

# Density, Viscosity and Dielectric Constant of Aqueous Solutions of Triglycine and Tetraglycine

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The density, viscosity, static dielectric constant and microwave dielectric loss of aqueous solutions of triglycine and tetraglycine have been studied at various temperatures from 0 to 60 °C.

A very similar electrostrictive effect is observed for triglycine and tetraglycine solutions, indicating a different conformational behaviour of the two peptides in solution. The density  $b$ -coefficients decrease with temperature and suggest solute-solvent interactions which are also supported by the viscosimetric results. The viscosity  $B$ -coefficients are always positive and the sign of  $dB/dT$  indicates that the two peptides are structure-breaking molecules.

The dielectric data show the existence of extended hydration sheaths with a different thermal stability for the two peptide molecules. The dielectric relaxation times and the activation enthalpies for these processes are estimated.

## Introduction

Oligopeptides have been found to be suitable models for conformational studies aimed at clarifying the structure of proteins [1].

However, in many physical and chemical respects the oligopeptides and their aqueous solutions show closer analogies with the amino-acids from which they are derived than with proteins, whose solution equilibria are controlled by a complex balance of forces.

A gradual change of the physical properties with the number of amino-acid residues in the oligomer is generally observed. For example, in the case of glycine and its derivatives, the dipole moment, dielectric relaxation time [2], solubility in solvents such as water [3], ethanol-water [4] and dioxane-water mixtures [5], adiabatic compressibility [6], ultrasonic adsorption [7], diffusional rate [8], activity coefficient [9], sedimentation rate [10] and refractive index [11] change gradually from glycine to the higher homopeptides.

Recently it was shown that the electrical conductivity of aqueous salt solutions containing amino-acids seems to be affected by ion interactions with polar amino-acid molecules as well as by water amino-acid molecular interactions [12]. Peptides have higher dipolar moments than simple amino-acids. With these we have therefore continued our

studies on aqueous biological solutions, considering specifically the density, viscosity and dielectric behaviour of triglycine and tetraglycine solutions.

The determinations were performed at several temperatures in order to detect possible temperature-structure effects.

## Experimental

Triglycine (BDH product, molecular weight = 189.17) was purified by crystallisation from water and acetone, after a preliminary filtration. The product was then dried under vacuum at 40–45 °C for four days.

Tetraglycine (Fluka product, molecular weight 246.22) was used without further purification. It was dried under the same conditions as triglycine. The tetraglycine solution gave a little insoluble residue which was removed and accurately weighed. The highest concentration reached with tetraglycine was about 0.09 mol/liter at 25 °C.

The solutions were prepared by weighing. Water with a conductivity of about  $0.6 \times 10^{-6} \Omega^{-1} \text{cm}^{-1}$  was used throughout.

In the viscosity and density measurements, the temperatures were controlled within 0.005 °C.

Tables 1 and 2 show the molal concentration  $M$ , density  $d$ , viscosity  $\eta$  and molal apparent volume  $\Phi$  of triglycine and tetraglycine solutions at 25, 35 and 45 °C.

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Table 1. Experimental values of density  $d$ , viscosity  $\eta$  and apparent molal volume  $\Phi$  of triglycine solutions.

$M \cdot 10^2$ mol kg <sup>-1</sup>	25 °C			35 °C			45 °C		
	$d$ g ml <sup>-1</sup>	$\eta$ cP	$\Phi$ cm <sup>3</sup> mol <sup>-1</sup>	$d$ g ml <sup>-1</sup>	$\eta$ cP	$\Phi$ cm <sup>3</sup> mol <sup>-1</sup>	$d$ g ml <sup>-1</sup>	$\eta$ cP	$\Phi$ cm <sup>3</sup> mol <sup>-1</sup>
1.105	0.99792	0.8947	112.2	0.99492	0.7233	111.4	0.99108	0.5994	113.4
2.199	0.99879	0.8988	110.9	0.99578	0.7269	111.0	0.99190	0.6027	113.9
3.379	0.99974	0.9032	109.9	0.99669	0.7307	111.2	0.99277	0.6058	114.4
4.363	1.00048	0.9075	110.7	0.99744	0.7343	111.5	0.99355	0.6087	113.3
6.148	1.00186	0.9149	110.8	0.99878	0.7403	112.0	0.99488	0.6138	113.5
10.225	1.00499	0.9325	110.9	1.00189	0.7547	111.9	0.99789	0.6255	113.8
14.265	1.00802	0.9511	111.3	1.00488	0.7694	111.7	1.00086	0.6370	113.9
18.690	1.01132	0.9729	111.5	1.00809	0.7857	112.7	1.00400	0.6495	114.4
25.650	1.01638	1.0099	111.8	1.01309	0.8123	113.1	1.00888	0.6694	114.8

