

CHROM. 8248

ELECTROPHORETIC THERMAL THEORY

I. TEMPERATURE GRADIENTS AND THEIR EFFECTS

J. O. N. HINCKLEY*

Veterans Administration Hospital, Tucson, Ariz. 85723, and University of Arizona, Tucson, Ariz. 85723 (U.S.A.); and Transphoresis Co., London (Great Britain)

(Received April 22nd, 1974)

SUMMARY

The effects of radial temperature gradients in circular-section columns and central-peripheral gradients in rectangular and annular columns, and lateral temperature gradients in both, normal to the electric field, are considered, taking into account the synergistic effects of gradients of resistivity due to negative temperature coefficients of resistivity of electrolyte solutions. Longitudinal temperature gradients and their interaction with radial temperature gradients in the steady and unsteady states are discussed. Consequences for distortion of separand bands, and limitations on apparatus design and choice of conditions are stated. Two methods of unlimited preparative scale-up are explained.

INTRODUCTION

In electrophoretic columns the exterior of the column wall may be well cooled and the coolant temperature controlled by a good thermostat. But even if the column wall is very thin and of high thermal conductivity, the wall and column lumen have finite thickness, and there are temperature gradients both across the wall and from center to periphery of the lumen. Such temperature gradients in the lumen due to Joule heating have been calculated^{1,2,22} as parabolic in the steady state. But that approach makes the simplifying assumption that the column lumen is a resistor of zero temperature coefficient of resistivity, which is not the case for aqueous electrolytes, whose resistance decreases by some 2%/°C. This means that the warmer center of the column will carry a higher current density than the periphery and the overall resistance will fall. For a given power dissipation, correction of temperature gradients to allow for this gives higher gradients, which are no longer parabolic^{3,4}.

While low wattages per unit length of column may involve a small correction, the error may approach 50% at higher levels with resultant increase of band distortion, convection, and denaturation of separand substances. Similar arguments apply to lateral and longitudinal temperature gradients. The interaction of the latter in steady and unsteady states becomes complex^{5,6}. The effects of temperature gradients

* Present address: Clinical Immunology Department, University of Arizona Medical Center, Tucson, Ariz. 85724, U.S.A.

are different in zone methods, isoelectric focusing⁷ and displacement methods^{8,9} such as transphoresis¹⁰ and isotachophoresis^{10,11}. These considerations, and results of calculations in the following papers¹²⁻¹⁴, were briefly reported in 1971³ at a symposium.

Two methods of unlimited preparative scale-up emerge^{6,15}. Lateral extension of flat-section and annular columns is possible. For transphoretic methods, an unsteady-state method is possible with any column section, particularly with counterflow.

TEMPERATURE GRADIENTS

Central-peripheral temperature gradients

Consider a uniform column of aqueous electrolyte perfectly cooled and thermostated at its exterior surface and imagine it to have an infinitely thin retaining wall. If the column is very long and communicates with large electrode vessels at either end, which pass a constant current, then in the steady state a temperature gradient will exist from center to periphery, longitudinally uniform at midpoints far from the ends. If we ignore or suppress thermal convection and other disturbing factors such as electro-osmosis and assume there are no internal solvent flows, this gradient will have bilateral symmetry for rectangular section columns and radial symmetry for columns of circular section. Annular section columns, where there is a large radius to radius-difference ratio, approximate to rectangular section columns where the shorter sides of the rectangle have been removed.

