March 2, 1971 R. E. MICHEL ET AL 3,567,611

TWO-STAGE ELECTROMAGNETOPHORESIS

Filed Sept. 16, 1968

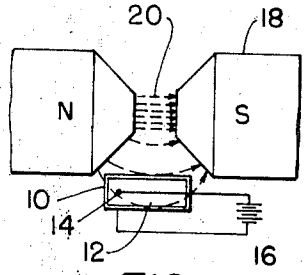

FIG. 1

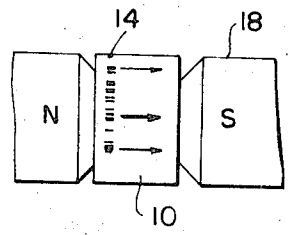

FIG. 5

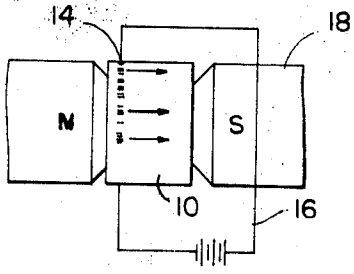

FIG. 2

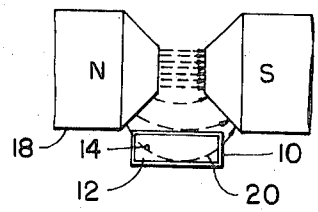

FIG. 4

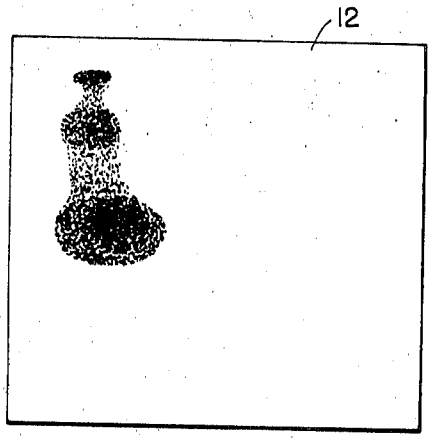

FIG. 7

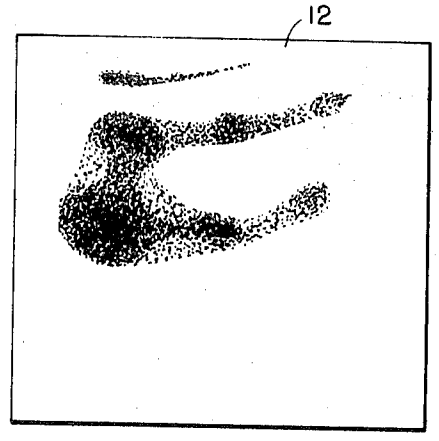

FIG. 6

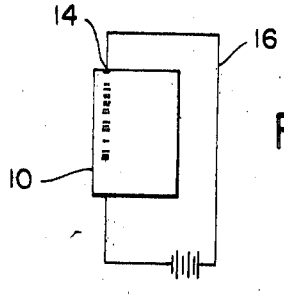

FIG. 3

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United States Patent Office 3,567,611
Patented Mar. 2, 1971

3,567,611

TWO-STAGE ELECTROMAGNETOPHORESIS

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Filed Sept. 16, 1968, Ser. No. 759,884
Int. Cl. B01k 5/00
U.S. Cl. 204—180 7 Claims

ABSTRACT OF THE DISCLOSURE

Material, such as human normal blood serum protein, is partitioned by two-stage electromagnetophoresis as a function of molecular paramagnetism. In the first stage the protein to be partitioned is positioned on a prism of gel and is electrophoresed to provide a first partition thereof in the direction of the applied electric field in accordance with physical and electrical characteristics thereof. Following the completion of the electrophoresis, the prism is placed in a non-uniform magnetic field and magnetophoresed. The proteins suspended in a partitioned state in the gel matrix as the result of electrophoresis are thus partitioned in a direction parallel to the magnetic field gradient as a function of the paramagnetism of the molecules of the protein.

BACKGROUND OF THE INVENTION

Field of the invention

The invention relates to partitioning techniques and refers more specifically to a method of partitioning human normal serum protein or the like by two-stage electromagnetophoresis as a function of molecular paramagnetism in which a material sample is electrophoresed and then magnetophoresed to provide displacement and grouping of molecules in accordance with their physical and electrical properties.

Description of the prior art

In the past physical partitioning of material samples has been carried out in laboratories by known methods, such as chromatography, electrophoresis which has been done in many ways, ultra-centrifugation, counter-current separation, density gradient separation, dialysis, and the like. Simultaneous electromagnetophoresis has also been attempted in the past, however the separation arose from the force on a charged particle moving in a magnetic field. It is not believed that two-stage electromagnetophoretic partition of human normal serum protein or the like has been previously attempted.

SUMMARY OF THE INVENTION

In accordance with the invention, two-stage electromagnetophoresis is accomplished by the method of first placing a sample of material to be partitioned on a gel prism and electrophoresing the sample, in a first stage, to provide a first partition thereof in accordance with the physical and electrical properties of the sample. The electrophoresed sample is then placed in a non-uniform magnetic field and magnetophoresed, in a second stage, to provide further partition of the sample in accordance with the paramagnetism of the molecules of the sample. The partition of the sample in the second stage is parallel to the magnetic field gradient.

