

[54] METHOD AND APPARATUS FOR CURTAIN ELECTROPHORESIS

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[51] Int. Cl. B01k 5/00

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

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[57]

ABSTRACT

A method and apparatus for curtain electrophoresis in which ionic species of a sample are separated by differential migration under the influence of an electric field gradient extending horizontally across a sheathed vertical curtain of a liquid medium. The sample enters the curtain, which includes buffer solution, adjacent the top of the curtain, and the sample fractions leave the curtain adjacent the bottom of the latter at different lateral locations with reference to the vertical center line of the curtain. The adverse effect of horizontal sample flow inequality on sample separation resolution, which inequality results in horizontal band broadening or spreading, is significantly reduced or eliminated. Such reduction is achieved by pulsing the electric field on and off in synchronization with the sample flow: the field is deenergized while the sample is entering the curtain and again while the sample is leaving the curtain.

4 Claims, 5 Drawing Figures

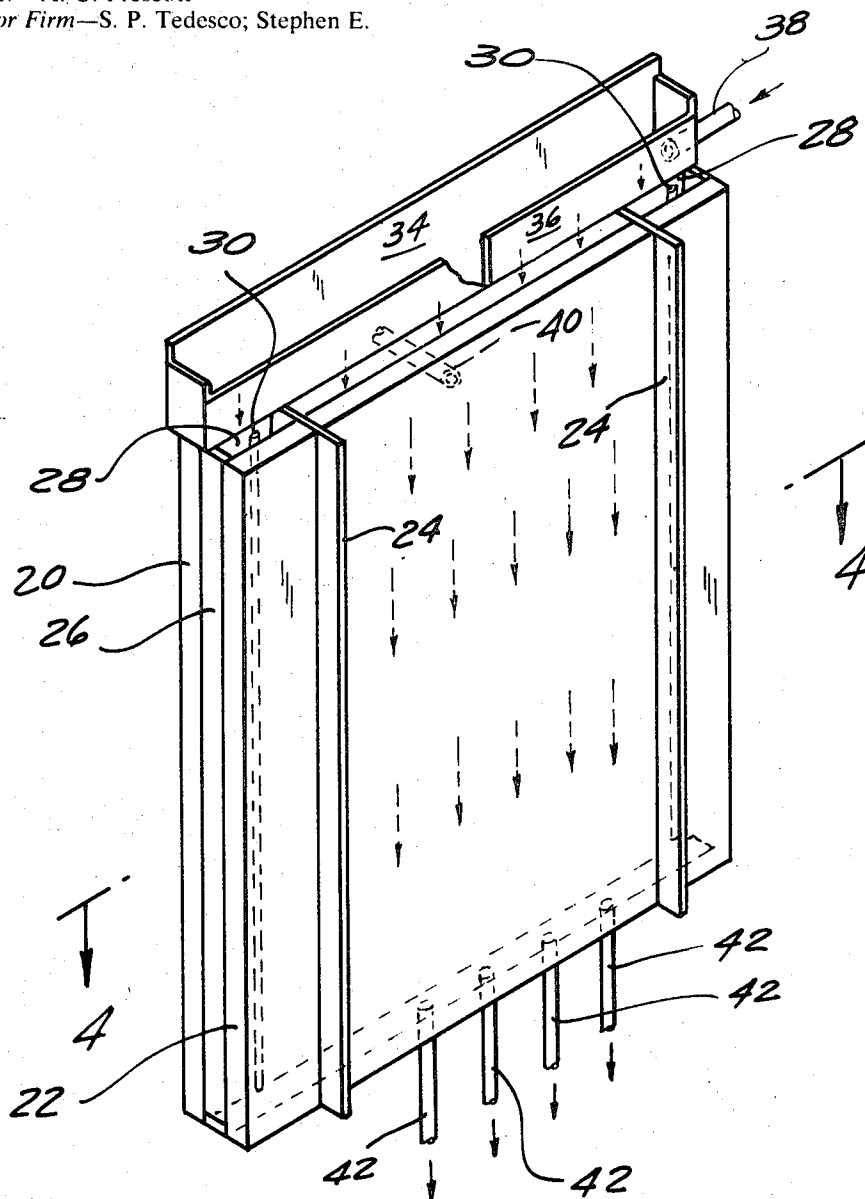


FIG. 1

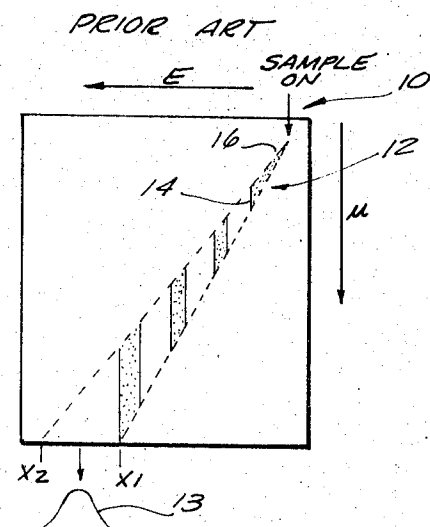


FIG. 2

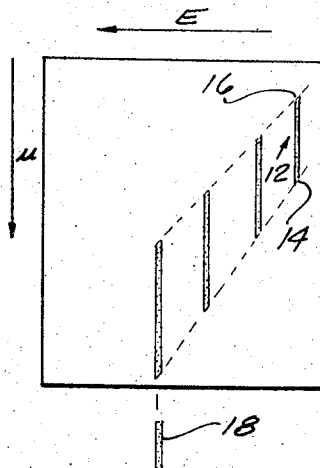


FIG. 3

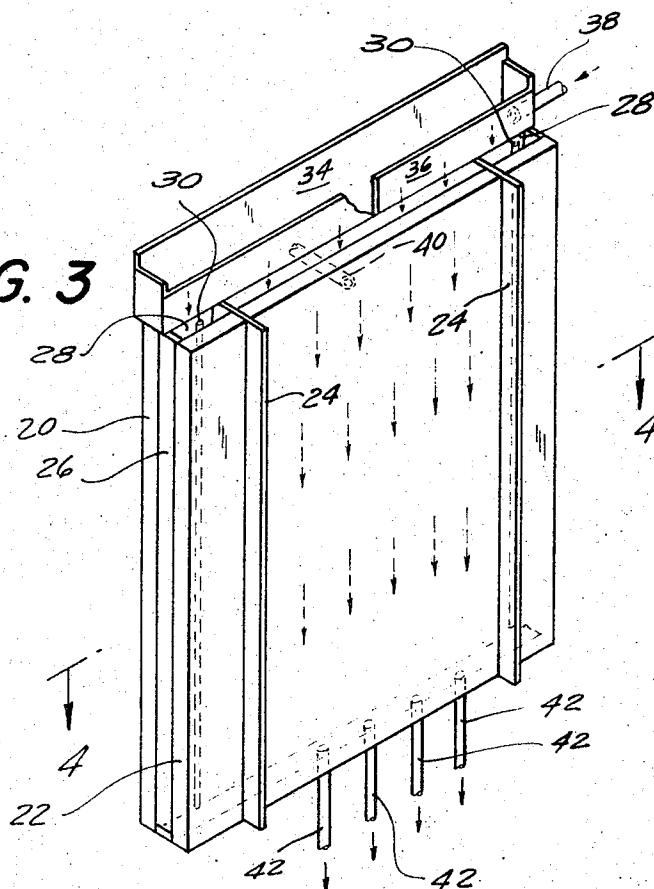


FIG. 4

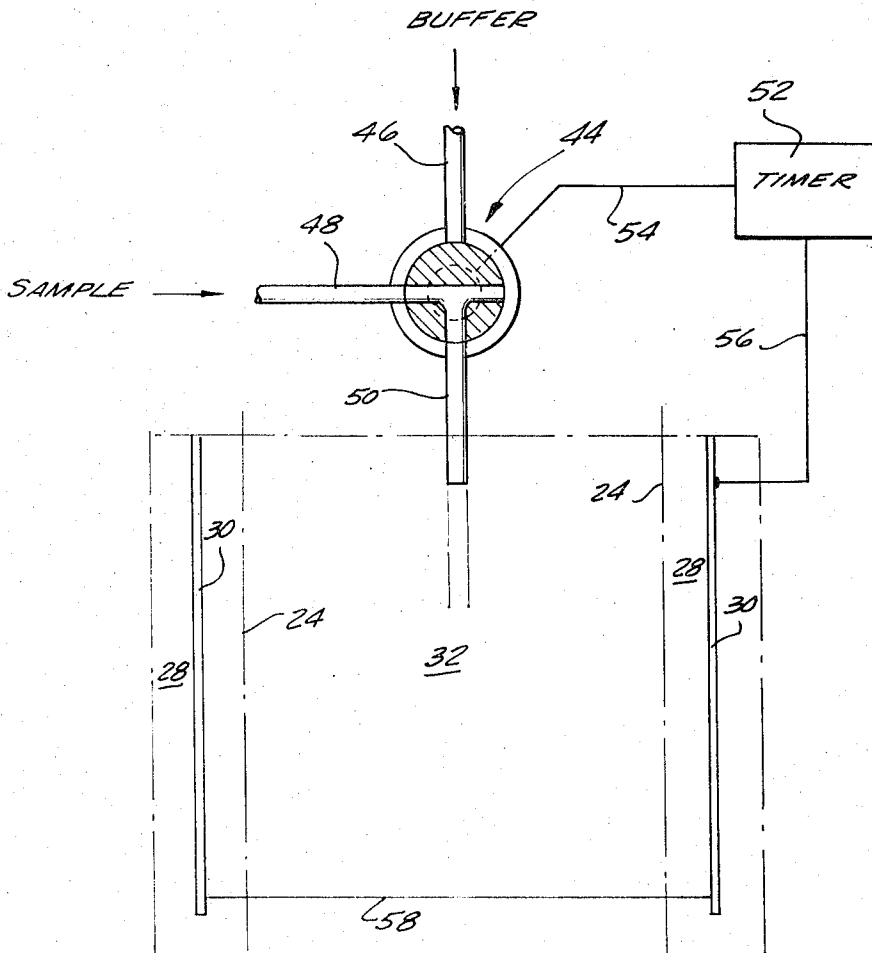
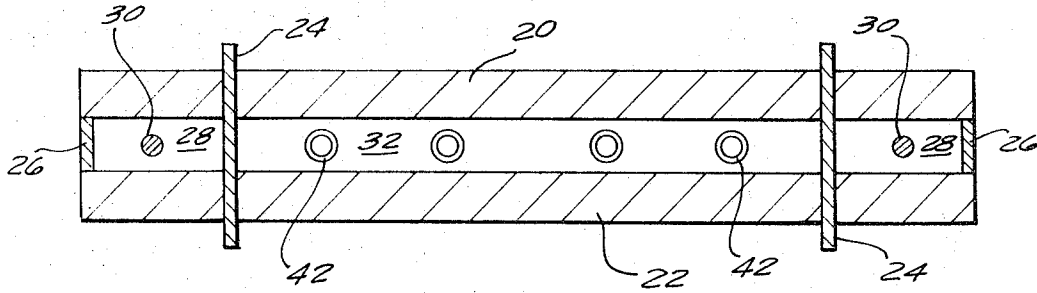


FIG. 5

