United States Patent [19]

Noll

[11] **3,764,041**

[45] **Oct. 9, 1973**

[54] **MICRODISPENSING PROCESS AND APPARATUS**

[75] Inventor: **Hans Noll**, Evanston, Ill.

[73] Assignee: **G. D. Searle & Co.**, Skokie, Ill.

[22] Filed: **Oct. 26, 1970**

[21] Appl. No.: **83,967**

[52] **U.S. Cl.** **222/1**, 23/259, 73/425.4, 222/132, 222/148

[51] **Int. Cl.** **G01n 1/00**

[58] **Field of Search** 222/1, 145, 132, 222/148, 149, 193, 420, 421, 488; 239/112, 424; 141/90, 91, 105; 73/425.4; 23/259

[56] **References Cited**

UNITED STATES PATENTS

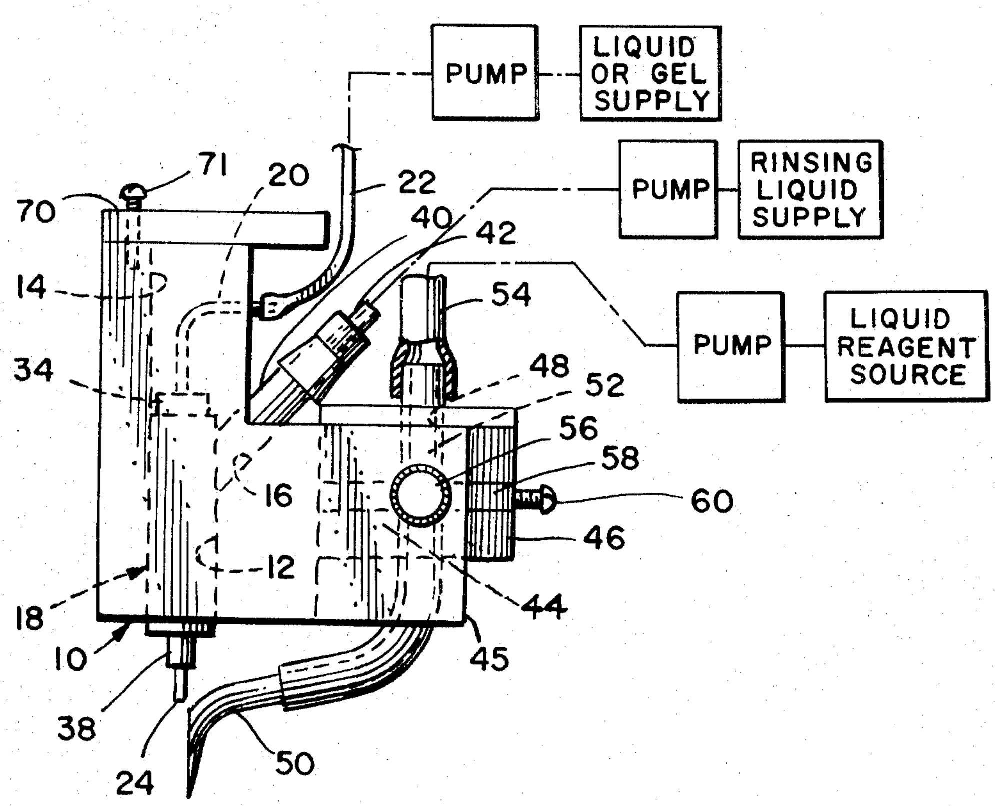
2,228,705	1/1941	Olson	239/424 X
3,386,683	2/1968	Rak	222/70 X
3,522,824	8/1970	Allen et al.	141/90
1,261,986	3/1918	White	222/148 X
2,526,331	10/1950	Copping	222/129.2

Primary Examiner—Robert B. Reeves
Assistant Examiner—David A. Scherbel
Attorney—Walter C. Ramm et al.

[57] **ABSTRACT**

A process and apparatus for the precise dispensing of very small volumes of liquids or gels wherein the means delivering such liquids or gels to collection vessels is rinsed at the end of each dispensing interval, thereby cutting a continuous stream of such materials into fractions of equal volume regardless of drop size. A process and apparatus which permits addition of reagent fluids to each of the collection vessels in conjunction with the dispensing of the fractions to be studied.

