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DISC ELECTROPHORESIS

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* *trademark*

These 1½″ long cylindrical columns of specially processed polyacrylamide gel resolve 20-30 proteins per run with human serum. Other large-molecular-weight ionic substances can also be separated. Developed over the last three years in the Cell Research Laboratory of Mt. Sinai Hospital in New York City, this powerful technique* opens new frontiers in protein research. Detailed technical data will soon appear in a leading scientific journal. That report will show how and why this remarkable medium, and the special way it is used:

—is *100 times more sensitive* than the routine starch gel technique (resolves .01 microgram of protein directly, even from large volumes of dilute protein solutions)

—is *more reproducible* than either paper or starch

—*does not react* with colorimetric reagents for protein, polysaccharides, lipids, or enzymes

—is *transparent* (especially important for high-sensitivity photometric studies)

—can be *adjusted* for preferential separation of molecules larger or smaller than typical proteins

—is *fast*: typically ½ hour vs 1 to 12 hours for paper or starch

—handles *many specimens* in minimum space

—is *simple* to understand and *easy* to operate

CANALCO has been exclusively licensed to produce instrumentation for this important new process. This folder briefly describes the technique and the equipment.

*patents applied for

THE TECHNIQUE

Key to the success of this technique is the advance preparation of the polyacrylamide gel, and the method of loading it in three stages into open-ended cylindrical glass tubes 5 mm i.d. x 70 mm long. First, the base of the tube is stoppered and it is half filled with a "hard gel" compound, which then polymerizes over 20 minutes. A shallow layer of "soft gel" compound is placed atop the hard gel and photopolymerized (15 minutes). Finally, a third gel mixture, which contains the specimen, is placed into the tube as a top layer and photopolymerized for a few minutes. Next, the top end of the glass tube is inserted from below into a receptacle in the bottom of a buffer tank.

The de-stoppered bottom of the tube is then lowered into a second buffer tank, and voltage is applied for approximately ½ hour. Since you can actually watch the "front" moving, you can check the timing of your run by the distance of migration. At the end of a run, the upper buffer tank is raised and the glass tubes are removed.

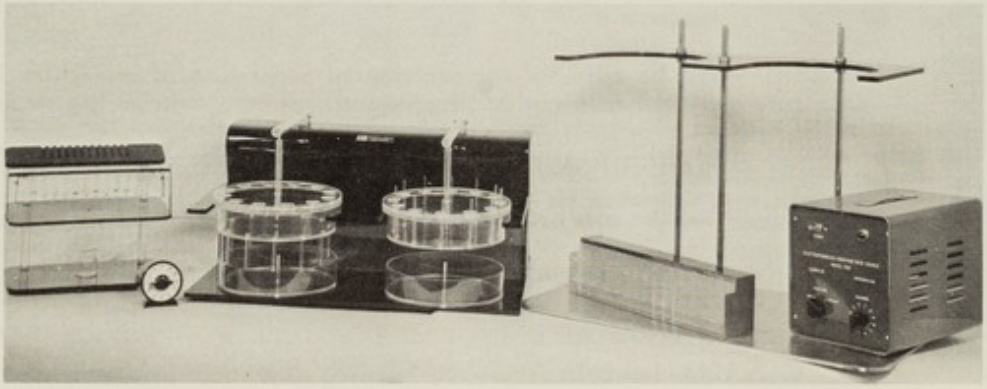
The gel column inside the glass tube is now removed from its tube and is dropped into a loose fitting glass holding tube for staining and destaining. Fixative and dye are pipetted into this holding tube. After the dye is taken up, all excess liquid is dumped and the gel column, still in its holder, is inserted into a destaining tank. Here it is electrophoretically cleared of all unbound dye in a few minutes.

The column may now be placed in a storage tube for visual examination and file, or else placed in the sample holder of the Microdensitometer to be photographed and/or scanned photometrically in order to produce a chart trace of the relative optical density and spacing of the various bands of protein.

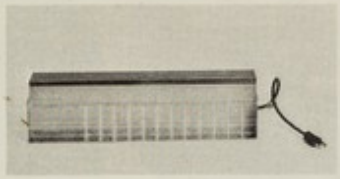
> The technique is called "Disc Electrophoresis" because the different proteins separate out in fine layers 1/10 mm or more thick which, in the column, resemble a stack of flat discs. The fatter discs can be physically separated by slicing if one desires, but most discs are too thin to slice with ease. Use of this technique for preparative purposes awaits development of larger columns handling larger amounts of protein.

THE EQUIPMENT

The basic unit has been designed to handle loading, polymerization, electrophoresis, staining, and destaining of twelve gel column tubes at a time, of the 5 x 70 mm size, each requiring typically a serum specimen of 3 microliter volume. The equipment is engineered to minimize operator motions and make it unnecessary to touch the gel columns or any chemicals with the fingers.



