butyric acid, the value of the ratio (log C_2/C_2^0)/C_3 being -0.20 for the former and -0.12 for the latter in dilute solution, a value of the same order as that calculated in Table I for α-aminocaproic acid. In their saturated solutions this ratio is reduced only to -0.14 for glycine and -0.19 for alanine, but to -0.06 for α-aminobutyric acid.

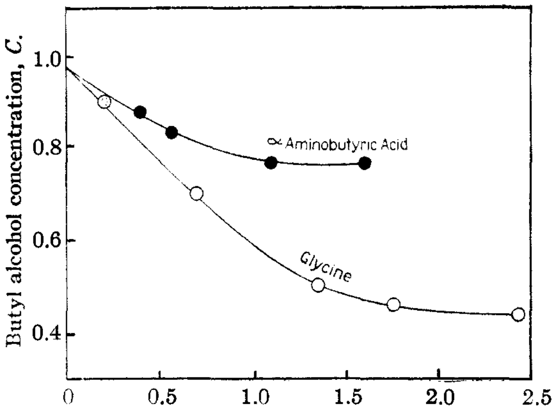

Fig. 3.—Influence of amino acids upon solubility of butyl alcohol in water.

The amino acids of still longer hydrocarbon chains, which in any case have far smaller solubilities in water, have far less influence on the solubility of butyl alcohol in water. The very profound influence of glycine and alanine in salting out butyl alcohol unquestionably accounts for the difficulties encountered by Dakin in his efforts to extract the monoamino monocarboxylic acids from gelatin, which contains 34% of these amino acids. Comparable difficulties might be expected with fibroin, which contains 65% glycine and alanine, whereas most other proteins thus far investigated contain not more than 11% amino acids of short hydrocarbon chain. It is apparently the length of the hydrocarbon chain that influences the solubility in alcohol–water mixtures of substances alike in other respects.

Summary

1. The distribution coefficients of α-amino acids between butyl alcohol and water have been measured at 25°.

2. The concentration in the water phase tends to be smaller and in the alcohol phase to be greater the longer the hydrocarbon chain.

3. Amino acids with branched chains tend to behave in butyl alcohol–water as in ethyl alcohol–water mixtures like smaller molecules than their straight chain isomers.

4. Butyl alcohol diminishes the solubility of amino acids, and amino acids diminish the solubility of butyl alcohol in water. The salting-out effect is greatest for the smallest amino acid, glycine.

5. Salting-out appears to diminish with increasing concentration presumably because of electrostatic forces.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL, AND THE DEPARTMENT OF ZOÖLOGY, HARVARD UNIVERSITY]

Studies of Multivalent Amino Acids and Peptides. III. The Dielectric Constants and Electrostriction of the Solvent in Solutions of Tetrapoles

BY JESSE P. GREENSTEIN, JEFFRIES WYMAN, JR., AND EDWIN J. COHN

I. Introduction.—Amino acids in the neutral state bear a positive $-NH_3^+$ and a negative $-COO^-$ group and the distance of separation of these charges determines predominantly the values of the dipole moment the dissociation constants and the amount of electrostriction of the solvent. The naturally occurring α-amino acids, with the exception of cystine, contain but one positive and one negative charge in the isoelectric state. This condition obtains moreover in the case of even the largest polypeptides so far prepared, containing respectively 18 and 19 simple amino acid residues.[1] These substances, although having a linear dimension comparable to the diameter of proteins, are nevertheless simple dipoles.[2] On the other hand, proteins must be considered to be multipoles. Egg albumin for example contains approximately 27 ammonium and 27 carboxyl groups.[3] The interpretation of protein behavior

[1] Fischer, *Ber.*, **40**, 1754 (1907); Abderhalden and Fodor, *ibid.*, **49**, 561 (1916).

[2] The length of peptides in the solid state is given roughly by multiplying the number of amino acid residues in the chain by 3.5 Å. [Astbury, *Trans. Faraday Soc.*, **29**, 193 (1933)]. The diameter of egg albumin according to Svedberg is 44 Å. [Svedberg, *Kolloid-Z.*, **51**, 10 (1930)].

