United States Patent

Strickler

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[54] CONTINUOUS ELECTROPHORESIS CELL WITH LATERAL PH GRADIENT

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[73] Assignee: **Beckman Instruments, Inc.**

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

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

[56] **References Cited**

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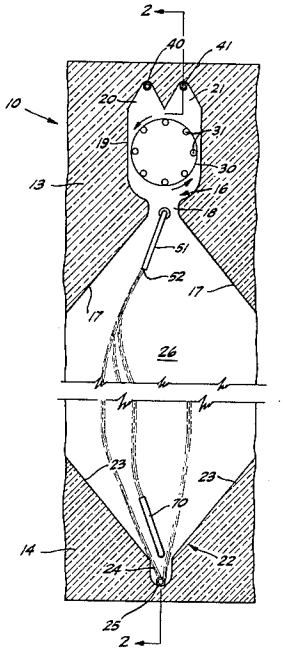
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[57] **ABSTRACT**

In a continuous electrophoresis cell, the present invention comprises means for establishing a lateral pH gradient in the electrolyte curtain whereby electrophoresis may be conducted at different lateral positions within the curtain, and in which, if desired, components may equilibrate at different lateral positions according to their isoelectric points. According to a first embodiment of the present invention, two curtain solutions of different pH are introduced continuously at laterally spaced positions. A gradient smoothing device is provided for partially mixing the two curtain solutions to such an extent that the plot of pH from one side of the curtain to the other is a continuous function, preferably approximately linear. According to a second embodiment of the invention, a hybrid arrangement permits either a two-curtain solution feed for pH effect exploration or a single curtain solution feed for conventional operation.

