fractions tested for formaldehyde and furfural as described above. Neither was detected.

## Discussion

The experiments described show that when either of the isomeric aloins is decomposed by treatment with borax followed by hydrochloric acid, formaldehyde is formed. The same result is obtained when sodium perborate is used, but in this case the acid treatment is unnecessary, indicating that the oxidizing action of the perborate can bring about the same change as the acid. Hydrochloric acid, without the preliminary borax treatment, cannot bring about the formation of formaldehyde.

These results show, also, that hydrolysis with borax produces little if any pentose, whereas with sodium perborate, presumably due, again, to the oxidizing action, pentose is formed in appreciable amounts as indicated by the formation of furfural after acidification and distillation. This is in agreement with the results of Goldner.[6]

## Summary

Some of the conditions for the formation of formaldehyde and of a pentose (detected by furfural formation) from the aloins have been determined.

(6) Goldner, *J. Am. Pharm. Assoc.*, **21**, 658 (1932).

ST. LOUIS, MISSOURI RECEIVED APRIL 13, 1942

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[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

# The Dielectric Investigation of Polypeptides. I. The Dielectric Increments of Amino Acid Polypeptides

BY W. P. CONNER,* R. P. CLARKE AND C. P. SMYTH

Structural discontinuities in the glycine polypeptides have been suggested by the condensation experiments of Pacsu[1] and others[2] with the peptide esters. The abnormality in the dissociation constants[3] and the unexpected increase in the rate of alcoholysis[4] of hexaglycine offered support to the view that the slightly low value for the dielectric increment of hexaglycine reported by Wyman[5] as compared to the linear relation between increment and the number of glycine residues found by him might be indicative of a structural change for this peptide. With this in mind, it was thought significant to examine further the question of this linear relation indicated by the excellent measurements of Wyman, and, at the same time, to determine the dielectric increments of other peptide combinations of glycine, alanine, and leucine with the object of observing the influence of substituent groups upon the incremental values of the glycine peptides.

* Research Assistant on Special Funds from the Rockefeller Foundation.

(1) Pacsu, *Nature*, **144**, 551 (1939).

(2) (a) Fischer, *Ber.*, **37**, 2501 (1901); (b) **39**, 453 (1906); (c) Curtis, *ibid.*, **16**, 734 (1883); (d) **37**, 1283 (1904); (e) *J. prakt. Chem.*, **37**, 173 (1888).

(3) Glasstone and Hammel, THIS JOURNAL, **63**, 243 (1941).

(4) Glasstone and Hammel, *ibid.*, **63**, 2003 (1941).

(5) (a) Wyman and McMeekin, *ibid.*, **55**, 908 (1933); (b) Wyman, *ibid.*, **56**, 536 (1934); (c) Wyman, *Chem. Rev.*, **19**, 213 (1936).

## Experimental

**Materials.**—The glycine polypeptides were prepared[6] according to the well-known Fischer methods as reviewed by Glasstone and Hammel[3] in their determination of dissociation constants of these peptides. Dr. E. J. Wilson[7] prepared the di- and tripeptides of glycine, alanine, and leucine following a modification of Fischer's general procedure. The purity of all the glycine polypeptides used in this investigation was checked by formol titrations carried out potentiometrically with the glass electrode. In all dielectric constant measurements exceptional care was taken to reduce the salt conductance of the water solutions. Each peptide was purified by dissolving it in a minimum of warm distilled water and then precipitating it with ethyl alcohol. This purification was repeated until the water solution of the desired peptide concentration could be readily balanced in the dielectric constant apparatus. The dioxane used in the calibration of the condenser cell was purified by fractional distillation over sodium hydroxide pellets, followed by six fractional crystallizations. The dioxane, so purified, had a melting point of 11.78° and a dielectric constant of 2.2131 ± 0.0008 at 25°. However, inasmuch as only mixtures of water and dioxane with high water content are critical in determining the cell constant, no attempt was made to maintain the hygroscopic dioxane in this high state of purity.

**Method.**—The dielectric constant measurements were made with a General Radio Company Twin-T impedance

(6) We are indebted to Mr. A. F. Chadwick for the tetraglycine, and to Mr. E. F. Hammel for the penta- and hexaglycine.

(7) Wilson and Pacsu, *J. Org. Chem.*, **7**, 126 (1942).

measuring circuit,[8,10] Type 821-A. A General Radio Company modulated oscillator, Type 684-A,[8] served as the frequency source and a Hallicrafters[9] "Super Defiant" communications receiver was used as the detector. The frequency range of 500 kc. to 30 mc. was available with the assembly.

The Twin-T circuit was completely calibrated in the manner suggested by Sinclair.[10] The resulting inductive and resistive constants were quite similar to those reported by him. However, slightly more consistent reproducibility was found when the actual inductances of the precision condenser and the cell were used instead of those determined by Sinclair's approximation. In addition, the linearity of the precision condenser was checked at a frequency of 50 kc. and the variation due to the worm gear drive was found to be less than $\pm 0.1$ $\mu\mu$fd in the central region of the scale. Because a small discontinuity in capacity was noted upon switching the bands of the Twin-T apparatus, dielectric measurements were carried out on only one frequency band at 25, 28, and 30 mc. This slight defect in the apparatus was later completely rectified by the General Radio Company.

