Oct. 22, 1968 L. TYBRING 3,406,635

PROGRESSIVE DILUTION SERIES APPARATUS

Filed Jan. 17, 1966 2 Sheets-Sheet 1

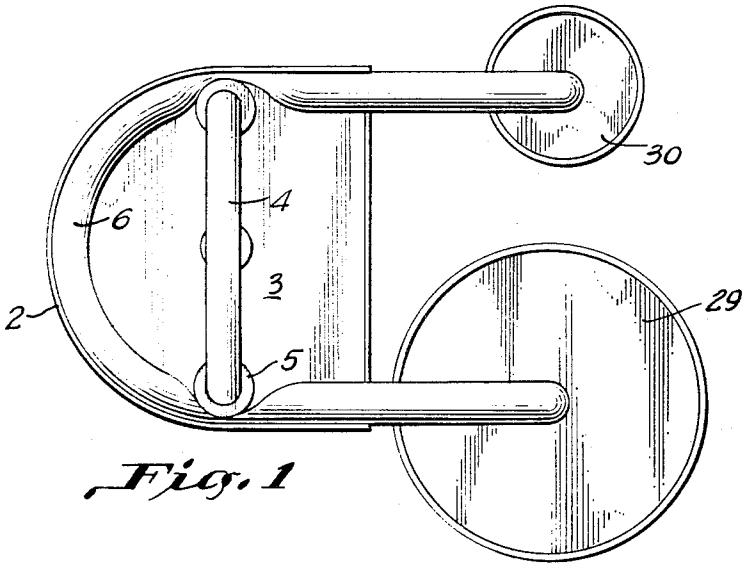

Fig. 1

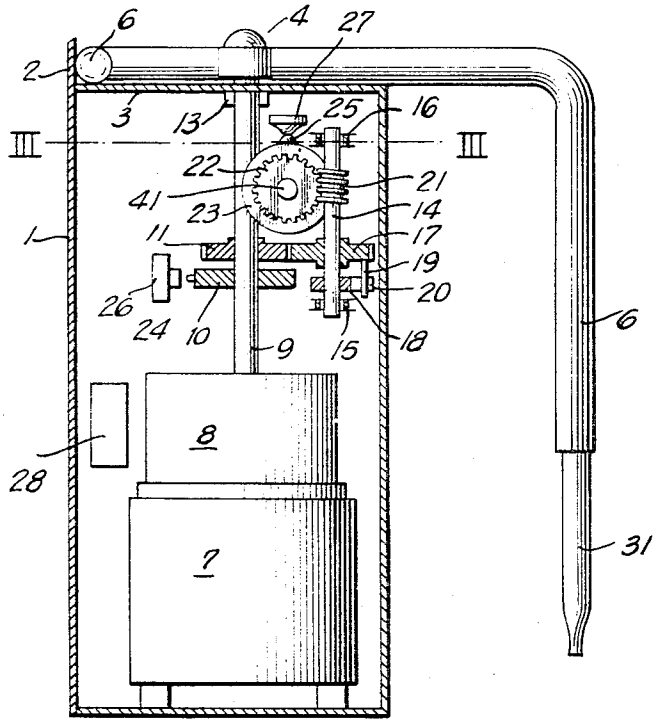

Fig. 2

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Fig. 4

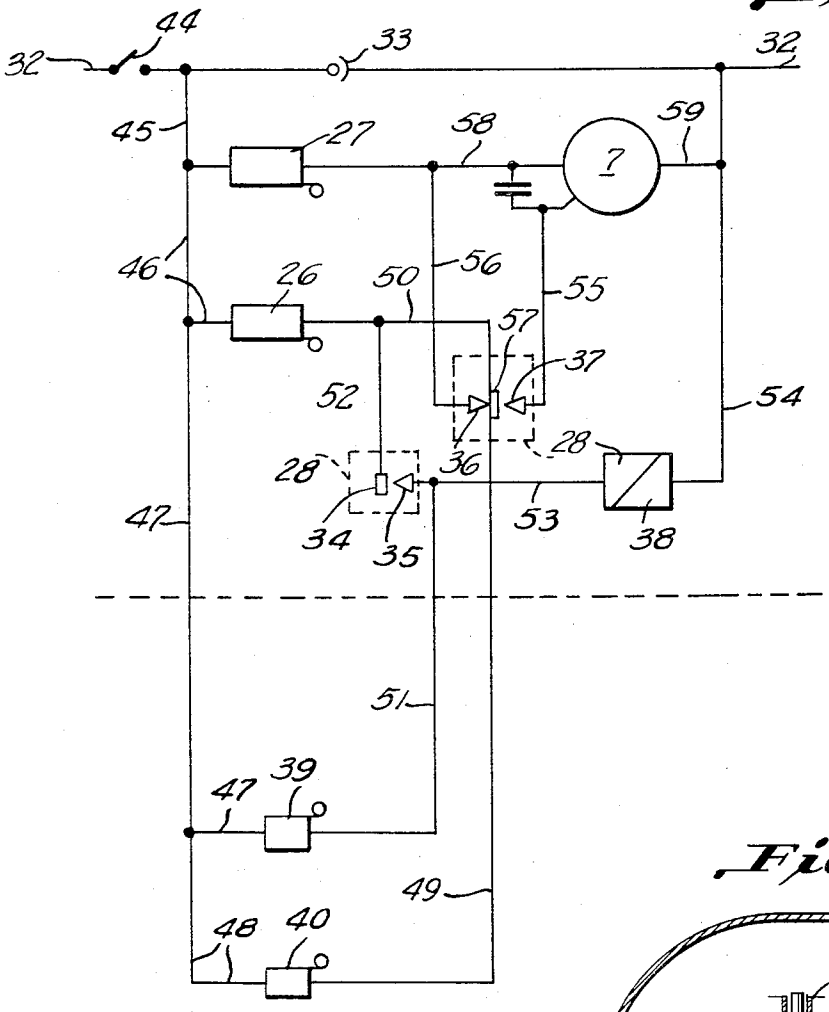
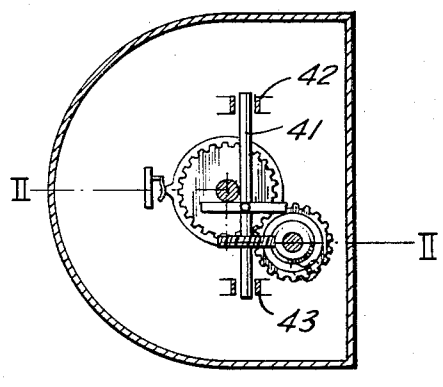


Fig. 3



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PROGRESSIVE DILUTION SERIES APPARATUS

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ABSTRACT OF THE DISCLOSURE

The apparatus consists of a rotating or peristaltic working piston pump inserted into a conduit for pumping volumetric quantities of a solution in one or the other direction through a conduit, one end of which forms a connection to a reservoir for diluent while the other end is free to be lowered into a container for basic or diluted solution, the pump being provided with means for terminating the pumping after a measured quantity has been transported towards the end of the conduit to be lowered into the basic or diluted solution and is a predetermined number of times greater than when transporting the liquid towards the reservoir.

In laboratories it is often required to produce a progressive-dilution series, i.e. a number or series of solutions in which a starting material is present in concentrations that are consecutive members of a geometrical progression. From a substance having the starting concentration A it may thus be desirable to produce a progressive-dilution series, the members of which have the concentrations A, $A \cdot 10^{-1}$, $A \cdot 10^{-2}$, $A \cdot 10^{-3}$ . . . and $A \cdot 10^{-n}$, respectively, n being an integer. It is not unusual that progressive-dilution series are required of the order of 5 to 10 members and in some kinds of experimental work the production of such series is a very frequently occurring task.

