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Elson et al.

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[54] **FLAT PLATE ELECTROPHORESIS**

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[52] **U.S. Cl.** ..... **204/299;** 204/180 G

[51] **Int. Cl.** **B01k 5/00**

[58] **Field of Search** ..... 204/180 G, 180 S, 299

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[57] **ABSTRACT**

A device for horizontal flat plate electrophoresis comprising in combination a horizontal plate defining a rectangular gel bed, surrounded by a ledge, the height of which defines the height of the gel, and by lateral walls, a cover adapted to rest on the ledge, fixing the thickness of the gel, two legs attached to the bottom of the plate and extennding along the entire width of the plate at each end, a slot being provided at each end of the gel bed and running the width of same said slots extending through the entire height of the leg, the cover being provided with a plurality of holes for the insertion of templates during the polymerization of the gel, and for the subsequent insertion of the samples. The gel bed can be divided into a plurality of separate channels, and openings for the insertion of samples are provided near the edge of the cover or at about its middle.

7 Claims, 2 Drawing Figures

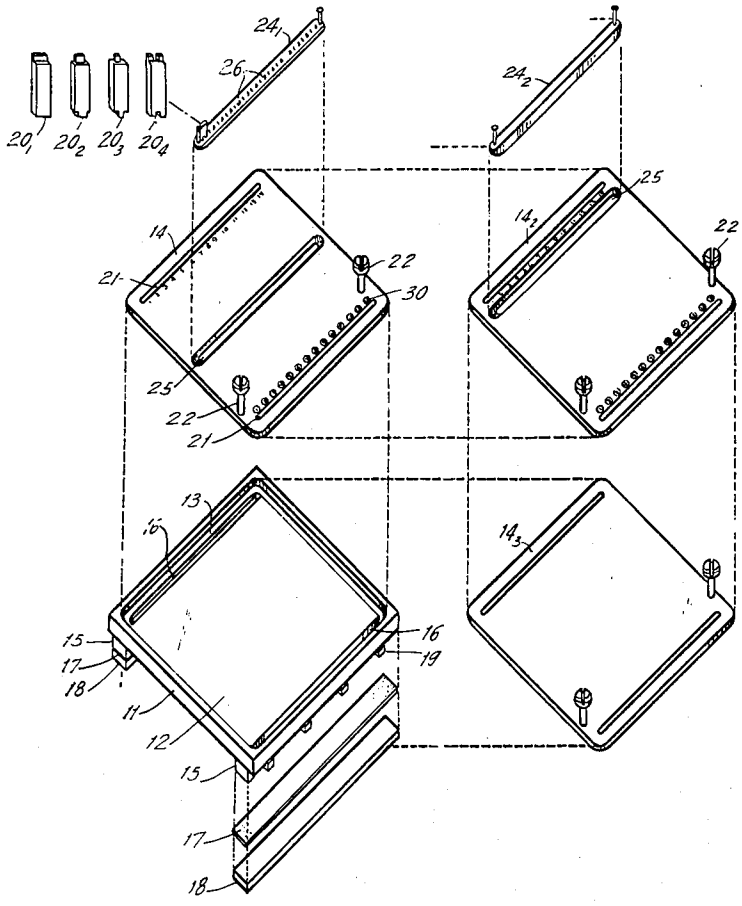


FIG. 1

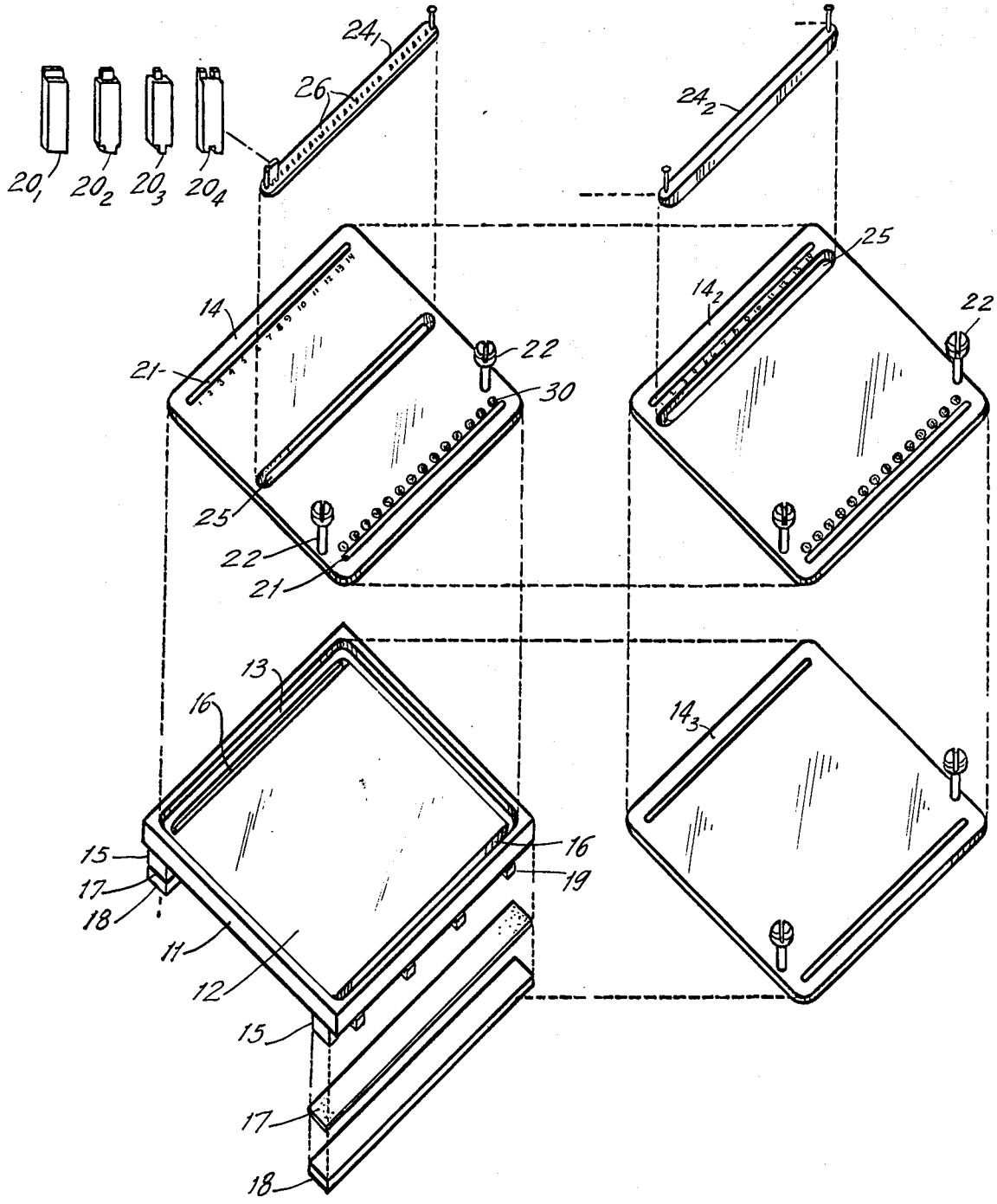
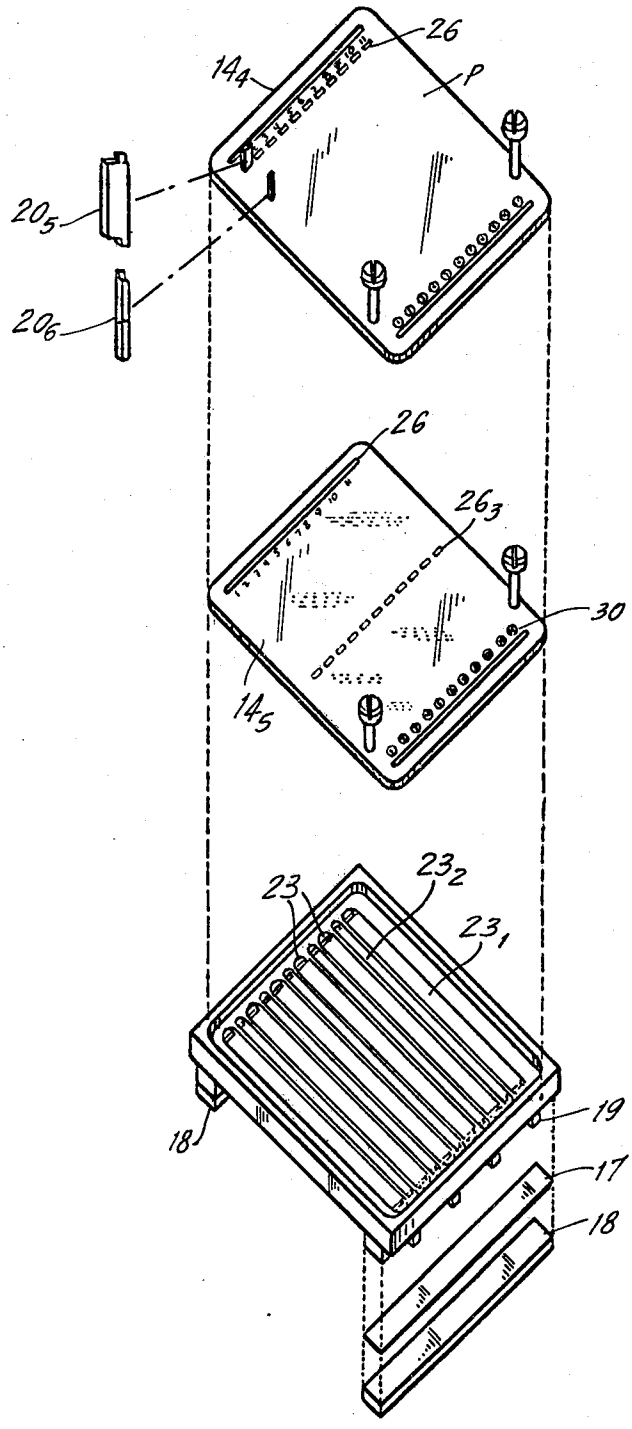

