[FROM THE DEPARTMENT OF ZOÖLOGY, HARVARD UNIVERSITY, AND THE DEPARTMENT OF PHYSICAL CHEMISTRY IN THE LABORATORIES OF PHYSIOLOGY, HARVARD MEDICAL SCHOOL]

The Dielectric Constant of Solutions of Amino Acids and Peptides

BY JEFFRIES WYMAN, JR., AND T. L. MCMEEKIN

I. Introduction

Ever since the introduction of the zwitter ion hypothesis in the papers of Küster[1] and Bredig[2] more than thirty years ago it must have been apparent that one of the outstanding properties of these ions would be their abnormally large electric moments, determined by the distance separating the oppositely charged acid and basic groups.[3] Although it is impossible to determine electric moments quantitatively in polar solvents, where if at all the existence of zwitter ions may be expected, it is nevertheless possible to make qualitative estimates of them from the values of the dielectric constant, and in the course of the last ten years a number of investigations of the dielectric constant of aqueous solutions of amino acids and related compounds have been carried out to test the validity of the hypothesis on this basis.[4] In spite of quantitative differences these results as a whole afford good evidence in favor of the zwitter ion concept.

In view of the interest of the question, the discrepancies among previous results, and above all the need for more extensive data, the present investigation was undertaken. This comprises a study of the dielectric constant of a number of amino acids dissolved in water, of one also in solution in alcohol water mixtures, and of aqueous solutions of the first six peptides of glycine. The results are of interest however not only in connection with the zwitter ion hypothesis but also on account of evidence they afford as to the configuration of the molecules.

II. Experimental Procedure

The method employed for the determination of the dielectric constant was one recently described by the writer,[5] based on the principle of resonance and yielding absolute values. The frequency was between about 1.5×10^8 and 6×10^7 cycles (in terms of wave length, between 2 and 5 meters) and was varied in a number of cases over a twofold range in view of

---

(1) Küster, *Z. anorg. Chem.*, **13**, 135 (1897).

(2) Bredig, *Z. Elektrochem.*, **6**, 33 (1899).

(3) Assuming the distance separating the amino and carboxyl groups in glycine to be 3 Å. in accordance with x-ray data, we obtain a value of about 14×10^{-18} e. s. u. for the electric moment. The largest moment reported for an un-ionized molecule is 7.1×10^{-18} in the case of 1,8-dinitronaphthalene, and this is several times the moment of most compounds.

(4) See Fürth, *Ann. Physik*, **70**, 63 (1923); Blüh, *Z. physik. Chem.*, **106**, 341 (1923); Walden and Werner, *ibid.*, **129**, 389 (1927); Hedestrand, *ibid.*, **135**, 36 (1928); Devoto, *Gazz. chim. ital.*, **60**, 520 (1930); **61**, 897 (1932). These results were obtained with several different methods at frequencies ranging from about 5×10^8 to 10^6 cycles per second.

(5) Wyman, *Phys. Rev.*, **35**, 623 (1930).

a possible dependence of dielectric constant on it. However, even in the case of the largest molecule studied (the hexapeptide of glycine), no evidence of dispersion was apparent, which is in accordance with an analysis of the data of previous investigators. The accuracy of the individual determinations of dielectric constant was about 0.1%. The measurements were, for the most part, made at 25°. In some cases, however, determinations were made at a variety of temperatures and the figures for 25° obtained by interpolation.

III. Preparation of Materials

The α-amino acids used in the investigation were recrystallized from commercial material from alcohol–water mixtures until the analytical results indicated a high degree of purity.

β-Alanine.—β-Alanine was prepared by treating β-bromopropionic acid with a large excess of ammonia. After removing ammonia, β-alanine was purified by recrystallization from alcohol, m. p. 198°; nitrogen, 15.80%.

β-Aminobutyric Acid.—β-Aminobutyric acid was prepared by the method of E. Fischer and Groh,[6] m. p. 188–190°; nitrogen, 13.50%.

γ-Aminovaleric Acid.—γ-Aminovaleric acid was obtained from the Chemistry Department of the University of Illinois. It was further purified by recrystallization from alcohol, nitrogen 12.0%.

ϵ-Aminocaproic Acid.—We are indebted to Dr. J. T. Edsall of the Harvard Medical School for the preparation of ϵ-aminocaproic acid. This substance was prepared by the method of Wallach[7] and melted sharply at 199–200°.

Glycine Peptides.—Peptides of glycine were prepared by the well known methods of E. Fischer.[6] Each of the peptides was reprecipitated from alcohol–water mixtures until salt free. Total and α-amino nitrogen determinations were made on each.

Glycine-dipeptide.—Total nitrogen found 21.00%, calcd. 21.21%; α-amino nitrogen found,[8] 10.96%, calcd. 10.60%.

