A RAPID METHOD FOR ISO-ELECTRIC POINT DETERMINATION

by

JOHN F. LARGIER

South African Council for Scientific and Industrial Research and the University of Cape Town Virus Research Unit, Department of Pathology; and Department of Chemistry, University of Cape Town (South Africa)

An important physical chemical property of a protein is its iso-electric point. Especially in multi-membrane electrodecantation[1] the accurate knowledge of iso-electric points is essential for the successful fractionation of proteins. It is also desirable that the determination of iso-electric points is based on electrophoretic mobility, since this property is used in defining the iso-electric point.

Recently POLSON[2] has described a cell in which the descending electrophoretic patterns of 4 samples can be observed simultaneously, and this cell is conventionally used to analyse different samples under the same conditions of pH, *etc.* The object of the work reported in this paper was to investigate the possibility of operating the apparatus with the same protein buffered at different pH's in each compartment. From the results it would be possible to extrapolate to zero electrophoretic mobility and thus obtain the iso-electric point both rapidly and accurately.

EXPERIMENTAL

The apparatus (Fig.1) is essentially the same as that described by POLSON[2]. The two electrode vessels communicate via two perspex adaptors with the four-compartment electrophoretic cell, a cellophane membrane being inserted between the lower adaptor and the bottom of the cell, and a short glass levelling section separating the cell from the upper adaptor. The only modification is a perspex slide between the upper adaptor and the levelling section. This slide has four rectangular holes corresponding to the four short compartments of the levelling section, which can then be isolated from the upper adaptor.

In the operation of the apparatus the lower adaptor and one electrode vessel are filled with relatively concentrated potassium chloride solution (1.5%), while the upper adaptor and the other electrode vessel contain dilute potassium chloride solution (0.15%). The four compartments of the electrophoresis cell are filled with the protein solutions, previously dialysed against buffer solutions of different pH and constant ionic strength, and the four compartments of the levelling section are filled with the corresponding buffer solutions. The concentrated potassium chloride solution is to prevent osmosis causing convection in the protein solutions, and the dilute potassium chloride solution is to provide sufficient density difference to establish a boundary between it and the buffer solutions. The pH's of the buffer solutions should all be on the same side of the

References p. 345.

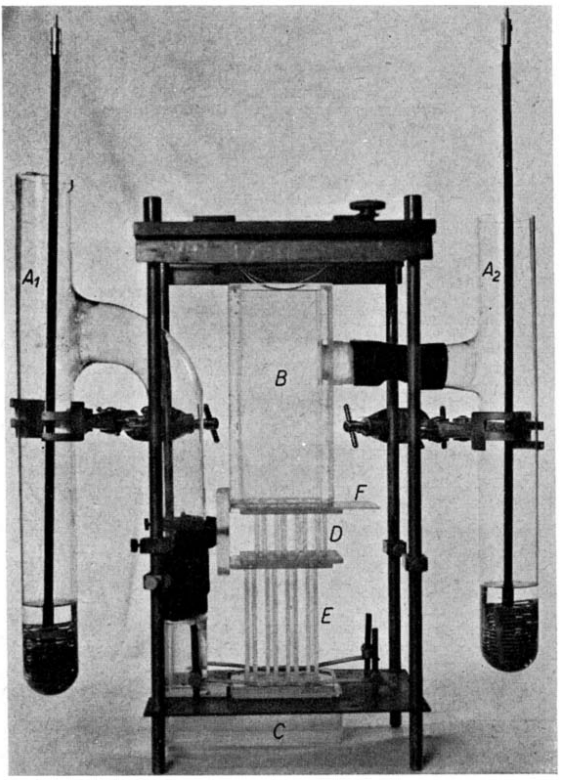

Fig. 1. Assembled four-compartment electrophoresis cell. A_1 and A_2 are the electrode vessels; B and C the upper and lower perspex adaptors; D the four-compartment levelling section; E the four-compartment analytical section, and F the perspex slide. The guides for the levelling section have been omitted.

iso-electric point since an ascending boundary could cause convection. The levelling section and the slide are moved so as to establish protein-buffer and buffer-potassium chloride solution boundaries respectively. The protein-buffer boundaries are lowered into suitable positions for observation and photography by means of a fine pipette with a bent tip[3], care being taken to remove as little of the buffer solution as possible. Using the Schlieren optical system initial photographs of the boundaries are taken before switching on the current. Photographs of all boundaries are taken at regular intervals (say 10 minutes).

