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Experimental and theoretical studies of hydrogen bonding in neat, liquid formamide

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Quadrupole coupling parameters (QCPs), χ_Q, for nitrogen, oxygen, the carbonyl deuteron, and both amide deuterons in neat, liquid formamide were measured via NMR relaxation time experiments as a function of temperature. At room temperature the experimental values obtained for nitrogen, oxygen, carbonyl deuteron, and *cis* and *trans* amide deuteron were 2.84, 9.18, 0.170, 0.280, and 0.233 MHz, respectively. These parameters and also the quadrupole coupling asymmetry parameters, η_Q, were calculated using standard *ab initio* self-consistent field methods at the 6-31G* level for eight different clusters of formamide molecules. The cluster sizes varied from one to six molecules and include linear and cyclic structures. At room temperature the theoretical calculations indicate that the dominant (95%) species is a six-membered ring of formamide molecules involving hydrogen bonding between the *trans* amide deuteron and the oxygen. This ring shows strong cooperative effects. The *ab initio* values obtained for the QCPs and the asymmetry parameters of this ring are 2.94 MHz and 0.394 for the nitrogen, 9.28 MHz and 0.402 for the oxygen, 0.182 MHz and 0.038 for the carbonyl deuteron, 0.285 MHz and 0.147 for the *cis*, and 0.221 MHz and 0.223 for the *trans* deuteron, respectively. These results are in excellent agreement with the experimental NMR results; both show clearly that primarily the *trans* amide proton is involved in hydrogen bonding. Other structural data from x-ray, electron, and neutron diffraction, low-frequency Raman and far-IR spectra are consistent with the conclusion that cyclic hexamers are the dominant species of liquid formamide under standard state conditions. © *1995 American Institute of Physics.*

I. INTRODUCTION

Hydrogen bonding plays a central role in the structure of peptides and proteins and is responsible for the native structure of proteins.[1] Much effort has been made using *ab initio* calculations of model systems to gain insight into the structure and dynamics of hydrogen bonding and the interactions that stabilize (or destabilize) hydrogen bonding and how it affects native protein structures (see, e.g., Ref. 2 and other papers cited therein). Experimental measurements are an important complement to such theoretical work, but the direct, unambiguous experimental measurement of hydrogen bonding is a difficult task. In solid state studies of the structure of formamide, cyclic dimers or chains of cyclic dimers have been postulated which involve hydrogen bonding of both amide protons. In earlier liquid studies both linear and cyclic dimers have been postulated, but the experimental data were insufficient to allow a more detailed interpretation. In both the experimental and theoretical work presented here, we show clearly that it is the *trans* amide proton that is involved in the hydrogen bonding and that in neat, liquid formamide at room temperature the dominant (95%) species is a cyclic hexamer of formamide molecules.

One of the more sensitive probes of solution state hydrogen bonding is the quadrupole coupling parameter (QCP), χ_Q, since it is dependent in a very sensitive fashion on the electronic structure near the nucleus. Several solid state NMR studies of single crystals of hydrated salts[3] and of single crystals of amino acids[4] have shown that the QCP is related to the inverse cube of the hydrogen-bond distance. Quite recently solution state NMR studies of water[5–7] and alcohols[8,9] have shown that the deuterium QCP depends in a sensitive way on the concentration and the temperature. The QCP, χ_Q, is the product of the nuclear electric quadrupole moment, eQ, and the electric field gradient (EFG) at the nucleus, eq, that is,

$$\chi_Q = eqeQ. \qquad (1)$$

Since the value of eQ for a given nucleus is a fixed parameter whose value is known with reasonable accuracy, χ_Q provides a measure of the electric field gradient, eq. This is discussed in more detail below. Nuclear magnetic resonance (NMR) relaxation time studies of quadrupolar nuclei offer a convenient way to measure the value of χ_Q since for a spin $I=1$ nucleus the NMR spin–lattice relaxation time, T_1 (or the corresponding relaxation rate, R_1), is given by

$$\frac{1}{T_1} = R_1 = \frac{3}{2}\,\pi^2\left(1 + \frac{\eta_Q^2}{3}\right)\chi_Q^2\tau_c, \qquad (2)$$

where η_Q is the asymmetry parameter of the electric field gradient tensor and τ_c is the rotational correlation time for the molecule. This relation is valid for the condition of "extreme narrowing" where $\omega_0\tau_c \ll 1$ (ω_0 is the NMR frequency of the nucleus being studied). If τ_c is known, then a measurement of the relaxation rate, R_1, provides a value for χ_Q.