17 Claims, 3 Drawing Figures



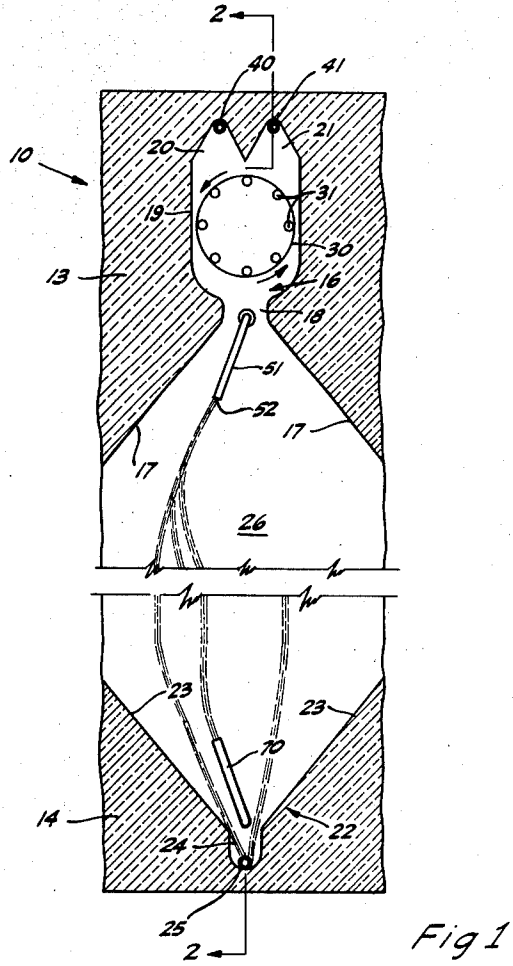


Fig 1

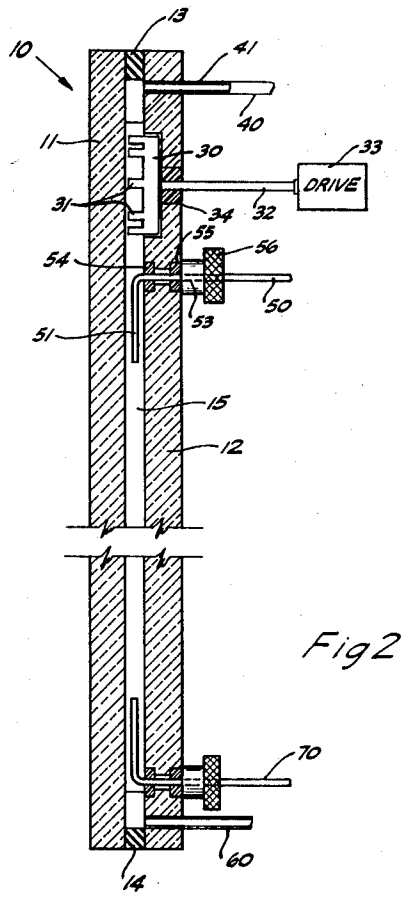


Fig 2

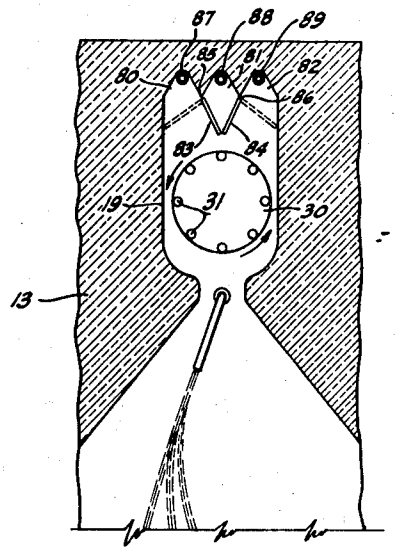


Fig 3

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CONTINUOUS ELECTROPHORESIS CELL WITH LATERAL PH GRADIENT

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to continuous electrophoresis and, more particularly, to a method and means for establishing a lateral pH gradient in the electrolyte curtain of a continuous electrophoresis cell.

2. Description of the Prior Art

Electrophoresis, in general, is the phenomenon of migration of charged particles or ions in a liquid carrier medium under the influence of an electric field. This phenomenon can be used to fractionate small particles or ionized components into separate bands dependent on their electrophoretic mobility or surface chemical properties.

In one form of continuous electrophoresis, a buffer solution or electrolyte is caused to flow freely as a thin film or curtain in an electrophoresis space between a pair of substantially flat plates of electrically insulating material mounted in substantially parallel, face-to-face relationship. The sample to be fractionated is injected continuously into the curtain in such a manner that it flows in a narrow band entrained within the electrolyte. An electric potential gradient is applied to the curtain at some angle to the flow, typically being perpendicular thereto. Such potential gradient causes the lateral separation of the sample components into various groups or components, in the form of a steady-state band pattern, depending upon various factors including the electrophoretic mobility of the respective components, the strength of the field, etc.

When continuous electrophoresis is used to explore the possibilities of fractionating a given mixture, an important parameter that needs to be varied is the composition of the curtain medium. It may be of interest to change the ionic strength, the cationic or anionic composition, the nature of the concentration of the surfactants present, etc. Probably the most important parameter that often needs to be varied is the pH of the curtain medium since the electrophoretic mobility of a component may vary markedly as a function of the medium pH.

In general, components that are in a more acid medium become more positive electrophoretically and vice versa. If they are initially negative, then they tend to become less negative or actually positive. For some types of particles, namely amphoteric particles, there is an especially strong dependence on pH. Amphoteric particles have two kinds of ionizable groups on the surface, one of which on ionization leaves a fixed positive charge, the other a fixed negative charge. Proteinaceous particles are an example, i.e. particles which have a surface which consists partly or wholly of protein. The charge on such particles depends on how acid or alkaline the medium is and there is a certain pH at which they are neutral. This pH value is called the isoelectric point. On the acid side of the isoelectric pH, the particle is positively charged whereas on the alkaline side of the isoelectric pH, the particle is negatively charged.

Under certain circumstances there may be two particles which, at one given pH, do not show any difference in electrophoretic mobility, but at a different pH have a significantly different mobility. In other words, it may be possible to separate some particles at a given pH whereas at another pH they could not be separated at all.

Another circumstance giving rise to the need for a pH gradient is the desire to perform isoelectric fractionations, i.e., electrophoretic separation of amphoteric substances or particles in which each component is allowed to reach an equilibrium pH position according to its isoelectric point.

Prior art attempts to vary the pH of the curtain medium for separation testing purposes have required separate curtain reservoirs for each desired pH value. In other words, in order to test the possibilities of separation at several different pH values, a corresponding number of reservoirs of substantial volume would be required with suitable valving to make the changeover from one reservoir to another. For the most carefully controlled results, the electrode rinse channel liquid would also need to be changed. This is not only inconvenient and cumbersome, but effectively limits the number of different pH values which can be conveniently tested.

The present invention also makes possible continuous isoelectric fractionation, while avoiding the use of density gradients and cumbersome pH gradient forming methods which have characterized earlier, non-continuous methods.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a technique for establishing a pH gradient laterally in the curtain medium, with sufficient curtain width available that electrophoresis can be conducted and tested when desired at several different lateral positions within the curtain.

