United States Patent [19]

Anderson et al.

[11] **3,927,826**

[45] **Dec. 23, 1975**

[54] **ROTOR FOR CENTRIFUGAL TESTING OF ELECTROPHORESIS GEL**

[75] Inventors: **Norman G. Anderson; John E. Caton, Jr.**, both of Oak Ridge, Tenn.

[73] Assignee: **The United States of America as represented by the Secretary of the Department of Health, Education and Welfare**, Washington, D.C.

[22] Filed: **Aug. 27, 1974**

[21] Appl. No.: **500,866**

[52] **U.S. Cl.** ..... **233/11**; 233/1 A; 233/26; 204/180 G

[51] **Int. Cl.²** **B04B 15/02**; B04B 1/04

[58] **Field of Search** ..... 233/16, 21, 26, 27, 28, 233/1 R, 11, 1 A; 204/299 R, 180 G

[56] **References Cited**

UNITED STATES PATENTS

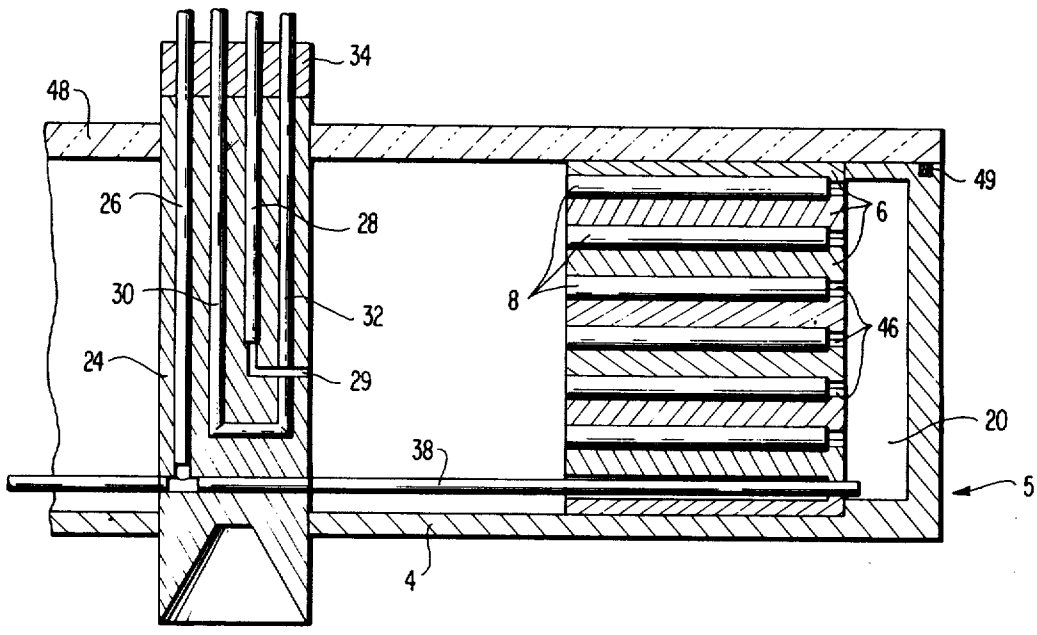
2,616,619	11/1952	Macleod	233/26 X
3,556,967	1/1971	Anderson	204/299 R
3,576,727	4/1971	Evatt	204/180 G
3,799,863	3/1974	Zeineh	204/180 G

Primary Examiner—George H. Krizmanich
Attorney, Agent, or Firm—Browdy and Neimark

[57] **ABSTRACT**

A rotor for the preparation of identical electrophoresis gels where the liquid is permitted to flow into a rotor bowl under dynamic conditions. The rotor comprises a cylindrical body, a sealable closure, a central core, and conduits within the core which communicate with the liquid system outside the rotor through a face-seal means. The rotor further contains a plurality of annular plates laminarly stackable and inserted into and removable from the rotor. Each plate has a multiplicity of radial grooves in a juxtapositionally matchable plate-to-plate relationship.