At the frequencies used it was essential that the cell have a minimum inductance. In addition, since the limiting factor in the size of the cell was not the capacitance but the conductance of the peptide solutions, the air capacity had to be kept as low as possible. A cell[11] which suited well the measuring circuit was constructed from copper blocks with Catalin insulation (Fig. 1). The metal walls and Catalin insulation were taper fitted so that no cement was necessary, thereby permitting the use of a wider variety of organic solvents. The Catalin itself is extremely insoluble in the common solvents. Since the liquid in the cell extended up the filling tube and thus was largely out of the electric field, the capacity of the cell did not vary appreciably with slight changes in the height of the liquid in the tube. A fairly constant level was obtained by filling the tube completely, inserting the copper plug and then wiping off the overflow. Throughout all measurements, the cell was thermostated by a water jacket at 25.00 ± 0.02°. For calibration, the cell capacity was considered to be a linear function of the dielectric constant of the liquid contained in it. The constants of the function were determined by the use of dioxane–water mixtures.[12] Deviations from linearity were noticed for mixtures with low water content. Since for this investigation only dielectric constants greater than that of water were important, the abnormality for low dielectric constants was disregarded. This abnormality resulted from the slight difference in the dielectric constant of pure dioxane as reported by Åkerlöf and Short and that measured here, along with the fact that the small inductance between the capacity across the Catalin and the capacity across the cell introduced a slight abnormality when these two capacities are of the same order of magnitude. The cell constant, $dC/d\epsilon$, was 3.118 $\mu\mu$fd and the invariant capacity was approximately 26.0 $\mu\mu$fd. A slight change in the invariant capacity was noted over long periods of time. The limiting specific conductance for which the apparatus could be balanced was approximately $1 \times 10^{-4}$ ohms$^{-1}$ cm.$^{-1}$.

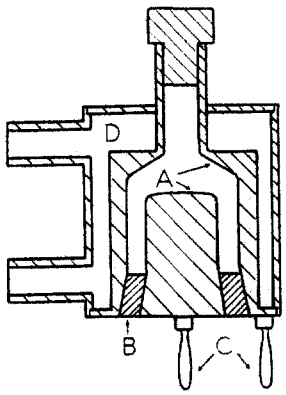


Fig. 1.—Cell for dielectric measurements: A, copper electrodes; B, catalin insulation; C, general radio plugs; D, water jacket.

Dielectric constant measurements were carried out according to the following scheme. With the cell removed, the Twin-T circuit was balanced with the precision condenser at 450.0 $\mu\mu$fd ($C_1$) and the conductance dial at zero. Either a minimum in the background noise or in the beat note from the beat frequency oscillator of the receiver was taken as the balance point. With the adjustment of the auxiliary condensers on the instrument itself, this could easily be set to $\pm 0.05$ $\mu\mu$fd. The cell was then inserted firmly into the banana plug sockets, and the circuit rebalanced. This second balance point was designated $C_2$. After the cell had been removed, the zero point balance was rechecked. To correct for the inductive effects in the cell and in the precision condenser, the following equations were used.

$$C_x = C_1 - C_2 + \Delta C_1 + \Delta C_2 + \Delta C_3 \quad (1)$$

$$\Delta C_1 = \frac{\omega^2 L_c C_1^2}{1 - \omega^2 L_c C_1} - \frac{\omega^2 L_c C_2^2}{1 - \omega^2 L_c C_2} \quad (2)$$

$$\Delta C_2 = -\frac{\omega^2 L_x C_x^2}{1 - \omega^2 L_x C_x} \quad (3)$$

$$\Delta C_3 = L_x (G_x')^2 \quad (4)$$

$$G_x' = G'' - 2\omega L'' G'' C_1 + R_c \omega^2 (C_2^2 - C_1^2) \quad (5)$$

where

$C_x$ = capacitance of cell in fd.
$\omega$ = $2\pi \times$ frequency
$L_c$ = inductance of the precision condenser = $6.14 \times 10^{-9}$ h.
$L_x$ = inductance of the cell and leads = $1.143 \times 10^{-8}$ h.
$G_x'$ = conductance of cell in mhos.
$G''$ = apparent conductance of cell in mhos as read from the conductance dial.
$L''$ = inductance of the conductance condenser = 2.4 $\mu$h.
$R_c$ = resistance of the leads to the conductance condenser in ohms = $3.4 \times 10^{-2}$ ohms.

Because of the small cell inductance, $\Delta C_3$ was, in general, neglected. Since $C_1$ was fixed at 450.0 $\mu\mu$fd, the first term of $\Delta C_1$ could be calculated once for all measurements. Since the second term of $\Delta C_1$ was small, it was determined with sufficient accuracy from a graphical plot of $C_2$ against $\omega^2 L_c C_2^2/1 - \omega^2 L_c C_2$. The remaining correction was plotted in the simplified form

$$\Delta C_2 = -\frac{\omega^2 L_x [C_1 - C_2 + \Delta C_1]^2}{1 + \omega^2 L_x [C_1 - C_2 + \Delta C_1]} \quad (6)$$

and the correction read directly from the graph as soon as $C_1 - C_2 + \Delta C_1$ had been determined.

(8) General Radio Company, 30 State Street, Cambridge, Massachusetts.
(9) The Hallicrafters, Inc., Chicago, Illinois.
(10) Sinclair, *I.R.E., Proc.*, **28**, 310 (1940).
(11) *Cf.* Ferry and Oncley, THIS JOURNAL, **63**, 272 (1941).
(12) Åkerlöf and Short, *ibid.*, **58**, 1241 (1936).

