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GEL ELECTROPHORESIS PROCESS

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FIG. 1.

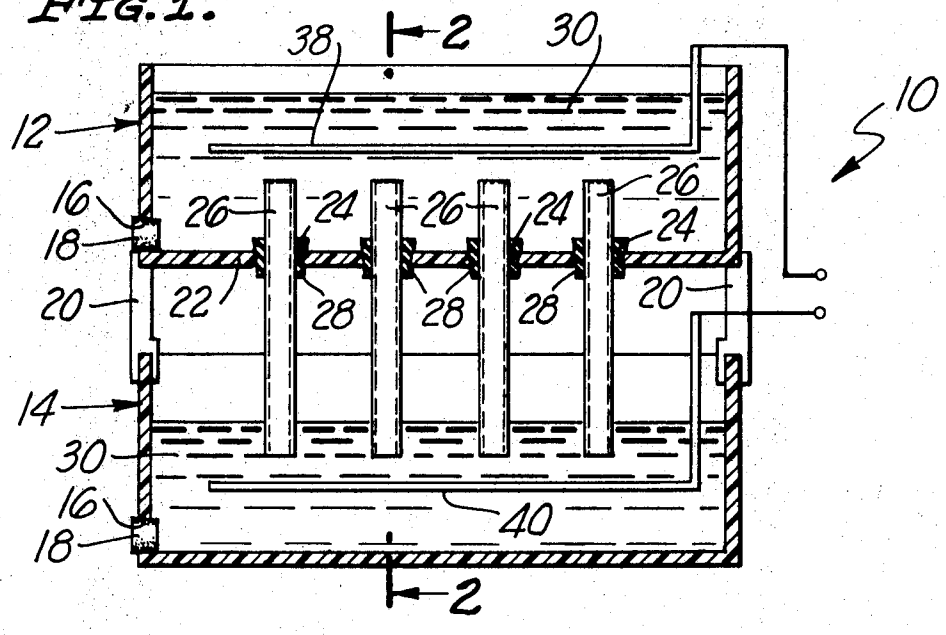
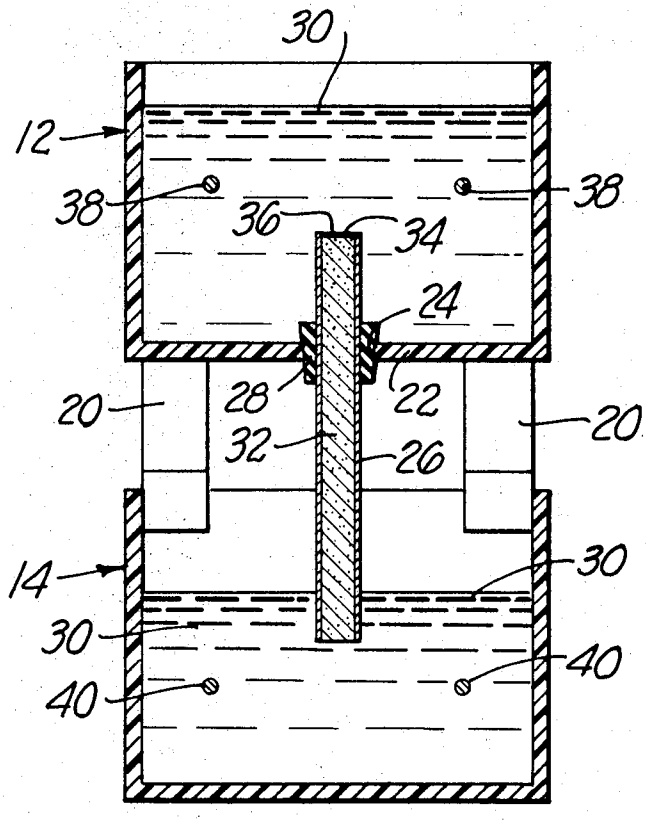



FIG. 2.

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# GEL ELECTROPHORESIS PROCESS

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## ABSTRACT OF THE DISCLOSURE

A gel electrophoresis apparatus and process are disclosed which are primarily intended to be used in separating blood into its components. The apparatus contains upper and lower electrolyte containers connected by at least one vertically extending tube containing a gel. Electrodes are located in the container and the containers are filled with an electrolyte connected through the tube. In the preferred manner of use whole blood is located on a carrier member containing an hemolysis agent and the member is located on the top of the gel within the tube; thereafter a potential is applied across the electrodes so as to cause migration of compounds within the blood within the gel structure in the tube.

