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Saravis

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[54] **ELECTROPHORESIS CHAMBER**

[75] Inventor: **Calvin A. Saravis**, Waban, Mass.

[73] Assignee: **Marine Colloids, Inc.**, Rockland, Maine

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[52] **U.S. Cl.** ..... **204/299**, 204/180 G

[51] **Int. Cl.** **B01k 5/00**

[58] **Field of Search** ..... 204/180 S, 180 G, 204/299, 300

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Primary Examiner—John H. Mack
Assistant Examiner—A. C. Prescott
Attorney—R. W. Furlong

[57] **ABSTRACT**

Electrophoresis apparatus with adjacent compartments arranged to hold buffer solution, means for supporting in position an impervious backing member having a gel layer on its upper surface, a cover with an aperture for applying samples and a layer of bibulous material fixed to the cover so as to be in contact with gel and the buffer solution when the cover is in position.

6 Claims, 4 Drawing Figures

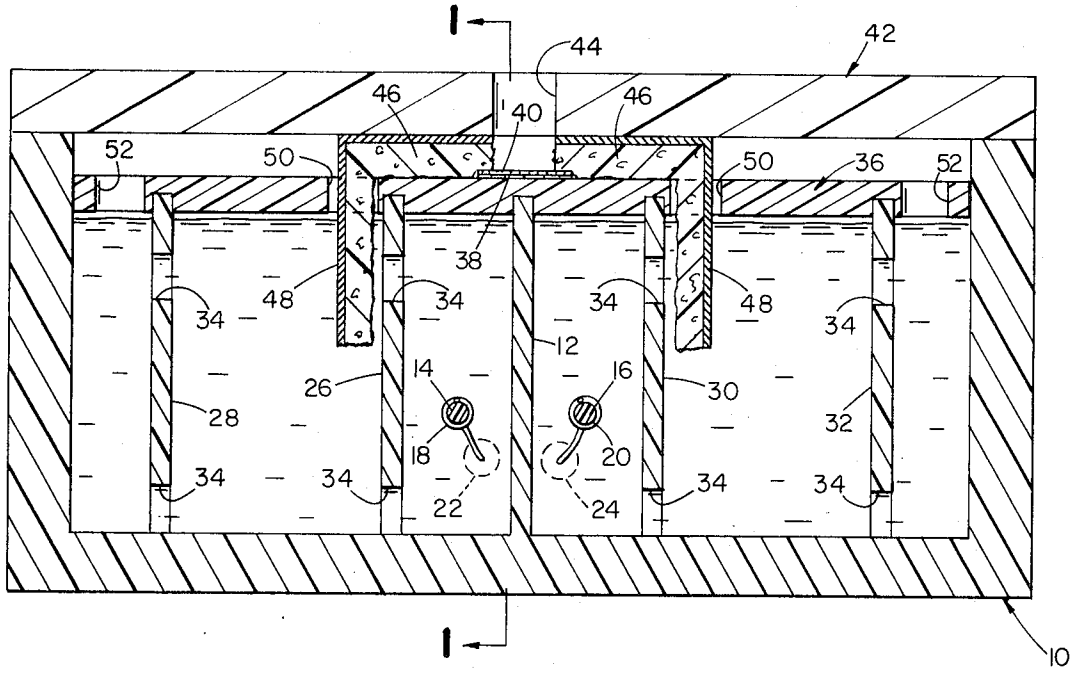
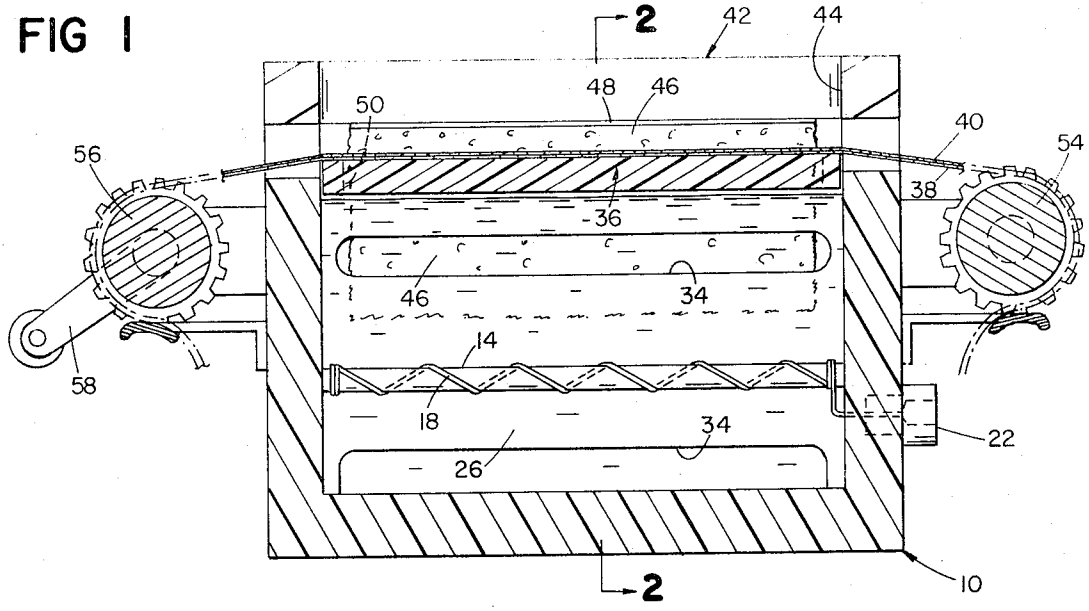