Glycine-tripeptide.—Total nitrogen found, 22.58%, calcd. 22.23%; α-amino nitrogen found, 7.64%, calcd. 7.41%.

Glycinetetrapeptide.—Total nitrogen found 22.84%, calcd. 22.77%; α-amino nitrogen found 5.39%, calcd. 5.69%.

Glycine-pentapeptide.—Total nitrogen found 22.95%, calcd. 23.10%; α-amino nitrogen found 4.68%, calcd. 4.62%.

Glycine-hexapeptide.—Total nitrogen found 23.50%, calcd. 23.38%; α-amino nitrogen found 3.68%, calcd. 3.89%.

IV. Results on Aqueous Solutions

It was found in all cases, in agreement with previous results of Hedestrand[4] and of Devoto,[4] that the dielectric constant of the solutions was, within the limits of experimental error, a linear function of the concentration, expressed in moles per liter at 25°, up to the highest concentration

---

(6) Fischer, "Untersuchungen über Aminosäuren, Polypeptide und Proteine," 1899–1906, Julius Springer, Berlin, 1906; Vol. II, 1907–1919, Julius Springer, Berlin, 1923.

(7) Wallach, *Ann.*, **312**, 171 (1900).

(8) The "found" value was obtained by multiplying the experimental value by 0.8. Abderhalden and Van Slyke [*Z. physiol. Chem.*, **74**, 505 (1911)] found that glycine peptides gave high α-amino nitrogen values, but if the values obtained were multiplied by 0.8, the result was very close to the theoretical value.

TABLE I

Substance	Range of concn. studied	Density at concn. c	Specific conductivity (ohms^{-1}) at concn. c	δ at 25° (author's value)	Other values of δ at temp. t
Glycine (NH_2CH_2COOH)	0.0–2.5	1.0569, $c = 2.0$		22.58	23.0, $t = 18^a$ 26.4, $t = 25^b$
α-Alanine ($CH_3NH_2CHCOOH$)	.0–0.8904		109×10^{-6}, $c = 0.8904$	23.16	23.57, $t = 18^a$ 6.4, $t = 19.6^c$
α-Aminobutyric acid ($CH_3CH_2NH_2CHCOOH$)	.0– .7355			23.16	
α-Aminovaleric acid ($CH_3CH_2CH_2NH_2CHCOOH$)	.0– .47	1.0051, $c = 0.329$		22.58	
β-Alanine ($NH_2CH_2CH_2COOH$)	.0–1.182			34.56	42.25, $t = 18^a$
β-Aminobutyric acid ($CH_3NH_2CHCH_2COOH$)	.0–0.7849	1.0182, $c = 0.7849$	308×10^{-6}, $c = 0.7849$	32.36	
γ-Aminovaleric acid ($CH_3NH_2CHCH_2CH_2COOH$)	.0– .1826	1.0023, $c =$ .1826	1725×10^{-6}, $c =$ .4566	54.8	
ϵ-Aminocaproic acid ($NH_2CH_2CH_2CH_2CH_2CH_2COOH$)	.0– .1335		1200×10^{-6}, $c =$ .890	77.5	
Glycine-dipeptide	.0– .1050	1.0030, $c =$ .1050	543×10^{-6}, $c =$ .1050	70.6	70 $t = 25^b$
Glycine-tripeptide	.0– .06523	1.0017, $c =$ .0622	1900×10^{-6}, $c =$ .06523	113.3	
Glycine-tetrapeptide	.0– .05962	1.00275, $c =$ .05962	4280×10^{-6}, $c =$ .05962	159.2	
Glycine-pentapeptide	.0– .02639	1.00012, $c =$ .02639	5310×10^{-6}, $c =$ .02639	214.5	
Glycine-hexapeptide	.0– .01315		4680×10^{-6}, $c =$ .01315	234.2	

[a] Hedestrand (4).

[b] Devoto (4).

[c] Walden and Werner (4).

studied (2.5 molar in the case of glycine). On this account it is unnecessary to list the individual values of the dielectric constant obtained and the data may be exactly expressed in terms of the increase of dielectric constant per mole of dissolved material, a quantity which, for brevity, we denote by δ, and which is accordingly defined by the relation

$$\delta = \frac{\epsilon - \epsilon_0}{c}$$

where ϵ is the dielectric constant of the solution, ϵ_0 that of water, and c the concentration of the ampholyte in moles per liter. The data so expressed for all the substances studied are given in Table I. In all cases where it was measured, the specific conductivity of the solutions and data on the density are also included; in addition, the results of previous observers, in so far as they exist, are added for comparison in the last column of the Table.[9]

The results on glycine were extended over a wider range of temperature than in the other cases and it was found that the variation of δ with temperature was remarkably slight, though definite. The values are given in Table II. The increase of δ with diminishing temperature may be regarded as further evidence, in addition to the fact that the results are independent of wave length, against the possibility of dispersion at these frequencies.

