples into two or more aliquots and, more particularly, for use in apparatus for chemical analysis of blood serum and other clinical samples and for counting particles in blood and such other samples.

It has been very common to collect blood serum samples by drawing about 5 ml. of blood from the patient into a syringe, emptying the contents of the syringe into a container, allowing the blood to clot, placing the container in a centrifuge and centrifuging the sample, then pouring off the serum into a further container and dispensing aliquots from this container.

This type of blood serum sample collecting and separating technique has important disadvantages including:

(a) The sample is stored at various stages in three or more containers, including a syringe, a collection tube and a dispensing tube, thus increasing the possibility of contamination and of causing sample loss due to sample adhering to the walls of the containers;

(b) The sample transfer process is time consuming and hence expensive.

A number of dispensing techniques have been described. For example, with a syringe type dispensing unit, the inlet tube of a syringe is manually or automatically dipped into the cup, sample is sucked into the syringe cylinder and then ejected through an exit tube into a reaction vessel or other desired container.

The disadvantages of the construction and method of operation of such prior art syringe dispensers are many and significant and include:

(a) The sample comes into contact with a relatively large surface area leaving a film of sample (or washing liquid if used). This film will contaminate the first part of the next sample, which must then be rejected to waste, with consequent loss of sample;

(b) If it is desired to change the volume dispensed it is necessary to physically change the syringe setting. This requires mechanical skill, and is time consuming;

(c) A syringe is a mechanical device with moving parts, and is susceptible to wear and breakdown;

(d) A drop of sample can readily lodge on the end of the inlet tube or the end of the exit tube. Any variation in volume of this drop will cause error.

An alternative dispensing technique is the peristaltic pump technique. Sample is sucked into tubing and pumped along tubing using a peristaltic pump to maintain liquid flow. This tubing can then be branched into further tubing of different diameters causing the sample to be segmented into aliquots and passed along these various tubing as selected.

The disadvantages of the construction and method of operation of such prior art peristaltic pump dispensers are also many and include disadvantage (a) of the syringe dispensing technique as well as:

(e) If it is necessary to change the volume dispensed, it is necessary to physically change pump tubing;

(f) A peristaltic pump is also a mechanical device with moving parts, susceptible to wear and breakdown;

(g) Peristaltic pump tubing can stretch with age causing drift in analytical results;

(h) Peristaltic pump tubing must be elastic, not readily permanently deformed and resistant to a wide range of solvents. There is no tubing material at present which fulfills all these requirements.

It will also be readily appreciated that methods and apparatuses for manual or automatic chemical analysis using these dispensing techniques such as previously known apparatus for automatic blood serum analysis will include the disadvantages of their respective dispensing technique.

Several particle counting techniques are described in the prior art, particularly for cell counting in hematology. The prior apparatuses most commonly used for cell counting use electrical and optical cell counting techniques, respectively. In the electrical technique, the diluted or treated blood sample is pumped between two electrodes placed in a capillary tube. As a cell passes through the tube, it alters the resistance between the electrodes, giving a current pulse, and the total number of pulses is a measure of the number of cells per unit volume. In the optical technique, the diluted or treated blood sample is pumped along a capillary tube which is irradiated with appropriate radiation. As the cell passes through the tube, it scatters radiation giving a pulsed absorption and/or scatter signal. The number of these radiation pulses is counted, giving a reading related to the number of cells per unit volume.

The disadvantages of these prior art apparatus are many and significant and include:

(a) The blood sample must be accurately dispensed into a diluent or lysing solution and an apparatus using syringe or peristaltic pump sample dispensing techniques with their before mentioned disadvantages;

(b) In order to accurately count particles it is necessary to isolate them from other particles and this is achieved in prior apparatus by passing the sample solution through a narrow aperture at the electrical or optical detector. This aperture should be as narrow as possible to give efficient particle isolation but its size is limited in practice by the necessity to avoid frequent blocking. The aperture is typically about ten times the particle size and this is too high to give highly accurate counting.

Accordingly, it is an advantage of this invention to provide an appaartus in which small volumes of blood can be collected, centrifuged, and the serum separated

from red cells and dispensed into one or more aliquots using substantially only one sample collecting container.

