175. ^{17}O-NMR. of Enriched Acetic Acid, Glycine, Glutamic Acid and Aspartic Acid in Aqueous Solution. II. Relaxation Studies[1])

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Summary

The ^{17}O-NMR. line widths of the enriched amino acids glycine, aspartic acid, glutamic acid and, for comparative reasons, acetic acid were measured in aqueous solution between pH 1 and 14. The ^{17}O-NMR. line-width maxima of glycine at pH $\approx$ 11 and acetic acid at pH $\approx$ 5 are shown to arise from traces of paramagnetic metal ions. The increase in line width in case of glycine on going from the zwitterion to the cation is attributed to an increase in the ^{17}O-quadrupole coupling constant. No evidence for an intermolecular or intramolecular association of glycine in the zwitterionic form was found. When the paramagnetic impurities were eliminated by addition of EDTA, both the longitudinal and transverse relaxation times remained unchanged on deprotonation of the amino group. The overlapping of the α,β- and α,γ-carboxyl resonances of aspartic and glutamic acid, respectively, created difficulties for their line-width analysis.

1. Introduction. – The pH-dependence of the ^{17}O-chemical shifts of enriched acetic acid, glycine, aspartic acid and glutamic acid has been outlined in the preceeding paper [1]. In this paper we present the results of transverse and longitudinal ^{17}O-relaxation time measurements of these compounds in aqueous solution and discuss their variation with pH. The study of molecular dynamics by ^{17}O-relaxation could be of considerable value since one generally encounters only one dominant relaxation mechanism, that due to interaction between the quadrupole moment of the ^{17}O-nucleus and the electric field gradients in the molecule [2]. Therefore, with the aid of an appropriate model, the measured relaxation times can be analyzed in terms of the molecular reorientation motions. This is of particular interest if the ^{17}O-quadrupole coupling constant has been obtained independently, *e.g.* by nuclear quadrupole resonance spectroscopy (NQR.) [3]. However, in a recent review on the multinuclear NMR. approach to amino acids and peptides [4] no mention of ^{17}O-NMR. was made. Since then, *Burger et al.* [5] have shown that

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^{14}N- and ^{17}O-relaxation can provide valuable complementary information concerning molecular reorientation in amide systems. *Valentine et al.* [6] used ^{17}O line-width data to discuss the intramolecular association of amino acids. The purpose of the present study is to gain more insight into the utility of ^{17}O as a molecular probe in biological systems. The fact that the O-atom participates directly in intra- and intermolecular H-bonds suggests that the use of ^{17}O-NMR. might be a productive approach. Finally, let us point out that in the course of the pH-titration studies of the amino acids and acetic acid we also analyzed other possible contributions to ^{17}O-relaxation, *i.e.* the effect of proton-exchange broadening and that of paramagnetic impurities.

2. Experimental. – The solvents and solutes used throughout this investigation as well as the procedures of ^{17}O-enrichment and sample preparation have been described previously [1]. The pH-values > 12 were measured by a high-resistance electrode (*W. Möller,* Zürich). In addition, several NMR. titrations have been performed in the presence of EDTA to eliminate the influence of paramagnetic metal ion impurities. Atomic absorption spectra were run on a *Varian* Model *1250* instrument for Mn^{2+} and Cu^{2+}. The concentrations were found to be $\leqslant 10^{-6}$M.

NMR. measurements. ^{17}O-NMR. spectra were recorded at 27.11 MHz using a *Bruker CXP-200* spectrometer equipped with either a high-power (90° pulse angle $\simeq$ 10 μs) or a high-resolution (90° pulse angle $\simeq$ 50 μs) probe head. No field/frequency-locking system was used. The conditions of acquisition and *Fourier* transformation were the same as outlined in the preceeding paper [1]. No proton decoupling was applied because of the intense heating of solutions containing 1M NaCl [7].