## BACKGROUND OF THE INVENTION

Electrophoresis is a well-known type of process commonly used to separate various different materials such as blood fractions. In electrophoretic process different materials move at different rates between two separate electrodes electrically connected by an electrolyte. This type of process has been widely recognized as being highly effective in separating various hard to separate type mixtures such as mixtures of various proteins.

As the art of electrophoresis has developed it has become known to separate the electrodes used in the process by a gel or gel-like composition containing the electrolyte used in connection with the process. When such a gel composition is employed at the close of electrophoretic operation the gel composition is removed from the equipment employed and is subjected to further treatment. Frequently such treatment involves separately identifying various compounds or fractions in various different areas of the gel or gel-like composition by various means which are unimportant to the present invention.

The use of such gel in electrophoretic separation although well recognized as being advantageous and desirable is somewhat limited by a number of different factors. One of these is the lack of equipment which can be satisfactorily used so as to obtain a plurality of separations of different starting materials simultaneously at a comparatively low cost. Another important factor pertains to the nature of the gel composition and the manner in which it is used. Frequently prior electrophoretic separation has involved the use of starch and similar gels which are relatively difficult and expensive to prepare in a satisfactory form for use with available equipment.

These factors can best be more fully understood by referring to the field of blood analysis. In order to determine the existence of an abnormal S-type anemia and other things it is desired to separate the blood of an individual by electrophoresis since this method of separation is comparatively simple and effective. However, the present methods of separation require a comparatively long period to obtain a comparatively few samples of separated blood from different individuals because of the types of equipment known for this purpose and complications pertaining to the gels used in the separations.



## SUMMARY OF THE INVENTION

The invention also has as one of its objects the teaching of a new and improved process for gel electrophoresis which overcomes various disadvantages and limitations of prior related processes as are briefly indicated in the preceding. An object of the invention is to provide a process of the type indicated which is relatively easy to carry out and which is extremely effective. Another related object of the invention is to provide for analyzing blood at a comparatively nominal cost a process having these characteristics.

An object of the present invention is also to provide a new and improved gel electrophoresis apparatus which can be easily and conveniently used. A more specific object of the invention is to provide an apparatus of this type which will make it possible to analyze a plurality of individual samples simultaneously and quickly. Related objects are to provide apparatuses as indicated which are comparatively simple, comparatively easy to construct, comparatively inexpensive and which are extremely efficient and practical for their intended use.

These various objects of the invention are achieved by providing an apparatus having upper and lower electrolyte containers, each containing electrodes electrically connected by at least one vertically extending tube containing a gel. When an electrolyte is located within each of the containers and a sample to be separated by electrophoresis such as an inert carrier disk containing whole blood is located upon the upper end of the gel within the tube and a potential is applied across the electrodes migration and separation of the fractions of materials within the sample being processed takes place within the gel. At the end of a desired period the apparatus can be disassembled and the gel containing the fractions taken out for separate analysis. This invention is much more detailed and comprehensive in many of its specific aspects than indicated by this brief summary.

## BRIEF DESCRIPTION OF THE DRAWING

The actual details of this invention and many specific advantages of it will be more fully apparent from a detailed consideration of the remainder of this specification, the appended claims and the accompanying drawing in which:

FIG. 1 is a side-elevational view of a presently preferred embodiment or form of an electrophoresis apparatus of this invention; and

FIG. 2 is a cross-sectional view of this apparatus at line 2—2 of FIG. 1.

It will be recognized that the accompanying drawing is not to be taken as limiting this invention in any respect inasmuch as on the basis of the disclosure embodied within this drawing and this specification those skilled in the art of electrophoresis will be able to construct differently appearing devices utilizing the invention set forth in the appended claims forming a part of this specification.

## DESCRIPTION OF THE INVENTION

The electrophoresis apparatus **10** of this invention illustrated in the drawing includes upper and lower electrolyte containers **12** and **14** formed out of an appropriate material which will be inert during the use of the complete apparatus **10**. Preferably they are formed out of an electrically non-conductive material. Both of these containers may have a conventional drain opening **16** which is normally closed by a cork **18** of rubber or similar material which is inert under the conditions of use of the complete apparatus **10**.

