

Some Improvements in Electrophoresis

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The introduction of the electrophoresis apparatus of Tiselius^{1, 2, 3} made available for the first time means for investigating in a convenient manner and with a reasonable accuracy the electrophoretic properties of proteins, enzymes and similar high molecular substances carrying an electric charge. Using this method it is possible not only to follow the migration of the substances in question without interrupting the experiment, but also to obtain rather accurate information about the composition of a mixture of high molecular electrolytes in terms of the electrophoretic properties of its components.

After its introduction the apparatus has found widespread use and some improvements have been described. Originally Toepler's schlieren method or Lamm's scale method were used for observing the migrating boundaries, *cf.* Tiselius and Kabat.⁴ Later two methods for directly recording the refraction gradient as a function of the position in the electrophoresis cell were published, *i. e.* the schlieren scanning method described by Longworth,⁵ *cf.* Longworth, Shedlovsky and MacInnes,⁶ and the modified Philpot method described by Svensson.^{7, 8} Recently Astrup and Helm⁹ have introduced a mirror method in the electrophoresis technic, thereby avoiding the very costly corrected schlieren lens. It is the purpose of this paper to describe some of our experiences with this method and further improvements of the apparatus.

THE OPTICAL ARRANGEMENT

The optical arrangement is shown in fig. 1. The camera arrangement (not shown in the figure) is the commonly used method according to Svensson (*l. c.*) including an objective (Hugo Meyer & Co., Görlitz, Aristoplanat 1:77,

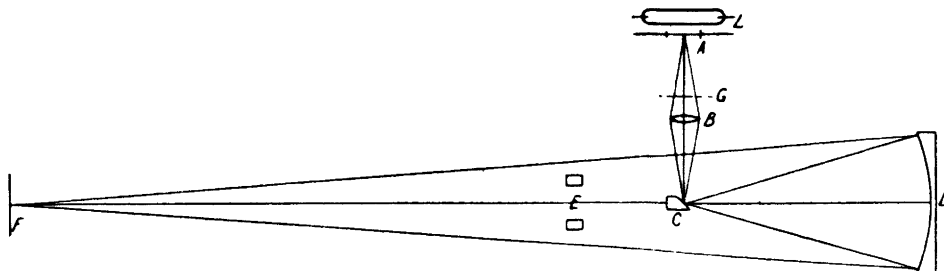


Fig. 1. Optical arrangement of the mirror method for observing the migrating boundaries. A horizontal slit (0.3×20 mm); B Voigtländer Anastigmat 1 : 2.5, $f = 5$ cm; C 90° reflecting mirror (aluminized); D aluminized elliptical mirror, diameter 168 mm, eccentricity 3.6; E electrophoresis cell; F inclined slit; L light source.

$f = 45$ cm) and a cylindrical lens (a cylindrical spectacle-lens of dioptry + 5 and 0).

As light source is used a Philips »Philora» Hg-lamp (H P 500). The high pressure tube is removed from the bulb and placed horizontally immediately behind the horizontal slit (distance from slit to axis of tube is ~ 10 mm). In use the tube is red hot and after burning for about 10 minutes the line of maximum light intensity lies a little over the center of the tube; this is to be kept in mind, when the tube is mounted. The horizontal part of the light arc is about 20 mm, with a diameter sufficient to give maximal light intensity during the slit within an angle of 12° — just what is needed. Under these conditions no condenser lens is needed for filling the elliptical mirror with light.

The yellow Hg lines may be used (Zeiss Monochromat Filter A), but the green line gives shorter times of exposure. This line is isolated by combining two filters from Schott & Gen., Jena, namely GG 14 and BG 20. Agfa »Isochrom» plates may be used, but Perutz »Silber-eosin» are better. A hard developer is used.

The objective B forms a diminished image in front of the reflecting mirror C. The size of this mirror allows it to be placed in the optical axis throwing no shadow on the two electrophoresis cells. The front side of the objective is turned against the slit.

The mirror D has ellipsoidal curvature with distances 622 mm and 2.250 mm to the foci, and forms an enlarged image of the horizontal slit on the inclined slit F.