13 Claims, 2 Drawing Figures



Patented Oct. 9, 1973 3,764,041

FIG. 1

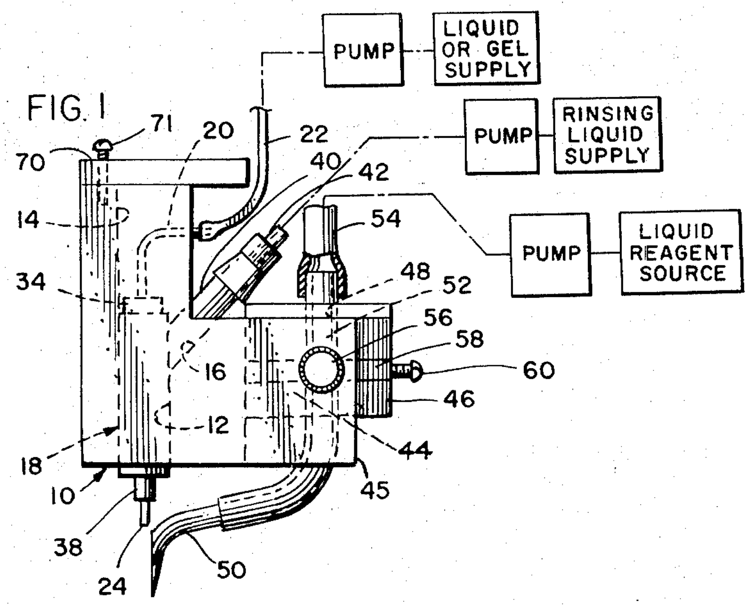
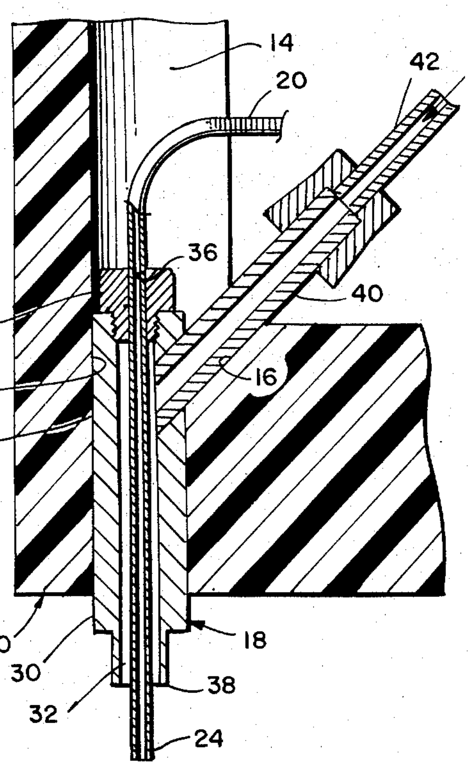


FIG. 2



INVENTOR:
HANS NOLL
BY
Alexander & Speckman
ATT'YS

MICRODISPENSING PROCESS AND APPARATUS

This invention relates to precision dispensing of liquids or gels and more particularly to improved apparatus and methods for use in laboratory fraction collecting and testing.

BACKGROUND OF THE INVENTION

An important operation in biomedical research and pharmaceutical manufacturing is the dispensing of identical volumes of liquids or gels into collection vessels or collectors by a process called fraction collection. In a typical operation of this type, small volumes of liquids or gels are dispensed mechanically and continuously into a series of collection vessels or tubes. The smallest quantity of liquid or gel that can be delivered by existing devices is determined by the size of the drop forming at the tip of the delivery tube or needle above the collection vessel. As a result, the obtaining of a precise fraction and the reproducibility thereof is frequently impossible.

Current fraction collectors designed for use in combination with chromatography, electrophoresis or ultracentrifugation procedures cut fractions by drop counting, by constant volume metering devices operated by mechanical siphoning or automatic valve action, or by interval timing. All of these methods, in which the liquid is allowed to drop into a series of tubes by gravity, are unsatisfactory for the collection of small equal fractions (0.5 ml and less), because (1) the desired fraction size does not correspond to a whole number of drops, (2) drops are lost between tubes at the moment of changing fractions, (3) the drop size changes with the concentration of solutes in the liquid, and (4) a relatively large and variable portion of the fraction volume remains at the tip of the delivery tube or needle. Moreover, the resolution often is determined by the smallness of the fractions that can be collected with precision. This applies to all procedures in which the detection and quantitative analysis of the substances that have been separated into narrow and closely spaced bands or zones must be carried out by discontinuous sampling. Thus, the separation into two closely spaced bands will only be detectable if a sufficient number of small fractions can be collected for analysis.

