

AN APPARATUS FOR HIGH-VOLTAGE PAPER ELECTROPHORESIS

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Paper electrophoresis is based on the differential migration of charged ions or particles in an electrical field. Since migration rates within a given time are proportional to the field strength, it is desirable to be able to use as high a voltage as experimentally possible in order to obtain the following immediate advantages: (1) economy in time, (2) high resolving power, (3) easier detection of components present in low concentration.

The definition of high-voltage technique implies the application of a potential gradient of, at least, 50 V/cm¹. To obtain the full benefit of the technique it is advisable, however, to aim at the use of potential gradients of 100 V/cm and more, though the difficulties in designing suitable equipment for this range become progressively greater with increasing voltage.

Several designs of high-voltage equipment have been described in recent years. Some are based on direct cooling of the paper strip by an organic solvent, immiscible with the sample and the aqueous background electrolyte solution, with and without cooling of the organic solvent, as suggested by MICHL², HEILMEYER *et al.*³, and TURBA *et al.*⁴. Others are based on cooling of the paper strip by plates of metal, glass or plastics which in turn are cooled by refrigerated brine or ordinary tap water. Designs incorporating one cooling plate, generally the bottom plate, have been described by BERBALK AND SCHIER⁵, WERNER AND WESTPHAL⁶, WIELAND AND PFLEIDERER⁷, and DOSE⁸. Designs incorporating two cooling plates (sandwich technique) for greatly enhanced efficiency have been proposed by MICHL⁹ and GROSS¹⁰⁻¹².

THEORETICAL CONSIDERATIONS

The movement of ions in an electric field is proportional to field strength and net charge carried:

$$\text{Field strength} = X = \frac{V}{l} \quad (1)$$

$$\text{Mobility} = u = \frac{d/t}{V/l}, \text{ or} \quad (2)$$

$$d = \frac{u \cdot V \cdot t}{l}$$

where d = distance travelled by ion, l = length of field, V = voltage, t = time.

The equations apply primarily to free electrophoresis and a certain correction factor for tortuous path in the stabilising medium has to be introduced for paper electrophoresis. Although the net charge can be altered by complexing agents and changes in pH, it is more convenient to increase migration rates by the application of higher voltage.

Diffusion

As a function of temperature, concentration, time and molecular weight, diffusion plays a significant part in paper electrophoresis, mainly by counteracting and reducing the separating efficiency of the technique. With a considerable reduction in time at high voltage, the diffusion effects are greatly repressed and the separating power increased, particularly for low-molecular weight compounds. This is helped by the maintenance of a steady, moderately low temperature. Small differences in mobilities are not obscured by the spreading and overlapping of zones. The sensitivity of the method is increased, as the detection and estimation of compact zones by colour reaction are extended to lower levels of concentration.

Heat dissipation

The passage of electric current through the moist, conducting filter paper is proportional to the voltage applied and causes generation of heat. The heat, according to Joule's law, increases with the square of the current and is given in calories per second by the formula:

$$Q = \frac{RI^2}{A} = \frac{VI}{4.185} \quad (3)$$

where Q = heat, R = electrical resistance of paper strip, I = current, and A = mechanical heat equivalent. ($A = 4.185 \cdot 10^7$ erg/cal.)

If the heat is not dissipated, the ensuing rise of temperature affects detrimentally the experimental conditions, since conductance and pH (to a lesser degree) of the background electrolyte solution, and mobilities of the ions under investigation are all temperature-dependent. To obtain steady conditions, it is essential that generated heat should be effectively dissipated so that there be no significant temperature rise during the experiment. The level of applied voltage is thus limited only by the efficiency of heat dissipation or cooling.

Cooling

Cooling can take place by evaporation, conduction or radiation, which latter plays no significant part in the designs discussed. Evaporation is the usual system, particularly in simple apparatus of the "moist chamber" type found so useful in low-voltage electrophoresis, but it leads to serious complications above a potential gradient of 10 V/cm. It is thus desirable that heat dissipation should occur predominantly by conduction. Metal plates in close contact with the heated surface (paper) are excellent conductors of heat and the coolant circulating in the plates provides a

suitable means of dissipation. The efficiency is somewhat reduced by the need for electrical insulation of the plates by glass, Polythene or other plastics. The heat exchange mechanism in this case is complicated because the heat has to pass the narrow air gap between paper and insulating layer, the thickness of the layer, another air gap, and finally the distance from metal surface to coolant, before it can be carried away. By choosing the thinnest possible insulating layer, yet one of sufficient dielectric strength, by cooling both surfaces of the paper and compressing the paper between two cooling plates under sufficient pressure to ensure close contact, a high degree of heat transfer efficiency can be achieved.

