

1

3,497,437

METHOD OF ELECTROPHORESIS

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2 Claims

ABSTRACT OF THE DISCLOSURE

A method of improving techniques of agar gel electrophoresis which consists of employing in the agar gel medium from about 0.5% to about 5% by weight polyethylene glycol.

This invention relates to a method of electrophoresis and, more particularly, to a method of electrophoresis employing an agar gel medium.

Electrophoresis is the movement in an electric field of charged particles in any medium, such as, for example, inorganic ions, proteins such as albumin, enzymes, hemoglobin, carbohydrates, blood serum, and the like. These charged particles, as constituents of particular mixtures, will migrate with different mobilities when the mixture in solution is placed in an electric field. While the current is flowing, the charged particles will move continuously at different speeds and separate into different groups, and thus provide an effective means for resolving the components of certain mixtures.

Various methods of electrophoresis are known, for example, the classical moving boundary method of Tiselius, paper electrophoresis, and methods employing liquid or solid buffer systems.

The present invention contemplates the use of an agar gel medium for the electrophoretic movement of charged particles. The general use of agar gel media in electrophoresis is known, as can be seen, for example, from U.S. Patents 2,843,540 and 3,062,731.

Agar is a dried hydrophilic colloid substance extracted from seaweed such as red algae (Class Rhodophyceae) and purified by various known means. It is capable of forming solid or semi-solid transparent gels of predetermined rigidity upon being cooled from the liquid solution form, which makes it useful as a medium for electrophoresis. In combination with various buffers, agar forms a relatively inert medium which has negligible harmful effect upon proteins, enzymes, and the like components of the mixtures to be analyzed.

In the conventional use of agar gel media for electrophoresis, the substance to be resolved into its components is placed in a slit in an agar gel film on a slide or other supporting means. By application of a suitable voltage gradient to the gel medium, the respective charged particles in the substance will migrate. After a predetermined period of time the various components are separated. The gel is then stained biochemically, soaked for about 6 to 12 hours and dried, or first fixed and then soaked for about 6 to 12 hours, stained and dried. The dried gel membrane can then be optically analyzed, as, for example, with a colorimeter or a scanning densitometer. In this method of electrophoresis, good separation of components is dependent upon the ability to close the sample slits completely. If closure is incomplete, current does not pass through the area of sample application.

In previous practice, the texture of the agar gel has been such that the slits could not be closed, resulting in a distortion in the migration of the charged particles from the slit. Moreover, the film when dried for analytical pur-

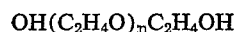
2

poses would not admit of easy removal from the slide tray and tended to crack. In addition, the long soaking period of 6 to 12 hours is time consuming and undesirable. But without using this long soaking period of conventional practice, the buffer and substrate salts would form crystal patterns in the dried membrane, rendering the membrane useless for quantitation by densitometry.

Accordingly, it is an object of the present invention to provide an improved agar gel medium for use in electrophoresis and the like.

In accordance with the present invention, an agar gel is improved for use in electrophoresis by incorporating in the gel medium from about 0.5% to about 5%, by weight of the medium, of polyethylene glycol having a molecular weight of from about 200 to about 20,000.

Polyethylene glycols are high molecular weight polymers which are generally produced by reacting ethylene oxide with ethylene glycol or water and have the following structure:



in which n represents the average number of oxyethylene groups. PEG 4000, which is a polyethylene glycol product having an average molecular weight of 4,000, is the preferred product of this group.

It has been found that the employment of polyethylene glycol in the hereinbefore stated concentration in agar gel media for electrophoresis substantially improves the texture of the gel such that after sample application the slits can be adequately closed. In addition, the substrate and buffer salts are rapidly eluted from the gel in the short fixing period, or in about a 5 to 10 minute soak in water instead of the usual 6 to 12 hour soak. The incorporation of polyethylene glycol in the agar gel makes the gel porous, allowing rapid elution of the buffer salts from the medium. Furthermore, the dried gel film can be readily removed from the slide without cracking, and the dried gel film remains soft and pliable for convenient use in subsequent analytical treatment.

Although it has been known to employ polyethylene glycol in agar used for culture media for the growth of fungi, as can be seen from U.S. Patent 2,437,766, it has not heretofore been known to employ polyethylene glycol to improve the texture of agar gel for electrophoresis such that sample application, elution of incorporated salts (buffer or substrate), and drying of the gel to a pliable transparent film are made practical.

The agar gel medium of this invention also generally employs a buffer system to maintain an essentially constant hydrogen ion concentration or pH. Any conventional buffer system can be employed, for example, phosphate and/or phosphoric acid, citrate-citric acid, acetate, borate, tris-glycine, tris-EDTA (ethylenediaminetetraacetic acid)-boric acid, barbital-barbituric, and the like buffer systems.