FIG 1


2
42
44
48
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50
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54
56
38
36
46
34
14
58
18
34
22
26
2
10

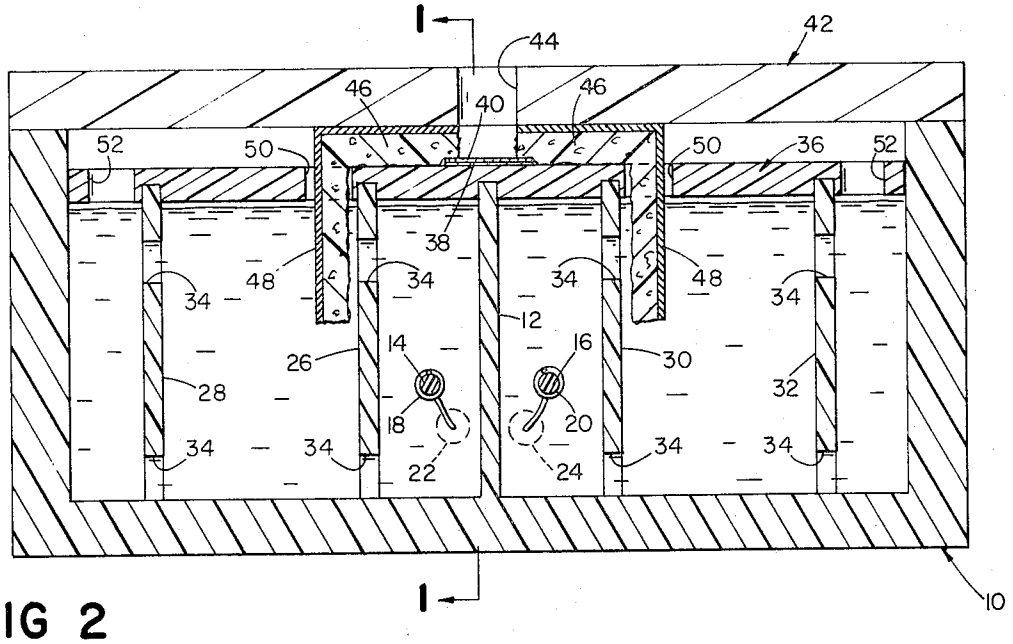
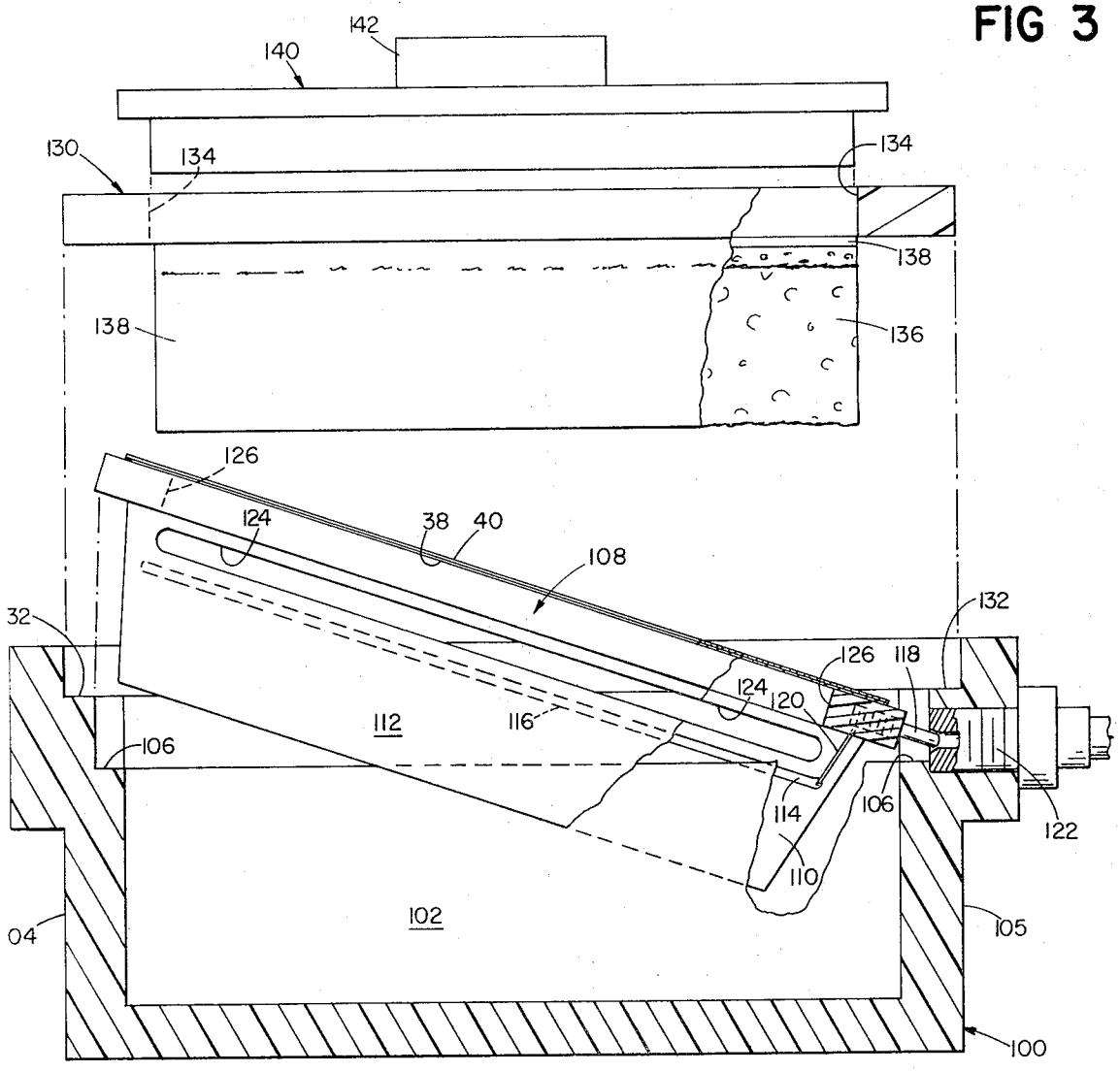