24
26
20_1
20_2
20_3
20_4
24_2
14
21
22
30
25
14_2
14_3
13
16
15
17
18
19
11
12

FIG. 2



FLAT PLATE ELECTROPHORESIS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to a novel device for horizontal flat plate electrophoresis; it can be used for one-dimensional and for two-dimensional runs and is used advantageously with polyacrylamide gel electrophoresis.

2. Description of the Prior Art

A number of instruments have been described for electrophoresis in flat gel slabs. Both vertical and horizontal arrangements have been employed, each having its own advantages and disadvantages. The vertical arrangement is amenable to the use of discontinuous gel and buffer systems; different slot-forming templates can be used interchangeably to vary the number and size of sample wells; sample loading is easily accomplished without opening the plates that enclose the gel; and the ends of the gel are in direct contact with the electrode buffers, obviating the need for filter paper or other connecting bridges. The horizontal instruments described lack these features but, having no upper buffer chamber, are simpler to construct and having the additional advantage that samples may be applied in the center of the gel as easily as at the end.

According to the invention, there is provided a horizontal apparatus that incorporates a number of the advantages of both types of instrument. The ends of the gel, bent at right angles to the horizontal body, make direct contact with the electrode buffers, so that neither a connecting bridge nor a water-tight upper buffer chamber is needed. Interchangeable plates enable sampels to be placed either at the end of the gel or in the center. Interchangeable removable templates afford a wide range of sample volumes and allow the sample to be applied without opening the instrument. The apparatus can be used for one- and two-dimensional electrophoresis and, in certain cases, two parallel samples can be run in the same two-dimensional gel, facilitating comparison. Its use is illustrated with ribosomal proteins.