Fractionation into smaller volumes can be achieved by pipetting (as in manual laboratory practice) by touching the tip of the pipette to the surface of the receiving vessel or tube or by immersing the tip below the surface of a diluent fluid present in the receiving vessel. Such a pipette and process is described in United States Pat. No. 2,991,647. The first method, however, entails obvious losses and the second causes contamination of subsequent vessels with the fluid present in the preceding tube. Both methods are cumbersome and difficult to adapt for automatic operation.

The problem of small volume collection fractioning can, of course, be alleviated by diluting the solution to be dispensed and thereby increasing the delivery volume so that it becomes large relative to drop size. There are, however, many applications in which this is not feasible, as in the continuous fractionation of inhomogeneous media (exemplified by density gradients and gels in centrifugation and electrophoresis) used for the separation and partitioning of substances.

With existing equipment it has not been possible to process by an automated operation density gradients of small volume (3–20 ml) with acceptable resolution and precision. A well-known commercial fraction collector accommodating liquid scintillation counting vials permits the automatic fractionation of density gradients of relatively large volume (over 20 ml), but the usefulness of such instruments is limited because of new low-volume, fast-centrifugation techniques and the need for independent addition of the required scintillation reagents.

DESCRIPTION OF THE INVENTION

My invention comprises a novel configuration of components providing for the precise dispensing of very small volumes of liquids or gels, thereby overcoming the limitations of automatic and manual fraction collecting mentioned above. The apparatus and process of my invention achieves this precision by removing even fractional drops from the tip of the tube or needle delivering the liquid or gel to the collection vessels by flushing this tip with a small volume of a suitable rinsing liquid added on command from a supply reservoir, immediately preceding the advance of the delivery tip to the next vessel. The rinsing liquid may comprise any liquid physically and chemically compatible with the fraction such as distilled water or suitable organic solvents. Where subsequent procedures require the addition of chemical reagents, the rinsing liquid may comprise or include such reagents.

My invention also comprises a delivery spout which may be operated so that reagents necessary for the liquid scintillation assay of collected fractions are dispensed simultaneously with the fractions, thereby eliminating time-consuming and inefficient separate addition thereof.

The invention is adaptable for automated or manual collection systems and, when used in conjunction with a commercial moving fraction collector, timers, and commercial pumps, makes possible a completely automatic system capable of processing a liquid or gel for radioassay or chemical testing. Substantial economies of time are thus achieved as well as greater accuracy and improved radioassay resolution, since resolution increases with the smaller size fractions obtainable by use of my invention.

Typical applications of the automatic microdispensing fractionation and processing system made possible by my invention are the processing of liquid density gradients and acrylamide electrophoresis gels for radioassay and the automatic chemical testing of fractions obtained from chromatographic columns, density gradients or acrylamide gels. The fractionation of gels by existing techniques is similar to the collection of liquids and subject to the same limitations. By applying the discontinuous flushing action and the reagent addition of my apparatus, the same increase in accuracy and savings in time is obtainable for the processing of gels.

Accordingly, it is an object of my invention to provide a process and apparatus for the precise dispensing of very small volumes of liquids or gels.

It is a further object of my invention to provide a process and apparatus of the character described and having means for the automatic and simultaneous addition of reagents which may be required for subsequent radioassay or chemical testing of samples collected during fractionation.

It is another object of my invention to provide an apparatus containing a liquid or gel microdispensing device in combination with a reagent dispensing device, such apparatus being adaptable to fit any existing automatic fraction collector.

It is a still further object of my invention to provide an apparatus and process combining microdispensing of liquids and gels with dispensing of reagents which substantially reduces the time required for fraction collecting and processing.

These and other important objects of the invention will become apparent from the following description taken in conjunction with the drawings illustrating a preferred embodiment wherein:

FIG. 1 is a front elevation view of a liquid or gel microdispensing and reagent dispensing apparatus embodying the invention, and

FIG. 2 is an enlarged vertical cross section view of the dispensing assembly of my microdispensing apparatus.

Referring specifically to FIG. 1, my liquid or gel microdispensing reagent dispensing apparatus comprises an L-shaped retainer block **10**, that attaches by means of a baseplate **70** to the lower portion of any known liquid or gel delivery head (not shown). The baseplate **70** has suitable slits for accommodating set screws **71** to enable adjustments of the apparatus in the direction of travel and laterally with respect to the center of the collection vessels, as required. The retainer block **10** has a vertical bore **12** whose upper portion is open-sided to afford a vertical channel **14** and an open-topped channel **16** communicating therewith to form an open acute-angle joint. The bore and two channels cooperate to removably support therein the liquid dispensing assembly **18**.

