

May 16, 1967

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3,320,149

ELECTROPHORESIS APPARATUS

Filed April 16, 1962

4 Sheets-Sheet 1

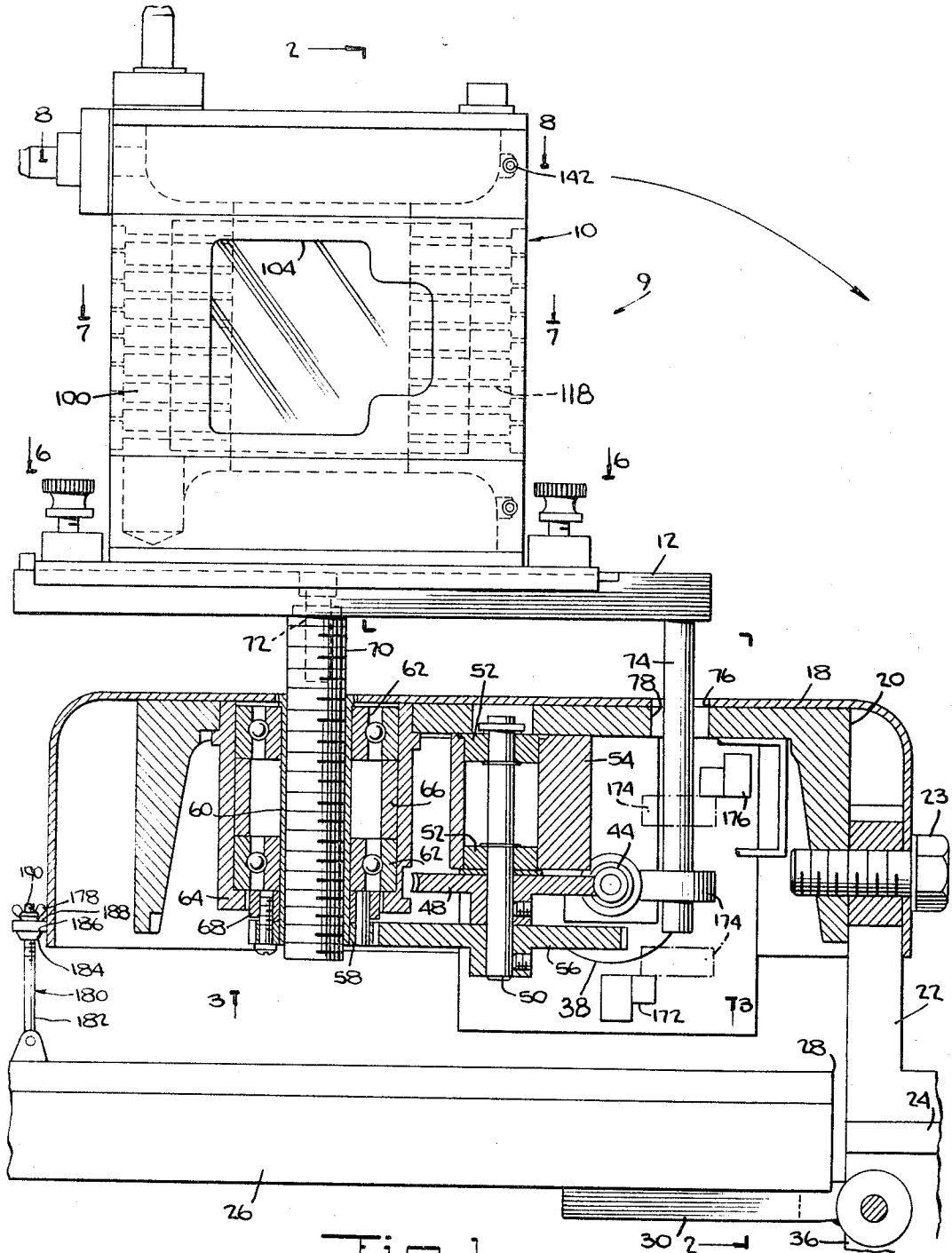


Fig. 1.

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Fig. 3.

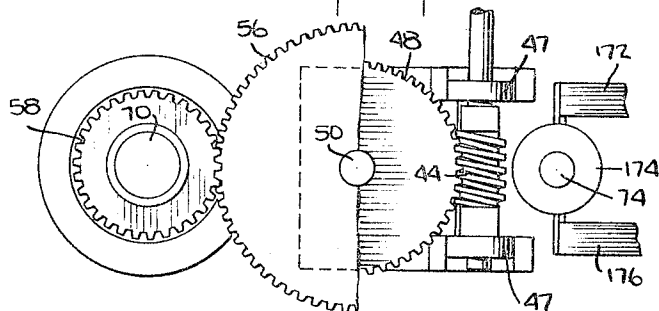


Fig. 4.