Briefly, and in accordance with a first embodiment of the present invention, two different curtain solutions of different pH, usually in the form of related buffer compositions, are introduced continuously into an electrophoresis space at laterally spaced positions. A gradient smoothing device, which may consist of a rotating wheel having spurs projecting into the curtain layer, is utilized for partially mixing the two curtain solutions to such an extent that the plot of pH from one side of the curtain to the other is a continuous function, which is preferably approximately linear, An adjustable tube permits injection of the sample in successive lateral zones where fractionation may be conducted or tested. According to a second embodiment of the invention, a hybrid arrangement permits either a two-curtain solution feed for pH effect exploration or a single curtain feed for conventional operation.

It is therefore an object of the present invention to provide a novel form of continuous electrophoresis apparatus.

It is a further object of the present invention to provide a method and means for establishing a lateral pH gradient in the electrolyte curtain of a continuous electrophoresis cell.

It is a still further object of the present invention to provide means for establishing a lateral pH gradient in an electrolyte curtain and means for testing electrophoresis at any one of several different lateral positions within the curtain.

It is another object of the present invention to provide means for establishing a pH gradient in the electrolyte curtain of a continuous electrolysis cell for conducting isoelectric fractionations therein.

It is still another object of the present invention to provide a method and means for varying the composition of the curtain medium in a continuous electrophoresis cell.

Still other objects, features and attendant advantages of the present invention will become apparent to those skilled in the art from a reading of the following detailed description of the preferred embodiments constructed in accordance therewith, taken in conjunction with the accompanying drawings wherein like numerals designate like parts in the several figures and wherein:

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is a front sectional view of a first embodiment of electrophoresis cell constructed in accordance with the teachings of the present invention;

FIG. 2 is a side sectional view of the apparatus of FIG. 1 taken along the line 2—2 thereof; and

FIG. 3 is a partial, front sectional view of a second embodiment of electrophoresis cell constructed in accordance with the teachings of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring now to the drawings and, more particularly, to FIGS. 1 and 2 thereof, there is shown a first embodiment of electrophoresis cell, including means for establishing a lateral pH gradient in the electrolyte curtain, which may be similar to the apparatus described in my U.S. Pat. No. 3,412,007 issued Nov. 19, 1968 for Continuous Flow Electrophoresis Ap-

paratus. Accordingly, only so much of such electrophoresis apparatus as is necessary for an understanding of the present invention will be described hereinafter.

The present electrophoresis cell, generally designated **10**, comprises two substantially flat plates **11** and **12**, suitably supported by means not shown, which are held in substantially parallel, face-to-face relationship. Plates **11** and **12** are separated by gasket or spacer means **13** and **14** sandwiched between plates **11** and **12** near the upper and lower ends, respectively. Plates **11** and **12** may be made of glass or any other suitable electrically insulating material. Spacers **13** and **14** may be fabricated of rubber or a similar material.

Spacers **13** and **14** are shaped so as to define an electrophoresis working space **15** between plates **11** and **12** which serves as a conduit for the flowing electrolyte. In the embodiment illustrated in FIGS. **1** and **2**, space **15** has a top section, designated generally by the reference numeral **16**, having the shape of a modified inverted Y. Inverted Y-shaped section **16** has a fan-shaped lower portion **17** which terminates at its upper end in a narrow channel **18**. Above channel **18**, section **16** of space **15** widens into the shape of a parallel-sided channel **19** which terminates at its upper end in a pair of side-by-side, inverted, V-shaped sections **20** and **21**.

The bottom section of working space **15**, designated generally by the reference numeral **22**, has the shape of a Y. Y-shaped section **22** has a fan-shaped portion **23** which terminates at its lower end in a narrow, parallel-sided channel **24** having a rounded apex **25** at the bottom thereof. An intermediate section **26**, defined by the lateral edges of plates **11** and **12** and having substantially parallel sides, interconnects top and bottom sections **16** and **22**, respectively, of working space **15**.

Positioned in channel **19** is a wheel **30**, the front face of which lies parallel to the interior face of plate **12**. The remainder of wheel **30** extends into a recess formed in plate **12**. The front face of wheel **30** supports a plurality of spurs **31** which project into working space **15**. Wheel **30** is connected to one end of a shaft **32** which extends through plate **12** and is connected to a suitable drive means **33**, for reasons which will be explained more fully hereinafter. Shaft **32** is rotatably supported by leakproof bearing means mounted in plate **12**. Such leakproof bearing means may be in the form of a plastic insert **34** mounted in a recess formed in the back of plate **12**.

