observed even after 2706 minutes of electrophoresis[6] (Fig. 3). Hence, it was possible to run the electrophoresis from two to five times longer in time without convection, with a corresponding increased resolution and multiplication of yield. Throughout the experimental runs, the sucrose–protein boundary near the bottom of the descending limb was observed to broaden upwards. Apparently, this is caused by diffusion and the electrophoretic "pile-up" arising from the decrease of the mobilities of the descending anions advancing into the sucrose boundary. In practice, the "pile-up" entering the solution to be isolated limits the duration of the run and the electrophoretic resolution attainable. However, by initially increasing the distance between the sucrose–protein gradient and the starting descending protein boundary, as is possible in the larger preparative cells, this limit in time and resolution may be still further greatly extended.

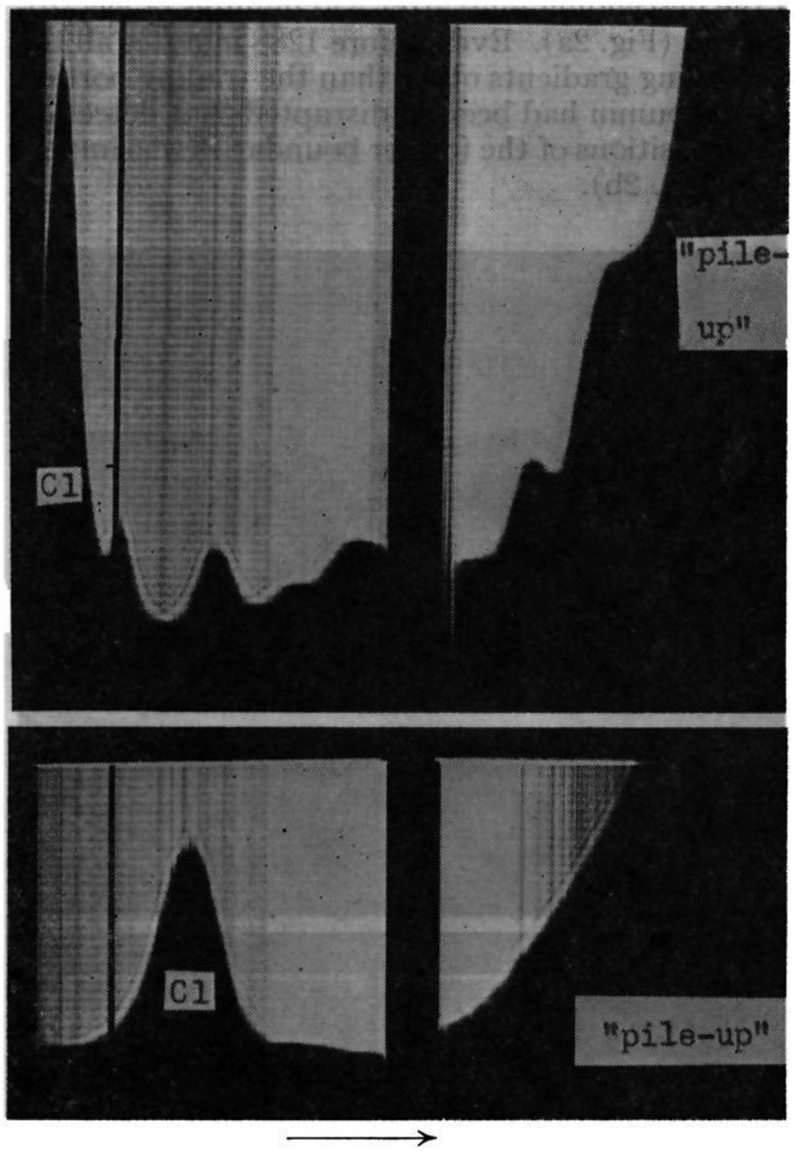

Fig. 3.—Descending patterns showing no convection in electrophoresis using the single sucrose gradient: (a) upper, 530 min.; (b) lower, 2706 min. (compensated boundaries as in text).