This steady-state gradient will be parabolic^{1,2} for resistors of zero temperature coefficient of resistivity and it is conceivable that an electrolyte system of special dynamic properties could be devised to conform with this, or even have a positive coefficient to reduce the parabolic effect, which is the case with most metallic resistors. Most aqueous electrolyte solutions resemble certain solid resistors, such as graphite and thermistor materials, in having a negative coefficient, becoming less resistive when hotter, which is the case for electrophoretic media. In the case of buffer electrolytes whose pK is temperature-sensitive, and saturated solutions in the presence of undissolved solute, the effective temperature change will be further altered. A special case is that of colloidal electrolytes¹⁶. Treating the simple case of a dilute salt, such as potassium chloride, and ignoring differential change of hydration and transport number, we may assume a change of resistivity of arbitrarily 2%/°C. Thus, central equivalent shells of electrolyte coaxial with the column will be less resistive than peripheral cooler shells and may be regarded as parallel but different resistors between and normal to two planes of different potential which provide the electric field, these shells being in electrical contact with adjacent shells and adjacent parts of themselves. The column may therefore be considered as divisible into any number of equipotential planes normal to all lines of current intensity. The more central resistive shells will carry more of the current density following the temperature gradient, which also becomes a gradient of ion speed since lowered resistance is due to higher mobility. Since power dissipation is dependent on the product of current and potential gradient, the power dissipation gradient follows the current density gradient. Since the higher central dissipation is further from the peripheral heat-sink, there will be a further temperature rise in central regions, leading synergistically to further increase of the current density and power dissipation gradients. At the steady state this tendency is exactly opposed by the increased heat loss by conduction through outer shells of so-

lution due to the increased temperature gradient. The calculation of these steady-state gradients involves the use of Bessel functions^{3,12-14} and is similar to Jakob's treatment of negative coefficient resistors¹⁷ if a correction having the effect of Dusinberre's second correction¹⁸ for current density gradient is written in. By rewriting the temperature coefficient of resistance, solutions containing the square root of -1 are avoided¹⁹, and the equations become amenable to digital computation. Derivation of these equations and a computer program for same are given in the following papers¹²⁻¹⁴. Those calculations do not take into account the palliative effects of the gradient of thermal conductivity of the column solution (Dusinberre's first correction¹⁸), which could be written in without difficulty, though the error is small²⁰.

The computed results have been graphically plotted (Fig. 1), using a thermostat temperature of 4° , for circular and rectangular section columns whose side ratio is 10:1. Curves for various ratios of internal and external diameter, assuming walls of fused quartz, have also been plotted for use in practical cases. Since, in common with the simple parabolic case, the absolute dimensions are immaterial and only the wattage per unit length and geometry of section is important, the central temperature