The formamide molecule is an excellent model compound for the study of solution state protein structure since it

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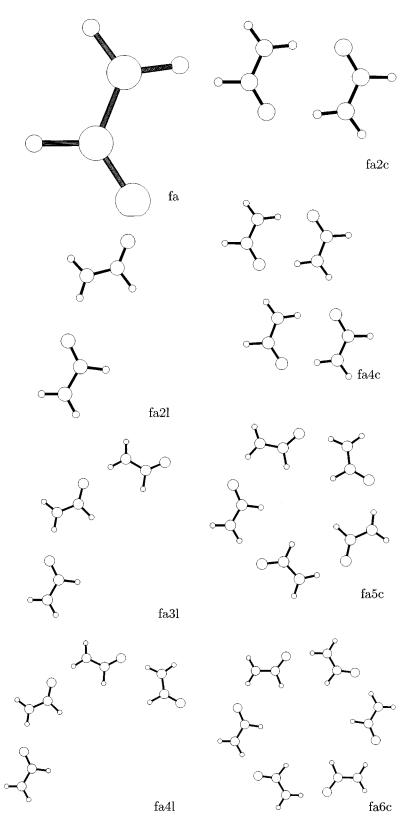[a)]Author to whom all correspondence should be sent.

FIG. 1. The eight different clusters considered for formamide $n = 1-6$ including linear and cyclic structures. The following labels are used to designate the eight different clusters: fa (formamide monomer), fa2l, fa3l, fa4l (linear dimer, trimer, and tetramer, respectively), fa2c, fa4c, fa5c, and fa6c (cyclic dimer, tetramer, pentamer, and hexamer, respectively); these labels are used here and in Tables I–III.

is small enough to be amenable to meaningful *ab initio* calculations and it is ideally suited for a number of NMR relaxation time studies. In the work reported here we have used proton, deuteron, carbon-13, nitrogen-14, nitrogen-15, and oxygen-17 to study the hydrogen bonding interactions for all six nuclei. Since the C–H bond is not significantly involved in hydrogen bonding, its bond distance is essentially independent of solvent and temperature. Given the bond distance,

TABLE I. *Ab initio* quadrupole coupling constants of deuteron C–D, nitrogen, oxygen, *cis* amide deuteron, D_c, and *trans* amide deuteron, D_t, in formamide (6-31G*).

Atom	fa	fa2l	fa3l	fa4l	fa2c	fa4c	fa5c	fa6c
D	0.1819	0.1799	0.1796	0.1795	0.1819	0.1824	0.1836	0.1831
		0.1844	0.1822	0.1819		0.1812		
			0.1849	0.1827				
				0.1851				
	0.1819	0.1822	0.1822	0.1823	0.1819	0.1818	0.1838	0.1831
N	3.882	3.706	3.661	3.644	3.060	2.735	2.955	2.920
		3.417	3.172	3.106		3.3887		
			3.329	3.063				
				3.301				
	3.882	3.561	3.387	3.279	3.060	2.789	2.955	2.920
D_c	0.2866	0.2834	0.2826	0.2825	0.2167	0.2379	0.2854	0.2853
		0.2887	0.2855	0.2851		0.2011		
			0.2891	0.2861				
				0.2892				
	0.2866	0.2861	0.2875	0.2857	0.2167	0.2195	0.2854	0.2853
O	10.291	9.838	9.795	9.782	9.350	9.070	9.249	9.257
		10.093	9.451	9.455		9.019		
			10.036	9.444				
				10.015				
	10.291	9.966	9.790	9.674	9.350	9.045	9.249	9.257
D_t	0.2919	0.2889	0.2880	0.2874	0.2908	0.2506	0.2217	0.2192
		0.2489	0.2409	0.2382		0.2887		
			0.2387	0.2286				
				0.2351				
	0.2919	0.2689	0.2559	0.2473	0.2908	0.2697	0.2217	0.2192

we can measure the molecular correlation time. Since, as shown in a previous study,[10] the motion of formamide is isotropic and on the NMR time scale the molecule is rigid and planar, the correlation time obtained from carbon-13 relaxation time measurements can be used to obtain values for the QCPs of the deuterium, nitrogen, and oxygen nuclei. This has been done for these nuclei over the temperature range 261–395 K.