1
44
42
46
40
46
52
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36
52
38
48
34
34
48
34
12
14
16
26
30
28
32
18
20
34
34
34
34
22
24
1
10

FIG 2

FIG 3


142
140
130
134
134
138
138
136
126
124
38
40
108
32
132
126
118
124
120
112
116
106
106
114
122
110
102
04
105
100

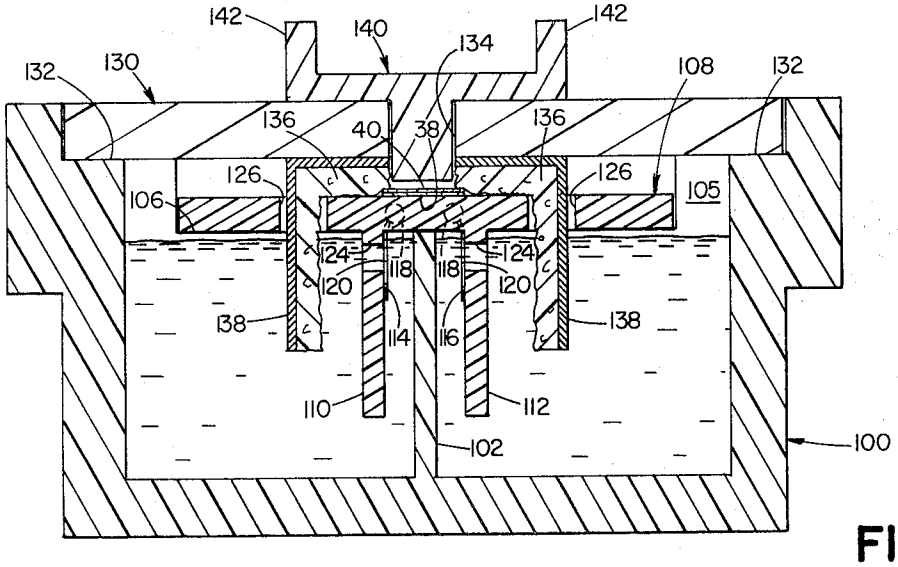

142
140
142
134
132
130
108
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136
40
38
136
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124
118
118
120
120
138
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114
116
110
112
102
100

