

then sublimed in a vacuum of 0.1 mm. at 160°. A white crystalline sublimate was recovered, m. p. 170.8–171°. The yield was over 90% of the theoretical.

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### Summary

By the use of radioactivity measurements, the vapor pressure of thorium acetylacetonate has been obtained at 100° as  $3.2 \pm 0.3 \times 10^{-4}$  mm. The gas-saturation method was employed, nitrogen being used, and from the latter the thorium compound was extracted and converted into thorium chloride by alcohol acidified with hydrochloric acid. A basic thorium chloride deposit was made on a platinum foil, and by means of an alpha count of this deposit, the molar quantity of sublimed thorium acetylacetonate was obtained.

CAMBRIDGE, MASS.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

## The Density of Pure Deuterium Oxide

BY HERRICK L. JOHNSTON

Tronstad and Brun<sup>1</sup> recently reported a value of  $d_{25}^{25}$  1.10750  $\pm$  0.00001 for the specific gravity of pure D<sub>2</sub>O at 25° and Swift<sup>2</sup> refers to a private communication from one of Tronstad's associates which revises this to  $d_{25}^{25}$  1.10764. Either of these figures is considerably below the value generally accepted at present, namely,  $d_{25}^{25}$  1.10790  $\pm$  0.00005, determined by Taylor and Selwood.<sup>3</sup>

Observations made several months ago in our own Laboratory confirm the conclusions of Tronstad and co-workers that the Princeton value for the density is considerably too high and agree quantitatively with Tronstad's revised value communicated to Swift.<sup>2</sup> At the same time they indicate definitely that Tronstad's published value of 1.10750 is somewhat low. We obtain the value  $d_{25}^{25}$  1.10763, which we regard as reliable to better than  $\pm$ 0.00005. We desire to report, here, our observations.

Our observations were made on two occasions incident to the preparation of some quantities of extremely pure D<sub>2</sub>O. In both instances we electrolyzed relatively large volumes of deuterium-rich water made alkaline with potassium hydroxide. The cells were equipped with Armco iron electrodes and were known to possess a separation factor,  $\alpha$ , toward the hydrogen isotopes, of a little more than 8. This factor had been determined repeatedly in the same and in similar cells during two years of service in the preparation of extensive quantities of heavy water.

This factor had proved constant with time and showed no significant variation either with current density or with deuterium concentration. The latter had been established with determinations in the concentration range 0.5 of 1% to about 60%.

In both electrolyses, which we will distinguish as Electrolysis A and Electrolysis B, the electrolytic gases were freed of moisture by passage through a trap cooled with a dry ice-ether mixture; passed through a small explosion trap of clean dry sand; recombined at a jet in an atmosphere of dry oxygen supplied from a commercial cylinder at a rate just sufficient to maintain oxygen in excess; and condensed out of contact with air. Successive fractions of the condensate were distilled, with a few crystals of pure dry potassium permanganate added to the still, and their specific gravity determined, at 27°, with an 11-cc. pycnometer of special design. Weighings were accurate to 0.1 mg., or better, and were significant to this figure. Buoyancy corrections were applied. This yielded specific gravities with a precision of  $\pm$ 0.00001. The residues in the cells, after electrolysis was carried as far as desired, were likewise distilled from the potassium hydroxide electrolyte to which a few crystals of dry potassium permanganate were added and, after a second distillation without addition of any reagent, were determined in the pycnometer. Precautions were taken, in all steps, to avoid contamination with atmospheric moisture.

In Electrolysis A we started with 350 cc. of 75% D<sub>2</sub>O and electrolyzed to a residual volume of 100 cc. before samples of combustion water were separately collected. A preliminary calculation indicated that the concentration of this 100 cc. in the cell should be about 99.5% D at this stage. Then a single fraction of 60 cc. was electrolyzed off and separately collected and the 40-cc. residue recovered without further electrolysis. In Electrolysis B the initial charge was 100 cc. of 95% D<sub>2</sub>O. One 40-cc. and two 20-cc. fractions of the combustion water were separately collected and the 20-cc. residue recovered. Volumes of

(1) L. Tronstad and J. Brun, *Trans. Faraday Soc.*, **34**, 766 (1938); cf. also, Tronstad, Nordhagen and Brun, *Nature*, **136**, 515 (1935).

(2) E. Swift, *THIS JOURNAL*, **61**, 198 (1939).

(3) H. S. Taylor and P. W. Selwood, *ibid.*, **56**, 998 (1934); **57**, 642 (1935).

samples electrolyzed were controlled accurately from a record of current against time.