The following examples will illustrate the invention in further detail although the invention is not limited to these specific examples. All percentages are by weight unless otherwise stated.

EXAMPLE 1

Agar gels containing about 2% by weight polyethylene glycol for serum protein electrophoresis are prepared by adding 1.2 gm. of Special Agar Noble, 2.0 gm. of PEG 4000, and 100 ml. of barbital buffer (sodium barbital-barbituric acid at pH 8.6, ionic strength 0.040) to a 500 ml. Erlenmeyer flask. The mixture is heated in a boiling water bath until a clear agar solution results. The temperature of the mixture is retained at the boiling temperature of water for 5 to 10 minutes to insure solution of the agar.

3

The agar solution is cooled from 100° C. to about 80° C. before pouring twenty-five milliliters of the solution into each of several trays. Gelling occurs below 50° C. The agar gels are then nested in groups of 10, sealed in plastic bags, and a portion are stored at room temperature and another portion at 5° C. If desired, the buffer constituent can contain sodium azide to prevent the growth of contaminants in the gel.

After gelling, slits are cut in the agar and buffer is absorbed out with filter paper strips. Open wells result which each accommodate approximately 5 microliters of blood serum or other sample to be tested. In this example, a blood serum sample is applied and allowed to perfuse into the surrounding gel for 2 minutes.

The slits are then closed to re-establish a continuous path in the gel for the flow of current; and open slit constitutes an undesirably break in the continuity of the gel. Closing the slits is accomplished by gently pulling the agar loose from one end of the gel plate. By means of this gentle pulling, the slits immediately close permanently. In an identical gel but without the polyethylene glycol, it has been found virtually impossible to close the slits.

The excess sample is blotted up and the gel is placed on the base unit. A current of 50 milliamperes (approximately 90 V.) passed through the gel for 70-80 minutes.

At the end of the electrophoresis the proteins are fixed by immersing the gel in an aqueous 7½% acetic acid solution. Fixation continues for 20 minutes, during which times the buffer salts are partially eluted from the gel. Equivalent amounts of other fixatives, for example, trichloroacetic acid and low molecular weight alkanols such as methanol, ethanol and isopropanol can be used in place of the acetic acid with comparable results.

After fixation the gel is allowed to imbibe in water for about 5 minutes. Then it is dried to a smooth, clear pliable film. These gels are optically scanned on a thin-film densitometer with excellent results. On identical gels but without the polyethylene glycol, a 6 to 12 hour soaking must follow the fixation process to yield suitable membranes. When these gels without the polyethylene glycol are not subjected to the extended 6 to 12 hour soaking, the resulting dried membrane contains a dense precipitation of buffer salt crystals. Attempts to dissolve these crystals from the dried membrane were unsuccessful; hence, these membranes were not suitable for quantitation in a transmission densitometer.

A substantial and significant difference has been found between dried gel films made from agars with polyethylene glycol and those without polyethylene glycol. Polyethylene glycol gels dry smooth, flat, and pliable; whereas, gels without polyethylene glycol, even after a 12 hour soaking, dry to a badly wrinkled, very fragile membrane. Gels without polyethylene glycol also are

4

generally difficult to remove from the tray after they have dried to a thin film whereas those with polyethylene glycol are easily removed from the tray.

EXAMPLE 2

When one gram of polyethylene glycol 6000 and ½ gram of polyethylene glycol 1000, respectively, are substituted for the polyethylene glycol 4000 in Example 1, substantially equivalent excellent agar gels for use in electrophoresis are obtained.

EXAMPLE 3

When one percent by weight and three percent by weight, respectively, of polyethylene glycol are substituted for the two percent by weight of polyethylene glycol in Example 1, substantially equivalent excellent agar gels for use in electrophoresis are obtained.

EXAMPLE 4

Hemoglobin and LDH (lacticdehydrogenase) isozyme samples are electrophoretically analyzed with agar gels containing 0.5% to 5% by weight polyethylene glycol in the same manner as the blood serum in Example 1 with excellent results.

As will be readily apparent to those skilled in the art, other examples of the herein-defined invention can be devised after reading the foregoing specification and claims appended hereto by various modifications and adaptations without departing from the spirit and scope of the invention. All such modifications and adaptations are included within the scope of the invention as defined in the appended claims.

What is claimed is:

1. In the method of electrophoresis employing a gel medium containing agar as a gelling agent, the improvement comprising incorporating in the gel medium from about 0.5% to about 5% by weight of the medium, of polyethylene glycol having a molecular weight of from about 200 to about 20,000.

2. The method of claim 1 in which the polyethylene glycol has an average molecular weight of about 4,000.

References Cited

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