ZONE ELECTROPHORESIS IN SILICA GEL AND SILICA PASTE*

FRANK F. DAVIS

Department of Agricultural Biochemistry,
Rutgers – The State University of New Jersey, New Brunswick, N.J. (U.S.A.)

(Received January 5th, 1960)

SUMMARY

Silica gel and silica paste have been used as anticonvection media in zone electrophoresis. Rapid methods for preparing gels and pastes containing the appropriate electrophoretic buffer, and their use in zone electrophoresis, are given.

INTRODUCTION

In 1946 CONSDEN, GORDON AND MARTIN[1] used freshly prepared silica gel as an anticonvection medium for the electrophoretic separation of various amino acid and peptide mixtures. A number of separations were effected, and material in the bands was readily recovered in good yield by drying and extracting the gel with water. CONSDEN AND GORDON[2] employed silica gel in the electrophoretic separation of peptides and amino acids from hydrolysates of wool. That silica gel has not been more widely used as an anticonvection medium in electrophoresis is probably due to the length of time required for the preparation of these gels, and their fragile nature. This paper reports a simple and rapid method for the preparation of silica gels containing appropriate electrophoretic buffers and their use in electrophoresis. For certain situations in which gels cannot be employed, the preparation and use of silica pastes as an anticonvection agent is given.

PROCEDURE

Electrophoresis apparatus

A modification of the apparatus described by CRESTFIELD AND ALLEN[3] is used. Plastic side and end strips coated with silicone stopcock grease on the surfaces of contact are employed to form electrophoretic troughs on the level glass plate of the apparatus 38 cm in length and 0.6 cm in thickness. The width varies from 5 to 15 cm, depending upon the particular application.

Preparation of silica sol

Silica sol is prepared by a modification of the procedure of PRAMER[4]. A solution that is 1 M in sodium metasilicate (6 % in SiO_2) and 10^{-3} M in sodium chloride is passed through a column of amberlite IR-120 in the hydrogen form. The silicic acid sol that emerges from the column has a pH of approximately 3, and will remain as

* Paper of the Journal Series, New Jersey Agricultural Experiment Station.

a sol in the refrigerator for at least two weeks. The sodium chloride is converted to hydrochloric acid, and the resultant low pH stabilizes the sol. At pH values near neutrality gelling occurs in 1 h or less at room temperature and in about one day at 4°.

Preparation of gels containing electrophoretic buffer

Electrophoretic buffers with a final ionic strength of 0.05 to 0.1 are commonly employed. One volume of silica sol is mixed with one volume of buffer that is twice the desired final concentration, and the solution is poured into the trough of the electrophoresis apparatus and allowed to gel. Gels from 2 to 6 mm in thickness have been used. The time required for gelling will depend upon the pH and ionic strength of the buffer and the temperature. A comprehensive investigation of the effects of pH, ionic strength, and temperature on the rate of gelling of silica sols has been made by PRAMER[4] whose work should be consulted for information on gelling time under various conditions. In the pH range from 5.0 to 8.5, and with an ionic strength of 0.05 to 0.1, the length of time required for the solution to gel varies froma few minutes to about 0.5 h. Gelling can be speeded by heating the solution.

Introduction of sample and electrophoresis

A slot is made in the gel for the introduction of the sample. Slots can be prepared by cutting out the gel or by incorporating a removable strip of plastic of the desired dimensions in the trough prior to casting the gel. Since the gel is rather fragile, and tends to stick to glass and plastic materials, care must be taken not to crack the gel during this operation. A method which has been found to be superior to either of the above methods is to use a thin-walled capillary tube attached to a vacuum line to aspirate material from the region where the inlay is to be made. Slots of 2 mm in width are easily made using this technique. Slots 3 to 10 mm in width and extending to within 1 cm of the sides of the slab have been used routinely. For a number of separate samples, small rectangular or circular openings can be made in the gel with the suction tube.

The pH of a liquid sample is adjusted to that of the electrophoretic buffer, and the sample is added to an equal volume of the sol. The solution is then poured into the slot, where it gels. In order to minimize syneresis and shrinking or swelling of the gel in the region of the inlay during electrophoresis, the ionic strength of the inlay should not vary markedly from that of the main body of the gel. A small amount of concentrated buffer can be added to dilute samples prior to mixing with the sol, and with samples of high ionic strength a more concentrated buffer can be employed in the main body of the gel. Solid samples, especially if they are of limited solubility, can be dissolved in sol that has been diluted with water or buffer.

