June 7, 1966 S. RAYMOND 3,255,100

ELUTION ELECTROPHORESIS

Filed Sept. 15, 1964 3 Sheets-Sheet 1

FIG. 1

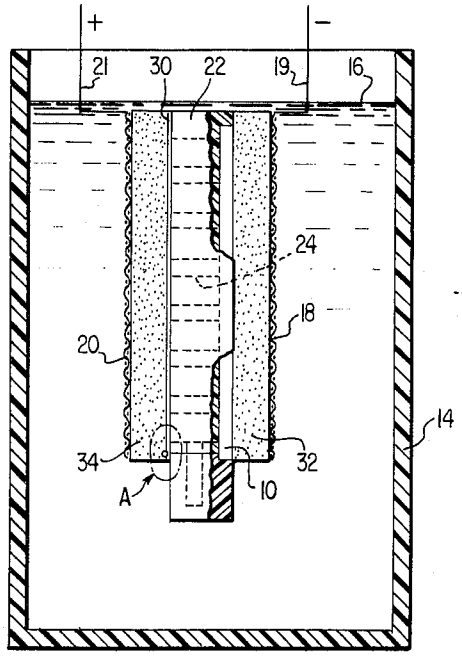
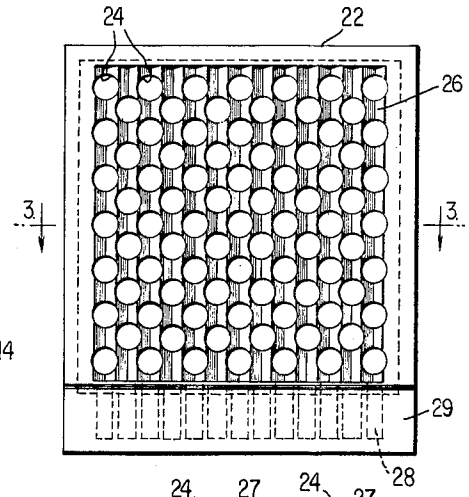


FIG. 2



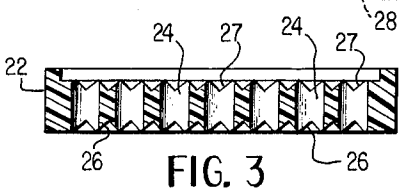


FIG. 3

FIG. 4

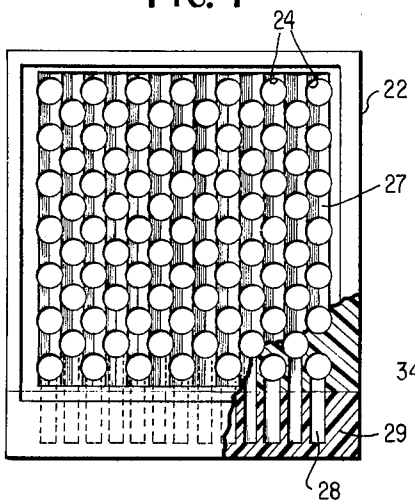


FIG. 5

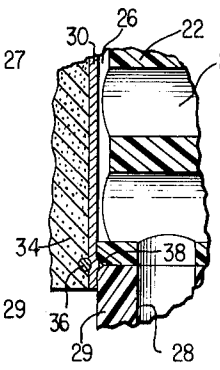
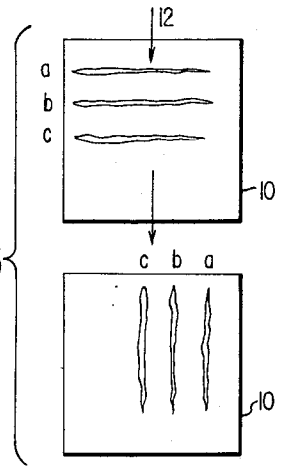