Two electrolyte solutions of different pH are introduced continuously into working space **15** via first and second tubes **40** and **41** which extend through plate **12**, tubes **40** and **41** being located at the apices of V-shaped sections **20** and **21** respectively. The magnitude of the pH difference may vary according to the application of the invention. A sample to be fractionated into its components is introduced or injected into working space **15** via suitable injection means such as a tubular element **50** which may be rotatably mounted in plate **12** in exactly the same manner as described in the beforementioned U.S. Pat. No. 3,412,007. As described therein, tube **50** is provided with a vertical section **51** lying in working space **15** and disposed substantially parallel to the interior faces of plates **11** and **12**. Vertical section **51** terminates in an injection tip **52** from which the sample flows into the electrolyte curtain. Tube **50** is also provided with a horizontal section **53** which is rotatably supported by leakproof bearing means mounted in plate **12**. Such bearing means may be in the form of a pair of plastic inserts **54** and **55** mounted in corresponding recesses formed in plate **12**. A wheel **56** secured to horizontal section **53** of tube **50** is provided for rotating tube **50**. Such wheel may be manually or mechanically operated.

As in the beforementioned U.S. Pat., tube **50** is mounted so that injection tip **52** lies at a point downstream of the electrolyte introduction points **40** and **41**. Preferably, injection tip **52** lies within the fan-shaped section **17** of working space **15** permitting tip **52** to be swivelled to substantially any point across the flowing electrolyte sheet. In addition, by rotatably mounting horizontal section **53** of tube **50** approximately at the vertex of fan-shaped portion **17**, vertical tube section **51** is always essentially parallel to the flow lines of the electrolyte, irrespective of its angular position. As a result of this geometry, there is minimal disturbance of the flow lines.

The electrolyte curtain is withdrawn or vented via a tube **60** which extends through plate **12** at apex **25** of channel **24**. In addition, means are provided for removing the sample components at a point downstream of sample injection tip **52**. According to the embodiment of FIGS. **1** and **2**, such sample removal means may be in the form of a tubular element **70** which may be constructed and rotatably mounted in the same manner as sample injection tube **50**. By varying the angular position of tube **70**, any sample band may be selectively removed. Since with this arrangement the extracted sample band is removed from the flowing film with substantially no change in its direction of flow, there is minimal disturbance of the flowing electrolyte sheet at the point of sample removal. In addition, by placing the rotatable mounting approximately at the vertex of fan-shaped portion **23**, tube **70** lies substantially parallel to the electrolyte flow lines irrespective of its angular position.

Means are also provided for applying an electric potential gradient across the electrolyte sheet flowing within working space **15**. Such means may conveniently be identical to that described in the beforementioned U.S. Pat. No. 3,412,007 so that no further discussion is necessary.

In operation, two electrolyte solutions of different pH are introduced continuously via tubes **40** and **41** at the top of gasket **13**. The two electrolyte solutions fan out in respective inverted V-sections and then flow downwardly in substantially parallel flow lines in channel **19**. Here the flow encounters gradient smoothing wheel **30** which is driven in rotation at some suitable speed by drive means **33**. By driving wheel **30** at some limited rate, spurs **31** act to transfer some of the left-side buffer solution to the right side, as well as vice versa. By a proper selection of the number of spurs **31** as well as the rotational speed of wheel **30**, wheel **30** partially mixes the two curtain solutions to such an extent that the plot of pH from one side of the curtain to the other is a continuous smooth function that may preferably be a straight line.

Below wheel **30**, the flow is again quiet and narrows into channel **18** where diffusion may exert a final smoothing effect. Below channel **18**, the electrolyte curtain diverges once again in fan-shaped section **17**. Thereafter, the curtain continues down the intermediate section **26** in parallel flow lines. Upon reaching bottom, Y-shaped section **22**, the flow lines converge toward apex **25** where the electrolyte is withdrawn or vented via tube **60**.

As stated previously, under certain circumstances, there may be two or more component bands which, at one given pH, do not show any difference in electrophoretic mobility, but at a different pH have a significantly different mobility. With such particle bands, it may be possible to separate the particles at one pH whereas at another pH they could not be separated at all. With the present apparatus, tube **50** may be scanned to inject the sample solution in successive lateral zones where fractionation may be tested until the pH which offers the greatest ability for fractionation is located. In this type of application of the invention, the two curtain solutions introduced into the cell may have widely different pH values.