A simple construction of the inclined slit is shown in fig. 2. It may be turned at different angles (α) with the vertical and $\tan. \alpha$ is marked on the

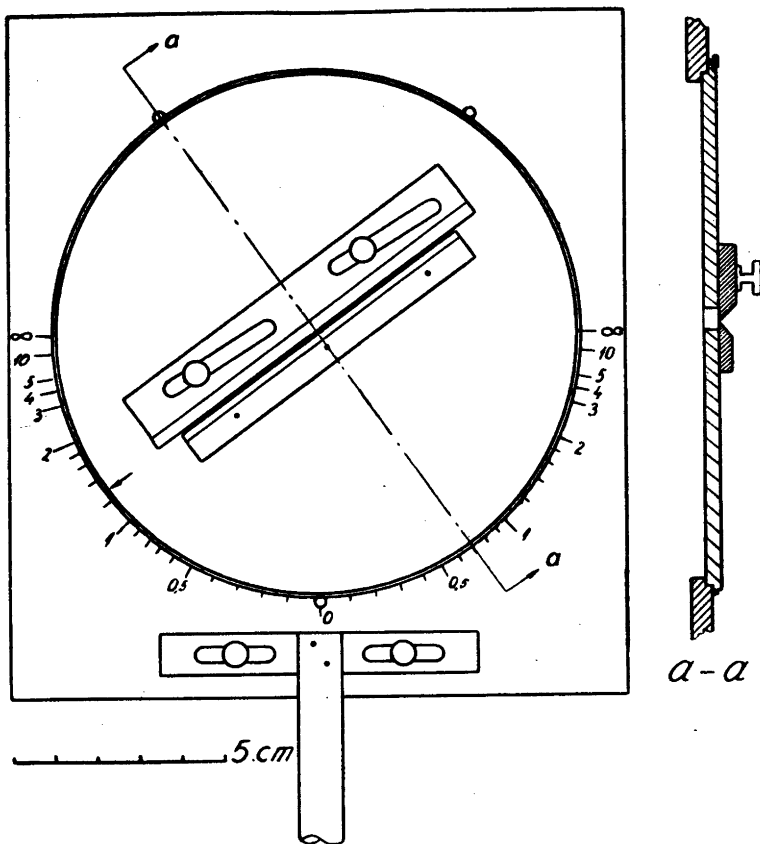


Fig. 2. Construction of the inclined slit.

slit. The areas of the maxima obtained on the photographic plate are proportional to $\tan. \alpha$, and it is thus easy to calculate the exposure times, when the angle is changed. The inclined slit may also be moved until 30 mm in horizontal direction, thus allowing several exposures on the same plate to be made.

The most important advantage of the mirror method over the usual method is that the elliptical mirror is far cheaper than a corrected lens of similar dimensions. A disadvantage is, that the arrangement is more susceptible to shaking. It may be mentioned that a mirror method also has been described for use in the ultracentrifuge.¹⁰

The waterbath containing the electrophoresis apparatus introduces some errors. The image is formed at a distance of, in our case, about 10 cm further

away from the cells, at which point the inclined slit must be placed. At the same time it becomes unsharp as pencils of light originating from the periphery of the elliptical mirror do not meet at the same points as pencils coming from the center of the mirror. This error may according to our experience be corrected by placing the inclined slit at some distance from the focus in the direction of the water bath, and the vertical slit at a larger distance from the objective B.

The water in the water bath must be kept clear, which is obtained by circulating the water through granulated charcoal (by means of a mammoth pump) at a speed of about 100 liters per hour. The charcoal is changed once a month.

THE ELECTROPHORESIS APPARATUS

In the original apparatus of Tiselius the electrode vessels have a comparative large surface area. Very small movements in vertical direction, as for instance produced by walking on the floor close to the apparatus, will suffice completely to destroy the experiment by liquid passing from one electrode vessel to the other through the cells. This is due to the small cross section of the cells in comparison with the surface area of the liquid in the electrode vessels. Tiselius proposed to decrease this surface area by placing an ebonite rod or stopper in the mouth of the vessels. In our experience we have had much trouble on this point, and we therefore prefer electrode vessels of the closed type described by Longworth and MacInnes¹¹ as later used also by Svensson.¹² These vessels also permit the emptying and filling of the apparatus to be carried out without removing it from the water bath.

As the correct dimensions are very important the construction of the apparatus is shown in fig. 3. The electrode vessels are made of »Duran» or »Pyrex» glass and the electrode consists of 50 g of 1 mm silver wire.

We always perform the filling of the apparatus, when it is placed in the water bath. When this filling is carried out at room temperature and the apparatus then placed in the water bath at about 0° the contraction of the solution in the lower closed compartment of the cells may allow liquid to pass in from the surrounding water bath, thus disturbing the experiment. After being placed in the water bath the lower compartment of the cells are filled with the solution under investigation (cooled in the water bath) and closed, whereupon the rest of the apparatus is filled with buffer. Buffer solution is sucked up to above the stopcock in the electrode tube, and 50 ml of saturated NaCl solution is filled in the funnels. The ground glass stoppers over the cells are placed in position and the salt solution in the funnels cautiously poured