FIG 4

ELECTROPHORESIS CHAMBER

This invention relates to electrophoresis, including immunoelectrophoresis, and pertains more specifically to improved apparatus for conducting a variety of electrophoretic reactions or separations.

Electrophoretic reactions or separations are conducted in thin layers of gel arranged to be in contact with electrically conductive liquids such as buffer solutions in which are immersed electrodes. The material to be reacted or separated is normally applied to the gel layer while an electric potential is applied to the electrodes. Because of the varying mobility of the molecules of the test material, certain of them are separated from others and/or are caused to enter into biophysical reactions in the gel layer. Because of the toxic or infectious nature of many of the materials employed, it is important that the operator or individual carrying out the procedure avoid contact with the materials as well as with the buffer solution itself.

The present invention provides apparatus for carrying out electrophoretic reactions and separations constructed and arranged so that the apparatus can be used repeatedly with minimum risk of the operator coming into contact with the test specimens or with any portion of the apparatus or of the electrical circuit with which the specimens or the buffer solution comes into contact. The procedures for using the apparatus and of replacing the gel layer after use are simple and efficient, facilitating use of the apparatus by operators with a minimum of training. The device is useful for all kinds of electrophoresis including, among others, counterelectrophoresis and immunoelectrophoresis.

In the drawings,

FIG. 1 is a view in cross-section showing one embodiment of the present invention;

FIG. 2 is a view in longitudinal section taken along line 2—2 of FIG. 1;

FIG. 3 is an exploded view in longitudinal elevation, partly broken away and in section, showing another embodiment in the process of assembly; and

FIG. 4 is a view in cross-section showing the embodiment of FIG. 3 after assembly.

As shown in the drawing, the apparatus of FIGS. 1 and 2 comprises a container **10** separated into two compartments by an imperforate median baffle **12.** The container and baffle are preferably constructed of a synthetic plastic material which is both electrically insulating and resistant to attack by the buffer solutions it is intended to contain. Extending across each compartment adjacent baffle **12** is a cylindrical mandrel **14, 16** also of electrically insulating plastic material on which is wound a spiral platinum wire electrode **18, 20** extending to terminals **22, 24** arranged to be connected to a suitable source of direct current (not shown). The spiral configuration of the wire facilitates elimination of bubbles which might otherwise interfere with conductivity. The electrodes are arranged to provide a short electrical path from one to the other during use.

Also extending across each compartment are additional baffles **26, 28, 30, 32,** each provided with slots **34, 34** adjacent top and bottom margins to provide for circulation of the buffer solution and providing pathways for ionic conductants.

Removably mounted on top of the baffles is a supporting element **36** also of plastic material serving to support a flexible plastic backing member **38** carrying bonded to its upper face a thin gel layer **40.** Supporting

element **36,** being supported by baffles **26, 28, 30,** and **32** is always maintained at the desired level in the container and serves as a firm base for maintaining the gel layer and its carrier in fixed position during application of the test material and operation of the device, ensuring uniform test conditions. The plastic backing member can be suitable water insoluble material such as a vinyl or acrylic polymer but is preferably transparent and preferably a polyester such as polyethylene terephthalate sold under the trade name Mylar or Cronar. The gel layer can be cross-linked dextran, polyacrylamide, or any other gel commonly used for this purpose, but is preferably agar or agarose. It may be a freshly prepared aqueous gel layer or one which has been previously dried and which is rehydrated either before or after being placed in the apparatus. When the backing member is transparent, the supporting element **36** is preferably opaque and black or other dark color to facilitate visual inspection of the gel layer during operation of the apparatus. Cover **42** which seats upon the walls of container **10** is provided with an aperture **44** positioned above gel layer **40** and has secured to its bottom adjacent opposite sides of aperture **44** a layer of bibulous material **46** from about ⅛ to ¼ inch thick in position to bear upon each opposing margin of gel layer **40** and extending laterally away from aperture **44** to a position beyond the margin of the gel layer. The layer of bibulous material may be any hydrophilic material such as cotton fabric, filter paper, regenerated cellulose open-celled sponge, or the like, capable of acting as a wick for the electroconductive liquid. Each layer of bibulous material thence extends downwardly supported by a rigid reinforcing plastic member **48** through appropriate slots **50** in support element **36.** In the embodiment shown, the apparatus is designed to accept backing member **38** in the form of a narrow elongated strip; additional slots **52** may be provided in support element **36** to accomodate other covers having layers of bibulous material more widely spaced so as to accomodate backing members and gel layers of different sizes and shapes. Sprocket **54** is mounted on an external wall of container **10** and take-up sprocket **56** is mounted at the opposite end of container **10,** being provided with crank handle **58,** and arranged to engage with appropriate sprocket holes in the margin of backing member **38.**