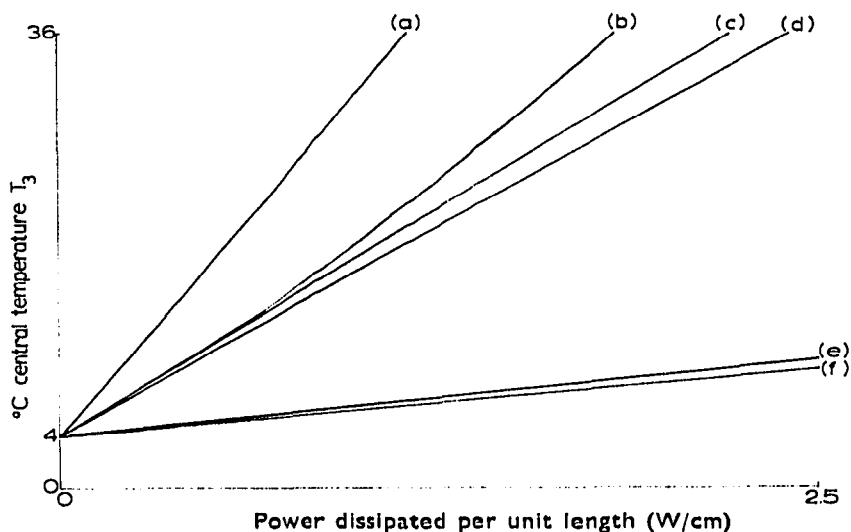


Fig. 1. The graph shows curves of temperature rise at the centre of an electrophoretic column, for a perfectly cooled and thermostatted exterior of 4° for various power dissipations per unit length of column from 0-2.5 W/cm. Curve (a) is for a filling of negative temperature coefficient of resistance electrolyte in a tube of outer to inner diameter ratio of 3.0. Curve (b) is similar, but the diameter ratio is 1.0, that is a wall-less tube. The wall is fused silica, the solvent water, and a 2% rise of mobility is assumed per $^\circ\text{C}$, such as might be approximated by dilute potassium chloride. The tube section is circular. Curves (c) and (d) have the same different diameter ratios as (a) and (b), respectively, but are for an electrolyte of zero coefficient, which is the parabolic case. Curve (e) is for a flat section tube (rectangle side ratio of 10:1) with a negative coefficient as previously, and a similar wall material. The total thickness to lumen thickness ratio is 1.2. Curve (f) is for a wall-less lumen of similar rectangular section side ratio. At this scale, and at this wattage range, the zero and negative coefficient cases virtually coincide. These curves were computed for the steady state, and the above are a few of many such too numerous to reproduce usefully on this scale. The wattages are real wattages, after correction for temperature and current density gradients, *i.e.* those to be found from measurement of voltage and current in practical cases, not the nominal ones obtained by calculation from cold resistivities. T_3 is the central temperature, as listed and used in refs. 12-14.

increase is obtainable for any system in which the nominal or effective wattage is known or calculable. Thus, the temperature rise in a tube of similar geometry of section is the same if the sectional area is ten times larger and the electrolyte diluted appropriately (roughly ten times). The graph shows that the error in using the simple parabola method increases non-linearly with wattage at high powers for circular section tubes. Thick walls produce larger errors, and have a greater effect on rectangular section tubes, and therefore annular ones. The more favorable configuration is clearly rectangular or annular, as in the parabolic case.

The unsteady state, where there is progressive warm-up of the column resistor, or cooling on reduction of the electric field strength, may also be treated by Bessel functions²¹, a requirement for the parabolic theory, and becomes more complex when the negative coefficient is written in. The general form of the rise in temperature and fall of resistance with time of the whole column on application of constant current may be obtained from a potentiometric detector in the column measuring the change of potential difference at two close points⁵, or with a point thermal detector^{8,9} or by measuring the overall voltage drop across the tube, and involves the thermal capacity of the column and lumen. The curves resemble those of Taylor for solid resistors²³, and those for passage of an ionic interface in displacement electrophoresis⁸. For negative coefficient resistors it is clearly prudent to use current-regulated power supplies, and voltage-regulated ones for positive resistors to avoid overheating if the cooling is inadequate.

The non-parabolic temperature-distance steady-state radial temperature gradients, which we have computed digitally for wall-less tubes, have further uses. If a smaller section of the tube is taken, by removal of a peripheral shell of lumen, this gives the radial gradient for a higher thermostat temperature at a lower wattage. For the zero coefficient case this wattage is directly proportional to the area of the section, and in negative coefficient cases is obtainable from integrals of temperature (current density) and area. Annular and rectangle-difference sections of the temperature profiles also apply to cases where the inner and outer lumen walls are at different temperatures, and inner walls supply heat at the rate of the (removed) lumen centre.

Longitudinal temperature gradients

These arise when there is relative movement of lumen contents and of a change of power dissipation and is a particular case of non-uniformity of column. Other non-uniformities of column may be due to cooling irregularities and construction of apparatus, including the case of a detector which interferes with the cooling locally. There are two types of relative movement of lumen and dissipation, which may also be combined, and may interact with other non-uniformities. First, if there is a flow of solution in the column, there is addition at one end of unsaturated thermal capacity. This leads to a linear longitudinal temperature gradient in the absence of cooling. If cooling of the lumen periphery is added, the longitudinal gradient is hybridised with unsteady-state radial gradients, and isothermal surfaces become a train of figures of revolution of different curves until the thermal capacity is saturated, when these surfaces become the coaxial cylindrical shells of the steady state. This applies to the use of counterflow of solvent in electrophoresis²⁴. In the second case, there is a traveling change of resistivity of the column resistor. In the absence of cooling there will be a change of the rate of temperature rise proportional to the resistance change,

which will become more complex and change continuously if the resistance change varies along its length. If peripheral cooling is added, combination with radial gradients gives a similar train of isothermal surfaces as in the first case. Examples of a moving change of resistance are found in zone electrophoresis²⁵, and displacement methods such as transphoresis and isotachophoresis, and therefore in the steady-state stacking stage of disc electrophoresis^{26,27}. In such cases, calculation of the unsteady-state radial temperature gradients at various depths gives the combined longitudinal and radial gradient if distance is substituted for time in the constant-current mode. Use of sheets of encapsulated cholesteric thermochromic liquid crystals across the lumen may render such gradients visible.