TABLE II

THE EFFECT OF TEMPERATURE ON THE DIELECTRIC CONSTANT OF SOLUTIONS OF GLYCINE IN WATER. RANGE OF CONCENTRATION STUDIED 0.0–2.0 MOLES PER LITER

Temperature, °C.	0	5	10	15	20	25
δ	23.80	23.43	23.14	22.94	22.78	22.58

V. Confirmation of the Zwitter Ion Hypothesis

In order to interpret the foregoing results we may assume with confidence that the greater the dielectric constant of a solution, of given molar concentration, in a given solvent, the greater the electric moment of the dissolved molecules, exactly as in the case of ideal solutions in non-polar solvents. More specifically, we will assume that the values of δ, defined above, correspond in magnitude with the values of the electric moments of the ampholytes, and base our considerations on these in what follows.

In accordance with this conception the foregoing results greatly strengthen the argument in favor of the predominance of zwitter ions in the cases studied. (a) In all the new instances, as well as the old, the values of δ are positive and much larger than in the case of any of the non-ampholytes thus far studied. For example, for most of these δ is negative, and even in the case of urea and related substances, which are exceptional in raising the dielectric constant of water, δ is not greater than about 4. It is interesting to observe in this connection that the anhydride of glycyl-

---

(9) Since Fürth's results failed to show a linear relation between dielectric constant and concentration, it is impossible to give a value of δ for comparison.

glycine, studied by Devoto, behaves like other non-electrolytes, in marked contrast to the peptide itself. It is also worth pointing out that the only amino acids so far investigated which have failed to produce an increase in the dielectric constant, namely the *o*-, *m*- and *p*-aminobenzoic acids studied by Hedestrand, are just those estimated by Bjerrum[10] and Ebert[11] on the basis of dissociation constants not to exist predominantly in the zwitter ion form. (b) The value of δ is very nearly the same for all four α-amino acids as, likewise, in the case of two β acids. A similar constancy was observed by Devoto among a large group of α-amino acids and Hedestrand found a close agreement between glycine and α-alanine. This suggests that the electric moments of these molecules in solution arise from their properties as zwitter ions and do not depend appreciably on specific chemical differences. (c) The same thing is also indicated by the progressive increase of δ with the distance separating the amino and carboxyl groups in the molecule. This is shown very impressively by the results on the γ- and ϵ-amino acids and by the series comprising the peptides of glycine. This latter series lends support to a previous interpretation given by the author[12] to observations on the dielectric constant of solutions of the protein zein in alcohol–water mixtures, where, owing to the great size of the molecule and the high viscosity of the solutions, the results are obscured by phenomena of anomalous dispersion.

VI. The Configuration of the Molecules

As already pointed out the present results afford evidence as to the configuration of the molecules. It would not be unnatural to suppose that the longer molecules, both the amino acids and the peptides, have a tendency to bend back upon themselves and possibly to form rings. This might be expected both in accordance with the tetrahedral model of the carbon atom, and on the general grounds that such a bending, bringing the positively and negatively charged ends of the molecule closer together, would diminish the electric moment and cause a reduction of the associated potential energy. It has, moreover, been suggested on more specific grounds by several observers.[13] In particular, Astbury and Woods[14] have concluded, on the basis of x-ray studies, that the protein molecules in unstretched wool fibers have a looped structure in which there is an alternation of five and seven numbered rings; on the other hand, in stretched wool fibers and in silk fibers under all conditions the molecules are stated to consist of an essentially linear arrangement of amino acids.

The present results appear to be definitely inconsistent with the idea of

---

(10) Bjerrum, *Z. physik. Chem.*, **104**, 147 (1923).

(11) Ebert, *ibid.*, **121**, 385 (1926).

(12) Wyman, *J. Biol. Chem.*, **90**, 443 (1931).

(13) Notable in this connection are observations of Svedberg that the majority of protein molecules subjected to a strong centrifugal force settle in accordance with Stokes' formula for spheres. See T. Svedberg, various papers since 1926 in THIS JOURNAL.

(14) Astbury and Woods, *Nature*, **126**, 913 (1930).

bending or ring formation in the case of the molecules studied in solution. The steady increase of electric moment with the length of chain separating the oppositely charged groups, which has been so clearly shown and which is taken as a strong argument in favor of the zwitter ion hypothesis, could only be expected and can indeed only be accounted for on the assumption of straight, rod-like molecules. If there were any considerable bending of the molecules, the carboxyl and amino groups would actually be expected to approach nearer together as the length of the chain is increased. For example, on the basis of the structure attributed by Astbury and Woods to the molecules of unstretched wool fibers the moment of glycylglycine should actually be less than that of glycine itself (in fact, about half).