The observed partition due to differential paramagnetic properties of the human normal serum proteins is another parameter by which isolation and characterization of the proteins is possible.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a top view of structure for accomplishing two-stage electromagnetophoresis in accordance with the method of the invention.

FIG. 2 is a side view of the structure illustrated in FIG. 1.

FIG. 3 is a side view of structure for accomplishing electrophoresis in a standard procedure which has been done in many ways.

FIG. 4 is a top view of structure for accomplishing the second stage of two-stage electromagnetophoresis in accordance with the method of the invention.

FIG. 5 is a side view of the structure illustrated in FIG. 4.

FIG. 6 is a pictorial illustration of the results of two-stage electromagnetophoresis practiced by the method of the invention.

FIG. 7 is a pictorial illustration of a control sample useful in illustrating the difference of two-stage electromagnetophoresis in comparison with electrophoresis alone.

DESCRIPTION OF THE PREFERRED EMBODIMENT

In accordance with the method of the invention two-stage electromagnetophoresis is accomplished by first filling a transparent plastic chamber **10**, open at both ends, with a polyacrylamide gel **12**. No spacer gel is used. Sodium persulfate is used to induce polymerization.

The method of providing the gel is fully set forth by Ornstein and Davis in their work, "Disc Electrophoresis I, Background and Theory," Ann. N.Y. Acad. Sci. 121:321–403, 1964, and "Disc Electrophoresis II. Method and Application to Human Serum Proteins," Ann. N.Y. Acad. Sci. 121:404–436, 1964, respectively.

At one side on the top surface of the rectangular prism of gel **12** in a slight depression 0.1 to 0.2 ml. of human normal serum protein **14** is deposited. The system is then electrophoresed through use of the electrical apparatus **16** for thirty to forty-five minutes using a current of thirty milliamperes with a voltage range of 130 volts to 320 volts delivered by a variable voltage power supply as a source of direct current.

Following the completion of the electrophoresis or first stage, the plastic chamber **10** with the gel **12** therein is placed for one hour in a non-uniform magnetic field generated by the electromagnet structure **18** where it is magnetophoresed. Typical values of the product of the magnetic field times the magnetic field gradient for the magnetophoresis, or second stage of the two-stage electromagnetophoresis, may be about twenty-five times 10^6 oersteds per centimeter squared.

As the result of the first stage or electrophoresis the human normal blood protein is separated vertically of the gel **12** in the chamber **10** into a plurality of separate layers of molecules having different physical and electrical properties, as shown diagrammatically in FIG. 3. The separation of the protein sample is evident when the gel is stained with a 0.1% amido-black solution and destained electrophoretically, as has been known in the past. A control sample of electrophoresed but not magnetophoresed protein is illustrated pictorially in FIG. 7.

Subsequent magnetophoresing of the electrophoresed protein sample produces movement of the partitioned protein sample suspended in the gel matrix parallel to the magnetic field gradient in which the gel is placed. The movement of the partitioned protein sample appears to be a function of paramagnetism of the individual molecules. Thus, the paramagnetic molecules undergo linear displacement in the non-uniform magnetic field (an established physical principle) whereby the serum sample is again partitioned, as shown pictorially in FIG. 6.

The partitioning of sample material by the method of two-stage electromagnetophoresis thereof is useful in diagnostic medical applications. For example, diagnosis of hemoglobinopathies and dysproteinemias may be possible using the two-stage electromagnetophoresis technique disclosed.

Two-stage electromagnetophoresis also may aid in physical partition problems occurring in biology and in the technology of the chemical and pharmaceutical industries. In addition, it is anticipated that in a more fundamental sense correlation will be found between new information about the structure of protein molecules resulting from two-stage electromagnetophoresis and a variety of diseases.

It is anticipated that the two-stage electromagnetophoresis disclosed herein may be used as a basic partition technique for other molecular systems (paramagnetic molecules with electrical charges) for which human normal serum proteins may be considered merely a general model. It is the intention to include all embodiments and modifications of the method of partition by electromagnetophoresis as are defined by the appended claims within the scope of the invention.

We claim:

1. The method of physical molecular partition, comprising separating a material sample by electrophoresis within a matrix in accordance with molecular physical and electrical properties thereof and additionally separating the sample by magnetophoresis within said matrix by placing it in a non-uniform magnetic field to produce movement of the molecules in a direction parallel to the magnetic field gradient in accordance with paramagnetism of the molecules thereof.

2. The method as set forth in claim 1 wherein the electrophoresis and magnetophoresis are carried out in two distinct and separate stages.

3. The method as set forth in claim 2 wherein the sample is electrophoresed before it is magnetophoresed.

4. The method as set forth in claim 1 wherein the material sample is human normal blood serum protein and the electrophoresis is accomplished in a polyacrylamide gel.

5. The method as set forth in claim 4 wherein the human normal blood serum protein is electrophoresed for thirty to forty-five minutes, using a current of approximately thirty milliamperes and a voltage variable between 130 and 320 volts.

6. The method as set forth in claim 1 wherein the magnetic field is such that the product of the magnetic field strength times the magnetic field gradient is approximately times 10^6 oersteds per centimeter.

7. The method as set forth in claim 6 wherein the electrophoresed sample is placed in the non-uniform magnetic field for approximately one hour.

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U.S. Cl. X.R.

204—299