Relaxation measurements. For single resonances an exponential line-broadening function was applied to the FID's before FT, hence line widths at half height of the absorption curves had first to be corrected [8] for the line-broadening factors (LB. = 50–75 Hz). The transverse ^{17}O-relaxation times were then obtained according to $T_2 = 1/\pi \cdot \Delta\nu_{1/2}$. Representative spectra were fitted to a *Lorentzian* line-shape function (*Bruker* DISNMR program). The values of $\Delta\nu_{1/2}$ from the best fit agreed with those of $\Delta\nu_{1/2}$ obtained by direct measurements to within ± 5%. The pH-dependent line width of glycine was measured at two different *Larmor* frequencies: *a)* at 27.11 MHz on the *CXP-200,* and *b)* at 12.2 MHz on a *Bruker HX-90* spectrometer operating in the FT-mode and with internal proton lock. Throughout, the contribution of the magnetic field inhomogeneity to the line width was < 5 Hz and within the experimental error (*cf.* the *Table*).

^{17}O-Longitudinal relaxation times were measured by the inversion-recovery method using a high-power probe. In the pulse sequence $(180° - \tau - 90° - T)_n$ sixteen values of τ were selected, T was > 5 T_1 of the amino acids and n = 100,000. The T_1 values were determined by a three-parameter nonlinear least-squares fit. They are considered reliable to ± 5%.

3. Theoretical background. – In diamagnetic solutions the ^{17}O-nucleus (I = 5/2) relaxes predominantly by the quadrupolar mechanism [2] [9]. In the motional narrowing limit, $\omega_0^2\tau_c^2 \ll 1$, which can be anticipated for small molecules in nonviscous liquids, the expression for the quadrupolar relaxation is given by *Equation 1,*

$$\frac{1}{T_{1Q}} = \frac{1}{T_{2Q}} = \frac{3}{125}\left(1+\frac{\eta^2}{3}\right)\left(\frac{e^2qQ}{\hbar}\right)^2\tau_c \qquad (1)$$

where ω_0 is the *Larmor* frequency of the ^{17}O-nucleus, τ_c the correlation time for isotropic molecular reorientation, $e^2qQ/\hbar$ the nuclear quadrupole coupling constant (NQCC.) and η the asymmetry parameter of the electric field gradient.

Relaxation of the ^{17}O-nucleus due to ^{17}O,^{1}H-scalar interaction will occur when the rate of proton exchange $1/\tau_e$ (where τ_e is the average life-time of either state)

approaches the order of magnitude of the coupling constant J. Then, the contribution of the scalar relaxation to T_1 is negligible, but the contribution to T_2 becomes [10]

$$\frac{1}{T_{2SC}} = \pi^2 J^2 \tau_e \tag{2}$$

In samples containing paramagnetic metal ions the ^{17}O-nucleus will be additionally relaxed by the dipolar and hyperfine interactions between the nucleus and the unpaired electrons [11]. The contribution of the dipolar interaction to the relaxation times is given by *Equations 3* and *4* [12] [13]

$$\frac{1}{T_{1D}} = \frac{2S(S+1)\gamma_I^2\gamma_S^2}{15r^6}\left(\frac{3\tau_d}{1+\omega_I^2\tau_d^2} + \frac{7\tau_d}{1+\omega_S^2\tau_d^2}\right) \tag{3}$$

$$\frac{1}{T_{2D}} = \frac{S(S+1)\gamma_I^2\gamma_S^2}{15r^6}\left(4\tau_d + \frac{3\tau_d}{1+\omega_I^2\tau_d^2} + \frac{13\tau_d}{1+\omega_S^2\tau_d^2}\right) \tag{4}$$

where γ_I and γ_S are the magnetogyric ratios of the nuclear spin I and electronic spin S, respectively, ω_I and ω_S are the corresponding *Larmor* frequencies and r is the distance between the nucleus and the metal ion. The correlation time τ_d is determined by three distinct times, where τ_r is the molecular rotation time of the

$$\frac{1}{\tau_d} = \frac{1}{\tau_r} + \frac{1}{\tau_s} + \frac{1}{\tau_m} \tag{5}$$

metal complex, τ_s is the electron spin relaxation time and τ_m is the mean life-time of the ligand in the bound state.