In operation of the device, the compartments are filled with suitable electroconductive liquids, e.g., buffer solutions such as an aqueous solution of sodium barbital to a level not exceeding that of support member **36.** The support element **36** is then put in place on top of the baffles and a strip of backing member carrying on its upper face a suitable layer of gel in a supplying reel (not shown) is led around sprocket **54** across the upper surface of support element **36** and over sprocket **56.** Cover **42** is then put in place with the layers of bibulous material **46** extending into the buffer solutions on opposite sides of baffle **12** and serving as a wick to transport the buffer solution into contact with the opposing margins of gel layer **40,** whence it permeates completely across the gel layer to provide an electroconductive path from one electrode to the other. The construction of the apparatus allows the wicks **46** to be applied simultaneously and uniformly to the margins of the gel layer **40** and avoids the necessity for the hands or fingers of the operator to come into contact with wicks **46** at any time. By employing a suitable ad-

hesive for securing the wicks to the bottom of cover **42** it is possible to remove and replace the wicks when and if desired. Moreover, because the level of the buffer solution in the compartments is below the level of gel film **40** on backing member **38,** and because slots **50** are wider than the combined thickness of bibulous material **46** and reinforcing member **48,** there is no possibility of a short circuit developing beneath the gel film. Measured quantities of the material to be tested are placed in spaced locations on gel layer **40** through aperture **44,** and the electrodes are connected to a suitable source of low voltage direct current (about 25 volts). After the desired reaction or separation has occurred, as may be determined for example by visual inspection through aperture **44** against the black background of supporting element **36,** crank handle **58** is used to advance the strip of backing material with the attached gel layer, the used gel layer stored if desired while the fresh layer is advanced beneath aperture **44** on top of support member **36.** Additional test materials are then introduced in the same manner.

In the embodiment shown in FIGS. **3** and **4,** open topped container **100** of synthetic plastic material is provided with a centrally-disposed imperforate baffle **102** dividing the container into two liquid-tight compartments. Each end wall **104, 105** of the container is provided with a recessed shoulder **106** adapted to support removably an end of supporting element or bridge member **108** which serves to support on its upper face backing member **38** with its gel film **40.** Bridge member **108** also supports two spaced apart baffles **110, 112** arranged to be on opposite sides of baffle **102** when the ends of the bridge member are supported on shoulders **106, 106.** Each baffle carries on its electrically nonconductive face nearest baffle **102** an electrode **114, 116** of a metal such as silver or platinum in the form of a thin narrow film or ribbon deposited for example by electroplating, sputtering or similar technique and bonded to the face of the baffle. In one end of supporting element **108** are mounted male bayonet electrical connectors **118, 118** each connected to an end of one electrode **114, 116** by a suitable wire conductor **120, 120.** Mounted in end wall **105** above shoulder **106** is a pair of female connectors **122, 122** in position to receive detachably the connectors **118, 118** when the bridge member is in position supported by shoulders **106, 106.** Connectors **122, 122** are connected by appropriate wires to a suitable source of direct electrical current (not shown). A slot **124** is provided near the upper edge of each baffle **110, 112** above electrodes **114, 116** to permit adequate circulation of buffer or electrolyte solution and provide a short electroconductive path from each electrode, and the lower end portions of baffles **110, 112** are cut away to permit insertion and removal of the bridge member as shown in FIG. **3.** A pair of slots **126, 126** is provided in bridge member **108** each disposed laterally outwardly of baffles **110, 112** respectively to permit a wick to pass through it into contact with the liquid.