Another advantage of this invention is to provide an apparatus in which an analytical sample held by capillarly action in a container can be manually or automatically inserted into a dispensing unit such that the sample forms part of a column of liquid comprising partly washing liquid so that the sample can be dispensed into one or more aliquots with minimum sample contamination and loss and with the dispensing unit washed in substantially the same operation.

Another advantage of this invention is to provide an apparatus in which an analytical sample in a container can be inserted into a dispensing unit such that the sample forms part of a column of liquid including a wash liquid and such that the sample containing section of the column provides a small fraction of the total resistance to flow so that changes in sample viscosity have a very small effect on liquid flow rate and hence dispensed volume.

Another advantage of this invention is to provide an apparatus for manual or automatic chemical analysis by molecular absorption spectroscopy, molecular fluorescence spectroscopy, and other techniques using the before mentioned sample dispensing technique.

Another advantage of this invention is to provide an apparatus for particle counting in which the liquid sample is converted into a fine stream whose diameter does not greatly exceed that of the largest particles being counted but without the liquid stream passing through a physical restriction of about the same dimension as this stream.

Another advantage of this invention is to provide an apparatus for particle counting in which the liquid sample is converted into a stream of small drops, each of which is not much larger than the largest particles being counted, and which will only very rarely contain more than one particle, and which is particularly suited to accurate particle recognition by optical techniques such as molecular absorption, scatter, optical scanning and holography.

Another advantage of this invention is to provide an apparatus for accurate semi-automatic or automatic blood cell counting.

Other advantages of the invention will be apparent from consideration of the following specification.

In order to portray the nature of the present invention, particular embodiments thereof will now be described by way of example and illustration only. In the following description reference will be made to the accompanying drawings in which

FIG. 1 is a diagrammatic cross-sectional view of a new and improved apparatus for collecting blood serum and other samples and for centrifuging and storing these samples.

FIG. 2 is a diagrammatic cross-sectional view of an apparatus for dispensing selected volumes of samples directly from part of the apparatus of FIG. 1.

FIG. 3 is a diagrammatic cross-sectional view of an alternative embodiment of the apparatus of FIG. 2.

FIG. 4 is a diagrammatic cross-esctional view of an apparatus for particle counting and separation.

Referring now to FIG. 1 of the drawings, the sample collecting, centrifuging and storing apparatus consists of two parts, a collecting device 1 and a storing and centrifuging device 2. The collecting device 1 is of convenient shape, for example, that shown in FIG. 1, with a flange 3 to facilitate handling and an elongated portion 4 which readily fits into device 2. In the elongated portion 4 of device 1 a generally central capillary tubing 5 of sufficient length and diameter to support a reasonable volume of sample such as about 50–200 microliters, typically about 100 microliters by capillary action is present. Thus device 1 is typically about 4–6 cm., preferably about 5 cm. long and the capillary tubing 5 is typically about 0.03–0.05 cm., typically about 0.04 cm. in diameter. The collecting device 1 is bevelled at both ends 6 and 7 to facilitate close fitting into the apparatus of FIGS. 2 and 3.

The other device 2 for storing and centrifuging is also of convenient shape for handling, for example, as shown in FIG. 1. It contains a generally central cylindrical hole 8 into which device 1 readily fits. For blood samples in clinical chemistry device 2 is designed so that the free space 9 below device 1 is of sufficient volume to contain all packed or clotted red cells after centrifugation.

In operation, device 1 only is dipped into the liquid sample so that sample is driven into tubing 5 by capillary action and, if necessary, gravity. Device 1 is then fitted into device 2 and, with blood sample in clinical chemistry, the assembly is placed in a centrifuge and the sample centrifuged. This causes red cells to move into space 9 leaving serum or plasma only in tubing 5. The two parts are separated and device 1 stored in further containers if necessary to prevent evaporation or fitted into the apparatus of FIG. 2 or 3. With samples not requiring centrifuging, device 2 can be conveniently used as a holder for device 1.

Both devices are fabricated from simple inexpensive material, say, polyethylene and may be easily discarded after use.

