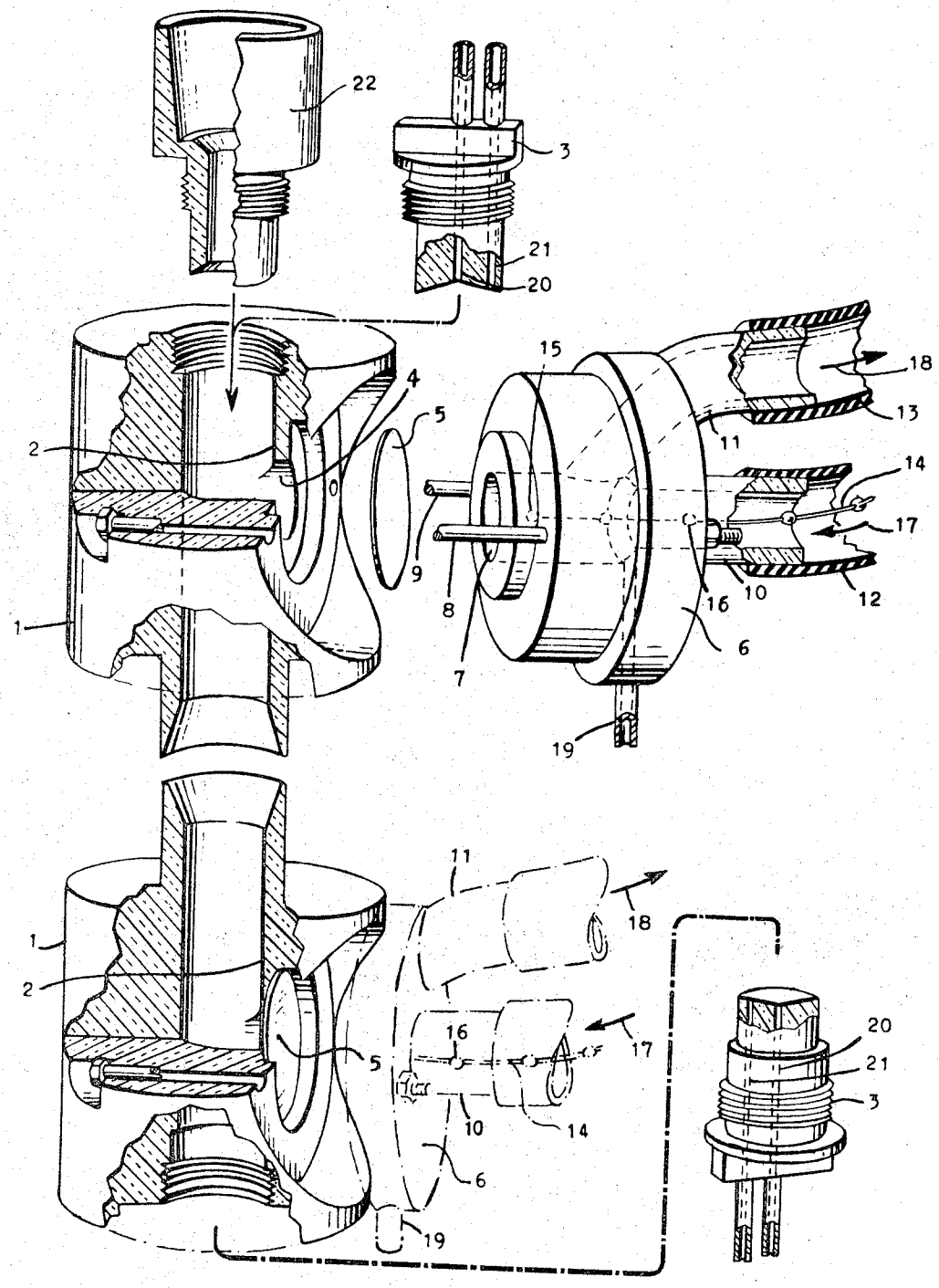
June 20, 1967 B. G. F. BERGRAHM 3,326,790

PROCESS AND APPARATUS FOR VERTICAL ZONE ELECTROPHORESIS

Filed Jan. 2, 1963



INVENTOR
BENGT GORAN FLEMMING BERGRAHM
BY
Stevens, Davis, Miller & Mosher
ATTORNEYS

United States Patent Office

3,326,790

Patented June 20, 1967

3,326,790

PROCESS AND APPARATUS FOR VERTICAL ZONE ELECTROPHORESIS

Bengt Goran Flemming Bergrahm, Stockholm, Sweden, assignor to Messrs. LKB-Produkter AB, Stockholm, Sweden

Filed Jan. 2, 1963, Ser. No. 248,959
Claims priority, application Sweden, Jan. 4, 1962, 82/62
6 Claims. (Cl. 204—180)

The present invention relates to electrophoretic methods and apparatuses and more specifically to improved apparatuses for the electrophoretic separation of high-molecular organic substances of a proteolytic character, and to a method for operating said apparatus.