The significant data are listed in Table I. The samples are numbered in column 1 for convenient reference and their measured densities given in column 3. Column 4 is the deuterium concentrations which correspond to these densities if Taylor and Selwood's value of  $d_{25}^{25}$  1.10790 for pure D<sub>2</sub>O be correct and column 5 gives the concentration computed on the basis of our own apparent limiting density of  $d_{27}^{27}$  1.10769. The final column gives the D concentrations to be expected for the several fractions on the basis of the cell factor of 8, and the volumes electrolyzed, and is independent, within wide limits, of the choice of  $d_{27}^{27}$  for pure D<sub>2</sub>O.

TABLE I

Sample	Description, cc.	Obs. $d_{27}^{27}$	% D ( $d_{27}^{27}$ ) 1.10796	% D ( $d_{25}^{25}$ ) 1.10769	% D Calcd.
Electrolysis A: initial 350 cc. of 75% D <sub>2</sub> O					
1	Condensate, 60	1.10781	99.86	100.13	99.93
2	Residue, 40	1.10768	99.80	99.99	99.9999
Electrolysis B: initial 100 cc. of 95% D <sub>2</sub> O					
3	Second condensate, 20	1.1070	99.2	99.36	99.39
4	Third condensate, 20	1.10771	99.82	100.02	99.975
5	Residue, 20	1.10768	99.80	99.99	99.9999

This computation was made using the equation

$$(D_i/D_f)^{\alpha-1} = (H/D)_i/(H/D)_f \quad (1)$$

in which  $(H/D)_i$  refers to the  $(H/D)$  ratio in the cell solution prior to electrolysis of any particular fraction;  $(H/D)_f$  is the corresponding ratio in the cell when this fraction is completed and  $(D_i/D_f)$  is the ratio of deuterium in the cell just before and just after electrolysis of the fraction.

We now believe that sample (1) may have contained a little potassium permanganate, carried over in the spray, or been subject to other inadvertent error. This electrolysis was not carried out with the object of determining the density of pure D<sub>2</sub>O and sample (1) was never repurified or its density rechecked. Sample (2) was rechecked, however, without significant change. The data from electrolysis B were obtained with especial care and with the object of checking the density of pure D<sub>2</sub>O. That these data are correct receives additional support from comparison of the entries in col. 5 with those calculated in col. 6 ( $\approx 0.00001$  in density corresponds to  $\approx 0.01\%$  in concn.).

We have considerable confidence in basing conclusions on the density of the residues. We wish to point out, by reference to the final column of the table, that samples (2) and (5) could not have differed significantly from 100% D<sub>2</sub>O before their removal from the cell. This would be true even if  $\alpha$  were very considerably under 8 in the final steps of electrolysis. The only uncertainty that can attach to these figures, therefore, outside of

the pycnometer precision, is in the consideration of possible contamination by atmospheric moisture during (1) removal from the cell; (2) distillations; or (3) in the process of filling the pycnometer. The latter is eliminated since the repeated distillation and determination with (2) made no significant change, and the distillation process can be eliminated since the only outlet to the atmosphere was blocked with a calcium chloride drying tube during distillation and the flask for the second distillation was the clean, dry receiver for the first. In view of reasonable precautions employed during the initial transfer and at all steps where atmospheric contamination was possible we think it improbable that the value  $d_{27}^{27}$  1.10769 can be sensibly in error.

A small correction is necessary for slight oxygen isotope abnormality in the residues. Since the water employed for electrolysis was equilibrated with carbon dioxide just beforehand, or at slightly earlier stages, we can take the initial O<sup>18</sup> concentration as normal.<sup>4</sup> The cells and electrodes are the same ones that we used previously to determine the electrolytic separation factor for oxygen<sup>5</sup> and we accordingly may employ equation (2) of our previous paper<sup>5</sup> with  $\alpha$  set equal to 1.008. This treatment yields a correction of  $-0.00001$  for either residue, so that we obtain  $d_{27}^{27}$  1.10768 for pure D<sub>2</sub>O with normal oxygen. Finally, we correct to  $d_{25}^{25}$  by use of the density ratio of normal water between the two temperatures of 25 and 27°<sup>6</sup> and that of D<sub>2</sub>O, between the same two temperatures, interpolated from the data of Lewis and Macdonald.<sup>7</sup> This yields a correction of  $-0.00005$  unit in specific gravity. Thence we obtain  $d_{25}^{25} = 1.10763$ . We believe that we are conservative in setting the uncertainty of this value at no greater than 0.00005 unit in sp. gr.

Data just published by Stokland, Ronaess and Tronstad [*Trans. Faraday Soc.*, **35**, 312 (1939)] yield the value  $d_{25}^{25}$  1.10764 referred to by Swift, but differ from those of Lewis and Macdonald with respect to the thermal expansion of D<sub>2</sub>O. This has, in particular, a considerable effect in the size of our correction from  $d_{27}^{27}$  to  $d_{25}^{25}$ . These new expansion data, in conjunction with the "Int. Crit. Tables" values for the thermal expansion of ordinary water, make this correction  $-0.00013$  unit in specific gravity instead of  $-0.00005$ . This reduces our  $d_{25}^{25}$  to 1.10755.