Referring now to FIG. 2, this shows an embodiment of an apparatus for dispensing samples directly from device 1 of FIG. 1. The apparatus consists of a pumping device which causes washing liquid to flow through tubing 5 at a constant rate, which pumping device is represented generally by 10, collector 1, and a dispensing unit represented generally by 11. Device 10 consists of a hollow barrel 12 of convenient shape with a nozzle 13, which can fit snugly into the bevelled entrance 6 to tubing 5. A plunger 14 moves within a cylindrical bore 15 containing wash liquid 16. Wash liquid 16 enters the cylinder 15 through an enrance port (not shown) and can leave through small exit ports 17 and 18 in the cylinder 15 or through tubing 5 and the dispensing device 11. Device 1 rests loosely in a hole 19 in a supporting mount or table elastically mounted to the supporting structure of the apparatus. The lower end of this device, 7, fits snugly into the bevelled surface 20 of dispensing device 11. This latter device consists of a mounting device 21 through which a further capillary tube 22 forms a needle 23 at the lower extremity. The needle can be vibrated at an appropriate frequency, say, 4 kHz., by any one of several alternative mechanisms, so as to cause any emerging liquid to form a stream of uniformly sized drops. This can be achieved by, for example, mounting needle 23 on a piezoelectric crystal and applying an AC voltage to the crystal, or by attaching the needle to the cone of a loudspeaker, or by attaching a magnet to needle 23 and sequentially attracting and repelling it with one or two AC driven electromagnets. In FIG. 2 a piezoelectric crystal 23A is depicted to effect vibration of needle 23. This dispensing device further consists of a charging electrode 24, which can be charged at any reasonable voltage between, say −500 v. and +500 v., and deflecting electrodes 24A and 24B, maintained at high positive and negative potentials, respectively, say, ±3 kv.

In operation, device 1 is placed in hole 19, and is then manually or automatically placed between devices 10 and 11. Device 10 is lowered so that nozzle 13 fits into entrance port 6, and then presses device 1 so that it in turn fits into entrance port 20. When sufficient pressure has been exerted to form pressure tight seals between the three devices plunger 14 descends, pumping wash liquid initially through ports 17 and 18, and then, when these ports are closed by the descending plunger, through tubing 5 and 22 to the atmosphere. Thus the sample followed by wash liquid is pumped out through the vibrating needle 23 where the liquid stream is converted into a stream of uniformly sized drops by the technique set forth above.

Thus, these drops can be charged at the desired voltage V_1, V_2, etc., applied between the liquid stream and charging electrode **24** so that drops are deflected by deflecting electrodes **24A** and **24B** as required to waste and into container(s) **26A**, **26B**, etc. The first part of the sample dispensed is usually contaminated by previously dispensed washing liquid and is deflected to waste. The bulk of the sample is uncontaminated and can be dispensed into container(s) **26A**, **26B**, etc., for subsequent addition of reagent, incubation, etc., in various apparatus for chemical analysis. Liquid dispensed thereafter will be either surplus sample, sample contaminated with wash liquid, or wash liquid, and these are deflected to waste.

It will be noted from the above description that sample and wash liquid are dispensed in substantially the same operation, that the sample passes along a very short length of tubing, typically about 7–15 cm., preferably about 10–11 cm., with minimal opportunity for contamination, and that the volumes of sample dispensed are electronically selectable.

Alternatively, the same result can be achieved by using the pressure pumping mechanism **10'** shown in FIG. 3 instead of device **10** of FIG. 2.

Referring now to FIG. 3, the washing liquid **15** is placed in vessel **27** and maintained under an appropriate pressure such as about 0.7–1 kg. wt. per sq. cm., preferably 0.8 kg. wt. per sq. cm. by gas, such as nitrogen, entering through tubing **25**. Liquid can leave vessel **27** through tubing **29** and pass through solenoid **30**, through further tubing **31** of device **10'**, tubings **5** and **22**, a second solenoid **32** to dispensing device **11**.

The tubings containing wash liquid **29** and **31** are of sufficiently narrow internal diameter, say 0.007–0.03 cm., typically 0.01 cm. and length of say 12–18 cm., typically 15 cm., or of sufficiently rough interior, for example, fitted with several gauze screens to obstruct liquid flow, so that the major resistance to flow occurs in this tubing.

Also, the dispensing needle **23** or other tubing through which the sample flows optionally has an irregular internal surface, for example, it could be slightly indented at, say, 6 places along its length so that sample flow is turbulent rather than laminar. The sample dispensed from needle **23** or other tubing then passes into a drop generator (not shown in FIG. 3) similar to that described in FIG. 2.