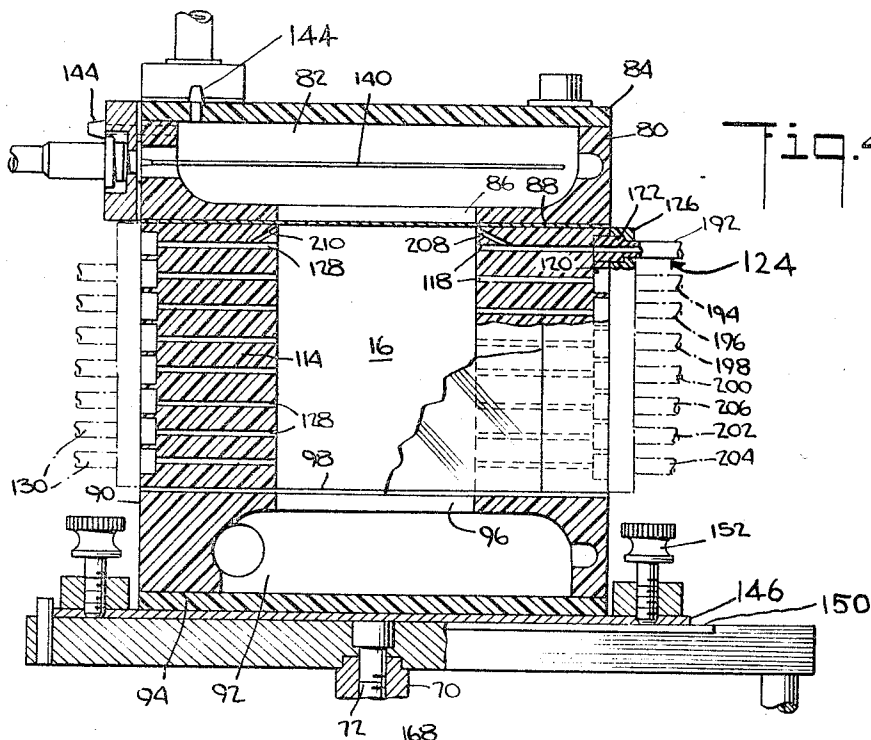
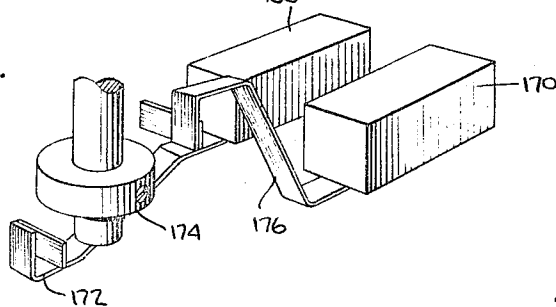


Fig. 5.



