GEL FRACTIONATOR

Filed April 1, 1970

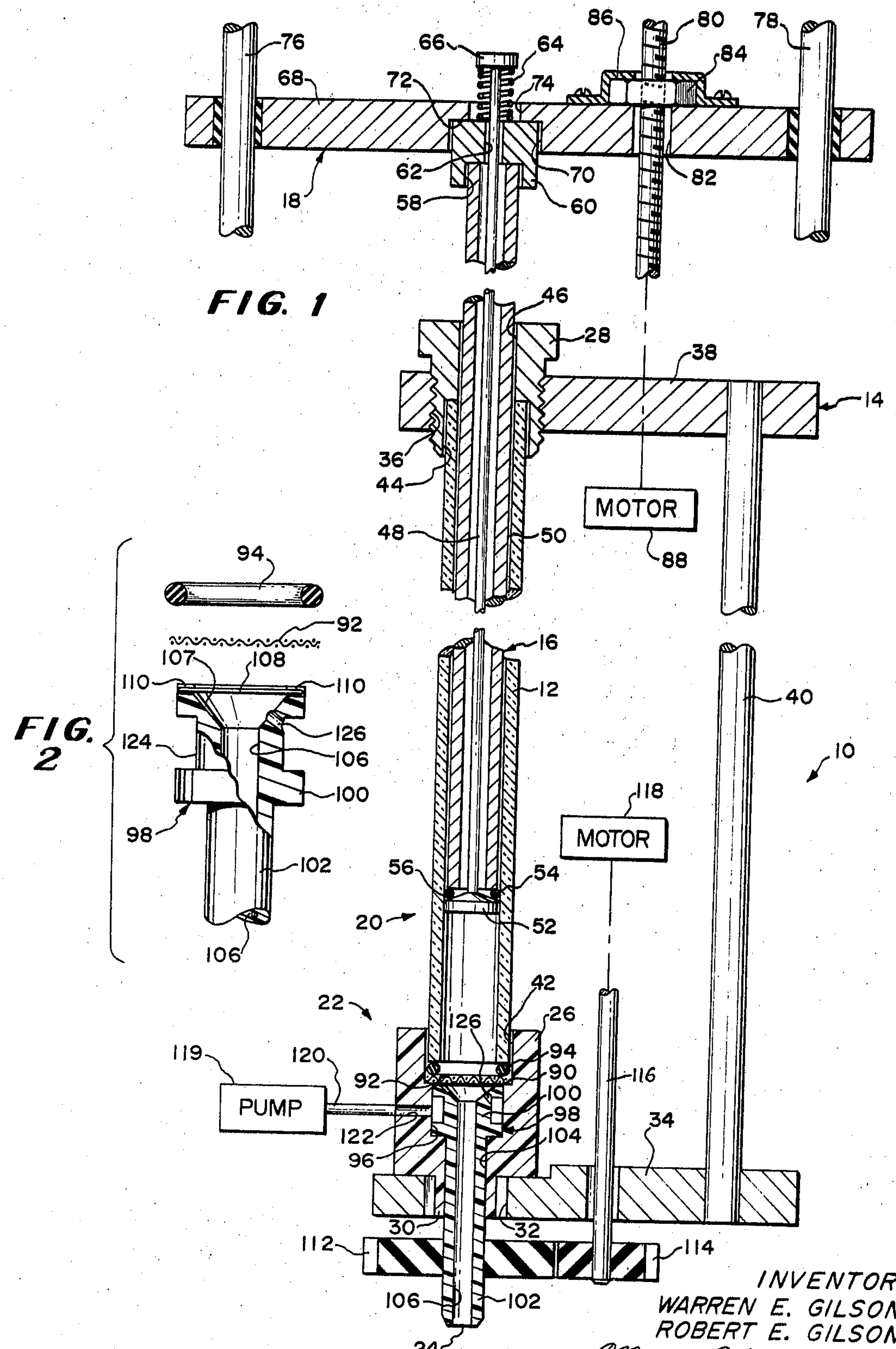

FIG. 1

FIG. 2

INVENTORS:
WARREN E. GILSON
ROBERT E. GILSON

By Mason, Kolehmainen, Rathburn & Wyss
Attorneys

United States Patent Office

3,664,593

Patented May 23, 1972

3,664,593

GEL FRACTIONATOR

Warren E. Gilson, 4801 Sheboygan Ave., and Robert E. Gilson, 4 Franklin Ave., both of Madison, Wis. 53705