A number of *ab initio* calculations and molecular dynamics studies have been carried out in order to learn more about the structural and dynamic properties of formamide.[11–24] None of this earlier work includes studies of the quadrupole coupling constants or asymmetry parameters for the liquid phase. We have used *ab initio* methods to calculate the QCP values of the different quadrupolar nuclei for eight different clusters varying in size from the monomer up to a cluster of six formamide molecules, including linear and cyclic structures. For all clusters the equilibrium structure and the total energies were computed. We have performed *ab initio* calculations with the 6-31G* basis set on 3-21G optimized geometries for all configurations.[25] From thermodynamic calculations at standard pressure we also calculated the equilibrium distribution of the formamide clusters at each temperature. Weighting the QCP values and asymmetry parameters with the cluster distribution at the desired tempera-

ture we obtained the average values for both parameters for a sample in the liquid phase.

II. COMPUTATIONAL METHOD

Ab initio calculations were carried out at the uncorrelated restricted Hartree–Fock (RHF) level for the eight formamide $(H_2NCHO)_n$ clusters $n=1-6$ shown in Fig. 1. We have used the following labels to designate the eight different clusters: fa (formamide monomer), fa2l, fa3l, fa4l (for linear dimer, trimer, and tetramer, respectively), fa2c, fa4c, fa5c, and fa6c (for the cyclic dimer, tetramer, pentamer, and hexamer, respectively); these labels are used in Fig. 1 and in Tables I–III. The EFG values for all quadrupole nuclei and clusters were calculated at the 6-31G* basis level, keeping the geometries of the clusters fixed at the values obtained at the 3-21G level[26] and restricting all molecules to a common plane. We have determined RHF/3-21G geometries and harmonic frequencies for each of the different clusters. This level of theory is sufficient to provide a consistent description of molecular geometries.

For smaller clusters $(H_2NCOH)_n$ with $n=1-2$ we also used 6-31G**, 6-311G*, and 6-311G** basis sets. Comparing the results of the different basis sets it was found that the 6-31G* level is already sufficient to provide accurate values

TABLE II. *Ab initio* asymmetry parameters of deuteron C–D, nitrogen, oxygen, *cis* amide deuteron, D_c, and *trans* amide deuteron, D_t in formamide (6-31G*).

Atom	fa	fa2l	fa3l	fa4l	fa2c	fa4c	fa5c	fa6c
D	0.0362	0.0385	0.0385	0.0385	0.0292	0.0218	0.0384	0.0385
		0.0283	0.0312	0.0318		0.0312		
			0.0271	0.0311				
				0.0269				
	0.0362	0.0334	0.0323	0.0321	0.0292	0.0265	0.0384	0.0385
N	0.0283	0.0484	0.0533	0.0555	0.3802	0.2905	0.3870	0.4025
		0.2281	0.2845	0.3024		0.4587		
			0.2759	0.3463				
				0.2910				
	0.0283	0.1383	0.2046	0.2488	0.3802	0.3746	0.3870	0.4025
D_c	0.1728	0.1711	0.1706	0.1703	0.2188	0.2048	0.1474	0.1466
		0.1575	0.1538	0.1526		0.2298		
			0.1545	0.1501				
				0.1536				
	0.1728	0.1643	0.1596	0.1577	0.2188	0.2173	0.1474	0.1466
O	0.0349	0.2014	0.2365	0.2495	0.3602	0.4592	0.3948	0.410
		0.1153	0.3092	0.3518		0.4973		
			0.1321	0.3375				
				0.1392				
	0.0349	0.1584	0.2259	0.2695	0.3602	0.4783	0.3948	0.410
D_t	0.1882	0.1862	0.1857	0.1857	0.1656	0.1883	0.2224	0.2240
		0.2111	0.2098	0.2096		0.1611		
			0.2183	0.2191				
				0.2212				
	0.1882	0.1987	0.2046	0.2089	0.1656	0.1747	0.2224	0.2240

for the components of the electric field gradient (EFG) tensor. This is true for the absolute values as well as for the trend in hydrogen bond strength. In addition to the calculations done using the bond distances obtained from the full optimized geometry, calculations were also carried out for all clusters using the experimentally (microwave) measured value of the C–H bond distance. Only for the C–H bond distance was the optimized value (108.36 pm) significantly different from the experimental one (109.8 pm).[27]

The EFG values and the asymmetry parameters were calculated for each nucleus in all clusters. As seen in Table I, for the same type of nucleus, the values for χ_Q and η_Q are different for each molecule in the linear clusters and the cyclic cluster with four molecules. In this event we calculated the average values. From thermodynamic calculations we obtained the distribution of the clusters at room temperature (298 K). The average EFG for each cluster was weighted with the liquid cluster distribution and used to calculate the

average QCP and asymmetry parameter values of formamide in the liquid phase.

