these regions appear to migrate along the surface of the adsorbent. This latter phenomenon was not observed in columns filled with weakly adsorptive, impervious materials such as sand and small glass beads.

The width of the bands of adsorbed substances formed in columns by electrophoresis depends upon the quantity of pigment present and upon the adsorptive capacity of the adsorbent. In experiments at room temperature with large columns, the bands of migrating pigments always exhibit well-defined boundaries throughout the mass of the adsorbent. The adsorbent thus serves to prevent mixing of the colored substances as well as to aid in their separation.

A pronounced electro-osmotic effect has been observed in all the experiments carried out thus far. Since the water usually moves toward the cathode, this electrode is placed at the top of the column so that the head of water may be adjusted to balance the electro-osmotic pressure. Passage of the water through the adsorbent also may be regulated, as customary in the Tswett method, by application of suction to the base or of pressure to the top of the column.

A great number of colored, water-soluble, organic substances such as nitro, hydroxy, amino, azo, carboxy and sulfonic compounds can be isolated from mixtures by electrophoresis upon Tswett columns. Combination of colorless organic compounds with colored, water-soluble radicals before adsorption would doubtless facilitate the separation of mixtures of colorless substances by the electrophoretic and chromatographic methods.[2]

Thus far, compounds separable by electrophoresis but inseparable by chromatographic adsorption have not been found. However, with columns of the same size and with equal quantities of pigments, electrophoresis often produces greater separation of the pigments than percolation alone.

The mixtures separated on Tswett columns by electrophoresis (the first compound named in each group remaining nearest the cathode) were: aminoazobenzene and indigo carmine, 3-nitro-4-aminoanisole and indigo carmine, methyl orange and methyl red, methyl orange and 2,6-dichlorophenol indophenol, methyl orange and methyl red and indigo carmine, picric acid and methyl orange. For the separation of these compounds, potentials ranging from 175 to 200 volts and

currents from 0.5 to 2 m. a. were used with columns ranging in size from 2 by 13 cm. to 3 by 23 cm. The quantity of each pigment adsorbed varied from 2 to 15 mg.

From weakly acid solutions, two forms of the indicators were separated on Tswett columns both by chromatographic adsorption and by electrophoresis. The forms present in acid solutions were much more strongly adsorbed than those present in weakly alkaline solutions. Indicators adsorbed from acid solutions were gradually converted into the isomers observed in alkaline solutions upon prolonged washing of the columns with water or upon extended passage of the electric current. These methods provide another means for investigation of the tautomerism of indicators.

Resolution of mixtures by adsorption upon Tswett columns depends upon the properties of the adsorbent, of the solvent and of the compounds in the mixture. Since the properties of the adsorbent vary with the method employed in its preparation and since the properties of the solvent are altered by small quantities of impurities, considerable experience is often required to adapt this effective adsorption technique to the isolation of new compounds from mixtures.

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The Temperature of Maximum Density of D$_2$O and of its Mixtures with H$_2$O

BY ELIJAH SWIFT, JR.

The temperature of maximum density of deuterium oxide has been reported as 11.4[1] and 11.6°,[2] the latter being the generally accepted value. This measurement was made on a sample of only 0.12 ml. volume in a small dilatometer, and the temperature taken as the temperature of maximum density was apparently that at which the meniscus was at its minimum position in the capillary. A temperature obtained in this manner is not the true temperature of maximum density, but what might be termed *the apparent temperature of maximum density in glass*, the difference between the two amounting to about 0.5° in the case of Pyrex.[3] It was hoped that a rede-

(2) H. H. Strain, THIS JOURNAL, **57**, 758 (1935).

(1) T. Takeuchi and T. Inai, *Japan J. Phys.*, **11**, 67 (1936).

(2) G. N. Lewis and R. T. Macdonald, THIS JOURNAL, **55**, 3057 (1933).

(3) "Pyrex" brand chemical glass.

termination of the true temperature of maximum density might allow a definite and significant value to be assigned to it.

The measurements necessary to determine this value were made in a 20-ml. pycnometer with a capillary of about 1 mm. diameter, with a single very fine scratch on the exterior.[4] The technique of the measurements consisted in filling the pycnometer to a point just above the mark and measuring the height of the meniscus above the mark at a series of temperatures close to the temperature of maximum density. The measurements were made with a travelling microscope reading to 0.005 mm., and the temperatures were measured with thermometers calibrated at the icepoint and at 5 and 11° against Bureau of Standards calibrated thermometers kindly loaned by Professor Grinnell Jones.

The observed heights of the meniscus were plotted against the temperature and a smooth curve drawn. This was then corrected for the thermal expansion of the glass,[5] the resulting curve then showing a minimum at the true temperature of maximum density. In order to check the accuracy of this method the temperature of maximum density of distilled water was measured. This was found to be 3.85 ± 0.1°, somewhat lower than the accepted value of 3.98, the difference probably being due in part to the presence of dissolved air, carbon dioxide, etc., since

TABLE I

TEMPERATURES OF MAXIMUM DENSITY OF D_2O–H_2O MIXTURES

N_{D_2O}	T	$T_{apparent}$	d^t_4
0.9985	11.1 ± 0.1	11.65 ± 0.1	1.10577
.9959	11.1	...	1.10522
.9353	10.8	...	1.09909
.7780	9.7	...	1.08264
.6051	8.7	9.2	1.06411
.3784	7.0	7.5	1.04019
.1866	5.6	...	1.01978
.0002	3.85	4.3	1.00000

(4) E. Swift, Jr., THIS JOURNAL, **61**, 198 (1939).
(5) This correction was obtained by finding the volume of the pycnometer at a series of temperatures and calculating the effect of the thermal expansion of the glass on the height of the meniscus from the volume of the capillary per unit length.

no attempt was made to remove all traces of dissolved impurities.

The temperature of maximum density of two different samples of nearly pure deuterium oxide was determined. The first was purified by chemical means and by distillation[4] and the second, loaned by Dr. Fred Stitt, was prepared from deuterium gas and atmospheric oxygen. The temperature of maximum density of the two samples was 11.1 ± 0.1°, there being no detectable difference between the two. It is probable that the temperature of maximum density of pure D_2O is not more than a few thousandths of a degree above this value. The temperature at which the meniscus was at its minimum position was 11.65°, in close agreement with the value of 11.6° reported by Lewis and Macdonald[2] as the temperature of maximum density.

The temperatures of maximum density of five mixtures of light and heavy water were also found by the same method. These mixtures behaved in the same manner as the pure components as far as could be told from these measurements and all showed temperatures of maximum density, the apparent temperature of maximum density being about half a degree above the true value in every case investigated. While the temperatures of maximum density of the mixtures were intermediate between those of the pure components, there was not quite a linear relationship between temperature and mole fraction, the temperatures being from 0.2 to 0.5° above the line.

The maximum densities of these mixtures were also measured and are included in the table.

Stokland, Ronaess and Tronstad have just published [Trans. Faraday Soc., **35**, 312 (1939)] a value of 11.23 ± 0.02° for the temperature of maximum density of D_2O as the result of a much more precise investigation than this one. While the value which they found is somewhat higher than the value obtained here, it is barely outside the limits of experimental error, and both results are in agreement in indicating that the results of earlier investigations were much too high.

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