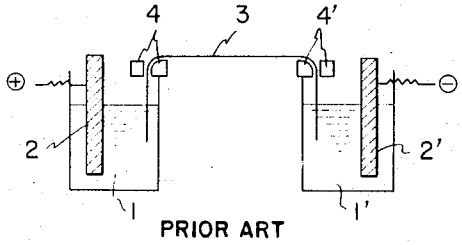
Dec. 5, 1967 TOMONORI IWATA 3,356,604

METHOD AND APPARATUS FOR MAGNETO ELECTROPHORESIS

Filed March 10, 1964

FIG. 1



PRIOR ART

FIG. 2

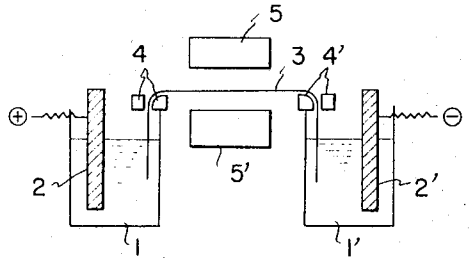
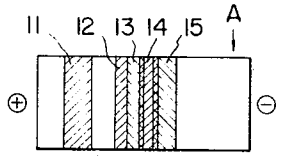
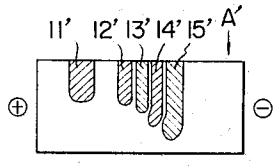


FIG. 3



PRIOR ART

FIG. 4



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METHOD AND APPARATUS FOR MAGNETO ELECTROPHORESIS

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12 Claims. (Cl. 204—180)

ABSTRACT OF THE DISCLOSURE

In chromatographic analyses using electrophoresis, the application of a magnetic field perpendicular to the receiver of the sample to be analyzed. The field thus applied averts overlapping of the separated parts. Such an overlap renders precise analysis difficult, if not impossible. The method and device are of particular value for the bidimensional separation or extraction of colloidal particles having very close mobility by dripping a colloidal mixture having components of different mobilities onto an electrolyte in a medium which forms a plane and causing electric as well as magnetic fields to act thereon.

This invention relates to techniques in electrophoresis and more particularly to improvements in electrophoresis technique. More specifically, the invention concerns a new method and apparatus for magneto-electrophoresis wherein electrophoresis procedure according to known practice is carried out in a magnetic field.

As is known, when a plus electrode (anode) and a minus electrode (cathode) are placed in an electrolyte in which a certain substance is dissolved or is suspended, and a direct current is passed through these electrodes, the substance migrates toward one of the electrodes, either toward the plus electrode (anaphoresis) or toward the minus electrode (cataphoresis). This phenomenon is the principle of the electrophoresis method, which is applied principally in research on colloidal solutions, being an indispensable technique for separation and analysis of minute bodies such as proteins and viruses. Specific methods and apparatus heretofore employed in this technique have had certain drawbacks as will presently become apparent.

It is a general object of the present invention to eliminate such drawbacks inherent in conventional practice and to provide a new and highly advantageous method and apparatus for electrophoresis.

The nature, principle, and details of the invention will be best understood by reference to the following description, taken in conjunction with the accompanying drawing in which like parts are designated by like reference characters, and in which:

FIGURE 1 is a schematic elevational view, in vertical section, illustrating one example of apparatus for carrying out an electrophoresis method according to known practice;

FIGURE 2 is a view similar to FIGURE 1, showing one embodiment of apparatus suitable for the practice of the magneto-electrophoresis method according to the invention;

FIGURES 3 and 4 are diagrams indicating the chromatographic patterns obtained, respectively, by a known electrophoresis method and by the magneto-electrophoresis method of the invention.

For a full understanding and appreciation of the nature and utility of the present invention, the following consideration, presented merely to establish a basis for comparison, of a known electrophoresis method is believed to be desirable and prerequisite.