The thermodynamic calculations were carried out with a computer program THERMO that implements a recently developed quantum cluster equilibrium (QCE) theory of fluids to be described in detail elsewhere.[48] Briefly, the QCE method employs the optimized geometries, harmonic frequencies, and binding energies to calculate an *ab initio* partition function for each cluster by the familiar methods of statistical thermodynamics. In the electronic partition function, calculated RHF/3-21G binding energies are corrected for effects of (i) basis set superposition error by full counterpoise calculations[49] and (ii) residual cluster–cluster interactions by a mean-field correction, proportional to system density. The condition of chemical equilibrium (equality of chemical potentials) in the canonical ensemble is then employed to find the cluster populations for the phase of lowest free energy at specified T, P. In the present case, the stable liquid phase at standard-state conditions is calculated to lie about about 11 kJ/mol below the low-density vapor phase. Macroscopic liquid formamide properties are calculated by weighting the properties of each cluster by the appropriate cluster populations. Further aspects of the T, P dependence of the QCE model and comparisons with experimental phase diagrams and quadrupole coupling data will be described in forthcoming papers.

TABLE III. Temperature population of chosen formamide cluusters at 298 K from thermodynamic calculations.

fa	fa2l	fa3l	fa4l	fa2c	fa4c	fa5c	fa6c
0.04	0.04	0.36	4.94	0.04	0.00	0.08	94.52

III. EXPERIMENTAL METHODS

The general expression for the quadrupole longitudinal relaxation rate, $R_1(Q)$, of any nucleus with any spin quantum number, I, in the extreme narrowing region is given by[28]

$$\left(\frac{1}{T_1}\right)_Q = R_1(Q) = \frac{3}{10}\,\pi^2\,\frac{2I+3}{I^2(2I-1)}\left(1+\frac{\eta_Q^2}{3}\right)\left(\frac{eQeq}{h}\right)_Q^2 \tau_c,$$

$$= \frac{3}{10}\,\pi^2\,\frac{2I+3}{I^2(2I-1)}\left(1+\frac{\eta_Q^2}{3}\right)\chi_Q^2\tau_c, \qquad (3)$$

where eQ is the nuclear quadrupole moment, eq is the principal axis component of the electric field gradient tensor, η_Q is the asymmetry parameter for the electric field gradient tensor, I the nuclear spin quantum number ($I=1$ for ^{2}H and ^{14}N and $I=5/2$ for ^{17}O), h is Planck's constant, and $\chi_Q = eQeq/h$ is the quadrupole coupling constant in units of s^{-1}. If the ^{15}N–H and the ^{13}C–H bond distances are known accurately, then the experimental value of the rotational correlation time, τ_c, can be obtained from the well-known longitudinal relaxation equations for nitrogen and carbon; they are

$$\left(\frac{1}{T_1}\right)_{(C)} = R_1(C) = \frac{4n}{3}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma_H^2\gamma_C^2\hbar^2 I(I+1)\,\frac{1}{r_{CH}^6}\,\tau_c,$$

$$(4)$$

and

$$\left(\frac{1}{T_1}\right)_{(N)} = R_1(N) = \frac{4n}{3}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma_H^2\gamma_N^2\hbar^2 I(I+1)\,\frac{1}{r_{NH}^6}\,\tau_c,$$

$$(5)$$

where γ_H, γ_C, and γ_N are the gyromagnetic ratios for proton, carbon, and nitrogen, respectively. I is the nuclear spin quantum number for the proton, r_{CH} and r_{NH} are the carbon–proton and nitrogen–proton bond distances, μ_0 is the permeability in a vacuum, $\hbar$ is Planck's constant divided by 2π and n the number of bonded protons. Because of the sixth power dependence of the relaxation rates on the C–H and N–H bond distances, it is important to use the correct values for r_{CH} and r_{NH}. For our work here we have used the values obtained from microwave experiments: the carbon hydrogen bond distance, $r_{CH}=109.8$ pm, the *trans* amide N–H bond distance, $r_{NH(t)} = 100.15$ pm and the *cis* amide N–H bond distance, $r_{NH(c)} = 100.16$ pm.[27] The experimental value for the N–H bond distance agrees well with the optimized geometry used in the SCF calculations, but the experimental value for the carbon–hydrogen bond distance is about one percent longer than the value of 108.36 pm which was obtained from the *ab initio* calculations for the C–H bond distance. We have carried out calculations for both values of the C–H bond distance.

