July 30, 1974 F. W. GODSEY, JR 3,826,734

APPARATUS FOR USE IN LIQUID SAMPLE ANALYSIS

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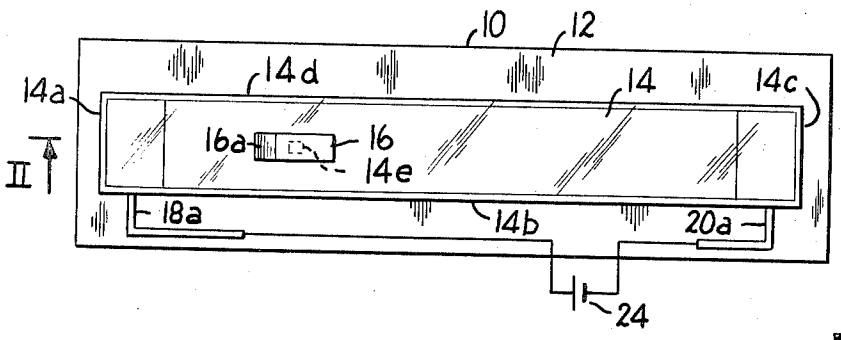


FIG. 1

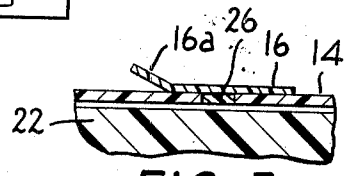


FIG. 3

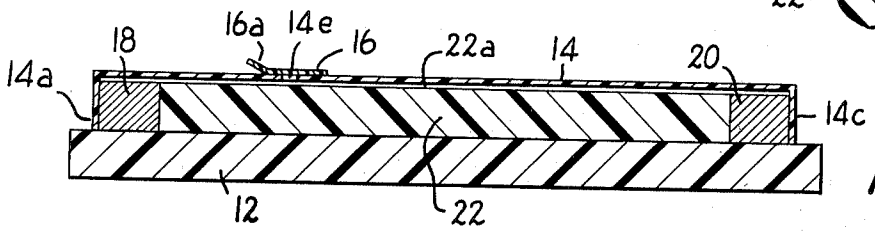


FIG. 2

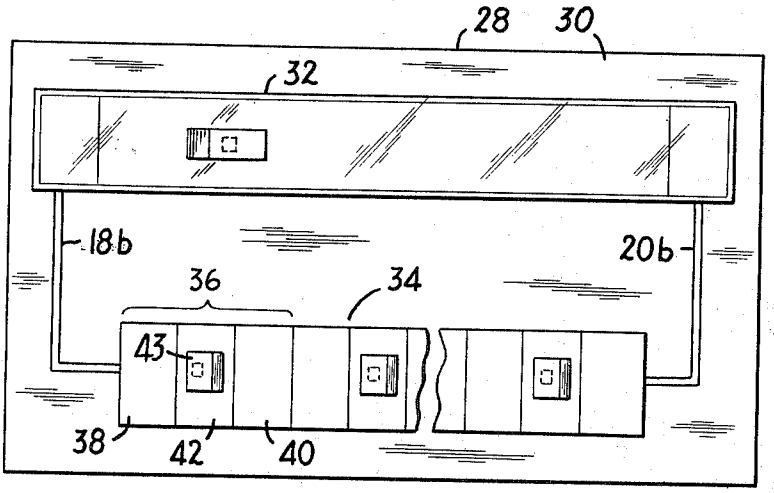


FIG. 4

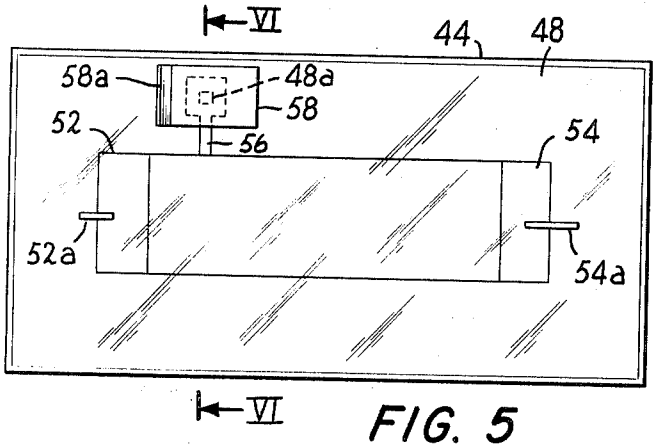


FIG. 5

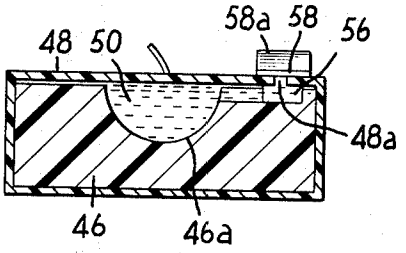


FIG. 6

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APPARATUS FOR USE IN LIQUID SAMPLE ANALYSIS

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ABSTRACT OF THE DISCLOSURE

An electrophoretic cell having an elongate electrolytic expanse is enveloped between an overlayer and a substrate, the overlayer including a closable opening through which a liquid sample may be applied to a preselected location on the elongate electrolytic expanse. The cell provides for separation of the constituents of the sample on application or preselected electric voltage to the cell electrodes.