14 Claims, 6 Drawing Figures



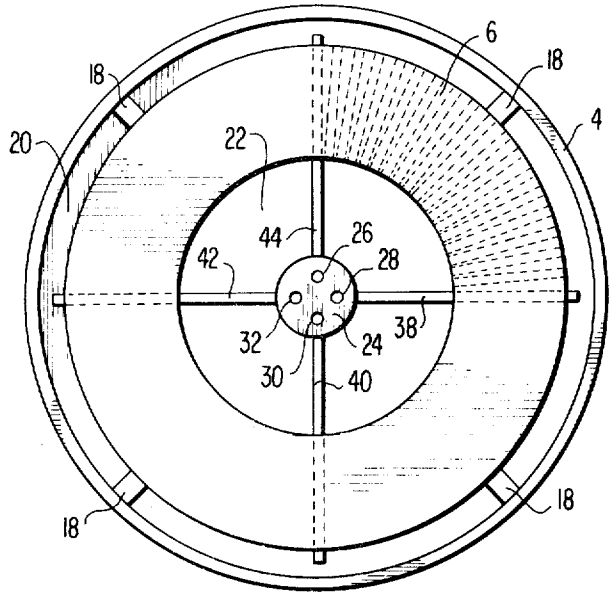


FIG. 1

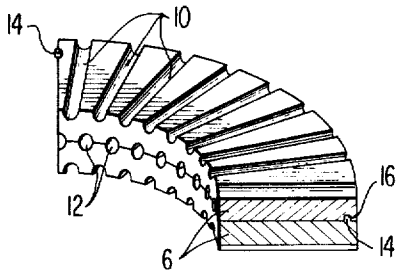


FIG. 3

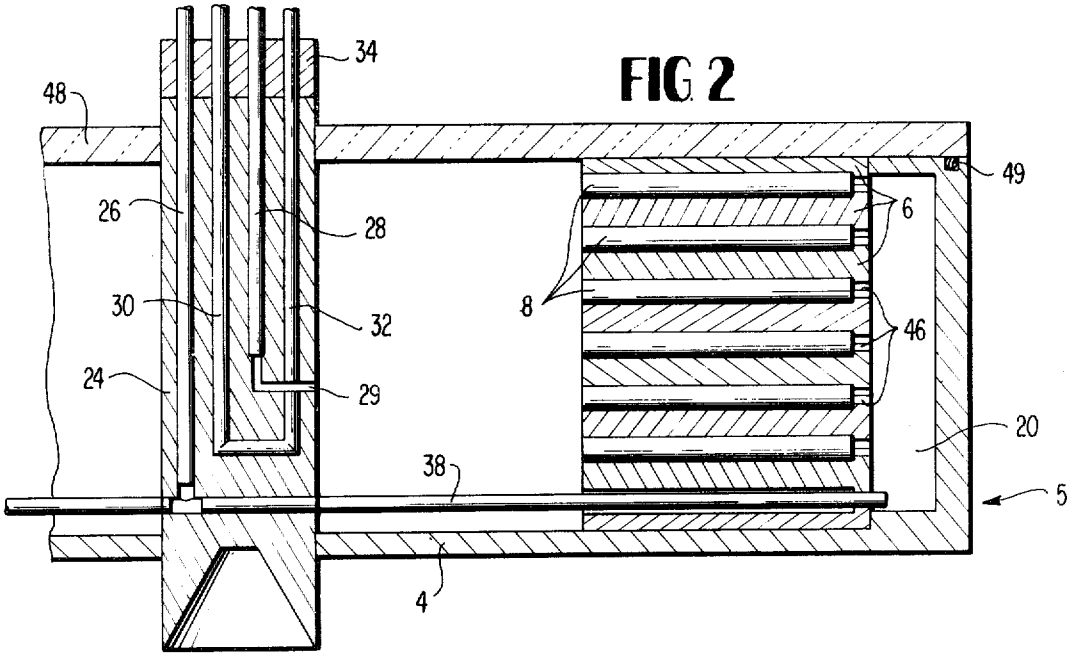