Since on the NMR time scale formamide is rigid and planar and the molecular motion is isotropic,[10] the above single correlation time is sufficient to describe the molecular motions for all of the dipolar vectors and the principal components of the various EFG tensors. In the deuteron case there is no question (even for anisotropic motion) that the dipolar correlation time τ_c and the correlation time for the principal axis component of the EFG tensor are equal since the C–H and the N–H dipolar vectors are coincident with the principal component of the EFG tensors. The deuterium asymmetry parameter, η_D, is assumed to be zero, since in all cases it is small (see Ref. 29 and Table II). No significant error in the deuteron QCP is caused by this assumption.

The values for the oxygen and nitrogen QCP are not so straightforward, since accurate values for η_O and η_N for the liquid phase are not available. The few experimental values available are relatively large. For the ^{17}O nucleus Burgar et al.[30] assumed $\eta_O=0.45$, which is a nominal asymmetry parameter for the carbonyl oxygen.[31] The value for the ^{14}N asymmetry parameter varies from 0.028 for microwave gas phase measurements[32] to 0.378 for NQR solid state data.[4] *Ab initio* calculations give a value of $\eta_N=0.056$ for a formamide monomer, which is close to the experimental gas phase value, and a value of $\eta_N=0.381$ for a cyclic dimer, which is close to the solid state result.[23] We thus estimate that neglecting η would result in a maximum error in the QCP value of about 3%.

Perhaps the key concern for formamide is the question of the correlation time for the motion of the principal axes for the nitrogen and oxygen EGF tensors. For nitrogen and oxygen the principal axes of the EFGs are perpendicular to the molecular plane[23,31] whereas the correlation time obtained from the dipolar measurements for the C–H and the N–H vectors lie in the molecular plane. The accuracy of the nitrogen and oxygen QCP values are therefore dependent upon the validity of our premise that formamide rotates isotropically. Our experimental observation that the C–H and N–H correlation times are equal over a relatively large temperature range is strong evidence for isotropic rotation in a neat liquid sample of formamide. This conclusion of isotropic rotation is also supported by the temperature behavior of Rayleigh relaxation times, τ_{Ray},[33] and shear viscosities, η_s.[34] For these reasons we are confident that the values obtained for χ_O and χ_N are accurate. The experimental values for all QCPs for the neat, liquid sample of formamide at 298 K[10] are given in Table IV.

IV. RESULTS AND DISCUSSION

The calculated QCP values and asymmetry parameters of the nitrogen, oxygen, carbonyl deuteron, amide *cis* and *trans* deuteron for all atoms and clusters are given in Tables I and II. The components of the quadrupole coupling tensors are determined by multiplying the components of the EFG tensor by the quadrupole moment of the deuteron (0.672 MHz/a.u.),[35] nitrogen (4.0415 MHz/a.u.),[23] or oxygen (6.058 MHz/a.u.).[36] The average values of the quadrupole coupling constants and asymmetry parameters for each cluster are listed in the same tables. The liquid cluster distributions from thermodynamic calculations at 298 K and 1 atm are given in Table III. Finally, the calculated QCP and η values for all quadrupole nuclei in liquid formamide are shown in Table IV together with the experimental NMR data and other available experimental data from literature for the solid and the gas phases.

For the carbonyl deuteron the calculated value for the QCP is 0.182 MHz which is somewhat higher than the observed experimental value of 0.170 MHz. Both values agree

TABLE IV. Effective quadrupole coupling constants, χ_Q, and asymmetry parameters, η_Q, for all quadrupolar nuclei in formamide in the liquid (calculation and experiment), solid and the gas phase.

