

April 23, 1974

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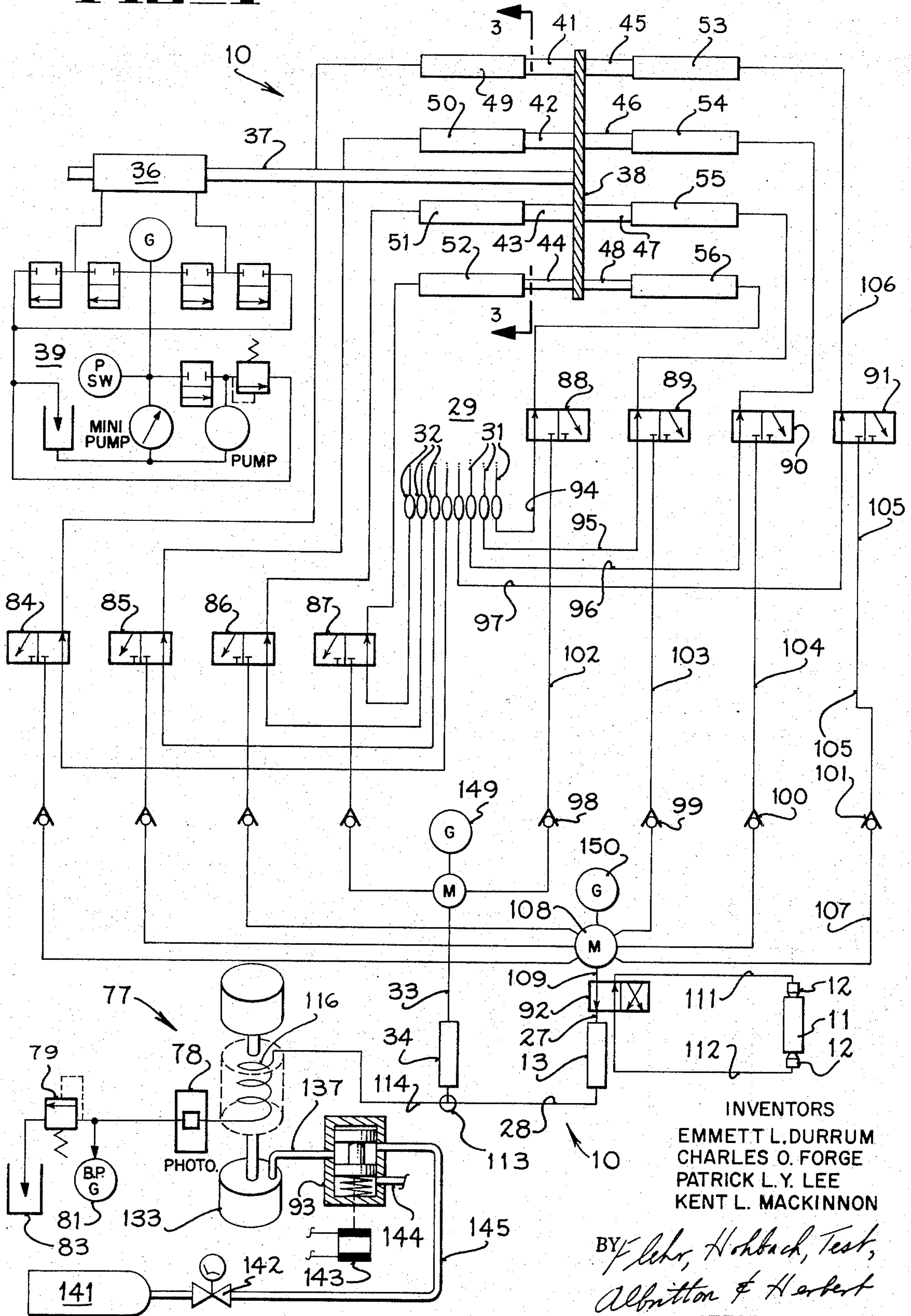
3,806,321