METHOD AND APPARATUS FOR CURTAIN ELECTROPHORESIS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to electrophoresis and relates more particularly to electrophoresis of the sheathed curtain type.

2. Prior Art

Band broadening of a sample species in the direction of separation of species, i.e., in the direction of the electric field gradient and at right angles to the direction of the curtain flow, has had a known adverse effect on sample separation resolution in curtain electrophoresis. The relative performance of a given electrophoresis system, that is, the degree of separation per time unit, is determined both by the extent of differential migration of adjacent sample species and by band broadening or spreading of each species. Optimum separation requires, among other parameters, minimum band spreading.

Factors which are known to contribute to band broadening in electrophoresis include, among others, molecular diffusion, thermal diffusion and convection, electroosmosis and inequality of sample flow apart from electroosmosis. The molecular diffusion factor is unavoidable. However, certain factors which contribute to band broadening, such as thermal diffusion and convection for example, may be lessened when circumstances indicate that the factor is significant. For example, if circumstances indicate that it would be desirable to raise the field voltage, the electrophoresis system may be cooled to avoid intolerable thermal conditions resulting from such voltage increase, and/or the thickness of the sheath from front to back may be reduced, with concomitant reduction in the thickness of the flowing medium in the sheath.

I contemplate a method and apparatus for curtain electrophoresis wherein the electric field gradient is pulsed in synchronization with flow of the sample to reduce or eliminate the contribution to band broadening of sample flow inequality, apart from electroosmosis, in circumstances indicating that such band broadening factor is significant.

SUMMARY OF THE INVENTION

One object of the invention is to provide an improved method and apparatus for curtain electrophoresis for the above-stated purpose.

It is further contemplated to provide such method and apparatus in which ionic species of a sample are separated by differential migration under the influence of an electric field gradient extending horizontally across a sheathed vertical curtain of a liquid medium. The sample enters the curtain, which includes a buffer solution adjacent the top of the curtain, and the sample fractions leave the curtain adjacent the bottom of the latter at different lateral locations with reference to the vertical center line of the curtain. The adverse effect of horizontal sample flow inequality on sample separation resolution, which inequality results in horizontal band broadening or spreading, is significantly reduced or eliminated. Such reduction is achieved by pulsing the electric field on and off in synchronization with the sample flows: the field is deenergized while the sample

is entering the curtain and again while the sample is leaving the curtain.