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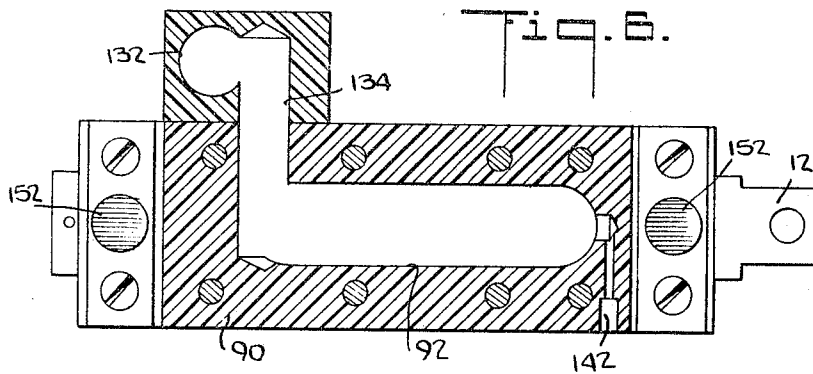
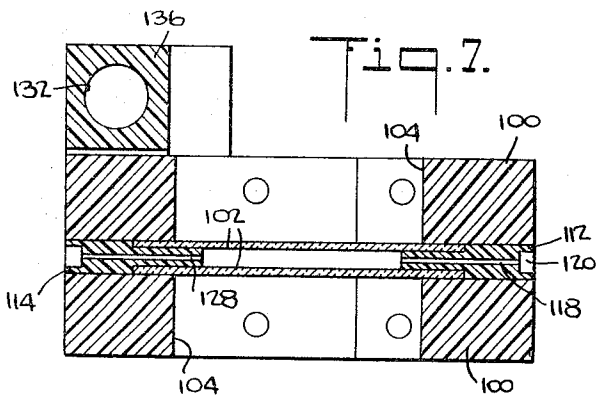
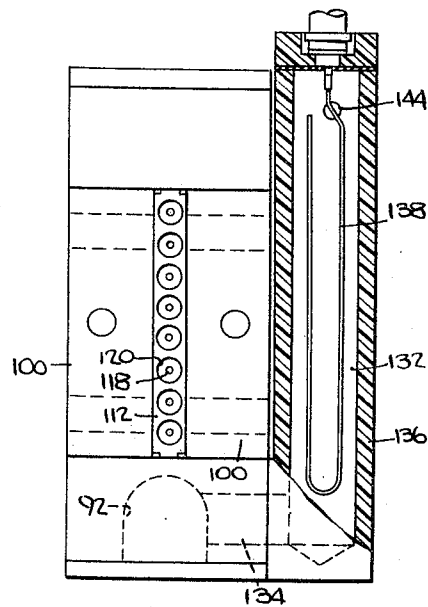
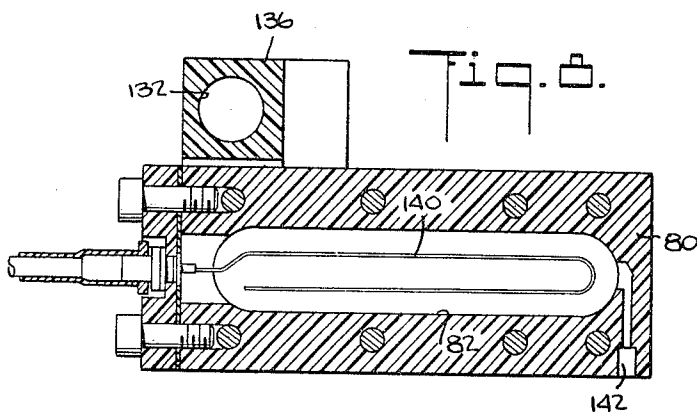
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ELECTROPHORESIS APPARATUS

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4 Sheets-Sheet 4



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ELECTROPHORESIS APPARATUS

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Filed Apr. 16, 1962, Ser. No. 187,515

6 Claims. (Cl. 204—299)

This invention relates to electrophoresis apparatus for the analysis of a sample liquid or of a series of samples of an ampholyte or a mixture of ampholytes, for example, but without limitation, a protein or a mixture of proteins, or other liquids whose constituents or some of them can be separated by electrophoretic action. While useful for other purposes, the apparatus of the present invention is especially valuable for the separation of the blood serum proteins, for instance, into the fractions albumin, alpha globulin, beta globulin and gamma globulin and their subfractions.

The fractionation of the plasma proteins by electrophoresis is referred to, for example, in Practical Psychological Chemistry by Hawk, Oser and Summerson, 12th edition, published in 1947 by The Blakiston Company, of Philadelphia, Pa. As there described, the electrophoretic separation of the proteins of a plasma sample is based on the difference in mobility of the various protein ions under the influence of a potential gradient, as a result of which the more highly charged albumin ions migrate at a faster rate than the globulin ions.

One of the objects of the invention is the provision of electrophoresis apparatus with improved means for moving the electrophoresis cell in a vertical rectilinear direction transversely of the light from a light viewing means to permit the latter to scan the liquid in the cell concurrently with the application of an electric potential across the liquid.

A further object is to mount the electrophoresis cell whereby it may be readily moved from its operating position to another position for the removal of air from the cell prior to the electrophoretic analysis operation.

Another object is to mount the electrophoresis cell in a manner which permits rapid and accurate adjustment of the position of the cell with respect to the light viewing means.

A further object is generally to provide electrophoresis apparatus of improved construction.

The above and other objects, features and advantages of the invention will be fully understood from the following description considered in connection with the accompanying illustrative drawings of the presently preferred embodiment of the invention.