FIELD OF THE INVENTION

This invention relates to apparatus for use in the analysis of fluid samples and more particularly to test apparatus for use in determining protein fractions in human body fluids such as blood.

BACKGROUND OF THE INVENTION

Various procedures are presently known for separating the protein fractions of blood serum for quantification thereof, e.g., salting-out of the fractions, precipitation of the fractions using predetermined concentrations of organic solvents, isoelectric, cationic and anionic precipitation, and the use of centrifuges and electrophoresis. As presently implemented, all of these methods are practiced with laboratory discipline and involve the collection of a sample of whole blood in the order of cubic centimeters, transportation of the collected sample to a laboratory facility, separation of blood serum from the whole blood sample and finally, the qualitative and quantitative analysis of the serum.

In the most widely practiced of the foregoing procedures, namely, that involving electrophoresis, the whole blood sample together with a clotting agent is placed in a centrifuge and the resulting blood serum is applied to a medium many inches in length and pre-wetted with an electrolyte of pH level sufficient to enable the medium to support the migration of protein fractions. The medium is disposed across a trough-like electrolyte container with the opposed medium end portions immersed in container electrolyte. An electric voltage of one hundred or more volts is applied between a pair of electrodes supported by the container and electrically connected by container and medium electrolyte. An electrical field is thus applied to the serum and induces migration of the protein fractions along the medium, fractions involving smaller molecules of higher electrical charge moving at a faster rate than other fractions involving larger molecules of lesser electrical charge. With the passage of time, the participating protein fractions of different type migrate to distinct zones having definite boundaries. Upon completion of a preselected time, the applied voltage is discontinued and the medium is subjected to treatment which fixes the protein fractions in their respective migration zones. Quantification of the separated and fixed fractions is attained by chromatography, i.e., by staining the medium and comparing color therein with a standard, or by passing the medium through a densitometer.

As is evident from the foregoing, the presently known procedures for blood serum analysis require apparatus not ordinarily available for use outside a laboratory and demand the discipline of a skilled technician. In this respect, in presently known electrophoretical apparatus, stringent control need be exercised in respect of the electrical current caused to flow through the electrolyte, on the one hand, to avoid overheating by excessive current and, on the other hand, to maintain current at a level such that sufficient electrical field strength exists for effective protein fraction migration.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide improved apparatus for use in the analysis of fluid samples.

It is a more particular object of the invention to provide improved electrophoretical apparatus for use in analyzing blood serum.

In the efficient attainment of these and other objects, the present invention provides electrophoretical apparatus usable in non-laboratory environment for the receipt of a fluid sample and for separating fractions thereof into distinct zones for quantification. In its particularly preferred form, such apparatus includes, on a common substrate, a blood serum protein fraction-discriminating cell disposed in a sealed container openable at the time of apparatus use and a further cell adapted to supply all operating power required by the protein fraction discriminating cell.

The foregoing and other objects and features of the invention will be evident from the following detailed description of preferred embodiments thereof and from the drawings wherein like parts are identified by like reference numerals throughout.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a plan view of one embodiment of apparatus in accordance with the invention.

FIG. 2 is a front elevational view of the FIG. 1 apparatus taken in section along the plane II—II of FIG. 1.

FIG. 3 is a partial view taken in section of an alternate form of the FIG. 1 apparatus.

FIG. 4 is a plan view of a second embodiment of apparatus in accordance with the invention.

FIG. 5 is a plan view of a third embodiment of apparatus in accordance with the invention.

FIG. 6 is a side elevational view of the FIG. 5 apparatus taken in section along the plane VI—VI of FIG. 5.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Test apparatus **10** of FIGS. 1 and 2 includes a substrate **12** to which is secured the marginal portions **14***a–d* of a film **14**. The substrate and film are comprised of chemically inert, electrically on-conductive materials. Film **14** is substantially transparent to light and the substrate may also have this characteristic. Film **14** includes an opening **14***e* extending therethrough for purposes discussed below. This opening is closed by removable sealing strip **16** preferably equipped with a tab **16***a* to facilitate removal thereof. First and second electrodes **18** and **20** are spacedly disposed on substrate **12** interiorly of film **14** and are separated by elongate means in the form of a strip member **22**. Electrodes **18** and **20** are preferably comprised of like or dissimilar metals and strip member **22** is typically a strip of paper, cellulose acetate or polyethylene foam, opposed end surfaces thereof preferably abutting the facing surfaces of electrodes **18** and **20**, respectively. In assembling the FIG. 1 apparatus, electrodes **18** and **20** are preferably formed on substrate **14**, e.g., by vacuum deposition or spraying techniques, and strip member **22** is treated to provide electrolytic connection of the electrodes of pH level supporting migration of the constituents of a liquid sample under study, e.g., the protein fractions of blood serum, upon application of preselected electrical voltage to the electrodes. In the case of blood serum, the