BRIEF DESCRIPTION OF THE DRAWINGS

5 In the drawings:

FIG. 1 is a diagrammatic view illustrating horizontal band broadening in curtain electrophoresis according to the prior art;

FIG. 2 is a similar view illustrating horizontal band width controlled in accordance with the invention;

FIG. 3 is a somewhat schematic, fragmentary view in perspective of curtain electrophoresis apparatus embodying the invention;

FIG. 4 is an enlarged sectional view taken on line 15 4—4 of FIG. 3; and

FIG. 5 is a diagrammatic view of controls for the sample flow and the electric field in accordance with the invention.

20 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In FIG. 1 illustrating band broadening of a cylindrical jet of a single sample species with reference to a curtain in accordance with prior art electrophoresis, the outline of a vertically arranged oblong curtain is indicated generally at 10. The flow of the curtain which is a liquid medium is represented by the arrow U. While in practice the sample is introduced into the upper portion of the curtain in the vertically central plane of the curtain and equidistant from the pair of usual non-illustrated electrodes at zero field gradient, for ease of illustration the sample is shown as introduced in the upper right corner of the curtain. In consequence, the illustrated portion of the electric field gradient is in the direction of the arrow E and may be assumed to be a negative gradient increasing in strength to the left and effecting leftward displacement of the sample species by pressure of the field thereon. Hence, the sample species is subject to velocity profiles in the vertical and horizontal directions as in practice.

The sample 12 is shown in FIG. 1 moving progressively within the curtain after its introduction thereinto, and the illustrated representation of its movement and broadening is intended to show typical band broadening in accordance with prior art electrophoresis apparatus only with reference to horizontal sample flow inequality, apart from electroosmosis. As the sample has a finite depth in the curtain and the curtain is conventionally sheathed by a non-illustrated wall structure including closely spaced plates opposing one another, the laminar sample flow in the center of the curtain is at a far greater velocity than the velocity of the sample flow along the wall structure, and this results in the aforementioned inequality of sample flow. Such inequality of flow occasions band broadening in the direction of separation of species, which is the horizontal direction, and has an adverse effect on separation resolution as previously indicated.

Such adverse effect on separation resolution is observable at the bottom of the curtain when the band leaves the curtain for typical analysis at that location, as by collection in a tube for later use. As indicated in FIG. 1, the leading edge of the sample leaves the curtain at point X1 while the trailing sample edge leaves at point X2. The sample band width on leaving the curtain is X2-X1. The curve 13 below the curtain represents the concentration profile of the sample band leav-

ing the curtain 10. Band broadening obviously interferes with the separation and/or collection of many bands of species, e.g., up to and in excess of fifteen bands in a single curtain.

With reference to FIG. 1 sample flow conditions in the curtain in additional detail, it is to be noted that the sample introduction to the curtain is of finite duration, the leading edge of the sample band being indicated at 14 and the trailing portion at 16. The sample has finite width at the time it leaves the non-illustrated sample injection tube, and subsequently the sample band is broadened by the aforementioned factors which are time dependent. It will be observed in FIG. 1 that migration of the sample species under the influence of the electric field occurs while the sample is introduced into the curtain, and that this migration effects leftward displacement and horizontal broadening of the sample band. Similarly, as the sample band leaves the curtain, the trailing portion of the band continues under the influence of the field to migrate leftward. Under such continuing influence, the band is broadened further as shown.

FIG. 2 illustrates the same sample species in the same curtain and shows how deenergizing the electric field, both on introduction of the sample to the curtain and again as the sample leaves the curtain, controls band broadening in accordance with the invention. The entire sample band 12, with leading edge 14 and trailing portion 16, is admitted to the curtain while the field is deenergized. Of course, during such sample introduction there is inequality of vertical sample flow resulting in vertical band broadening but this does not adversely effect sample separation resolution. When the entire sample is in the curtain, the field is reenergized and the sample species band then commences lateral migration under the influence of the field. Such migration continues until the species band is about to commence leaving the curtain. At such time the field is again deenergized until the entire band has left the curtain. The band 18 below the curtain represents the flow of the sample species from the curtain, and it can be seen that the last-mentioned band is very much narrower, indicating much better separation resolution, than the base of the curve 13 of FIG. 1. To compensate for lost sample migration time during deenergization as aforesaid of the electric field, the field voltage may be increased.