DESCRIPTION OF THE PRESENT INVENTION

The present invention relates to a novel device for horizontal flat plate electrophoresis. The novel device can be used for both one- and two-dimensional runs. It is advantageously used with polyacrylamide gel electrophoresis, which has become one of the most widely used techniques for the analytical separation of biological macromolecules.

The novel device according to the present invention is described by way of example only with reference to the following detailed description and with reference to the enclosed drawings, in which:

FIG. 1 is a schematical exploded view of a device according to the invention, illustrating a number of different embodiments;

FIG. 2 is a schematical exploded view of another device according to the invention.

The device according to the invention is designed for horizontal flat plate gel electrophoresis in one or two dimensions. It consists essentially of a box and a cover, the space between these defining the gel volume. In the following, there are set out two versions, Version A defining a continuous rectangular gel bed; Version B defining a gel bed divided into a plurality of individual elongated gel strips.

The following description contains by way of example definite dimensions. These relate to certain laboratory models made and tested. The dimensions are clearly illustrative and not to be construed in any limitative manner.

Version A is shown in FIG. **1.** The box comprises a perspex plate **11,** 17 cm square and 1.2 cm thick, into which is machined a 14 cm × 14 cm square gel bed **12,** and a surrounding ledge **13,** 1 cm wide and 2 mm higher than the bed. A cover **14** rests on the ledge, fixing the thickness of the gel at 2 mm. Two perspex legs **15** 1.8 cm wide and 1.6 cm high are permanently fixed at right angles to the bottom of the plate and these extend the entire width of the plate of 17 cm, one at each end. At each end of the gel bed **12** there is a slot **16** 2 mm wide running the width of the gel bed, and extending downward through the entire height of the leg, which is open at its bottom.

In use, the slots **16** are closed off at the bottom of the legs with a rubber strip **17** supported by a perspex strip **18** held in place by rubber bands stretched back and forth across it and anchored to lugs **19** on both sides of the leg. A cover, **14** (see below) is placed on the ledge **13,** completely covering the gel bed, and is clamped in place with spring clips, which are not shown. Templates **20** are inserted into the slots **21** in the cover and are pressed down to touch the bottom of the gel bed (see below). The gel bed and leg slots are filled with gel mixture through one of the slots **21** in the cover, through which air also escapes. The apparatus now contains a continuous gel 2 mm thick that extends from the bottom of one leg through the gel bed to the bottom of the other leg. After the gel has polymerized, the templates are removed, leaving holes in the gel for the samples. (In case of a pre-run without sample, the templates are left in place.) The perspex and rubber strips are removed from the legs, exposing the two ends of the gel. The apparatus is placed on two rectangular electrode vessels filled with buffer, with the plate resting horizontally on the opposing rims of the two vessels and each leg dippling down into the buffer of a different vessel so that the gel is in contact with the buffer. The samples are pipetted through the template slots into the holes left by the templates, and the electrophoretic run is made.

At the end of the run the cover is gently disengaged from the gel by removing the clips and by turning the screws **22** which press on the ledge **13.** This allows the cover to be raised slowly in a controlled manner and prevents the gel from tearing. The gel should be lubricated during this operation by introducing water or another suitable liquid through the slot **21.** After removal of the cover, a knife or spatula is moved around the periphery of the gel, and the lubricated gel is cut loose from its extensions into the legs, gently disengaged from the gel bed with the aid of a spatula, and lifted out.

A second version (B) of the apparatus is shown in FIG. **2.** It resembles version A except that the gel bed is divided into eleven separate channels 23_1 and 23_2, each 14 cm long, 2 mm deep and either 1.0 or 0.6 cm wide, respectively. The slots leading from the gel bed through the legs are also separate.