 $d_{\text{H}_2\text{O}} = 0.99707 \text{ g/cm}^3$  at 25 °C $\eta_{\text{H}_2\text{O}} = 0.8903 \text{ cP}$  at 25 °C $d_{\text{H}_2\text{O}} = 0.99406 \text{ g/cm}^3$  at 35 °C $\eta_{\text{H}_2\text{O}} = 0.7194 \text{ cP}$  at 35 °C $d_{\text{H}_2\text{O}} = 0.99024 \text{ g/cm}^3$  at 45 °C $\eta_{\text{H}_2\text{O}} = 0.5963 \text{ cP}$  at 45 °CTable 2. Experimental values of density  $d$ , viscosity  $\eta$  and apparent molal volume  $\Phi$ , of tetraglycine solutions.

$M \cdot 10^2$ mol kg <sup>-1</sup>	25 °C			35 °C			45 °C		
	$d$ g ml <sup>-1</sup>	$\eta$ cP	$\Phi$ cm <sup>3</sup> mol <sup>-1</sup>	$d$ g ml <sup>-1</sup>	$\eta$ cP	$\Phi$ cm <sup>3</sup> mol <sup>-1</sup>	$d$ g ml <sup>-1</sup>	$\eta$ cP	$\Phi$ cm <sup>3</sup> mol <sup>-1</sup>
0.553	0.99765	0.8930	147.2	0.99461	0.7219	151.2	0.99073	0.5985	153.5
0.659	0.99774	0.8940	147.2	0.99471	0.7227	151.1	0.99085	0.5992	153.4
1.136	0.99822	0.8971	147.1	0.99519	0.7246	151.1	0.99132	0.6011	153.4
1.418	0.99848	0.8987	147.0	0.99547	0.7261	151.0	0.99156	0.6019	153.3
2.044	0.99910	0.9022	146.9	0.99599	0.7289	150.9	0.99214	0.6045	153.2
2.344	0.99938	0.9937	146.9	0.99639	0.7304	150.9	0.99243	0.6060	153.2
2.851	0.99988	0.9045	146.9	0.99689	0.7337	150.8	0.99292	0.6079	153.1

The  $\Phi$  values have been calculated using the equation

$$\Phi = \frac{1000}{M} \frac{d_0 - d}{d d_0} + \frac{M_2}{d} \quad (1)$$

where  $d_0$  is the density of water and  $M_2$  the solute molecular weight. Table 3 shows the coefficients  $b$ ,  $f$ ,  $B$  and  $D$  of the equations

$$d = d_0 + b M - f M^2, \quad (2)$$

$$\eta/\eta_0 = 1 + B M + D M^2. \quad (3)$$

For tetraglycine solutions the quadratic terms of (2) and (3) have been neglected because their determination is too uncertain at very low concentrations.

The coefficients  $B$  found by Orttung [11] ( $B = 0.05587$  and  $0.095$  for triglycine and tetraglycine, respectively, at 25 °C) differ from the data in Table 3. Tsangaris and Martin [13] found a lower  $B$  value than that reported in Table 3 at 35 °C.

The measurements of the dielectric constant of aqueous solutions of triglycine and tetraglycine

(0.33 and 0.022 molar, respectively) were made between 100 kHz and 1 MHz with an HP mod. 4270 A automatic capacitance bridge, and between 1 MHz and 10 MHz with an HP mod. 4815 A Vector Impedance Meter.

At 10 GHz, measurements were carried out using a microwave X band circuit with a TE<sub>011</sub> cavity resonator.

