Recently it has been shown that s-RNA is a compact macromolecule of 100 Å length and 20 Å diameter[8,9]. The shape is represented by an ellipsoid of axial ratio 6:1 (ref. 9) and the volume is of the order of 30000 Å^3. For a spherical and unhydrated macromolecule of 30000 Å^3, one can estimate a relaxation time (ρ_h) equal to $0.22 \cdot 10^{-7}$ sec at 20° by Formula 3.

$$\rho_0 = \frac{3\eta V}{kT} \tag{3}$$

The difference between that value ($0.22 \cdot 10^{-7}$ sec) and the experimentally found one ($0.28 \cdot 10^{-7}$ sec) indicates a departure from the spherical symmetry which should be expected for an ellipsoid of revolution of axial ratio 6:1. On the other hand hydration of RNA in solution which is not taken into account by Formula 3 will also tend to increase the relaxation time.

The fact that the sedimentation behavior of RNA–dye complex ($s^0_{20,w} = 3.6$ S) was very similar to that of s-RNA ($s^0_{20,w} = 3.65$ S), indicates that little or no structural change was associated with the incorporation of the dye.

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1 F. PERRIN, *J. Phys. Radium*, 7 (1926) 390.
2 G. WEBER, *Biochem. J.*, 51 (1952) 145.
3 J. CHURCHICH, *Arch. Biochem. Biophys.*, 97 (1962) 574.
4 R. W. HOLLEY, J. APGAR, B. P. DOCTOR, J. FARROW, M. A. MARINI AND S. H. MERRILL, *J. Biol. Chem.*, 236 (1961) 200.
5 P. C. ZAMECNIK, M. L. STEPHENSON AND J. F. SCOTT, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 811.
6 W. L. GLEN, N. M. J. SUTHERLAND AND F. J. WILSON, *J. Chem. Soc.*, (1936) 1484.
7 Y. SVESKNIKOV, P. I. KUDYASHOV AND L. A. LOMAREVA, *Opt. i Spektroskopiya*, 9 (1960) 107.
8 M. SPENCER, W. FULLER, M. H. F. WILKINS AND G. L. BROWN, *Nature*, 194 (1962) 1014.
9 S. W. LUBORSKY AND G. L. CANTONI, *Biochim. Biophys. Acta*, 61 (1962) 481.

Received April 29th, 1963

Biochim. Biophys. Acta, 75 (1963) 274–276

SC 2300

A simplified apparatus for vertical gel electrophoresis

Zone electrophoresis has been widely used for investigation of biologic compounds, in particular proteins, by virtue of its adaptability—for example, a variety of media can be used to stabilize the moving boundaries of the convection currents of the electrolyte solutions. SMITHIES[1] described a technique of zone electrophoresis in starch gel that combines advantages of low adsorption characteristics of starch, gel filtration, and the additional convenience of detection of substances by a number of procedures. A new synthetic medium for zone electrophoresis, polyacrylamide gel, was reported to be effective in resolving components of serum proteins and to have certain advantages over other media[2,3]. Although the technique of preparation of the polyacrylamide gel is relatively easy, attention to detail must be maintained, since variations in amount of catalyst, pH, and ionic strength of buffers greatly affect the formation of the gel. Further, the apparatus should be air-tight and the gel in close contact

with cooling plates, since adequate cooling during electrophoresis is necessary. The principle and application of the method may be found elsewhere[3].

This report describes a simple and easily constructed apparatus for vertical gel electrophoresis using polyacrylamide gels, somewhat similar to that used by SMITHIES[4]. This model has proved convenient for resolution of proteins on a preparative scale.