Three types of cover **14** are used with version A (FIG. **1**). Type 14_3 is employed only from two-

dimensional runs, described below. Types **14** and **14_2** are used for both analytical and preparative runs, and are identical except that the samples are placed near one end of the gel with **14_2** and in the center of the gel with **14.** For analytical runs, a template carrier **24_1** is fixed in the carrier slot **25.** Template carrier **24_1** contains 14 template slots **26,** each of 6 × 2 mm. The templates **20** made of, e.g., polyethylene or teflon, have a rectangular cross section of 6 × 2 mm to fit the slots **26.** The last protruding 2 mm of template **20,** i.e., the part that enters the gel, is shaped in rectangles of different size, 2, 3, 4 or 6 mm long and either 1 mm wide (at one end of the template) or 2 mm wide (at the other end). For a gel 2 mm thick, the corresponding sample volumes range from 4 to 24 μl. Fourteen samples of defined volume can thus be run in a single gel. Another template **20_4,** with two protrusions at either end, forms two sample holes, each 2 × 2 mm allowing up to 28 samples to be run simultaneously. Identical or different templates may be used. Unused template slots are plugged with a 6 × 2 mm template inserted so that its flat end is flush with the under surface of the cover and does not enter the gel. For perparative runs, the template carrier **24_1** is replaced by a single perspex template **24_2** defining a sample bed 2 mm wide and 12 cm long, accommodating a sample of 0.5 ml in a gel of 2 mm thickness. In some cases it is feasible to increase the template width to as much as 6 mm, raising the sample volume to 1.5 ml.

The covers **14** of instrument B, shown in FIG. **2,** are identical with covers **14** and **14_2** of A, except that the template slots are cut directly into the cover **14.**

In both A and B, number **30** from 1 to 14 and from 1 to 11, respectively, are engraved into the underside of the cover and become embossed on the gels. When the gels are removed, the numbers make it easy to orient the gel properly, to identify each sample in gel slabs from instrument A, and to identify each gel strip from instrument B. This makes it possible to process (e.g., stain and destain) a number of strips together in the same vessel without confusing them. When the sample is placed in the center of the gel, the numbers at each end, oriented oppositely with respect to the center or otherwise marked to distinguish one from the other, identify the anode and cathode ends.

In two-dimensional electrophoresis, the first run is made in one channel of instrument B. (A duplicate sample can be run in a parallel channell to monitor the run.) The strip is then placed in the gel bed of instrument A parallel to the slots **16,** anywhere along the length of the gel bed, provided that it does not cover either of the slots **16.** The ends of the gel are trimmed so as to leave a small space between each end of the gel and the wall of the gel bed. Cover **14_3** (FIG. **1**) is clamped in place and the second gel is poured, embedding the first gel in it. A run is carried out and after the run, the gel is removed as described above.

When the two-dimensional pattern is suitable, it is possible to run two samples in the same gel, facilitating comparison. In the first dimension, with instrument B and cover **14_5,** one sample is placed in the regular template slot **26** with template **20_5** or **20_3.** A second template slot, and template **20_6** are provided for the same gel strip, two or three cm closer to the center of the gel. The samples may be slightly displaced from each other laterally to give, in the first dimension, two separate patterns lying side by side but displaced from each other longitudinally or else the two samples may share the same migration path. Both arrangements have given satisfactory results.

The major advantages of the instruments are their simplicity, ease of use and versatility. The various covers and templates afford a wide range of sample volumes, both for analytical and preparative purposes. No difficulties are encountered with unusually soft or hard gels. Samples may as easily be placed in the center of the gel as at the end, using the template slots **26_3** of cover **14_5,** allowing the simultaneous handling of components that migrate in opposite directions. Instrument A is suitable for the simultaneous analysis of a large number of samples in a single gel slab. With instrument B, it is possible to pour only as many gel strips as are needed for a small number of samples, to use gels of different porosities in different channels (when passing from one porosity to another, it is well to wait half an hour, to prevent the possibility of mixing of the different gels), and to run different channels for different periods of time (electrophoresis can be stopped in a single channel by interrupting the run and gouging the gel out of the leg slots of the channel to be stopped, after which the run is resumed).