The arrangement and method of the invention, without special modification, may be used also for isoelectric fractionations. The magnitude of pH difference between the two curtain solutions introduced into the cell may be selected according to the range of isoelectric points found in the components of the sample. The more acidic of the two solutions will be introduced on that side of the cell which is closest to the anode, giving a pH gradient which diminishes in pH value toward the anode. Thus, a component introduced into the curtain near the cathode side and having a negative charge would migrate toward the anode, becoming meanwhile less negatively charged as it migrates into a more acid environment. When it reaches a position where the curtain pH equals its isoelectric pH value, it has zero charge and ceases to migrate with respect

to the liquid. Except for electroosmotic effects which may impart a certain lateral movement to the liquid itself, the band, on reaching its equilibrium position, will thereafter descend vertically in the cell until it reaches the lower Y-shaped converging section. It will be seen that in isoelectric fractionations, the lateral position of sample injection is in principle unimportant to the separation, since each component will migrate, as required, to right or left, to find its isoelectric position.

The gradient smoothing device may be other than the wheel **30** shown. For example, a laterally vibrating or oscillating comb device may serve the same purpose. In addition, a portion or side band of each curtain solution may be allowed to descend, unmixed with the central region, at the respective outer edges of the electrophoresis working space **15**, just inside the membranes. This will isolate the working region from pH change due to a possibly different pH of the electrode rinse electrolyte. As an alternative, the rinse solutions on the two sides may be different, each matching the curtain medium on the respective side of the working region.

Referring now to FIG. **3**, there is shown an alternate embodiment of the present invention in the form of a hybrid arrangement which permits either a two-curtain solution feed for pH effect exploration or a single curtain feed for conventional operation. The embodiment of FIG. **3** is identical to the embodiment of FIGS. **1** and **2** except that top **16** of working space **15** is in the shape of three, side-by-side, inverted V-sections **80**, **81** pivotable **82**. A pair of pivotable weirs **83** and **84** are positioned in channel **19**, weirs **83** and **84** being pivotable around points **85** and **86**, respectively, located at the contact points between inverted V-sections **80** and **81**, and **82**, respectively. The embodiment of FIG. **3** also includes three electrolyte inlet tubes **87**, **88** and **89** located at the apices of V-sections **80**, **81** and **82**, respectively.

With weirs **83** and **84** in the position shown in solid lines in FIG. **3** and with two curtain solutions of widely differing pH introduced through tubes **87** and **89**, the operation is identical to that previously described with respect to the embodiment of FIG. **1** and **2**. On the other hand, with weirs **83** and **84** pivoted outwardly as shown in phantom in FIG. **3** and with a single curtain solution introduced through tube **88**, conventional operation is permitted. In this latter case, smoothing wheel **30** would remain stationary.

Instead of two different curtain solutions of widely differing pH being used, two different concentrations or types of cations, anions, etc. may be injected. In addition, to determine just what the compositional or pH distribution across the curtain actually is, the curtain, in the absence of sample, can be "scanned" stepwise by the fraction collection tube **70** and a plot made by measuring the curtain composition or pH.

It can therefore be seen that in accordance with the present invention there is provided a method and means for establishing a lateral pH gradient in the electrolyte curtain of a continuous particle electrophoresis cell, with sufficient curtain width available that electrophoresis can be tested, if desired, at several different lateral positions within the curtain, and permitting isoelectric fractionation, if desired. In accordance with a first embodiment of the present invention, two different curtain solutions of different pH may be introduced continuously into the electrophoresis space at laterally spaced positions. A gradient smoothing device, such as wheel **30** having spurs **31** projecting into the curtain layer, is utilized for partially mixing the two curtain solutions to such an extent that the plot of pH from one side of the curtain to the other is a continuous, preferably approximately linear, function. Adjustable tube **50** permits injection of the sample in successive lateral zones where fractionation may be tested. According to a second embodiment of the invention, as shown in FIG. **3**, there is provided a hybrid arrangement which permits either a two-curtain solution feed for pH effect exploration and isoelectric fractionation or a single curtain solution feed for conventional operations.

While the invention has been described with respect to the preferred physical embodiments constructed in accordance therewith, it will be apparent to those skilled in the art that various modifications and improvements may be made without departing from the scope and spirit of the invention.

I claim:

1. In a continuous particle electrophoresis apparatus having a pair of spaced, parallel plates defining an electrophoresis working space therebetween, the improvement comprising:
- means for introducing into said working space, at a first position, a first curtain solution;
- means for introducing into said working space, at a second position which is laterally spaced from said first position, a second curtain solution which is different from said first curtain solution; and
- movable means located within said working space adjacent said positions for partially mixing said first and second curtain solutions.