The contribution of the hyperfine interaction is given by *Equations 6* and *7*

$$\frac{1}{T_{1H}} = \frac{2S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2 \frac{\tau_h}{1+\omega_S^2\tau_h^2} \tag{6}$$

$$\frac{1}{T_{2H}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2 \left(\tau_h + \frac{\tau_h}{1+\omega_S^2\tau_h^2}\right) \tag{7}$$

where A is the hyperfine coupling constant for the nucleus I and τ_h is given by *Equation 8*.

$$\frac{1}{\tau_h} = \frac{1}{\tau_s} + \frac{1}{\tau_m} \tag{8}$$

Chemical exchange will also influence the effective relaxation of the ^{17}O-nucleus. Only rapid exchange conditions are of interest in the present work. Let us consider that the ^{17}O-nucleus exchanges between two environments A and B

with different intrinsic relaxation rates, T_{2A}^{-1} and T_{2B}^{-1}. If the rates of exchange τ_A^{-1} and τ_B^{-1} are large with respect to the chemical shift difference and the relaxation rates ('fast exchange narrowing'),

$$\tau_A^{-1}, \tau_B^{-1} \gg |\omega_A - \omega_B|, T_{2A}^{-1}, T_{2B}^{-1} \quad (9)$$

the observed relaxation rate is the weighted average of the contributions from the two sites *(Eqn. 10)* [14], where P_A and P_B are the populations of the respective sites.

$$\frac{1}{T_2} = \frac{1}{T_1} = \frac{P_A}{T_{2A}} + \frac{P_B}{T_{2B}} \quad (10)$$

While still under conditions of rapid exchange, but before the sharpening of the resonance is completed *(Eqn. 11)*, the longitudinal relaxation rate averages over

$$\tau_A^{-1}, \tau_B^{-1} > |\omega_A - \omega_B| \quad (11)$$

the sites as before, however the transverse relaxation rate is given by *Equation 12* [14]

$$\frac{1}{T_2} = \frac{P_A}{T_{2A}} + \frac{P_B}{T_{2B}} + P_A^2 P_B^2 (\tau_A + \tau_B)(\omega_A - \omega_B)^2 \quad (12)$$

and depends therefore on the actual frequency-shift difference between the sites.

In the case of exchange between A and a dilute species B, $P_B \ll P_A$, and in the fast exchange narrowing limit *(Eqn. 9)* the transverse relaxation rate is given by *Equation 13*. The extent of line broadening obtained when B is for example a

$$\frac{1}{T_2} = \frac{1}{T_{2A}} + \frac{P_B}{T_{2B}} \quad (13)$$

paramagnetic complex can be expressed as in *Equation 14* and is directly pro-

$$\frac{1}{T_{2P}} = \frac{1}{T_2} - \frac{1}{T_{2A}} = \frac{P_B}{T_{2B}} = P_B \left(\frac{1}{T_{2D}} + \frac{1}{T_{2H}}\right) \quad (14)$$

portional to the relaxation rate of the nucleus in the bound ligand.

4. Results. – *Titration of acetic acid. Figure 1* shows the pH-dependence of the ^{17}O-line-width of 0.1 M acetic acid in aqueous solution containing 1 M NaCl. The line width was measured to be ≈108 Hz at low pH, then passed through a maximum of ≈123 Hz near the pK_a of the carboxyl and reached a value of ≈98 Hz at high pH. To decide if the maximum was due to an incomplete 'wash out' of the $^1J(OH)$-coupling (see *Eqn. 2* and *Discussion*) a further titration was carried out with deuteriated acetic acid in ^2H_2O. It can be seen *(Fig. 1)* that the line-width behavior of CH_3COO^2H is very similar to that of CH_3COOH, with a maximum near the