The electrophoresis apparatuses used for the present are suffering from a number of objectionable disadvantages which limit their utility and render the operation more or less cumbersome. This is especially true of the methods for electrophoretic separation carried out in vertical columns the electrolyte of which has been stabilized, by packing with a supporting medium of an inert material such as starch, powders of cellulose or other materials, agar or various types of gels, against recombination due to uncontrolled convection. Because of the relative complexity of the apparatuses hitherto used the assembling and disassembling of the apparatus prior and subsequently to an operation has been time-consuming and laborious. This is especially the case when it is desired to establish the stabilizing medium in the form of a density gradient or in the form of a dextran-gel (such as the product "Sephadex" marketed by AB Pharmacia, Uppsala, Sweden).

Furthermore, in the known apparatuses electrodes of a relatively intricate design are utilized, requiring, for instance, the insertion of electrodes or electrode wires into U- or S-shaped glass tubes filled with an electrolyte and communicating more or less indirectly with the ends of the packed column.

From a theoretical point of view the electrode arrangement should be of such design that it can be applied in close proximity to the base and top of the separation column. In this way the distance between the ends of the electrodes will be approximately the same as or slightly larger than the effective length of the separation column, whereas in conventional U- or S-tube column electrophoresis apparatuses it is not possible to obtain the same electrophoretically favorable relation when calculating the quotient of the distance between the electrodes and the effective length of the separation column, which quotient in the latter case will be approximately two or more. Furthermore, for practical reasons, the electrode arrangement should be constructed so as to enable the use of different column types of various lengths. In addition to that the construction should permit free access to the top and base of the column before, during and subsequently to the performance of the electrophoresis operation, without unintentional distortion of the stabilized volume portion within the separation column. The aforementioned drawbacks are eliminated in the apparatus being a subject of the present invention.

According to the invention a device for supplying an electrolyte to one end of an electrophoresis column is provided, comprising a connecting block substantially in the form of a T-junction, said connecting block having centrally disposed therein an open-ended vertical passage, one opening of which being adapted to be connected with one end of the electrophoresis column and the other opening of which being provided with a replaceable stopper means to permit free access to the interior of said column, a horizontally extending passage connecting said first passage with the interior of a lateral electrode connecting member comprising a housing having a front wall and a back wall, the front wall of which is detachably secured to said connecting block, a diaphragm being interposed between the orifice of said horizontal passage and said electrode connecting member to prevent free liquid flow through said passage, said electrode connecting member having centrally located therein a substantially horizontal bore constituting an electrode chamber to be filled with an electrolyte, said horizontal bore bifurcating into two passages extending through the back wall of the housing and connecting said electrode chamber with an electrolyte supply tubing and an electrolyte return tubing, respectively, and a bore electrode terminating inside said electrode chamber adjacent to said diaphragm.

A practical construction of the present invention will now be described, by way of example only, with reference to the accompanying drawing, which is a perspective view, partly in section, of the essential parts of an electrophoresis apparatus according to the invention.

In the drawing a connecting block **1** has centrally located therein an open-ended vertical bore **2**, one opening of which is adapted to be connected to the end of a separation column (not shown) and the other opening of which is provided with a replaceable stopper means **3** to permit free access to the interior of said column. At right angles to the vertical bore **2** the connecting block has a horizontally outwardly extending bore **4** the vertical bore **2** of the connecting block forms a proper continuation of the separation column. Said vertical bore **2** of the connecting block, according to the electrophoretic technique and the type of column used, most frequently is stabilized but may also contain a non-stabilized volume portion of the electrolyte, whereas the electrolyte contained in the separation column proper always is stabilized by means of a capillary (or density gradient) supporting medium. By applying a suitable stopper in the bottom opening of the lower connecting block and in the top opening of the upper connecting block, respectively, it is possible to close the separation column in a manner which permits an easy accessibility not only before and subsequently to an electrophoresis operation but also in the course of the electrophoresis proper.

The mouth of the horizontal bore **4** of the connecting block **1** is covered by a semi-permeable membrane **5** which prevents liquid flow as well as a migration of high-molecular components therethrough whereas the ions of the electrolyte can pass freely.