The cooling efficiency will also depend greatly on the choice of suitable materials and the design of plates, particularly with regard to flatness of surfaces.

Choice of materials of construction

The material chosen for cooling plates must fulfil several conditions. It must be of high thermal conductivity and, preferably, of high dielectric strength, *i.e.* afford excellent electrical insulation. However, these two properties cannot be found together, since, as a rule, a material of good thermal conductivity is also one of good electrical conductivity, and vice versa. Glass is almost as good an insulator as any of the plastic materials. Its dielectric strength per 0.001 in. is about 700 V against 500 V for P.V.C. (polyvinylchloride), 1000 V for Teflon (polytetrafluoroethylene) and 1000 V for Polythene (polyethylene). However, its mechanical weakness in thin layers puts it at a serious disadvantage in comparison with the plastics which can be used as insulating films. Its thermal conductivity at $1.7 \cdot 10^{-3}$ cal/cm²/°C/cm is admittedly better than that of Polythene (or similar material) at $7 \cdot 10^{-4}$, but still very far below the conductivity of a metal such as aluminium at $5 \cdot 10^{-1}$.

In view of the contrasting properties, a combination of the high insulating strength of a film of plastics with the good heat-conducting property of a metal plate would appear to offer the best solution, provided the thinness of the film is kept to a minimum limited only by the insulating capacity under the voltage conditions required. It must be remembered, however, that the dielectric strength of plastics is dependent on the temperature and time of exposure and is usually decreasing with increasing temperature. Weakening or failure of insulation can often be caused by mechanical or chemical breakdown of the material. It is therefore imperative to maintain the temperature of the film at a moderate level and avoid local overheating leading to ultimate breakdown of the insulation. If the cooling is efficient, the temperature of the film can be kept within 1-2° of that of the coolant.

Experience has shown that a 0.01 in. thick film of Polythene (British Visqueen Ltd.), or similar material, is suitable for experiments employing potential gradients of 100-160 V/cm. It must be added, however, that the energy throughput and the type of background electrolyte solution used are important factors in deciding the performance of insulating films. A strong, highly-dissociated buffer, like ammonium carbonate or ammonium citrate solution, with consequent relatively high current consumption at a given voltage puts a heavier demand on insulation than

e.g. a mixed acetic acid-formic acid solution. In this case, the energy which can be dissipated per unit area (W/cm^2) without leading to a significant temperature rise will be the governing factor.

Cooling efficiency

Water or refrigerated brine can be used to carry away the heat from the plates, the most effective way being circulation of the coolant in a system of channels milled out of the plates. If a refrigerating unit is used, the coolant is best re-circulated by a suitable pump.

The present design uses as a coolant tap water without refrigeration and re-circulation. The quantity of water required to dissipate the average energy throughput can be calculated from the following considerations: supposing the temperature of the cooling water to be 12° , the energy throughput 1500 W and the amount of energy converted to heat as 20% , i.e. 300 W or 258 kcal/h , then a flow of 720 l/h will be sufficient to prevent any significant rise in temperature. Measurements have shown the average temperature differences between inflow and outflow to be within 1° . With a lower current consumption and water temperature, the conditions will be even more favourable.

The effect of efficient cooling is shown by the constancy of current consumption during the run, giving thus a certain indication of constancy of temperature, ionic strength, moisture and potential difference, all important factors ensuring steady conditions in the paper strip.

A vital point in the calculation mentioned is the assumption that heat transfer from the heated surface (paper) to the coolant is uninterrupted and almost instantaneous. This is, however, not the case and thus the results can only be approximate.

Air pressure

The pressure applied to the top plate need be only moderate. Measurements have shown that a pressure of 1.5 lb./sq.in. is sufficient to ensure good contact between paper, Polythene film, and cooling surface, without undue interference with the proper moisture level of the paper strip. Increasing pressure leads to less moisture, lower current consumption, lower migration rates, and deterioration of cooling efficiency. At pressures of $6\text{--}8\text{ lb./sq.in.}$, lack of sufficient moisture leads to inefficient cooling of the cellulose fibres instead of the solution between the fibres. A good control of air pressure will ensure high reproducibility of the results.