FIG. 6



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ELUTION ELECTROPHORESIS

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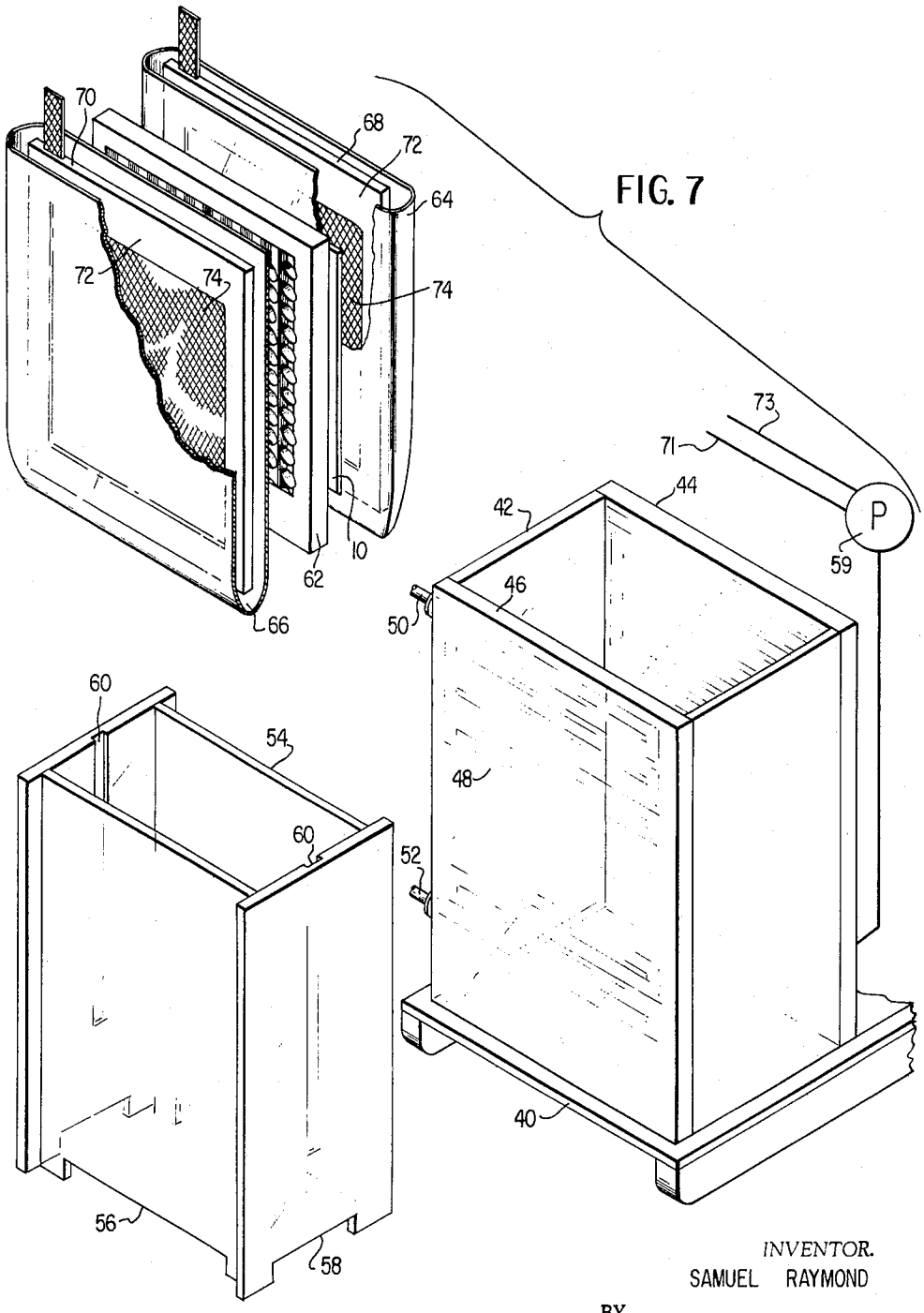