Filed Apr. 1, 1970, Ser. No. 24,640
Int. Cl. B02c *19/00*
U.S. Cl. 241—60 10 Claims

ABSTRACT OF THE DISCLOSURE

A gel fractionator includes a cylinder for holding a column of gel, together with a piston assembly for advancing the column through the cylinder toward a fractionating apparatus. The piston assembly includes an air release valve operable from the exterior of the cylinder for releasing trapped air from above the gel column. The fractionating assembly includes a cutting screen engaged by the advancing column for severing the advancing column in a generally axial direction into subdivisions or segments. A rotating wire beneath the screen severs the subdivisions of gel as the gel advances. The wire is mounted across the mouth of a funnel which receives the severed gel particles, and the funnel includes structure for directing a stream of liquid at the underside of the screen for flushing the severed particles. The fractionating operation is carried out by repeatedly advancing the column a predetermined distance, pausing, and carrying out several discrete flushing operations to remove all severed particles.

The present invention relates to improvements in an apparatus and in a method for fractionating a column of gel.

A gel fractionator is a device for dividing a cohesive column of gel material into a series of individual samples or fractions. Such devices are useful in connection with known analytical procedures wherein a material to be analyzed, such as for example a protein or other macromolecular mixture, is dispersed by electrophoresis or the like within a column of a suitable gel material, such as a polyacrylamide gel. The column is divided into sequential individual fractions during the fractionating process, and the fractions are individually analyzed as by determination of radio-activity in a scintillation counter or the like.

The traditional method used for subdividing a gel column into fractions is simple manual slicing with a knife or blade. This method yields satisfactory results in that very little zone distortion is introduced—i.e. the distribution of the sample along the column is accurately reflected in the contents of the sequence of individual samples. The difficulty with manual slicing, of course, is that it is excessively tedious and time consuming.

In order to overcome the necessity for manual slicing of the gel column, it has been proposed to provide a mechanical gel fractionator capable automatically of dividing a gel column into fractions. U.S. Pat. No. 3,451,629, Maizel describes a mechanical fractionator wherein a column of gel is forced continuously through a restricted orifice and carried away in a continuous liquid stream to be deposited in a series of containers. Although representing an improvement in convenience over manual methods, this device has been found to introduce an undesirable amount of zone distortion in the resulting fractions. It is believed that this difficulty arises because the column of gel is reduced greatly in cross section as the gel is forced or extruded through a restricted orifice, resulting in undue distortion of the column.

Important objects of the present invention are to provide an improved fractionating method and apparatus; to

provide a gel fractionator overcoming the disadvantages of known devices; to provide a device and a method wherein zone distortion is overcome or minimized; and to provide an improved gel fractionator having a novel air release arrangement.

In brief, a gel fractionator constructed in accordance with the principles of the present invention includes a cylinder for holding a column of gel and a piston assembly for advancing the gel in the cylinder toward a fractionating assembly including first cutting means for subdividing the column into axially oriented subdivisions or extensions and second cutting means for severing the subdivisions or extensions. During the cutting operations, the cross section of the gel column is not substantially reduced. Means are provided for flushing the severed gel particles from the region of the cutting means. In accordance with the novel method of the present invention the gel is advanced a predetermined distance and then stopped during a delay period in which the cutting oepration is completed. Following the delay period, several separate sequential flush operations are carried out fully to wash the severed gel particles from the cutting region.

The present invention together with the above and other objects and advantages may be better understood from consideration of the embodiment of the invention illustrated in the accompanying drawing wherein:

FIG. 1 is a fragmentary, broken, partly sectional and partly diagrammatic view of a gel fractionator constructed in accordance with the present invention; and

FIG. 2 is a greatly enlarged, fragmentary, partly sectional, exploded view of one portion of the apparatus of FIG. 1.

Having reference now to the drawing, there is illustrated a gel fractionator designated as a whole by the reference numeral **10** and constructed in accordance with the principles of the present invention. In general, the gel fractionator **10** includes a cylinder **12** held in a relatively fixed position by a support structure **14** and adapted to contain a unitary, cohesive column of gel to be divided into individual samples or fractions. A relatively movable piston assembly generally designated as **16** is moved by a piston driving structure generally designated as **18** thereby to move the gel column within the cylinder **12**. The piston assembly **16** includes a novel air release valve generally designated as **20** for permitting the release of air trapped between the column and the piston assembly **16**. The column of gel is moved by the piston assembly **16** toward a novel fractionating assembly generally designated as **22** and serving to divide the advancing gel column into fractions which are discharged from an outlet **24**.