Lateral temperature gradients

If there is a difference of cooling or heat dissipation towards one wall of the column, bilateral and radial symmetry no longer apply, and the regions of higher temperature, power dissipation, and migration speed are shifted in the direction of the hotter side. This may arise at inflexions of an electrophoretic tube, where the inner part of the inflexion is a shorter and more used current path, for which Martin and Everaerts suggested thermal banking compensation⁸, using a lateral externally imposed temperature gradient to counteract it. It may also occur in free solution electrophoresis where the separand is significantly different in density to adjacent solution and is relatively displaced to one wall of the tube by gravity⁶. Such displacement is seen in zone electrophoresis of proteins and particles and may be relieved by rotation of horizontal columns². This has also been seen in transphoresis^{6,28}.

APPLICATION OF TEMPERATURE GRADIENTS TO ELECTROPHORESIS

In zone electrophoresis, isoelectric focusing, and displacement methods, radial temperature gradients, as calculated with correction for current-density gradients induced by Joule heating, indicate that previous calculations of central temperature rise underestimate its size. This means that zones will bow more than previously estimated and the bow will not be parabolic. If the resistance difference between separand and buffer electrolyte is negligible, previous theory of zone bowing²⁹ will require further refinement. If there is a resistance difference, theory probably becomes too complex to be useful, as unsteady longitudinal gradients will be superimposed. The graph in Fig. 1 shows that rectangular and annular section tubes should be even more favorable thermally than was previously thought to be the case¹, for high power dissipations, a conclusion particularly applicable to preparative-scale electrophoresis in wide columns.

In isoelectric focusing the radial temperature gradients will require more convective stabilization by density gradients or viscous colloids than simple theory would indicate, and progressive denaturation at the center would be more rapid by virtue of the higher temperature. Increased theoretical migration speeds would increase separand return to the isoelectric band as much as diffusion from the bands would increase, but if the generation and stability of ampholyte regions is not altered the system is largely self-stabilizing.

Inflected columns, causing spreading of separand over a greater length of column due to differential migration speeds, become less favorable still and should

be avoided, as should column constrictions and dilatations, being antiseparative in all cases. Similar arguments apply to local cooling imperfections. Use of one discontinuity to oppose another may be palliative,⁸ but is difficult to arrange really well.

In transphoresis radial temperature gradients give rise to a stable bow of the ionic interface, rather than progressive bowing found in zone methods, and the three-dimensional interface structure must therefore have properties which are self-restorative⁶ in ways other than its already stated control of diffusion⁸, since a current density gradient would involve faster migration of central compartments. This bow limits the accuracy of analytical quantitation by a high-resolution detector since the volume of the bow is not easily ascertainable, except empirically⁵. The longitudinal temperature gradient in transphoresis would be unimportant if there was no change of the mobility ratio of co-ion and counter-ion with temperature. But since most analysand ions of interest are complex and require buffering counter-ions, there is indeed a temperature effect and therefore a progressive change of Kohlrausch-regulated³⁰ concentration of the following separand compartment, through the temperature gradient, impairing quantitation. This is complicated by interaction of longitudinal and radial temperature gradients, whose unsteady state treatment is complex.

In isotachophoresis, where a co-running train of continuous mobility spectrum spacer ampholytes migrate at the same speed as the separand⁵, temperature gradient alteration of concentration will be added to already existing pH and concentration gradients and the effects of these ampholytes on quantitation and resolution can only be studied empirically.

Thermometric effects will also increase for unsteady states, such as beginning and end of an electrophoretic run, unless solutions are preheated to start with to a temperature matching the mean volume temperature of the column contents, with adjustment for differential wall expansion, and kept at the run temperature at the end, by substitution of a.c. for the electrophoretic d.c. current. Judicious choice of tube wall material, or composite walls may help. Suppression of thermometric effects, until quantitation or sample removal, is desirable for accuracy and controlled work, and to prevent remixing of regions due to relative fluid or gel movement. Working at about 4°, the expansion plateau of water, is desirable for thermometric and convective reasons, and the graph of Fig. 1 has therefore been based on that temperature.