These equations are all approximations, but they represent adequately the data in this frequency range. The approximations involving the conductance determinations are the least precise, but further accuracy was not warranted inasmuch as such data were unimportant for this investigation. Since individual values of $C_x$ corrected to zero frequency differed at the most by $\pm 0.1$ $\mu\mu$fd for a capacity change of 300 $\mu\mu$fd for the three frequencies used, the relative accuracy of the dielectric constant determinations was approximately 0.04%.

## Experimental Results

The values of the dielectric increment $\delta$, the change of the dielectric constant of the solution per mole of solute per 1000 g. of water, are recorded in Table I for the peptides investigated in this report. These results represent the values of $\delta$ in a region of frequency lower than that of dispersion. As the observed dielectric constants were linear functions of the concentrations, they are not reproduced. The dielectric constant of any of these solutions is the sum of the value for water, 78.54, plus the product of the increment by the concentration.

TABLE I

DIELECTRIC INCREMENTS AT 28 MC. AND 25°

| Substance[c] | Concentrations, moles/1000 g. $H_2O$ | Observed | Reported |
|---|---|---|---|
| Glycine | 0.4 –0.8 | 22.65 ± 0 .03 | 22.58[a] |
| Diglycine | .05– .1 | 70.4 ± 0.4 | 70.6[a] |
| Triglycine | .02– .06 | 114.5 ± 0.3 | 113.3[a] |
| Tetraglycine | .02– .06 | 165.8 ± 1.0 | 159.2[a] |
| Pentaglycine | .01– .03 | 202.2 ± 2.3 | 214.5[a] |
| Hexaglycine | .00153 | 240 ±25 | 234.2[a] |
| Alanine | .2 – .6 | 23.20 ± 0.05 | 23.16[a] |
| Leucylglycine | .05– .15 | 65.7 ± 0.3 | 68.4 ± 1.0[b] |
| Leucylalanine | .03– .06 | 57.7 ± 0.5 | |
| Alanylglycylglycine | .02– .06 | 117.4 ± 0.2 | |
| Leucylglycylglycine | .01– .06 | 120.4 ± 0.3 | 120.4 ± 1.2[b] |
| Alanylleucylglycine | .01– .05 | 124.5 ± 1.2 | |
| Glycylleucylalanine | .01– .04 | 110.5 ± 0.7 | |

[a] Wyman and McMeekin, THIS JOURNAL, **55**, 908 (1933). [b] Greenstein and Wyman, *ibid.*, **58**, 463 (1936). [c] All the preparations used were racemic mixtures.

The errors reported here are based upon the reproducibility of independent measurements. Since the method employed does not measure absolute dielectric constants, the absolute errors, based on the accuracy of the dielectric constants of the calibration mixtures, are somewhat greater than those reported, the additional error in the increment being about 0.3%. Since no density determinations were made, the concentrations expressed here are molal and not molar concentrations. However, by use of the apparent molar volumes of these peptides, it can easily be shown that for these concentrations the two increment values, molal and molar, agree well within the error of the experiment, except in the case of glycine, where the higher concentrations employed would cause the molar increment to differ appreciably from the molal, being 23.3 instead of 22.58. An increment value, 174.2 ± 1.0, was obtained for two different samples of tetraglycine. As this value was out of line with those for the other peptides, it was discarded in favor of the lower value in Table I obtained subsequently from closely agreeing measurements on two other samples. The reason for the discrepancy is not apparent in view of the supposed purity of all the samples, but its existence indicates the possibility of an error as large as 10 units in this value. Of the two newly measured samples, one had been kept for some time out of contact with air, while the other had been exposed to the moisture of the atmosphere. The excellent agreement of the two indicated that hydrolysis had not occurred in the exposed sample. A solution of tetraglycine maintained at 100° for some hours showed a rapid falling off in the increment value.

## Discussion of Results

The agreement of the $\delta$-values reported in Table I and those reported by Wyman and his co-workers is very close. However, in the case of tetraglycine and pentaglycine, there appear to be differences beyond the experimental errors. If these polypeptides undergo dispersion with a single relaxation time and with a critical wave length equal to that to be reported in a subsequent paper then the incremental values measured at two meters, the approximate wave length used by Wyman, will appear less than those reported here because of dispersion. The reduction will amount to 3% for triglycine, 5% for tetraglycine and 7% for pentaglycine. Comparison of the corrected values with those reported here still shows significant discrepancy.

In Fig. 2, are plotted the incremental values of the glycine polypeptides against the number of glycine residues. Unfortunately, an accurate determination of the incremental value for the hexapeptide was impossible because of the low solubility of that substance. The equilibrium saturation concentration of this sample was 0.0015 molal. By evaporation of a large volume of the saturated solution an 0.007 molal solution was obtained. However, inasmuch as the solute readily precipitates out, the solution was much too unstable for dielectric constant determinations.