FLUID SAMPLE ANALYSIS SYSTEM

Filed Sept. 2, 1971

2 Sheets-Sheet 1

FIG 1



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2 Sheets-Sheet 2

FIG 2

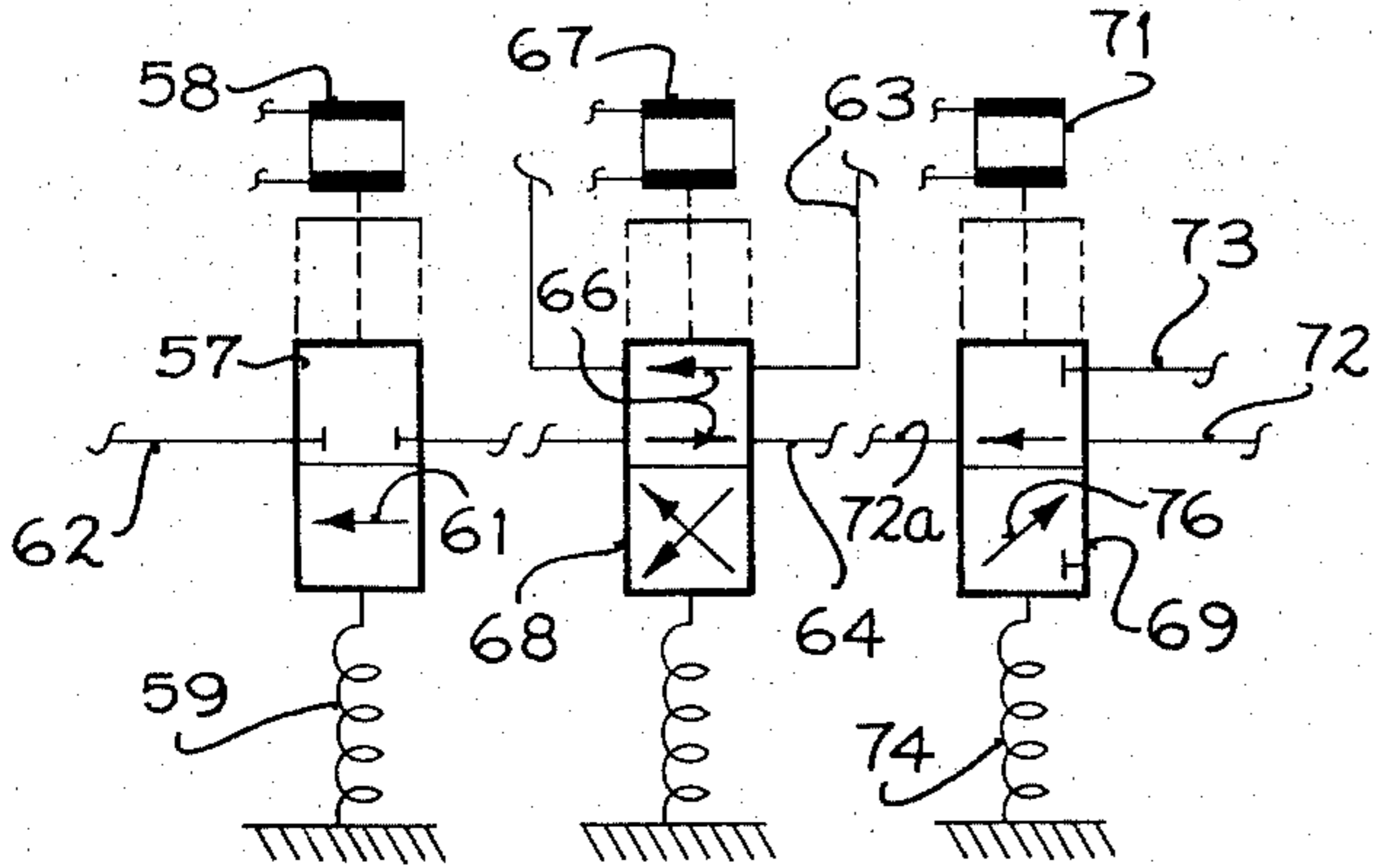


FIG 3

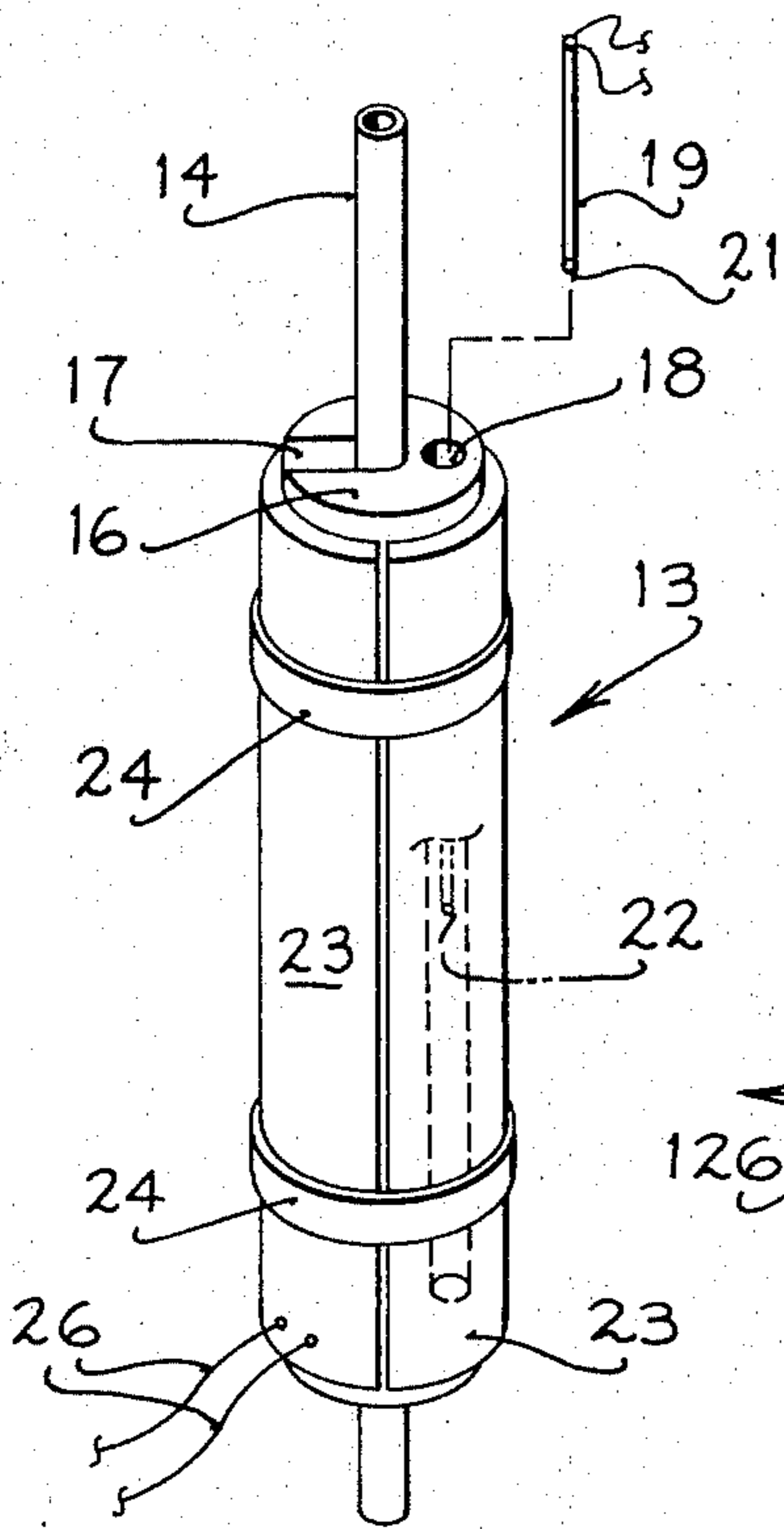
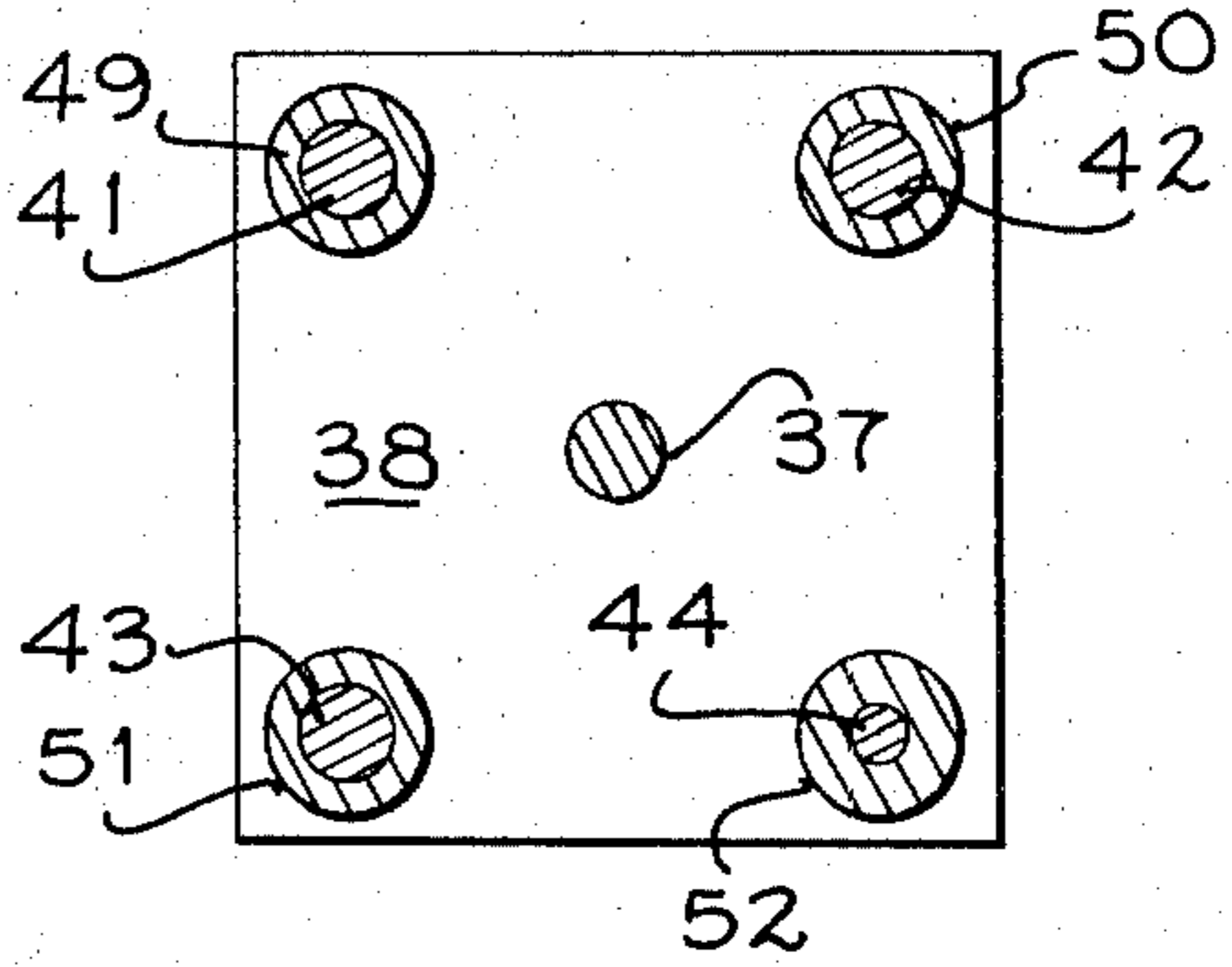