electrolyte may comprise sodium diethylbarbiturate buffer, known commercially by the trademark "Veronal" and having a pH of 8.6. Such treatment of the strip member may comprise pre-wetting the strip throughout or applying electrolyte to surface **22***a* thereof to provide a film of electrolyte thereon, constituent migration desirably occurring in such electrolyte film and not within the strip member. Film **14** may be fabricated so as to provide nominal clearance between itself and surface **22***a*. Since the film is secured to the substrate along its marginal portions, the film comprises a containment layer for the electrophoretic cell comprised of electrodes **18** and **20** and the elongate means therebetween. By virtue of the seals between members **12** and **14** and between **14** and **16**, loss of electrolyte is minimized and apparatus shelf life is enhanced.

Various alternative modes of assembling the apparatus of FIGS. 1 and 2 may be employed. For example, the assembly of elements may be accomplished with strip member **22** in dry state, electrolyte being added prior to securement of sealing strip **16** to film **14** or at the time of use of the apparatus.

Conductors **18***a* and **20***a* extend from electrodes **18** and **20**, respectively, through film **14** to provide for the connection of a voltage source **24** to the electrodes.

In use of the apparatus of FIGS. 1 and 2, sealing strip **16** is peeled away from film **14** at the time of use and pre-measured quantity of blood serum, less than a drop as contrasted with above-discussed cubic centimeters laboratory quantity, is applied to opening **14***e* of the cell containment layer. After a short time period, the residue of the drop is wiped away from opening **14***e*. At this time, conductors **18***a* and **20***a* are connected to voltage source **24**, which provides a voltage of magnitude sufficient to provide the field intensity (volts/cm.) throughout the electrolyte film on strip member **22** required for protein fraction separation.

The elongate means supporting protein fraction separation in the apparatus of FIGS. 1 and 2 may comprise a strip member of several centimeters in length, as contrasted with the separation supporting means in prior laboratory-type apparatus of many inches in length. Accordingly, to attain sufficient field intensity, e.g., 20 volts per centimeter, apparatus of FIGS. 1 and 2 requires a voltage source providing tens of volts rather than the hundreds of volts required in prior laboratory-type apparatus. Strip member width is likewise reduced from that of the prior laboratory apparatus. The apparatus of FIGS. 1 and 2 provides for the clear separation of protein fractions in shortened migration distances and in consequently shortened time periods. Such separation occurs on completion of a time period predeterminable in accordance with the distance between electrodes **18** and **22**, the voltage provided by source **24**, the electrolyte employed, and like factors influencing migration. Following separation, the elongate means is subjected to fixing, and may thereafter be stained and inspected by chromatography whereby each separated fraction is quantized.

Apart from such staining technique, the invention contemplates optical reading of the separated constituent magnitudes. In this connection, substrate **12**, film **14** and strip member **22** may all be comprised of material substantially transparent to light whereby light diffraction through the apparatus, attributable to protein fraction magnitude, provides a basis for quantizing.

As discussed above, blood serum quantity employed in samples analyzed by the apparatus of the invention is quite small. As a result, a separation of the blood serum sample from whole blood may be effected by the use of filter means integral with the test apparatus. Referring to FIG. 3, microporous member **26** may be disposed in the aperture **14***e* of film **14**. Where whole blood is applied to the member, clotting thereof occurs in the member and only the serum passes therethrough to strip member **22**. Such microporous member may incorporate a clotting agent to enhance the clotting of whole blood therein.

Test apparatus **28** of FIG. 4 includes on its substrate **30** an electrophoretic cell **32** constructed as in the case of the electrophoretic cell of the apparatus of FIGS. 1 and 2. While in the apparatus of FIGS. 1 and 2, conductors **18***a* and **20***a* were connected to a voltage source **24**, not integral with apparatus **10**, corresponding conductors **18***b* and **20***b* of apparatus **28** are connected to a galvanic cell **34** disposed on substrate **30**. The FIG. 4 apparatus is accordingly self-contained as respects all elements needed in providing for the separation of the constituents of a liquid sample. In the particular application of the apparatus to blood serum analysis, galvanic cell **34** is required to apply to conductors **18***b* and **20***b* a voltage in the order of tens of volts, as discussed above, and is thus preferably comprised of a plurality of discrete cells connected in series circuit between conductors **18***b* and **20***b* each discrete cell providing a complement of such total voltage. In the illustrated embodiment of apparatus **28**, three such cells are illustrated each being commonly structured. By way of example, cell **36** may include first and second electrodes **38** and **40**, respectively fractions of milligrams of carbon and zinc spacedly disposed on substrate **30** with suitable electrolytic connection therebetween. Such electrolytic connection may be provided by the use of an electrolyte-absorbent member **42** comprised of paper or the like. The electrolyte for activating cell **36** may be introduced into member **42** at the time of use as is illustrated schematically by removable sealing member **43**.