At the time of maximum resolution and yield (2706 minutes), the boundaries of the sucrose-containing runs were compensated to exclude all but the lagging portion of the slowest protein (conalbumin 1) from the top half section of the descending limb (Fig. 3b). This isolated solution had a volume of 3.2 ml. and a protein concentration of 0.6%.

In order to test the purity of the isolated conalbumin 1, the removed solution was dialyzed at the same concentration against sodium phosphate buffer, 0.1 $\mu$, *p*H 5.80 and analyzed in the 2-ml. open cell of the Perkin–Elmer electrophoresis apparatus, Model 38.[7] The isolated conalbumin 1 exhibited an average mobility of $-0.24 \times 10^{-5}$ cm.$^2$/volt/sec. (*cf.* reported values[4,8]), and appeared electrophoretically homogeneous under these conditions (Fig. 4).

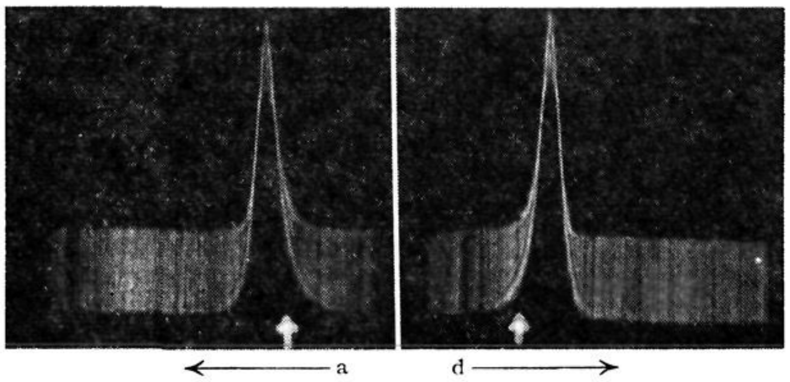

Fig. 4.—Electrophoretic analysis after 240 min. at 6.4 volts/cm. of conalbumin 1 isolated by preparative single sucrose gradient electrophoresis. Vertical arrows indicate starting boundaries.

An interesting example of the role of a large, steep gradient was encountered during the electrophoretic isolation of $\gamma$-globulin from human serum, where the $\beta$-anomaly spike acted as an effective convection barrier.

In summary, a modified method of descending boundary preparative electrophoresis has been presented. Involved is the use of a single, large, steep sucrose gradient as a barrier to the convection arising from the entrance of descending protein boundaries into the bottom section of the electrophoresis cell. The procedure permits substantially increased electrophoretic resolution, with corresponding gains in the yield and potential purity of the isolated fraction.[9]

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[6] In order to avoid the entrance of sodium chloride from the electrodes into the cell, it was necessary at 1400 minutes to move temporarily the cell out of alignment and to refresh the solutions in both buffer vessels.

[7] Manufactured by Perkin–Elmer Corp., Norwich, Conn.

[8] J. A. Bain and H. F. Deutsch, *J. Biol. Chem.*, **172**, 547 (1948).

[9] Recent experiments have attested to the analytical value of the new method. The prolonged duration of the electrophoresis of mixtures thus made possible permits the discovery of slow components which are unresolved by conventional electrophoresis.

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## Purification and Manipulation of Samples of Alkali Metals[1]

BY GEORGE W. WATT AND DONALD M. SOWARDS

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Frequent need for the preparation of a considerable number of standard solutions of alkali metals (or alkali metal amides) in liquid ammonia has led to the development of a simplified procedure for the simultaneous preparation of several samples of twice distilled metal contained in fragile Pyrex glass ampoules. The method is simple, rapid and

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[1] This work was done in connection with studies supported in part by the Atomic Energy Commission, Contract AT-(40-1)-1639.

adaptable to the preparation (in Pyrex) of small samples of any thermally stable substance that distils *in vacuo* at temperatures not greatly in excess of 500°. By use of Vycor, presumably higher boiling substances could be handled similarly.