Turning now to the structure of the electrophoresis system shown FIGS. 3-5, a pair of plate parts 20, 22 of dielectric material are vertically arranged in slightly spaced apart and opposing relation to another. The space between the plate parts is vertically partitioned conventionally by a pair of dialysis membranes 24, and the plate parts are provided with two side edge seals 26 therebetween. This construction and arrangement provides two side compartments 28 housing suitably mounted vertically arranged electrodes 30, respectively. Between the compartments 28, there is a compartment 32 which provides a conventional sheath for the electrophoresis curtain. The usual buffer solution is supplied to the interior of the sheath and to the electrode compartments 28 as by a spill-over trough, for example. The trough, which is arranged horizontally, has a vertical back wall 34, a shorter front wall 36 over which the solution spills along its horizontal extent, end walls and a bottom wall. Liquid buffer solution, which forms a salt bridge between the electrodes 30 through the

membranes 24, may be supplied to the trough through an inlet tube 38.

The bottoms of the compartments 28, 32 may be open for drainage therefrom. As shown in FIG. 3, a sample inlet 40 is formed in plate part 20 opening into the upper central portion of the compartment 32. In the illustrated form of the invention, the sample fractions separated from one another by differential migration in the field gradient between the electrodes 30 are not analyzed by being scanned with a spectrometer as they leave the curtain, but are collected for later use in numerous tubes, only four such tubes being shown and indicated at 42. Each tube 42 has the inlet end thereof extending upwardly between the respective lower edges of the plate parts 20, 22 in the compartment 32. If desired, the tubes 42 may convey the separated sample species therein for delivery to respective ones of analysis manifolds, not shown, in a continuous-flow type of automated analyzer.

In FIG. 5 illustrating the controls for pulsing the electric field on and off in synchronization with the sample flow, a solenoid-operated three-way valve is indicated generally at 44. The valve has a buffer solution inlet arm 46, a sample inlet arm 48 and an outlet arm 50. The outlet arm 50 is connected to the inlet 40 (FIG. 3) in the plate 20. The arm 46 is supplied with the usual buffer solution under pressure, and the arm 48 is supplied with sample under pressure. The valve 44 is controlled by a timer 52 through lead 54. The energization and deenergization of the electrodes 30 is also controlled by the timer. The electrodes 30 may be electrically connected in series and for this purpose one of the electrodes is shown connected to the timer by a lead 56, the electrodes being interconnected by a lead 58.

In operation, the valve 44 is actuated by the timer 52 to the valve position shown in FIG. 4 to admit sample to the electrophoresis curtain while simultaneously deenergizing the electrodes 30. The timer 52 next simultaneously actuates the valve 44 to shut off the sample supply to the curtain and admit buffer solution through the arm 46 to pass into the electrophoresis curtain through the valve, while energizing the electrodes 30. The sample in the curtain moves progressively there-through in the electric field and species of the sample are separated into respective bands by differential migration in the field. As the bands of species commence leaving the curtain at different times depending on the lateral displacement of the bands, it is necessary to deenergize the field before the first band commences to leave the curtain and to maintain the field deenergized until the last band has left the curtain. The timer 52 deenergizes the electrodes during this period. The timer may then initiate the next cycle of operation by placing the valve 44 in the condition of FIG. 4. In this manner a series of samples may be sequentially separated into sample components.

While only one form of the invention has been illustrated and described, it will be apparent, especially to those versed in the art, that the method and apparatus for curtain electrophoresis may take other forms, and are susceptible of various changes in details without departing from the principles of the invention.

What is claimed is:

1. In a method of curtain electrophoresis, wherein a sample is introduced into a sheathed curtain of buffer solution in an electric field for separation of at least one

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sample constituent by differential migration, which constituent forms a sample band laterally displaced with reference to the remainder of the sample when exiting from the curtain; the improvement of deenergizing the electric field during the interval that the sample is introduced into the curtain and again during the interval that the sample band exits from the curtain.

2. A method as defined in claim 1, further including collecting said sample constituent as it exits from said curtain.

3. A method as defined in claim 1, wherein: a series of samples are sequentially introduced into said curtain

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and said electric field is pulsed in phase with the sample flow to deenergize said field during the interval that each sample is introduced into the curtain and again during the interval that the sample exits from the curtain.

4. A method as defined in claim 3, wherein: each sample is separated in said curtain into a plurality of constituents forming sample bands laterally displaced with reference to one another, and collecting said sample constituents as they exit from the curtain.

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