FIG 4

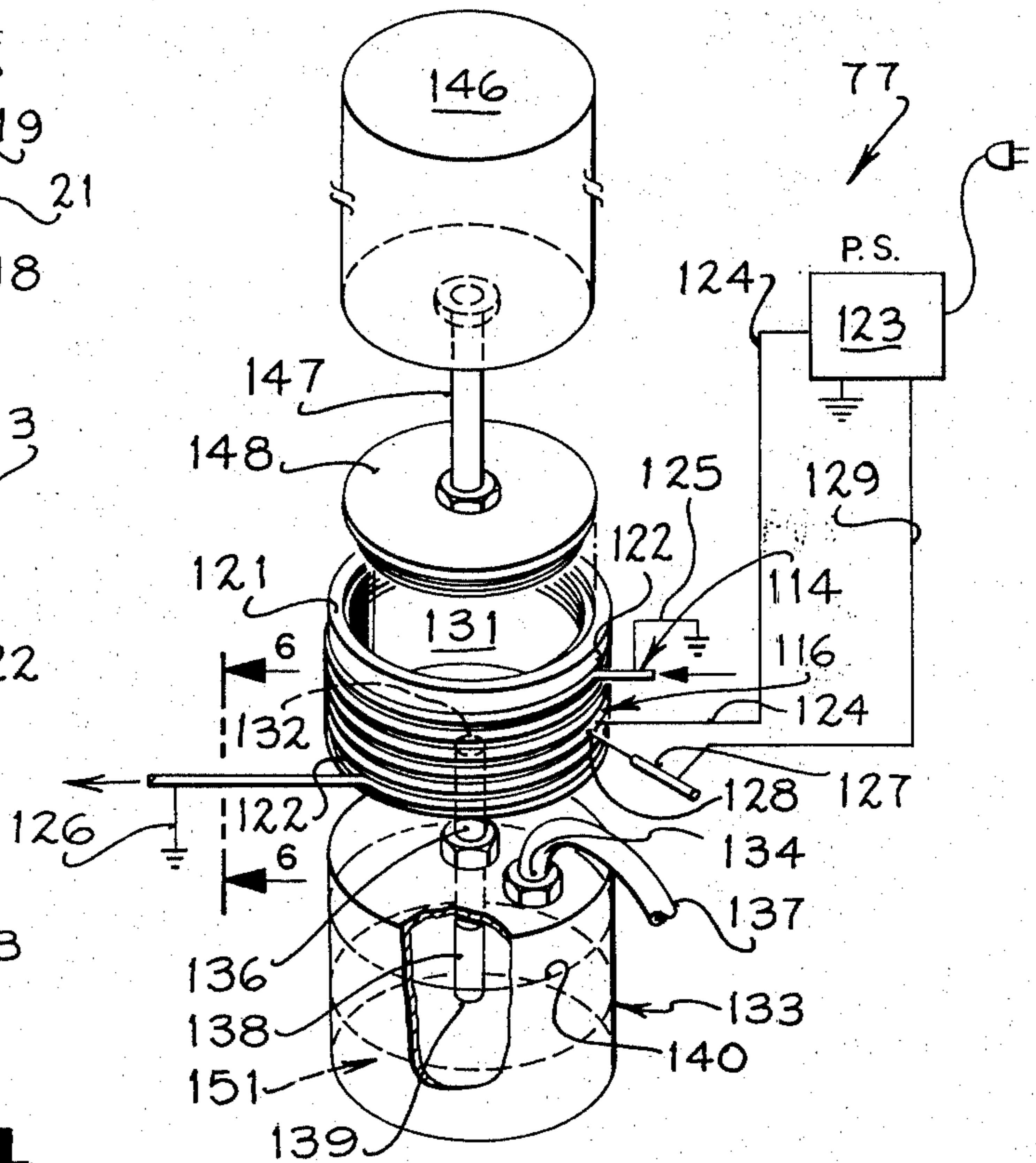


FIG 5

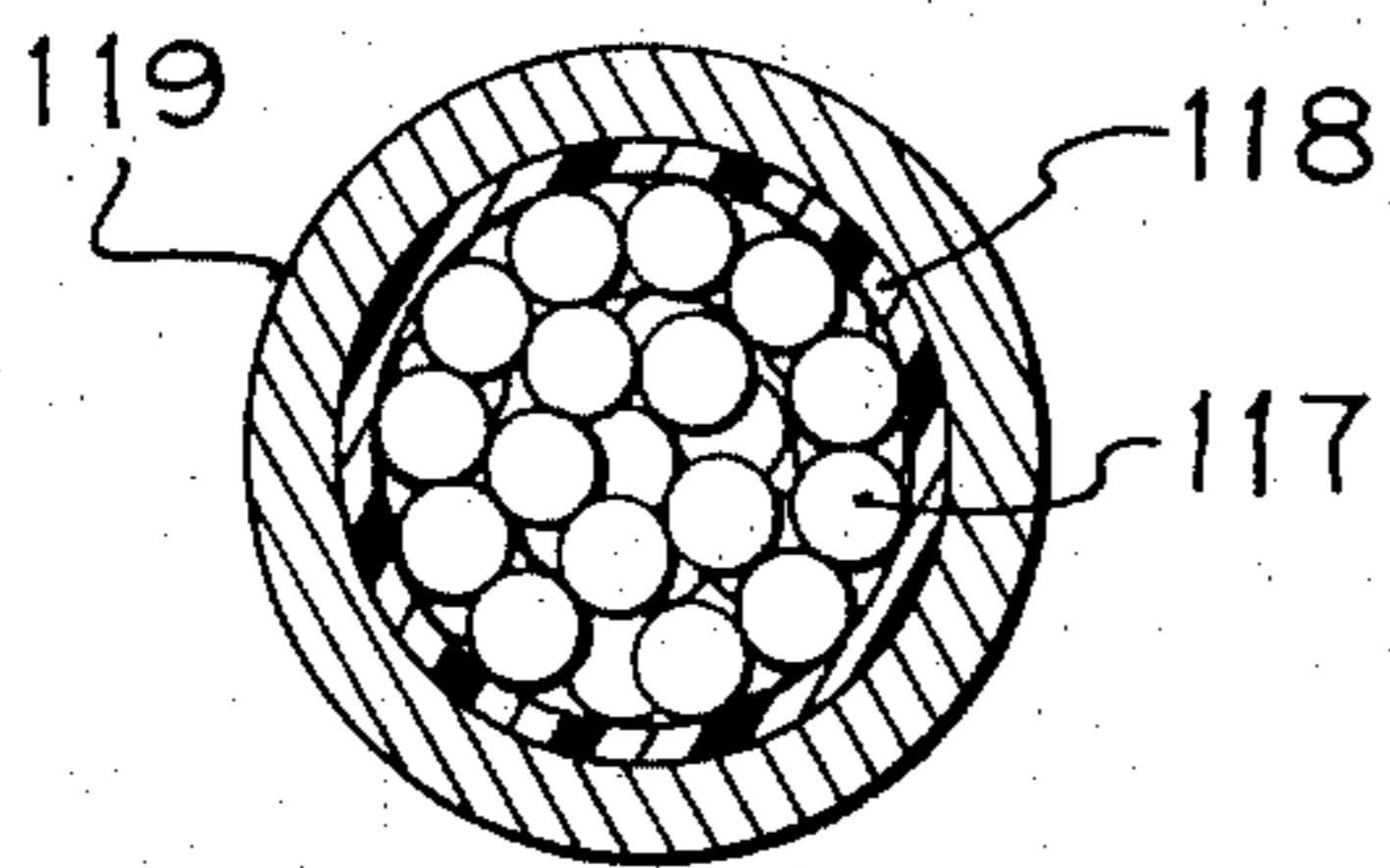


FIG 6

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1

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FLUID SAMPLE ANALYSIS SYSTEM

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U.S. Cl. 23—253 R

15 Claims

ABSTRACT OF THE DISCLOSURE

A system for analyzing the constituents of liquid sample of a type wherein buffers or other sample carrier liquid are applied to the sample characterized by an ion exchange column having input and output flow passages for passing the sample and buffers therethrough together with means for supplying sample and buffer to the input flow passage of the column under substantial positive pressure. The column contains ion exchange material for separating the sample constituents on a time basis. A reagent supply line under substantial positive pressure discharges reagent into the eluent of the ion exchange column for mixing reagent and eluent. Further, means in the reagent supply line provide a back pressure resistance to passage of fluid therethrough substantially matched to the back pressure resistance provided by the ion exchange column.

This system is further characterized by means for mixing the eluent and reagent in a manner which serves to intermingle reagent and eluent by lateral deflections of their travel along their flow paths while inhibiting axial extension of portions of the body of mixed material traveling along the flow path so as not to upset the timed relation of the different successive constituents of sample (separated out of the ion exchange column at different times). In addition, the system is further characterized by a reaction coil in the form of a length of tubing disposed in heat transfer relation about a support element adapted to receive an emergency supply of coolant, the tubing being arranged to be electrically heated in order to heat the contents thereof to provide reaction between eluent and reagent therein.

BACKGROUND OF THE INVENTION

This invention pertains to a fluid sample analysis system for analyzing the constituents of a liquid sample of a type wherein buffers or other sample carrier liquids are applied to the liquid sample and a proportionate amount of reagent is mixed with the buffered sample whereby suitable means, such as an electrophotometer device using principles of chromatography can provide indications identifying the constituents of the liquid sample. The system is particularly useful in separating the components of a mixed amino acid sample using a cation exchange resin by sequential elution.

Heretofore, the separation of the constituents or components of a mixed amino acid sample has been accomplished by feeding the sample to a column of ion exchange resin composed of materials, such as a fixed matrix of divinyl benzene cross-linked polystyrene or the like, to which is attached a negative functional group, commonly sulphonic acid. The sample is typically puddled on top of the resin column and then buffered sequentially by a number of buffering solutions or other sample carrier liquids. The sulphonic group of resin attracts the positively charged amino acids, each of which has a characteristic affinity thereto. Thereafter, the individual amino acid components are sequentially eluted from the ion exchange column and thereby appear in inverse order to the degree of resin affinity for each component. The eluent

2