	Liquid calculation χ_Q (MHz)	η_Q	Liquid experiment χ_Q (MHz)	η_Q	Solid χ_Q (MHz)	η_Q	Gas χ_Q (MHz)	η_Q
D	0.182	0.038	0.170[a]	⋯	⋯	⋯	⋯	⋯
D_c	0.285	0.147	0.280[a]	⋯	0.198[b]	0.14[b]	⋯	⋯
N	2.941	0.394	2.84[a]	⋯	2.27[c]	0.378[c]	3.852[d]	0.028[d]
O	9.282	0.402	9.18[a]	⋯	10.20[e]	0.45[e]	⋯	⋯
D_t	0.221	0.223	0.233[a]	⋯	0.198[b]	0.14[b]	⋯	⋯

[a]From Ref. 10.
[b]From Ref. 39. Deuteron quadrupole coupling constant obtained from Acetamide.
[c]From Ref. 4.
[d]From Refs. 38, 40, 41.
[e]From Ref. 31. $\eta = 0.45$ is a nominal asymmetry parameter for the carbonyl group oxygen-17 quadrupole coupling constant.

within the limits of experimental error. These results are in good agreement with the value of 180 kHz expected for an sp^2 C–D bond.[37]

For the amide deuterons the *ab initio* values are 0.221 MHz (*trans*) and 0.285 MHz (*cis*). These values agree well with the experimental values of 0.233 and 0.280 MHz at 298 K. Both the experimental and the theoretical values for the *cis* and the *trans* deuterons show a significant difference of about 50 kHz. The QCP value for the *cis* deuteron is almost totally invariant as a function of cluster size and is clearly not involved in hydrogen bonding; its values for clusters found in the liquid phase at 298 K are very close to the theoretical value for the formamide monomer. This value is probably very close to the gas phase value. Theory also predicts that the dimer and tetramer cyclic configurations should be present in only trace amounts ($<0.04\%$) at room temperature. Both of these predictions are in accord with the experimental results which show clearly that the *cis* deuterons are not hydrogen bonded. Thus, theory and experiment both indicate clearly that cyclic dimers are not present in neat, liquid formamide at standard state conditions.

For the *trans* amide deuteron, theory and experiment are again both in accord with a cyclic hexamer structure, but in sharp contrast to the *cis* amide deuteron, the QCP value for the *trans* deuteron is a function of cluster size, as shown in Table I. Indeed, the only theoretically calculated value for the average *trans* deuteron QCP value that is even close to the experimentally measured value is the one for the cyclic hexamer, 0.222 MHz.

For the nitrogen quadrupole coupling constant we calculate a value of 2.94 MHz which compares well with our experimentally measured value of 2.84 MHz from NMR relaxation time experiments. Both values agree within experimental and theoretical errors and lie between the known values of about 2.27 MHz for the solid state[4] and 3.6–3.85 MHz for the gas phase.[38–41] We are aware of only one other comparison of experimental[4] and calculated[23] values of QCPs in the condensed phase. Palmer calculated values for χ_N and η_N for the central formamide molecule in a group of six formamide molecules in a crystallographic configuration that consisted of groups of cyclic dimers with hydrogen bonding for both the *cis* and *trans* amide deuterons and for

two hydrogen bonds to the oxygen atoms. Although only dimerlike structures were considered, Palmer's values of 2.757 MHz for χ_N and 0.474 for η_N are reasonably close to the solid state NMR results of 2.273 MHz and 0.378, respectively. Since solid state NMR results were available only for nitrogen, he considered only that nucleus in his calculations.

The oxygen QCP obtained from the calculation is 9.28 MHz. This value is likewise in good agreement with the NMR result of 9.18 MHz. For the oxygen nucleus in formamide no literature data for gas or solid state are available. A QCP of about 10.20 MHz was obtained for the carbonyl group oxygen from liquid crystal splitting of urea.[31]

Only for cyclic hexamers are the theoretical and experimental values in excellent agreement for all five QCPs. This is compelling evidence that the hexamer clusters are an accurate description of the molecular structure of formamide in the liquid phase.