[3] Cohn, *Physiol. Rev.*, **5**, 349 (1925).

therefore cannot be deduced from the behavior of dipoles. Moreover, the effect of the charges on the protein molecules will depend upon their distribution. Accordingly we have undertaken the study of multipoles synthetically prepared so that their structure may be the better known.

In this communication we shall consider two tetrapoles, lysylglutamic acid and ϵ,ϵ'-diamino-di-(α-thio-*n*-caproic acid). Both neutral molecules bear two positive and two negative charges, the distribution of which results in extremely water-soluble molecules which have a considerable effect upon both the orientation of solvent molecules and the dielectric constant of the solution.

The lysylglutamic acid and ϵ,ϵ'-diamino-di-(α-thio-*n*-caproic acid) were prepared, respectively, according to the methods of Bergmann, Zervas and Greenstein[4] and of Greenstein.[5] α,α'-Dithiodiacetic acid was prepared by the method of Biilmann.[6]

II. **The Apparent Molal Volumes.**—Solutions of amino acids have higher densities than those of uncharged molecules containing the same chemical groups. The densities of the solutions of various concentrations of the tetrapoles are given in Table I, as well as their apparent molal volumes.

TABLE I

APPARENT MOLAL VOLUMES AT 25°

Concn., C	Density, ρ	Apparent molal volumes Obsd. Φ	Calcd. Φ
	Lysylglutamic acid		
0.0860	1.00593	172.67	
.1720	1.01476	172.85	
.3440	1.03223	173.49	
.6718	1.06269	178.03	
	ϵ,ϵ'-Diamino-di-(α-thio-*n*-caproic acid)		
0.0400	1.00101	226.50	226.76
.0625	1.00319	227.04	227.00
.125	1.00925	227.52	227.50
.25	1.02127	228.20	228.20
.50	1.04492	229.30	229.19
	α,α'-Dithiodiacetic acid		
0.0625	1.00136	113.76	113.68
.125	1.00559	114.32	114.16
.250	1.01406	114.52	114.55
.500	1.03075	115.14	115.14
.8037	1.05061	115.90	115.62
1.6075	1.10347	116.32	116.62

The naturally occurring tetrapole, cystine, is by virtue of its extreme insolubility in water unsuited to molal volume investigations. The role of the disulfide linkage in cystine as well as in the more soluble compound prepared from two molecules of ϵ-aminocaproic acid may, however, be ascertained from the molal volumes of dithiodiacetic acid, which has been prepared for the purpose.

The apparent molal volume of acetic acid may be considered as the sum of the volume occupied by the CH_2 group, 16.3 cc., and by the remainder of the molecule common to all aliphatic acids, and having the volume of 35.0 cc. per mole.[7] Adding 18.9 cc. for the second carboxyl group and 13.2 cc. for a CH group yields 83.4 cc. for all the elements of the dithiodiacetic acid molecule save the –S–S– linkage. At the lowest concentration, at which density measurements were carried out, namely, 0.0625 molar, the apparent molal volume of dithiodiacetic acid was 113.76 cc. Subtracting 83.4 cc. gives 30.4 cc. for the two sulfur atoms, which is in good agreement with the estimate of 15.5 cc. per mole of sulfide sulfur previously reported.[8]

The apparent molal volume, Φ, of strong electrolytes is generally considered to vary with the square root of the concentration.[9,10] Weak electrolytes such as acetic acid do not obey this law, but it has been suggested that Drucker's[11] measurements upon mono-, di- and trichloroacetic acids appear to do so, having values for $\delta\Phi/\delta\sqrt{C}$ of 1.55, 3.12 and 6.00, respectively.[12] Dithiodiacetic acid also appears to obey this law, its apparent molal volume being given within the error of the measurements by the equation

$$\Phi = 113.2 + 2.7\sqrt{C}$$

The coefficient by which the square root of the concentration is multiplied is of the same order for the sulfur as for the chlorine in substituted acetic acid molecules.