If the pK temperature sensitivity of the system, the pH or the gel properties can be so chosen as to minimise the apparent effect of temperature on separand net mobility, or even reverse it, then thermal limitations are less severe, and bowing is not a problem.

Such reversal of bow for proteins in agar gels is discussed by Wieme³¹, particularly for hemoglobin. Similar phenomena have also been seen in transphoresis and isotachophoresis^{6,32,33} with some proteins and dyes, using agarose and polyacrylamide. It also occurs with Ampholines (LKB)³⁴, and whatever their mechanism such effects appear to require the presence of gel, and seem dependent on the way it is prepared and handled. Trailing effects of weak anions in agar gels was noted by Kendall *et al.*³⁵ in pioneer work on transphoresis.

UNLIMITED SCALE-UP FOR PREPARATIVE PURPOSES

For preparative purposes, the use of thermally favourable rectangular or

circular section columns suggests methods of almost unlimited scale-up of production capacity. Thus the rectangular section, whose width is far greater than its thickness, may be compacted into a corrugated or spiral section. The annulus may be of very large internal radius or may be likewise convoluted to render the coolant facility more manageable. A minor disadvantage may be the more elaborate introduction of sample in non-circular sections in zone methods. For transphoretic and focussing methods this is less troublesome as these systems are self-correcting, the former particularly with counterflow of leader electrolyte¹⁵.

This leads to another concept of cooling which may allow major scale-up of column bore of any section, for preparative or other purposes. In transphoretic methods, the longitudinal temperature gradient due to separand compartments, considered in a frame moving with the interface, depends on the power dissipation per unit length and the rate of presentation of unsaturated thermal capacity of tube and contents. If the latter can be increased per watt at a given migration speed, by diluting the system and enlarging the bore in proportion, then it may be possible to run the separand train in that part of the column which suffers only the initial small portion of the temperature gradient. This use of unsteady-state gradients may be extended in the case of balancing counterflow (which is meaningful only in transphoretic methods) to the longitudinal gradient in the leader and separand, where the separand may occupy most of the column, with commensurate power saving per unit of product. Since this is now an internal form of cooling, the walls may be insulated, and radial gradients abolished, so there is no longer a thermal restriction of bore, but one of length. In practical apparatus this suggests use of transphoretic three-dimensional interface self-correction to maintain flat bands, and fine woven monofilament flow-smoothing meshes, single or duplex, at either end of a wide, short column, to induce a substantial element of plug flow and suppression of the Stokes parabolic flow profile. The system is self-stabilising for a given voltage and a wide range of constant counterflow: Increase of voltage merely shifts the position of the bands, as does a small change of counterflow. Using a voltage-stabilised power supply, a too low counterflow shifts the bands to the leader end of the column, current and potential gradient are reduced, and a new stable position is found. The convenience of such a system for intermittent introduction and removal of separands using stationary plumbing is attractive¹⁵. Apparatus has been constructed using these principles, and will be the subject of a future report. Methods using radial fields and counterflows with transverse flows, or radial transverse flows in annular section columns, have been described for continuous preparative use¹⁵.

A third method, which is restricted to batch production, should be mentioned. Brownstone³⁶ did zone electrophoresis in a gel cylinder whose side walls were poorly cooled, the heat loss being more from large end-faces³⁷, and found reduced radial temperature gradient profiles. This principle is also implicit in the methods described above, where both electrode vessels and their flows may be cooled and thermostatted. In zone electrophoresis there is no self-correction of zone-bowing, unless a sieving or conductivity^{19,40} gradient is introduced. Difficulties of removing sample from the gel remain.

ACKNOWLEDGEMENTS

This work was supported in part by NASA Contract NAS 8-29566 and also

draws on prior work of Transphoresis Co., London, Great Britain. Thanks are due to D. H. Jones, who urged the use of digital computation, programmed and computed values, from which Fig. 1 was plotted with the help of J. F. Brown, who checked values manually. Thanks are due also to R. B. Hall for advice and arranging access to computing facilities through Brunel University.