Dispensing assembly **18** comprises a hollow needle or small-diameter conduit **20** which is connectable at its upper end, as by suitable tubing **22**, to a liquid or gel supply and which terminates at its bottom in a dispensing tip **24**. A cylindrical tube or casing **30** having an internal diameter greater than the external diameter of the needle **20** is positioned in sheath-like manner over the needle **20** to provide an annular passageway **32** therearound. Passageway **32** is closed at its upper end by a removable cap member **34** having a central aperture **36** which supports the needle **20** coaxially within the passageway **32**. The open bottom end **38** of the casing **30** is positioned above the dispensing tip **24** for reasons which will subsequently become apparent.

A tubular side arm **40** projects at an acute angle from the casing **30** and communicates with the passageway **32**, said side arm being connectable, as by suitable tubing **42**, with a rinsing liquid supply and flushing pump. It should thus be noted that the entire assembly **18** is supported by the contour-accommodating bore **12** and channels **14** and **16** so that the same may be readily removed for purposes of cleaning, maintenance and replacement.

The foot of the L-shaped block is formed with a vertical channel or cut-out defined by a vertical wall **44** and opening to the facing edge **45** of the foot. This channel is adapted to slideably and removably receive an inset block **46** which carries, through a vertical hole **48** provided therein, a curved delivery spout **50** having a vertical section **52**. Delivery spout **50** is connectable, as by suitable tubing **54**, to a scintillation liquid reagent source and a pump. Lateral positioning of the delivery spout **50** is adjustable by means of a set screw **56** adapted to track in a horizontal groove **58** formed in a side wall of inset block **46**, while vertical and radial positioning of the spout is adjustable by means of a set screw **60** carried by the inset block and adapted to bear against vertical section **52** of the spout. The spout thus may be positioned precisely with relation to dispensing tip **24** and the receiving vessels as dictated by the particular conditions obtaining.

In the operation of my microdispensing and reagent dispensing process, the drops of liquids to be dispensed and collected form at the tip **24** of the needle **20** above the collecting vessels. At the pre-set interval in each dispensing cycle, the flushing pump is activated to pump a charge of rinsing liquid through the side arm to and into the passageway **32** where it flows downwardly and circulates around the needle **20** to emerge at the casing bottom **38**. Upon emergence, the rinsing liquid surrounds and flows down the protruding dispensing tip **24** of the needle **20** in a stream, flushing from the tip the drop or fractional drop formed thereat. The relative diameters of the needle **20** and passageway **32** are in some measure a function of the surface tension of the rinsing liquids and are determined to prevent unwanted flow of the rinsing liquids between flushing actions.

The invention makes possible the development of a totally automated system for use in any of the analytical procedures described hereinabove. In such a system, the microdispensing and reagent dispensing apparatus is combined with a commercial fraction collector, which moves the collecting vessels relative to a stationary delivery system or which moves the delivery head over a rack of collecting vessels. The microdispensing apparatus will be interposed between the delivery system and the collecting vessels. Also included are such known elements as a reagent supply and a reagent pump, a rinsing liquid supply and pump, a first timing means regulating the collecting interval, and a second timing means regulating the delivery of the rinsing liquid and the scintillation reagent, when required. It should of course be appreciated that where the particular procedure involves chemical tests of the fractions, the rinsing liquid may contain, or comprise solely, the reagent or reagents required.

For proper operation of the automated system described, the flushing and reagent pumps are of the one-shot repetitive-action type. The action of the pumps is synchronized with the advancement of the delivery head or collecting vessels depending on the fraction collector used in such a way that the pumping action is triggered and the liquid addition completed immediately before advancement to the next collection vessel. This has been accomplished by using the signal of the first timing means, such as an interval timer normally controlling the advancement to the next collecting vessel in the standard fraction collecting unit, and feeding the signal into a secondary timing means, such as a delay or slave timer, which in turn actuates the pumps. The slave timer is set for an interval that is a few seconds shorter than the collection interval. Hence, with each advancement to the next collecting vessel, the slave timer is started. At the end of the interval, or a few seconds before the next advancement, the slave timer actuates the pumps and the rinsing liquid and/or reagent are introduced into the microdispensing and reagent dispensing apparatus.

The retainer block and inset block preferably are constructed of a rigid, lightweight, easily shaped or cut material such as metal, plastic or wood in order to properly support and maintain in position the various dispensing means. The dispensing assembly **18** may be made of any suitable non-reactive and easily cleanable material such as stainless steel or glass.