The volume occupied by the –S–S– linkage can perhaps most accurately be determined by sub-

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(4) Bergmann, Zervas and Greenstein, *Ber.*, **65**, 1692 (1932).

(5) Greenstein, *J. Biol. Chem.*, in press (1935).

(6) Biilmann, *Ann.*, **339**, 356 (1905).

(7) Cohn, McMeekin, Edsall and Blanchard, THIS JOURNAL, **56**, 784 (1934).

(8) Traube, *Chem. und chem.-techn. Vortr.*, **4**, 255 (1899).

(9) Gucker [*Chem. Rev.*, **13**, 111 (1933)] contends that "the data of Perman and Urry [*Proc. Roy. Soc.* (*London*), **A126**, 44 (1929)] show that the apparent molal volumes and compressibilities of sucrose and urea are linear functions of $c^{1/2}$ over the entire experimental range," but this view is disputed by Redlich and Klinger [*Monatsh.*, **65**, 137 (1934)] who contend that the square root law "is characteristic only of the apparent molal volume of strong electrolytes." The square root law certainly does not apply to such strong electrolytes as the salts of certain weak acids and amino acids containing any appreciable number of non-polar CH_2 groups.

(10) Geffcken, *Z. physik. Chem.*, **155**, 1 (1931).

(11) Drucker, *ibid.*, **52**, 641 (1905).

(12) Cohn, *Science*, **79**, 83 (1934).

tracting the volume of the other groups in dithiodiacetic acid from its apparent molal volume at infinite dilution, which yields 29.8 cc.

The apparent molal volume of the ϵ,ϵ'-diaminodi-(α-thio-n-caproic acid) may be estimated from the dithiodiacetic acid by the addition of 8 CH_2 groups and the substitution of 2 NH_2 groups for hydrogen in the ϵ-position. The estimated volume 252.9 is appreciably greater than the observed apparent molal volumes. These, as in the case of dithiodiacetic acid, also appear to vary with the square root of the concentration according to the equation

$$\Phi = 225.8 + 4.8\sqrt{C}$$

Equations of this type are employed in this paper merely for interpolation and extrapolation for Φ_0 without any theoretical implication as to why this form of equation should obtain. Subtracting the difference between the observed and calculated values of Φ_0 yields 27.1 cc. This difference may be ascribed to electrostriction of the solvent estimated to be 13.3 cc. for dipoles when the amino group is in the α-position, and 18.0 cc. when in the ϵ-position.[7] In the tetrapole that we have now studied the value is slightly larger than that for two α-amino acids, 26.6 cc., and considerably lower than that for two ϵ-amino acids, suggesting mutual interference of the groups.

In the case of cystine this interference might be still larger. Assuming electrostriction characteristic of α-amino acids, its apparent molal volume—not readily measurable because of its insolubility—may be taken to be 128.4 cc. per mole.

The apparent molal volume of lysylglutamic acid may be estimated from elements of volume previously considered.[7] Taking the apparent molal volume of a terminal NH_2 and COOH group as 40.7 cc., and additional NH_2 and COOH group, an amide, two CH and six CH_2 groups yields 211.5 cc. The observed value at the lowest concentration studied was 172.7. The electrostriction of the solvent is therefore estimated to be 38.8 cc. We have previously estimated that the electrostriction was 20 cc. for one amino plus one carboxyl group at infinite distance and dilution. The above result suggests that the charged groups of lysylglutamic acid affect the solvent as though they were widely separated from each other. The small inner dipole of lysylglutamic acid may be considered to be glycylglycine. The electrostriction due to this molecule previously has been estimated to be 16.1 cc. per mole. Assuming the charged groups of the other dipole—constituted of the ϵ-amino group of lysine and the γ-carboxyl group of glutamic acid—to be as widely separated from each other as possible yields 36.1 cc., a result close to, but smaller than, that observed at the lowest concentration studied.