In operation, collector **1** is placed in hole **19** as before, pumping device **10'** is lowered so as to form a pressure tight seal between devices **10'**, **1** and **11**. Solenoid **30** is opened to allow pressure of build up along tubings **31** and **5**, then solenoid **32** is opened allowing sample then wash liquid to be dispensed as above. The fact that tubings **29** and **31** gives rise to the major part of total resistance to flow prevents variations in sample viscosity from greatly affecting liquid flow rates. For example, if the only resistance arose from laminar sample flow, the Poiseuille equation for flow through a cylinder would apply, and an increase of, say, 25% in sample viscosity would cause an about 25% drop in sample flow rate. If, however, a sample requiring, say 0.07 kg. wt. per sq. cm. net pressure to cause a flow rate of 1 ml. per minute is placed in series with a column of wash liquid of constant viscosity requiring 0.7 kg. wt. per sq. cm. net pressure to maintain the same flow rate, then a 25% change in sample viscosity would cause an about 2% change in flow rate.

Also, the irregular internal surface of the dispensing needle will prevent laminar flow, and the Poiseuille equation will no longer apply. If this tubing can be indented or otherwise treated so that it is hydrodynamically equivalent to a large number of small cylinders, say ten, placed in series then flow rates are almost independent of viscosity.

If desired, the mounting device **21** including tubing **22** can be placed between the collector **1** and solenoid **32** such that the sample also flows through this tubing. This has the disadvantage of giving the sample a greater length of tubing to pass through, and hence increasing washing problems, but the advantage of allowing a reasonable volume of wash liquid to pass through tubing **5** before the sample passes through, so that any surging or instability associated with the starting of liquid flow will affect the dispensing of wash liquid rather than of the sample.

Further, the same results could be achieved by using a collector **1** fitted with a solid piston, and using the pumping mechanism **10'** to mechanically push the piston down the collector tube **5** (by mechanically moving the whole pumping unit). The dispensing needle **23** could then be washed out by inserting an unused collector **1** without the solid piston, and pumping solvent through the needle **23** as above.

There are several optional variations on these apparatus. For example, if collector **1** does not hold sufficient sample for particular analyses, it can be fitted with multiple tubes **5A**, **5B**, **5C**, etc., each of which can support a reasonable volume of sample, say, 50 microliters by a capillary section. Thus with ten tubes **5A**, **5B**, **5C**, etc. to **5J** the collector can hold 0.5 ml.

FIG. 4 illustrates a further embodiment of the present invention which enables accurate particle counting and particle separation. Referring now to this figure, the apparatus typically consists of a reagent or diluent dispensing unit, represented generally by **33**, typically consisting of one or more reagents in flasks, and which can be pumped under pressure to a dispensing unit **11A**, a sample dispensing system **11B**, each of the type shown **11** in FIGS. 2 and 3, mixing or reaction vessels **34A**, **34B**, etc., and a further dispensing mechanism, generally represented by **36**, typically consisting of a mechanically driven inlet tube **37**, a solenoid **38** and a dispensing needle **39**. The apparatus is further fitted with an optical detector system, generally represented by **41**, which could consist of a light source **42**, focussing lens **43**, condensing lens **44**, optical filter **45** and photodetector **46**. This is an absorption type system, but optical detection and recognition systems based upon measurement of scatter, optical scanning and holography may also be used.