From the observations on the change in the quality factor of the cavity containing the polypeptide solutions, values of the dielectric loss  $\epsilon''$  were calculated. The cavity perturbation method was applied. The temperature of the samples was defined within 0.1 °C and was varied between 0 and 60 °C.

The low frequency dielectric constant measurements are summarized in Table 4. Note that no dielectric dispersion was observed within the frequency range 100 kHz to 10 MHz so that the quoted values must be considered as static dielectric constants. In the same frequency range Sakellaridis et

	Triglycine			Tetraglycine		
	25 °C	35 °C	45 °C	25 °C	35 °C	45 °C
$b$ g cm <sup>-3</sup> molal <sup>-1</sup>	0.0788	0.0778	0.0767	0.0992	0.0957	0.0940
$f$ g cm <sup>-3</sup> molal <sup>-2</sup>	0.0139	0.0144	0.0159			
$B$ (molal <sup>-1</sup> )	0.425	0.467	0.478	0.647	0.679	0.682
$D$ (molal <sup>-2</sup> )	0.386	0.146	0.042			

Table 3.  
Density and viscosity  
coefficients in Eqs. (2)  
and (3).

Table 4. Static dielectric constant of triglycine and tetraglycine aqueous solutions.

$T$ (°C)	5	10	15	20	25	30	35	40	45	50	55
Triglycine 0.33 M	117.2	114.6	112.2	109.9	107.1	104.6	101.8	99.0	95.4	91.4	87.2
Tetraglycine 0.022 M	89.0	87.2	85.2	83.3	81.2	79.2	77.5	75.7	74.2	73.0	71.7

al. [14] have observed small changes in the permittivity of aqueous solutions of diglycine at low concentrations. Such variations are too small to be detected with our experimental set-up.

## Discussion

It may be obvious that the densities of tetraglycine solutions are higher than those of triglycine solutions and, consequently, that the  $b$  coefficients of tetraglycine are higher than those of triglycine (Table 3).

Nevertheless, it is interesting to see that these coefficients increase linearly as the number of amino-acid residues increases, (Fig. 1). The apparent molal volume  $\Phi_0 = (M - 1000b)/d_0$  also changes linearly showing an evident additivity.

Traube [15] predicted that the apparent molal volume of organic molecules in aqueous solutions

is the result of the single molecular contributions of the functional groups present, plus a co-volume of 13 ml per mole for each solute.

Traube computed that the  $-\text{COOH}$ ,  $-\text{CH}_2$ ,  $-\text{CO}-\text{NH}-$  and  $-\text{NH}_2$  functional groups occupy volumes corresponding to 18.9, 16.1, 20.0 and 7.7 cm<sup>3</sup>/mole respectively.

Considering that the polyglycine molecules differ from one other by a  $-\text{CONH}-$  group, the linearity of  $b$  and  $\Phi_0$  is not surprising.

However, the  $\Phi_0$  values are lower than the  $V_T$  values predicted by Traube (Table 5). This behaviour is not unique to glycine oligomers, but is generally observed for charged solutes in water and has been attributed to a general electrostrictive effect, i. e. to a volumetric contraction of water molecules surrounding polar solutes. The electrostriction,  $E = V_T - \Phi_0$ , increases with the complexity of the oligomers because not only the terminal

Table 5. Density coefficient  $b$ , apparent molal volume  $\Phi_0$ , Traube volume  $V_T$ , electrostriction  $E$  and viscosity coefficient  $B$  of glycine and polyglycine solutions at 25 °C.

	molecular weight	$b$ g cm <sup>-3</sup> mol <sup>-1</sup>	$\Phi_0$ cm <sup>3</sup> mol <sup>-1</sup>	$V_T$ cm <sup>3</sup> mol <sup>-1</sup>	$E$ cm <sup>3</sup> mol <sup>-1</sup>	$B$ molal <sup>-1</sup>
(Glycine)	75.07	0.031978 <sup>(1)</sup>	43.22 <sup>(1)</sup>	55.7	12.5	0.16 <sup>(1)</sup>
(Diglycine)	132.12	0.056007 <sup>(1)</sup>	76.34 <sup>(1)</sup>	91.8	15.5	0.35 <sup>(1)</sup>
(Triglycine)	189.17	0.0789	110.6	127.9	17.3	0.425
(Tetraglycine)	246.22	0.0992	147.5	164.0	16.5	0.647

<sup>(1)</sup> H. D. Ellerton, G. Reinfelds, D. E. Mulcaby, and P. S. Dunlop, J. Phys. Chem. **68**, 398 (1964).  
 $\Phi_0 = (M - 1000b)/d_0$ .