2. In a continuous particle electrophoresis apparatus according to claim **1**, the improvement wherein said means for mixing comprises:
- a wheel, the front face of said wheel lying parallel to the interior face of one of said plates, the remainder of said wheel extending into a recess in said one plate;
- a plurality of spurs, said spurs being supported by said front face of said wheel and extending into said working space; and
- means for rotating said wheel about an axis which is perpendicular to said front face.

3. In a continuous particle electrophoresis apparatus according to claim **1**, the improvement further comprising:
- means for injecting a sample solution into said working space at a point downstream of said curtain solutions introduction points.

4. In a continuous particle electrophoresis apparatus according to claim **3**, the improvement wherein said means for injecting a sample solution into said working space includes means for selecting the lateral position of sample solution injection.

5. In a continuous particle electrophoresis apparatus according to claim **3**, the improvement wherein said means for injecting a sample solution into said working space comprises:
- a tubular element having a first section lying in said working space and disposed substantially parallel to the interior faces of said plates and a second section extending through one of said plates perpendicular to said first section, said tubular element being pivotable about said second section thereof.

6. In a continuous particle electrophoresis apparatus according to claim **1**, the improvement wherein said first and second curtain solutions have different pH values and said means for partially mixing said curtain solutions is operative to mix said solutions to such an extent that the plot of pH across the electrophoresis curtain is a substantially smooth, continuous function.

7. In a continuous particle electrophoresis apparatus according to claim **6**, the improvement wherein said continuous function is substantially linear.

8. In a continuous particle electrophoresis apparatus according to claim **6**, the improvement further comprising:
- means for injecting a sample solution into said working space at a point downstream of said curtain solutions introduction points.

9. In a continuous particle electrophoresis apparatus according to claim **8**, the improvement wherein said means for injecting a sample solution into said working space includes means for selecting the lateral position of sample solution injection.

10. In a continuous particle electrophoresis apparatus according to claim **1**, the improvement further comprising:
- means for introducing into said working space, at a third position which is intermediate said first and second positions, a third curtain solution; and

means located within said working space for selectively blocking the flow of either said third curtain solution or said first and second curtain solutions.

11. In a continuous particle electrophoresis apparatus according to claim **10**, the improvement wherein one end of said working space is in the shape of three, side-by-side, inverted V-sections, wherein said third curtain solution is introduced at the apex of the middle V-section, wherein aid first and second curtain solutions are introduced, respectively, at the apices of the remaining V-sections, and wherein said means for blocking comprises:

first and second pivotable weirs which are pivotable around first and second points located at the intersections between said V-sections.

12. In a continuous particle electrophoresis apparatus according to claim **10**, the improvement further comprising:

means for injecting a sample solution into said working space at a point downstream of said curtain solutions introduction points.

13. In a continuous particle electrophoresis apparatus wherein an electrolyte curtain flows in substantially parallel flow lines in an electrophoresis working space, the improvement comprising:

movable, mechanical means for establishing a lateral, substantially smooth, continuous pH gradient in said curtain; and

means for selectively injecting a sample solution into said curtain at any lateral position thereof.

14. In a continuous particle electrophoresis apparatus according to claim **13**, the improvement wherein said means for establishing a lateral pH gradient in said curtain comprises:

means for continuously introducing into said working space at laterally space positions two curtain solutions of different pH; and

means for partially mixing said two curtain solutions.

15. In a continuous particle electrophoresis apparatus according to claim **14**, the improvement wherein said means for partially mixing said two curtain solutions comprises:

a rotatable wheel, said wheel including a plurality of spurs which extend into said curtain solutions; and

means for rotating said wheel at a limited rate.

16. A method for performing isoelectric fractionations using an electrophoresis apparatus provided with an electrophoresis working space having an anode and a cathode positioned adjacent to its opposite sides for applying an electric potential gradient across a curtain flowing through said space comprising the steps of:

providing two curtain solutions of different pH;

introducing the more acidic curtain solution into said working space on the side thereof closest said anode;

introducing the more alkaline curtain solution into said working space on the side thereof closest said cathode;

mixing said curtain solutions to establish a lateral pH gradient in the curtain flowing through the working space; and

injecting a sample solution into said curtain.

17. A method as set forth in claim **16** wherein the magnitude of the pH difference between aid curtain solutions encompasses the range of isoelectric points in the components of said sample solution.

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