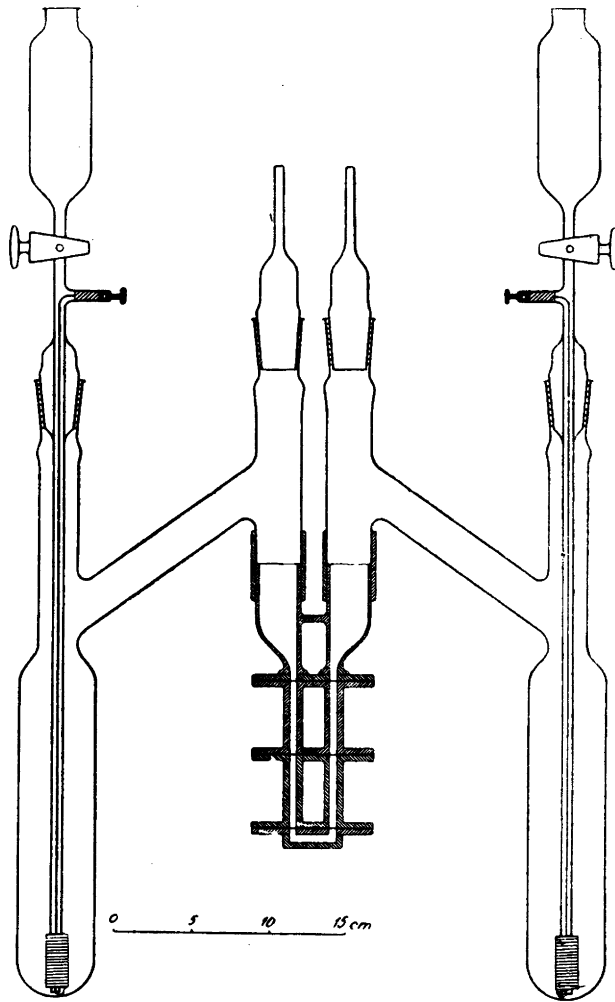


Fig. 3. Design of the electrode vessels.

down over the electrode, whereby the glass stoppers are filled and closed, *e. g.* by means of glass stopcocks (not shown).

In this manner it is possible to prepare for a new experiment in 8—10 minutes, and to run about 15 experiments without removing the apparatus for greasing of the cells. This relates to qualitative experiments, for the quantitative determination of the migration velocity greasing must be carried out more often in order to secure complete insulation of the cells from the water bath.

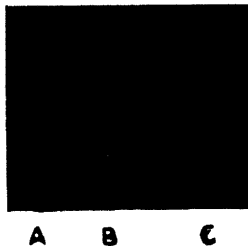


Fig. 4. A: 0.3 % chondroitin polysulfuric acid (K-74) after 7 minutes electrophoresis, $\tan. \alpha = 0.5$ (T-251).
 B: 0.3 % chondroitin sulfuric acid after 7 minutes, $\tan. \alpha = 0.5$ (T-252).
 C: A solution containing 0.2 % K-74 and 0.2 % chondroitin sulfuric acid after 15 minutes, $\tan. \alpha = 0.5$ (T-265).

The apparatus has been used for orientating investigations of various kinds, thus in a study of polysaccharide sulfuric acids where it has been found very convenient.

The cellulose-, chitin- and starch polysulfuric acids, prepared according to Astrup, Galsmar and Volkert¹³ were investigated. These substances are all highly charged colloidal electrolytes with a high mobility; they were used as sodium salts and 15–45 mg were dissolved in 15 ml of phosphate buffer of pH = 6.7 and ionic strength 0.1. The electrophoresis were carried out with 0.018 ampere during 7–15 minutes. Examples are shown in the paper mentioned. In this manner it is easy to get information about the contents of high molecular electrolytes in the preparation. It was expected, that also the purity of the preparation could be determined in this way, but it was not found possible to differentiate between the cellulose sulfuric acid ester *insoluble* in concentrated salt solutions and the *soluble* ester prepared according to Astrup and Piper,¹⁴ although the soluble substance yields less viscous solutions and inhibits the clotting of blood to a lesser degree than the insoluble substance does. This probably may be the case if the charge per unit of cellulose is the same, while the number of units per molecule is smaller for the soluble substances. Still more unexpected it was, that also a mixture of chondroitin sulfuric acid with a chondroitin polysulfuric acid (active as inhibitor for the blood-clotting) (K-74) did not separate during the electrophoresis (fig. 4).

These results mean that in some cases the results of electrophoresis experiments must be judged with caution. Probably experiments under more varied conditions would yield the information wanted. Compare similar results in electrophoresis of insulin preparations by Hall.¹⁵ Also the boundary anomalies studied by Svensson¹⁶ interfere with the results.

Information about the details of electrophoresis investigations may be found in the comprehensive papers of Longsworth^{17, 18} and Svensson.¹⁹

SUMMARY

1. A method using an elliptical mirror in the optical arrangement of the Tiselius electrophoresis apparatus is described.
2. Electrode vessels of the closed type allowing rapid handling of the apparatus are designed.
3. Experiences in the use of the electrophoresis apparatus are mentioned.

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