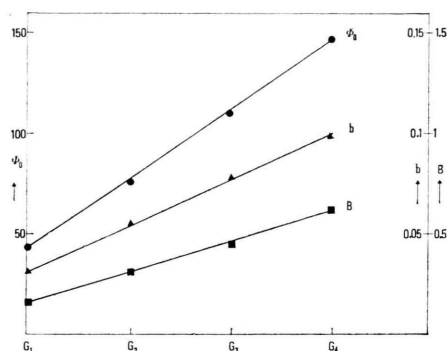


Fig. 1. Partial molar volume  $\Phi_0$  ( $\text{cm}^3 \text{mol}^{-1}$ ) density coefficient  $b$  ( $\text{g cm}^3 \text{mol}^{-1}$ ) and viscosity coefficient  $B$  ( $\text{molal}^{-1}$ ) vs. number of glycine peptides  $G$ .

charged groups but presumably even peptide groups take part in electrostriction. Therefore it is unexpected that the  $E$  values of triglycine and tetraglycine are so similar.

According to Craig et al. [16] polyglycine chains must be more flexible than other polypeptides because of the absence of side chains and strong steric hindrance and, on the basis of spectroscopic data, Destrade et al. [17] proposed a torsional conformation stabilized by intramolecular hydrogen bonds for diglycine and triglycine in water. It may therefore be supposed that our two peptides tend to adopt a spherical shape, reducing the contact surface with the surrounding water molecules. The reduced water-solute interaction may be responsible for the reduced electrostrictive effect.

The coefficients  $b$  are influenced by temperature changes.

At corresponding concentrations, the densimetric increments are higher at low temperature, both for triglycine and tetraglycine solutions. This behaviour may be correlated with structural interactions between the oligopeptide and water molecules.

Glycine and its oligopeptides are considered to be "solvent breaking" and presumably their effects are stronger on highly structured solvents, i. e. at low temperatures.

Moreover, assuming tetraglycine to be more active than triglycine, the density coefficient  $b$  of the former must change more than that of the latter as the temperature changes.

In fact,  $b_{45^\circ} - b_{25^\circ}$  equals 0.0054 and 0.0021 for tetraglycine and triglycine, respectively.

The above suggestion is supported by the viscosimetric results.

The coefficients  $B$  are positive and even  $dB/dT$  is positive. Analogous behaviour is exhibited by several solutes considered water structure breakers [13].

The viscosimetric behaviour of triglycine and tetraglycine does not conform with the prediction of the Einstein equation

$$\eta = \eta_0 (1 + a V g) \quad (4)$$

where  $\eta$  and  $\eta_0$  are the viscosity of the solution and solvent, respectively,  $g$  the concentration of the solute and  $V$  the specific volume of the spherical solute.

In the case of triglycine, for example, the density of dry solute is 1.57 grams per cubic centimeter [18]. Substituting the experimental  $\eta$  and  $g$  values in Eq. (4) gives a coefficient  $a$  of 4.4 instead of the theoretical value 2.5.

The deviation from the Einstein equation may be due to the shape of the solute, which is presumably ellipsoidal and not spherical, or to the above mentioned structural effects. It may be even due to solvation effects which produce an increase in the hydrodynamic size of the solute, not taken into account by the Einstein formulation.

The dipole moment of the triglycine and tetraglycine molecules in aqueous solution can be deduced from the equation [19]

$$\mu = 3.30 \delta^{1/2} \quad (5)$$

where  $\delta = (\epsilon_s - \epsilon_w)/c$  is the dielectric increment;  $\epsilon_s$  and  $\epsilon_w$  are the static dielectric constants of solution and pure water, respectively;  $c$  is the molar concentration.

The dipole moments of triglycine and tetraglycine were found to be about 32 to 25 D and 40 to 36 D, respectively, as the temperature is increased. Note that these decreases may be meaningless, owing to the assumptions involved in deriving (5).