is then typically reacted with ninhydrin reagent to form a compound which absorbs light in a predetermined wave length region. A prior system is arranged to direct the mixture through a flow cell past a colorimeter detector (for example, a photocell) which, when used with selected optical filters, is quantitatively responsive to that particular wave length.

The colorimeter detector or other electrophotometer is connected to a recorder which plots the response on a suitable medium, as on a chart. Quantitation is performed by time integrating each peak of the chart, each peak having a characteristic position and configuration dependent upon the amino acid type thereof.

The foregoing technique has characteristically required an excessive amount of time, for example, on the order of several hours for protein hydrolyzates, and even longer for physiological fluids.

Thus, there has been a need for a system wherein the constituents of the sample can be separated at a much faster rate without sacrifice of reliability.

SUMMARY OF THE INVENTION AND OBJECTS

In general, a system for analyzing the constituents of a liquid sample wherein buffers or other sample carrier liquids are applied to the sample has been provided to include an ion exchange column having input and output flow passages for passing a sample and buffers therethrough. The column contains a flow impeding material serving to provide, at the input flow passage, a substantial back pressure resistance to fluid flow therethrough. In addition, means for supplying sample and buffer to the input flow passage under substantial positive pressure serves to feed buffered sample through the column. A reagent is supplied to the eluent of the column via a reagent supply line also under substantial positive pressure. The reagent supply line includes means for providing a back pressure resistance to fluid flow therethrough in a manner to be substantially matched to the back pressure resistance to fluid flow through the ion exchange column as measured at its input end.

Since fluid compressibility and fluid viscosity can each be widely different between the two subsystems which respectively include the reagent and ion exchange columns, the term "matched" is used above and herein in the sense that back pressure resistance of the reagent flow is established such that, at a given piston velocity of rod 37 (see below) the product of volume compressibility factor times back pressure is maintained approximately equal for both the reagent and buffer paths.

Finally, means for mixing the reagent and eluent for analysis further downstream in the system have been provided.

Preferably, the means for mixing reagent and eluent comprises a means forming a flow path to receive both the reagent and eluent and means in the flow path for intermingling reagent and eluent by lateral deflections of their travel along the flow path while simultaneously inhibiting axial extension of portions of the body of mixed material traveling along the flow path. In addition, in supplying the sample and buffers to the ion exchange column and reagent to its supply line under substantial positive pressure a positive displacement means supplies buffer and sample to the ion exchange column and reagent to its supply line in a fixed ratio by volumetric flow rate while applying substantial positive pressure to the column and impeded reagent path, the back pressure resistance to fluid flow through both the supply line and the ion exchange column remaining substantially matched as noted above.