### Summary

Electrolysis of relatively large volumes of deuterium-rich water, at The Ohio State University, has yielded residues with limiting density close

(4) Cf. footnote (5) of W. H. Hall and H. L. Johnston, *THIS JOURNAL*, **57**, 1515 (1935).

(5) H. L. Johnston, *ibid.*, **57**, 484 (1935).

(6) "International Critical Tables," Vol. III, p. 25.

(7) Lewis and Macdonald, *THIS JOURNAL*, **55**, 3057 (1933).

to  $d_{27}^{27}$  1.10769. Correction for a slight abnormality in oxygen isotope ratios and for the difference in thermal expansions of H<sub>2</sub>O and D<sub>2</sub>O yields  $d_{25}^{25}$  1.10763.

Deuterium concentrations of recombined fractions taken in the electrolysis are in good agreement with values calculated from measured separation coefficients for hydrogen isotopes determined with the same cells, if the above figure is

taken to be the specific gravity of pure D<sub>2</sub>O, but are inconsistent with Taylor and Selwood's  $d_{25}^{25}$  1.10790.

The experimental observations indicate that Tronstad and Brun's published value  $d_{25}^{25}$  1.10750 is definitely low but yield very close agreement with the revised value  $d_{25}^{25}$  1.10764, attributed to them.

COLUMBUS, OHIO

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## Heavy Oxygen Exchange Reactions of Proteins and Amino Acids

BY WHITNEY H. MEARS AND HARRY SOBOTKA<sup>1</sup>

Exchange reactions with the heavy oxygen isotope O<sup>18</sup> which recently has become available<sup>2</sup> promise to become valuable and helpful in the elucidation of a number of structural problems. The tendency of simple organic compounds to exchange, or not to exchange, the oxygen in various functional groups may be recognized in exploratory experiments. Hence, one may deduce the types of oxygen linkage in molecules, whose structure is still questionable, from their exchange behavior against water with different O<sup>18</sup> abundance. Other applications of heavy isotopes are the study of reaction mechanisms<sup>3</sup> and the earmarking of organic substances in metabolism studies. The use of O<sup>18</sup> along the latter line of research presupposes the possibility of isolating oxygen from its compounds, a task much more difficult in the case of oxygen than with other elements, as the disintegration of organic materials must be carried out, in the case of O<sup>18</sup>, in the absence of extraneous oxygen to avoid dilution. The development of a convenient hydrogenation method must precede investigations of this nature (*cf.* ref. 9). Studies on compounds earmarked with O<sup>18</sup> must be based upon findings on oxygen exchange behavior.

Studies on O<sup>18</sup> exchange with proteins and their derivatives are reported in this paper. There are

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(2) J. R. Huffman and H. C. Urey, *Ind. Eng. Chem.*, **29**, 531 (1937).

(3) I. Roberts and H. C. Urey, *THIS JOURNAL*, **60**, 880, 2391 (1938).

at least half a dozen functional groups containing oxygen in proteins, namely, (1) the peptide bond

$$-\text{CO}-\text{NH}- \left( \text{or } \begin{array}{c} \text{OH} \\ | \\ >\text{C}-\text{N}< \end{array} \right.$$

under the assumption of cyclization), (2) alcoholic hydroxyl groups as in serine, hydroxyglutamic acid, hydroxyproline, (3) phenolic hydroxyl groups as in tyrosine, (4) free carboxyl groups with two oxygen atoms in aspartic and glutamic acid, (5) amide groups in asparagine and glutamine, (6) —OPO(OH)<sub>2</sub> in phosphoproteins, and finally water of hydration, as in crystals, or possibly bound otherwise in the hollow of globular proteins. If only one or certain types of such oxygen atoms were subject to exchange, one would obtain information regarding protein structure, since it would be possible to accomplish such exchanges without denaturing the protein.

Exploratory experiments were carried out with a number of derivatives and potential fragments of protein. Model substances for the various oxygenous groups in protein, in which we observed no oxygen exchange, comprise glycylglycine, the simplest peptide (1), tyrosine as a model for a phenol (3), and benzamide for the —CONH<sub>2</sub> group (5). The stability of oxygen in peptide and amide linkages toward exchange can only be observed at moderate acidities where these bonds themselves are stable. The stability of aliphatic —OH (2) toward oxygen exchange over a wide range of hydrogen ion concentration was taken for granted on the basis of numerous precedents with alcohols, etc.

In the first runs with individual amino acids O<sup>18</sup> exchanges up to 21% of the amount theoretic-