### Experimental

**Apparatus.**—The distillation unit shown in Fig. 1 (I) is constructed of Pyrex glass; the seal at D is indented on both sides to leave a slit about 1 cm. wide connecting B and C. The assembly is connected to a vacuum system and inert gas supply *via* a two-way stopcock and ground glass joint attached at I. The storage ampoules are shown in Fig. 1 (II); they are made by pulling 5–7-mm. Pyrex tubing to capillary size (J), blowing a thin-walled bulb (K), and making a U-bend near L.

**Procedure.**—For the distillation of potassium, for example, 1–1.5 g. of the commercial product immersed in xylene is cut into cubes which will pass through D. These are placed in the boiler A via I–G–C–D–B. Six (or more) weighed, dry ampoules are placed in H so that the tips J rest at the bottom of F. The unit is then attached to the vacuum line and pumped at *ca.* 0.03 mm. pressure for 1 hr. Heat is applied with a low flame until the metal is melted; if, during outgassing, the metal is heated too rapidly, it spatters vigorously and may bump out of the boiler. Low-boiling impurities may now be removed to G; otherwise, the potassium is distilled to B. While A is kept warm, metal at B is redistilled to C. By warming CE and tapping the unit gently, the metal at C runs into the filling well F. With the metal in F molten, the connection to the vacuum line is closed and dry oxygen-free nitrogen, helium or other suitable inert gas (supplied from a tank or high-pressure ballast) is admitted until the pressure is just in excess of atmospheric pressure. This operation must be performed rapidly in order to prevent solidification of the metal in the capillary tubes. After cooling, the bulbs are removed and sealed off at L. If metal remains in J, it may be removed with alcohol. By weighing the bulb and stem, the potassium content is determined by difference.

**Results.**—In three typical cases, weights of potassium contained in ampoules and determined as described above were found to be 76, 55 and 49 mg. These samples were dissolved in liquid ammonia to provide solutions having concentrations of the order of 50–75 millimolar and were titrated potentiometrically[2] with standard ammonium bromide solution. The weights of potassium calculated on the basis of the titration data were 74, 53 and 48 mg., respectively.

The alkali metal samples prepared in this manner appear to be entirely free of oxide and/or other contaminants. Over a period of 12 hr., one may prepare as many as two dozen ampoules of metal and devote only about 4 hr. to the actual manipulations. Although slight variations in sample weights usually result, this is sometimes advantageous when it is desired to select specific weights of metal. By carefully matching the capillary (J) diameters, the weights of metal in bulbs from a multiple filling operation have been held constant to within 10 mg. Alternatively, a spread of weights over a desired range may be realized.

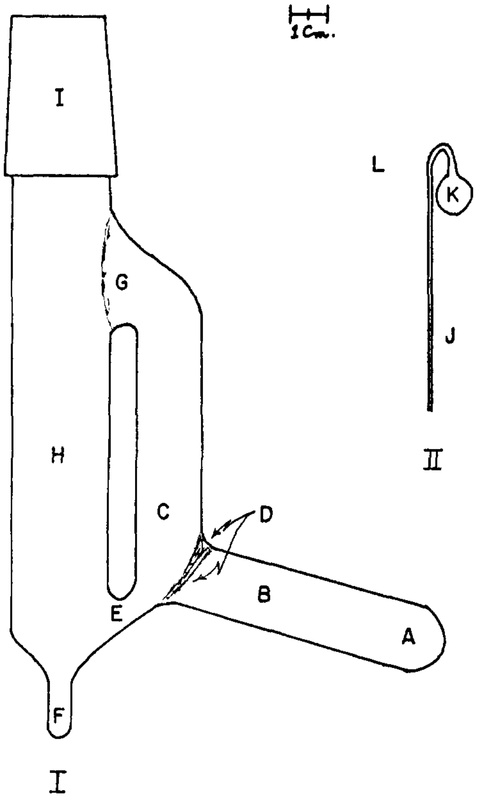

Fig. 1.—Apparatus for the purification of alkali metals.

In addition to alkali metals (other than lithium), samples of cadmium have been prepared satisfactorily by this procedure.

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[2] G. W. Watt and J. B. Otto, Jr., *J. Electrochem. Soc.*, **98**, 1 (1951).