Electrophoresis

The plastic strips that form the ends of the trough are removed after gel formation and filter paper pads that extend into the buffer vessels are laid on the ends of the gel. The pads are covered with a single thickness of polyethylene film up to the point where the pad contacts the gel in order to minimize evaporation from the paper. A cover plate that rests on the side strips and remains a few millimeters above the gel is placed on the apparatus, and electrophoresis is commenced. The field strength that has been employed is in the range of 10 to 20 V/cm. Generally, it is advisable

to begin the electrophoretic run at lower voltages, as heating, syneresis, swelling and shrinking of the gel in the region of the inlay are aggravated by higher voltages.

Electro-osmosis is slightly less in silica gel than in paper at pH values up to 8. Above pH 8, electro-osmosis in the gel increases rapidly, until at pH 10 it is about three times that observed in Whatman No. 1 paper. Caffeine is used as an indicator of electro-osmosis and is detected by examining the slab with a short-wave u.v. light in a darkened room. After the run is completed, substances may be located within the gel by various methods. Those that absorb u.v. light can be detected by examination of the gel with an appropriately shielded u.v. light. The gel can be sprayed or stained, or a piece of filter paper that has previously been moistened with water and partially dried can be placed in contact with the surface of the gel for a short period of time and the bands printed off onto the paper. The paper is then dried and the location of the bands determined by the appropriate technique.

The use of silica at pH values some distance from neutrality

Silicic acid sols gel at extremely slow rates at pH values less than 5 or greater than 8. Electrophoresis is carried out in silica paste in these regions. A suitable paste can be prepared by either of the following procedures.

1. An amount of sol, three times the quantity that would be used in preparing the slab of gel, is gelled in a beaker by the addition of a component of the electrophoretic buffer that is approximately neutral in pH (for example: sodium monochloroacetate, in the case of monochloroacetic acid–sodium monochloroacetate buffer of pH 2.3). The gel is stirred with a motor-driven stirrer until smooth and the remaining component of the buffer is added to give the desired final pH and ionic strength. The silica gel, now in the form of a thin paste, is centrifuged (2300 $\times$ g, 5 min) and the supernatant liquid is decanted. The thickened paste, which contains three times the SiO_2 content of the original gel, is stirred with a spatula to break up lumps and is spread in the electrophoretic trough.

2. CAB-O-SIL M-5 (Godfrey L. Cabot, Inc., Boston, Mass.), a colloidal form of silica, is stirred with complete electrophoretic buffer until a stiff paste is formed. Approx. 12 g of CAB-O-SIL are required /100 ml of buffer. This paste is then spread in the trough.

A slot is formed in the paste for introduction of the sample. The sample, in solution, is mixed with an equal volume of paste and poured into the slot. The paste in the inlay can be thickened by the addition of a small amount of CAB-O-SIL. If a wide slot is used, the sample can be made up as a thick paste by the addition of CAB-O-SIL prior to addition to the slot.

Removal of substances from the gel or paste

Nucleotides and amino acids, the substances which have been investigated in this work, are easily removed from the silica by any of three methods. The gel may be dried in air or in an oven and the nucleotide or amino acid extracted with a small amount of water. The gel and paste can be lyophilized and the material extracted, a procedure that should be useful when working with labile substances. Material can be recovered in a more dilute form by homogenizing the undried gel or paste with a few volumes of water and centrifuging the solid residue.

Comparison of gel and paste

The silica gel is more transparant than the paste, is free of air-bubbles and is more uniform in texture. It is easily cracked and cannot be repaired if broken. The smoothness and clarity of the gel permit bands to be easily detected by scanning or other techniques. The paste is less brittle than the gel and if cracking or other accidents occur, they can be repaired by working the paste with a spatula.

For large scale separations with silica paste, it is advantageous to place the top lid in contact with the paste. The trough is completely filled with paste and a glass cover plate is tilted from the side onto the surface of the paste. The plate is removed after the run is completed by sliding it sideways off the paste.

RESULTS AND DISCUSSION

Fig. 1. (left) shows a large-scale separation in silica paste of the four major ribonucleotides. The bands were printed off onto filter paper which was then photographed using a short-wave u.v. light. Paste in the region of the bands was removed from the slab, dried in an oven at 55°, and the nucleotides extracted with water. Recoveries were as follows: cyticylic acid 81 %, adenylic acid 93 %, guanylic acid 94 %, and uridylic acid 96 %. The rather low recovery of cytidylic acid is probably due to losses in the region of the soft inlay.