A lateral electrode connection member coaxial with the horizontal bore **4** of the connecting block comprises a housing **6** having centrally located therein a substantially horizontal bore **7**, constituting an electrode chamber. The mouth at the front wall of said housing is detachably fastened to the aforementioned connecting block **1** by means of bolts **8**, **9** or the like, said semi-permeable membrane **5** being interposed therebetween. The horizontal bore (electrode chamber) of said electrode housing **6** bifurcates outwardly approximately in the form of a lying Y the branches **10**, **11** of which emerge at the back wall of the housing and communicate by means of flexible tubes **12**, **13** with an electrode vessel filled with a buffer solution (not shown).

A bare electrode wire **14** extends from the electrode vessel through one of the tubes (for instance the tube **12**) and terminates inside said electrode chamber **7** with a bead-shaped enlargement **15** to prevent damage of the membrane from the point of the wire. A number of beads **16** of insulating material in spaced relation is provided to prevent the wire from contacting the inner wall of the tube.

Because of the possibility of uniting the connecting block **1** and the lateral electrode connecting member **6**

into close (liquid-tight) contact with each other it is possible to establish an open tubular communication between the two electrode vessels attached to the upper and the lower electrode housing of the electrophoresis apparatus, respectively: electrode vessel I–double tubing I–lateral electrode connecting member I–connecting block I–separation column–connecting block II–lateral electrode connecting member II–double tubing II–electrode vessel II. (Upper electrode system=I; lower=II.) However, this open tubular communication is interrupted in two places by the aforementioned semi-permeable membranes **5** interposed between the connecting block **1** and the lateral electrode connecting member **6** of the upper and lower electrode assemblies, respectively. Thus, the separation column by means of semi-permeable membranes is separated from the electrode and electrode vessel system, whereas the electrical conductivity of the system is nevertheless maintained because of the ability of the ions to migrate through the membranes.

An important and novel feature according to the present invention resides in the fact that by means of the double tubular communication with the respective electrode vessels it is possible by means of a pump to establish a circulating buffer flow from the electrode vessel via one of the branches **10** of the electrode connecting member to the electrode chamber **7** and back to the electrode vessel via the second branch **11** as indicated by the arrows **17, 18.**

The presence in one of the branch ducts of an electrode wire **14**, the end of which terminates adjacent to said semi-permeable membrane **5** inserted between the connecting block **1** and the lateral electrode connecting member **6**, enables the attainment of some of the substantial electrophoretic benefits outlined above.

The aforementioned stopper means **3** may suitably be provided with a passage or passages **20, 21** to permit the introduction or removal of buffer solutions or proteolytes. Another stopper means is shown at **22** which may be used when it is desired during the electrophoretic separation to make provisions for a mechanical pumping action through the column system.

It hardly needs to be emphasized that the material from which the parts of the apparatus are fabricated must be inert to the liquids contacting the surfaces thereof. Examples of suitable materials are quartz, glass or synthetic resins wherein, if necessary, the surfaces coming into contact with the liquids may be coated with an insoluble highly inert material such an silicone or polytetrafluoroethylene resins.

The electrophoresis apparatus according to the present invention makes it possible to use different techniques in the establishment of various types of stabilizing media. (To stabilize the column against convection it is preferred to establish a capillary system or a combined capillary-density gradient system within the bore of the lower connecting block and the separation column, respectively.)

The novel construction of the electrophoresis apparatus according to the present invention which permits free access to the separation column in the course of an electrophoresis operation largely extends the electrophoretically potential separation possibilities as compared with an electrophoresis effected in a vertical-column electrophoresis apparatus of a conventional design. Furthermore, the connecting member **1** with the laterally attached electrode connecting member **6** which houses the electrode system and constitutes in itself the electrode chamber is of such a design that the drawbacks accompanying the electrophoretic separation as a result of the electrochemical processes at the electrodes (principally the formation of acids, bases and gases) are eliminated.

The improved electrophoresis apparatus being a subject of the present invention in its construction fulfills the theoretical as well as the practical desiderata. The novel apparatus provides for a simple means for the withdrawal

of the separated substances. Thus, in the emptying of the column it is not necessary to consider the non-stabilized volume portions on the electrode side of the membrane system within the bore **7** of the electrode connecting member.

In the course of an electrophoretic separation the surface of the buffer solution contained in the open electrode vessels should be maintained at the same level as the top of the separation column, preferably higher.

After the completion of an electrophoretic separation the buffer solution contained in the cavities of the electrode assembly may be drawn off through a drainage tube **19** provided for this purpose at the bottom of the housing **6** of the electrode connection member.