For separation of substances such as proteins and viruses, the method generally employed is that of placing a substance such as a protein or a virus, by dripping, onto a starch liquid, a starch paper, or a filter paper containing a starch liquid and carrying out electrophoresis. In one example of apparatus, as illustrated in FIGURE 1, suitable for the practice of this method, a filter paper is used. The two ends of a piece of filter paper **3** positioned by guides **4** and **4'** are immersed in electrolytic baths **1** and **1'** provided with electrodes **2** and **2'**, respectively.

In actually separating out a sample by means of this apparatus, the sample is caused to drip in a linear pattern onto the filter paper **3** after the electrolyte has spread over and is adhering uniformly to the filter paper **3**, and a D-C voltage is impressed across the electrodes **2** and **2'**. A chromatograph obtained by this method is illustrated in FIGURE 3, in which a sample consisting of blood serum protein has been applied by dripping onto the paper at A, and migration has been carried out. The regions **11**, **12**, **13**, **14**, and **15** respectively correspond to albumin, α_1-globuline, α_2-globuline, β-globuline, and γ-globuline, and their respective ranges indicate the ranges in which the respective constituents exist.

The separation of the respective constituents is accomplished by means of this chromatograph, and qualitative and quantitative analyses are carried out. In the case wherein there are great differences in mobility, the separation between parts corresponding to the constituents, for example, the separation between albumin **11** and γ-globuline **15**, is substantially good. However, in the case wherein the mobility differences are small, a thorough analysis cannot be accomplished. For example, in the parts of α_2-globuline **13** and β-globuline **14** in FIGURE 3, even if separation is nominally possible, the overlapping part is excessive, and, moreover, the density gradient is gradual. For this reason, when the separated parts are extracted in a material form and caused to undergo immunochemical reaction, mutual contamination is excessive, and satisfactory analysis cannot be accomplished.

Moreover, in some cases wherein the content is low, there is superimposition on the overlapping parts of constituents in close proximity and even qualitative analysis, not to mention quantitative analysis, is difficult.

It is a specific object of the present invention to overcome the above described difficulties and to provide a new and practical method and apparatus for magneto-electrophoresis whereby separation of substances in mutually close proximity, particularly separation of constituents of low content, and analysis thereof are made possible. The invention, which is based on the principle of carrying out electrophoresis in a magnetic field, will now be described with respect to a preferred embodiment thereof as shown in FIGURES 2 and 4.

Referring to FIGURE 2, the example of apparatus shown therein differs from conventional apparatus in that it has magnetic field generating devices **5** and **5'** so disposed and adapted to establish a magnetic field perpendicularly to the span of the filter paper **3** between the electrolytic baths **1** and **1'**. The remainder of the apparatus is similar to that shown in FIGURE 1.

When phoresis is carried out by means of an apparatus of this arrangement, the various constituents are subject to a first force due to electrolysis and, simultaneously, to a second force due to the magnetic field, the second force being in a direction perpendicular to that of the first force, and migrate on the filter paper surface also in a direction perpendicular to the electric field.

In order ot indicate more fully the nature and details of the invention, the following example of typical procedure according to the magneto-electrophoresis method of the invention is set forth, but it should be understood

that this example is presented as illustrative only, and that it is not intended to limit the scope of the invention.

Blood plasma of a guinea pig was dialyzed with a buffer solution of the same concentration as a buffer solution of synthetic polyacrylamide gel, and approximately 0.005 ml. of the plasma so dialyzed was placed in and caused to adhere to a slit provided in one part of a plate of approximately 1 mm. thickness molded from synthetic polyacrylamide gel and was subjected to electrophoretic separation. That is, the synthetic polyacrylamide gel was used in place of the filter paper **3**. Platinum wire was used for the electrodes **2** and **2'**, and a buffer solution of boric acid (pH 8.1) was used for the electrolyte. The magneto-electrophoretic conditions were as follows: voltage across the ends of the gel plate, 100 volts; electrophoresis current, 7 ma.; magnetic field strength, approximately 8,000 gausses; and phoresis time, two hours. Upon completion of the magneto-electrophoresis, the gel plate was steeped for approximately two hours in a solution of 45 percent methanol and 5.5 percent acetic acid in which solution 0.01 percent of a protein dyeing solution has been dissolved, and, in order to remove surplus dyeing solution, the plate was washed with methanol acetic acid solution until the background became transparent.