In addition to the above, and according to the invention, the system further includes a reaction coil for re-

ceiving both the reagent and eluent of the ion exchange column in a manner whereby they will be mixed with considerable lateral interaction and intermingling while axial extension of the body of mixed material will be inhibited. In this manner, short "blocks" of mixed material will travel through the system one after another in isolation of each other so as to be positively identified by the electrophotometer means located in the flow path. In addition, as each block of mixed material passes through the reaction coil provided herein, the mixed material will be thoroughly and effectively heated due to the lateral deflection of the material while means have also been provided for immediately quenching an overheated block of mixed material in the event of power failure, blockage of flow of material in the system, or other conditions which could cause the material to be heated for too long a period in the reaction coil as might cause polymerization of the mixed material.

In general, it is an object of the present invention to provide an improved system for analyzing constituents of a liquid sample and particularly to provide such a system in which a great number of fluid samples can be analyzed over a relatively short period of time.

Another object of the invention is to provide an improved means for handling a series of buffered sample portions in succession free of axial mixing therebetween so as to promote a "block flow" of each buffered sample portion through the system.

Another object of the invention is to provide an improved reaction coil of a type wherein each block of buffered sample is heated by novel heating means and free of danger of overheating for prolonged periods.

The foregoing and other objects of the invention will become more readily evident from the following detailed description of a preferred embodiment.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 discloses, in diagrammatic form, a system according to the invention;

FIG. 2 constitutes an enlarged showing of the valving conventions employed in the diagram in FIG. 1 for purposes of explanation;

FIG. 3 shows a transverse section view of four syringe components employed in the system of FIG. 1 and figuratively taken along the line 3—3 of FIG. 1 for showing proportional diameters of the syringe plungers thereof;

FIG. 4 is a perspective view showing an ion exchange column of a type as employed in the system of FIG. 1 and also of a type suitable for utilization as a reagent column employed in the system of FIG. 1;

FIG. 5 is a perspective exploded view showing a reaction coil taken from the system of FIG. 1; and

FIG. 6 is a transverse section view taken along the line 6—6 of FIG. 5.

DESCRIPTION OF A PREFERRED EMBODIMENT

A system 10 for analyzing the constituents of a liquid sample of a type wherein buffers or other sample carrier liquids are applied to a liquid sample is shown in FIG. 1. A sample holding unit 11, such as a previously loaded vial open at each end and carried between fittings 12 at its ends, serves to dispose the liquid sample in a position whereby it can be injected into an ion exchange column 13. Preferably, the unit 11 includes inert previously packed material, such as boro-silicate glass beads of the order of 22 to 40 microns diameter, for providing flow resistance believed to promote "block" flow as described below.

Ion exchange column 13 basically consists of a length of very small tubing 14 having an internal diameter, for example on the order of 0.007 inch. Tubing 14 is packed with a suitable ion exchange resin of a type as used presently in the art.

The tubing column 14 is disposed within an isothermal metallic block 16 having a readily heat transmissive char-

acteristic, such as aluminum. Block 16 includes a slot 17 for receiving tubing 14 as well as a bored hole 18 extending a substantial distance along block 16 for receiving a thermister probe 19 whereby a thermister bead 21 can be disposed deeply into block 16 as shown by the phantom line position 22. In this manner, the temperature at that point in block 16 can be readily detected electronically.

Means for heating block 16 includes the electric heater blanket 23 which is held in its wrapped position by means of a pair of elastic rings 24. Conductive leads 26 may be used to connect an appropriate power supply for properly heating the isothermal block 16. The temperature of block 16 is readily transferred to tubing 14 by means of a heat conductive paste (not shown) of conventional type applied to the exterior of tubing 14. Ion exchange column 13, accordingly, is considered to include input and output flow passages 27, 28 at its upper and lower ends respectively (FIG. 1) for receiving and eluting liquid material.

A supply 29 of chemicals, such as buffers, distilled water, or reagents, such as ninhydrin, etc., are maintained in reservoirs (not shown) to be coupled to the system by lines 31 and suitable bubble traps 32, whereby a source of chemicals may be provided for the system operation as described further below.

Means are provided for withdrawing fluid from the chemical supply and for discharging same under substantial pressure, on the order of 2,000 to 3,000 p.s.i., to the input flow passage 27 of ion exchange column 13 and to supply a reagent, such as ninhydrin, along a supply line 33 to a ninhydrin column 34 (of a construction corresponding to that shown in FIG. 4 for ion exchange column 13). Both columns 13 and 34 are arranged to retain their previously packed material therein by suitable means, such as a five micron mesh stainless steel screen disposed at the bottom and top of the column so that each resin-filled column can be handled as a unit prior to assembly in the system without loss of its resin.

In lieu of resin, column 34 can employ other suitable inert pervious material, such as fine glass beads, of comparable size to provide the desired flow impedance.

The means for supplying the chemical liquids to the two columns 13, 34 in predetermined proportionate amounts includes a double acting hydraulic actuator 36 equipped with a piston rod 37 attached at its outer end to a drive plate 38 of suitable rigid material, such as steel. The hydraulic control system 39 serves to move drive plate 38 between advanced and retracted positions to the right and left as shown in FIG. 1.

Drive plate 38 is further attached to the plunger elements 41-48 disposed in opposed relation in pairs and distributed uniformly around plate 38 as shown best in FIG. 3. However, for schematic purposes, the syringes 49-56 in FIG. 1 are arranged as shown for ease in explanation of operation of the system. Each plunger element moves in and out of a related cylinder in the manner of a syringe 49-56. Accordingly, as best shown in FIG. 3, it will be readily evident that the liquid displaced by the plunger elements in syringes 52, 56 will be somewhat less than the liquid displaced by the other syringe elements.

It is intended that the various syringe elements, depending upon the chemicals with which they are to be used, shall be of a selected displacement so as to supply a predetermined proportionate amount of liquid in a fixed ratio with respect to the liquid which is supplied by the other syringes.

The three schematic conventions used in FIG. 1 to show the valve activity for valves employed in the system is best explained with respect to the three examples, shown in FIG. 2. In FIG. 1 all valves are de-energized.

Accordingly, in FIG. 2, the left hand valve element 57 is shown in a position as would be represented when its associated solenoid 58 controlling same is in its de-energized state. A spring 59 is shown for moving valve element 57 to its de-energized position so as to block feed line 62.

Accordingly, when solenoid 58 has been energized, valve element 57 is drawn into a position so as to align its port, represented by the arrow 61, with a feed line 62. Upon de-energizing solenoid 58, spring 59 serves to move valve element 57 so as to interrupt the fluid path along line 62.

Having in mind the foregoing explanation of the left hand valve unit shown in FIG. 2, it should be readily evident that in the center valve unit of FIG. 2 a pair of supply lines 63, 64 can be arranged to carry fluid in the direction shown by the arrows 66. Upon energizing solenoid 67, the lower portion of the valve control unit 68 will move into the position previously occupied by the upper end of unit 68 so as to form a cross-over connection between the lines 63, 64.

The right hand example shown in FIG. 2 represents a valve element 69 operated by a solenoid 71 shown in its de-energized state. In that condition, the upper portion of the diagrammatic valve element 69 serves to form a fluid connection continuing line 72 while blocking off further passage of fluid in a line 73. On the other hand, energizing solenoid 71, serves to move valve element 69 upwardly against the force of spring 74 whereby a fluid connection is made from the left hand side of 72a of line 72 via the port represented by arrow 76 to connect 72a with line 73.