Cooling plates

A very important point is the quality of the plate surfaces. Utmost attention to the finishing of the surfaces to an overall flatness of 0.001 in. is essential for uniform cooling and avoidance of warm spots responsible for distortion of migrating zones and breakdown of the insulation. Systematic temperature gradients over the whole area must also be avoided. This is best done by the choice of an effective channelling system for the coolant, preferably a double-countercurrent flow, smoothing out any differences in temperature between inflow and outflow.

Mechanism of hydrodynamic flow

With evaporation reduced to a negligible minimum, shifts of liquid in the paper are due to electro-osmosis and capillary flow caused by small differences in temperature along the length of the strip. There is a certain non-uniformity of flow, with rate of flow increasing slightly from cathode to anode, due to this composite effect. The liquid flow gradient is dependent on several factors such as pH, ionic strength, time, temperature, potential gradient, and moisture content of the paper. In the interest of high reproducibility of results it is advisable to minimise the hydrodynamic flow in the paper, although the inherent electrokinetic phenomenon of electro-osmosis cannot be eliminated. The choice of a suitable moisture content in the paper and the use of cellophane sleeves are helpful in this respect.

Measurements have shown that a moisture content of 130–140% of electrolyte solution on oven-dry paper and uniform wetting produce dependable conditions. The use of cellophane sleeves¹³ to restrict the influx of liquid from the buffer vessels into the separating area, greatly assists in stabilising the liquid level in the paper for the relatively short duration of the high-voltage experiment.

DESIGN OF APPARATUS

The considerations set out above and the need for a heavy-duty, compact, safe, efficient, yet simple, apparatus led to the adoption of the design of a double-cooled, pressure-regulated sandwich-type of model.

The apparatus consists of the following parts: (1) 2 cooling plates, (2) pneumatic uniform-pressure device, (3) lifting device for top plate, (4) buffer vessels containing the electrodes, (5) safety cage. In addition, there are various instruments, 2 safety electrical cut-outs and an acoustic warning system.

Fig. 1 shows diagrammatically a view of the apparatus in the closed and operating position. The cooling plates, D and E, are made of aluminium alloy, NP5/6M, (British Aluminium Co. Ltd.) and their overall dimensions are 22 × 13 × 1 in. The cooling faces are precision-ground to a flatness with a tolerance of 0.001 in. over the whole area. The ends of the plates are cut out so as to form on closure a cavity on either end. Two thick pads, H, of buffer-soaked filter paper are pressed into the cavity at either end and, by sandwiching the emerging strip, G, help to minimise temperature gradients arising from less efficient cooling at these points. One of the pads forms the electrical bridge to the power supply, by being extended into the buffer vessel, Q. It is advisable to wrap the pads tightly in cellophane so as to reduce evaporation and minimise the hydrodynamic flow from the buffer vessels into the strip. This is also helped by the level of the strip being considerably higher than that of the electrolyte solution in the vessels. A minimum plate thickness of 1 in. is governed by the necessity for a system of ¼ in. wide and ⅜ in. deep grooves in the plates to allow for a rapid and efficient water circulation, and by the rigidity required to avoid warping or bending of the plates on compression. A double-counter-current system of flow has been chosen for each plate so as to avoid any temperature gradients. Total water flow is 12 l./min.