While the invention has been described in relation to a preferred embodiment thereof, it will be apparent to those skilled in the art that the structural details are capable of wide variation without departing from the principles of the invention.

I claim:

1. Apparatus for dispensing precise volumes of a fluid which is a member of the group consisting of a liquid and a gel comprising:

a dispensing conduit connectable to a reservoir of said fluid and terminating in a dispensing tip;

means adjacent to at least a segment of the dispensing conduit, providing a passageway, and communicating with a rinsing liquid supply,

dispensing pump means communicating with said dispensing conduit for delivering a predetermined volume of said fluid dropwise through said dispensing tip, and

discontinuous flushing pump means communicating with said passageway for delivering a rinsing liquid at said dispensing tip and adapted to be actuated when delivery of said predetermined volume is substantially complete so as to flush from said dispensing tip fluid collected thereon; whereby the rinsing liquid flushes from the dispensing tip any fluid collected externally thereon to reproducibly complete the volume of fluid dispensed.

2. Apparatus according to claim **1** in which said conduit comprises a hollow needle and said first-mentioned means comprises a cylindrical tubular casing having an inner diameter greater than the outer diameter of said needle,

said needle being positioned within said casing so that at least the dispensing tip extends outwardly from the bottom thereof.

3. Apparatus according to claim **2** in which the flushing pump means delivers the rinsing liquid under pressure and the difference between the inner diameter of the casing and outer diameter of the needle is such as to be effective to prevent flow of the flushing liquid between flushing cycles.

4. Apparatus according to claim **3** in which said flushing pump means is provided with a hollow side arm projecting from the casing and communicating with said passageway at a point adjacent to a top portion of said passageway.

5. Apparatus according to claim **4** and comprising further a cap member sealing the top portion of said passageway and accommodating said needle therethrough to maintain the same coaxially within the casing.

6. Apparatus according to claim **2** and comprising further reagent dispensing means for dispensing a reagent during the dispensing cycle of the liquid or gel responsive to a pre-determined signal.

7. A process for obtaining precise test fractions from a reservoir containing a fluid which is a member of the group consisting of a liquid and a gel characterized by the steps of:

dispensing dropwise a predetermined volume of the fluid for a predetermined through a small-diameter conduit having a dispensing tip and into a collecting vessel, and

intermittently delivering at said dispensing tip when the dispensing of said predetermined volume is substantially complete a rinsing liquid flushing from the dispensing tip and into the collecting vessel any fluid collected on said dispensing tip and thereby reproducibly completing each collecting cycle.

8. The process of claim **7** wherein the rinsing liquid comprises a reagent for use in chemical analysis of the test fraction.

9. The process of claim **7** further characterized by the step of dispensing into the test fraction a liquid scintillation reagent simultaneously with the delivery of the rinsing liquid.

10. In an apparatus for continuously and automatically obtaining precise test fractions from a liquid or gel reservoir for further analysis and including a rack containing a plurality of collection vessels, a delivery head mounted above said vessels and means for moving delivery of liquid or gel from one vessel to the next at a pre-determined dispensing cycle, the improvement comprising:

a supporting block attached to said head;

a small-diameter conduit having a dispensing tip;

a tubular casing of larger inner diameter sheathing a portion of said conduit and providing an annular passageway therealong;

means connecting the conduit to the casing in coaxial relationship so that the dispensing tip extends beyond the bottom of the casing;

a side arm projecting from the casing and communicating with said passageway;

means in said block cooperating with said conduit, casing and side arm to support the same in readily removable relationship;

means connecting said side arm to a flushing liquid source;

pump means for delivering flushing liquid to the side arm; and

timer means for automatically actuating the pump means at a pre-determined time in each dispensing cycle,

whereupon the flushing liquid flows through said passageway and rinses from the dispensing tip any liquid or gel collected thereon to complete each test fraction.

11. Apparatus according to claim **10** in which the difference between the inner diameter of the casing and the outer diameter of the conduit is such as to be effective to prevent flow of the flushing liquid between flushing cycles.

12. Apparatus according to claim **11** in which the flushing liquid comprises a reagent for use in chemical analysis of the test fractions.

13. Apparatus according to claim **10** and comprising further a reagent spout;

means for removably and adjustably connecting said reagent spout to the supporting block;

means connecting the reagent spout to a liquid scintillation reagent source; and

second pump means for delivering the scintillation reagent to said spout;

said timer means automatically actuating said second pump means to dispense into the test fraction simultaneously with the delivery of the flushing liquid a fixed amount of the liquid scintillation reagent for use in radioactive assay of the test fractions.

* * * * *