The apparatus is operated as follows when it is decided to count the number of particles per unit volume in a given sample, for example, the number of red blood cells per cubic millimeter in blood. The reagent dispensing unit **33** is used to dispense a given volume, say 1 ml. of diluting fluid into vessel **34A**, using drop generating technique with dispensing unit **11A** described above in FIG. 2 with charging electrodes **24** and deflecting electrodes **24A** and **24B**. Vessel **34A** is then moved mechanically to a position below the sample dispensing system **11B** where the required volume of sample is added to the vessel. This sample mixes with the reagent, or is stirred mechanically if necessary, and the vessel is moved to a further dispensing position (vessel **34C**, FIG. 4), where an inlet tube **37** is mechanically lowered into vessel **34C**. The chamber **35** is maintained at a sufficient pressure, say, 0.35 kg. wt. per sq. cm. such that liquid in vessel **34C** can be pumped to needle **39**, which can be vibrated so as to form appropriately sized drops. After inlet tube **37** is immersed in the sample-diluent mixture **48C**, solenoid **38** is opened allowing the mixture to flow through needle **39** equipped with vibrating means, such as a piezoelectric crystal **39A**. The needle is preferably vibrated at a frequency such that two streams of drops are formed, a primary stream **49**, consisting of drops **49A**, **49B**, **49C**, etc., whose diameter is approximately equal to the exit diameter of needle **39**, and a subsidiary stream **50**, consisting of drops **50A**, **50B**, **50C**, etc., whose diameter is much less than the exit diameter of the needle **39**. These two streams can be formed by vibrating the needle at frequencies other than those giving maximum instability of the liquid stream emerging from needle **39**. The exit diameter of needle **39** can thus be selected such that it is much larger than the largest particle passing through it, say, 100-micrometer for blood cell counting, and the diameter of drops **50A**,

50B, 50C, etc., can be chosen such that any one drop will contain no more than one of the particles being counted, say, 10 micrometers for counting 10-micrometer diameter white cells in blood.

Thus any one drop 50A, 50B, 50C, etc., will either not contain the particle of interest, or it will contain this particle, and drops containing particles will contain very little extraneous material. This greatly simplifies the problems of particle recognition.

The apparatus of FIG. 4 with further minor additions can be used for particle separation. A charging electrode 40 is placed below needle 39 and maintained at a sufficient potential, say, +400 v., to induce a charge in all drops leaving this needle. Some or all of these drops pass through the optical detector system which can sense the presence of unwanted particles. The stream then passes between two electrodes 47B and 47C, which can be pulsed at a high positive and a high negative potential, respectively, say, ±1,000 v. When the optical detector system senses the presence of the particle type to be separated in, say drop 50X, FIG. 4, electrodes 47B and 47C are switched from zero potential to plus and minus 1,000 v., respectively, when particle 50X reaches the position shown as 50X'. This drop is then deflected by the electric field (drop 50X") and collected in a separate container, thus achieving the desired separation.

While particular examples of the application of the principles of the present invention have been described, it will be appreciated that many modifications are possible without departing from the scope of the present invention.

What is claimed is:

1. A method for dispensing discrete analytical liquid sample contained as blood liquid columns free of sediment in a capillary collector tube comprising fitting and sealing said capillary collector tube containing said liquid column between a pumping device including a column of wash liquid and a drop generator means, pumping said wash liquid under pressure to force said analytical liquid sample out of said capillary collector tube followed by said wash liquid without loss or leakage into and through said drop generator means wherein a selected volume of said analytical sample is dispensed into a container and said analytical sample contaminated with said wash liquid and said wash liquid is dispensed to waste.

2. The method claimed in claim 1 wherein the resistance to liquid flow in said column of wash liquid is the major part of the resistance to flow of the wash liquid and the analytical liquid sample, thereby preventing variation in analytical liquid sample from greatly affecting liquid flow rates.

3. A method for chemical analysis of a liquid analytical sample collected in a container in accordance with claim 1 wherein selected volume of reagent is mixed with said collected liquid analytical sample and physical properties of the product of the reagent and sample are analyzed.

4. An apparatus for dispensing analytical liquid sample comprising a capillary collector tube which is bevelled at both ends, said capillary collector tube being removably fitted and sealed between a pumping device including a column of wash liquid and a drop generator means, whereby said analytical liquid sample can be pumped followed by said wash liquid through said drop generator for collection of said analytical liquid sample in a container while dispensing said analytical liquid sample contaminated with said wash liquid and said wash liquid to waste.

5. In a capillary tube apparatus for collecting, storing and centrifuging in a capillary collector tube discrete analytical samples to separate liquid from sedimentary phases the improvement which comprises providing for said capillary collector tube a capillary collector tube holder means in which said capillary collector tube readily fits and from which it is readily removable, there being present in said capillary collector tube holder a gap of free space below the lower extremity of said capillary collector tube to permit collection of sediment therein upon centrifugation.

6. The capillary tube apparatus claimed in claim 5 wherein said capillary collector tube is bevelled at both ends, thereby facilitating fitting and sealing at both ends in a dispensing apparatus.

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U.S. Cl. X.R.

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