The apparent molal volume of lysylglutamic acid changes extremely little with concentration in dilute solution in contrast to the behavior of the two sulfur-containing molecules studied. Linear extrapolation yields an estimate of 172.4 for Φ_0. Despite this small change of Φ with concentration lysylglutamic acid is an extremely dense molecule. Its partial specific volume is equal approximately to the larger peptides of glycine; but, unlike the latter, it is extremely soluble. The ϵ,ϵ'-diaminodi-(α-thio-n-caproic acid) has a somewhat higher partial specific volume, though a far smaller one than the molecules of ϵ-aminocaproic acid of which it is constituted.

III. **Dielectric Constant.**—The very exceptional properties of these substances in regard to dielectric constant are indicated by the measurements reported in Table II. From these data it may be seen that the dielectric constant is linear in the concentration of the solute. This is in accordance with previous observations of other amino acids and peptides in polar solvents;[13] but the values of δ, the slope of the dielectric constant concentration curve, are both very large, namely, 131 for dithio-diamino-dicaproic acid and

TABLE II

DIELECTRIC CONSTANT AT 25°

Concn., moles per liter	Dielectric constant Obsd.	Calcd.
ϵ,ϵ'-Diamino-di-(α-thio-n-caproic acid)		
		$\epsilon = 78.54 + 131\ C$
0.0000	78.54	78.54
.0423	83.71	84.08
.0771	88.54	88.64
.1084	92.43	92.75
.1506	98.23	98.26
.1926	103.96	103.75
Lysylglutamic acid		
		$\epsilon = 78.54 + 345\ C$
0.00000	78.54	78.54
.02546	87.15	87.33
.05035	95.98	95.89
.06792	101.81	101.94
.06719	101.86[a]	101.74

[a] After recrystallization.

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[13] Hedestrand, *Z. physik. Chem.*, **135**, 36 (1928); Devoto, *Gazz. chim. ital.*, **60**, 520 (1930); **61**, 897 (1932); Wyman and McMeekin, THIS JOURNAL. **55** 908 (1933).

345 for lysylglutamic acid. The latter figure represents the greatest δ value thus far recorded, well exceeding that of 290 for the heptapeptide of glycine.[14]

Wyman and McMeekin[13] have pointed out that in the peptide series δ increased by approximately 46 for each additional amino acid in the chain from the value 23 characteristic of the α-amino acids. On the other hand, among the amino acids which they and Devoto have studied, δ increased by approximately 13.3 for each CH_2 group separating the positively charged ammonium from the negatively charged carboxyl group. Since the increase in length of the chain due to the addition of one CH_2 group is known to be close to 1.26 Å. and the length of each glycine unit in the peptides is 3.5 Å., as determined by x-ray studies on the solid material, this indicates that δ increases by between 10.5 and 13, or taking the average by approximately 11.5 per Ångström unit in the length of the chain separating the amino and carboxyl groups in the molecules.

Inasmuch as, in the case of zwitterions, the electric moment is proportional to the actual distance between these charged groups, this relation would be readily intelligible provided one assumed that the molecules were rod-like structures free from bending and that δ were a linear expression of the moment of the dissolved material. This was the interpretation first tentatively suggested (Wyman and McMeekin[13]). Both these assumptions are however to some extent artificial, more especially the latter. The interpretation of dielectric constants in strongly polar solutions is a very difficult matter. Undoubtedly the Clausius–Mosotti relation breaks down, but it would be more natural to think of the dielectric constant, and consequently of δ, in terms of polarizations than of electric moments. Evidence has indeed recently been presented,[14] based on studies of amino acids in a wide range of polar solvents, that the volume polarization p of a strongly polar liquid is given by the expression[15]

$$p = (\epsilon - 1)/3$$

Barring relatively minor corrections involving the density, this would mean that δ is a linear expression of the polarization of the solute molecules and consequently not of their electric moment itself but of its square. This interpretation of δ is in no way inconsistent with the original observations of Wyman and McMeekin on the monoamino monocarboxylic acids and peptides but affords the basis of an alternative explanation to that originally proposed. According to it the electric moment, and consequently the distance between the acid and basic groups in these straight chain compounds, should be proportional not to the number of atoms in the intervening length of chain as in the case of a rod-like structure but to the square root of this number.

