

March 19, 1968

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3,374,166

VERTICAL GEL ELECTROPHORESIS APPARATUS

Filed Nov. 30, 1964

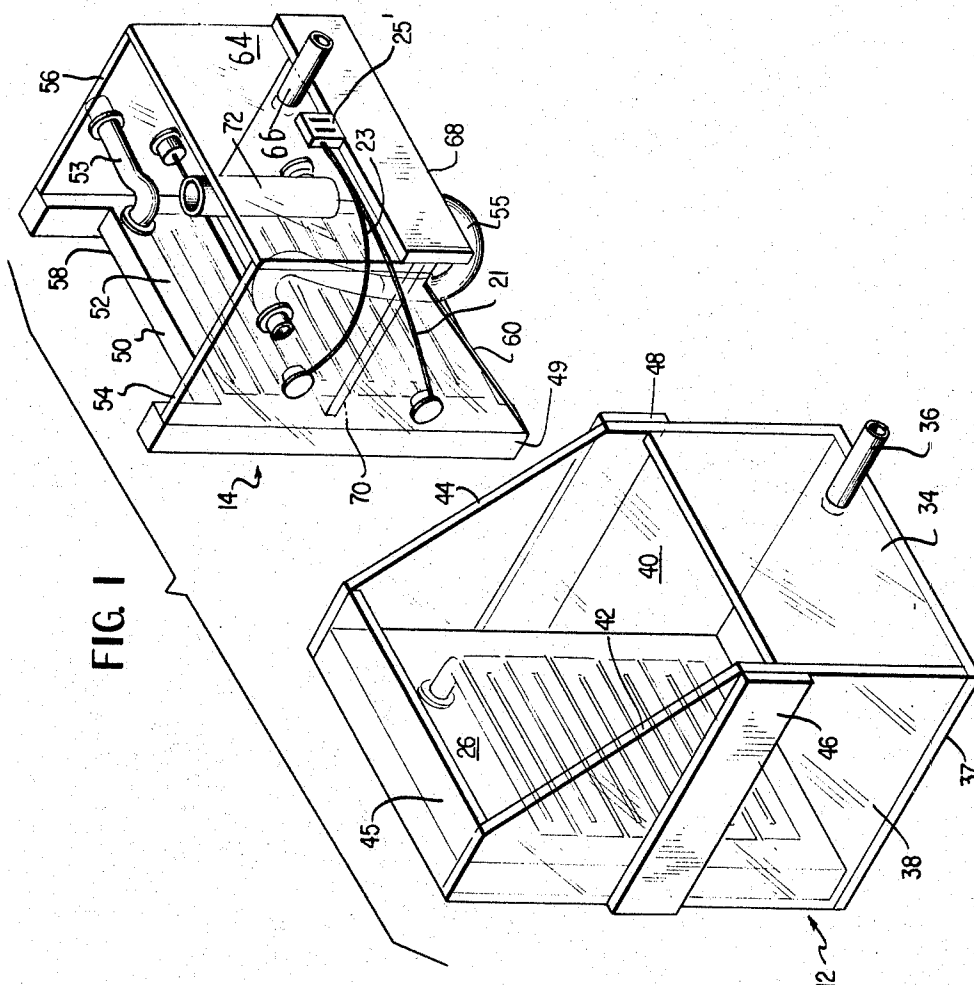


FIG. 1

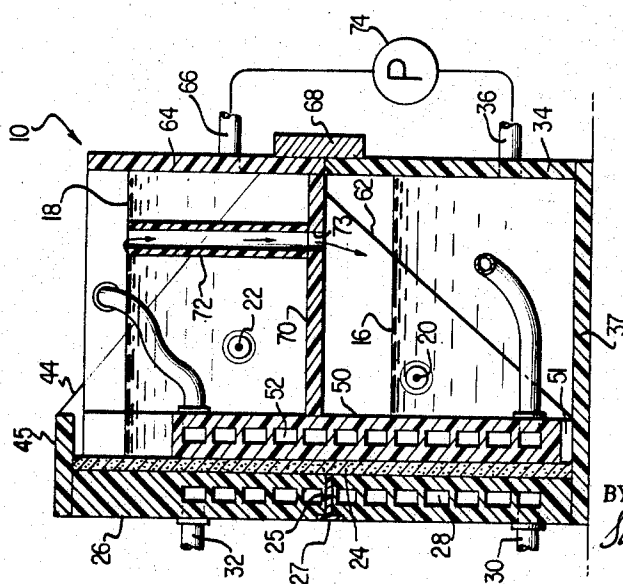


FIG. 2

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3,374,166 VERTICAL GEL ELECTROPHORESIS APPARATUS

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Filed Nov. 30, 1964, Ser. No. 414,654

Claims priority, application Great Britain, Sept. 21, 1964,
38,461/64

7 Claims. (Cl. 204—299)

This invention relates to the art of electrophoresis and particularly to improvements in apparatus for practicing vertical gel electrophoresis.

Electrophoresis, generally, relates to the separation of a complex substance into its component fractions by procedures based upon the migration or mobility of electrically charged fractions in a direct current electric field. With an electric field developed between two spaced electrodes and a substance placed therein, the variously charged components or constituents of the substance move or migrate toward the respective electrodes of opposite charge and the respective components will move with different mobilities, i.e., at different rates. Thus separation may be accomplished on the various component fractions. This separation may take place on a suitable support medium such as acrylamide gel saturated with a buffer solution. Each component separated by the electrophoresis procedure is subject to a qualitative and quantitative analysis and has, therefore, provided a useful tool in laboratory analysis of various substances such as albumin, enzymes, hemoglobin, carbohydrates, blood serum, etc., as well as various inorganic ions.

Vertical gel electrophoresis refers to electrophoresis wherein the component fractions migrate under the influence of an electric field on a gel and in a vertical direction. Vertical gel electrophoresis is generally known in the art as taught in U.S. Patent 3,129,158. This invention relates to improvements in the vertical gel apparatus of the known prior art.