FIG. 2

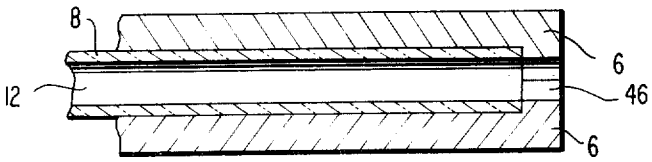


FIG. 4

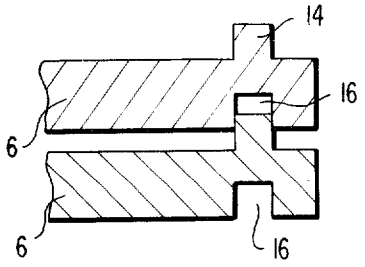
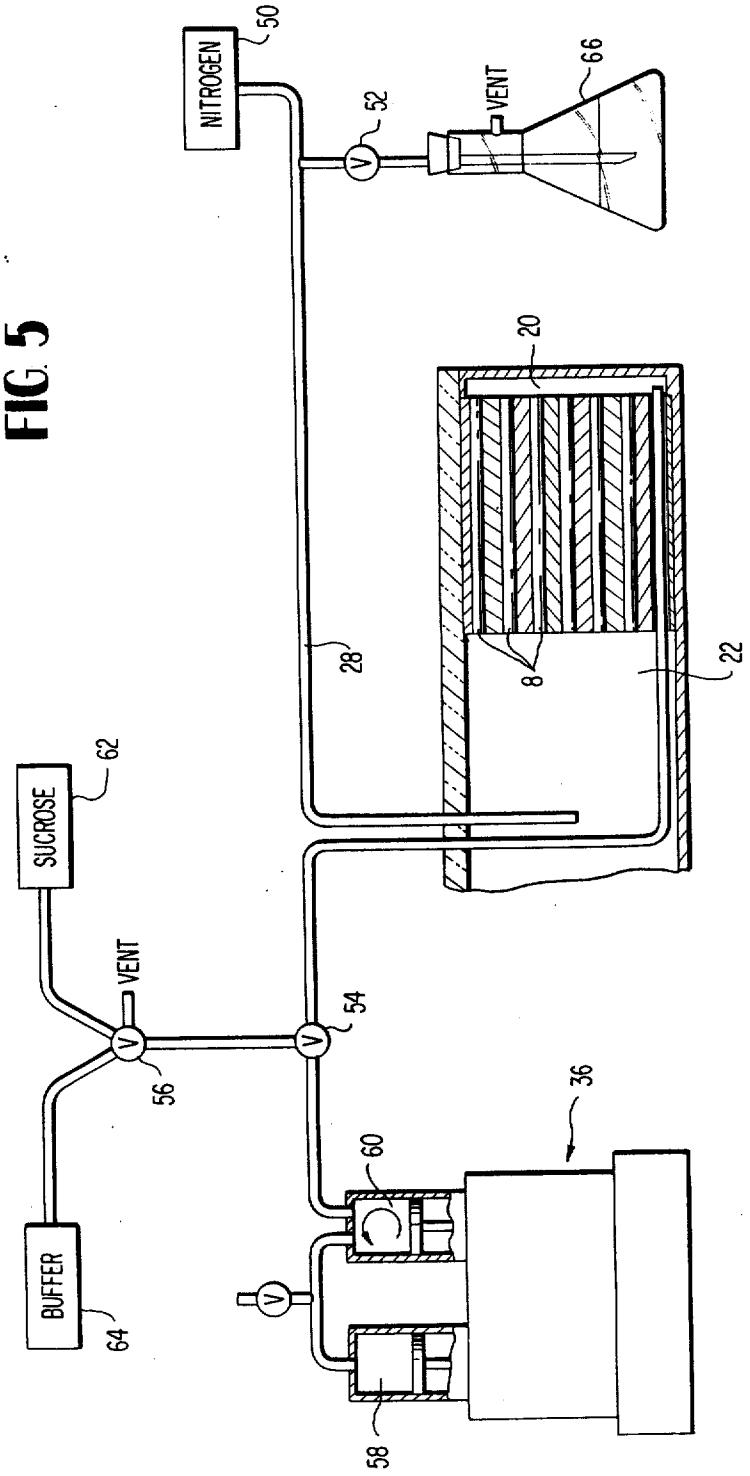