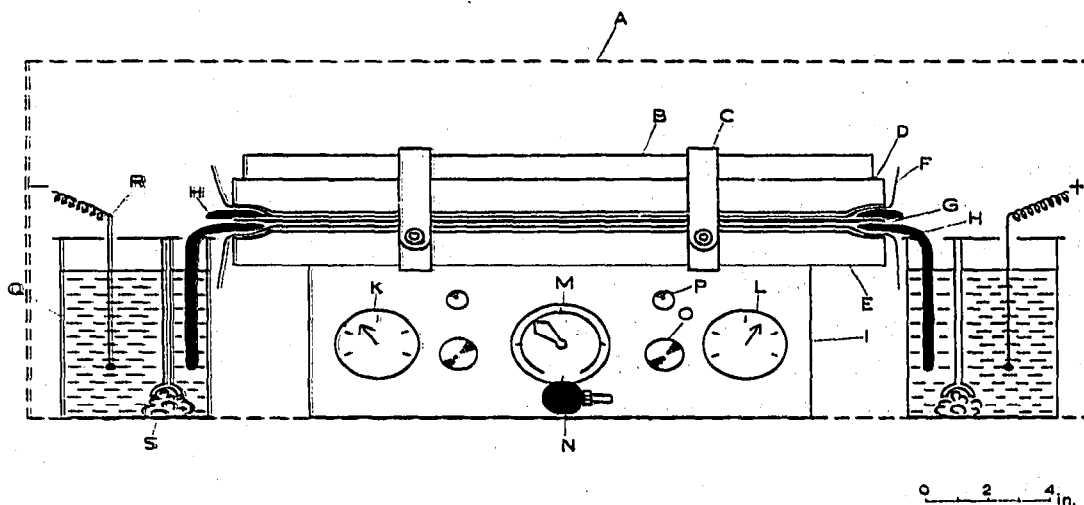


Fig. 1. Diagram of apparatus, front view. Key: A = Safety cage; B = Pneumatic pressure device; C = Retaining bracket; D = Top cooling plate; E = Bottom cooling plate; F = Insulating film; G = Paper strip; H = Thick paper pad; I = Instrument panel; K = Air pressure gauge; L = Water flow gauge; M = Timing device; N = Knob for plate locking device; O = Pressure reducing valve; P = Neon indicator light; Q = Buffer vessel; R = Platinum electrode; S = Cotton wool plug.

In addition, the channelling systems in the two plates are arranged counter-current to one another so that the smoothing-out of any still existing temperature differences is brought about in this way as effectively as possible, though the heat transfer between top and bottom plates is appreciably diminished by the interposed insulation. The channelling system adopted is shown in Fig. 2.

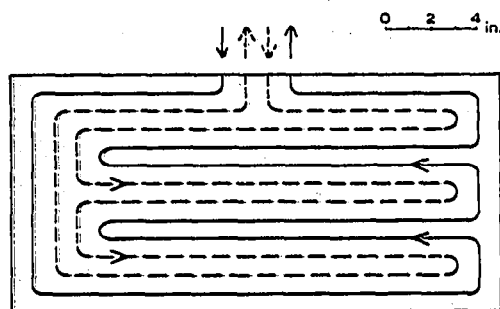


Fig. 2. Channelling system in cooling plate; arrows indicate direction of water flow.

Pressure control

The uniform-pressure control device, B, (Fig. 1) rests on top of the upper plate and consists of an inflatable plastic air cushion approx. 20×13 in. (Pneumatic Tent Co. Ltd., Dorking, Surrey), contained in a casing made of steel sheet, $\frac{1}{4}$ in. thick, and joined through an inlet tube to a compressed air supply. On closure of the top plate the two retaining brackets, C, are engaged by suitable studs and the cushion inflated to the desired pressure so as to produce a uniform, strictly reproducible pressure over the whole area of the paper strip. Incorporated instruments such as an air pressure gauge, K, pressure reducing valve, O, and taps enable strict control over the pressure.

Buffer vessels

The buffer vessels are made by heat welding of Polythene sheet, $\frac{1}{4}$ in. thick, and $13 \times 5\frac{1}{2} \times 6$ in. in dimensions, each holding about 4.5 l of electrolyte solution. The big volume was chosen so as to minimise the effects of pH changes due to electrolytic action. Covers are made of Perspex for easy fitting of electrodes. The vessels stand on narrow drip trays (Polythene) to prevent spilled electrolyte solution from making contact with the metal casing.

The vessel is divided into two compartments of uneven dimensions, the smaller one to accommodate the paper pad, H, acting as electrical bridge, and the larger one to hold the electrode, R. Communication between the two halves is afforded by a narrow horizontal, $\frac{1}{2}$ in. wide slit, $\frac{1}{4}$ in. from the bottom, plugged with cotton wool and acting only as a physical barrier.

Electrodes

Platinum wire, 18 S.W.G., 6 in. long, horizontally extended between 2 vertical holders made of Perspex, dipping to about 2 in. below the liquid level in the vessel and connected to water-proof sealed terminals in the cover, was found to provide a clean and durable electrode. Dropping of the liquid level below the electrodes during an experiment must be avoided. Filling the liquid in each vessel to the marked required height before each experiment makes the use of a special levelling device superfluous.

Lifting and arresting mechanism

The heavy weight of the plate (28 lb.) makes the provision of some lifting arrangement both for speed and convenience of operation necessary. The design evolved is shown diagrammatically in Fig. 3.