The correction for electro-endosmosis is determined by substituting dextran for the protein and carrying out the electrophoresis under identical conditions. The distances of migration of the protein boundaries, at each time-interval, are measured on the photographic plate by means of a micro-comparator and corrected for electro-endosmotic flow. These are plotted against pH and extrapolated to zero migration in order to obtain the iso-electric point.

The systems used to investigate the practicability of this method were rabbit serum albumin (RSA) in sodium phosphate/citric acid buffer and in acetate buffer, as well as human serum albumin (HSA) in acetate buffer. Both proteins were prepared by multi-membrane electrodecantation.

RESULTS

Sodium phosphate/citric acid buffer

Numerous experiments were carried out in this buffer system with RSA. The pH's of the buffer solutions were usually within 1 or 1.5 units of the iso-electric point, since the relationship between pH and the distance of migration is linear in the immediate vicinity of the iso-electric point. The experiments with dextran showed that the electro-endosmotic flow is independent of pH over the range involved and was directly proportional to time (Fig. 2).

The results of a typical determination are recorded and extrapolated to zero mobility in Fig. 3. All the buffers used were prepared from 0.2 molar Na_2HPO_4 and 0.1 molar

References p. 345.

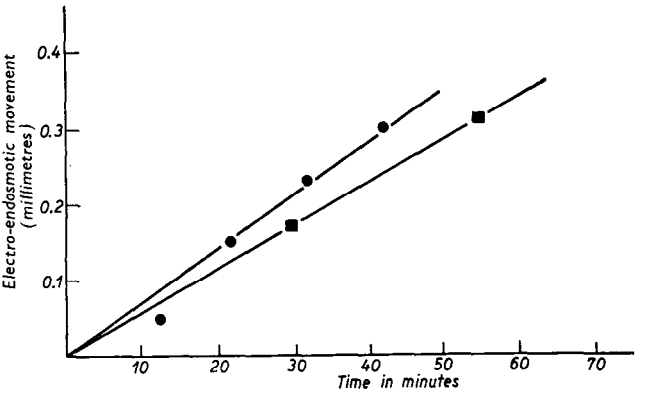

Fig. 2. Correction for electro-endosmotic flow. Squares refer to sodium phosphate/citric acid buffer and dots to acetate buffer.

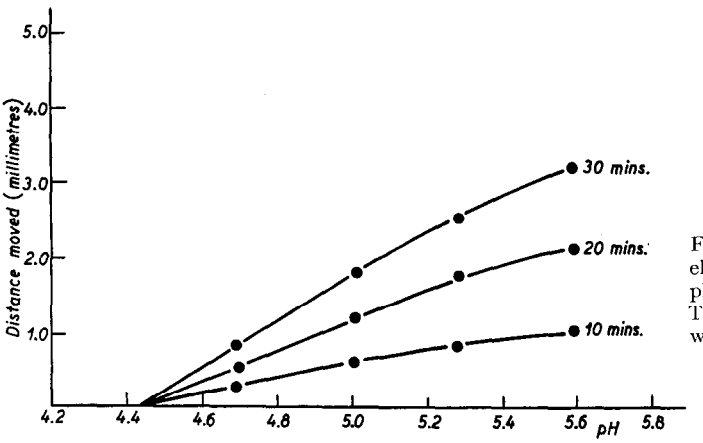

Fig. 3. Determination of iso-electric point of RSA in sodium phosphate/citric acid buffer. The relative distances moved were determined at 10, 20 and 30 minutes after starting.

citric acid and diluted to constant ionic strength ($\Gamma/2 = 0.22$). The iso-electric point of R.S.A. determined in this buffer is pH 4.44.

Acetate buffer

The iso-electric points of both HSA and RSA were determined in sodium acetate/acetic acid buffer of constant ionic strength ($\Gamma/2 = 0.1$). The correction for electro-endosmosis was similar to that with the previous buffer system and is recorded in Fig. 2. The results of HSA and RSA are recorded and extrapolated in Figs. 4 and 5 with iso-electric points of pH 4.76 and 4.89 respectively. The former agrees fairly well with that recorded by BALDWIN *et al.*[4] in the same buffer (pH 4.71). In the case of the RSA the pH of one of the buffer solutions was below the iso-electric point and hence these values were excluded from Fig. 5.

All the experiments on sodium phosphate/citric acid buffer were with a potential gradient of 3.0 volts/cm, while those in acetate buffer were with a potential gradient of 4.3 volts/cm.

In both buffer systems the pH's in the four compartments started to decrease after approximately 40 minutes. This was shown by a final sampling of the compartments and also by the fact that the later curves (not shown in Figs. 3 to 5) cut the zero-mobility line at progressively higher pH's.

References p. 345.