FIG. 6

FIG. 5


NITROGEN
50
52
VENT
66
20
28
8
22
62
SUCROSE
VENT
54
56
36
60
BUFFER
64
58

ROTOR FOR CENTRIFUGAL TESTING OF ELECTROPHORESIS GEL

FIELD OF THE INVENTION

This invention relates to a rotor for the preparation of a number of electrophoresis gels identical with respect to size, shape, and density in composition profiles.

BACKGROUND OF THE INVENTION

It is well known in the prior art that electrophoretic techniques may be performed in conjunction with screening new-born infants for genetic disorders; thus facilitating early discovery of these diseases. However, clinical electrophoretic analysis of large numbers of samples is presently laborious and time-consuming. In order to obtain meaningful high resolution of which this method is capable of yielding, extreme care is essential in preparing identical gels. These gels are normally prepared by gravity dispensing of gel forming liquids in vertically positioned tubes, where the dispensing is in the form of concentration gradient or non-gradient. The gradients are difficult to duplicate under these conditions and a meniscus tends to form at the upper surface which must be eliminated later.

This type of preparation is discussed in "Disc Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis" by H. Rainer Maurer, published by Walter de Gruyter, **1971.** This book describes a method for the preparation of a spacer gel and also a separation gel which must be mixed in order to create an acrylamide gradient gel. As is shown on pages 52 and 53 of this text, this method must be done by hand and the use of a high-speed electrophoretic technique is impossible. In addition, the gels which are produced are not always of high resolution nor are they identical.

SUMMARY OF THE INVENTION

It is an object of the present invention to overcome the defects of the prior art as mentioned above.

It is a further object of this invention to provide a device by which gels may be mass produced having identical characteristics as to size, shape and chemical profile.

It is yet another object of this invention to provide a high speed method for the preparation of electrophoresis gels.

BRIEF DESCRIPTION OF THE DRAWING

For a better understanding of the invention, a possible embodiment thereof will now be described with reference to the attached drawing, it being understood that this embodiment is exemplary and not limitative.

FIG. **1** is a plan view of a rotor with plate assembly;

FIG. **2** is a partial cross-section of a typical rotor with plates and cover in place;

FIG. **3** is a perspective view of two quarter-sectioned grooved annular plates;

FIG. **4** is an enlarged cross-section of two grooved plates containing a gel-casting tube;

FIG. **5** is a diagram of the rotor and gradient pump assembly; and

FIG. **6** is a partial cross-sectional view of two grooved plates showing the hole and pin combination.

DETAILED DESCRIPTION

The rotor plate assembly is shown in FIGS. **1** and **2** which includes a rotor **5** made of a bowl **4** which may be of anodized aluminum. The bowl may be of any desired size, the bowl illustrated being of about 16 inches in diameter and 2½ inches deep. Into this bowl **4** are placed a plurality of doughnut-shaped annular plates **6,** each plate having a plurality of radially extending semicircular shaped radial grooves **10** on both their top and bottom surfaces. Two such plates mesh together in a juxtapositionally matchable plate-to-plate relationship so as to form a layer. As illustrated, each layer contains 72 cylindrical columns **12** lying along the radii of the rotor. Each of these columns is adapted to hold a pyrex glass tube **8** which may be approximately 7 millimeters in outside diameter and 9 centimeters long. The outer end of each of these cylindrical columns **12** is restricted so that the tube **8** cannot be thrown against the rotor wall when it is spinning. For structural purposes, each row of glass tubes **8** will be aligned in such a manner so as to ensure that each tube is not directly above (or below) a tube in an adjacent layer. In other words, grooves **10** on a top surface are located between two grooves on a lower surface. Four glass tubes are omitted from the bottom layer in order to accommodate the conduit lines **38, 40, 42** and **44** which deliver the incoming solution to the periphery of the rotor **20** via the feed tube **26.** Between the apertures of the tube **46** and the rotor wall, there is a space **20** which may be approximately 2 millimeters to allow the incoming solution to equilibrate both radially and vertically before being forced into the tubes **8.**