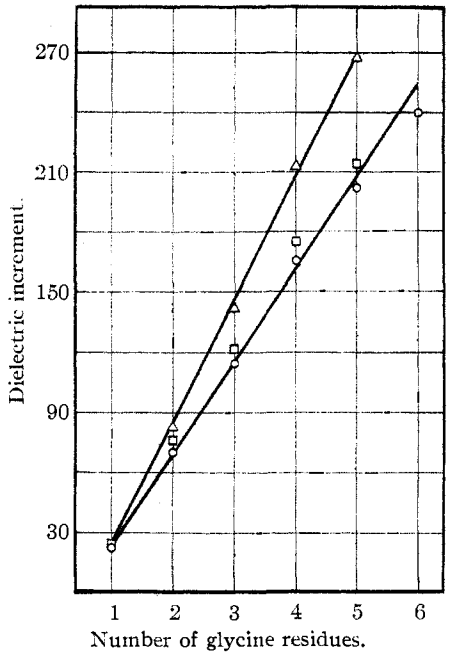


Fig. 2.—Dependence of dielectric increment upon number of glycine residues. Circles, uncorrected increments; squares, increments corrected for volume; triangles, increments corrected for solvent effect.

Although the rather inaccurate value 240 ± 25 for the increment could be measured, the error in the determination is too large to warrant any conclusion concerning the structure of the hexapeptide molecule.

The increment values reported here are not immediately proportional to the polarization of the solute molecules but must be corrected for the volume which the solute molecules occupy. Following Wyman's designation,[13] we have

$$\delta = \beta P - \gamma V \qquad (7)$$

in which $P$ and $V$ are the molar polarization and volume of the solute and $\beta$ and $\gamma$ are constants, $\beta$ is independent of the solvent and $\gamma$ is a function of the solvent. The correction, $\gamma V$, may be estimated by calculating the reduction in the dielectric constant of water occurring upon the introduction of a mole of holes equal to the molar volume of the solute. From values of $d\epsilon/dp$, it appears that the dielectric constant of water at 25° may be well represented by[14]

$$\epsilon - 1 = 77.8\,\rho \qquad (8)$$

in which $\rho$ is the density of water. From values of the apparent molar volumes, $\phi$, either observed or calculated from atomic volumes, reductions in the dielectric constants of the water solutions are estimated for the following molecules in Table II. The agreement between the calculated and observed[5c] values of the increments for molecules not forming zwitterions lends support to this method of estimation.

(13) Wyman, *J. Phys. Chem.*, **43**, 143 (1939).
(14) Kyropoulos, *Z. Physik*, **40**, 507 (1926).

TABLE II

ESTIMATED INCREMENT VALUES FOR MOLECULES NOT IN THE ZWITTERION FORM

| Substance | δ (calcd.) | δ (observed) |
|---|---|---|
| 2,5-Dioxypiperazine | −5.7 | −10 |
| Phenol | −5.9 | − 6.6 |
| Aniline | −6.0 | − 7.6 |
| Biuret | −5.0 | − 6.3 |
| Urethan | −4.5 | − 4.2 |
| Hydantoin | −5.4 | − 6.4 |
| Nitromethane | −2.4 | − 2 |
| *o*-Dihydroxybenzene | −6.1 | − 6 |
| *m*-Dihydroxybenzene | −6.1 | − 6 |
| *p*-Dihydroxybenzene | −6.1 | − 6 |

It must be emphasized that this correction is at best only a crude estimation. Yet it is hoped that it corrects partially for the differences in molecular volume among the peptides. Obvious uncertainties in this approach lie in the application of the pressure function of the dielectric constant as determined for densities slightly greater than unity to densities very much less than unity, and, in addition, the substitution of a mole of holes of dielectric constant unity for a mole of holes of dielectric constant about 2.5. Moreover, it is noted that the observed negative increments are not completely linear with the molar concentration of the solute.

In Table III are listed the volume corrections and the resulting total incremental value, which

TABLE III

VOLUME CORRECTIONS AND CORRECTED DIELECTRIC INCREMENTS

| Substance | $\phi$ | $\gamma V$ | Total | $\frac{9}{2000}\frac{n_1}{v_2}P_2$ |
|---|---|---|---|---|
| $G_1$ | 43 | 3.0 | 25.6 | 3.3 |
| $G_2$ | 77 | 5.7 | 76.1 | 6.0 |
| $G_3$ | 113.5 | 7.9 | 122.4 | 8.8 |
| $G_4$ | 150 | 10.1 | 175.9 | 11.7 |
| $G_5$ | 186 | 12.1 | 214.4 | 14.5 |
| $G_6$ | 223 | 14.1 | 254 | 17.4 |
| A | 60.6 | 4.3 | 27.5 | 4.7 |
| L | 108 | 7.4 | 32.4[a] | 8.4 |
| LG | 143.0 | 9.8 | 78.2 | 11.1 |
| AG | 94.5 | 6.5 | 77.5[a] | 7.4 |
| LA | 159.0 | 10.6 | 68.3 | 12.4 |
| $\phi$AG | 173.6 | 11.4 | 68.1 | 13.6 |
| LGG | 178.5 | 11.7 | 132 | 13.9 |
| ALG | 195 | 12.5 | 137 | 15.2 |
| AGG | 130 | 8.8 | 126 | 10.1 |

[a] Uncorrected values taken as those listed by Wyman.[5c]

is the sum of the measured δ-value and the volume correction, for zwitterions of interest for this paper. The substances are indicated by abbreviations: φ for phenyl, A for alanine, L for leucine, $G_1$ for glycine, $G_2$ for diglycine, etc. The total δ-values for the glycine polypeptides are also plotted as squares in Fig. 2. Since the volume correction is proportional to the number of glycine residues, the only change of the linear relationship is in the slope of the line.