The basis for a decision between these two opposing points of view is afforded by a consideration of the present results. It is clear that were we to adopt the first interpretation the moment of lysylglutamic acid, calculated from its δ value, should be the same as that of a simple monoamino monocarboxylic acid in which the distance between the acid and basic groups exceeds that in glycine by $(345 - 23)/11.5$ Å. = 28 Å. The corresponding figure in the case of diamino-dithiodicaproic acid is $(131 - 23)/11.5$ Å. = 9.4 Å.

Now the distance between the amino and carboxyl groups in glycine itself is close to 3 Å., for if the positive charge of the ammonium group has its center in the nitrogen atom, its distance from the center of the carboxyl carbon atom is 2.4 Å. To this must be added the distance of this carbon atom from the locus of the negative charge of the carboxyl group. Pauling and Sherman[16] estimate that "in the acetate group the angle α has the value $124 \pm 3°$, equal to the value $125 \pm 5°$ found for formic acid; we may conclude that this value of the bond angle in carboxyl groups is probably retained in all aliphatic acids" (p. 344). The interatomic distance from the carboxyl carbon to the two oxygens Pauling and Sherman give as 1.29 Å. The linear component will, therefore, be $1.29 \cos 124°/2 = 0.6$ Å., and the total distance from the center of the oxygens to the center of the nitrogen atom will be approximately 3 Å. This is a lower limit. Provided the locus of the positive charge is in the orbit of the rotating hydrogens and that these are not attracted within the sphere of the nitrogen atom, then the total distance would be 3.2 Å. Along quite independent lines, on the basis of our studies on the solvent action of neutral salts on glycine in solvents of

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(14) Wyman, THIS JOURNAL, **56**, 536 (1934).

(15) For a system of n components of total molar polarizations $P_1, P_2, \ldots P_n$ this may be written as $(\epsilon - 1)/3 = (C_1P_1 + C_2P_2 + \ldots + C_nP_n)/1000\rho$, where ρ denotes density and the C's denote concentrations in moles per liter. The electric moment of a molecule is still to be related to the total polarization P in the usual way, *i. e.*, $\mu = 0.0127 \times 10^{-18} \sqrt{(P - P_0)T}$ in which P_0 is the optical polarization and the T is the absolute temperature.

(16) Pauling and Sherman, *Proc. Nat. Acad. Sci.*, **20**, 340 (1934).

low dielectric constants,[17] Kirkwood estimates the dipole distance in glycine to be 3.17 Å.[18] We will for our purposes adopt the latter value of 3.17 Å.

The moment of lysylglutamic acid should therefore on the basis of the original interpretation of δ by Wyman and McMeekin[13] be the same as that of a monoamino monocarboxylic acid in which the charges are separated by a distance of (3.17 + 28) Å. = 31.17 Å. In the case of the dithio compound the corresponding distance is (9.4 + 3.17) Å. = 12.57 Å. The equivalent moments are 149×10^{-18} and 60×10^{-18} e. s. u., respectively.

Let us now, for purposes of comparison, consider what values for the moments of these compounds may be expected on the basis of our knowledge of molecular structure. Both molecules, as zwitterions, contain two positive and two negative charges, and the moment of each may be regarded as the vector sum of two constituent dipole moments. In the case of lysylglutamic acid the maximum possible moment evidently corresponds to the extended structure shown in Fig. 1. Here the two constituent dipoles may be chosen conveniently as those involving in one case the pair of centrally located charges and in the other case the two terminal charges. The distance between the central charges may be expressed as the sum of the dipole length of glycine, 3.17 Å., plus 3.5 Å. for the joint length occupied by a CONH and a CH group, or 6.67 Å. in all. The corresponding moment is 31.8×10^{-18} e. s. u. and this makes an angle of approximately 58° with the other dipole involving the terminal charges. The distance between these terminal charges on the other hand may be written as