The annular plates **6** are oriented with respect to its rotational alignment by any suitable means such as a pin and hole system **14** and **16.** Each plate, along its outer diameter, contains a pin **14** placed on the top surface and a hole **16** opening into the bottom surface. Thus, placing the pin of one of the plates into the hole of another of the plates, would automatically allow the plates **6** to be aligned with one another forming the cylindrical spaces **12** into which the glass tubes **8** are placed.

To ensure axial alignment of the annular plate assembly, four ribes **18** are placed on the inside wall of the rotor bowl **4** and protrude into the periphery of the rotor **20.** The plates **6** are positioned flush against these ribs **18** and a tight, snug fit is thus maintained. Additionally, a pin is provided in the bottom of the rotor bowl **4** to fit in a hole in the bottom plate to guarantee the axial alignment.

A column **24,** in the middle of the central core **22,** contains four tubes **26, 28, 30** and **32.** Tubes **30** and **32** are the input and output cooling tubes which permit the circulation of cold water to ensure that the rotor and centrifuge do not overheat. The feed tube **26** is connected to delivery conduits **38, 40, 42** and **44** to introduce the liquid gel solution from the gradient pump **36** to the header periphery **20** and then into the glass tubes **8.** Vent tube **28** has an aperture **29** in the central core **22** which is used in the purging of the system to ensure that the gel solution will not interact with any oxygen. To maintain this closed system, the column **24** contains an A-type seal **34** at its top and the bowl **4** is sealed by means of an O-ring **49** around the outer diameter of the bowl and is compressed between this bowl **4** and a cover **48,** which may be made of 1 inch thick plexiglass.

The rotor is mounted on and powered by a standard International Model PR-2 centrifuge. Although this centrifuge can spin the rotor at a rate of up to 2,000 rpm, optimum results are obtained in the 800–925 rpm range.

The entire rotor, gradient pump and venting assembly is depicted in FIG. **5**. A gradient pump **36** is provided with two chambers **58** and **60**, the latter of which contains a stirring mechanism. The passage of a gel solution, buffer solution **64**, and sucrose mixture **62** to the rotor **5** is controlled by the opening and closing of three-way stopcocks **54** and **56**. Another stopcock **52** allows nitrogen **50** to purge the rotor assembly or to be vented to a vent assembly **66** containing water.

The polymerized gel solution is prepared in the following manner: To ensure that no oxygen is present in the rotor **5** when the gel solution is introduced, the assembly is purged in a backward direction with nitrogen **50** during which stopcock **52** is closed and stopcocks **54** and **56** are opened to vent the rotor. While the system is being purged, the gradient pump **36**, which is jacketed and maintained at ice water temperatures, is filled with the proper amounts of a solution, such as acrylamide, to be polymerized. This pump **36** contains two chambers **58** and **60** with chamber **58** containing a higher concentration of acrylamide than chamber **60**.

After the purging, rotor **5** is accelerated to the desired speed and the vents on stopcock **54** and **56** are connected to the container of gel buffer **64**. After the nitrogen source **50** is clamped and stopcock **52** is opened to vent assembly **56**, a predetermined amount of gel buffer **64** is pumped into the rotor **5**. This buffer is to ensure that oxygen does not react with the gel gradient and also to adjust the pH of the solution to approximately 8.5. The buffer tris-hydroxymethyl amino methane-HCL was used in this method. However, any suitable buffer may be employed in this regard.