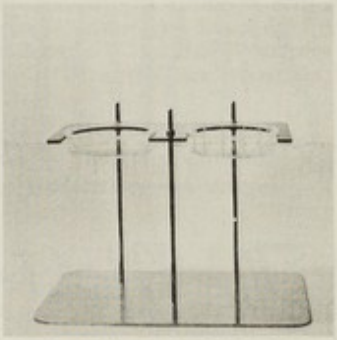
(1) The Loading and Polymerizing Rack



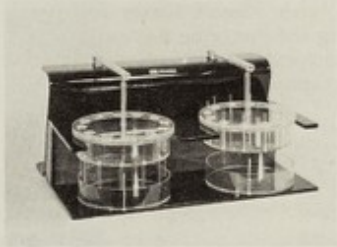
Twelve stoppered tubes are placed here for initial loading and chemical polymerization of the "hard gel", and subsequently for loading and photopolymerizing the "soft gel" and the sample-containing gel. A built-in fluorescent lamp provides energy for photopolymerization.

(2) The Holding Rack

The electrophoresis buffer tank and the destaining tank are placed here for convenient insertion and removal of the tubes. (Shown with electrophoresis tank fully loaded with tubes, and the destaining tank without any tubes but with all hole plugs in place. Tube is inserted with hole plug in place, then hole plug is removed. System runs equally well with one or more tubes inserted.



(3) The Central Bath Assembly



Shown on left with top buffer tank open (electrode raised and destaining tank closed (electrode lowered). All metals or liquids carrying an electric charge are physically shielded from human contact, or safety-switched so that current is off when charged objects are exposed.

(4) The Staining-Destaining Rack

Accepts up to twelve glass holding tubes into which the gel columns fit loosely (rack shown partially filled). After columns are stained, the rack is rotated around its long axis, so that the excess dye and fixative liquids are dumped into the base, whence they drain out through a center hole into a receptacle below. The glass holding tube is then inserted manually into the bottom of the destaining tank pictured in paragraph 3 above. Individual tube receptacles for both the Staining-Destaining Rack and the buffer and destaining tanks are numbered, so that specimens can be kept track of throughout the procedure.

(5) The Constant-Rate Power Sources

Model 300

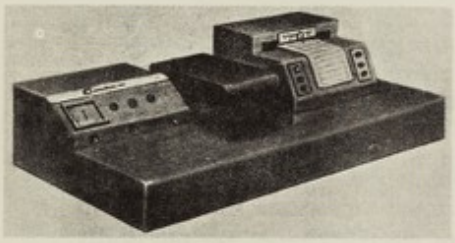
Two supplies are available, the *Model 300* which makes up to 300 V available to put 2½ milliamps of current consistently through each gel column during electrophoresis, and 12½ milliamps during destaining.

This unit is adequate for most clinical and many research applications. For more versatile use of the equipment, the *Model 1400* is offered. This unit provides the same basic 2½ and 12½ ma per column, plus smoothly adjustable current up to 150 ma in one column if desired, with 1400 volts available for high-speed separations. Both power supplies are current stabilized to plus/minus 2%. Both have a selector switch for "Electrophoresis" and "Destain", which routes the current to the appropriate pair of electrodes and adjusts it to the appropriate ampere level.

Model 1400

The Model 1400 Constant-Rate Power Source is useful also with paper and other types of electrophoresis of non-CANALCO origin, where higher current values or better current regulations may be desired.

(6) The Model E Microdensitometer



This unit has been especially designed to accept the cylindrical gel column, pass a beam of light through it in such a way as to project a true flat image of the column upon a screen, along which an apertured photocell is scanned by motor drive. The varying electrical signal created by the photocell as it passes over the light and dark bands of the image is recorded on the Y axis of a strip-chart recorder. An optical train, with several lenses and mirrors provides a means of focusing the image on a ground-glass viewing screen which is parfocal to the photocell aperture and to the 4 x 5″ photographic film. Dimensions 24″ wide x 16″ deep x 10″ high.

★ Accessory Kit

a. Filling droppers
b. Special hypodermic syringe for mixing serum and soft gel.
c. One gross storage bottles, with loading rack.
d. Column plugs.
e. Degassing syringe.
f. Column removing wire.
g. Rinse bottle.
h. Timer.
i. Tank holding fork.

★ Chemical Kit

a. Hard gel solution #1
b. Hard gel solution #2
c. Hard gel solution #3
d. Soft gel solution #1
e. Soft gel solution #2
f. Soft gel solution #3
g. Buffer mixture.
h. Specimen stain.
i. Tracking dye.

Note: *Techniques and machinery are now being developed to mass-produce the glass tubes, factory-loaded with "hard gel" and polymerized, to be packaged and shipped in hundred lots to the user. This will save user time and will help to standardize results between laboratories by eliminating the effect of differences in polymerizing and handling techniques and storage conditions. Reproducibility of results from this chemical system are excellent when the gel is handled in a consistent manner. Availability of the prepackaged gel columns will be announced when ready. Until then, pre-mixed chemical solutions and powders are available in suitable containers, both for original installation and resupply.*

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