Since the ends of the gel make direct contact with the electrode buffers when the plate is horizontal, there is no need for a vertical arrangement or for cloth or filter paper bridges. There are no special requirements for the electrode vessels. Ordinary plastic refrigerator storage boxes and suitably bent stainless steel rods make satisfactory vessels and electrodes.

After destaining, it is often useful to dehydrate the gel by placing it in 55–60% aqueous ethanol. This causes the gel to shrink to about a quarter of its original size within about a day, with one change of solvent, sharpening and intensifying the stained zones and making the gel stiffer and more resistant to tearing. At higher ethanol concentrations, the gel also becomes opaque and white, increasing the contrast between stain and background. The ethanol concentration required for this varies with the nature of the gel and must be found empirically. For gels of acrylamide and bisacrylamide concentrations of 7.5% and 0.2% or 20 and 0.2%, 75 – 80% ethanol has been satisfactory. Gels of 18 and 0.5% have been damaged by this treatment but have responded well to 65 – 70% ethanol. Dehydrated gels may be removed from the ethanol bath, air-dried, and stored for long periods without special precautions. During drying, the corners of the gel should be weighted down to prevent curling. Alternatively, if excess moisture is removed from one side of the gel with an air stream, the partially dried side adheres to cardboard and the gel dries flat and remains mounted on the cardboard. Dehydrated gels may be rehydrated in 7% acetic acid, where they regain their original size and transparency within an hour or two.

The optimal conditions for a gel-buffer system must be found empirically. If migration is faster in the middle of the gel than at the sides, the effect can be reduced or eliminated by lowering the temperature (doing the run in a cold room instead of the laboratory) and by reducing heat production by lowering the voltage (the duration of the run is increased accordingly). It seems best to regulate the voltage rather than the current. Particularly in second-dimensional runs, where no equilibrating pre-run is possible, the current may drop considerably during the run. At constant voltage, this

does not significantly affect migration and progressively reduces heat production. If the current were kept constant, the voltage would rise, increasing heat production and also increasing the migration rate in a way not always fully controlled. Since there may be an appreciable voltage drop within the buffer vessels, the voltage should be adjusted with an external voltmeter whose electrodes are placed close to the ends of the gel.

We claim:

1. A device for horizontal flat plate electrophoresis comprising in combination,

a horizontal plate having an upper and a lower surface and surrounded by lateral walls, the upper surface of the plate and the lateral walls defining a rectangular gel bed;

a step of predetermined height in each of the lateral walls thereby defining a ledge;

a cover adapted to rest on the ledge and thereby define the thickness of the gel bed;

a plurality of holes in the cover for the insertion of templates during polymerization of the gel to define predetermined holes of predetermined volume in the gel and for insertion of samples of predetermined volume in the holes;

two legs attached to the lower surface of the plate at opposing sides thereof and coextensive with the sides to which the legs are attached; and

a slot through the plate positioned over each leg, extending for the full length of the leg, and extending through the entire height of the leg.

2. The device of claim **1** wherein the horizontal plate defines a square gel bed.

3. The device of claim **2** wherein the gel bed is divided into a plurality of separate channels, defining individual gel strips, separate from each other.

4. The device of claim **2** wherein the holes in the cover for the insertion of templates are located near one of the edges of the cover and about the middle of the cover.

5. The device of claim **2** further comprising templates of varying predetermined volume so as to define in the gel bed space for predetermined vaying samples of predetermined varying volume.

6. The device of claim **2** wherein the gel bed is divided into a plurality of separate channels, defining individual gel strips, separate from each other, and wherein two of the holes in the cover are located above the same channel, one of which is positioned near one of the slots and the other hole is offset toward the center of the cover.

7. The device of claim **6** further comprising templates in each of the two holes, each of the templates being of a different shape.

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