$$3.17 + 3.5 + (6 \times 1.26) \text{ Å.} = 14.23 \text{ Å.}$$

which corresponds to a moment of 68×10^{-18} e. s. u. The maximum possible moment of lysylglutamic acid is therefore 89×10^{-18} e. s. u. That this moment, involving the configuration shown in Fig. 1, may indeed actually be realized is consistent with the results on electrostriction, which indicate that the charges are widely separated.

The ϵ,ϵ'-diamino-di-(α-thio-*n*-caproic acid), on the other hand, may be regarded as the combination of two simple dipoles, each an ϵ-amino acid. If these two constituents are parallel and point in the same direction, the moment of the molecule must evidently be a maximum and equal to twice that of an ϵ-amino acid. But the distance between the amino and carboxyl groups in an ϵ-amino acid is $(3.17 + 4 \times 1.26)$ Å. = 8.21 Å. and the corresponding moment is 39.2×10^{-18} e. s. u. The greatest possible value for the moment of the molecule is therefore 78.4×10^{-18} e. s. u. For any other orientation of the constituent dipoles such as might be expected in the view of possible rotation about the –S–S– linkage and in the light of the observed electrostriction the moment would be of course less than this.

In view of these considerations therefore the original interpretation involving the conception of δ as a linear expression of the moments of the solute molecules and of a straight rod-like structure for the monoamino monocarboxylic acids and peptides studied by Wyman and McMeekin[13] may be ruled out. Although it is not inconsistent with our knowledge of the ϵ,ϵ'-diamino-di-(α-thio-*n*-caproic acid) molecule, it demands a moment nearly twice as great as the maximum possible moment based on our knowledge of molecular structure in the case of lysylglutamic acid.

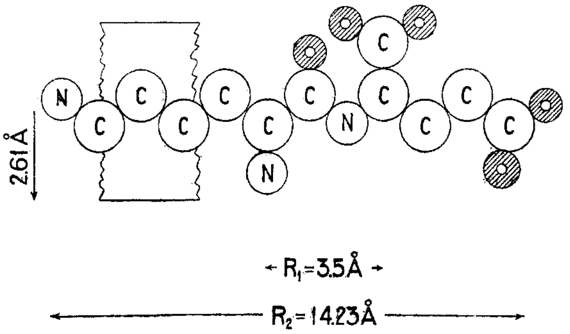

Fig. 1.

On the other hand, the alternative point of view, according to which δ is linear in the polarization, gives results in good accord with our knowledge of the two molecules. If we use the expression $p = (\epsilon - 1)/3$ to calculate the moments from the data in Table II we arrive at the values 78×10^{-18} e. s. u. and 49×10^{-18} e. s. u. for lysylglutamic acid and the dithio compound, respectively. The former value is thus slightly less than what would be expected (89×10^{-18}) for the extended model shown in Fig. 1, which we have reason to believe may be an approximate representation of the actual structure; the latter value would correspond to a considerable amount of bending in the ϵ,ϵ'-diamino-di-(α-thio-*n*-caproic acid) such as may also be expected.

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(17) Cohn, *Naturwissenschaften*, **20**, 663 (1932).

(18) Kirkwood, *J. Chem. Physics*, **2**, 351 (1934).

In conclusion it may be pointed out that the conception that the length of the straight chain monoamino monocarboxylic acids and peptides is proportional to the square root of the number of atoms in the chain, involved in our interpretation of δ as linear in the polarization, agrees with the results of recent statistical calculations of the configuration of long chain molecules by Eyring[19] and by Werner Kuhn.[20]

Summary

1. Molecules having two positively charged ammonium and two negatively charged carboxyl groups in the isoelectric condition have been studied. In one molecule the two dipoles are of the same length, in another the one dipole is far longer than the other.