The influence of substituent groups upon the corrected dielectric constant increment values can arise in three ways: the substituent group may change the proportion of zwitterions present by helping or hindering ionization in a manner similar to the variation in strengths of organic acids; the substituent group, because of its volume, may modify the solvent effect upon the apparent dipole moment of the molecule; or the substituent group may actually modify the real dipole moment of the zwitterion.

The presence of substituent groups undoubtedly influences the ratio of the zwitterion form to the un-ionized form as equilibrium constant measurements indicate.[3,15] However, in all cases the equilibrium is already so far in the direction of the dipolar form that the un-ionized molecules are present in negligible amounts. Thus it would seem that this method of approach cannot explain the rather large effect of substituents upon the dipole moments.

The substitution of a difficultly polarizable hydrocarbon chain for easily polarizable water at the side of a dipolar ion would in general increase the observed moment, and, consequently, increase the dielectric increment value for the substance. One would expect that, the longer the hydrocarbon chain, the greater would be the increase in the moment. Thus may be explained the influence of the methyl and isobutyl groups upon the δ-value for glycine, diglycine, and triglycine in the series of Table IV, in which are used the abbreviations, G, A, L for glycine, alanine, and leucine or their corresponding radicals when they appear in compounds. The more closely the hydrocarbon group approaches an end, rather than a side position, the less will be its effect in elevating the incremental value.

The presence of a hydrocarbon side group in the middle of a peptide chain would be expected to increase the observed δ-value both because of reduction of the solvent effect and because it would tend to make closed-up configurations less probable as a result of steric hindrance. Using the data of Greenstein and Wyman for the substances not reported here and the volume corrections listed in Table III (no difference being recorded for a permuted species, AG and GA), the following δ-values are obtained. No value is reported for

TABLE IV

| | | | | | |
|---|---|---|---|---|---|
| G | 25.6 | GG | 76.1 | GGG | 122.4 |
| A | 27.5 | AG | 77.5 | AGG | 126 |
| L | 32.4 | LG | 78.2 | LGG | 132 |

TABLE V

| | | | |
|---|---|---|---|
| GA | 78.3 | LA | 68.3 |
| AG | 77.5 | Gφ A | 81.8 |
| GL | 84.4 | φAG | 68.1 |
| LG | 78.2 | | |

alanylleucine. The symbol φ represents the phenyl radical. In the first four substances, a hydrocarbon group attached to the chain increased the δ-value probably by reduction of the solvent effect. In comparing leucylglycine and leucylalanine, it is noted that the presence of the methyl group of leucylalanine in the middle of the side chain, as the result of steric effects, forced those configurations in which the isobutyl group takes an end-on position to predominate. Thus the δ-value is a compromise between the increase caused by the methyl group and the reduction caused by the end-on isobutyl group. The peptides Gφ A and φAG have the same relationship found for GA and AG and for GL and LG, the hydrocarbon on the side elevating the incremental value and that on the end reducing it. Consideration of molecular models shows that, because of the carbon valence bond angle, the toluyl group will more readily take an end-on position than will the isobutyl group and, hence, it appears reasonable that the δ-values for the toluyl peptides will be somewhat lower than those of the isobutyl peptides.

In the case of the tripeptides (GGG, 122.4; ALG, 137; GLA, 123), the prediction from solvent effects does not follow the experimental determinations. One would expect that GLA would have a larger δ-value than alanylleucylglycine for the end-on position of the hydrocarbon groups is not possible in glycylleucylalanine.

The direct influence upon the actual dipole moments of dipolar ions of substituent side chains may arise from a redistribution of the

(15) Edsall and Blanchard, THIS JOURNAL, **55**, 2337 (1933).

electrical charges within the molecule as a result of inductive effects. However, induction in purely hydrocarbon chains is slight because of the low polarizability of these groups. Moreover, if induction were the major cause of differences in values, a hydrocarbon group at the end position of a molecule would be more effective in increasing the moment than a hydrocarbon in the central region of the molecule. In addition, the change of charge on the hydroxyl oxygen arising from hydrocarbon substituents as calculated from the the ratio of acid strengths[16] of the substituted and unsubstituted amino acids is much too small to change appreciably the dipole moments of the molecules of glycine, alanine, and leucine.

Thus, it is concluded that the most important effect of side chains upon the dipole moments of the polypeptides is that of modification of the solvent effect itself. A complete quantitative approach to the problem cannot be made without more certain knowledge of the shapes of the peptides and of the dielectric properties of the solvent in the immediate vicinity of the polar molecule. However, that there is an enormous solvent effect of water upon the apparent dipole moment of a molecule arbitrarily taken as an ellipsoid of revolution with the dipole at the center is indicated by the calculations of Higasi[17] for which good agreement with experiment has been found for solvents of dielectric constants much lower than water. For a ratio of major axis to minor axis of 2:1, straightforward application of the theory leads to the result that the apparent dipole moment is approximately one-half the real moment; that is, the measured increment may be 400% in error. If conclusions concerning the nature of the peptide molecules are to be obtained from increment measurements, some more satisfactory attempt must be made to determine the solvent effect in highly polar solvents.

A more complete calculation of the influence of the solvent upon the measured dielectric increments of spheroidal dipolar molecules may be obtained in a manner suggested by Kirkwood[18] in his investigation of the dielectric polarization of polar liquids. From a classical statistical mechanical approach, Kirkwood has arrived at a general formula for the dielectric constant of polar mixtures.

(16) Ri, Magee aud Eyring, unpublished.