The chromatograph of the sample so obtained is shown in pattern form in FIGURE 4, in which the reference character A' designates the position where the sample is adhering, and the designations **11'**, **12'**, **13'**, **14'** and **15'** correspond respectively to the designations **11**, **12**, **13**, **14**, and **15** of FIGURE 3. The principal characteristics of this chromatograph indicated in FIGURE 4 are that each constituent is concentrated toward a direction perpendicular to the direction of electrolysis and that the boundaries between neighboring parts are distinct, and the resolution is good. The cause of this phenomenon is not clear, but it appears to be very similar to the phenomenon whereby, in the case of only an electric field with cataphoretic discharge, the boundaries between, for example, Ne-Ar-Hg are of continuous character and are not distinct, but in the case when a magnetic field perpendicularly intersecting the electric field is established, the boundaries become discontinuous and distinct.

That is, in the above described chromatograph obtained in accordance with the present invention, the various constituents become concentrated, and the connected parts become discontinuous, wherefore the separation and extraction of the samples is facilitated. Moreover, the separation and analysis of micro-elements, that is, constituents of extremely low content, which were heretofore almost impossible, become possible by the method and apparatus of this invention.

While the embodiment of the invention has been described hereinabove with respect to only the method in which filter paper or gel plate is used, it will be obvious that the invention can be applied also to the method wherein a vessel is used. Furthermore it will be obvious that the samples to be measured are not limited to those such as proteins and viruses and that the invention can be applied to other inorganic and organic substances.

Thus, as will be apparent from the foregoing disclosure, the magneto-electrophoresis technique according to the present invention is effectively applicable to a wide range of uses.

It should be understood, of course, that the foregoing disclosure relates to only a preferred embodiment of the invention and to a typical example of application and that the invention is intended to cover all changes and modifications of the examples of the invention herein chosen for the purposes of the disclosure, which do not constitute departures from the spirit and scope of the invention as set forth in the appended claims.

What is claimed is:

1. A method of bidimensionally separating and individually extracting, respectively, components from a solution containing a plurality of charged colloidal particles of different mobilities which comprises dripping said solution in a uniform linear pattern onto an electrolyte contained and held in a medium kept in a horizontal plane, passing a direct current between a pair of electrodes along the horizontal plane of said medium in a direction transverse to said linear pattern and simultaneously applying a magnetic field in a direction perpendicular to the plane of the medium whereby said particles are separated.

2. The method as defined in claim **1**, wherein said medium is filter paper.

3. The method as defined in claim **1**, wherein said medium is starch paper.

4. The method as defined in claim **1**, wherein said medium is filter paper containing starch liquid.

5. The method as defined in claim **1**, wherein said medium is a gel plate.

6. The method as defined in claim **1**, wherein said colloidal solution is constituted of proteins.

7. The method as defined in claim **1**, wherein said colloidal solution is constituted of viruses.

8. A device for magneto-electrophoresis which comprises an absorbent supporting medium to hold an electrolyte in a horizontal plane; means for generating an electric field to cause phoresis along the plane of said electrolyte formed by said medium; and means to generate a magnetic field in a direction perpendicular to said electric field and said plane of the electrolyte.

9. The device as defined in claim **8**, wherein said medium is filter paper.

10. The device as defined in claim **8**, wherein said medium is starch paper.

11. The device as defined in claim **8**, wherein said medium is filter paper containing starch liquid.

12. The device as defined in claim **8**, wherein said medium is a gel plate.

References Cited

UNITED STATES PATENTS

2,016,442	10/1935	Kilgus	204—155
3,140,714	7/1964	Murphy et al.	204—180

FOREIGN PATENTS

252,454	10/1962	Australia.

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