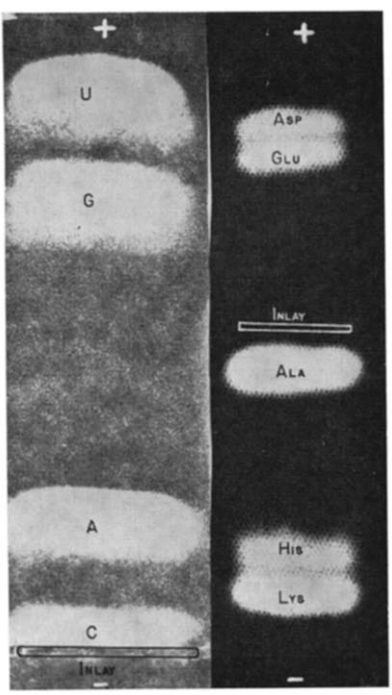

Fig. 1. Separation of ribonucleotides and amino acids by electrophoresis in silica paste and gel. Left: 20 mg each of uridylic acid (U), guanylic acid (G), adenylic acid (A) and cytidylic acid (C). Conditions: Silica paste as the anti-convection medium; 0.05 *M* formate buffer, pH 3.4; 38 × 10 × 0.6 cm slab; 12 V/cm for 4 h; glass plate in contact with the upper surface. Right: Separation of 5 mg each of aspartic acid, glutamic acid, histidine and lysine, and 7.5 mg of alanine in silica gel. Conditions: 0.1 *M* acetate buffer, pH 5.5; 38 × 8.5 × 0.2 cm slab; 12 V/cm for 2 h.

Fig. 1. (right) shows a separation in silica gel of a mixture of five amino acids. The bands were printed off onto filter paper and visualized with ninhydrin. In a second experiment, 5-mg quantities of aspartic acid, alanine and lysine were separated under similar conditions. A print was made and the bands were removed, dried at 55°, and the amino acids extracted. Recoveries, as determined by the ninhydrin method of MOORE AND STEIN[5], were as follows: aspartic acid 96 %, alanine 99 %, and lysine 95 %.

Electrophoresis in silica gel or paste would appear to be a useful method for the purification of small amounts of materials, owing to the speed of separation, the ease with which substances can be recovered from the silica, and the lack of contamination of the separated substances with material from the anticonvection medium. These procedures should also be adaptable to quantitative techniques, as the gel or paste do not retain substances of low molecular weight that are added to them.

GORDON *et al.*[6] report that proteins cannot migrate electrophoretically through the pores of silica gel, and these results have been confirmed in this laboratory with ribonuclease, cytochrome *c*, serum albumin, and hemoglobin, all of which remain at the site of application in the gel or paste. However, the ribonucleic acid from yeast that is soluble in 1 *M* sodium chloride and which has been shown to be of about 9 nucleotides in length[7] does migrate in the gel without restriction, as compared to its mobility on paper. If, as suggested by GORDON *et al.*[6], proteins are unable to migrate in silica gel due to their large size, it would appear that in the molecular weight region somewhat above that of the soluble RNA (about 3,000) electrophoretic fractionations might be achieved by molecular sieve effects in which the size and shape of a molecule as well as the charge density would dictate its rate of movement in the gel. Such sieving effects have been noted in the migration of certain proteins in starch gels of high concentration[8].

ACKNOWLEDGEMENTS

This work has been supported by research grants from the National Institutes of Health, United States Public Health Service, the National Science Foundation, and by an Amalie Muller Memorial Grant for Cancer Research from the American Cancer Society.

REFERENCES

1 R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *Biochem. J.*, 40 (1946) 33.
2 R. CONSDEN AND A. H. GORDON, *Biochem. J.*, 40 (1950) 8.
3 A. M. CRESTFIELD AND F. W. ALLEN, *Anal. Chem.*, 27 (1955) 422.
4 D. PRAMER, *Appl. Microbiol.*, 5 (1957) 392.
5 S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 211 (1954) 907.
6 A. H. GORDON, B. KEIL, K. SEBESTA, O. KNESSL AND F. ŠORM, *Coll. trav. chim. tchécoslov.*, 15 (1950) 1.
7 F. F. DAVIS, A. F. CARLUCCI AND I. F. ROUBEIN, *J. Biol. Chem.*, 234 (1959) 1525.
8 H. G. KUNKEL AND R. TRAUTMAN, in M. BIER, *Electrophoresis*, Academic Press, New York, 1959, p. 236.