NOTE ADDED IN PROOF

Temperature gradients were measured by Porath²², using particulate columns for which correction factors were applied. Conditions and materials used make comparison with our results difficult. Close correspondence with calculated parabolas was found, though the difference of shape between those and our Bessel curves for circular sections may not be easily detectable thus. Indirect confirmation may be obtainable using say a thick thermistor rod. However, measurements in electrolytes require reduction of convection. Very uniform gels of known controlled properties are a possibility. Centrifugal stabilization by rotation about the column long axis might be tried. Another solution would be to use the effectively zero gravity of spacecraft, such as NASA's projected Shuttle, for which continuous transphoretic preparators have been proposed¹⁵. It is hoped to present experimental work later.

The work in this and the following papers¹²⁻¹⁴ was done in London in 1971-1972, and briefly reported then³ and later⁴⁻⁶. Its purpose was to help specify design and performance limits of transphoresis⁴⁻⁶ (and isotachophoresis^{4-6,11}), as opposed to zone separators⁴⁴, to aid calculation of stable and incipient three-dimensional shapes of transphoretic ionic interfaces⁶, to be of use secondarily in direct method transport number determination¹⁰, and generally for negative coefficient resistors and analogous systems. Such considerations also underlay the author's endorsement of transphoresis as the method of choice in space^{15,43}. The work we now present was therefore circulated in 1973 for study and use to former colleagues, who endorsed and extended it, as outlined elsewhere³², to comprehend the unsteady state^{5,6}, in two more recently submitted papers^{38,39}, likewise on both circular and rectangular section columns. While many basic features are common to the two treatments, readers may wish to study other significant apparent differences of inference revealed by their later work, in order to reconcile our and their approaches and tacit assumptions as best they may. Thus for the same central temperature rise, in circular and rectangular cases, we did not find that increased relative wall thickness gave higher power dissipations, nor that internal radius or dissipation depended on voltage gradient alone nor that there was a maximum lumen section (since we purposely left concentration and resistivity unspecified³), nor that dissipation was less in rectangular than in circular sections. We did not apply the field strength determinant^{5,8,41,42} of unidimensional interface sharpness^{8,41,42} directly to the real three-dimensional case^{5,6,43}, nor did we equate stable interface convexity with ionic velocity gradients of a uniform electrolyte column. Their use of the unsteady state for rapid separation is implicit in methods discussed herein and elsewhere^{6,15}, and relates to Hjertén's similar proposals for zone electrophoresis². While thick walls may confer some useful gain in electrical efficiency, by acting as thermal insulators and thereby raising mean lumen temperature and conductivity, it would seem preferable to achieve this with thin walls and a higher coolant temperature, particularly in the thermally discontinuous systems of transphoresis and isotachophoresis.