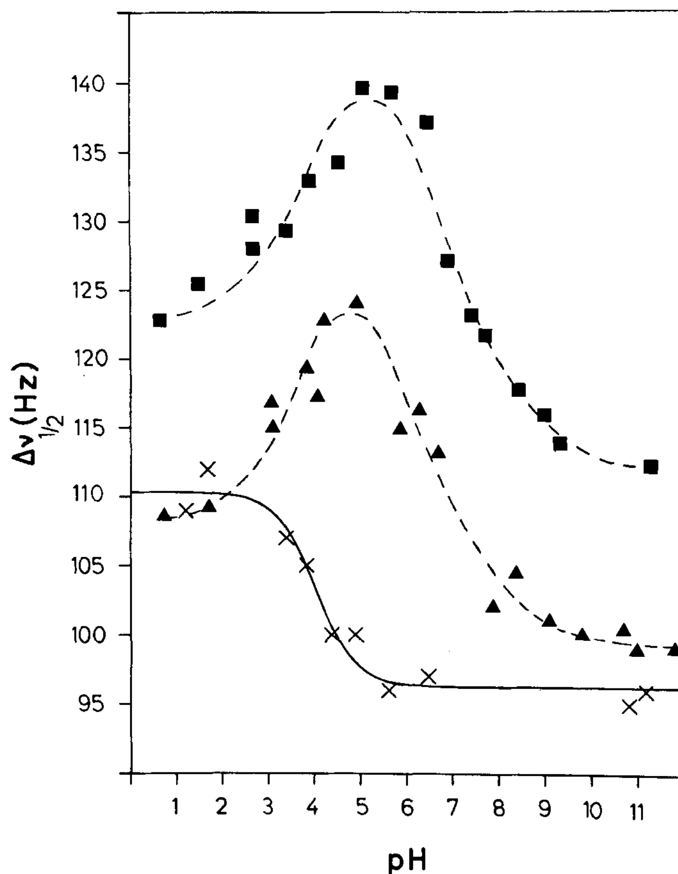

Fig. 1. *The pH-dependence of the ^{17}O-NMR.-line-width of 0.1 M acetic acid measured at 27.11 MHz and 40° in H_2O (▲) and in ^2H_2O (■) in the presence of 1 M NaCl* (The experimental points (×) correspond to the solution of acetic acid in H_2O after addition of 0.0004 M EDTA. The solid line corresponds to a nonlinear least-squares fit of the points (×) to an equation analogous to *Equation 1* in [1] expressed for the line-width variables. The line-width titration parameters are listed in the *Table*)

pK_a of the carboxyl and an increase in the line width of ≈15 Hz relative to that measured at low pH.

The titration of the 0.1 M solution of acetic acid in 1 M aqueous NaCl was repeated after addition of 0.0004 M EDTA. This did not change the ^{17}O-chemical shifts of acetic acid within experimental error. However, a dramatic change in line-width dependence was observed *(Fig. 1)*. The maximum of the line width near the pK_a disappeared and the curve followed a simple one-proton titration equilibrium in rapid exchange *(Eqn. 10)* with an inflection point at the pK_a. The line widths obtained for the two ionization states of acetic acid by a nonlinear least-squares fit are given in the *Table*.

Table. *^{17}O-NMR.-Line-widths of acetic acid and glycine in the different ionization states*[a])

	Line width $\Delta\nu_{1/2}$ (Hz)[b])		
	[c])	[d])	[e])
Acetic acid	108	98	
	110[f])[g])	96[f])[g])	
	102[h])	92[h])	
	123[i])	113[i])	
Glycine	205[g])	132[g])	
	226[k])[g])	137[k])[g])	138[k])

[a]) Measured in 0.1 M solutions in H_2O which contained 1 M NaCl; T = 40°. [b]) Estimated accuracy ±2%. [c]) Line width of the protonated carboxyl group at acid pH. [d]) Line width of the deprotonated carboxyl group. [e]) Line width of the deprotonated carboxyl group after deprotonation of the α-amino group at basic pH. [f]) Obtained after addition of 0.0004 M EDTA. [g]) Calculated from nonlinear least-squares fits of one-proton titration curves [1] to the experimental data. [h]) Measured in H_2O without added NaCl. [i]) Measured in ^2H_2O. [k]) Obtained after addition of 0.002 M EDTA.

Titration of glycine. In contrast to the behavior of acetic acid, the ^{17}O-line-width of the carboxyl absorption of glycine showed a broad minimum between pH 4 and 7, with a subsequent increase at both lower and higher pH-values *(Fig. 2)*. Qualitative agreement is found with the results reported by *Valentine et al.* [6], an absolute comparison of the line widths being impossible due to differences in the temperature, concentration and ionic strength [15]. However, when the titration was continued in the high pH-region we observed a broad maximum centered at pH ≈ 11, followed by a decrease in line width of ≈ 60 Hz up to pH 14 *(Fig. 2)*. To evaluate if this maximum was due to a slow exchange process between the zwitterionic and anionic forms of glycine, we studied the line-width dependence from pH 7 to 12 at two different magnetic field strengths and found it completely field-independent *(Fig. 2)*. Thus, the difference in line width (and T_2) at neutral and basic pH had to be explained by other means. T_1-measurements gave similar values for the carboxyl O-atoms (2.4 ± 0.1 ms at pH 6.1 and 2.3 ± 0.1 ms at pH 10.7). This indicated the presence of paramagnetic impurities with long spin electronic relaxation (see *Discussion*). Addition of 0.002 M EDTA to the original solution showed clearly the disappearance of any line-width variation in the neutral and high pH-region (*Fig. 2* and the *Table*).