The movable top plate, D, is fixed to a horizontal, U-shaped levering device, U, the curved frontal portion of which is formed by a rubber-insulated stainless steel handle for convenient and safe manipulation. One pair of strong (0.19 in. diam.,

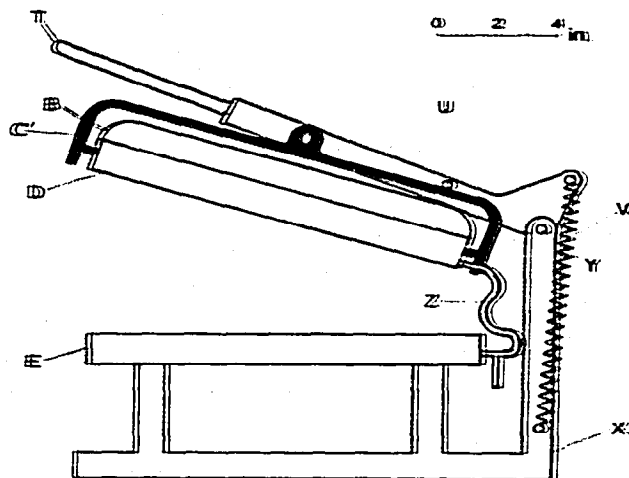


Fig. 3. Diagrammatic side view of the lifting device. Key (see also Fig. 1): T = Handle; U = Lever; W = Steel spring; X = Stand; Y = Pivoting pin; Z = Heavy rubber tubing; C' = Leveling bracket.

65 lb./in. extension) non-fatiguing steel springs, V, (International Spring & Engineering Co. Ltd., London) is attached eccentrically to either open end which turns about a pivoting pin, Y, set in a rigid pillar, X, forming part of the steel stand. Overtipping is prevented by a fixed stop.

Upward movement of the plate is assisted by the counterbalancing action of the moderately extended springs, whilst in the downward movement any abrupt drop due to gravity is braked and smoothed out by the resistance of the slightly more extended springs, the difference in extension between the two positions being not more than $\frac{1}{4}$ in. Oil-filled dashpots assist in making the movement smooth.

Safety devices

The use of high voltage requires strict precautions to eliminate the risk of fatal accidents. To ensure the safety of the operator the apparatus is enclosed in a safety cage consisting of a base and back of steel sheet, sides of expanded metal (metal mesh) and front and cover of Perspex for visibility. The cage is suitably earthed and incorporates two micro-switches connected to the input side of the electrical circuit to ensure that the apparatus can be used only when the cover is closed.

In addition, there is a device combining an acoustic warning system (buzzer) with an automatic electrical cut-out. This is activated by the pressure of the cooling water and set so that below a certain pressure it comes into operation, draws the attention of the operator to insufficient cooling, prevents overheating, and thus saves the experiment and equipment even if the water supply should fail.

Insulation

The choice of Polythene film of appropriate thickness (0.01 in.) for electrical insulation in preference to other plastic films such as P.V.C., Teflon etc. was prompted by the excellent electrical properties, pliability, lack of moisture absorption and inexpensiveness, which made handling and replacement in case of a breakdown simple and convenient. An occasional breakdown of the insulation, however, cannot be entirely prevented, since repeated mechanical handling, creasing, and overheating, particularly at points of less efficient cooling, *i.e.* ends of plates, under conditions of high current density reduce the dielectric strength of the material and thus bring about a breakdown in the form of pinholes in the weakest places. This is usually accompanied by a rattling noise, which serves as a warning to switch off the current supply and replace the damaged film before continuing the experiment. If the warning is unheeded and the situation allowed to continue for several minutes, the pinholes are widened to sizable slits and may finally lead to a short circuit and significant damage. This is the main reason why direct insulation of the plates by coating was rejected. Damage to the insulation in this case means a tedious and expensive repair, putting the equipment out of use for some time.

Electrical power unit

The power requirements, rather than the maximum attainable voltage, determine the design. The convenience of working with wide strips of paper entails the need

for a powerful electrical supply, as the current consumption increases proportionally with the width of the strip. A compromise solution has been found whereby an effectively high voltage could be produced together with the sufficient amount of current required for use with buffer systems of varying ionic strength, composition and degree of dissociation.

The power unit, the circuit diagram of which is shown in Fig. 4, is capable of supplying 500 mA at 5 kV, although in practice a current consumption of over 350 mA would present some difficulties in heat dissipation.