After the gel buffer is pumped into the rotor, stopcock **54** is adjusted so that the gradient pump **36** and the rotor **5** are connected and the gradient solution is pumped into the feed tube **26** light end first. Once the gradient pump **36** is activated, the solution contained in chamber **60** is directly pumped into the rotor assembly while the higher concentrated solution contained in chamber **58** is pumped into chamber **60**. Since this chamber **60** is constantly being stirred, the concentration in this chamber of the acrylamide solution slowly increases. This solution is pumped into feed tube **26** and then into the delivery conduits **38, 40, 42** and **44** which transports the solution to the header periphery **20** of the rotor assembly. Due to the centrifugal action of the spinning rotor **5**, this solution is forced from the header pierphery **20** into the outer ends of the glass tubes **8** and outwardly displaces the lighter buffer solution already in these tubes **8**. This buffer solution then acts as a layering solution protecting the gel from contact with any oxygen. As the pump **36** continues to supply gradient solution to the rotor, the lighter fluid already present in the rotor **5** is displaced inwardly in the tubes towards the rotor axis enabling the tubes to fill equally. When the contents of the gradient pump **36** have been expelled into the rotor **5**, stopcocks **54** and **56** are actuated to cut off the gradient pump **36** and to connect the solution **62** to the rotor so that enough of a solution **62** containing, for example, 20% sucrose, is pumped into the spinning rotor to feed all the gel solution into the tubes and out of the periphery space **20**. The seal **34** may then carefully be disconnected from the spinning rotor, and the refrigeration unit of the centrifuge is turned off and the rotor is allowed to spin at room temperature for several hours or overnight to complete the polymerization of the gels. These gels are subsequently removed by dismantling the plates **6** and removing the glass tubes **8** which are stored in refrigerated jars at 4°C. under the gel buffer containing 0.1% sodium azide.

It should be noted that while a system for producing gradient gels has been described, the rotor assembly **5** is not so limited. If the same concentration of solution were placed in both chambers **58** and **60**, the gradient pump **36** would pump in only one concentration of solution and thereby making a homogeneous set of gels.

Although the present invention has been described with a certain degree of particularity, it is understood that the present invention has been made by way of example and that changes in details of construction may be made without departing from the spirit thereof.

What is claimed is:

1. An apparatus for preparing identical electrophoresis gels comprising:

a rotor having a generally cylindrical body;

means for rotating said rotor;

a plurality of stacked plates, each plate having a multiplicity of radial grooves in its upper and lower surfaces each groove so sized and shaped as to form one half of a cylinder, each pair of said plates being in a juxtapositionally matchable plate-to-plate relationship so as to form a plurality of gel casting tubes therebetween, said plates having an outer radius smaller than that of said rotor so that a space is formed between said stack of plates and the inner surface of the outer wall of said rotor; and

means to feed a liquid solution to said space;

whereby as liquid is fed to said space while said rotor is rotated, said liquid solution is forced inwardly from said space into said gel casting tubes.

2. A rotor according to claim **1**, wherein each of said gel casting tubes contains a glass cylinder.

3. A rotor according to claim **1**, wherein said rotor further includes a means for orienting said plates with respect to alignment during rotation.

4. A rotor according to claim **1**, wherein said rotor further includes a means of axial alignment of said annular plates.

5. A rotor according to claim **1**, wherein said means for feeding the liquid solution includes a gradient pump.

6. An apparatus according to claim **1** wherein said rotor further contains a central core, said liquid solution being fed into said rotor through said central core.

7. An apparatus according to claim **1** wherein said rotor is sealed by a sealable closure.

8. An apparatus according to claim **1** further comprising a means for maintaining an inert atmosphere in said rotor.

9. An apparatus according to claim **1** further comprising means for cooling said rotor during rotation.

10. A method for the preparation of identical electrophoresis gels comprising the steps of:

spinning a rotor by;

pumping a gel solution into a plurality of gel casting tubes radially arranged in said rotor;

additionally spinning said rotor for a sufficient time to allow said gel solution to polymerize in said tubes.

11. A method according to claim **10** wherein said rotor is sealed from the atmosphere and an inert atmosphere is maintained in said rotor.

12. A method according to claim **10** wherein said gel is a gradient gel.

13. A method according to claim **10** wherein a buffer solution is pumped into said tubes before said gel solution, so as to form an inner seal for each tube.

14. A method according to claim **13** wherein a sucrose solution is pumped into said rotor after said gel solution to act as an outer seal.

* * * * *