These containers are preferably held one above another as shown by means of conventional braces **20** so that a flat bottom **22** in the upper container **12** is located horizontally. The upper container **12** has in its bottom **22** at least one opening **24**. Preferably a plurality of these openings **24** are used so that the entire apparatus **10** may be used so as to simultaneously analyze a number of different samples. Any opening **24** which is not used as the entire apparatus **10** is being employed for its intended purpose may be closed by a cork such as the cork **18**.

Each of the openings **24** contains an identical hollow tube **26** which preferably has a uniform cross-sectional configuration and which is located so as to extend vertically between the interiors of the containers **12** and **14**. These tubes **26** are held in place by rubber or similar grommets or hollow corks **28** which are inert under the conditions of use of the entire apparatus **10**. These grommets **28** seal the bottom **22** so that an electrolyte **30** may be located within the upper container **12**. Preferably the same electrolyte **30** is located within the lower container **14** in such a manner that these containers are electrically connected through the tube **26**.

The flow of the electrolyte **30** from the container **12** and to the container **14** is however prevented by locating within these tubes **26** a porous, permeable gel-like composition **32** suitable for making an electrophoretic separation. Preferably the gel **32** within each of the tubes **26** has a completely flat top **34** extending perpendicular to the wall of the tube **26** within which it is located. Such a flat top **34** is considered to facilitate separation by making it possible for the fraction separate to travel and form in bands which extend directly across the interior of the gel **32** at right angles to the walls of a tube **26**.

During the use of the apparatus **10** preferably a small sample **36** of a composition being fractionated by electrophoresis is located directly across the top **34** of the gel **32** in a tube **26**. By virtue of the fact that the top **34** is preferably spaced from the top of the tube **26** by comparatively short distance such a composition to be analyzed and may be readily located in place so that it does not get distributed throughout the electrolyte **30** in the upper container **12** so as to interfere with the separation of other samples.

Such a composition to be analyzed such as a fluid composition such as blood is preferably located on a small wafter or disk **36** of an inert carrier material such as filter paper. It will be recognized that other carriers can be used instead of filter paper and that the sample need not be blood. It will also be recognized that such an inert carrier may be dispensed with, and that a sample can be located directly on the gel **32**. Such a disk **36** should preferably fit closely within the interior of a tube **26** so as to aid in maintaining a uniform migration across the interior of the tube **26** within the gel **32** as the apparatus **10** is used.

After the disk **36** is in place, an electric potential is applied across an anode **38** and a cathode **40** located within the upper and lower containers **12** and **14**, respectively. Such an anode and cathode **38** and **40** preferably are formed of a material which is inert during the use of the apparatus **10**. These electrodes may be supported in place in any convenient manner such as by the use of an adhesive **42** securing them to the walls of the containers **12** and **14**. Preferably the electrodes **38** and **40** are located equidistant from the tops and bottoms of, respectively, all of the tubes **26** so that a plurality of samples **36** may be separated at substantially the same rate. If desired several of the electrodes **38** and **40** may be used as indicated in FIG. 2 so that there is substantially equal potential differential between them across all portions of all of the tubes **26** during the use of the apparatus **10**.

As an electric potential is applied as indicated the gels **32** will tend to filter out materials which are too large to pass within their pores adjacent to the tops **34**. Simultaneously various fractions will travel downwardly along the lengths of the tubes **26**. After a desired period, when



on the basis of experience or visual observation, it has been determined that an adequate separation has been obtained, the current may be turned off, the apparatus **10** disassembled and the gels **32** within various individual tubes **26** taken from these tubes for further examination and analysis.

In practicing the present invention in the separation of blood into its fractions it is preferred that the gel **32** used in the individual tubes **26** may be an acrylamide gel of a porous character containing the electrolyte **30** used in the complete apparatus **10**. With such a gel an effective separation of blood fractions may be obtained. A suitable electrolyte for use with blood fractionation may contain 96.8 grams tris (hydroxymethyl) aminothane, 12.48 grams disodium ethylenediaminetetracetic acid, 7.36 grams boric acid, and 5.2 liters of water.

An acrylamide gel for use with this electrolyte can be made by the method set forth in an article by Raymond and Makamichi entitled, "Electrophoresis in Synthetic Gels," appearing on pages 23–30 of the publication, Analytic Biochemistry 3, 23–30 (1962). In the interest of brevity the entire disclosure of this article is incorporated herein by reference. From this article it will be seen that an acrylamide gel for use with this invention is made by mixing an acrylamide compound with the electrolyte and a catalyst so as to form a composition which will set up to the ultimate gel structure. Such a composition will normally adhere to the interior of a tube such as a tube **26** on setting up.