If desired the fractionator **10** may be mounted directly on the carriage structure of a fraction collector, in which case the fractions may be deposited directly from the outlet **24** into a series of vials or containers (not shown) disposed successively beneath the outlet **24**. Conveniently, the cylinder **12** comprises a glass cylinder, and if desired, may constitute the same cylinder in which the gel column and sample are subjected to a preceding electrophoresis operation. This arrangement avoids the necessity of transferring the column of gel from a different cylinder in order to carry out the fractionating operation.

The cylinder **12** is held in a relatively fixed position by the support structure **14** including a lower holder **26** and an upper holder **28**. Lower holder **26** includes a neck portion **30** loosely received in an opening **32** in a lower mounting bracket **34**, while the upper holder is threaded into an opening **36** in an upper bracket **38**. In order to maintain the brackets **34** and **38** rigidly in a parallel spaced arrangement corresponding to the length of the cylinder **12**, a support rod **40** is fixed to each of the brackets **34** and **38**.

In order to mount the cylinder 12 holding a column of gel and a dispersed sample, the lower holder 26 is first positioned as illustrated on the lower bracket 34. The column 12 is then slipped through the opening 36 and into a recess 42 in the lower holder 26. The upper holder 28 is then threaded into the opening 36, the upper end of the cylinder 12 being received in a recess 44. The upper holder 28 is threaded into the upper bracket 38 until the cylinder 12 is held firmly in position.

After the cylinder 12 is mounted, the piston assembly 16 is inserted. The piston driving structure 18 is moved upwardly as viewed in FIG. 1 to its uppermost position to provide clearance for insertion of the piston assembly 16 through an opening 46 in the upper holder 28 and into the top of the cylinder 12.

In accordance with a feature of the present invention, the piston assembly 16 includes the novel air release valve 20 for releasing air that may be trapped in the cylinder 12 between the upper end of the gel column and the lower end of the piston assembly. Referring more specifically to the construction of the piston assembly 16, there is provided an inner piston rod 48 received within an outer piston sleeve 50. The air release valve 20 is made up of an enlarged piston head member 52 of the rod 48, a resilient sealing gasket 54, and the lowermost end or edge 56 of the outer piston sleeve 50.

Ordinarily the valve 20 assumes its illustrated position wherein the gasket 54 is held in sealing relation between the edge 56 and the head 52. Since the upper surface of the head 52 is sloped or cone-shaped, the gasket 54 is also forced outwardly into sealing relation against the inner wall of the cylinder 12. In this position, air and gel is prevented from leaking or extruding upwardly past the piston assembly 16. However, the release valve 20 can be opened from the exterior of the cylinder 12 in order to permit the release of trapped air. The upper end of the piston sleeve 50 is received in a recess 58 in a piston cap element 60, while the piston rod 48 extends outwardly of the cap 60 through a smaller diameter recess 62. A spring 64 held in compression between the top of the cap 60 and the underside of a push button or enlargement 66 carried at the top of the rod 48 normally squeezes the gasket 54 between the edge 56 and the head 52.

When the piston assembly 16 is first inserted into the cylinder 12, air may be trapped above the column of gel and below the piston head 52. The push button or enlargement 66 is pressed toward the cap 60 against the force of the spring 64 to move the head 52 away from the edge 56 and to free the gasket 54. In this position air can pass through a clearance provided between the head 52 and the wall of the cylinder 12 and around the gasket 54. Air is then free to pass to atmosphere through the clearance between the piston sleeve 50 and the inner wall of the cylinder 12. When the head 52 engages the top of the gel column and the trapped air is fully vented, the push button 66 is released and is returned by the spring 64 to the position wherein the air release valve 20 is fully closed by the force applied to the gasket 54 between the edge 56 and the head 52. The sealing force is increased when a driving force is applied to the piston sleeve 50 during the fractionating process.

The piston driving structure 18 serves to force the piston assembly 16 into the cylinder 12 thereby to move the gel column toward the fractionating assembly 22 and outlet 24. Although various types of driving structures may be used, in the illustrated arrangement there is provided a drive plate 68 including a recess 70 receiving the cap 60 and including a shoulder 72 for applying a force by way of the cap 60 to the piston sleeve 50. A reduced diameter recess 74 freely receives the push button 66 and the spring 64.