Titration of aspartic acid and glutamic acid. The line widths could not be evaluated for the dicarboxylic amino acids owing to the strong overlapping of the resonances. To separate the resonances a *Gaussian*-exponential resolution-enhancement function [1] [16] was applied to the FID which after FT no longer gave *Lorentz*ian line shapes (*cf. Fig. 1* in the preceeding paper [1]).

5. Discussion. – *The ^{17}O-line-width of glycine at neutral pH.* Some controversies can be found in the literature concerning the conformational and aggregational state of amino acids in aqueous solution [17–19]. Early (^{13}C-T_1)-measurements reported the possibility of intermolecular association of amino acids at the isoelectric point [17]. Reinvestigation, however, revealed no significant dependence of the relaxation times of the carboxyl C-atoms upon either pH or concentration [19].

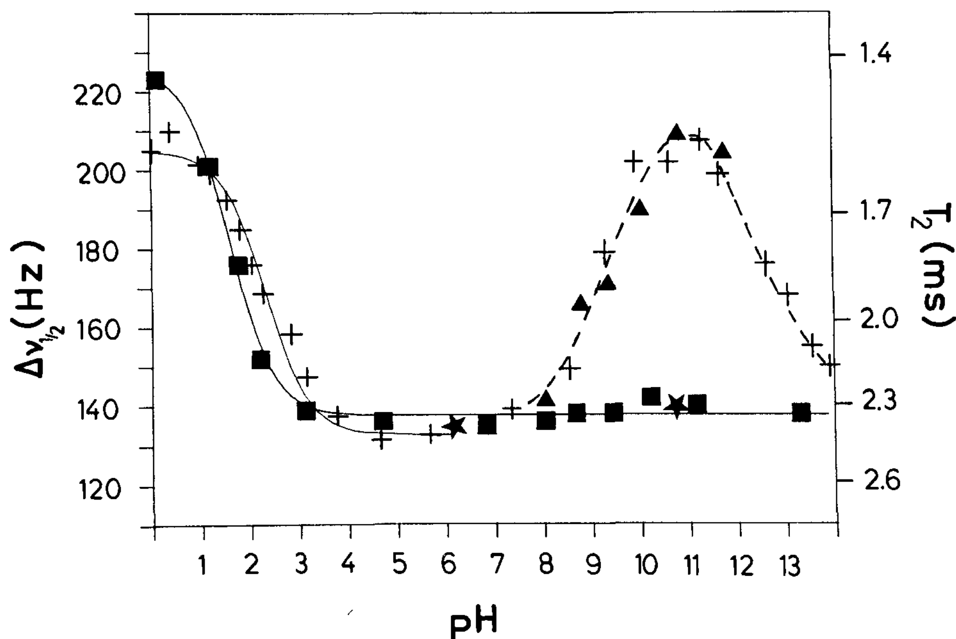

Fig. 2. *The pH-dependence of the* ^{17}*O-NMR.-line-width of 0.1* M *glycine in* H_2O *which contained 1* M *NaCl; temperature = 40°;* (+), *measured at 27.11 MHz;* (▲), *at 12.2 MHz;* (■), *at 27.11 MHz after addition of 0.002* M *EDTA* (The dashed lines were drawn to follow the experimental points. The solid lines correspond to a nonlinear least-squares fit to an equation analogous to *Equation 1* in [1] expressed for the line-width variables; (★) are T_1 values (ms) measured at 27.11 MHz)

Our ^{17}O-NMR. results for glycine show a minimum line width at neutral pH *(Fig. 2)*. The value of ≈125 Hz compares well with that of ≈98 Hz observed for the acetate ion where the monomeric state is found in aqueous solution [20]. Thus, at the concentration, ionic strength and temperature used, an intermolecular association of glycine is highly improbable.