However, if the dipole moments of different polyglycine molecules are compared at the same temperature, it is seen that these values, especially that for tetraglycine, appear to be markedly lower than those obtained from crystallographic data [18].

Evidently, the peptide molecules take up a curvilinear shape in solution, and this the more so, the larger the number of glycine units in the molecule.

Similar behaviour was observed by other authors in glycine and diglycine solution [14, 20] confirming the Destrade and Garrigou-Lagrange [17] torsional model.

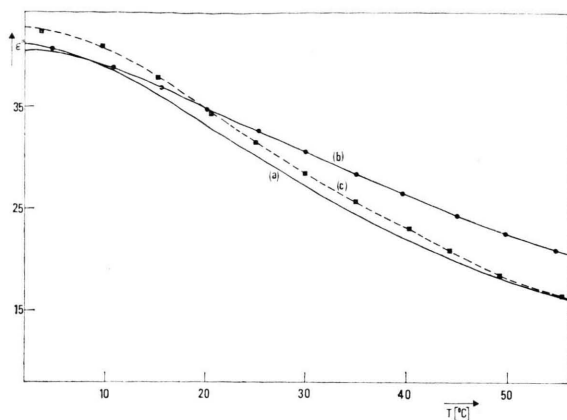


Fig. 2. Dielectric loss  $\epsilon''$  of aqueous solutions of triglycine and tetraglycine as a function of temperature at 10 GHz; curve (b) — Triglycine 0.33 M, curve (c) — Tetraglycine 0.022 M, curve (a) — dielectric loss of pure water.

The dielectric loss,  $\epsilon''$ , of triglycine and tetraglycine solutions at 10 GHz as a function of temperature is shown in Figure 2.

The dielectric loss of pure water at the same frequency and in the same temperature range is also reported for comparison.

As can be seen, the two solutions under test behave very differently. The relaxation times were obtained with an analysis similar to that of Sandus and Lubitz [21]. In the whole temperature range the relaxation times are found to be larger for the triglycine solutions than for pure water, while for the tetraglycine solutions this difference approaches zero as the temperature is increased.

Evidently, this circumstance must be related to solute-solvent interactions, since the relaxation frequency of the solutes occurs in the  $10^8$  Hz region.

The observed dielectric loss can be justified on the basis of a simple model developed by Pottel and Kaatz [22].

The assumption is, that  $N$  water molecules in each hydration sheath relax with a time  $\tau_s = p \tau_w$ , different from that of the bulk water  $\tau_w$ .

The frequency variation of the dielectric loss of the solution can be written as

$$\epsilon'' = \frac{(1-q)\omega\tau_w(\epsilon_s - \epsilon_\infty)}{1 + \omega^2\tau_w^2} + \frac{q\omega\tau_s(\epsilon_{s,s} - \epsilon_\infty)}{1 + \omega^2\tau_s^2}, \quad (6)$$

where  $(\epsilon_s - \epsilon_\infty)$  and  $(\epsilon_{s,s} - \epsilon_\infty)$  are the dielectric increments of pure water and water around the solute molecules, and  $q = Nc/c_0$ , where  $c$  and  $c_0$  are the solute and solvent molarities.

Our experimental data are incomplete for a full utilization of this relaxation model. Nevertheless some interesting features can be obtained if a rather specific assumption about the dielectric increments is introduced.

Assuming that the two dielectric increments have the same value (equal to that of pure water), the characteristic parameters  $p$  and  $q$  can be estimated from  $\epsilon''$  data as a function of temperature.

This assumption seems to be reasonable in the limit of the approximation of the model, since the dielectric increment of water is little dependent on structure change and arrangement of water molecules.

The analysis can be carried out plotting  $q$  vs.  $p$  at a fixed temperature.

For triglycine solution, the curves intercept one another approximately at values of  $p \cong 2.6$  and  $q \cong 0.2$ .

This trend seems to show that the solute-solvent interaction is independent of temperature, in the range investigated, and it can be deduced that about 30 molecules of water are organized around a peptide molecule.

This value is in agreement with those obtained by Pottel and Kaatz for aqueous solutions of heterocyclic  $N$  compounds.