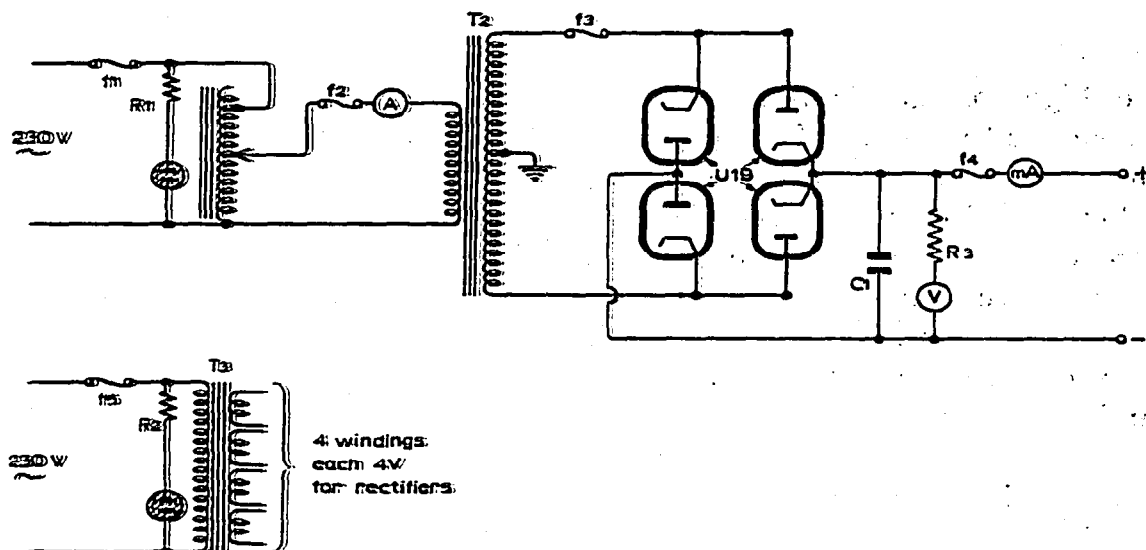


Fig. 4. Circuit diagram for a 5 kV power unit. Meters: A = 0-10 A, A.C. 50 \sim ; mA = 0-500 mA, m/c, V = 0-5000. Resistances: R₁ = 220 k Ω ; R₂ = 220 k Ω ; R₃ = 5 M Ω 10 W. Transformers: T₁ = 100R Variac; T₂ = 230 V in, 2250 ϕ 2250 V, 500 mA out; T₃ = 230 V in, 4 V 4A, four times, high insulation. Condenser: C₁ = 3 mfd, 8000 V, D.C. Fuses: f₁ = 10A; f₂ = 10A; f₃ = 500 mA; f₄ = 500 mA; f₅ = 1A.

A stand-by unit of a maximum rating of 150 mA, 10 kV has in the past proved to be insufficient for experiments using strips wider than 8 in., even in weak electrolyte solutions such as used for the separation of amino acids and peptides.

The ratings of the component parts given in the diagram are not necessarily the best, but were often selected on account of availability and convenience of price. A Variac of a higher output rating would be advisable. The rectifying valves, transformers and condensers have performed well over more than two years and only few replacements were required.

A special smoothing unit was tested but finally omitted as not essential. The set of fuses placed in several critical positions in the circuit has proved to be ample protection against sudden damage. The power unit is, of course, carefully earthed.

RESULTS

With efficient cooling, uniform pressure, and evaporation reduced to insignificant proportions, current constancy and thus steady conditions of temperature, ionic

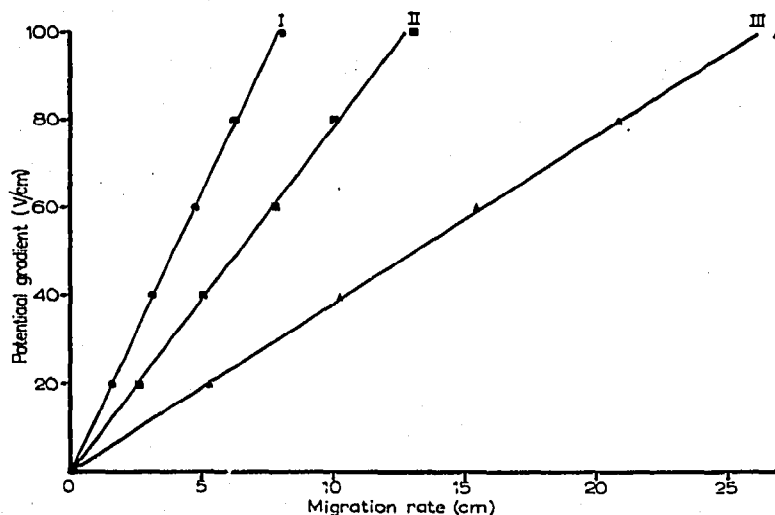


Fig. 5. Migration rates of amino acids as a function of potential gradient. Experimental conditions: Whatman No. 3 MM paper, $12 \times 22 \frac{1}{2}$ in., pH 2.0, 0.75 *M* formic acid solution, 10° , 20–100 V/cm, 1 lb./sq.in., 30 min. I = tryptophan; II = leucine; III = lysine.