The structure of liquid formamide has been the subject of several earlier experimental studies. Our finding that at room temperature the dominant species (95%) is a six-membered ring of formamide molecules involving hydrogen bonding between the *trans* amide deuteron and the oxygen is in accord with data from x-ray, electron, and neutron diffraction, low frequency Raman and far-IR spectra. Ohtaki *et al.* have investigated the structure of liquid formamide using x-ray diffraction.[20] On the basis of their experimental results alone, the authors could not decide whether cyclic dimers or linear chains were the dominant structure, but in accord with theoretical calculations, they favored linear chains. If one looks carefully at their experimental results, the only intermolecular distance the authors could determine was the N···O distance of about 305 pm compared to 280 pm in the six membered ring. Their *ab initio* calculations were helpful only in excluding the existence of cyclic dimers, in agreement with what we find in our population distribution at room temperature. Another x-ray study by De Sando and Brown[42] also gave an N···O distance length of 305 pm.

Kálmán[43] *et al.* have investigated liquid formamide via electron, neutron, and x-ray diffraction methods. These authors obtained for all three methods an intermolecular distance of about 290 pm for the N···O distance and 190 pm for

the O···H distance. Our values for the six-membered ring are 280 and 180 pm, respectively.

Wiesmann *et al.* performed a neutron diffraction study.[44] From molecular pair correlation functions they obtained a distance of 420 pm between the centers of the molecules (the carbon atoms of formamide). On the basis of this data, the authors postulated a cyclic dimer. However, given their experimental uncertainties, a model where one of the molecules is rotated by 180 around its C–N bond, resulting in only one hydrogen bond, could not be excluded. The distance between neighboring carbon atoms in our six-membered ring is 418 pm, in good agreement with the above result. Wiesmann *et al.* also found a maximum in the pair correlation function about 800 pm, which is close to the 725 and 837 pm distances to the second and third neighboring carbon atoms in the ring. An ESCA experiment by Siegbahn *et al.* indicated chainlike structures.[45] In a comparison of low frequency Raman spectra with far-IR spectra, Nielsen, Lund, and Praestgaard[46] concluded that chain structures and not cyclic dimers are dominant species. These studies rule out small cyclic structures, such as the dimer, but appear to leave open the possibilities that the ''chainlike structures'' belong to large closed rings, such as a hexamer.

At this point it is clear that there is substantial experimental evidence which is consistent with a liquid structure which consists of six-membered rings of formamide molecules in the liquid state. Both our experimental quadrupole coupling results and our *ab initio* calculations indicate clearly that cyclic dimers which involve hydrogen bonding of the *cis* amide deuterons can be ruled out. Our results and the x-ray and neutron experimental results all give intermolecular distances that are in good agreement and are in accord with a six-membered ring. Although some of the other results in the published literature favor linear structures of different sizes up to four molecules, the work we report here is the first to demonstrate that a six-membered ring of formamide molecules is the most probable structure.

The asymmetry parameters, η_Q, for the quadrupolar nuclei in formamide cannot be measured experimentally via the liquid state relaxation time experiments. However, they are generally assumed to lie between the gas phase and the solid state values. Our calculated value for η_N in liquid formamide is 0.394. The value for η_N for the monomer and higher clusters varies between 0.028 and 0.402. The experimental values for η_N are 0.028 for the gas phase[32] and 0.378 for the solid state.[4] *Ab initio* calculations from the literature are $\eta_N=0.056$ for a formamide monomer, $\eta_N=0.381$ for a cyclic dimer, and $\eta_N=0.474$ for a group of six formamide molecules in the crystallographic orientation.[23] The *ab initio* value which we obtain for the monomer is exactly the value found from the microwave study. The calculated value of 0.394 for the liquid phase is close to the solid state experimental asymmetry parameter. Both, the QCP and the η_N values for the liquid are closer to the solid values than to the values for the gas.

Our calculated asymmetry parameter for oxygen in the liquid phase is 0.394. The calculated values for the different clusters vary from 0.0350 to 0.478. The high value of 0.478 was found only for the cyclic structure with four molecules

which theory predicts is not present in significant amounts in the liquid. Experimental values for the gas and the solid phase for oxygen in formamide are not available. The important point here is that the *ab initio* calculations are an accurate guide to the values of the nitrogen and the oxygen asymmetry parameters. The calculated values of approximately 0.394 and 0.402, respectively, show that their neglect in the experimental evaluation of the quadrupole coupling constants is reasonable and would lead to errors of less than 3% in the determination of the QCP values. As mentioned above, the values for all of the deuterium asymmetry parameters are negligibly small (from 0.04 to 0.224). Even the highest value of 0.224 would lead to an error of less than 1% in the QCP value obtained from a NMR relaxation time experiment. It is interesting to note that the large changes in the oxygen and nitrogen asymmetry parameters tend to obscure somewhat the changes in the QCP values as measured by the NMR relaxation times. Fortunately, neglect of the asymmetry parameters introduces errors of only a few percent.