The usual way of producing progressive-dilution series is by using a pipette, which is rather laborious. It consists in extracting with a smaller pipette a predetermined portion of the basic solution, after which a portion of diluent is extracted from a suitable reservoir by means of a pipette, the volume of which is $p-1$ times the volume of the first one, if the rate of dilution is to be p. The measured portion of the basic solution is then mixed with the measured portion of the diluent. Then a measured portion of the solution thus produced is extracted by means of another pipette of the small size, and another measured amount of the diluent is extracted with the larger pipette, after which the liquids are mixed. Again, a portion of the thus produced mixture is extracted by means of still another pipette of the smaller volume, and a portion of the diluent is extracted by means of the larger pipette, the liquids are mixed and so on. Thus, for each step in the dilution series a further clean pipette of the smaller volume must be used and in some cases also a further pipette for each portion of the diluent to be extracted. That is the case if complete emptying of the larger pipette is achieved by using it for the stirring. Alternatively, the larger pipette used for measuring the diluent may be kept clean, but in this case special care must be taken with regard to emptying it so that the same dilution ratio is obtained each time, which takes a considerable time. Anyhow, for each progressive-dilution series a great number of pipettes is necessary, which pipettes must be cleansed and dried and perhaps sterilized before they can be used anew.

The invention aims at providing an apparatus for the production of progressive-dilution series which apparatus

is simple and by which the above-mentioned drawbacks are avoided.

The apparatus according to the invention consists in a rotatingly or peristaltically working piston pump inserted into a conduit and designed for pumping volumetrically quantities of a solution in one or the other direction through the conduit. One end of the conduit forms a connection to a reservoir for diluent while the other end is free to be lowered into a container for basic or diluted solution. The pump is provided with means for terminating the pumping after a measured quantity has been transported, which quantity when the pump works to transport the liquid in a direction towards the end of the conduit adapted to be lowered into the basic or diluted solution, which end is above and in the following designed as the free end, is a predetermined number of times greater than when the pump is working to transport the liquid in a direction towards the reservoir for the diluent. The volume transported in the latter case between the termination points thus defined will be designed in the following as the unit volume.

The apparatus works in the following way: When the conduit is filled with the diluent and one end of the conduit is in connection with a reservoir for the diluent, and the other end, the free end, is lowered into a container with the basic solution, one or more unit volumes are first sucked into the conduit, whereby a corresponding amount of diluent is simultaneously delivered to the reservoir containing the reserve portion of diluent. Thereupon the pumping terminates and the free end through which the basic solution has been sucked in, is transferred to a container for receiving diluted solution. Now pumping is reversed and the predetermined number of unit volumes is pumped into said container. Firstly, the basic solution just sucked up leaves the conduit and then a number of unit volumes of the diluent corresponding to the desired total volume. On being pumped out the two liquids mix and before the free end of the conduit is removed from the just formed diluted liquid and by reversing and starting the pump one or more unit volumes of the mixture are sucked into the conduit, whereupon the end of the conduit is transferred to an empty container serving to take up the next dilution step of the series, the pump is reversed and the predetermined number of unit volumes is given off. The process continues in this manner until a series of liquids with increasing degree of dilution has been obtained, the last of which has the highest degree of dilution required for the experiment in view.

As it will be seen it is possible by using the apparatus to achieve that the mixing with diluent owing to the pump pressure takes place spontaneously, that the apparatus does not need any separate rinsing during the process of producing a series of progressive dilution, as the free end through which the basic solution or the last formed diluted solution is taken up will always be rinsed properly by the passage of the diluent through it, that the pipettation takes place automatically and that the apparatus is always ready for use as long as the same diluent is used. If a different diluent is to be used for another progressive-dilution series the apparatus is readily rinsed with the new diluent by just pumping the same through the conduit.

Furthermore the apparatus is very well suited for automatic production of progressive-dilution series as the containers for the diluted liquid may be placed on a conveyor or a turning table, so that another container automatically replaces the former one after each dilution operation.