The assembled apparatus is shown in Fig. 1 and the three basic components (I, II and III) formed with plexiglass are illustrated in the diagram, Fig. 2. Strips of plexiglass were placed along the edges of cooling plate of I to form a tray into which the gel solution can be poured prior to polymerization. This is usually done with the I and III components assembled (AA) and laid horizontally. The multiholed rectangular plate at the end of the tray I provides support for sponge strips. In the

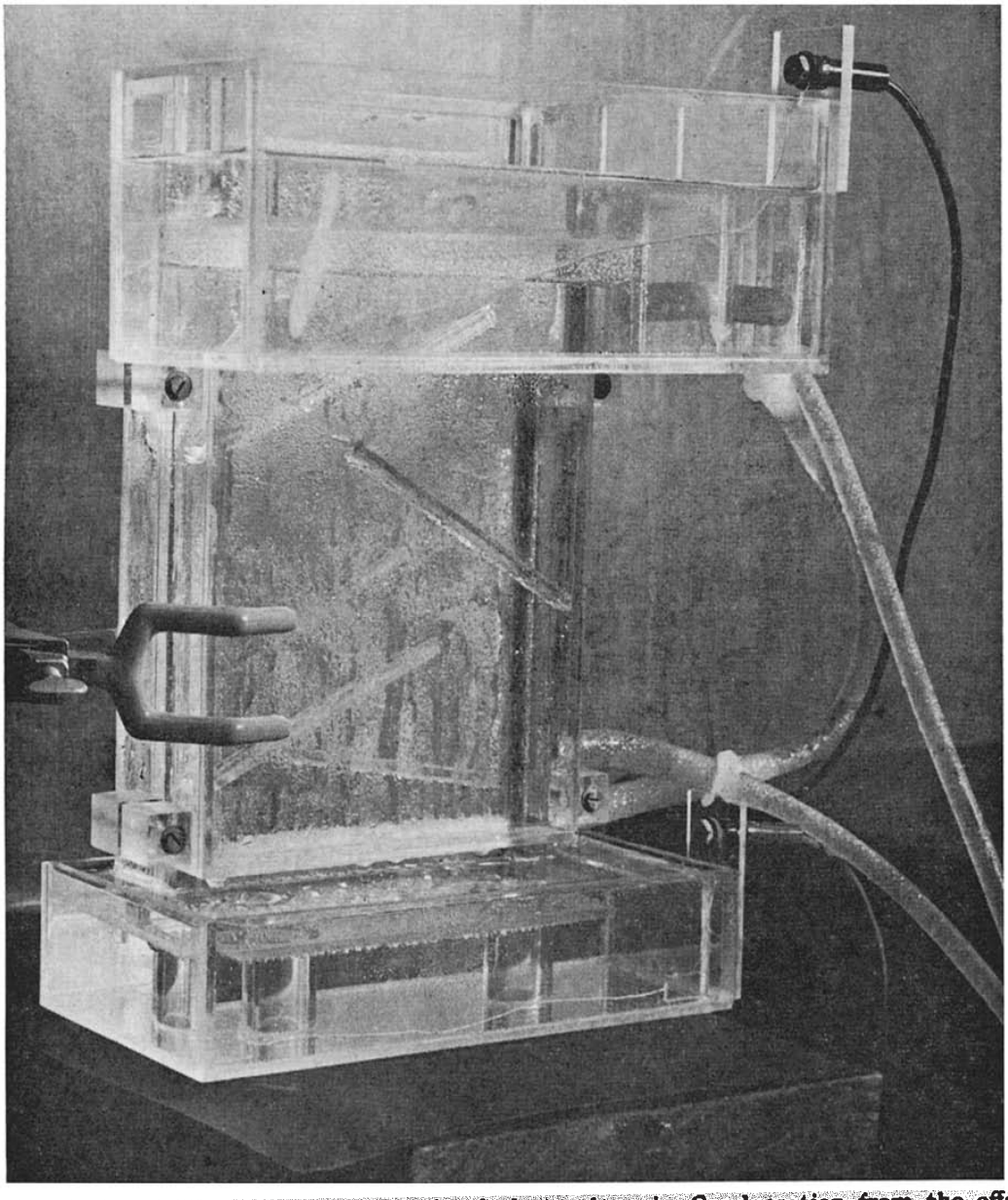

Fig. 1. Assembled apparatus for vertical gel electrophoresis. Condensation from the effects of cooling is evident on the surface of the plastic and the tubing which carry circulating cold water. The slanted baffles are to reduce streaming of circulating water.