For convenience in illustration the movement of the valve elements described in regard to FIG. 2 is shown as moving vertically whereas in FIG. 1 some valve elements move vertically and others horizontally. Thus, it is to be understood that the elements are merely shown to move between advanced and retracted positions.

Means are provided for receiving the eluent from ion exchange column 13 and the discharge from ninhydrin column 34 for mixing these two bodies of liquid and reacting them by passage through a reaction coil assembly 77. The output from reaction coil assembly 77 is then fed to an electrophotometer or colorimeter device 78 of suitable construction for optically identifying the constituents of each successive "block" or body of mixed material eluted from coil assembly 77.

Finally, a back pressure relief valve 79 is disposed immediately downstream of a back pressure gauge 81 for establishing a desired degree of back pressure in the system sufficient to keep any bubbles in solution in the material in flow path 114 and coil 116. A collection vessel 83 serves to collect the overflow material passing through the system.

Suitable means, such as manually operated switches (not shown), of a conventional nature have been provided whereby each of the valves 84-93 may be operated to carry out the steps in various amino acid analysis runs or other analyzing runs on given liquid samples.

For example, if it is assumed that the hydraulic control system 39 serves to operate actuator 36 to drive piston rod 37 leftwardly as shown in FIG. 1, fluid material in each of the right hand four lines 31 leading from the chemical supply will be drawn into syringes 53-56 by virtue of the condition of the valves 88-91 positioned as shown. Then, during the reverse stroke, selected ones of valves 88-91 can be shifted under a suitable control program whereby the fluid material from syringes 53-56 will be discharged either to column 13 or 34 or (where valves 88-91 have not been shifted) cycled back to supply 29.

Thus, the discharge of syringes 53-56 occurs under movement of plate 38 and piston rod 37 to the right as shown in FIG. 1. At such time, it is to be understood that suitable known means serves to provide the programming and conditioning of valves 88-91 to their desired state so that only those chemicals which are desired to be discharged move either (via valve 88) to the reagent supply line 33 (via line 102, check valve 98 and a fluid manifold element M) or (via valves 89-91) to the ion exchange column 13 via manifold 108.

Syringes 49-52 function in the same manner as described

above relative to syringes 53-56 for both loading of chemical material and discharge thereof.

Assuming for the moment that the liquid in syringe 53 constitutes the first buffer to be applied to a liquid sample contained in sample holding unit 11, valve 91 will be conditioned leftwardly so as to transfer the material discharging from syringe 53 via line 106, line 105, check valve 101, line 107, buffer manifold 108, and subsequently via line 109 to valve element 92. In the event that the solenoid (not shown) associated with valve 92 is energized (see FIG. 2), the control element 68 (FIG. 2) will be disposed in a position whereby the buffer material will be discharged along the line 112, via fitting 12 to sample holding unit 11 where the liquid sample contained in unit 11 will be urged outwardly and upwardly along line 111 and transfer into input line 27 leading to ion exchange column 13.

Assuming that one of valves 88-91 has been energized to feed liquid to column 13 or 34, during the rightward advance of the drive plate 38, the liquid in the column will be under substantial pressure on the order, for example, of 2,000 to 3,000 p.s.i. and each of valves 88-91 may be variously operated in order to dispense predetermined amounts of chemical to be successively applied to the sample now moved to the top of ion exchange column 13 by the above described operation of valve 91. Pressure is relieved, however, whenever valves 88-91 are operated so as to simply return the chemicals to their source 29, i.e., recirculated between syringe and source.

The perviously packed resin material in ion exchange column 13 causes a flow impedance generating a substantial back pressure resistance to fluid flow through that particular column. It is readily evident that the longer the column extends, the greater will be the back pressure resistance to liquid flow therethrough.

It has been observed that a high degree of reliability and repeatability can be achieved in the results from a system of the above type by introducing a means in the reagent supply line 33 for providing a back pressure resistance to fluid flow therethrough substantially matched to or at a selected optimum relation with the back pressure resistance encountered at the input 27 of ion exchange column 13. In the present instance, a ninhydrin column of the type disclosed above is provided and packed with a flow impeding material which is substantially inert to the liquid employed in the system, for example, resins of the type typically employed in ion exchange columns. An objective in providing a flow-impeding ninhydrin column in addition to the ion exchange column in the system is to provide a suitable means for developing a back pressure resistance to fluid flow which is proportional to the applied volumetric flow rate. It is has been observed that an extended column packed with pervious material serves to inhibit fluid flow in such a manner, and the impedance of the flow through the ninhydrin column can be readily matched to that of the ion exchange column. Thus, the ninhydrin column 34 provides a means forming an impeded path for injecting a reagent into the flow path defined by line 114 while the hydraulic actuator and syringe arrangement provides a positive displacement means for supplying buffer and sample to the ion exchange column as well as reagent to the impeded path thus formed. The optimum flow impedance can be closely adjusted by employing the heater blanket 23 so as to vary the viscosity of fluid in column 34.

The fixed ratio of diameters of plunger elements 41-48 for the syringes shown clearly establishes a fixed ratio by volumetric flow rate for the supplying of the various liquids into the system. At the same time, a very substantial positive pressure is applied to the column. Means for mixing the eluent from column 13 and reagent from column 34 includes the T-connection 113 and flow path line 114 for receiving both the reagent and eluent. Means have been provided in the flow path 114 for intermingling

reagent and eluent by laterally deflecting their travel along flow path 114 while inhibiting axial extension of portions of each body or block of mixed material traveling along the flow path.

Flow path 114 further includes a spirally wrapped coil 116 having an internal diameter of the order of 0.030 inch and containing a perviously packed inert material, such as glass beads, having diameters in the range of between 0.007 to 0.019 inch, i.e., somewhat larger than in the sample holding unit 11, since their function in coil 116 is intended to permit mixing of fluid.

As best shown in FIG. 6, the cross section of coil 116 discloses small beads 117 of suitable inert material, such as glass contained within a tubing 118 of suitable inert material, such as tetrafluoroethylene resin as sold under the trademark Teflon, enclosed within a covering or sheath 119 of stainless steel having a heating resistance on the order of conventional heater wire elements.

A generally non-conductive but readily heat transmissive annular support element 121, such as an anodized aluminum cup, is formed with a helical groove 122 into which coil 116 is laid.

As noted above, the outer conductive covering 119 of coil 116 is characterized by a sufficient coefficient of electrical resistance so as to provide heating of fluid material within the tubing when electrically energized as by means of the variable power supply 123 connected to sheath 119 by means of lead 124 while both ends of coil 116 are grounded as, for example, at leads 125, 126. Lead 124 contacts sheath 119 substantially midway between its ground points 125, 126 so as to provide two parallel equally heated branches to the circuit branching from the mid-point along the length of sheath 119.

Means are provided for directly sensing the temperature of the covering or sheath 119 so as to adjust power supply 123 to vary the heating of the tubing in response to changes in the temperature of the sheath 119. Accordingly, a thermister probe 127 carries a thermister bead 128 into direct engagement with the outer surface of sheath 119 so as to immediately sense any change in temperature effected thereat by means of power supply 123.