It is desirable in an electrophoresis cell to obtain uniform patterns of the separated components which are easily interpretable and this in turn is dependent on the same conditions existing in the cell. One of the conditions is the strength and character of the buffer solution. If the strength, composition or pH of the buffer solution changes during the electrophoresis the results are not as uniform as would be desirable. I have found that such changes can be prevented either by (a) making the buffer chambers very large and filling them with a large volume of buffer, (b) periodically removing the partially changed buffer from the buffer chambers and replacing it with fresh buffer, (c) continuously recirculating the buffer at a sufficiently rapid flow rate from one buffer chamber to the other, (d) provision of diffusion baffles to keep electrode charges away from the electrophoresis matrix, or (e) use of electro-chemically reversible electrodes. This invention provides improvements in electrophoresis apparatus by providing means for recirculating the buffer solution from the upper chamber to the lower chamber during electrophoresis to maintain the strength, composition and pH of the buffer substantially uniform in both buffer solution containing chambers during the electrophoresis separation. Thus, the electrophoresis cell can operate using smaller volumes of buffer solution and providing a much more compact and satisfactory apparatus than to produce more nearly uniform and reproducible results than that of the known prior art.

The prior art disclosed electrodes separately positioned and attached to two separable parts of the apparatus so that, when assembly of the parts was effected, any malposition or mismatching of the parts resulted in a variable spatial relationship of the electrodes, and consequently

a variable intensity and distribution of the electric field between them. Furthermore, the attachment of the two electrodes to separable parts of the cell required a separable electrical connection to each such electrode, with consequent increase in the uncertainty of proper electrical connection and actual danger in operation of the cell because of the high voltages applied. This invention comprises attaching both electrodes to a single rigid component of the cell and providing a single bipolar means for making electrical connection to both electrodes simultaneously. It has been found that attaching both electrodes to the outer component of the cell is inadequate to provide a fixed and constant intensity and distribution of the electric field, since this is governed in large part by the position of the internal member of the cell. It is therefore preferable to attach both electrodes to the inner cell member.