vertical position the apparatus rests on a rectangular plate which extends from the bottom edge of cooling plate I. The rubber gaskets (shaded in diagram) on each side of the gel tray I and plexiglass strips cemented on the inner surface of cooling plate III, shown in insert AA, provide a tight seal, which can be adjusted by four screws inserted into the lateral rectangular blocks (shaded in diagram). The bottom buffer reservoir, II, contains two blocks for support of the gel tray. Cooling plate III has an upper buffer reservoir which maintains continuity with the upper edge of the gel. The slot former, IV, shown for one large sample application, is a rectangular plate with a lip along one edge (section B). A longitudinal slot former cemented to plate III can be used for immunodiffusion studies. The dimensions given in Fig. 2 are not critical, although a model of this size has been found useful for preparation and isolation.

The assembled apparatus is laid horizontally and the space between plates I and III is completely filled with the gel solution without air bubbles. The slot former is immediately inserted vertically across the space at the top of the cooling plate III, with the lip projecting into the space between the two cooling plates. When the gel

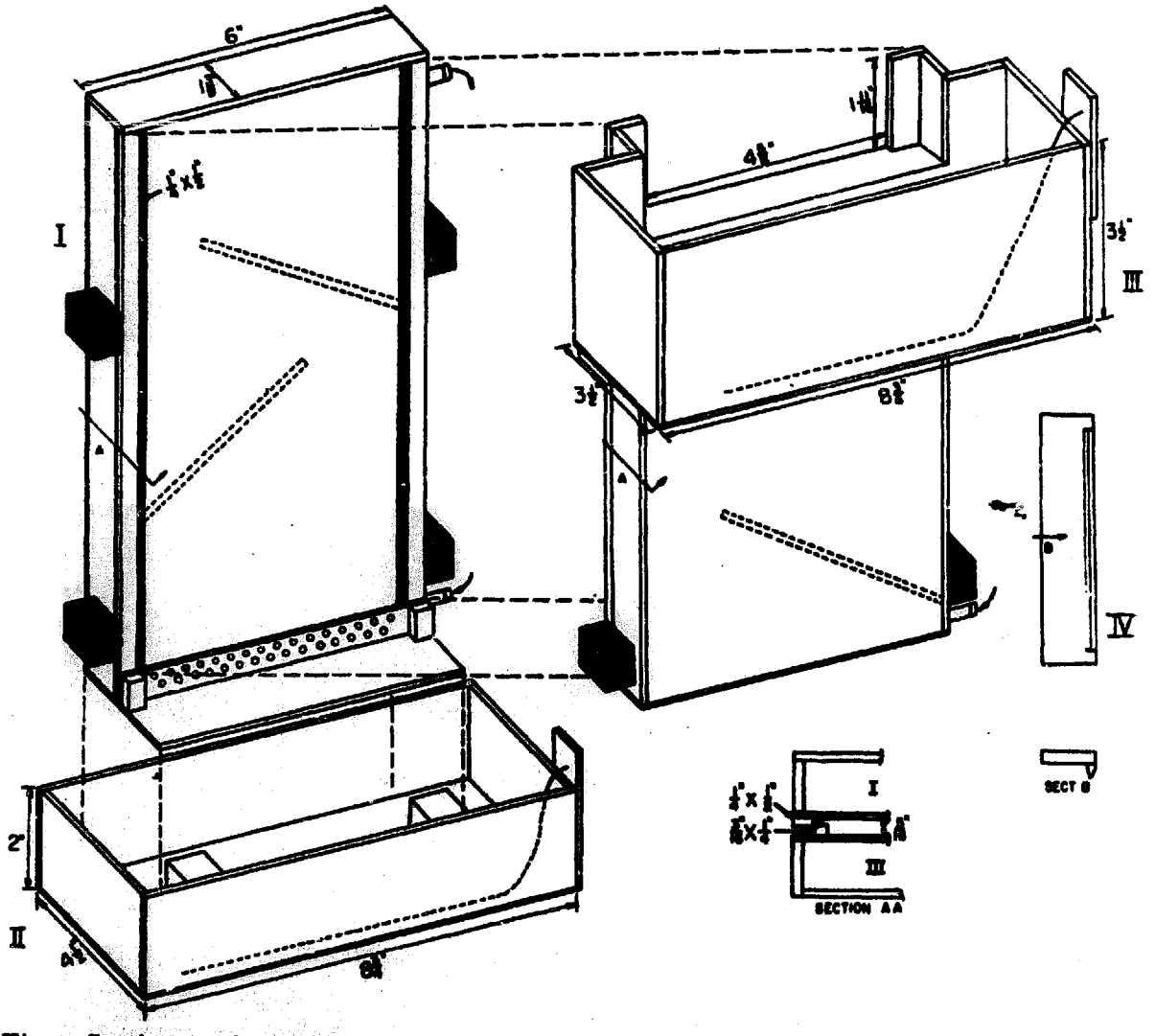

Fig. 2. Sections of the apparatus for gel electrophoresis in diagram. The dimensions are expressed in inches due to availability of plastic in this scale. Sections I and III are cooling plates and form the tray for the gel. Section I has two longitudinal strips of rubber tubing which act as a gasket, as in AA. Section IV, B is the slot former for preparative work. This can be redesigned for multisample technique.

has set, the ungelled solution behind the slot former is aspirated and discarded. The apparatus is then carefully placed on the stand in the bottom buffer reservoir without disturbing the gel, and cold water is circulated through the cooling plates. The slot former is slowly removed and the slot is cleaned with a fine spatula to eliminate any threads of gel remaining in the slot. After insertion of the sample the electrodes are connected to a power supply and electrophoresis is begun. On completion of the experiment the