In the drawings:

FIG. 1 is a side elevational view, partly in section, of the apparatus according to the present invention;

FIG. 2 is a vertical sectional view of the apparatus taken on line 2—2 of FIG. 1 and on a smaller scale than FIG. 1, with certain parts being illustrated diagrammatically;

FIG. 3 is a bottom plan view, with portions cut away, taken on line 3—3 of FIG. 1;

FIG. 4 is a vertical sectional view, on a larger scale, taken on line 4—4 of FIG. 2;

FIG. 5 is a perspective view of part of the apparatus;

FIG. 6 is a horizontal sectional view, on a larger scale, taken on line 6—6 of FIG. 1;

FIG. 7 is a horizontal sectional view, on a larger scale, taken on line 7—7 of FIG. 1;

FIG. 8 is a horizontal sectional view, on a larger scale, taken on line 8—8 of FIG. 1; and

FIG. 9 is an end elevational view, partly in section, taken in the direction of arrow 9 of FIG. 1.

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Referring now to the drawings in detail, the apparatus comprises an electrophoresis cell 10, which is made of electrically non-conducting and chemically inert material, and is mounted on a platform 12 for vertical up and down rectilinear movement transversely of a thin, horizontal stationary beam of ultra-violet light 14 for scanning of the liquid in chamber 16 of the cell. The platform is mounted above a housing 18 having a frame member 20 which is secured to an L-shaped bracket 22 by bolts 23. The horizontal leg of the bracket is suitably secured to a platform 24 which is adapted to carry a sample supply device and pump (not shown) for supplying a series of buffer solutions, of different specific gravities, respectively, and the samples to chamber 16 for analysis of said samples.

The apparatus is supported on a table 26 which is provided with a cut-out 28 that permits the apparatus to be rotated in a clockwise direction, as viewed in FIG. 1, for a purpose which will be more clearly understood hereinafter. To permit this rotational movement the apparatus is hinged to the table along the edge of the cut-out 28. The hinge connection comprises a pair of laterally spaced hinged plates 30 which are secured to the under-surface of the table along the edge of cut-out 28. Each plate has a pair of laterally spaced hinge pin support members 32 which carry a stationary hinge pin 34. A pair of laterally spaced lugs 36 extend downwardly from platform 24 and are mounted for rotational movement on the hinge pin between members 32.

The mechanism for moving platform 12 and the electrophoresis cell 10 thereon in a vertical up and down rectilinear direction is mounted within housing 18 and comprises a reversible motor 38 which is suspended from the frame member 20 by a bracket 40 having a centrally located opening 42. The motor is an alternating current synchronous capacitance motor of a well known type. The drive shaft of the motor is connected to a worm 44 by a coupling 46 and the ends of the worm are journaled in bearings 47 which are supported in brackets 47a suspended from the frame member 20. The worm drives a worm wheel 48 which is fixed to a rotary shaft 50 journaled in bearings 52 which are mounted in the frame member 54. A gear 56 is fixed to shaft 50 and is in mesh with a gear 58 which is suitably secured to the lower end of an internally threaded vertical sleeve 60. The sleeve is journaled in bearings 62 which are supported in frame member 64. The bearings are separated from each other by a spacer sleeve 66 and a spacer sleeve 68 is connected to gear 58. An externally threaded vertical screw 70 is threaded in sleeve 60 and the upper end of the screw extends above the top of housing 18 and is secured to platform 12 by screw 72.

A rod 74 extends downwardly from the platform 12 through openings 76 and 78 provided in housing 18 and frame member 20, respectively. During the operation of motor 38, sleeve 60 is rotated about its vertical longitudinal axis which tends to rotate screw 70 about its respective longitudinal axis. Rotation of screw 70 and platform 12 about the vertical longitudinal axis of the screw is prevented by the engagement of rod 74 with the parts of housing 18 and frame member 20 at openings 76 and 78, respectively. Accordingly, rotation of sleeve 60 causes screw 70 to move in a vertical up and down rectilinear direction so that platform 12 and the electrophoresis cell 10 also move in a vertical up and down rectilinear direction.

The electrophoresis cell 10 comprises an upper block 80 having a chamber 82 for an electrolyte. The top of the chamber is covered by a plate 84 and the bottom of the chamber is provided with a passage 86 which is separated from the top of chamber 16 by a semi-permeable membrane 88 of cellophane or other suitable material.

