amount of reduction may be controlled and generally it is kept close to the average percentage mentioned.

Reference

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Simplified Preparative Electrophoresis at Room Temperature[1]

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Many attempts have been made to adapt the separation of electrically charged ions like proteins to simple equipment operable at room temperature. Membranes, sand barriers, gels, and jellies have been used to immobilize the material and thus prevent convection from mixing the protein in a solution, P, with the adjacent buffer solution, B. Tiselius (*1*) points out that aside from optical observations his method increases the potential gradient in the **U**-tube without undesirable heat convection, simplifies sampling, and avoids disturbing electrolytic processes.

The equipment for the preparative method described here costs less than $50. It suitably embodies the advantages of the Tiselius method just mentioned, and is especially adaptable for use at room temperature without any temperature control. The technique employs a modification of the classical **U**-tube with large side vessels for electrodes (carbons or reversible electrodes). Tail-hole stopcocks are used for withdrawing samples at the side, or a two-way stopcock at the bottom of the **U**-tube is employed for forming the boundary and draining the electrophoretically purified fractions from either side of the **U**-tube. The method depends upon controlling solutions B and P so that they will have different densities, viscosities, pH's, and conductances as follows: solution B (supernatant) will have a high coefficient of viscosity at the pH of the isoelectric point of the protein contained in solution P, to be immobilized in the lower part of the **U**-tube; solution B will have a density considerably less than solution P, so that as the boundary P-B is formed, a well-defined boundary, stable enough for preparative electrophoresis results. Typical solutions used by the writer and his co-workers (*2*) for the past year for the separation of trifidin and artefolin (*1*), the unpigmented fractions of giant and dwarf ragweed extracts, have been: B = 40% glycerol at pH 6.8; P = 50% glycerol extract of ragweed pollen. The electrical conductance of solutions B and P in the separation should be regulated so that as little electrolyte as convenient is in P, with a suitable amount in B, to control the pH and drop in potential at the beginning of the separation. For example, in the case of ragweed extracts, no saline was added to the ragweed to be fractionated in solution P. The 40% glycerol in B contained M/15 phosphate buffer at pH 6.8. Thus initially the main drop in potential was across the material to be separated and fractionation was facilitated. A striking experiment demonstrating this technique may be made by having solution B at about pH 6.8 employing M/15 phosphate buffer in 40% glycerine, with solution P, a mixture of hemoglobin and T-1824 (a negatively charged dye), in 50% glycerine. At this pH, the isoelectric point of hemoglobin is electrophoretically fixed at the boundaries, whereas the dye T-1824 migrates out of the mixture to the anode, leaving the hemoglobin with a fairly sharp boundary at the negative side after 24 hr.

It may be emphasized that our method utilizes Newtonian or truly viscous liquids. The convection ordinarily resulting from the application of 450 volts to a **U**-tube, 60 cm long with an internal diameter of 1.0 cm, at room temperature is avoided by the use of only high viscosities, not plasticities. No membranes, jellies, or similar mechanical devices are required. The temperature in the laboratories at Cold Spring Harbor during the summer of 1948 varied considerably over 24 hr but did not disturb the boundaries for preparative purposes during 4-day electrophoretic separations. The apparatus can be readily used in an ice chest. If room temperature does not destroy the material to be studied, it is preferable not only because of simplicity but also because the mobilities are greater at this temperature.

TABLE 1

COMPARISON OF FORMATION OF CLASSICAL TISSUES BOUNDARIES AND THOSE OF THE MODIFIED TECHNIQUE

Tiselius method	Method reported herein
Conductance B* = conductance P†	Conductance B >> conductance P
Viscosity B = viscosity P	Viscosity B < viscosity P
Density B = or slightly less than P	Density B < density P
pH of B = pH of P	The pH of B at or close to isoelectric point of protein to be separated in solution P
Temperature controlled at or near 4° C	Room temperature fluctuations—no temperature control

* Supernatant solution.
† Lower protein solution. Note that both P and B have Newtonian flow.

Table 1 summarizes the differences in preparative procedures with the classical method of Tiselius and the method herein described.

The fractionation experiments based upon this technique will soon be reported in detail (*2*). The early experiments were done with C. Reiter and M. Loebl.

References

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