In a preferred embodiment of the apparatus according to the invention the volume of the conduit, including the inserted pump volume, is larger than the unit volume. Hereby it is obtained that the basic liquid or diluted liquid

sucked up fills only part of the conduit and that nothing of these liquids escape at the other end which would result in that the said other end of the conduit had to be transferred to a container for basic or diluted liquid thus pumped out, which would be impractical. Likewise, if the volume of the conduit, including the inserted pump volume was to have the same size as the unit volume, a diffusion of the diluted material into the diluent might occur, whereby said diluent would be contaminated and part of the substance contained in the measured amount of liquid might be lost, whereby the progressive-dilution series would be unreliable. It is even preferable that the volume of the conduit is several times that of the unit volume which permits more than one volume to be taken up and consequently a variety of dilution rates can be obtained. In many cases it is also preferable that only the foremost part of the conduit, i.e. the part lying between the diluent and the pump space, is used for taking up a unit volume of the basic liquid or the diluted liquid, or for taking up a few unit volumes. Hereby a reliable cleaning of the apparatus during the working is ensured.

The apparatus according to the invention is very simple and cheap when the pump is a rotating piston pump of the valveless type, adapted for being driven with alternate directions of rotation, preferably by means of an engine, the direction of rotation of which can be shifted. As examples of rotating piston pumps of the valveless type which can be used according to the invention, may be mentioned a rotary vane pump, a gear pump or a flexible-hose pump. On the other hand, nothing prevents the use of a piston pump with valves, but these must be reversible so that the pump can work both ways. By using a valveless pump, however, certain drawbacks are avoided such as faults owing to possible insecure closing of the valves, and that the valves owing to their complicated form may retain impurities or undiluted liquid and thus cause contamination or inaccuracy in the progressive-dilution series. In the case of microbiological works the valves may furthermore give rise to infection from one series to the other. When the piston pump is valveless the measured volume is determined by the rotating angle of the rotor and the pumping direction is controlled by the direction of rotation of the rotor. Both are easily adjusted either mechanically or electrically.

As compared with other rotating piston pumps of the valveless type a flexible-hose pump has the advantage that the piston moves on the outside of the hose and the closing operation is performed merely by compressing said hose. Thus, it is only the hose which comes into contact with the measured and transported liquid and the pump space and the conduit may form a continuous jointless system with smooth walls which is exceedingly easy to keep clean by rinsing. As mentioned above, when the apparatus is working a constant rinsing with the diluent takes place in connection with each dilution operation, and consequently a pump of the last-mentioned type needs practically no special cleaning.

The apparatus according to the invention where the pump is a rotating piston pump of the valveless type, for instance a flexible-hose pump, may preferably be so constructed that the rotor shaft carries or is in constant transmission engagement with a cam wheel having one or more cams serving to discontinue the rotation of the rotor shaft after transportation of a unit volume. Hereby a reliable measuring of the unit volumes is obtained. As is well known a flexible-hose pump has dead points; by making the moment for cutting off the rotation of the rotor shaft coincide with the moment when the piston passes one of the said dead points, it is possible to obtain an extraordinary exactitude of measuring.

In order to make such an apparatus particularly easy to manage according to the invention the rotor shaft may be in transmission engagement with a second cam wheel by means of a one-way connection, for instance a ratchet connection; said second cam may be provided with one

or more cams which serve to discontinue the rotation of the rotor shaft after transportation in the direction towards the container for diluted liquid has taken place, the transmission ratio between the first and the second cam wheel being decided by the dilution ratio and the ratio between the number of cams on the two cam wheels. Hereby the apparatus can be worked solely by starting the rotor shaft in one or the other direction, said direction deciding whether the rotor shaft moves in steps corresponding to the measuring of a unit volume or in steps corresponding to the measuring of a total volume which is a predetermined number of times larger than the unit volume.