Cover **130** the edges of which are received and supported by shoulders **132** inset in the walls of container **100** is provided with a central aperture **134** in position to overlie gel film **40** on supporting element **108** when the device is assembled and is otherwise similar to the cover **42** of the first embodiment, being provided with two wicks of bibulous material **136, 136** supported on reinforcing members **138, 138** secured to the bottom of cover **130** in position to extend through slots **126, 126** of supporting element **108,** and having their adjacent upper edges spaced from each other in position to lie in contact with opposing marginal portions of gel film **40.**

A closure **140** having upstanding handles or finger grips **142, 142** is arranged to fit into aperture **134** of cover **130** to seal it against dust and to reduce evaporation of electrolyte during use.

In use of the device the two compartments are filled to the desired level with suitable buffer solution and supporting element **108** is placed in position while joining connectors **118, 122** as shown in FIG. **3.** Gel film **40** with its backing **38** may be mounted in position along the center of the upper surface of supporting element **108** either before or after the supporting element is put in place, whereupon cover **130** is positioned with its edges supported by shoulders **132** and with the margins of wicks **136, 136** in overlying contact with opposing marginal portions of gel film **40.** The dimensions and clearances of the various components of the device are such that when supporting element **108** is in place with its ends resting on shoulders **106** and with connectors **118** and **122** engaged, the lower surfaces of wicks **136,** which are resilient after swelling with liquid buffer solution, are in gentle but firm contact with the upper marginal surfaces of gel film **40,** also swollen with the liquid, thus providing a continuous electrical path from one electrode to the other by way of the buffer solution, the wicks and the gel film.

Samples to be tested are placed on the surface of gel film **40** in the desired locations and are subjected to analysis by application of an appropriate electrical current to the electrodes, as in the embodiment of FIGS. **1** and **2.** Some or all of the container, bridge member, cover, and closure can be made of transparent plastic material to facilitate observation of the sample and of the interior of the container. Desirably the surface of supporting element **108** directly beneath gel film **40** on its backing member **38** can be opaque and of some color such as black to provide a contrast of color of the sample and to facilitate observation of the latter.

It will be appreciated that the construction of the present invention facilitates changing the buffer solution and the gel layer with a minimum risk of contamination to the operator because there is no necessity of touching or handling the bibulous materials or the buffer liquid.

I claim:

1. In electrophoresis apparatus comprising a pair of adjacent compartments each constructed and arranged to hold a supply of buffer solution,

an electrode in each said compartment in position to be submerged in the respective supply of buffer solution and means for connecting said electrodes to a source of electrical potential, and

means for supporting in position above the surface of said supplies of buffer solution an impervious backing member having an electrophoresis gel layer on its upper surface,

the improvement comprising a cover for said compartments having an aperture positioned over the location of said gel layer arranged to permit introduction therethrough of material to be subjected to electrophoresis, said cover having secured to its bottom adjacent each opposite side of said aperture a layer of bibulous material in position to bear

upon each opposing margin of said gel layer, each said layer of bibulous material extending laterally away from said aperture to a position beyond the margin of said underlying gel layer, thence downwardly into one of said compartments, each said layer of bibulous material being constructed and arranged to come into contact with its respective supply of buffer solution.

2. Apparatus as claimed in claim 1 including means for advancing said backing member with its gel layer along said supporting means.

3. Apparatus as claimed in claim 1 in which said supporting means for said backing member extends over said compartments and is provided with slots through which said layer of bibulous material extends downwardly.

4. In electrophoresis apparatus comprising a pair of adjacent compartments each constructed and arranged to hold a supply of buffer solution,

the improvement comprising removable means for supporting in position above the surface of said supplies of buffer solution an impervious backing member having an electrophoresis gel layer on its upper surface, said supporting means including a pair of electrodes and means for supporting each said electrode in each of said compartments in position to contact the respective supply of buffer solution and means for detachably connecting said electrodes to a source of electrical potential, and a cover for said compartments having an aperture positioned over the location of said gel layer arranged to permit introduction therethrough of material to be subjected to electrophoresis said cover having secured to its bottom adjacent each opposite side of said aperture a layer of bibulous material in position to bear upon each opposing margin of said gel layer, each said layer of bibulous material extending laterally away from said aperture to a position beyond the margin of said underlying gel layer, thence downwardly into one of said compartments, each said layer of bibulous material being constructed and arranged to come into contact with its respective supply of buffer solution.

5. Apparatus as claimed in claim 4 in which the means for supporting each said electrode comprises a baffle extending downwardly into each compartment.

6. Apparatus as claimed in claim 5 in which the detachable electrical connection means comprises a connection between said support means and the wall of the compartment.

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