When such gels set up they do not form a flat surface under normal conditions because of factors which are unrelated to the present invention. As a result of this, it is preferred to form the individual tubes **26** used with this invention while the gel composition is still in a liquid state by casting this composition into the tubes **26** to just below the tops of these tubes, and then placing on the surfaces of the composition before it is set up a quickly evaporating substance which is lighter than the gel composition. Particularly satisfactory results have been achieved using as such a substance anhydrous diethyl ether. Upon the setting of the gel and the natural evaporation of such a substance, a top of the ultimate gel **32** in a tube **26** will be flat so as to achieve the results indicated in the preceding. A tube **26** prepared in this manner may be stored prior to use.

The tubes **26** created in this manner normally have at the tops of these tubes a comparatively small space which is capable of reeciving a sample such as the sample **36** and holding it in place so that effective electrophoretic separation may take place without interference with the separation taking place in other adjacent tubes. As indicated in the preceding it is normally preferred that the composition to be analyzed be located on an inert member such as a filter paper disk **36** fitting closely within the tube **26**. Such a composition may be held on such an inert carrier **36** by either absorption or adsorption or by physical entrainment.

When the present invention is to be used in the analysis of blood, it is preferred that such an inert carrier **36** be coated with and/or contain an appropriate hemolysis agent. Such a compound may be the well-known compound saponin; however, preferred results are achieved by using common detergents which will in solution cause fats to dissolve. Such hemolysis agents may be placed on an inert carrier **36** as indicated by simply dipping such a carrier in such an agent and then drying it prior to use.

When the disc **36** is such a coated carrier it may be coated with blood by being dipped into such blood. After any excess is wiped off its exterior, it may be directly located on the surface **34**. After a comparatively small period of time the hemolysis agent within the disc **36** will cause hemolysis of the blood cells, liberating hemoglobins. Then, as the apparatus **10** is used in its intended manner as indicated in the preceding, cell walls from this blood will tend to be filtered out by the gel **32** and the hemo-

globins will move in the individual tubes **26** as previously described.

During the use of the apparatus **10** the voltage applied across the electrodes **38** and **40** and the current density within the tubes **26** can, of course, be varied depending upon a variety of factors such as the material being separated, the numbers and dimensions of the tubes, the nature of the gel within the tubes, the nature of the electrolyte employed and the like. For this reason it is not considered that an understanding of this invention requires a detailed description of the current and voltage density used. Such matters will vary from application to application and can be easily determined by routine experimentation. However, in order to aid in understanding this invention, it can be indicated that with the electrolyte indicated in the preceding and tubes 8 mm. O.D., 4" long filled with an acrylamide gel, satisfactory results have been achieved using a voltage of about 100 volts D.C. and a current density in each tube of from about 2 to about 3.6 ma.

From a detailed consideration of the aforegoing, those skilled in the art to which this invention pertains will realize that the apparatus herein described is an efficient, relatively inexpensive, relatively easy to use piece of equipment which readily accomplishes its intended function. They will also realize that by following the procedure herein indicated blood and other materials may be easily and conveniently separated into fractions.

I claim:

**1.** A process for separating a mixture of blood compounds into individual compounds by electrophoresis which comprises:

locating said mixture of blood compounds within an inert carrier member containing a compound capable of promoting hemolysis at the top of a vertically extending tube containing an acrylamide gel capable of permitting the movement of different compounds through said gel at different rates when a potential is applied between the ends of said tube;

locating an electrolyte at both ends of said tube and applying an electric potential between the ends of said tube through the use of electrodes in contact with said electrolyte at each of the ends of said tube.

**2.** A process as claimed in claim **1** wherein said compound is a detergent.

**3.** A process as claimed in claim **1** wherein said member is a paper member.

**4.** A process as claimed in claim **1** wherein:

said gel within said tube has a flat top extending perpendicularly to the interior of said tube;

said mixture is whole blood;

said member is a paper member; and

said paper member containing said whole blood is located at the top of said tube on said flat surface.

**5.** A process as claimed in claim **4** wherein said compound is a detergent.

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U.S. Cl. X.R.

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