In the following the invention is described in greater detail by describing an apparatus which is an embodiment of the invention, reference being made to the drawing, in which

FIG. 1 shows the embodiment in question, seen from above, together with a reservoir for diluent and a container for basic liquid or diluted liquid,

FIG. 2 the same apparatus, partly in longitudinal section,

FIG. 3 horizontal section through the apparatus in FIG. 2, along the line III—III, and

FIG. 4 a diagram showing the electric connections in the apparatus according to FIGS. 1–3.

In FIGS. 1, 2 and 3 the reference number **1** is a house with cylindrical rear wall **2**, a plane front and a flat roof **3**. In continuation of the cylindrical rear wall there is an edge reaching up above the roof, said edge forming support for a conduit consisting of a tube **6** of elastic pliable material, such as for instance rubber or plastic. One end of the tube **6** is connected to the liquid in a reservoir **29** for the diluent, the other, which is provided with a nozzle **31** is lowered into a container **30** which may either be a container containing the basic liquid from which a progressive-dilution series is to be produced by means of the diluent in the reservoir **29**, or an empty container, in which diluted liquid is to be filled in and from which a measured amount of the thus diluted liquid is again to be sucked up for use in the next step in the progressive-dilution series.

4 is a rotor of the kind known from flexible-hose pumps which on the ends of motor arms carries rollers **5**, which during the passage along the cylindrical rear wall **2** compresses the tube **6** so that it closes securely against passage of liquid. The rotor arms **4** with the rollers **5** thus form the pistons of the flexible-hose pump. The rotor is carried by a shaft **9**, which is driven by a motor **7** through a gearbox **8**. The shaft is supported in the roof **3** by a bearing **13**. The rotor shaft **9** carries a spur-gear wheel **11** and a cam wheel **10** which on its circumference has a cam **24**. The gear wheel **11** and the cam wheel **10** are mounted on the rotor shaft **9** in fixed connection therewith. During the rotation of the rotor shaft the cam **24** gets into engagement with the contact of a micro-switch **26** of the normally-closed type, which opens when its contact engages the cam **24**. The gear wheel **11** is in constant engagement with another gear wheel **17**, which is freely rotatable on a shaft **14** parallel with the rotor shaft and mounted in journals **15** and **16** carried on brackets mounted on the walls but not shown in the drawing. The spur-gear wheel **17** carries adjacent to the circumference thereof a pin **19** upon which a ratchet **2 0**is rotatable. The ratchet **20** is in engagement with a ratchet wheel **18** mounted on the shaft **14** in fixed connection therewith. By rotating the gear wheel **17** in one direction the shaft **14** must accordingly follow driven by the ratchet **20**, whereas when the gear wheel **17** is turned in the opposite direction, the shaft **14** remains unmoved, kept still by friction means not shown on the drawing. The shaft **14** further carries a worm drive **21** in fixed connection therewith and in engagement with a worm gear **22** carried on a shaft **41**. The shaft **41** further carries a cam wheel **23** with a cam **25**. The worm gear **22** and the cam wheel **23** are in fixed

connection with the shaft **41**. A micro-switch **27** of the normally-closed type is mounted on a bracket not shown in such position that it gets in engagement with the cam **25**, when the cam wheel **23** is rotated. By such engagement the micro-switch **27** opens. In some convenient place, for instance as shown, a relay **28** having a coil, an armature and a closing and a shifting contact as later on described, are placed in the housing **1**.

In the following the ratchet and the ratchet wheel are assumed to be oriented in such a manner that the shaft **14** is rotated when the rotor shaft **9** is moved in clockwise direction in FIGS. 1 and 3, i.e. when liquid is pumped from the reservoir **29** to the container **30**.

The electric connections are not shown in FIGS. 1–3 but illustrated in the diagram shown in FIG. 4.