FIG. 7

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ELUTION ELECTROPHORESIS

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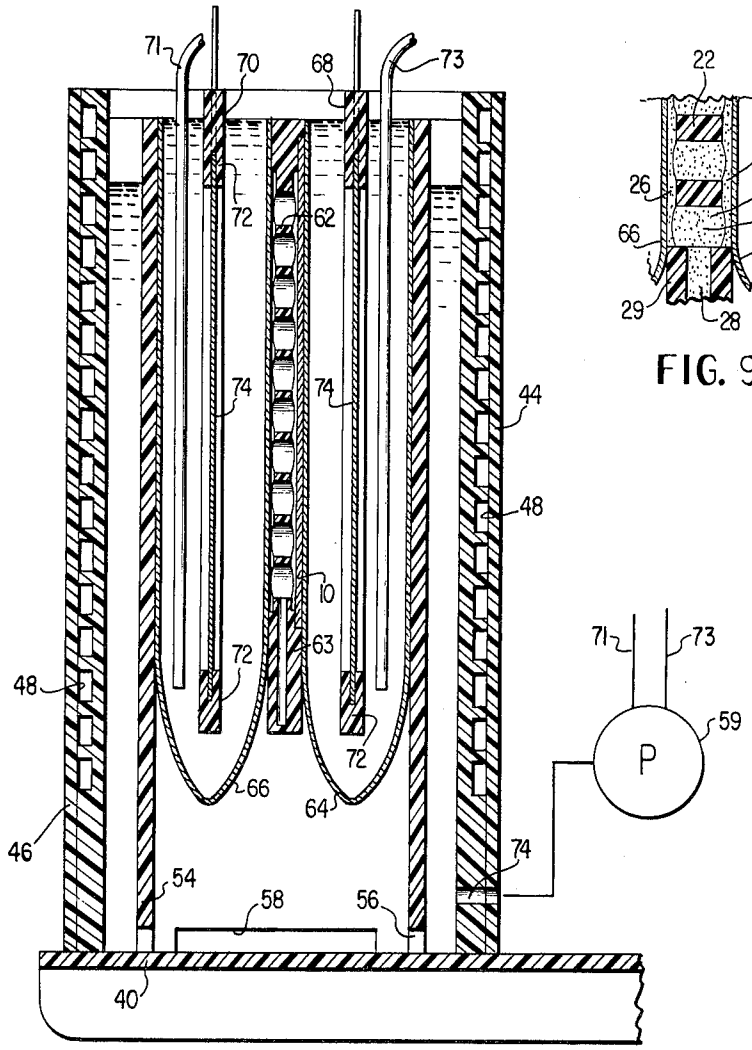

FIG. 8

FIG. 9

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3,255,100

ELUTION ELECTROPHORESIS

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Filed Sept. 15, 1964, Ser. No. 396,664
7 Claims. (Cl. 204—180)

This invention relates to improvements in the art of electrophoresis, and more particularly relates to a method and apparatus for elution convection 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 component fractions 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, after a suitable time separation of the various component fractions is accomplished. This migration and separation may take place on suitable support mediums such as a sheet of absorbent material-like filter paper or on gel such as acrylamide saturated with a buffer solution. Each component separated by the electrophoresis procedure is subject to a qualitative and quantitative analysis while on its support medium 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.

Electrophoresis generally may be practiced by the apparatus shown in my prior patent, 3,047,489, or the prior patent to myself and my brother, 3,129,158, or other types of apparatus known in the art.

In the prior known electrophoresis, each component may be analyzed while on its support medium and this type of electrophoresis may be considered as analytical electrophoresis. However, many times it is desirable and necessary to collect the isolated component fraction in its pure state and without other substances such as its support medium and without change in the component. The isolated component could then be put to further use. It is to this type of electrophoresis that this invention is directed and to distinguish it from the prior known analytical electrophoresis it is termed "elution convection electrophoresis."

After the component fractions of a complex substance are separated by analytical electrophoresis while migrating in the direction of current between two electrodes while on a support medium, it is many times desirable to isolate the separated components from the support for further use. It is the object of this invention to provide a method and apparatus for preparing isolated components by themselves and without change by an electrophoresis procedure.

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 a schematic illustration of the apparatus of this invention placed in a buffer solution-containing tank which is shown in section.

FIG. 2 is a side elevation of a separation grid used in the apparatus of this invention for keeping the separated components isolated from one another.

FIG. 3 is a sectional view of the grid taken along line 3—3 of FIG. 2.

FIG. 4 is a side elevation showing the reverse side of the grid from that shown in FIG. 2 with a portion of the grid shown in section.

FIG. 5 is a detail view of a portion of the grid and adjacent components shown as detail A in FIG. 1.

FIG. 6 is an illustration of the separated components of a complex substance after practicing analytical electrophoresis thereon and illustrates how the support medium is rotated prior to practicing preparative electrophoresis of this invention.

FIG. 7 is a perspective exploded view of a preferred embodiment of the apparatus of this invention.

FIG. 8 is a sectional view taken through the assembled apparatus shown in FIG. 7.

FIG. 9 is a detail view of a portion of the separation grid illustrating another method of practicing the invention.