In prior designs of electrophoresis cells for vertical gel electrophoresis, the space into which the gel is poured frequently had paths of leakage which permitted waste of gel-forming solution. In many designs of cell it is not obvious how such solution escapes, since the walls of the gel space extend above the level of the solution. I have found that capillary spaces between component parts of such cells, particularly along the edges of gaskets, permit such leakage. The present improvement in construction has the object of eliminating such leakage. An inner part is provided which fits entirely within the walls of the outer part, with spacing between the two sufficiently great to eliminate all capillary paths. The design therefore no longer requires any gaskets to make it leak-free.

In prior designs of electrophoresis cell, the cooling plates, which must comprise electrically non-conducting material, had a tendency to warp or bend owing to the inherent low strength of such materials as compared with, for example, steel. I have found that stiffening members of appropriate shape can, for practical purposes, completely prevent such bending and warping.

Still another feature of this invention is the provision of means whereby samples may be introduced into the gel slab at a distance from the exposed end thereof.

Other objects of the invention will be pointed out in the following description and claims and illustrated in the accompanying drawings, which disclose, by way of example, the principle of the invention and the best mode which has been contemplated of applying that principle.

In the drawings:

FIG. 1 is an exploded perspective view of the vertical gel apparatus of this invention showing the two component parts; and

FIG. 2 is a sectional side elevational view taken through the vertical gel apparatus assembled from the two component parts.

A vertical gel apparatus 10 includes an outer assembly 12 and an inner assembly 14, constructed of suitable electrically insulating material such as a machinable plastic. When these two assemblies, as shown in FIG. 1, are assembled in the manner illustrated in FIG. 2 they define a lower buffer solution containing tank 16, an upper buffer solution containing tank 18. Adapted to be positioned within the buffer solution in each tank are electrodes 20 and 22. Electrodes 20 and 22 are attached solely to inner member 14, and connecting insulated wires 21 and 23 are shown leading to a bipolar connector 25' shown in FIG. 1. Further, when assembled the two component parts define therebetween a vertical passageway 24 adapted to contain a gel support medium for the electrophoresis. This gel may be initially cast while the outer assembly 12 is in a horizontal position as disclosed in U.S. Patent 3,129,158.

The outer assembly 12 includes a back wall 26 with

coolant passages 28 therein connected with suitable tubing connections 30 and 32. A front wall 34 is provided with external tubing 36. A bottom floor 37 supports the front and back walls as well as side walls 38 and 40. The side walls have a triangular shaped upper edge at 42 and 44 to add to the strength and rigidity of the assembly and maintain the back wall cooling plate in flat shape. Clamp straps 46 and 48 are provided at the midpoint of the outer component part 12. A short top wall 45 is utilized to hold the gel during the casting thereof. A slot 25 extends through the wall 26 between adjacent cooling channels 28. This slot allows a sample of a complex substance to be inserted into the gel between the ends thereof. A close fitting plug 27 is provided to plug the slot 25 and is removable from the outside.

The inner assembly 14 includes a water cooled back wall 50 having coolant passages 52 therein connected by suitable tubing 53, 55 to a source of coolant. Side walls 54 and 56 are provided and they extend beyond the water cooled back wall 50 to provide a recessed area 58 extending vertically in the assembled component and providing the gel passageway 24. Thus, the gel passageway is defined by the two side walls 54 and 56 as well as the back wall 50 of the inner assembly 14 and by the back wall 26 of the outer assembly 12. This arrangement prevents undue leakage of the gel being cast or gelled in passageway 24 as it eliminates butt joints at the edge of wall 50. The side walls 54 and 56 are provided with triangular bottom edges 60 and 62 for the same purpose as edges 40 and 42. Front wall 64 of the inner assembly 14 has external tubing 66 and a clamping strap 68 secured thereto so that this clamping strap will cooperate with clamping straps 46 and 48 and allow the assembly to be held together by a suitable clamp. A floor 70 is liquid tight to hold the buffer solution in the upper tank 18. There must be a space between floor 37 and the bottom edge of inner cooling plate 50. To provide said space, plate 50 is provided with two spacers 49 and 51, one at each corner of said bottom edge.