(17) Higasi, *Sci. Papers. Inst. Phys. Chem. Research* (Tokyo), **28**, 284 (1936).

(18) Kirkwood, *J. Chem. Phys.*, **7**, 911 (1939).

$$\epsilon - 1 = 9/2 \sum_{k=1}^{v} c_k P_k \tag{9}$$

$$P_k = \frac{4\pi N}{3}\left[\alpha_k + \frac{\mu_k \cdot \bar{\mu}_k}{3kT}\right] \tag{10}$$

where $N$ is the Avogadro number; $c_k$ is the concentration in moles per cc. of the component $k$, and $P_k$ its molar polarization; $\mu_k$ is the dipole moment in the solution of a molecule of the $k$th component; and $\bar{\mu}_k$ is the total moment of any finite macroscopic spherical specimen polarized by a fixed molecule of the $k$ type, when immersed in a medium of the same dielectric constant. Equation (9) is exact except for the use of the Onsager approximation of the local field for the optical contribution to the dielectric constant and the simplifying assumption that $P_k/v_k$ is large in comparison to unity where $v_k$ is the molar volume of component $k$. It is well to restate that $\mu_k$ is not equal to the moment of the molecule in the gas phase, but is the total moment of the molecule in solution as modified by its own polarization in the dipole field of its environment.

To make use of Eq. 9, $\bar{\mu}_k$ must be calculated. For a spherical molecule, $\mu_k \cdot \bar{\mu}_k$ reduces to $\mu_k^2$ if the microscopic dielectric constant at the surface of the molecule is not different from the macroscopic dielectric constant, and if short range orientating forces are assumed to be non-existent. Kirkwood has indicated an approximate treatment of the scalar product for the case of water in which short range orientating forces are of major importance.

The effect of the spheroidal nature of the dissolved molecule upon $\mu_k \cdot \bar{\mu}_k$ may be calculated with the assumption that the spheroidal molecule is immersed in a fluid of completely uniform macroscopic dielectric constant in the following manner. The moment $\bar{\mu}_k$ is the total moment induced in a spherical specimen by any one of the molecules of the $k$th component held at a fixed position, the spherical specimen being sufficiently large that the homogeneous polarization resulting from the reaction field produced by the outer boundary is zero in any finite region of the specimen. Thus by neglecting the fact that the dielectric constant of the specimen does not become uniform for a few thousand molecular diameters away from the fixed molecule, $\mu_k$ is given by

$$\bar{\mu}_k = \mu_k + \int_{\lambda_0}^{\infty} P_\mu \mathrm{d}v \tag{11}$$

where $\lambda_0$ defines the volume of the fixed spheroidal molecule and $P_\mu$ is the polarization of the speci-

men outside of the spheroid and is equal to $-((\epsilon - 1)/4\pi) \nabla \psi_{(\lambda_0,\infty)}$, $\psi_{(\lambda_0,\infty)}$ being the electrostatic potential in the interior of the specimen. It can be shown that $\bar{\mu}_k$ is equal to $\mu_k$ if $\lambda_0$ is taken as a sphere.

Proof of Eq. 11 may be found according to Kirkwood's[18] approach. The advantage of defining $\bar{\mu}_k$ is in the elimination of the polarization due to the outer surface of the very large volume of polar liquid. If we define as has Kirkwood, $\overline{M}$ the total moment of a large spherical sample of the polar liquid of radius $R$, then

$$\overline{M} = \overline{M}(R, r_0) + \int_v^{v_0} P dv \qquad (12)$$

where $P$ is equal to $-((\epsilon - 1)/4\pi) \nabla \psi_i$, $\psi_i$ being the electrostatic potential in the region between $v_0$ and $v$, and $\overline{M}(R, r_0)$ is the total moment of a spherical region of radius, $r_0$, with a fixed elliptical molecule at its center. However, we assume here that $r_0$ is so large that not only the local dielectric constant approaches the macroscopic dielectric constant at $r_0$, but also the electrostatic potential due to the fixed elliptical molecule attains a radial dependence. The second assumption is permissible, for the electrostatic potential of a prolate spheroidal molecule with charges at the foci has the spatial dependence of $\eta/(\lambda^2 - \eta^2)$, where $\eta$ and $\lambda$ are the customary confocal elliptical coördinates. If $r_1$ and $r_2$ are the distances from a point in space to the respective foci, and $R_0$ is the interfocal distance

$$\lambda = (r_1 + r_2)/R_0 \qquad (13)$$

and

$$\eta = (r_1 - r_2)/R_0$$

The function $(\eta/\lambda^2 - \eta^2)$ at large distances approaches the radial dependence $1/r^3$, where $r$ is measured from the point $(\lambda, \eta = 1, 0)$.

Now with $\overline{M}(R, r_0)$ having dependence only upon $R$ and $r_0$, the equations derived by Kirkwood for spherical molecules hold exactly for spheroidal molecules. Thus

$$\bar{\mu} = \lim_{\substack{r_0 \longrightarrow \infty \\ R/r_0 \longrightarrow \infty}} \overline{M}(R, r_0) \qquad (14)$$

Therefore $\bar{\mu}_k$ is the moment induced by a fixed molecule, $k$, in an infinite spherical specimen immersed in an infinitely larger sample of the same dielectric constant. Thus the surface polarizations at the two outermost surfaces are eliminated, and Eq. 11 holds in which $\psi_{(\lambda_0,\infty)}$ is determined by only the one surface bounding the molecule itself and the fact that it vanishes properly at infinity.