In order to maintain the orientation of the drive plate 68 and to permit the drive plate to be moved reciprocally relative to the support structure 14 and cylinder 12, the plate is slidably mounted on a pair of parallel slide bars 76 and 78.

A driving force is applied to the drive plate 68 by means of a threaded drive shaft 80 held against axial movement and extending through an opening 82 in the plate. The shaft 80 threadedly engages drive nut 84 held on the plate 68 by means of a suitable holding bracket 86. Drive shaft 80 is rotated by means of a suitable driving connection indicated by a broken line to a suitable drive motor 88 illustrated schematically in FIG. 1. Rotation of the shaft 80 causes movement of the drive nut 84 axially along the shaft and concurrent movement of the plate 68 along the slide bars 76 and 78. When the plate 68 is moved toward the supporting structure 14, the piston assembly 16 is moved into the cylinder 12 to advance the gel column toward the fractionating assembly 22.

In accordance with an important aspect of the present invention the fractionating assembly 72 carries out a novel fractionating operation on the column of gel advanced toward the assembly 22 by the piston assembly 16 operating within the cylinder 12. Advantageously, the cross sectional area occupied by the advancing column of gel is not greatly restricted in carrying out the fractionating operation and excessive extrusion of the gel and the resulting zone distortion are avoided. In accordance with the invention, the advancing column of gel is first subdivided by severing the gel in substantially axial directions to produce a plurality of generally axially oriented portions or extensions of the gel column. The subdivisions are then severed from the gel column by cutting in a substantially radial direction. The severed gel segments are flushed from the cutting area and are delivered through the outlet 24.

Referring now more specifically to the structure of the fractionating assembly 22, the lower holder 26 includes the recess 42 for receiving the lowermost end of the cylinder 12, which recess 42 also defines a shoulder 90 upon which is mounted a first cutting structure 92 arranged substantially normal to the axis of the cylinder 12 and thus substantially normal to the path of advancement of the gel column. In the illustrated arrangement the cutting member 92 comprises a screen which, for example, may be an 80 mesh screen. The screen 92 is held against the shoulder 90 by means of a gasket 94 sandwiched between the screen and the lower end of the cylinder 12.

Disposed directly beneath the screen 92 in a reduced diameter recess 96 of the lower holder 26 is a gel severing and flushing unit generally designated as 98 and serving both to sever the gel subdivisions produced by advancing the gel through the screen 92 and to flush the severed subdivisions from the region of the screen 92. As illustrated in greater detail in FIG. 2, the unit 98 includes a body portion 100 mounted for rotation in the recess 96 together with a stem portion 102 extending through a further recess 104 in the stem portion 30 in the lower holder 26. The unit 98 includes an axially oriented central passageway 106 communicating the outlet 24 with the region of the screen 92. The uppermost end of the passageway 106 comprises a cone-shaped funnel portion 107 having a mouth located beneath the screen 92 for receiving severed portions of the gel column.

Supported across the mouth of the funnel portion of the unit 98 is a second cutting means movable through a plane parallel to and just below the screen 92. The second cutting means comprises a wire 108, the ends of which are attached to the mouth of the funnel 107 as by inserting the ends of the wire into notches 110 (FIG. 2).

Movement of the wire 108 is caused by rotating the gel severing and flushing unit 98. For this purpose, the stem portion 102 carries a drive gear 112 driven by a drive pinion 114 mounted on a rotatable shaft 116. As indicated by a broken line in FIG. 1, the shaft 116 is drivingly coupled for rotation by a suitable drive motor 118. Operation of the motor 118 causes rotation of the stem 102 and thus of the unit 98 and accordingly causes the wire 108 to rotate beneath the screen 92 and thereby carry out a cutting operation on the advancing gel subdivisions. In the illustrated arrangement, for example, the unit **98** is rotated continuously throughout the fractionating operation at about twenty-five revolutions per minute. Continuous rotation, however, is not necessary to the method of the invention.

Since the subdivided and severed gel segments may tend to stick to the advancing gel column and to the screen **102** and/or wire **108**, the fractionating assembly **22** includes novel provision for flushing the segments from the region of the screen **92**. A suitable liquid pressurized by a pump **119** flows through a conduit **120** to a passage **122** in the lower holder **26** communicating with the recess **96**. The outer peripheral wall of the body portion **100** of the unit **98** includes an annular recess **124** (FIG. 2) communicating with passage **122**. When pressurized fluid is channeled from the pump **119** to the recess **124**, the fluid is directed by a passage **126** extending through the body **100** from the recess **124** to the surface of the cone **107** and toward the underside of the screen **92**. The pressure of the fluid and the diameter of the passage **126** are chosen so that the fluid squirts upwardly against the screen. As the unit **98** is rotated, the fluid is directed over the entire cutting area thereby efficiently and completely to carry away severed gel particles.