In general, this invention provides a method and means for isolating and collecting pure components from their supporting medium after they have been separated by analytical electrophoresis. This is accomplished by placing the supporting electrophoresis medium with the separate zones of previously separated components thereon in a further electrophoresis cell with the direction of current in such cell at an angle to the plane of the supporting medium to remove the separated components from their support. After the separated components have been removed from their support and are migrating under the influence of the electric field, they are kept separated by a suitable separating block, their path of movement is intercepted by a substantially vertical dialysis membrane, then they accumulate at this membrane without passing through it, then the accumulated separated components on the membrane fall gravitationally in a convection current into separate collection receptacles.

Referring to the drawings for a more particular description of the invention, in FIG. 6 there is shown an electrophoresis support medium **10** which may be a slab of acrylamide gel or the like having thereon three zones of separated component fractions *a*, *b*, and *c* of a complex substance. These fractions having been previously separated from a complex substance in an electrophoresis process by passing direct current between electrodes generally in the direction of the arrows **12** for a suitable period of time. Such may be accomplished by means known in the art such as shown in my prior mentioned patents. The different components of the initial complex substance migrate at different rates under the influence of electrophoresis and they may be separated on the gel support using the apparatus of my prior Patent 3,047,489 or the process and disclosed apparatus of my Patent 3,129,158.

After the components *a*, *b*, and *c* are separated by electrophoresis into separate zones, the suporting medium which may be a gel slab **10** is removed from the apparatus and turned at an angle to the direction of original migration. Therefore, it is turned to the position as shown in the lower half of FIG. 6. At this position, the separated components *a*, *b*, and *c* extend generally vertically. With the gel slab **10** in the position shown in the lower half of FIG. 6 it is placed in another electrophoresis cell in a buffer solution and an electric direct current field is applied in a direction generally normal or perpendicular to the plane of the slab **10**.

As schematically illustrated in FIG. 1, the apparatus includes a tank **14** containing buffer solution **16** to a suitable level therein. A pair of wire grid electrodes **18** and **20** are connected to opposite poles **19** and **21** of a source of D.C. so that a D.C. field is established therebetween. The electrophoresis support medium **10** with the separated components *a*, *b*, and *c* extending generally vertically is placed against a separating grid **22** with the

components facing and abutting one side of the grid. The grid has a plurality of holes **24** arranged in slightly overlapping vertical columns as shown in FIGS. 3 and 4 and extending completely through the grid **22**. The pattern of holes **24** also slightly overlaps horizontally providing an arrangement similar to a honeycomb. This arrangement of the holes **24** will not leave any blank spaces between adjacent fractions. On the side of the grid opposite that where the gel slab **10** is placed there are a plurality of component draining vertical grooves **26** as shown for example in FIG. 3. Similar vertical grooves **27** are provided on the face of the grid adjacent the gel slab **10**, and these groves allow for natural convection circulation of the buffer solution. Alternatively, grooves **26** could be much deeper to provide the same function. As a further alternative external curents of buffer solution may be applied eliminating the need for grooves **27**. At the bottom of each vertical drain groove **26** there is a collecting receptacle **28**. These collecting receptacles **28** may be provided in a separate block **29** which is secured to the grid block **22** as shown in FIGS. 4 and 5. On the back or grooved surface of the grid **22** there is a dialysis membrane **30** which allows the electric field and buffer solution to pass therethrough but will not pass the separated components.

Suitable pressing means are provided adjacent the electrodes **18** and **20** and in the FIG. 1 embodiment these pressing means may be sponge blocks **32** and **34**. Sponge block **32** presses the slab **10** against one face of the grid while pressing block **34** presses the dialysis membrane **30** against the other side of the grid.

As an additional precaution and for sealing purposes a sealing means may be provided at the bottom of the dialysis membrane and this sealing means includes a rubber rod **36** and a groove **38** in the receptacle block. The pressing of the sponge **34** assures a seal at the area of the groove **38** in rod **36** so that separated fractions will not leak out from inside the membrane **30**.

The assembly of electrodes, sponges, membrane matrix and gel slab are held together by suitable mechanical means not completely shown in the diagrammatical illustration of FIG. 1.