A buffer drain tube 72 extends upwardly from the floor 70 to a distance equal to the desired height of the buffer solution in tank 18. The tube 72 provides a path of fluid communication through holes 73 in floor 70 to the lower buffer solution containing tank 16. External tubing 36 and 66 from the lower and upper buffer solution containing tanks are connected to a pump 74 for the recirculation of buffer solution. The recirculation is from the lower tank 16 to the upper tank 18 where it overflows through the drain tube 16 and continually recirculates. This continuous recirculation during an electrophoresis run prevents alterations in buffer strength, composition or pH thereby maintaining more uniform conditions in electrophoresis cell and providing a more uniform and easily interpretable pattern than heretofore available.

In operation the unit is first positioned with the back walls 26 and 50 horizontal and gel is poured into passageway 24. After gelling the assembly is turned upright to the position shown in FIG. 2 and the tanks 16 and 18 filled with buffer solution. A substance to be separated is placed across the gel at a suitable point. The electrodes 20 and 22 are connected to a source of D.C. and the pump 74 is started to recirculate the buffer solution. Under the influence of the electric field while on the permeable gel the components of the substance to be separated migrate at different rates and after a suitable period of time the electrophoresis may be stopped, the gel with the migrated separated components may be removed and then analyzed or interpreted.

While there have been shown and described and pointed out the fundamental novel features of the invention as applied to the preferred embodiments, it will be understood that various omissions and substitutions and changes

in the form and details of the device illustrated and in its operation may be made by those skilled in the art without departing from the spirit of the invention. It is the intention, therefore, to be limited only as indicated by the scope of the following claims.

What is claimed is:

1. A vertical gel electrophoresis apparatus of the type including a lower buffer solution chamber capable of holding liquid buffer solution, an upper buffer solution chamber capable of holding liquid buffer solution, a wall of each buffer solution chamber defining a gel passageway, the gel passageway being in fluid communication with liquid buffer solution in the buffer solution chambers and, when said passageway is filled with impermeable gel free flow of buffer solution from the upper chamber to the lower chamber is prevented by said gel, an electrode in each buffer solution chamber positioned below a normal level for buffer solution therein, each electrode including means for connection to a source of D.C. current, the upper and lower buffer solution chambers and the gel passage walls being constructed of a material having electrically insulating properties at least on the surface thereof, and means for holding the upper and lower buffer solution chambers in rigid assembled relationship, the improvements comprising, an internal drain tube extending from the bottom of the upper tank upwardly a distance equal to a desired level of buffer solution in the tank, external tubular connections establishing fluid communication between the two tanks and pump means for recirculating buffer solution from the lower tank through the external tubular connections to the upper tank and then draining back to the lower tank.

2. A vertical gel electrophoresis apparatus as defined in claim 1 wherein the buffer solution carrying tanks and gel containing passageway are provided by outer and inner assemblies and characterized by the electrodes for both buffer solution tanks being carried by one of the assemblies.

3. A vertical gel electrophoresis apparatus as defined in claim 2 in which the assembly carrying both electrodes is the inner assembly.

4. A vertical gel electrophoresis apparatus as defined in claim 2 and further characterized by a clamping strap at the center of the outer and inner component parts for clamping the outer and inner component parts in correctly assembled position.

5. A vertical gel electrophoresis apparatus as defined in claim 2, wherein walls of the two assemblies defining two walls of the gel containing passageway have cooling passages therein for the circulation of coolant and characterized by the inner of the two assemblies providing in addition the other two walls of the gel containing passageway.

6. A vertical gel electrophoresis apparatus as defined in claim 2 characterized by triangular shaped portions side walls on the outer and inner assemblies.

7. A vertical gel electrophoresis cell as defined in claim 2 further characterized as having a slot means piercing an outer wall of said outer assembly between cooling channels thereof, said slot means being adaptable for the introduction of samples into the gel at a distance from an end thereof.

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