Tetraglycine solution show a markedly different behaviour.

The curves  $q$  vs.  $p$  at various temperatures don't intercept and show a minimum; as the temperature is increased, the minimum corresponds to lower values of  $q$  and higher values of  $p$ . On the basis of these curves, it is possible to conclude that the solute-solvent interactions in tetraglycine solution are strongly dependent on temperature since at the lowest temperatures almost 200 water molecules per peptide molecule must be considered in an organized structure, and this number approaches zero as the temperatures increased.

From a plot  $\ln \tau_s$  vs.  $1/T$ , the activation enthalpy of the relaxation process of water molecules in the sheath around the solute molecules can be also calculated.

For the samples studied, values of about  $3 \div 4$  kcal/mole were estimated. In a similar way it is possible to obtain the activation enthalpy for the viscous process, and the values so derived are equal



to those of the relaxation processes to within the experimental errors. This circumstance strongly suggests that electric reorientation of organized water and viscous flow are closely related processes at molecular level, as previously pointed out for diglycine solutions by Aaron and Grant, who considered the dielectric relaxation of solute molecules.

Tait et al. [23] found three different relaxation times for solutions of monosaccharides, corresponding to relaxation of bulk water, solute and water in primary hydration sheaths. In our case the activation enthalpies were almost equal, which indicates that they must be attributed to ordinary water and water of the secondary hydration sheath.

- [1] G. Nemethy and M. P. Prinz, *Macromolecules* **5**, 755 (1972).
- [2] E. J. Cohn and J. T. Edsall, *Proteins, Aminoacids, and Peptides*, Reinhold, New York 1943.
- [3] P. K. Smith and E. R. B. Smith, *J. Biol. Chem.* **135**, 279 (1940).
- [4] M. S. Dunn and F. J. Ross, *J. Biol. Chem.* **125**, 309 (1938).
- [5] Y. Nozaki and C. Tanford, *J. Biol. Chem.* **246**, 2211 (1971).
- [6] S. Goto and T. Isemura, *Bull. Chem. Soc. Japan* **37**, 1697 (1964).
- [7] G. G. Hammes and C. N. Pace, *J. Phys. Chem.* **72**, 2227 (1968).
- [8] L. G. Longworth, *J. Amer. Chem. Soc.* **74**, 4155 (1952); **75**, 5705 (1953).
- [9] E. E. Schrier and R. A. Robinson, *J. Solution Chem.* **3**, 493 (1974).
- [10] W. G. Martin, C. A. Winkler, and W. H. Cook, *Can. J. Chem.* **37**, 1662 (1959).
- [11] W. H. Orttung and J. M. Orttung, *J. Phys. Chem.* **74**, 2143 (1970).
- [12] B. Sesta and G. Di Schino, *Gazzetta Chim. Ital.* **105**, 1119 (1975).
- [13] J. M. Tsangaris and R. B. Martin, *Arch. Biochem. Biophys.* **112**, 267 (1965).
- [14] P. U. Sakellaris and E. K. Karageorgopoulos, *Z. Naturforsch.* **29a**, 1834 (1974).
- [15] J. Traube, *Samml. Chem. U. Chemtechn. Vortr.* **4**, 255 (1899).
- [16] M. Burackik, L. C. Craig, and J. Chang, *Biochemistry* **9**, 3293 (1970).
- [17] C. Destrade and C. Garrigou-Lagrange, *J. Mol. Structure* **31**, 301 (1976).
- [18] Landolt-Börnstein, *Structure Data of Organic Crystals*, N.S. III/5a, Springer-Verlag, Berlin 1971.
- [19] J. T. Edsall and J. Wyman, *Biophysical Chemistry*, New York 1958.
- [20] M. W. Aaron and E. H. Grant, *Brit. J. Appl. Phys.* **18**, 957 (1967).
- [21] O. Sandus and B. B. Lubitz, *J. Phys. Chem.* **65**, 881 (1961).
- [22] C. R. Pottel and U. Kaatz, *Ber. Bunsenges. Phys. Chem.* **73**, 437 (1969).
- [23] M. J. Tait, A. Suggett, F. Franks, S. Ablett, and P. A. Quickenden, *J. Sol. Chem.* **1**, 131 (1972).