For a prolate spheroidal molecule, the electrostatic potential $\psi_{(\lambda_0,\infty)}$ has been expressed by Kirkwood[19] in terms of confocal elliptical coordinates for a dipole consisting of two charges $+e$ and $-e$ at the foci of the generating ellipse.

$$\psi_{(\lambda_0,\infty)} = \frac{4\mu_k}{\epsilon R_0^2} \frac{\eta}{\lambda^2 - \eta^2} \left[\frac{1}{\lambda_0}\left(\lambda_0 - \frac{(\lambda_0^2 - 1)}{2} \ln \frac{\lambda_0 + 1}{\lambda_0 - 1}\right)^{-1}\right] \qquad (15)$$

where $R_0$ is the interfocal distance, $1/\lambda_0$ is the eccentricity of the ellipsoid, and $\epsilon$ is the dielectric constant of the external medium. This equation is an approximation, being more accurate for solutions of high dielectric constant and limiting eccentricities, either unity or zero. For solutions of lower dielectric constant $\epsilon$ and for a cavity of dielectric constant $\epsilon_0$, the electrostatic potential is better expressed as

$$\psi_{(\lambda_0,\infty)} = \frac{4\mu_k\sigma}{R_0^2} \frac{\eta}{\lambda^2 - \eta^2} \left[\frac{1}{\lambda_0}\left\{(1 - \sigma)\left[\lambda_0 - \frac{(\lambda_0^2 - 1)}{2} \ln \frac{\lambda_0 + 1}{\lambda_0 - 1}\right] + \frac{\sigma}{\lambda_0}\right\}^{-1}\right] \qquad (16)$$

where $\sigma$ is equal to $\epsilon_0/\epsilon$. This may be simplified by the introduction of $\mu_k^*$, the value of which may be found by comparison with the previous equation.

$$\psi_{(\lambda_0,\infty)} = \mu_k^* \frac{4}{R_0^2} \frac{\eta}{\lambda^2 - \eta^2} \qquad (16a)$$

In the evaluation of the integral of Eq. 11 it is noticed that, as a result of the cylindrical symmetry of the electrostatic potential, there is no total induced moment perpendicular to the dipole axis. Thus one need consider only the component of the electric intensity in the direction of the dipole.

Evaluation of the definite integral of Eq. 11 shows that

$$\bar{\mu}_k = \mu_k + u\mu_k^* (\epsilon - 1)\left[\frac{\lambda_0(\lambda_0^2 - 1)}{2} \ln \frac{\lambda_0 + 1}{\lambda_0 - 1} - \lambda_0^2 + \frac{2}{3}\right] \qquad (17)$$

in which $u$ is a unit vector in the direction of the dipole. Examination of the second term of this expression, which represents the moment induced in the surrounding liquid by a fixed dipolar molecule, shows that the factor in the brackets is always negative, varying between zero and $-1/3$ for eccentricities of zero and unity. In addition, for a high dielectric constant of the surrounding medium, $\mu_k^*$ ranges from $3/2\ \mu_k/\epsilon$ to $\mu_k/\epsilon$ for the same variation

(19) Kirkwood, *Chem. Rev.*, **24**, 233 (1939).

in eccentricity. Since for a prolate spheroid the second term is always negative, the moment induced in the solvent always opposes that of the dipolar molecule; *i. e.*, $\bar{\mu}_k \leq \mu_k$.

In comparing Eq. 17 with that suggested by Higasi[17] for computing the solvent effect for polar molecules dissolved in non-polar solvents, it is noted that the terms enclosed in the brackets are exactly equal to the quantity which he designates as $A$. Since $\sigma = \epsilon_0/\epsilon$ is almost unity, a fair approximation being given for non-polar solvent by $n^2/\epsilon = (1.4)^2/2.3$, $\mu_k^*$ is about 85 ± 5% of $\mu_k$, the uncertainty being dependent upon $\lambda_0$. We may compare the results of the two treatments by the equations

$$\mu_{\text{Higasi}} = \mu_{\text{gas}}\left[1 + (\epsilon - 1)\,\text{A}\,\frac{3}{\epsilon + 2}\right]$$

$$\mu_{\text{eq. (17)}} = \mu_{\text{gas}}\left[1 + \frac{(\epsilon - 1)}{2}\,\text{A}\,(0.85)\right]$$

The factor $^1/_2$ occurs in the second expression because the actual calculated moment is proportional to the square root of $\mu_k\bar{\mu}_k$. Thus from the equations in this paper, $(\Sigma\mu_i/\mu)$ calcd. is about 45% lower than those reported in Table VII of Higasi's paper.[17] Such a reduction agrees well with the experiments in all cases except chlorobenzene, for which neither calculation is satisfactory. Undoubtedly, the assumption of prolate spheroidal shape with the dipole charges at the foci is a rather poor approximation for this molecule. It should be pointed out that both of these treatments of the solvent effect neglect the Onsager reaction field which, in general, acts to increase the molecular moment, about 10% for spherical molecules dissolved in non-polar solvents.