In this way, oscillations in the power supply are held to an inconsequential minimum since the sensing of a change in heating of the tubing is virtually instantaneous with respect to the application of increased power applied to the circuit. Feedback to the variable power supply 123 is supplied by a lead 129. Variable power supplies of conventional and known construction are readily adaptable to control by the output of changes in resistance occasioned by temperature changes as affect thermister beads in the manner shown and, accordingly, detailed description of such control circuitry is not believed required for those skilled in the art.

The provision of a previously packed coil of beads as described above serves to promote what has been referred to as "block flow" of discrete bodies of eluent and reagent so that they may be mixed thoroughly without axial extension of their end portions as would otherwise cause a loss in discrimination in the detection and identification of such materials by the electrophotometer 78. It has further been observed herein that such "block flow" is promoted by the provision of glass beads or other previously packed inert material in coil 116 to cause the body of mixed material to be deflected laterally of its path by tumbling the liquid material in and around the glass beads obstructing the path and in this manner causes the body of mixed material to engage the heated side wall of coil 116 to a greater degree than would otherwise be achieved. In this manner, a more uniformly heated mixture has been observed to be obtained than heretofore, and it is believed that in this way improved discrimination among the constituents may be obtained by the disclosed system.

Further, by locating the electrical heating means immediately adjacent tubing 118, it will be readily evident that immediate heating response is obtained and permits the use of a feedback temperature-detecting system of the kind disclosed.

Finally, means have been provided for quickly quenching the heat of the mixed materials disposed in coil 116 in the event that they should remain in the coil too long as, for example, by means of a power failure or other flow blockage condition which might, through prolonged heating, cause the polymerization of the mixed materials. Thus, the central portion of support element 121 forms a hollow receptacle 131 formed within the support element for admitting liquid upwardly therein via an inlet port 132. Coil 116 is disposed in heat transfer relation with respect to the walls of receptacle 131. A liquid reservoir 133 is filled partially (up to water line 140) by passing water or other coolant, into receptacle 131 for draining downwardly via ports 132, 136. An air pressure inlet 134 and air pressure line 137 serve to force the coolant upwardly as now to be described.

A flow passage formed by means of the tubing or pipe 138 is coupled to the inlet port 132 and disposed and adapted to originate at one end 139 at a point substantially beneath the surface level 140 of the cooling liquid in the reservoir. Outlet 136 supplies the cooling liquid from reservoir 133 to receptacle 131 via inlet port 132. A source of gas, such as compressed air, under pressure, is continuously stored in the tank 141 (FIG. 1) and coupled via a reducing valve 142 so as to direct pressure into the upper part of reservoir 133 for forcing the cooling liquid into receptacle 131.

The pressure is applied to reservoir 133 only under certain conditions as, for example, where there is a power failure and, accordingly, a solenoid 143 is arranged to operate the spool within valve 93 between each of two conditions. The condition of solenoid 143 during normal operation of the system is to be energized and to hold the spool of valve 93 downwardly against spring pressure so as to vent supply line 137 to atmosphere via passage 144. On the other hand, when there is a power failure or other operative condition which serves to deenergize spring to its upward position so as to interconnect supply solenoid 143, the spool in valve 93 is driven by the valve line 137 with the gas pressure line 145 thereby causing a portion of the liquid in reservoir 133 to be forced upwardly into receptacle 131, lowering the liquid level to line 151.

Thus, there has been provided means for interconnecting the pressure source to the reservoir so as to displace liquid therefrom into the receptacle to dispose a cooling liquid into heat transfer relation with the heat transmissive walls of support element 121 for cooling same and also for cooling the coil 116 disposed in heat transfer relation to the outer side wall of element 121.

It is to be observed that the lower end 139 of tubing or pipe 138 is spaced somewhat clear of the bottom of reservoir 133 so as to divide the liquid therein into a first and second portion, one portion being above the end 139 and the other portion being below end 139. In this manner, the pressure applied to liquid in reservoir 133 serves to feed a predetermined volume of liquid upwardly into receptacle 131 while retaining a reserve of relatively cool liquid in reservoir 133. Subsequently, when valve 93 is conditioned to vent reservoir 133 to atmosphere, the warmer liquid within receptacle 131 will return downwardly into the relatively cool liquid reserve in the bottom of reservoir 133 and thereby be quickly cooled. In this manner, in the event that a second emergency should occur relatively closely following the first, it can be assured that there will be an adequate supply of thoroughly cooled liquid in reservoir 133 so as to permit a second operation of the quenching system.

Finally, means forming a closed overflow chamber 146 and a fluid flow path in the form of the tubing 147 ex-

tending upwardly from the top closure 148 of receptacle 131 forms an adequate storage for the successive flooding of receptacle 131 by a relatively larger volume reservoir 133 than shown. As cooling liquid is forced upwardly through receptacle 131, it will overflow upwardly into chamber 146. As chamber 146 fills, air or other gas trapped within chamber 146 will become compressed. Ultimately, upon venting valve 93 to atmosphere, it will be readily evident that the compressed gas contained in chamber 146 serves to quickly purge chamber 146 and receptacle 131 of cooling liquid to send it quickly returning into reservoir 133.

From the foregoing, it will be readily evident that there has been provided a novel system and apparatus for running a series of analyses of fluid samples with highly improved repeatable results and discrimination.

It will also be readily evident that the length and type of previously packed material contained within the reagent to supply line 33 can serve to provide a substantially matched or optimum relationship between the pressure on the reagent supply line 33 as measured by gauge 149 and the pressure detected by gauge 150 indicative of the pressure at input 27 on the ion exchange column 13.

Discrete identification of each eluent derived from a common sample is obviously maintained by means of the discrete block flow handling effected by utilization of the lateral deflection of the materials in each discrete body of mixed liquid traveling along the path 114 as by means of the glass beads located within reaction coil 116. In this way, axial merging of successive blocks of mixed eluent is precluded whereby the electrophotometer means 78 can readily discriminate between successive ingredients being examined.

Finally, manual manipulation of switches can readily serve to control the various valve units shown in the diagram in FIG. 1 so as to carry out any selected sequence of operations.

In addition, by means of the hydraulic drive operating a plurality of syringes in the manner shown, it is possible to provide reliable fixed feeding ratios as between the various chemicals required to be injected into the system. Thus, it is believed that this portion of the system also contributes substantially to the enhanced results obtained.

We claim:

1. In a system for analyzing the constituents of a liquid sample of a type wherein buffers or other sample carrier liquids are applied to the sample, an ion exchange column having input and output flow passages for passing said sample and buffers therethrough, means for supplying sample and buffer to said input flow passage under substantial positive pressure, said column containing an ion exchange material, means for supplying a reagent to the eluent of said column, the last named means including a reagent supply line, and means in said reagent supply line for providing a substantial positive back pressure resistance to fluid flow therethrough substantially matched to the back pressure resistance provided by said ion exchange column and means for mixing said reagent and eluent for analysis downstream.

2. A system for analyzing the constituents of a liquid sample according to claim 1 in which said means in said reagent supply line comprises means for developing a back pressure resistance to fluid flow proportional to applied volumetric flow rate.

3. A system for analyzing the constituents of a liquid sample according to claim 2 in which the last named means comprises a column of perviously packed material serving to inhibit fluid flow therethrough, the impedance thereof substantially matching that of said ion exchange column.

