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Zone Electrophoresis in a Glass Powder Column *

Preliminary Report

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Stabilization by gels and powders has been much used in earlier electrophoresis studies with the moving boundary method. Electro osmosis, however, was found to make accurate mobility measurements impossible. Later on the method was applied again for preparative purposes. Kendall and Crittenden [1] used electrophoresis in a capillary system with the aim to separate isotopes. More recently Brewer, Madorsky and Westhaver [2] enriched one of the potassium isotopes electrophoretically in a packing of glass wool, cotton, glass beads *etc.* The method has also been applied to biochemical problems. Cooledge [3] separated albumin and globulin in a glass wool column, Consden, Gordon and Martin [4] made separations of amino acids and peptides in silica jelly, and later Gordon, Keil and Sebesta [5] reported similar effects with proteins in agar jelly. Butler and Stephen [6] studied the electrophoresis of amino acids in a horizontal asbestos column. Electrophoresis on filter paper has lately been described by several authors, see for example Cremer and Tiselius [7]. Recently Svensson and Brattsten [8] described an apparatus for complete and continous electrophoretic separation, with glass powder as the stabilizing principle. The apparatus described here has also glass powder as stabilizing agent, but is intended to be an analytical instrument.

The principle of the apparatus is shown in Fig. 1. The center glass tube is loaded with the capillary system, glass powder, and connected with the electrode tubes. The system is filled with the buffer in question and a "zone" of the solution to be investigated is made up in the glass powder column.

* It seems practical to distinguish between "boundary electrophoresis" as performed in the common moving boundary apparatus, and "zone electrophoresis" where the migration of more or less completely separated zones is studied, usually by application of some immobilizing medium to prevent convection.

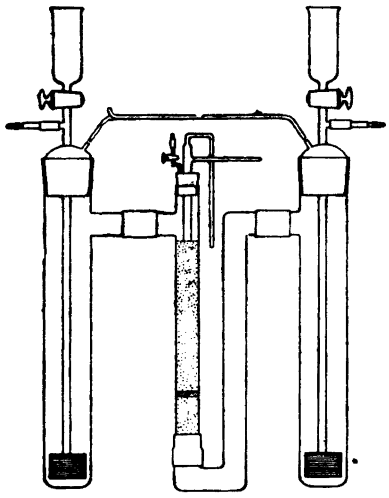

Fig. 1. The principle of the apparatus. The two horizontal tubes which makes it possible for the electro-osmotic flow to develop freely.

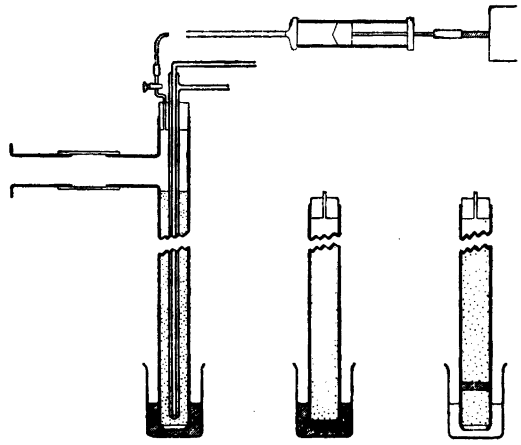

Fig. 2. Filling the apparatus. The central cooling tube is depicted in the left drawing.

When a potential is applied between the electrodes, charged molecules and particles migrate in the electrical field in the tube. If more than one electrophoretic component is present in the original zone, this will split up during the migration into a number of zones corresponding to the number of components.

An essential part of the apparatus is of course the capillary system. The ideal system should have, a low zeta-potential, low adsorptive power and a uniform structure. Those properties of a capillary system suitable in electrophoresis are discussed by Svensson and Brattsten [8]. The present apparatus uses almost the same capillary system as that described by Svensson and Brattsten. However, since the present system requires very uniformly sized particles, the glass powder has been fractionated extensively. Some preliminary attempts to get a glass powder with spherical particles have been carried out.

The electro-osmotic flow is usually a disturbing factor if a closed system is used. However, if the system is open and the glass powder has sufficient porosity to allow the electro-osmosis to develop without convection, the phenomenon has no influence on the electrophoretic separation and practically none on the sharpness of the zone boundaries. This "open" system can be established by the introduction of two horizontal tubes (in the same level)

each connected with the upper tube on the electrode joint (Fig. 1). The buffer in the vessels ends with a meniscus in the horizontal tubes respectively (the diameters must be small enough). Now an electro-osmotic flow may occur without altering the hydrostatic properties, since the two menisci move perpendicular to the gravitational field. This movement is easily measured and then also the direction and extent of the electro-osmotic flow.

For a sharp separation the temperature gradient in the tube with the capillary system during the experiment is of great importance. Temperature differences arising from the current between the peripherial and the central parts of the tube, make the zones parabolic in shape because of the great mobility temperature coefficient. In order to decrease these differences a cooling tube is placed in the center of the tube (Fig. 2). The thermostat water is forced through the cooling tube and back into the thermostat. It is not necessary to work at the density maximum of water to secure stability of the zones in this apparatus.

The analytical power of the apparatus is adversely influenced by the unsharpening effects at the zone boundaries (diffusion *etc.*) and by the height of the zone at start. In the latter case this is dependent upon the amount of material necessary for chemical analysis after the separation. Most of the experiments have been carried out with the solution under investigation in osmotic equilibrium with the buffer (dialysis). In some cases a special technique has been used to sharpen the zone boundaries. By continuous dilution of a buffer brought into the tube with the glass powder by the sucker-system (Fig. 2), a conductivity gradient is formed in the electrophoresis tube. When a zone migrates in this gradient, the lower parts of the zone move in a stronger field than the upper. This effect tends to press the zone together and make the boundaries sharper.