In accordance with an important object of the invention the fractionation of gel with the apparatus **10** is carried out in a novel sequence of steps assuring minimal zone distortion. In carrying out a fractionating operation with a container located beneath the outlet **24**, the first step is to advance the piston assembly through **16** through a predetermined increment of distance, which may for example be one millimeter. This causes the gel column to be advanced into engagement with the first cutting means, i.e. the screen **92**. The screen **92** severs the advancing gel in an axial direction to produce gel subdivisions or "worm-like" extensions of the gel column. After the gel has been advanced through an increment of distance, a pause of several seconds duration is provided in order to allow the gel fully to ooze through the screen. During this pause, the unit **98** may be continuously rotating in order that the wire **108** severs the subdivided gel extensions. Alternatively, if desired, the unit **98** may be operated only briefly near the end of the pause.

In order to assure complete flushing, and prior to the next incremental advance of the piston assembly **16**, a flushing operation is carried out. Preferably several distinct flushing operations are provided by providing a pulse of fluid through the passage **126**, pausing briefly, and applying a subsequent pulse of fluid. It has been found that three or four distinct flushing operations during rotation of the unit **98** produce very complete removal of gel particles from the fractionating apparatus **22**. The particles and the liquid drop through the passage **106** and out of the outlet **24** and into the container or vial. Prior to repeating the process a next container is located beneath the vial.

Although the present invention has been described with reference to the illustrated embodiment, it should be understood that the details thereof do not limit the invention as defined in the following claims.

What is claimed and desired to be secured by Letters Patent of the United States is:

1. A gel fractionator comprising a cylinder for holding a column of gel having a cross-sectional area, a piston means slidable within the cylinder for moving the column axially in the cylinder, first cutting means posi-

tioned in the gel path and engaged by the advancing column for subdividing the gel into a plurality of axially oriented sections having substantially the same combined total cross-sectional area as the cross-sectional area of said column of gel, and second cutting means movable across the gel path for severing end portions from said axially oriented sections.

2. The gel fractionator of claim **1**, said first cutting means comprising a wire mesh screen arranged in a plane intersecting the gel path.

3. The gel fractionator of claim **1**, said second cutting means comprising a wire movable in a plane intersecting the gel path.

4. The gel fractionator of claim **3**, said first cutting means comprising a wire mesh screen arranged generally parallel to said plane of movement.

5. Gel fractionating apparatus comprising a cylinder for holding a gel column; a screen disposed at one end of said cylinder, piston means for forcing the column through said screen thereby to subdivide the column, cutting means movable in a plane substantially parallel with said screen for severing the gel column divisions, means for washing the severed subdivisions from the screen, and means for collecting the severed subdivisions.

6. The apparatus of claim **5**, said collecting means comprising a funnel disposed beneath said screen.

7. The apparatus of claim **6**, said cutting means comprising a wire extending across the mouth of said funnel, and means for rotating said funnel.

8. The apparatus of claim **7**, said washing means comprising an opening through the wall of said funnel directed at said screen.

9. A gel fractionator comprising a cylinder for holding a column of gel, a piston means slidable within the cylinder for moving the column axially in the cylinder, first cutting means positioned in the gel path and engaged by the advancing column for subdividing the gel into a plurality of axially oriented sections, and second cutting means movable across the gel path for severing end portions from said axially oriented sections, said piston means nicluding valve means for releasing air from between said piston means and the column, and valve operating means disposed externally of said cylinder.

10. A piston assembly for forcing a column of gel material through a cylinder comprising an inner member including an enlarged head, an outer sleeve surrounding said inner member, said head and sleeve having diameters smaller than the inside cylinder diameter, a resilient gasket sandwiched between said head and the end of said sleeve, resilient means forcing said head and said sleeve toward one another and against said gasket and forcing said gasket into sealing relation against said cylinder wall, and said inner member being movable against the force of said resilient means to free said gasket and permit the release of air past said head.

References Cited

UNITED STATES PATENTS

622,048	3/1899	Hunsinger	146—61 X
3,525,374	8/1970	Fitch	146—176 X

GRANVILLE Y. CUSTER, JR., Primary Examiner

U.S. Cl. X.R.

241—90, 166; 146—61