4. A system for analyzing the constituents of a liquid sample according to claim 1 further including means for providing a positive fixed ratio by volumetric flow

rate of the buffer and reagent as they are supplied while simultaneously applying substantial pressure to each.

5. In a system for analyzing the constituents of a liquid sample of a type wherein buffers or other sample carrier liquids are applied to the sample, an ion exchange column having input and output flow passages for passing said sample and buffers therethrough to be discharged along a flow path for analysis downstream, means forming a reagent column having input and output flow passages for passing reagent therethrough, a reagent supply line coupled to supply reagent to the last named said input, means in said reagent supply line for providing a substantial back pressure substantially matched to that of the exchange column, the last named output flow passage serving to inject reagent into said flow path, and positive displacement means for supplying buffer and sample to said ion exchange column and reagent to said reagent column in a fixed ratio by volumetric flow rate while applying substantial positive pressure to said column and impeded path.

6. A system for analyzing the constituents of a liquid sample according to claim 1 wherein the last named said means for mixing includes means forming a flow path to receive both said reagent and stationary eluent, and means in the flow path for intermingling reagent and eluent by lateral deflections of their travel along said flow path while inhibiting axial extension of portions of the body of mixed material traveling along said flow path.

7. In a system for analyzing the constituents of a liquid sample of a type wherein buffers or other sample carrier liquids are applied to the sample and a reagent is mixed with the buffered sample, an ion exchange column discharging an eluent of a buffered sample, a reagent supply line, means in said reagent supply line for providing a substantial back pressure substantially matched to that of the exchange column, and means for mixing reagent and said eluent, the last named means forming a flow path for receiving said eluent and reagent and stationary means in the flow path impeding the flow of and serving to intermingle reagent and eluent by lateral deflections of their travel along said flow path while inhibiting axial extension of portions of the body of mixed material traveling along said flow path.

8. A system for analyzing the constituents of a liquid sample according to claim 7 wherein the last named said means for mixing includes a length of tubing forming a flow path to receive both said reagent and eluent, inert beads filling a portion of said flow path for laterally deflecting reagent and eluent to mix these materials in their travel along said flow path while inhibiting axial extension of the end portions of the body of mixed materials traveling in said tubing.

9. A system for analyzing the constituents of a liquid sample according to claim 8 further including a generally non-conductive but readily heat transmissive annular support element, said length of tubing including an outer conductive covering of material having a sufficient coefficient of electrical resistance to provide heating of fluid material within said tubing when electrically energized, said tubing being disposed and carried by said support element, power supply means electrically coupled to said covering for heating same and said fluid material within said tubing, and means directly sensing the temperature of said covering for adjusting said power supply means to vary the heating of said covering in response to changes in the temperature of said covering.

10. A system according to claim 9 wherein said tubing is wrapped as a coil about said support element in heat transfer relation thereto.

11. In a system for analyzing the constituents of a liquid sample of a type wherein a buffered sample is mixed with a reagent before flowing to an analyzing station, means for mixing said reagent and buffered sample comprising a length of tubing forming a flow path to re-

ceive both said reagent and buffered sample, said tubing including glass beads therein for laterally deflecting reagent and buffered sample to mix these materials in their travel along the tubing while inhibiting axial extension of the end portions of the body of mixed materials traveling in the tubing, a generally non-conductive but readily heat transmissive annular support element, said length of tubing being carried by said support element, electrical conductor means adjacent said tubing and wrapped about said element, said electrical conductor means having a sufficient coefficient of electrical resistance to provide heating of fluid material within said tubing when electrically energized, power supply means electrically coupled to said conductor means for heating same and said fluid material within said tubing, and means directly sensing the temperature of said conductor means for adjusting said power supply means to vary the heating of said conductor means in response to changes in the temperature thereof.

12. In a system for analyzing the constituents of a liquid sample of a type wherein a buffered sample is mixed with a reagent before flowing to an analyzing station, means for mixing said reagent and buffered sample comprising a length of tubing forming a flow path to receive both said reagent and buffered sample, said tubing including glass beads therein for laterally deflecting reagent and buffered sample to mix these materials in their travel along the tubing while inhibiting axial extension of the end portions of the body of mixed materials traveling in the tubing, a generally non-conductive but readily heat transmissive annular support element, said length of tubing being carried by said support element, electrical conductor means adjacent said tubing and wrapped about said element, said electrical conductor means having a sufficient coefficient of electrical resistance to provide heating of fluid material within said tubing when electrically energized, power supply means electrically coupled to said conductor means for heating same and said fluid material within said tubing, means directly sensing the temperature of said conductor means for adjusting said power supply means to vary the heating of said conductor means in response to changes in the temperature thereof, means for quickly quenching the heat of the mixed body of materials in said tubing comprising a hollow receptacle formed within said support element for receiving liquid therein, said coil being disposed in heat transfer relation with respect to the walls of said receptacle, a liquid inlet port formed in said receptacle, a liquid reservoir having an inlet and outlet, a flow passage coupled to said inlet port of the receptacle and disposed and adapted to originate at one end at a point substantially beneath the surface level of liquid in said reservoir, the outlet of said reservoir being coupled to supply cooling liquid from said reservoir to said receptacle via its said liquid inlet port, a source of gas under pressure coupled to lead into said reservoir for forcing the liquid therein into said receptacle, and means for interconnecting said pressure source to said reservoir to displace the liquid therefrom into said receptacle thereby to bring a cooling liquid into heat transfer relation with the heat

transmissive walls of said support element for cooling same and said coil.

13. In a system for mixing a buffered sample with a reagent while heating the mixed body of liquid, the combination comprising a hollow receptacle forming an annular support body, the material of the walls of said body being readily heat transmissive, a length of tubing for receiving said buffered sample and reagent and wrapped as a coil in heat transfer relation to said support body, means for heating the liquid body of material within said tubing, and means for quickly quenching the heated material including a liquid reservoir having an inlet and outlet, a liquid inlet port formed in said receptacle and coupled to a flow passage disposed and adapted to originate at one end at a point substantially beneath the surface level of liquid in said reservoir, the reservoir outlet being coupled to supply cooling liquid from said reservoir to said receptacle via its said liquid inlet port, a source of gas under pressure coupled to lead into said reservoir for forcing the liquid therein into said receptacle, and means for interconnecting said pressure source to said reservoir to displace the liquid therefrom to said receptacle thereby to bring a cooling liquid into heat transfer relation with the heat transmissive walls of said support body element for cooling same and said coil.

14. A system for mixing buffered sample and reagent according to claim 13 further comprising means disposing said one end of the first named flow passage intermediate the level of liquid in said reservoir and the bottom of said reservoir to divide the liquid into a first portion above said end and a second portion below said end, means forming an overflow chamber and a fluid flow path from said receptacle to said chamber, and means for displacing one of said portions into said receptacle upon operation of said interconnecting means whereby to leave a substantial reserve portion of liquid in said reservoir for quickly cooling returning liquid of said one portion.

15. A system according to claim 14 wherein said overflow chamber is formed as a closed chamber for containing a gas compressible upon entry of liquid into said chamber whereby said compressed gas within the overflow chamber serves to more readily discharge liquid from within the chamber.

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