gel may be sliced or stained as such with a suitable dye (amido black 10 B). For preparative work, marker strips are sectioned longitudinally from the center and the lateral margins, and are stained. The background of the marker strips is destained and the strips are approximated to the unstained portions of the gel. The latter are then sectioned into selected horizontal segments. The fractionated material can be isolated from the unstained gel sections by extraction with buffer in a tissue grinder, centrifugation, and dialysis.

Electrophoretic patterns of bovine serum albumin obtained with the apparatus described were similar to those reported with commercially available equipment[5]. 50–150 mg of protein mixtures can be fractionated on the apparatus and approx. 65–70 % of the material recovered.

The apparatus has also been used successfully to resolve and isolate a family of four glycoproteins obtained from bovine aorta from material presumably "homogeneous" after repeated zone electrophoresis on starch blocks[6]. Glycoproteins in the polyacrylamide gum have been stained with PAS, and distinguished from other types of proteins.

Supported by grants from the United States Public Health Service (H2942) and the Strickland Memorial Fund.

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[1] O. SMITHIES, *Biochem. J.*, 61 (1955) 629.
[2] S. RAYMOND AND L. WEINTRAUB, *Science*, 130 (1959) 711.
[3] S. RAYMOND AND YI-JU WANG, *Anal. Biochem.*, 1 (1960) 391.
[4] O. SMITHIES, *Biochem. J.*, 71 (1959) 585.
[5] W. J. EVANS, W. B. CARNEY, J. M. DECHARY AND A. M. ALTSCHUL, *Arch. Biochem. Biophys.*, 96 (1962) 233.
[6] B. RADHAKRISHNAMURTHY, A. F. FISHKIN, G. J. HUBBELL AND G. S. BERENSON, *Federation Proc.*, 22 (1963) 2252.

Received March 16th, 1963

SC 2275

Thermal transitions in different mammalian collagen-bearing structures

Collagen always occurs naturally in association with small quantities of non-collagenous protein and varying percentages of mucopolysaccharide, especially hyaluronic acid and the chondroitin sulphates. In its purest form, tendon, there is less than 1 % by dry weight of polysaccharide. Mammalian skin contains about 1 % polysaccharide but approx. 20 % lipid, again on a dry weight basis. At the other extreme