REFERENCES

- 1 Q. P. Peniston, H. D. Agar and J. L. McCarthy, *Anal. Chem.*, 23 (1951) 994.
- 2 S. Hjertén, *Chromatogr. Rev.*, 9 (1967) 122.
- 3 J. O. N. Hinckley, in E. Reid (Editor), *Methodological Developments in Biochemistry*, Vol. 2, Longman, London, 1973, Ch. 21, p. 201.
- 4 J. O. N. Hinckley, *Anal. 72 Symp., Imperial College, London, 1972*; in supplement to *NAS 8-29566 Rep.*, in preparation.
- 5 J. O. N. Hinckley, *Biochem. Soc. Trans.*, 1 (1973) 574.
- 6 J. O. N. Hinckley, *Clin. Chem.*, 20 (1974) 973.
- 7 H. Haglund, *Methods Biochem. Anal.*, 19 (1971) 1.
- 8 A. J. P. Martin and F. M. Everaerts, *Proc. Roy. Soc. London, Ser. A*, 316 (1970) 493.
- 9 D. Peel, J. O. N. Hinckley and A. J. P. Martin, *Biochem. J.*, 117 (1970) 69P.
- 10 J. O. N. Hinckley, in E. Reid (Editor), *Methodological Developments in Biochemistry*, Vol. 2, Longman, London, 1973, Ch. 21, p. 207.
- 11 H. Haglund, *Sci. Tools*, 17 (1970) 1.
- 12 J. F. Brown and J. O. N. Hinckley, *J. Chromatogr.*, 109 (1975) 218.
- 13 J. F. Brown and J. O. N. Hinckley, *J. Chromatogr.*, 109 (1975) 225.
- 14 D. H. Jones and J. O. N. Hinckley, *J. Chromatogr.*, to be submitted.
- 15 J. O. N. Hinckley, report to M. Bier and NASA, 1972; AIAA Paper 74-664, Amer. Inst. Aeronaut. Astronaut., New York, 1974.
- 16 N. K. Adam, *Physical Chemistry*, Oxford Univ. Press, London, 1956, p. 601.
- 17 M. Jakob, *Trans. Amer. Soc. Mech. Eng.*, 70 (1948) 25.
- 18 G. M. Dusinger, *Trans. Amer. Soc. Mech. Eng.*, 70 (1948) 28.
- 19 J. Porath, *Biochim. Biophys. Acta*, 22 (1956) 151.
- 20 J. F. Brown and M. Coxon, personal communications, 1972, 1973.
- 21 H. S. Carslaw and J. C. Jaeger, *Conduction of Heat in Solids*, Oxford Univ. Press, London, 1959, p. 266.
- 22 J. Porath, *Ark. Kemi*, 11 (1957) 161.
- 23 L. S. Taylor, *J. Res. Nat. Bur. Stand.*, 38 (1947) 137.
- 24 A. K. Brewer and S. L. Madorsky, *J. Res. Nat. Bur. Stand.*, 38 (1947) 137.
- 25 F. M. Everaerts and W. M. L. Hoving-Keulemans, *Sci. Tools*, 17 (1970) 25.
- 26 L. Ornstein, *Ann. N.Y. Acad. Sci.*, 121 (1964) 321.
- 27 B. J. Davis, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
- 28 S. Hjertén, in E. Reid (Editor), *Methodological Developments in Biochemistry*, Vol. 2, Longman, London, 1973, Ch. 4, p. 47.
- 29 H. C. Cox, J. K. C. Hessels and J. M. G. Teven, *J. Chromatogr.*, 66 (1972) 19.
- 30 F. Kohlrausch, *Ann. Phys. Chem. Leipzig*, 62 (1897) 209.
- 31 R. J. Wieme, *Agar Gel Electrophoresis*, Elsevier, Amsterdam, 1965, pp. 118, 192.
- 32 M. Bier, J. O. N. Hinckley, A. J. K. Smolka, M. J. Binder, M. Coxon, T. W. Nee, M. O. Scully, H. S. T. Shih and R. S. Snyder, *Proc. 3rd Space Process. Symp. Skylab Results*, 2 (1974) 729.
- 33 B. Bakay, personal communication, 1973.
- 34 P. J. Svendsen, *Sci. Tools*, 20 (1973) 1.
- 35 J. Kendall, and W. West, *J. Amer. Chem. Soc.*, 48 (1926) 2619.
- 36 A. D. Brownstone, in E. Reid (Editor), *Methodological Developments in Biochemistry*, Vol. 2, Longman, London, 1973, Ch. 2, p. 13.
- 37 J. St. L. Philpot, *Trans. Faraday Soc.*, 36 (1940) 38.
- 38 M. Coxon and M. J. Binder, *J. Chromatogr.*, 101 (1974) 1.
- 39 M. Coxon and M. J. Binder, *J. Chromatogr.*, 107 (1975) 43.
- 40 H. Haglund and A. Tiselius, *Acta Chem. Scand.*, 4 (1950) 957.
- 41 T. W. Nee, *J. Chromatogr.*, 93 (1974) 7.
- 42 M. Coxon and M. J. Binder, *J. Chromatogr.*, 95 (1974) 133.
- 43 M. Bier, J. O. N. Hinckley, A. J. K. Smolka and R. S. Snyder, *Protides Biol. Fluids, Proc. Colloq.*, 22 (1975) 673.
- 44 J. O. N. Hinckley, *Electrophoresis*, in *MTP Int. Rev. Sci., Phys. Chem. Ser. 2*, Vol. 13, Medical & Technical Publishing Co., Oxford, in press.