The lower part of the cell is formed by a lower block 90 having a chamber 92 for and electrolyte. The bottom of the chamber is closed by a cover 94 and the top of the chamber is provided with a passage 96 which is separated from the bottom of chamber 16 by another semi-permeable membrane 98. A pair of laterally spaced frame members 100 are positioned between the membranes and the confronting laterally spaced sides of the blocks are each secured, as by cementing, to a quartz plate 102 which are laterally spaced from each other to form the sides of chamber 16. Each frame member 100 is provided with an opening 104 (FIGS. 1 and 2) and the light exit end 106 of the light viewing means 108 is positioned, at one side of the chamber 16, in opening 104, and the light receiving end 110 of the light viewing means is positioned in the opening of the other frame member at the other side of the chamber.

The ends of chamber 16 are each formed by vertically extending inlet and outlet plates 112 and 114, respectively, and the plates are suitably secured, as by cementing, to the adjacent sides of the quartz plates 102 and frame members 100 (FIG. 7). The upper and lower electrolyte blocks 80 and 90, respectively, and intermediate frame members 100 of the cell are held together by screws 116. The plates 112 and 114 are identical in construction. The inlet plate 112 is provided with a series of vertically spaced horizontally extending passages 118 provided with recesses 120 to receive the nipple ends 122 of the inlet tubes 124 for the sample and buffer solutions of different specific gravities, respectively. The nipple ends of the tubes are held in their respective recesses by the coupling plate 126 and it will be understood that the tubes and their nipple ends are made of a soft vinyl material or other suitable material which can be resiliently compressed by tightening the coupling plate 126 against the adjacent end of the electrophoresis cell to connect the tubes to the corresponding passages. The outlet end of the cell is constructed identically to the inlet end of the cell except that the upper outlet passage 128 is not provided with an outlet tube 130 but is left exposed to the atmosphere to prevent the occurrence of undue pressure fluctuations in the electrophoretic chamber 16. In addition, outlet passage 128 provides an outlet for the flow of any excess liquid from the chamber. It is to be observed that corresponding inlet and outlet passages are at the same level. The cut-outs 104 of the members 100 have the shape indicated in FIG. 1 to permit some of the inlet passages 118 to be seen for observing the incoming streams, especially the sample stream, and to provide sufficient material in the blocks for the ends of the screws 116.

The lower electrolyte chamber 92 is connected to an electrode chamber 132 by a passage 134 and the electrode chamber is formed in a vertical extending arm 136 provided along the side of the cell at its outlet end. A platinum electrode 138 extends longitudinally in chamber 132. The upper electrolyte chamber 82 is provided with a similar electrode 140 and it will be understood that the electrodes are connected to a source of D.C. voltage of suitable value, say 300 volts. Each of the electrolyte chambers 82 and 92 are provided with suitable inlet and outlet openings 142 and 144, respectively, for passage of the electrolyte through the respective chambers.

It will be understood that the electrolyte is supplied from a suitably vented container (not shown) and is pumped through suitable tubing to the inlets of the electrolyte chambers and is pumped from said chambers through their respective outlets back to the container, as shown and described in the U.S. Patent application of Leonard T. Skeggs, Ser. No. 156,583 filed Dec. 4, 1961, and assigned to the assignee of the present application.

Pursuant to another feature of the invention, the electrophoresis cell 10 is mounted for adjustable horizontal movement transversely of the light beam 14 for correct positioning of chamber 16 with respect to said light. For

this purpose, the cross section of the upper part of platform 12 is in the form of a T, as best seen in FIG. 2, and the bottom of the cell is provided with a metal slide 146 secured to the bottom of the cell by the screws 116.

The slide is provided with laterally spaced longitudinally extending grooves 148 (FIG. 2) which slidably engage the upper edges 150 of the platform. The ends of the slide are each provided with locking screws 152 which are adapted to engage the top of the platform. By loosening the screws, the electrophoresis cell can be adjus-
10 tably moved horizontally on the slide to its correct position and locked in said position by tightening the screws.