The quadrupole coupling constants and asymmetry parameters for the different nuclei vary from cluster to cluster and they vary as a function of position of the host molecule within a given cluster. These quantities change between values found in the monomer (in which the QCP and η values are similar to those found in the gas phase) to values found in the cyclic hexamer, which are more similar to the values typically found for solid samples. These differences for the QCP values are about 30% for the nitrogen, 10% for the oxygen, and 30% for the *trans* deuteron which is involved in hydrogen bonding. Clearly, the QCP is a very sensitive indicator of changes in hydrogen bonding and electronic structure. The changes in the asymmetry parameters for the different clusters are even larger. In the nitrogen and oxygen case the values vary dramatically by about a factor of 10.

It is most interesting to note that there is a wide variation in the quadrupole coupling constants and asymmetry parameters for specific nuclei within a cluster. For the linear structures of formamide one observes a clear cooperative effect. Consider, for example the variation in the values of the QCP for the *trans* amide deuteron nucleus as a function of its position in the linear tetramer. The QCP values for the hydrogen bonded deuterons are much lower than is the case for the linear dimer. A still lower value is seen for the cyclic hexamer. The same trend is seen for all other nuclei. Note also that the QCP value for hydrogen bonded nuclei is a function of the position of the formamide molecule in the in the chain. The cooperative effects are similar in nature to those found recently in $(HCN)_n$ clusters.[47] Experimentally, we measure the average value of the QCPs. Using the temperature distribution calculations (see Table III) and the *ab initio* values for the various QCP values we have calculated the average QCP values. As can be seen from Table IV, these values are in excellent agreement with the experimental values.

The thermodynamic propensity for cyclic hexamers can be traced to cooperative binding energy effects that parallel (and are reflected in) the measured quadrupole coupling shifts. The cooperativity of hydrogen bonding arises essentially from intermolecular delocalizations of ''donor–

acceptor'' type[50] that are a characteristic feature of general B···H–A interactions, but are ignored in the ''electrostatic'' picture of hydrogen bonding based on pairwise-additive potentials. In the language of natural bond orbital (NBO) analysis[51] these interactions are of $n_O \rightarrow \sigma^*_{NH}$ type, representing delocalization from the ''donor'' oxygen lone pair n_O of one monomer into the unfilled ''acceptor'' N–H antibond σ^*_{NH} of an adjacent monomer. Such donor–acceptor interactions are intrinsically cooperative, since a monomer that participates as a donor in one H-bond interaction (acquiring partial cationic character) becomes an enhanced acceptor for a subsequent H bond to another monomer, extending the coordination chain. The cooperative linear chain develops appreciable positive charge at one end and negative charge at the other ($\sim$4% of an electronic charge ($0.04e$), as reflected in the distinctive QCP values at chain termini), incurring a thermodynamic energy penalty associated with creation of the supramolecular charge–transfer dipole. However, this penalty can be avoided by connecting the two ends into a closed loop, provided the chain is sufficiently long to avoid substantial nonlinear O···H–N bond ''strain,'' as in the cyclic pentamer or hexamer. Although such large, highly ordered clusters are opposed by entropic factors at higher temperatures (and essentially disappear in the vapor phase), they are found to be the dominant species of the liquid region, stabilized by cooperative enhancements that are of the same order of magnitude as the dimer H-bond energy itself.

In summary, it has now become apparent that NMR relaxation studies of quadrupole coupling interactions, in conjunction with *ab initio* cluster calculations, can provide a remarkably direct probe of the actual cluster species present in H-bonded liquids. These studies provide strong evidence for the surprising prediction of *ab initio* quantum cluster equilibrium theory that liquid formamide under steady-state conditions is dominated by *supramolecular assemblies of cyclic hexameric form*, with each monomer linked in a cooperative bicoordinate *trans* pattern (···HNCO···) to its nearest neighbors. The resulting picture stands in stark contrast to the expectations of a simple electrostatic model of H bonding, or to liquid simulation models based on pairwise-additive potentials. Moreover, it suggests that a true account of amide hydrogen bonding in protein folding and related biophysical phase transitions will involve a more complex interplay of electronic and structural cooperativity patterns than have commonly been considered.

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