strength, conductance, pH, moisture and potential gradient, are attained after a very short time (a few minutes). Under these conditions a linear relationship should obtain between migration rate and voltage applied, and likewise with respect to time of electrophoresis. The constancy of the current is a good indication of steady conditions prevailing in the paper strip. Figs. 5 and 6 show the results obtained, using the equipment at up to 5 kV and an energy throughput of up to 0.8 W/cm². The values plotted are migration rates corrected for the electro-osmotic and hydrodynamic effects established by measuring the displacement of various sucrose spots across the whole area of the strip. Cellophane barriers were used to minimise fluid flow.

The three amino acids chosen cover a wide range of mobilities. Linear movement with respect to potential gradient, in this case up to 100 V/cm, seems clearly establish-

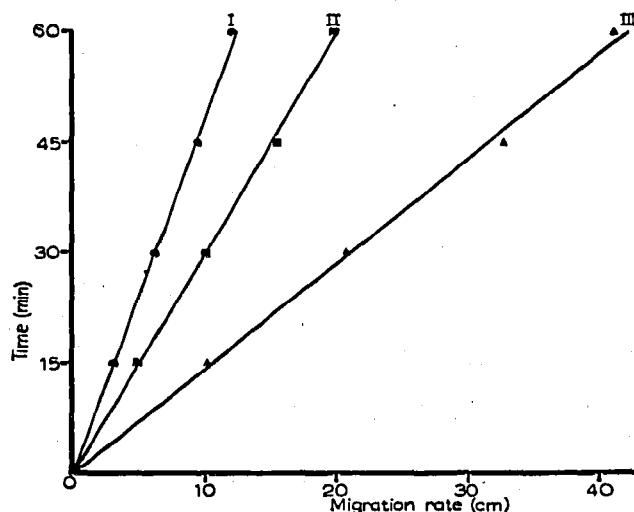


Fig. 6. Migration rates as a function of time. Experimental conditions: as in Fig. 5, 80 V/cm, 160 mA.

ed. In another series of experiments, using narrower strips and potential gradients up to 160 V/cm, a linear relationship could still be found.

The experiment carried out at 80 V/cm shows a substantially linear migration with time although the slight scattering of the later points on the lysine line may indicate some temperature disturbance near the cathodic end of the strip. The time limit of one hour is set by the length of the strip and the migration rate of the fastest compound. Distances linear with time can be assumed to indicate a uniform potential gradient along the length of the strip.

It would therefore appear that the conditions essential for the measurement of electrophoretic mobilities, even at high potential gradients, are amply provided by the general design of the apparatus, and that for ordinary separation experiments a high degree of reproducibility can be expected, even under less strictly controlled conditions. The latter has, in fact, been achieved in separations of complex mixtures of amino acids^{12,14}, of organic acids¹⁵ and aliphatic amines¹⁶ with a reproducibility for individual runs within 3%. The good reproducibility and high resolution aid substantially in the isolation and identification of unknown constituents.

DISCUSSION

There are several advantages in using high-voltage electrophoresis. Since the rate of diffusion is independent of the applied voltage and dependent on time, a significant reduction in the time of the electrophoretic run, for the same distance of migration, is bound to minimise the adverse effects on resolution of diffusion, particularly in the case of low-molecular weight compounds. The control of hydrodynamic flow in the strip over short periods is also much easier. The economy in time is substantial.

Attempts to apply high voltages are met, however, by a serious limitation, *viz.* the generation of heat. Efficient heat dissipation is the most important factor in solving the problem and any useful design of high-voltage equipment must be judged primarily by this criterion. The voltage and current level is limited by the heat-dissipating capacity of the apparatus.

Liquid heat exchangers can only dissipate energy¹ up to 0.2 W/cm² and have certain objectionable features such as evaporation of solvent with resultant fire and health hazards. Solid heat exchangers, on the other hand, are far more effective, provided they are good thermal and bad electrical conductors. However, as a rule, material of good thermal conductivity is also of good electrical conductivity. Glass is a good insulator but a poor heat conductor, especially when used in a thickness to impart sufficient mechanical strength. The same applies to plastics. Cooling plates made of plastics are probably able to cope satisfactorily with an energy throughput of up to 0.1 W/cm², depending on the design.