2. Diamino-dithio-dicaproic acid is composed of two molecules of ϵ-aminocaproic acid combined at the α-carbon atoms by sulfur linkage. Both hydrocarbon chains terminate in an ammonium and a carboxyl group. Electrostatic attraction results in twisting of the chains, and consequently in a relatively small effect on the dielectric constants of solutions and on electrostriction of the solvent.

3. Lysylglutamic acid, a peptide of a diamino and a dicarboxylic amino acid may be considered as constituted of a small dipole, glycylglycine, and a longer one between the ϵ-amino group of lysine and the γ-carboxyl group of glutamic acid. Electrostatic repulsion between the two positively charged ammonium groups on one side of the peptide linkage and between the two negatively charged carboxyl groups on the other results in a rod-shaped molecule, in which the charged groups are widely separated from each other and the influence on the dielectric constant increment of the solution and the electrostriction of the solvent are maximal.

BOSTON, MASS. RECEIVED JANUARY 12, 1935

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[19] Eyring, *Phys. Rev.*, **39**, 746 (1932).

[20] Kuhn, *Ber.*, **67**, 1526 (1934).

[CONTRIBUTION FROM THE FRICK CHEMICAL AND PALMER PHYSICAL LABORATORIES OF PRINCETON UNIVERSITY]

Electrolytic Concentration of Oxygen Isotopes

BY P. W. SELWOOD, HUGH S. TAYLOR, J. A. HIPPLE, JR., AND WALKER BLEAKNEY

As soon as it became apparent that deuterium oxide could be isolated by prolonged electrolytic decomposition of ordinary water, efforts were made to determine whether the density change should all be ascribed to deuterium or in part to the heavier oxygen isotopes. Experiments by Lewis and Macdonald,[1] Selwood and Frost,[2] and Bleakney and Gould[3] showed that no appreciable concentration of O^{18} had taken place even in water of the maximum specific gravity 1.1079.[4] Papers by Washburn, Smith and Frandsen,[5] and by Greene and Voskuyl[6] have indicated, however, an appreciably rapid concentration of O^{18}, and in a recent paper by the late Dr. Washburn and E. R. and F. A. Smith[7] the major part of the density change of ordinary water on preliminary electrolysis is ascribed to heavy oxygen concentration rather than to heavy hydrogen.

The purpose of the present work was to clear up these discrepancies and to determine whether electrolysis is a possible method for the separation of oxygen isotopes even if carried to extreme lengths.

The reason[8] why the earlier workers found no heavy oxygen enrichment in heavy water becomes obvious on consideration of the concentration method used in practice. It is customary, from time to time during the process, to carbonate the concentrated alkali solutions with carbon dioxide and to distil off the partially enriched water. This results in equilibration of the oxygen isotopic ratio to, or nearly to, that of the carbon dioxide used. Any heavy oxygen enrichment is, therefore, defeated as often as carbon dioxide is used.

Experimental

In the present work carbonation was eliminated and neutralization of the solutions prior to dis-

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[1] Lewis and Macdonald, *J. Chem. Phys.*, **1**, 341 (1933).

[2] Selwood and Frost, THIS JOURNAL, **55**, 4335 (1933).

[3] Bleakney and Gould, *Phys. Rev.*, **45**, 281 (1934).

[4] Taylor and Selwood, THIS JOURNAL, **56**, 998 (1934). Since this figure was originally published, the writers have on numerous occasions checked it with comparatively large samples of heavy water, and now have confidence in adding another digit, d^{25}_{25} for D_2O = 1.10790 ± 0.00005, the oxygen isotope ratio being normal.

[5] Washburn, Smith and Frandsen, *Bur. Standards J. Res.*, **11**, 453 (1933).

[6] Greene and Voskuyl, THIS JOURNAL, **56**, 1649 (1934).

[7] Washburn, Smith and Smith, *Bur. Standards J. Res.*, **13**, 599 (1934).

[8] Taylor, THIS JOURNAL, **56**, 2643 (1934).