The ^{17}O-line-width minimum has been previously explained [6] by a decrease of the molecular tumbling time *(Eqn. 1)* attributable to a reduction in hydration and intramolecular association of glycine in its zwitterionic state. The same proposition was also made by *Blomberg et al.* [21] from the ^{15}N-NMR. chemical shifts of the cation and the zwitterion. Assuming that an interaction exists between the charged carboxyl and amino groups in the zwitterionic form of glycine, a change in pH in either direction would cause the collapse of the intramolecular complex and hence give an increase in line width reaching a constant value at both ends of the pH-scale. However on titrating to very high pH we observed a maximum at pH≈11, a result difficult to reconcile with the zwitterionic model of *Valentine et al.* [6]. In fact we shall see below that the line width attributable to the glycine anion is equal to that of the zwitterion. Our conclusion that no intramolecular interaction exists for glycine in aqueous solution, is in accordance with the previous interpretation of the ^{17}O-chemical shifts [1].

The ^{17}O-line-width of glycine at high pH. Several explanations are possible for the line-width behavior of glycine at high pH, *a)* the proton exchange between the zwitterionic and anionic forms, *b)* the exchange between different hydrated species, *c)* a pH-dependent aggregation of the zwitterionic and/or anionic forms, and *d)* contamination of the solution with paramagnetic impurities. A chemical-shift averaging between the ^{17}O-resonances in $H_3N^+CH_2COO^-$ ($\delta = 270.5$ ppm) and $H_2NCH_2COO^-$ ($\delta = 267.4$ ppm) could justify the different values of T_1 and T_2 observed at pH 10.7 *(cf. Eqn. 12)*. The same mechanism has been suggested from the pH-dependence of the ^{15}N-NMR. line-shape and nuclear *Overhauser* enhancement in glycine [22]. The ^1H-decoupled ^{15}N-resonance broadened at pH 8.3, then vanished into the baseline at pH ≈ 11 and finally returned at pH 13.5, a behavior which is in very close agreement with that of the ^{17}O-resonance *(Fig. 2)*. There are however two arguments against the possibility of chemical-exchange broadening. Firstly, although the pH determines not only the equilibrium concentrations but also the rate of proton exchange [23], the maximum line width would still be expected to be near but no higher than the pK_a of the amino group (≈ 9.4) where the relative concentrations of the exchanging species are equal. This is in contrast to our observation, the maximum being at pH ≈ 11. Second, since $T_2 < T_1$ at pH 10.7, a square dependence of the transverse relaxation rate upon the field strength is expected *(Eqn. 12)*. We found the line width of glycine field-independent *(Fig. 2)* and must therefore conclude that the high pH-broadening cannot be due to a diamagnetic exchange process *a* or *b*.

A pH-dependent aggregation *c* of glycine is ruled out also for the reason of $T_2 < T_1$ at pH 10.7. Since the expected degree of aggregation is small the extreme narrowing condition should be fulfilled and, according to *Equation 1*, $T_1 = T_2$ is imposed.

Paramagnetic impurities. Addition of the chelating agent EDTA to the glycine solution produced one constant value for the ^{17}O-line-width between pH 5–14 *(Fig. 2)*. This proves unambiguously that the line broadening which existed before in this pH-range must arise exclusively from traces of paramagnetic metal ions. Complexation of these ions with the carboxylate anions of glycine increases the line width at pH values >7 when the ions are in rapid exchange between the free and the bound state. Indeed, fast exchange narrowing conditions *(Eqn. 10)* must be fulfilled since the line width was independent of the field strength *(Fig. 2)*. Obviously, at high pH the hydroxyl group competes with the glycine anion for the metal ion, this probably being the cause of the line-width maximum at pH ≈ 11.

Despite the very sensitive effect of these paramagnetic ions on the ^{17}O-line-width, the change in chemical shift observed after EDTA addition was <0.5 ppm throughout the pH-region and therefore negligible. This behavior is typical of species with long electronic relaxation times such as $\tau_s \approx 10^{-9}$–10^{-8} s for Cu^{2+}- and Mn^{2+}-ions [24]. These ions also allow us to explain the difference in the T_1 and T_2 values observed at pH 10.7. According to *Equations 3* and *4* a ratio $T_{1D}/T_{2D} = 1.17$ from the dipolar relaxation is expected in a 4.7 T magnetic field [11]. However, since τ_s is sufficiently large so that $\omega_s^2 \tau_h^2 \gg 1$, from *Equations 6* and *7* a hyperfine contribution to only $1/T_{2H}$ but not to $1/T_{1H}$ is obtained, in agreement with our results.