A combination of an excellent heat-conductor such as a metal plate with a very thin film of highly insulating plastic material such as Polythene (polyethylene) gives satisfactory results, particularly when the heated paper strip is compressed between two plates for good contact. An apparatus designed on this principle and

described above has proved to be of high cooling efficiency, making the dissipation of 1 W/cm^2 possible by using a flow of tap water of 12 l/min at 12° . This design makes it essential to have uniform, reproducible, moderate pressure exerted on the paper strip, since several important conditions such as moisture, conductance, uniform potential gradient and current consumption are governed by the pressure. The advantage of this arrangement over single-plate cooling is quite considerable. A recently described apparatus⁹ based on bottom plate (copper) cooling with insulating film and a refrigerated coolant, or water, was described as capable of dissipating only about 0.1 W/cm^2 .

It is often desirable to use the highest possible potential gradient allowed by the heat-dissipating capacity in order to obtain the maximum benefit from the technique. Very small differences in mobilities are insufficient for separations, unless a fairly large distance is travelled within a short time. Good examples to prove this point were the separation of sorbitol from mannitol¹⁷ and of the alkali metal ions K, Rb, Cs from one another¹⁸ carried out on a similar, though smaller apparatus.

The present dimensions, though suitable both for analytical and preparative work (using a thick sort of filter paper), impose certain limitations on the important two-dimensional work. It is thought that square cooling-plates, $22 \times 22 \text{ in.}$, would be more convenient, though the power requirements may present a difficult problem.

The method of using a coolant refrigerated to a temperature of just above 0° , as stipulated by designs employing glass or plastics for solid heat-exchangers, is not without serious disadvantages. Although the cooling is improved by the lower temperature of the coolant, the viscosity of the electrolyte solution is increased and the mobilities of the ions are significantly reduced. Consequently, the migration time or the applied voltage will have to be increased to produce the same path length within the given time of experiment, thus reducing the intrinsic value of the technique. Experience over several years with tap water has given convincing proof that, even with seasonal temperature fluctuations from $5\text{--}20^\circ$, water provides an inexpensive, ample and convenient means of cooling. It is, of course, necessary to compare mobilities at the same temperature, and for this purpose provision for measuring the temperature of the water entering and leaving the cooling system has to be made. The estimated temperature in the strip is about $1\text{--}2^\circ$ higher, as measurements with inserted thermocouples have indicated.

The smooth performance of the apparatus is also dependent on the dielectric strength of the insulating film under the experimental conditions. At present, using Polythene film, it is found that occasional breakdowns due to overheating, and mechanical wear at the edges of the cooling plates (mostly the anodic side) cannot be entirely eliminated. Occasional flaws in the material may also contribute to it. It is, however, hoped that in the not too distant future improvements in the quality and dielectric strength, or the appearance of new plastics, will considerably reduce the risk of insulation failure.

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SUMMARY

1. An apparatus for high-voltage (> 50 V/cm) paper electrophoresis has been developed, with efficient cooling and high degree of reproducibility of results as the major features of the design.

2. A sandwich-type with double-cooling of the paper strip by a system of thinly insulated solid heat-exchangers (aluminium alloy plates), with tap water as coolant, was found most efficient with respect to heat-dissipating capacity.

3. A special double-countercurrent channelling system in the plates minimises the possibility of temperature gradients.

4. Polythene (polyethylene) film of 0.01 in. thickness has proved itself as a suitable, convenient and inexpensive insulation.

5. The apparatus is provided with a pneumatic uniform-pressure device for evenness of cooling and control of moisture.

6. A lifting device is incorporated for convenient handling and safety devices ensure both the safety of the operator and the prevention of damage to the equipment.

7. The results obtained at potential gradients of 100 V/cm and energy throughputs of up to 1 W/cm² show a straight-line relationship of migration rate with respect to potential gradient and time. The degree of reproducibility of individual runs is high and usually within 3%. The steady-state conditions obtainable in the apparatus make the measurement of migration rates, even in background electrolyte solutions of relatively high ionic strength (> 0.2), both convenient and reliable. Difficult separations of complex mixtures of various groups of low-molecular weight compounds such as amines, amino acids, sugars, inorganic cations, organic acids etc. can be accomplished with a high degree of resolution within a comparatively very short time.

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