As mentioned hereinabove, most of the conventional apparatuses for vertical-column electrophoresis are suffering from a disadvantage as a result of the electrochemical processes at the electrodes (formation of acids, bases and gases) since in the known apparatuses it is not possible in a simple way to prevent the accumulation of the acid and alkaline products formed at the electrodes without affecting the stabilized volume portion in the separation column. Hence, it has previously been necessary to construct the electrode system for the purpose of, primarily, partly compensating the acid and base formation by the provision of large electrode vessel arrangements and by having the ends of the electrode bars ending at a relatively long distance from the ends of the separation column and, secondarily, constructing the apparatus in such a way that the gas formed freely can leave the electrophoresis apparatus through the non-stabilized system.

One of the great advantages attainable with the apparatus according to the present invention is the fact that when using the apparatus in a common manner without circulation of the buffer solutions within the respective electrode systems, the electrodes are located in close proximity of the top and bottom of the separation column, and in a manner so as to permit free access to the column not only during and subsequently to an electrophoresis operation but also in the course thereof.

Furthermore, because of the possibility of circulating the buffer solution past the connection with the top and bottom of the separation column, respectively, it is possible in a simple way continuously to "compensate" the acid- and base-formation. In this system it is, of course, also possible to vary the volume of the non-stabilized portion within the electrode vessel system.

By a controlled intermixing of the buffer solutions contained in the electrode vessels it is possible in the solution of biological separation problems additionally to improve the electrophoresis in a favorable sense. Thus, cations as well as anions are all the time migrating through the electrophoresis apparatus and the risk of the ion depletion easily occurring in a conventional vertical-column electrophoresis (i.e. non-circulating and non-intermixed buffer solutions) is still more prevented.

Further, by providing a third buffer solution contained in a vessel at the side of the anode or cathode vessels, and connecting said third buffer solution as a non-stabilized volume portion to the bottom or top of the separation column, by establishing a mechanically controllable buffer flow through the separation column in its entirety or through a part thereof (according to the construction of the separation column), in the course of the electrophoresis it is possible to influence the migration of the proteolytes to be separated in the direction desired, i.e. towards the anode or towards the cathode. If, for instance, it is desired to direct the buffer solution to the anode, the third non-stabilized volume portion is connected to the cathode end of the separation column. The buffer solution supplied to the separation column is withdrawn at the bottom of the separation column.

While the invention herein has been described with respect to particular embodiments, those skilled in the art

will appreciate that various modifications within the scope of the invention may be derived from the teachings herein. Thus, by the invention the scientist has to his disposal an apparatus of a flexible design permitting the use of various types of columns and arrangements consistent with the actual electrophoretic problems to be solved. Accordingly, this invention is not to be restricted unduly and is to be understood to extend all modifications which flow from the teachings herein.

Having now described the invention, what I claim as new and desire to secure by Letters Patent, is:

1. A device for supplying an electrolyte to one end of an electrophoresis column, comprising a connecting block substantially in the form of a T-junction, said connecting block having centrally disposed therein an open-ended vertical passage, one opening of which being adapted to be connected with one end of the electrophoresis column and the other opening of which being provided with a replaceable stopper means to permit free access to the interior of said column, a horizontally extending passage connecting said first passage with the interior of a lateral electrode connecting member comprising a housing having a front wall and a back wall, the front wall of which is detachably secured to said connecting block, a diaphragm being interposed between the orifice of said horizontal passage and said electrode connecting member to prevent free liquid flow through said passage, said electrode connecting member having centrally located therein a substantially horizontal bore constituting an electrode chamber to be filled with an electrolyte, said horizontal bore bifurcating into two passages extending through the back wall of the housing and connecting said electrode chamber with an electrolyte supply tubing and an electrolyte return tubing, respectively, and a bare electrode terminating inside said electrode chamber adjacent to said diaphragm.

2. A device as claimed in claim 1, in which said electrode comprises a bare wire extending through one of said buffer tubings.

3. A device for supplying a buffer solution to one end of an electrophoresis column packed with a supporting medium, comprising a cylindrical connecting block substantially in the form of a T-junction made out of a material inert to the liquids contacting the material, said connecting block having centrally disposed therein a vertical open-ended bore, one opening of which terminates with a tubular member adapted to be connected with one end of the electrophoresis column and the other opening of which is provided with a replaceable stopper means to permit free access to the interior of said column for the insertion and removal of said supporting medium, a radially extending bore connecting said vertical bore with the interior of an electrode connecting member comprising a housing having a front wall and a back wall, the front wall of which is detachably secured to said connecting block, a semi-permeable membrane being interposed between the orifice of said radially extending bore of the connecting block and the front wall of the electrode housing to prevent high-molecular components from entering the interior of said electrode housing, said electrode housing having, coaxially with said radially extending passage of said connecting block, a central cylindrical electrode chamber to be filled with a buffer solution, a first passage extending through the back wall of the housing and connecting said electrode chamber with a buffer supply tubing, a second passage extending through the back wall of the housing and connecting the side wall of said electrode chamber with a buffer return tubing, and electrode means comprising a bare wire extending through said buffer supply tubing and terminating inside said electrode chamber with a bead-shaped enlargement adjacent to said semi-permeable membrane, and a downwardly extending drainage tube for draining off the buffer solution subsequently to the completion of an electrophoresis operation.