The light viewing means 108, which is diagrammatically illustrated herein, comprises essentially a source of ultra-violet light 154, a tube 156 containing suitable lenses 158 and a prism 160 which is mounted for rotary adjustment to obtain the desired wave length for the light, for example 280 mμ. A tube 162 containing suitable focusing lenses is positioned to receive light from the prism and transmit it through its exit end 106 in the form of a thin horizontal beam to the chamber 16 of the cell. After the light passes through the chamber, it is transmitted through the receiving portion 110 of the light viewing means to a photomultiplier tube 164 which operates a recorder 166 of the current-ratio null-balancing type for recording the results of the electrophoretic separation of the constituents of the substance in the sample. The stylus of the recorder is operated by the movable tap of the slide wire of the recorder and it will be understood that the deflection of the stylus is directly proportional to the concentration of the constituents of the substance in the sample. It is to be observed that the light beam 14 is normal to the quartz windows of chamber 16. During the vertical up and down movement of the cell for the scanning operation, the resulting recording provided by the recorder 166 is a measure of the ultra-violet absorption of the liquid in chamber 16 and is plotted on the chart paper of the recorder against the vertical position of the light in reference to its position in the chamber.

A pair of switches 168 and 170 controls the up and down movement of the cell by reversing the direction of the current flow in the windings of motor 38 in conjunction with the operation of a reversing relay (not shown) as explained in detail in the aforementioned U.S. patent application Ser. No. 156,583. The switches are suspended from frame member 20 and switch 168 has its operating arm 172 in the path of downward movement of a collar 174 which is secured to rod 74. Switch 170 has its operating arm 176 in the path of upward movement of the collar 174 so that when the cell is in its uppermost position, switch 170 is operated to reverse the direction of rotation of the motor to move the cell downwardly into its lowermost position where the collar engages the actuating arm 172 of switch 168 to reverse the direction of rotation of the motor and move the cell back to its uppermost position.

Before operating the apparatus for the electrophoretic separation of the constituents of the substance in the sample, the electrophoresis cell 10 is tilted in a clockwise direction, as viewed in FIG. 1, to remove all the air from the electrophoresis chamber 16 and the electrolyte chambers 82 and 92. This can be readily accomplished by loosening the wing nut 178 of the clamp 180 which holds the apparatus with the electrophoresis cell in a vertical position. The clamp comprises a rod 182 which is pivoted at its lower end to the top of table 26. The upper end of the rod is provided with an enlarged part 184 which has a horizontal upper surface 186 against which rests the bottom horizontal surface of a bifurcated part 188. The bifurcated part is secured to the side of housing 18 and has an open ended slot through which the threaded end 190 of rod 182 extends. This threaded end of the rod carries the wing nut 178 which is clamped

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to the upper surface of part 188 for holding the cell in a vertical position during the electrophoretic separation operation thereof.

With the cell in an inclined position with at least some of the outlet passages 128 above the inlet passages 118, the various liquids are pumped through their respective inlet passages 118 and are transmitted through chamber 16 to the outlet passages 128, it being understood that the upper outlet passage is left exposed to atmosphere. The electrolyte is also pumped through their respective chambers 82 and 84. After removal of the air, the apparatus is returned to its normal position with the electrophoresis cell in a vertical position and is clamped in said position by clamp 180. The apparatus is now in condition for operation for the electrophoretic separation of the constituents of a substance in the sample which is introduced into chamber 16.

Briefly described, the pumps for transmitting the liquids to chamber 16 and the electrolyte to their respective chambers are operated and a series of buffer solutions, of different specific gravities, are introduced into chamber 16 through inlet tubes 192, 194, 196, 198, 200, 202 and 204, respectively, and the sample is introduced into the chamber through inlet tube 206. After a sufficient time has elapsed for adequate flushing and charging of chamber 16, the pumps are stopped and the solutions are allowed to come to rest for about 15 seconds before electrophoresis operation is begun.

The electrodes are energized and a vertical potential is applied across the liquids in chamber 16 which provides the potential gradient necessary for the electrophoretic separation of the constituents of the substance in the sample introduced into chamber 16 through tube 206. The pattern resulting from the electrophoretic separation of the constituents of the substance, and the migration of the separated constituents or ions to different levels in the chamber through the density gradient provided by the electric potential across the buffer liquids, is viewed by vertically scanning the liquid in chamber 16, as explained above. The results of the scanning operation are recorded by the recorder 166.

After the completion of the scanning operation, the pumps are again actuated and the scanned liquids are transmitted from the cell and a new series of buffer solutions and sample is introduced into the cell for repetition of the operation. The repetition of the operation of the apparatus is preferably controlled automatically by a programmer, as explained in the aforementioned U.S. application Ser. No. 156,583 filed Dec. 4, 1961.

A passage 208 (FIG. 4) extends between upper passage 118 and the bottom of membrane 88 and a similar passage 210 extends between the membrane and the upper outlet passage 128. During the operation of the apparatus, passage 208 directs a portion of the liquid along the undersurface of the membrane which helps remove any air that tends to remain at said surface.