In operation, upon the application of a relative low voltage D.C. curent to the electrodes, a field is established and electrophoretically active material components *a*, *b*, and *c* (which are electronegative) migrate from the support **10** into the holes in the grid block **22** in a direction toward the positive electrode **20** with dialysis membrane **30** positioned in their path on the opposite face of the grid **22**. The voltage between the electrodes is preferably less than 5 volts per cm. of electrode spacing to prevent the migrating components from sticking to the blocking membrane **30**. The still separated pure components with the buffer solution flow by gravity downward in the vertical channels or grooves **26** until they are stopped by the seal at the bottom of the grooves and then they flow into the separate receptacles **28**. Grooves **27** allow for recirculation of the buffer without the separated components. Thus, the sample receptacles **28** will contain the various separated components. To remove the components from the receptacles **28**, the grid **22** is separated from the receptacle block **29** or a pipette or the like may be inserted into the receptacle to remove the pure components.

FIG. 7 shows a preferred form of the apparatus in an exploded perspective view. In this form there is a suitable support **40** for a liquid holding tank **42**. The tank has hollow side walls **44** and **46** having therein coolant passage **48** for the passage of coolant through tubes **50** and **52** for the purpose of temperature control of buffer solution in the tank. Sitting inside the tank is a generally vertical support box **54** having relieved portions **56** and **58** in the bottom of its walls to allow circulation of buffer solution (by external pump **59**) and including a center slot or groove **60** in both edges to support the grid block. Box **54** is only an example of one way to hold the assembly and other suitable arrangements could be used. Similarly, instead of the coolant passages **48** suitable external cooling means could be utilized. The separating grid block **62** may be the same or substantially the same as the block **22** in the FIG. 1 embodiment.

In the FIG. 7 embodiment, the pressing means for pressing the electrophoresis support medium against the matrix and for pressing the membrane against the matrix takes a different form, namely they are bags of dialysis membrane material such as a cellulose acetate film. More particularly, bags **64** and **66**, shown partly cut away in FIG. 7 for the sake of clarity, completely enclose electrode assemblies **68** and **70**. These bags are filled with buffer solution causing each bag to press against the matrix or separating grid **62** from both side faces thereof. However, prior to filling the bags the electrophoresis matrix or support medium **10** is placed against the face of the grid and is supported on support step **63** of the grid block **62**.

The electrode assemblies **68** and **70** are substantially identical so only one will be described. Each electrode includes a frame **72** of Plexiglas or the like in which is embedded a grid type electrode such as a perforated stainless steel sheet **74**, having suitable connecting terminals.

Circulating pump **59** circulates buffer solution to the inside of bags **64** and **66** through suitable tubes **71** and **73**. The solution flows into both bags simultaneously in parallel streams directed to the bottom of the bags. Then the solution flows upwardly and overflows the bags. The buffer solution which overflows from the bags into the tank **42** and is recirculated to the pump through passage **74** and this continuous recirculation of buffer solution is very beneficial as it accomplishes the following:

(a) It keeps the bags filled to a height above the level of buffer in the tank **42**. Without pumping, the liquid buffer would gradually dialyze out of the bags until the levels were equal inside and out.

(b) It remixes the anode and cathode buffer solution, thus neutralizing any pH or concentration changes which result from electrode reactions within the bags.

(c) It provides adequate heat transfer. Heat is generated by passage of the electric current through the electrodes, bags, gel slab, and separating grid. At normal operating currents and voltages, the heat is far too much to be removed by direct conduction; convection transfer of heat is impossible in the gel; the temperatures are not high enough for radiant heat transfer, and no evaporation can take place in the totally confined gel and matrix. Therefore, heat must be removed either by reducing the current and voltage (thereby prolonging the separating time unduly) or by pumping in cooled buffer and removing warmed buffer by overflow.

(d) Finally, continuous pumping allows us to use a construction in which there can be mass leaks from the bags **64**, **66**. It is not easy to get leak-proof bags of dialysis membrane, and those presently available are not very large. But by using sheets of membrane held in place by gaskets, the apparatus can be designed for any size desired. Gaskets, however, entail leaks, and these, if not too great, can be overcome by sufficiently rapid pumping into the bags.

In operation of the FIG. 7 and 8 embodiment the electrophoretic support medium **10** with the zones of separated components, *a*, *b*, and *c* extending generally vertically thereon is placed against one side face of the grid block **62** as shown in FIGS. 7 and 8 and the grid is slid into groove **60** in the holding box **54** which in turn is placed in the tank **42**. The two electrode assemblies are inserted together with their encircling bags **64** and **66** and the bags are filled to overflowing with buffer solution thereby covering the electrodes, the bags being made of dialysis membrane material to prevent the fractions from passing therethrough while allowing the buffer solution

to pass. Then the entire tank **42** is filled with buffer solution to a level somewhat below the level of the buffer solution in the bag so that a hydrostatic head exists which acts to hold the face of one bag against the groove side of the matrix and also acts to press firmly but without excessive pressure on the gel slab **10** on the other side of the grid. The pump **59** is started and the electrical connections are made to start the elution convection.