DESCRIPTION OF THE APPARATUS

The tube with the capillary system has an inner diameter of 20 mm, length of 200 mm, and has a glass filter plate in one end. The cooling tube has an outer diameter of 8 mm. The electrophoresis tube has rubber connections on one side and on the other rubber-glass with the electrode tubes as shown in Fig. 1. The electrodes and electrode tubes are the same as used in the standard Tiselius moving-boundary apparatus.

The glass powder has to be fractionated very carefully. After boiling with nitric acid, the powder is added in portions to a vertical glass cylinder with water flowing rapidly from the bottom (through a glass filter). The width of the cylinder was 80 mm and the velocity of water flow about 50 ml/sec. The

smaller glass-particles follow the streaming water and the heavier remain in the tube. With the aid of a siphon the powder flowing above the middle in the cylinder is sucked off after ten minutes. The remaining heavy particles are removed. This operation is repeated three times for the sucked off powder. The remaining "middle-fraction" is again put in the cylinder. The flo wof water in the cylinder is now made more rapid, and the water is directed to fall down in another vertical cylinder with weak counterflow of water. Only the heaviest particles of the middle fraction can reach the bottom of this last cylinder.

This last heavy fraction is used and the fractionated glass powder is put into the tube. In order to get a better close packing the tube is vibrated during filling with a commercial "massage vibrator". The cooling tube is centered manually during the addition of the glass powder. This method of centering is somewhat inaccurate and another arrangement is desirable. During the filling of the powder the liquid must always cover the glass powder, if not, air-bubbles may arise. In order to avoid gas-bubbles in the capillary system the buffer should be boiled before use.

The filling procedure with the solution under investigation is shown in Fig. 2. The syringe driven by a syncronous motor siphons buffer from the upper end of the tube with a speed of 0.5 ml, and the solution surrounding the lower part of the tube (in a beaker) flows gently through the glass filter plate up into the glass powder. When the solution-zone has reached a height of about 3 mm, the filter is washed with buffer. Then the latter surrounds the lower part of the tube (in the beaker) and the syncronous motor is started again. When the zone has migrated to a suitable level (about 3 or 4 cm) in the tube the lower rubber connection is put on. This operation must not force buffer into the tube. To avoid this the rubber has to be either somewhat greater in diameter than the tube or thin enough to be rolled over it. In the first case a tight connection must be assured.

For the localization of the zones no suitable optical method is now available. However, a sampling technique similar to that used in chromatography (see for example Stein and Moore [9]) has proved to be of great value for this apparatus, especially when the experimental arrangements can be made simpler. The solution is pressed downwards out of the column and is collected in successive portions in a rack of sample tubes, the movement of which is synchronized with that of the solution passing through the column. In this way the separated zones are collected, and the distribution of material in the column after the separation may be determined by analysis (according to some suitable method). During the sampling one of the electrode vessels is detached. (Fig. 3).

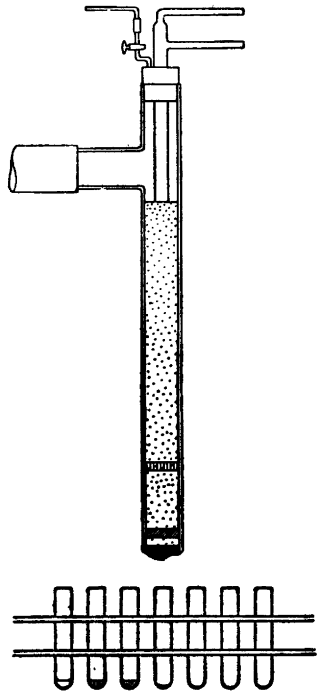

Fig. 3. Sampling after the electro-phoretic separation.

Several preliminary experiments have been carried out with the apparatus, both with organic indicators and protein pigments. A mixture of phycoerythrin and phycocyan in a phosphate buffer of ionic strength 0.025 and pH 7.6 is easily separated.

The described principle has proved applicable to the separation of some colored ions and some proteins. However, much more work is necessary to make the apparatus a good analytical instrument. The capillary system must be improved. A refined conductivity gradient technique would perhaps give still better results. A method for direct observation of the zones should be of great value.

SUMMARY

An apparatus for electrophoretic analysis is described, in which glass powder is used as a capillary system. A thin "zone" of the solution to be investigated is made up in the glass powder. This original zone will split up during the experiment in a number of zones corresponding to the number of electrophoretic components. The fractions are collected with a sampling technique similar to the one used in chromatography.

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REFERENCES

1. Kendall, J., and Crittenden, E. D. *Proc. Nat. Acad. Sciences* **9** (1923) 75.
2. Brewer, A. K., Madorsky, S. L., and Westhaver, J. W. *Science* **104** (1946) 156.
3. Coolidge, T. B. *J. Biol. Chem.* **127** (1939) 551.
4. Consden, R., Gordon, A. H., and Martin, A. J. P. *Biochem. J.* **40** (1946) 33.
5. Gordon, A. H., Keil, B., and Sebesta, K. *Nature* **164** (1949) 498.
6. Butler, J. A. V., and Stephen, J. M. L. *Nature* **160** (1947) 469.
7. For references see Cremer, H. D. and Tiselius, A. *Biochem. Z.* **320** (1950) 273.
8. Svensson, H., and Brattsten, I. *Arkiv Kemi* **1** (1950) 401.
9. Stein, W. H., and Moore, S. *J. Biol. Chem.* **176** (1948), 337.

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