As explained in the aforementioned U.S. patent application, Ser. No. 156,583, the electrolyte for chambers 82 and 92 is preferably a buffer solution which, by way of example, is prepared from a stock buffer solution made by dissolving 425 grams of tris (hydroxymethyl) aminomethane, 42.1 grams of ethylenediamine tetraacetic acid, and 32.4 grams of boric acid in a quantity of distilled water to make up 700 ml. of the solution. The buffer is made from the stock buffer by mixing 532 ml. of the stock buffer solution with 180 ml. of stock phenylmercuric acetate and 8150 ml. of distilled water. The stock phenylmercuric acetate is prepared by dissolving 1 gram of phenylmercuric acetate in 1 liter of distilled water.

The different specific gravities of the several buffer solutions which are transmitted into and out of the electrophoretic chamber 16 are derived by adding sucrose or other uncharged molecules, for example glycerine, to the above described buffer to increase its specific gravity.

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The following table is a preferred non-limitative example of the buffer solutions supplied to chamber 16 through the designated tubes:

Tube	Percent Sucrose	Buffered Sucrose, ml.	Buffer, ml.
192-----	0	0	3,000
194-----	42.5	1,075	126
196-----	0	0	3,000
198-----	25.0	630	570
200-----	0	0	3,000
202-----	47.5	1,360	0
204-----	47.5	1,360	0

The sucrose solution for providing the buffers of different specific gravities is a stock 50% sucrose solution prepared by dissolving 2200 grams of sucrose (highest purity) in distilled water in a quantity to make a final volume of 4400 ml. The resulting solution is treated with decolorizing carbon and then filtered. Phenylmercuric acetate is added as a preservative. For this purpose 176 mg. of phenylmercuric acetate is dissolved in a few milliliters of boiling water and this solution is stirred into the sucrose solution. The sucrose solution as thus prepared is substantially non-absorptive of ultra-violet light measured at 280 mμ which is the wave length of the scanning light.

The buffered sucrose solution of the above table is prepared by mixing 168 ml. of the above described stock buffer solution with 33 ml. distilled water and 400 ml. of the above described stock 50% sucrose solution. It will be understood that the buffers supplied to cell 10 through the tubes specified in the above table are prepared by mixing the buffers and the buffered sucrose in the indicated quantities.

The sample liquid which is supplied to the cell through tube 206 is diluted with a 64% sucrose solution which is prepared by dissolving 320 grams of sucrose in a quantity of distilled water to make up 500 ml. of the solution. It is decolorized by treatment with carbon and is preserved by adding a solution of 20 mg. of phenylmercuric acetate in a few milliliters of water. Two parts of this sucrose solution added to one part of the sample liquid, by volume, provides the sample with 42.7% sucrose so that the density of the liquid sample in chamber 16 is less than the densities of the buffers introduced into the chamber below the sample through tubes 202 and 204.

While I have shown and described the preferred embodiment of my invention, it will be understood that the invention may be embodied otherwise than as herein specifically illustrated or described, and that certain changes in the form and arrangement of parts and in the specific manner of practicing the invention may be made without departing from the underlying idea or principles of this invention within the scope of the appended claims.

It will be understood that he light viewing means 108, diagrammatically illustrated in FIG. 2, has not been described in detail since it does not, per se, form part of the present invention. As indicated above, the function of the light viewing means is to provide a thin horizontal band of light which is transmitted to the vertical electrophoresis chamber for the scanning of the liquid therein. The parts of the light viewing means, such as the lenses, prism and filter, through which the light passes, are made of material which transmits ultra-violet light, for example quartz. The light viewing means is illustrated and described in the copending patent application of Seymour Rosin, Ser. No. 187,709, filed April 16, 1962, now U.S. Patent No. 3,226,556, concurrently with the present application, and assigned to the assignee of this application.