4. An apparatus for electrophoretically separating a liquid mixture of components having different electrophoretic migration velocities, in which the liquid mixture is moving through a stabilized column and under the influence of an electric voltage is separated into components, comprising an open-ended elongated column, a first and a second connecting block substantially in the form of a T-junction detachably connected with the ends of said column, each of said connecting blocks having centrally disposed therein, coaxially with said column, an open-ended bore, the mouth of which opposite that connected with said column being provided with replaceable stopper means to permit free access to the interior of said column, a radially extending bore communicating between said vertical bore and the interior of a lateral electrode connecting member comprising a housing having a front wall and a back wall, the front wall of which is detachably secured to said connecting block, a diaphragm being interposed between the orifice of said radially extending bore and said lateral electrode connecting member to prevent free liquid flow through said bore, the housing of said electrode connecting member having an electrode chamber provided with two passages extending through the back wall of the housing and connecting said electrode chamber with a buffer supply and a buffer return tubing, respectively, and electrode means comprising a bare wire extending through said buffer supply tubing and terminating inside said electrode chamber adjacent to said diaphragm.

5. An apparatus for electrophoretically separating a liquid mixture of components having different electrophoretic migration velocities, in which the liquid mixture is moving through a stabilized column and under the influence of an electric voltage is separated into components, comprising an open-ended tubular vertical stabilized column moistened with a buffer solution, an upper and a lower connecting block substantially in the form of a T-junction detachably connected with the upper and lower ends of the column, respectively, each of said connecting blocks having centrally disposed therein, coaxially with said column, a substantially vertically extending open-ended cylindrical buffer chamber filled with a buffer solution, the opening opposite that connected with said column being provided with replaceable stopper means to permit free access to the interior of said column for the insertion and removal of said supporting medium, a radially extending passage communicating between said buffer chamber and the interior of an electrode housing having a front wall and a back wall, the front wall of which is detachably secured to said connecting block, a semi-permeable membrane being interposed between the orifice of said radially extending passage and said electrode housing to prevent high-molecular components from entering the interior of said electrode housing, said electrode housing having coaxially with said radially extending passage of said connecting block, a central cylindrical electrode chamber filled with a buffer solution, a first passage extending through the back wall of the housing and connecting said electrode chamber with a buffer supply tubing, a second passage extending through the back wall of the housing and connecting the side wall of said electrode chamber with a buffer return tubing, said electrode means comprising a bare wire extending through said buffer supply tubing and terminating inside said electrode chamber with a bead-shaped enlargement adjacent to said semi-permeable membrane, and a downwardly extending drainage tube for draining off the buffer solution subsequently to the completion of an electrophoresis operation.

6. A process for electrophoretically separating a liquid mixture of components having different electrophoretic mobilities, comprising the steps of establishing a substantially vertical supporting column of a capillary system in a degassed buffer solution providing upper and lower semi-permeable diaphragms, arranging the diaphragms laterally of the vertical longitudinal direction of said column with their surfaces in a substantially ver-

tical position, providing electrodes terminating adjacent to said diaphragms on a side thereof separated from said column, introducing the liquid mixture to be separated into the column, circulating from a first source to a first buffer solution past the electrode side of said upper diaphragm and from there back to said source, circulating from a second source a second buffer solution past the electrode side of said lower diaphragm and from there back to said second source, and applying a voltage over said electrodes.

References Cited

UNITED STATES PATENTS

2,247,065	6/1941	Pauli et al.	204—180
2,713,818	7/1955	Herbold	204—180
2,888,392	5/1959	Grassman et al.	204—299

OTHER REFERENCES

Haglund, Acta Chemica Scandinavica, "Zone Electrophoresis in a Glass Powder Column," No. 4, 1950, pp. 957–962.

Heftmann, Chromatography, 1961, pp. 269–270.

JOHN H. MACK, *Primary Examiner.*

JOHN R. SPECK, *Examiner.*

G. BATTIST, E. ZAGARELLA, *Assistant Examiners.*