Use of the Fig. 4 apparatus parallels that of the FIGS. 1 and 2 apparatus discussed above, the application of electrical voltage to electrophoretic cell **32** of FIG. 4 being accomplished by activating galvanic cell **34**. In the galvanic cell form illustrated schematically, activation occurs on addition of electrolyte to all the individual units, e.g., **36**. Where the galvanic cell is provided beforehand with electrolyte, means may be incorporated for providing selective connection of the galvanic cell to the electrophoretic cell to control application of voltage to the electrophoretic cell.

Referring to FIGS. 5 and 6, apparatus **44** comprises a substrate **46** having a surface depression **46***a* covered by cell containment layer **48**. Such depression defines a trough-like receptacle for an electrophoretic cell including electrodes **52** and **54** and elongate means comprising a body of electrolyte **50**. Layer **48** includes therein an opening **48***a* in communication with a channel **56** extending to depression **46***a*. Layer opening **48***a* is closed by removable sealing member **58** having tab **58***a*. On removal of member **58**, the sample is applied to layer opening **48***a* and thence through channel **56** into the electrolyte in depression **46***a*. Upon application of voltage to electrode conductors **52***a* and **54***a*, separation of the sample constituents occurs in electrolyte **50** between the electrodes.

In the apparatus of FIGS. 4 and 5, the dimensions of depression **46***a* are selected with a view toward minimizing mixing of the sample with the electrolyte and toward avoiding any migration of the sample constituents in the absence of applied voltage. The mobilities of sample constituents are increased from sample constituent mobilities in the previously-discussed embodiments of apparatus in accordance with the invention in view of the increased migration-supporting electrolyte area. Accordingly, lessened time for sample analysis is required. The greater ratio of surface area to volume in the apparatus of FIGS. 4 and 5 as compared to the previously-discussed apparatus serves effectively to reduce temperature rises from electrical sources. In addition, lesser sample volume is required. Where substrate **46** and cell containment layer **48** are comprised of materials substantially transparent to light, the aforementioned optical reading of sample constituents may be practiced upon completion of constituent migration.

It is to be appreciated that various changes may be made in the foregoing particularly disclosed preferred embodiments of apparatus according with the invention.

For example, any of the vast number of materials having the aforementioned characteristics may be used in respect of the apparatus substrates, cell containment members, sealing strips, electrodes, electrolytes and the like. Immobilizing additives, such as gelling agents, for the electrolytes are within the contemplation of the invention. Accordingly, the particularly disclosed embodiments are intended in an illustrative and not in a limiting sense. The true spirit and scope of the invention is defined in the following claims.

What is claimed is:

1. A self-contained electrophoretic cell comprising a chemically inert, electrically non-conducting substrate member, a pair of electrodes fixed to said substrate member, a thin strip member disposed on said substrate member between and in electrical contact with said electrodes, said strip being operable to support an electrophoretic medium, and a chemically inert electrically non-conducting transparent film overlying said strip and said electrodes and having its marginal portions sealed to said substrate member, said film having an opening operable to permit introduction of a sample to said strip and closure means operable to seal said opening before and after sample introduction.

2. A cell as defined in claim 1 wherein said strip member is wetted throughout by an electrolyte prior to sealing said film.

3. A cell as defined in claim 1 including a pair of conductors in electrical connection with said electrodes and extending to the exterior of said cell.

4. A cell as defined in claim 3 wherein said conductors are adapted for electrical connection with an outside voltage source.

5. A cell as defined in claim 3 including a self-contained voltage source in electrical contact with said electrodes through said conductors.

6. A cell as defined in claim 1 wherein said strip member comprises paper, cellulose acetate or polyethylene.

7. A cell as defined in claim 1 wherein said substrate member is a material substantially transparent to light.

8. A cell as defined in claim 1 including filter means disposed in said film opening and operable to remove a constituent of a sample during introduction of the sample to said strip.

9. A cell as defined in claim 8 wherein said filter means is operable to remove whole blood constituents other than those constituents comprising blood serum.

10. A cell as defined in claim 9 wherein said filter means comprises a microporous material incorporating an agent for clotting whole blood constituents in said material.

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U.S. Cl. X.R.

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