I claim:

1. Electrophoresis apparatus, comprising:
 - (a) an electrophoresis cell having a chamber provided with light permeable portions for the passage of light through the liquid in said chamber,
 - (b) means for applying an electric potential across the liquid in said chamber,

- (c) light viewing means operable in relation to said chamber for examining said liquid to provide a quantitative indication of the components of a substance in said liquid,
- (d) means mounting said cell for rectilinear vertical movement transversely of the light from said light viewing means to permit said light viewing means to scan said liquid during the application of said electric potential, 5
- (e) said mounting means comprising a vertical internally threaded rotary sleeve, 10
- (f) a screw threaded in said sleeve for relative movement with respect thereto,
- (g) means connecting the bottom of said cell to the upper end of said screw, and 15
- (h) means operatively connected to said screw to prevent the rotation thereof so that rotation of said sleeve results in rectilinear vertical movement of said screw and rectilinear vertical movement of said cell transversely of the light. 20
2. Electrophoresis apparatus, comprising:
- (a) an electrophoresis cell having a chamber provided with light permeable portions for the passage of light through the liquid in said chamber,
- (b) means for applying an electric potential across the liquid in said chamber, 25
- (c) light viewing means operable in relation to said chamber for examining said liquid to provide a quantitative indication of the components of a substance in said liquid, 30
- (d) means mounting said cell for rectilinear vertical movement transversely of the light from said light viewing means to permit said light viewing means to scan said liquid during the application of said electric potential, 35
- (e) said mounting means comprising a vertical internally threaded rotary sleeve,
- (f) a screw threaded in said sleeve for relative movement with respect thereto, 40
- (g) means connecting the bottom of said cell to the upper end of said screw,
- (h) means operatively connected to said screw to prevent the rotation thereof so that rotation of said sleeve results in rectilinear vertical movement of said screw and rectilinear vertical movement of said cell transversely of the light, 45
- (i) a reversible motor operatively connected to said screw for the rotation thereof, and
- (j) switch means in the path of movement of said screw rotation prevention means for operating said motor and reversing the direction thereof in response to predetermined upper and lower positions, respectively, of said cell for movement thereof up and down in said rectilinear vertical direction. 50
3. Electrophoresis apparatus, comprising:
- (a) an electrophoresis cell having a chamber provided with light permeable portions for the passage of light through the liquid in said chamber,
- (b) said cell having a series of inlet openings disposed laterally of each other at different levels and in communication with said chamber for transmitting a series of liquid streams to said chambers, 60
- (c) said cell having a series of outlet openings horizontally spaced from said inlet openings and disposed laterally of each other at different levels and in communication with said chamber for transmitting a series of liquid streams therefrom, and 65
- (d) means mounting said cell for movement into a position inclined from the vertical so that at least one of said outlet openings is at a level above said inlet openings for removing air from said chamber by transmitting liquid to said chamber while said cell is in said inclined position. 70

4. Electrophoresis apparatus according to claim 3, further including:
- (e) light viewing means operable in relation to said chamber for examining said liquid to provide a quantitative indication of the components of a substance in said liquid,
- (f) a horizontal platform mounted for rectilinear vertical movement,
- (g) means mounting said cell on said platform for adjustable movement in a horizontal rectilinear direction transversely of the light from said light viewing means, and
- (h) means for moving said platform in said vertical rectilinear direction so that said chamber of said cell is moved in said direction transversely of the light from said light viewing means to permit said light viewing means to scan said liquid.
5. Electrophoresis apparatus, comprising:
- (a) an electrophoresis cell having a chamber provided with light permeable portions for the passage of light through the liquid in said chamber,
- (b) means for applying an electric potential across the liquid in said chamber,
- (c) light viewing means operable in relation to said chamber for examining said liquid to provide a quantitative indication of the components of a substance in said liquid,
- (d) a horizontal platform mounted for rectilinear vertical movement,
- (e) means mounting said cell on said platform for adjustable movement in a horizontal rectilinear direction transversely of the light from said light viewing means,
- (f) means for moving said platform in said vertical rectilinear direction so that said chamber of said cell is moved in said direction transversely of the light from said light viewing means to permit said light viewing means to scan said liquid during the application of said electric potential,
- (g) said platform moving means comprising an internally threaded vertical sleeve mounted for rotation about its longitudinal axis,
- (h) a screw threaded in said sleeve for relative movement with respect thereto,
- (i) means connecting one end of said screw to said platform,
- (j) said platform having a member connected thereto to prevent rotation of said platform and thereby prevent rotation of said screw during the rotation of said sleeve, and
- (k) a reversible motor operatively connected to said sleeve for rotation thereof.
6. An electrophoresis cell according to claim 3, wherein said light permeable portions of said electrophoresis cell comprise a pair of laterally spaced quartz side walls for the passage of ultraviolet light therethrough; and light viewing means including an ultraviolet light source and an ultraviolet light detector for viewing through said quartz walls the contents of said cell.

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