In regard to the question of the rod-like form of the molecules it is of interest to consider exactly how far δ is in fact proportional to the distance between the oppositely charged groups in the zwitter ion, assuming the ion to be straight. This is illustrated by the two accompanying figures. In Fig. 1 values of δ for the amino acids studied are plotted against this distance, expressed in terms of the number of carbon atoms (n). In Fig. 2 values of δ for the peptide series, including glycine itself, are plotted against the number of glycine units (N) in the molecule.

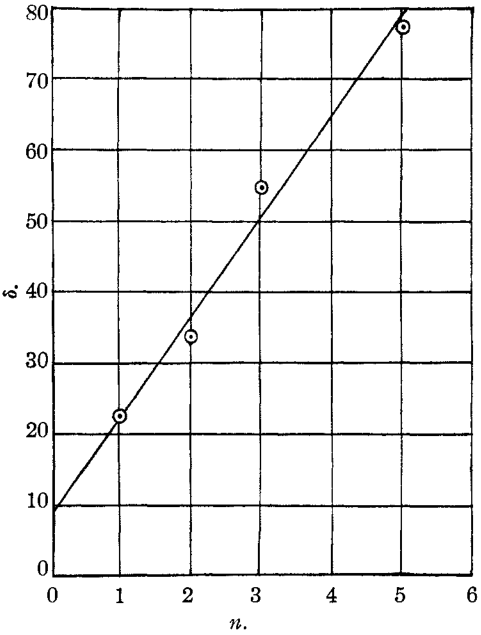

Fig. 1.

VII. Results on Glycine in Alcohol–Water Mixtures

On the basis of a consideration of dissociation constants in alcohol, Ebert[11] has estimated that the formation of zwitter ions must be greatly reduced in this solvent. In regard to this point, it is of interest, therefore, to consider the data on the dielectric constant of solutions of glycine in ethyl alcohol–water mixtures. The data were obtained by the authors using the same method employed for aqueous solutions. Owing to the decrease of solubility of glycine with increasing concentration of alcohol, the measurements were not extended beyond 60% alcohol. The results are given in Table III.

TABLE III

Per cent. alcohol	δ
0.0, ϵ_0 = 78.54	22.58
20.0, ϵ_0 = 69.96	22.47
40.0, ϵ_0 = 59.69	21.67
60.0, ϵ_0 = 47.88	20.56

These figures show a surprisingly small change of δ with change of the percentage alcohol in the solvent, which would indicate that, even in the case of 60% alcohol, the zwitter ion predominates. It must not be overlooked, however, that since estimates of the ratio of zwitter ions to unionized molecules is of the order 1000 to 1, or 10,000 to 1, a many-fold change in the ratio would still leave the zwitter ion predominant.

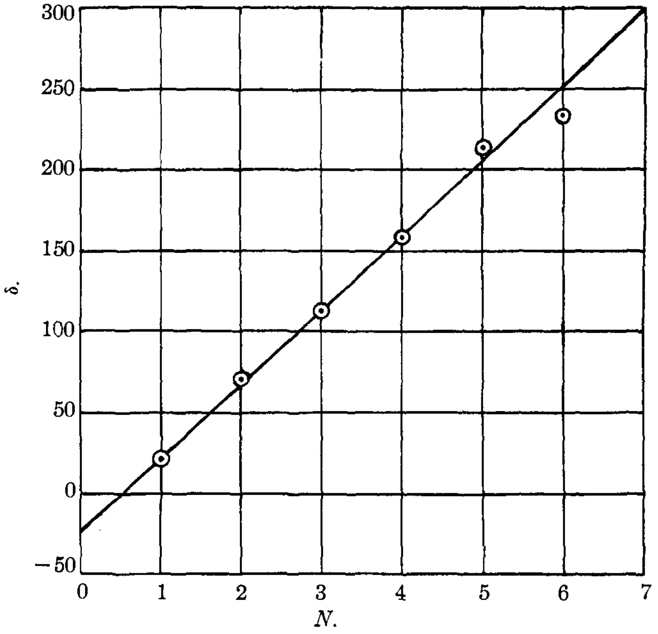

Fig. 2.

VIII. Summary

Measurements of the dielectric constant of a variety of amino acids of the α, β, γ and ϵ types and of five peptides of glycine, in aqueous solution, afford additional evidence in favor of the zwitter ion hypothesis. The results indicate that all the molecules studied are rod-like structures without tendency to bending or formation of rings. Measurements of solutions of glycine in alcohol–water mixtures indicate that in these cases too the zwitter ions are predominant.

CAMBRIDGE AND BOSTON, MASSACHUSETTS

RECEIVED AUGUST 9, 1932
PUBLISHED MARCH 7, 1933