Another method of accomplishing preparative electrophoresis within the scope of this invention is illustrated in FIG. 9. In this method the electrophoresis gel slab with the initially separated components thereon is laid on a flat surface such as a table. The separating grid such as grid **22** or **62** is placed on top of the slab and aligned so that the vertical grooves **26** coincide with the zones or pattern of separated components on the gel slab **10**. The grid **22** is then pressed into the gel slab **10**. Because of the pliable nature of the gel, it is forced into the holes **24** of the grid. If the web of the grid between the holes is sufficiently narrow all of the gel can be squeezed into the hole thus subdividing the gel slab into a series of plugs filling or partially filling the holes **24** in the grid **22**. This is accomplished by laying out the holes in a honeycomb pattern. Then, the grid carrying the plugs of gel is inserted into the vertical holder and a dialysis membrane is applied to the face of the separating matrix having the vertical grooves and is held thereagainst either by the flexible porous sponge **34** of FIG. 1 or by the bag **66** of FIG. 7 while the electric field is passed through the gel in a direction causing the components to migrate toward the dialysis membrane and the separated components then flow by gravity down the vertical grooves **26** into the collecting receptacles **28** as explained before.

While there have been shown and described and pointed out the fundamental novel features of the invention as applied to the preferred embodiment, 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.

I claim:

1. An apparatus for elution electrophoresis on components separated by analytical electrophoresis on a planar support medium, the apparatus comprising; a tank for containing a buffer solution, means for supporting the separated components on their planar support medium within the tank, a pair of generally planar electrodes sandwiching the faces of the support medium carrying the separated components, means for passing current from the electrodes to cause the components to migrate in buffer solution from their support medium towards one of the electrodes, mechanical means for fluid channelizing of separated components simultaneously, the mechanical means including a membrane permeable to an electric field but impermeable to the separated fractions and a dividing block, and means for simultaneously collecting the separated components.

2. A method of elution electrophoresis comprising;

(a) separating constituents of a composition by electrophoresis on a planar type support whereby different components migrate different distances on the support in a predetermined direction under the application of electric potential,

(b) removing the separated components from the planar support by subjecting them to an electric field in a buffer solution, the field directed generally at an angle to the plane of the support,

(c) keeping the removed components separate from each other, and

(d) collecting the separately removed individual components.

3. A method of elution-electrophoresis comprising separating components of a complex substance by electrophoresis on a planar type support whereby different components of the substance migrate different distances to form a plurality of zones on the support under application of a D.C. electric field, positioning the planar support with the separated components thereon so that the zones of the components extend generally vertically, removing the separated components from the planar support by subjecting them to a D.C. electric field while in a buffer solution with the field directed generally at an angle to the plane of the support, keeping the removed constituents separate from one another, and collecting the separate and now removed individual constituents.

4. A process for preparing pure concentrated component fractions from a complex substance of an electrophoretically active material, the process comprising; first, separating the fractions of the complex substance into generally parallel zones by electrophoresis in a planar electrophoretic medium, then electrophoretically eluting the separate fractions in the parallel zones while the fractions are transported in a buffer solution by an electric field passing through the planar electrophoretic medium at an angle to the plane medium, keeping the eluted fractions in buffer solution separated from each other, and concentrating and collecting the eluted fractions in separate zones by transporting the eluted fractions by a separate current of buffer solution for each such fraction.

5. A process as defined in claim **4** further comprising interrupting electrophoretic migration of the eluted fractions during the elution step by a means permeable to the electric field and impermeable to the eluted fractions and external to the planar electrophoretic medium, and in which the concentrating and collecting of the eluted fractions is accomplished by buffer solution positively circulated flowing in a direction generally parallel to the separated zones.

6. A process as defined in claim **4** further comprising interrupting the electrophoretic migration of the eluted fractions during the elution step by a means permeable to the electric field and impermeable to the eluted fractions, and in which concentrating and collecting of the eluted fractions is accomplished by convection currents of buffer solution.

7. A process as defined in claim **6** in which the parallel zones are disposed in generally vertical position during the elution step.

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