In this diagram the reference number **32** shows a phase line of an electric current distribution network. In the phase line is inserted a glow discharged lamp **33** and a main circuit breaker **44**. From this an electric wire **45** is carried to the switch **27**, a connection **46** to the other switch **26**, a connection **47** to a foot-operated switch **39** of the normally-open type, which can be closed by pressing the pedal and a further connection **48** to another foot-operated switch **40** of the same kind. The relay **28** mentioned above consists of a coil **38**, which is activated through the connection **53** and an armature having two contacts **34** and **57**, the former closing a contact **35** when activated, the latter breaking a contact **36** and closing a contact **37** when activated. From the foot-operated switch **40** a connection **49** is carried to the movable contact **57** from which a connection **50** is carried to the switch **26**. From the foot-operated switch **39** a connection **51** is carried to the fixed contact **35** and from the movable armature contact **34** a connection **52** is carried to the switch **26**. From the switch **27** a connection **58** is carried to the motor **7** and from the fixed contact **37** a connection **55** is carried to another terminal of the motor **7**. A third terminal of the motor **7** is connected with the other end **54** of the phase line **32** and the same applies to the spool **38** which is connected to the motor **7** through a connection **54**.

When the foot-operated switches **39** and **40** are opened, the motor **7** has current though the connections **45** and **58** if the switch **27** is closed, and in this case the motor continues running clockwise until this switch is opened by the cam **25** of the cam wheel **23** getting in engagement with the microswitch **27**. When hereby the motor has been stopped and the foot-operated switch **39** is pressed, the coil **38** is activated through the connections **51** and **53** and thus pulls its armature with the movable contacts **34** and **57**. Hereby the said contacts **34** and **57** are displaced to the right and close the current though the connection **46**, the normally-closed switch **26**, the fixed contact **37** and the connection **55**, which causes the motor **7** to start and rotate the rotor shaft in anticlockwise direction. If the foot switch **39** is then immediately closed the motor continues running until the cam **24** breaks the micro-switch **26**. The relay **28** is thereby inactivated. After this the motor is able to restart solely by pressing either the foot switch **39** or the foot switch **40**. If the foot switch **39** is pressed, the same will happen as above described, viz. the motor gets current through the connection **55** and accordingly rotates in such a direction that the rotor moves in anti-clockwise direction. Thereby a unit volume is sucked in from the container **30** and the motor stops again after having moved the rotor one turn. This can be repeated a few times, more or less dependent on the volume of the conduit **6**, since the amount of liquid sucked in must not be allowed to fill the said conduit or get too close to the end of the conduit connected with the diluting agent contained in the reservoir **29**.

If, on the other hand, the foot switch **40** is pressed, although the motor will start, the relay **28** is not activated. Accordingly, the current passes the connections **48** and **49**, the contacts **57** and **36**, the connections **56** and **58** to the motor **7**. The motor now runs in a direction opposite the one in which it ran before, i.e. the rotor moves clockwise. Accordingly the ratchet **20** turns the ratchet wheel **18** and thus drives the shaft **14** and the worm gear **22** and the cam wheel **23**, so that the cam **25** gets thrown out of engagement with the micro-switch **27** which accordingly closes. Now, the foot switch **40** can be released without the motor stopping, the motor getting now current through the connection **45**, the micro-switch **27** and the connection **58**. Now the motor cannot stop until the cam **25** has made one turn so that it gets once more into engagement with the micro-switch **27**. However, even this circumstance is not sufficient to stop the motor. The cam wheel **10** rotates also and has greater velocity. Most of the time the cam **24** is accordingly out of engagement with the micro-switch **26**, which is closed most of the time and allows current to pass through the motor, through the connection **50**, the contact **57**, the contact **36**, the connection **56** and the connection **58**. For this reason the motor is kept running in a direction which causes the rotor to move clockwise until the cam **24** of the cam wheel **10** has got into engagement with the micro-switch **26**. Thus, it is the sharper breaking by means of this faster moved cam which determines the point at which the motor **7** stops and not the less sharp breaking by means of the engagement of the cam **25** with the micro-switch **27**.