By differentiating the approximate Eq. 9 with respect to $c_1$, the concentration of the dipolar molecule, the dielectric increment, $d\epsilon/1000\ dc_1$, may be obtained. Recalling that, for a binary mixture of concentrations $c_1$ and $c_2$, in moles per cc. and molar volumes $v_1$ and $v_2$

$$c_1v_1 + c_2v_2 = 1 \qquad (18)$$

we see that

$$\frac{d\epsilon}{1000\ dc_1} = \frac{1}{1000}\left(\frac{9}{2}P_1 - \frac{9}{2}\frac{v_1}{v_2}P_2\right) \qquad (19)$$

Since the first term is proportional to the polarization of the dipolar molecule and the second is proportional to its volume and to the polarization of component 2, water in this system, the equation may be reduced to that used by Wyman, Eq. 7. Actually, $P_1$ and $P_2$ are functions of the concentrations by virtue of $\bar{\mu}_k$. However, for these low solute concentrations, since the dielectric constants of the solutions are already high, the variation of $\bar{\mu}_k$ with dielectric constant is extremely small. The volume correction of the increment value, $\frac{9}{2000}\frac{v_1}{v_2}P_2$, has been compared with that obtained from Eq. 8 in the last column of Table III. In view of the approximate character of the treatments, the agreement is very good. Neglecting the optical polarization of the dipolar molecule in comparison to that due to the permanent dipole moment, we find that

$$\delta_{\text{corr.}} = d\mu_k\bar{\mu}_k \qquad (20)$$

where $\delta_{\text{corr.}}$ is the value of the increment after the volume correction has been applied and $d$ is a constant independent of $\epsilon$ or $\mu_k$.

In arriving at knowledge concerning the free rotation about the valence bonds of the dipolar ions from a plot of the increment against the number of glycine residues, the increment value used should be that one determined solely by the square of the dipole moment of the peptide in free space, that is, dipole moments should be involved, not increments. However, because of the variation in shape of the peptides as reported in a later paper, the increment values determined in solution are in error on two counts: first, that $\bar{\mu}_k$ is not equal to $\mu_k$ and, second, that $\mu_k$ is not equal to the dipole moment of the molecule in free space but is somewhat larger because of its polarization due to the field of the orientated molecules surrounding it. The polarization due to the reaction field may be large even for a spherical molecule, about 20% for water.[18] However, it probably does not vary greatly with the shape of the molecule within the limits of the shape factors of the first five peptides, unity to two.

In Table VI are listed the increment values corrected by means of Eqs. (17, 20) so as to eliminate dependence upon molecular shape and, thereby, eliminate the first of the errors in the values. The shape factors $k$ are then determined from dispersion measurements as will be reported elsewhere.

TABLE VI

| Peptide | $k = a/b$ | $\lambda_0 = \frac{k}{\sqrt{k^2-1}}$ | $\frac{\bar{\mu}_k}{\mu_k}$ | $\delta + \gamma V$ | $\delta'$ |
|---|---|---|---|---|---|
| $G_1$ | | | | 25.6 | |
| $G_2$ | 1.27 | 1.62 | 0.921 | 76.1 | 82.7 |
| $G_3$ | 1.58 | 1.29 | .856 | 122.4 | 143.0 |
| $G_4$ | 1.83 | 1.19 | .823 | 175.9 | 214.0 |
| $G_5$ | 2.08 | 1.14 | .799 | 214.4 | 268.0 |

The value $\delta'$ so corrected shows, in Fig. 2, linear dependence upon the number of glycine residues. It is concluded that the dipole moment $\mu$ in solution also increases as a linear function of the square root of the number of glycine residues. For completely free rotation about the valence bonds in long polypeptide chains, one would expect that the moment in free space would be proportional to the square root of the number of glycine residues. However, Kuhn,[20] in his calculation of the mean square distance between the charged ends of a dipolar molecule, taking into account the attraction between the charged ends and assuming a value of 1.5 Å. for the closest approach of the charged groups, has shown that the linear relationship does not hold for the shorter molecules, but is valid only for very long chains. For short chains, the mean square distance increases more rapidly than the number of valence bonds up to a limiting linear relationship. Thus it is surprising that these smaller peptides reported here exhibit true increment values which are closely linear in the number of glycine residues. Moreover, in solution, because of the reaction field, a completely flexible molecule should be stabilized in a slightly extended configuration, the ease of extension increasing with the number of chain links. One might expect, then, an additional increase in slope in the increment–peptide residue curve with increasing numbers of peptide residues.

These considerations, together with the dispersion measurements,[21,22] indicate a marked degree of rigidity in the peptide molecules. However, the uniform increase in increment value implies a large measure of freedom to attain a random distribution of the molecules among the possible orientations about the valence bonds, the potential barriers between configurations being relatively high but not prohibitive for crossing.

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## Summary

An apparatus employing the Twin-T circuit has been used to measure, in the 10-meter wave length region, the dielectric constants of aqueous solutions of twelve amino acid polypeptides in order to obtain the corresponding dielectric increments. The increment values obtained are, for the most part, in satisfactory agreement with such as have been previously determined.

The effect of molecular volume upon the apparent increment values has been investigated and a method of correction has been developed for the effect of the solvent when the solute molecules approximate prolate spheroids in shape. Application of the corrections raises the increment values, which, for the polyglycines, show some deviation from the approximate linear dependence upon the number of glycine residues previously observed. The results are interpreted as still consistent with sufficient rotational freedom within the molecules to permit of an approximately random distribution of the orientations about the valence bonds in the molecules. It is concluded that, in the various di- and tripeptides investigated, the principal effect of side chains upon the molecular dipole moments is that of modification of the solvent effect.

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(22) Marcy and Wyman, THIS JOURNAL, **63**, 3388 (1941).