The ^{17}O-line-width of glycine at low pH. The line-width dependence at low pH corresponds to the rapid exchange conditions between the anionic and the zwitterionic states *(Eqn. 10)* and is independent, within experimental error, of EDTA *(Fig. 2)*. The curve shows an increase in line width on going to low pH with an inflection point around the pK_a of the carboxyl group. No exchange broadening due to ^{17}O, ^{1}H spin-spin coupling *(Eqn. 2)* is observed.

The ^{17}O-line-width of acetic acid. The line-width behavior of CH_3COO^2H was found to be similar to that of CH_3COOH with a pronounced maximum at $pH \approx 5$ *(Fig. 1)*. Since the $^1J(O^2H)$ coupling constant is reduced by a factor $\gamma_H/\gamma_{^2H} = 6.51$ with respect to $^1J(OH)$, an elimination of the exchange broadening would be expected in ^2H_2O if $^1J(OH)$ was the dominant broadening factor *(Eqn. 2)*. The results show clearly that the contribution of $^1J(OH)$ to the ^{17}O-line-width of acetic acid is negligible, and is in contrast to that observed for the case of water [25] and alcohols [26]. The constantly higher line width of CH_3COO^2H as compared to that of CH_3COOH parallels the viscosity increase in going from H_2O to ^2H_2O (24% at 25° [25]).

However, addition of EDTA revealed clearly *(Fig. 1)* that this maximum originates from paramagnetic impurities in solution showing a similar pH-dependence to that of glycine *(Table)*. A ^{13}C-NMR. study [27] of the relaxation of the carboxyl C-atom of acetic acid found a maximum of $1/T_1$ in the same pH-region when Cu^{2+}-ions were added to the solution. We can therefore adopt the earlier explanation of the pH-dependence of $1/T_1$ in terms of the binding competition of acetic acid between protons, Cu^{2+}- and OH^--ions [27]. We conclude that *paramagnetic metal ion impurities less than 10^{-6} M can dominate the transverse relaxation of the ^{17}O-nucleus.* This effect has to be accounted for whenever O-atoms can undergo direct bonding with metal ions.

Influence of the NQCC on the ^{17}O-line-widths. NQR. measurements as well as SCF-MO-calculations have recently been performed [28] to determine the NQCC's of several O-functional groups. The calculated value obtained for formic acid is 12.8 MHz compared to 10.5 MHz for the formate ion [28]. Calculation of the asymmetry parameters gave divering results ($\eta = 0.06$ for formic acid and $\eta = 0.51$ for its anion), however the weight of η in the determination of the line width *(Eqn. 1)* is small. Assuming a similar tendency for the NQCC's of acetic acid and glycine and their anionic forms, a reduction of the line width in the anion relative to the acid is expected in agreement with the experimental results *(Table)*. Whether the observed reduction is exclusively due to the alteration of the NQCC or whether there is a further contribution, *e.g.* due to a change in the molecular correlation time by a different hydration of the ionic forms, needs further investigation. Since the (^{13}C-T_1)- and (^{15}N-T_1)-values of glycine were found to be constant in the pH-range studied [19] [29] a change of τ_c does not appear important. An independent determination of the NQCC's in *Equation 1* is desired. It should be emphasized however, that values obtained by NQR. are not necessarily the same as in the liquid state since in the solid state new forms of inter- and intramolecular interaction are to be expected [30] [31]. The evaluation of the NQCC's of amino acids oriented in liquid crystals is now in progress.

The ^{17}O-line-widths of aspartic acid and glutamic acid. The overlapping of the α,β- and α,γ-carboxyl[3]) resonances of aspartic acid and glutamic acid, respectively, did not permit an analysis of the line-width data. Substitution of the α-C-atom increases the bulk of the molecule and leads to a marked broadening of the resonances compared to glycine [15]. A qualitative inspection of the α- and γ-resonances of glutamic acid at pH 3 did not show large differences in their relative line widths (*cf. Fig. 1* in [1]). Although we can now simulate line shapes obtained after multiplication of the FID by a *Gaussian*-exponential function [32] we feel that ^{17}O-NMR. line-width measurements of polycarboxyl amino acids and oligopeptides need either the use of very high field for sufficient resolution or a selective enrichment of the various O-containing groups.

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