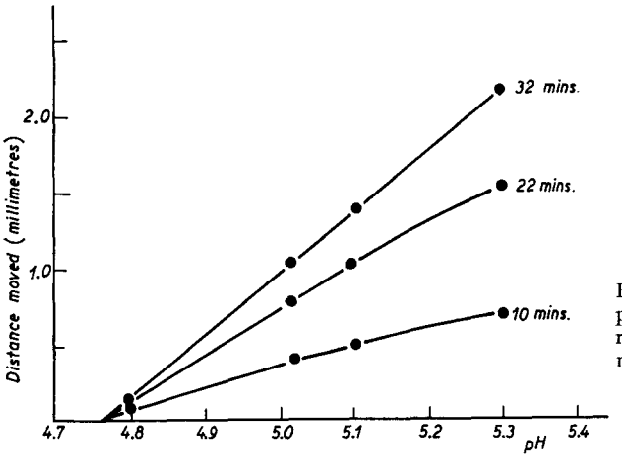

Fig. 4. Determination of iso-electric point of HSA in acetate buffer. The relative distances moved were determined at 10, 22 and 32 minutes after starting.

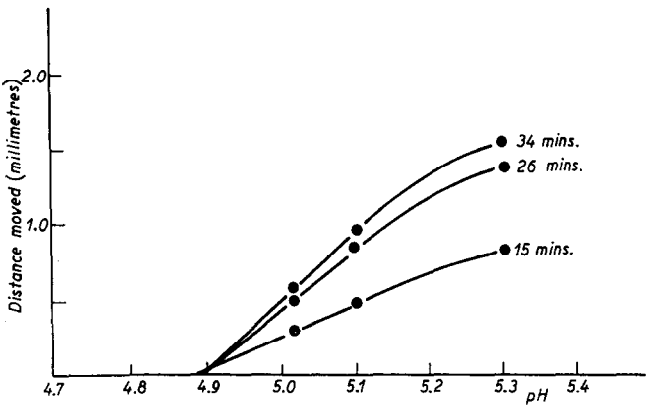

Fig. 5. Determination of iso-electric point of RSA in acetate buffer. The relative distances moved were determined at 15, 26 and 34 minutes after starting. Values at pH 4.80 are not recorded since an ascending boundary was found.

DISCUSSION

The results of the above experiments demonstrate the effect of the buffer ions on the iso-electric point of RSA, which shifts from pH 4.44 in sodium phosphate/citric acid buffer to pH 4.89 in acetate buffer. This shift is probably due to differences in the binding of the buffer ions and also their effect on the ionisation constants of the dissociable groups of RSA[5].

This method of determining iso-electric points is shown to be both accurate and rapid. The amount of correction for electro-endosmotic flow need only be re-determined when one uses a new buffer system or different ionic strength. A valuable modification to the apparatus would be the lengthening of the levelling cell, since a longer column of buffer would enable the duration of electrophoresis to be increased without any pH change occurring in the cell compartments. This would be especially valuable in the case of proteins with lower mobilites.

ACKNOWLEDGEMENT

The author would like to thank Dr. A. POLSON for his encouragement during the course of this work.

References p. 345.

SUMMARY

An accurate and rapid method of determining iso-electric points, by means of a four-compartment electrophoresis cell, is described. Using this method the iso-electric points of human serum albumin and rabbit serum albumin were determined in different buffers.

RÉSUMÉ

Une méthode précise et rapide de détermination des points isoélectriques, au moyen d'une cellule à électrophorèse à quatre compartiments, est décrite. A l'aide de cette méthode, les points isoélectriques de la sérumalbumine humaine et de la sérumalbumine du lapin ont été déterminés dans différents tampons.

ZUSAMMENFASSUNG

Eine genaue und rasche Methode zur Bestimmung der isoelektrischen Punkte mit Hilfe einer vierteiligen Elektrophoresezelle wird beschrieben. Unter Benützung dieser Methode wurden die isoelektrischen Punkte menschlichen und Kaninchenserumalbumins in verschiedenen Pufferlösungen bestimmt.

REFERENCES

1 A. POLSON, *Biochim. Biophys. Acta*, 11 (1953) 315.
2 A. POLSON, *Biochim. Biophys. Acta*, 13 (1954) 451.
3 A. POLSON, *Onderstepoort J.*, 20 (1945) 159.
4 R. L. BALDWIN, P. M. LAUGHTON AND R. A. ALBERTY, *J. Phys., and Colloid Chem.*, 55 (1951) 111.
5 R. A. ALBERTY, *The Proteins*, edited by H. NEURATH AND K. BAILEY, 1953, Chap. 6.

Received January 31st, 1955