As it appears from what has been said above the apparatus works in the manner intended by activating only the foot contacts **39** and **40**. If, for instance, the transmission ratio between the cam wheel **10** and the cam wheel **23** is 1:10, pressing of the foot switch **39** would cause a unit volume of the liquid contained in the container **30** to be sucked in. When, next, the foot switch **40** is pressed totally 10 unit volumes of fluid consisting of 1 volume of the liquid sucked in from the container **30** and 9 volumes diluting agent will be delivered to the container **30**. In this manner a progressive-dilution series with the dilution ratio 1:10 is attained. If a progressive dilution series in which the dilution ratio is 1:5, i.e. 2:10 is required, the foot switch **39** must be pressed twice before the foot switch **40** and so on.

The volume of the conduit **6** depends on its length and said volume can thus always be so adjusted that it surpasses sufficiently the number of unit volumes which it can be required to suck into the conduit to obtain a required dilution ratio.

The magnitude of the unit volume depends on the sectional area of the conduit **6** and can be adjusted merely by replacing the conduit with another conduit having a different cross-section but the same wall thickness.

The velocity of pumping should be so adjusted that a thorough mixing of the liquid to be diluted with the diluting agent is attained at the delivery of the liquid to the container **30**. A means for thus adjusting the pumping velocity is to use a suitable velocity of the rotor shaft. A suitable velocity may for instance be attained by using a gear motor running 60 revolutions per minute, which motor may be a synchronous motor. Hereby it is attained that the motor stops immediately on breaking the current and that the velocity is always the same. By way of example of a suitable motor may be mentioned the Burger-motor designated RSM 65 provided with gear E.

I claim:

1. Apparatus for producing progressive-dilution series comprising a volumetric pump inserted in a conduit one end of which is connected to a container for diluting agent, the other end being free and adapted to be lowered into basic fluid or diluted liquid, as the case may be, contained in a container for such liquid, means for activating the said pump in one or the opposite direction and means for automatically discontinuing such pumping after the transportation of a unit volume when the transportation takes place in direction towards the container for diluent, and means for automatically discontinuing trans-

portation after a plurality of unit volumes have been transported when transportation takes place in the direction towards the said free end of the conduit.

2. Apparatus according to claim 1 in which the volume of the conduit including the pump space surpasses the unit volume.

3. Apparatus according to claim 1 in which the volume of the conduit including the pump space surpasses a plurality of unit volumes.

4. Apparatus according to claim 1, in which the pump is a flexible-hose pump the rotor of which is driven by a motor, the direction of rotation of which is shiftable.

5. Apparatus according to claim 4, in which the means for discontinuing the pumping action consist of cams activating electric switches for breaking the circuit of the motor, said cams being carried by cam wheels in transmission engagement with the shaft of the motor of the flexible hose pump, one cam wheel being carried by the rotor shaft and the other by an auxiliary cam wheel shaft being in one-way engagement with the rotor shaft and driven a plurality of times slower than the said rotor shaft.

6. A method of producing a progressive dilution series containing a number of solutions in concentrations that are consecutive members of a geometrical progression which comprises maintaining a supply zone of basic solution from which a progressive dilution series is to be produced, pumping one or more unit volumes of basic solution from said supply zone, simultaneously pumping in the same a corresponding amount of diluent to a reserve zone for diluent, transferring the one or more volumes of basic solution pumped from the supply zone to a receiving zone for receiving diluted solution, now reverse pumping the predetermined number of unit volumes into the receiving zone for diluted solution, then pumping out the two liquids in the last mentioned zone to mix them, collecting the mixed solutions, and then repeating the stated cycle of operations on further amounts of liquids.

7. The method of claim 6 in which a single pumping system is utilized in reverse pumping operation.

References Cited

UNITED STATES PATENTS

2,035,159	3/1936	Henry	103—149
2,097,028	10/1937	Gates	103—149
2,105,200	1/1938	Phelps	103—149
2,662,666	12/1953	Lamport	103—149
3,055,551	9/1962	Johnson	103—149
3,234,796	2/1966	Leonards	103—149
3,277,356	10/1966	Kraft et al.	103—149

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