Amino Acid Chemistry in Solution: Structural Study and Vibrational Dynamics of Glutamine in Solution. An ab Initio Reaction Field Model

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The self-consistent reaction field (SCRF) theory was used to study structural and vibrational features of the amino acid L-glutamine in solution. Raman and infrared spectra of this molecule in solutions of H_2O and D_2O were recorded and measured. The bands were firstly assigned on the basis of the isotopic shifts. An ab initio quadratic force field at the 6-31+G* level was achieved. The calculation simulated a polar solvent by placing the molecule in an ellipsoidal cavity surrounded by a continuum dielectric. The theoretical results, in terms of structural parameters, vibrational frequencies and descriptions, and infrared intensities, were in satisfactory agreement with the experimental data.

Introduction

The study of the vibrational spectra of amino acids outlines an interesting problem from both theoretical and experimental points of view. The strongly polar topology of these molecules gives rise to intermolecular interactions in condensed phases, which dramatically influences their vibrational dynamics and structural properties. Therefore, in order to investigate the vibrational spectra of an amino acid in solid state or in solution, interactions with the solvent molecules cannot be ignored.

In a theoretical treatment of such a problem, solvent molecules can be directly added to the solute molecule, thus allowing for specific solute−solvent interactions. However, when ab initio quantum-chemical approaches are employed, only a few solvent units can be included because of the high computational cost. In addition, nonspecific and long-range interactions are not correctly described. Thus, the polarizable continuum models (PCM)[1,2] have became the most succesful methods to simulate solvent effects.

The present work is aimed at the study of the amino acid glutamine in aqueous solution. This is one of the most abundant amino acids in free form, and it occupies a central position in nitrogen metabolism.[3] Prior to this paper, a vibrational study of solid glutamine was reported[4] that was supported on the basis of a normal coordinate calculation from semiempirical methods.[5] Here we report infrared and Raman spectra of this amino acid in solutions of H_2O and D_2O. The theoretical support was obtained by using a PCM approach in which the solvent effect is simulated by a continuum dielectric. This approach has been adapted to be used with an extensive number of quantum-chemical methods, and we have used that implemented in the GAUSSIAN series of programs.[6]

This paper has been organized as follows: first we give the calculation and experimental methods. Then we discuss the obtained results in three sections. Firstly, the molecular structure is discussed and compared to some experimental data. Secondly, we analyze the vibrational spectra of glutamine and its ND_3^+,ND_2 deuterated derivative in solution, proposing a tentative assignment for the observed bands. Finally, we present the results of the normal coordinate calculation in terms of frequencies, normal modes, and quadratic force field. In the last section we outline our conclusions.

Experimental and Computational Methods

We have used L-glutamine (99% purity), furnished by Sigma-Aldrich, without further treatments. Deuteration was carried out as described elsewhere.[4] Aqueous solutions were prepared by using tridistilled water and deuterium oxide (99.9 atom % D, Aldrich). Raman spectra of the solutions at room temperature were recorded on a Jobin Yvon Ramanor U1000 spectrometer. An excitation radiation wavenumber at 514.5 nm was used as generated by a Spectra Physics argon-ion laser. The spectra were all reached working at 500 mW of laser power and with a minimum of 20 accumulations each. The best resolution obtained was 1 cm^{-1}. Infrared spectra at room temperature between 1700 and 1100 cm^{-1} were recorded on a Perkin Elmer 1760X Fourier transform infrared spectrometer purged with dry Ar gas. Demountable cells with calcium fluoride windows were used to record the spectra in aqueous solution. A mean of 50 scans with 2 cm^{-1} spectral resolution were accumulated for each case.

Solute−solvent electrostatic interactions were simulated employing the continuum model of Rivail et al.[7−9] In this model, the liquid is assimilated to a continuum characterized by a dielectric constant (78.4 for water). The quantum system is then placed in an ellipsoidal cavity whose volume is obtained by means of an empirical relation.[10] The electrostatic interaction is calculated using a multipolar moment expansion (up to sixth order) and introduced in the Hamiltonian of the system. The analytical derivatives of this electrostatic term have been derived leading to an efficient geometry optimization procedure.[11] Geometry has been optimized using the redundant coordinates algorithm.[12] Cartesian force constants in solution have been calculated at the fully optimized geometry using analytical second derivatives.[13] All the calculations have been carried out using the GAUSSIAN 94 package of programs[6] at the 6-31+G* level.[14] The 6-31+G* basis set, which includes polarization functions on all the atoms and diffuse functions on all the atoms

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TABLE 1: SCRF/6-31+G* and Experimental[a] Structural Parameters for Glutamine Molecule[b]

bond	calc	expt	angle	calc	expt	dihedral angle	calc	expt
C_1-C_2	1.563	1.540	$C_2-C_1-O_6$	115.0	118.7	$O_6-C_1-C_2-N_9$	−177.5	167.0
C_1-O_6	1.229	1.240	$C_2-C_1-O_7$	117.2	114.6	$O_7-C_1-C_2-N9$	5.0	−15.6
C_1-O_7	1.243	1.262	$C_1-C_2-C_3$	116.5	110.3	$C_2-C_3-C_4-C_5$	−68.2	175.5
C_2-C_3	1.541	1.528	$C_1-C_2-H_8$	105.3	108.7	$C_4-C_3-C_2-N_9$	81.8	66.1
C_2-H_8	1.082	1.097	$C_1-C_2-N_9$	110.5	110.2	$C_1-C_2-N_9-H_{10}$	−28.4	44.6
C_2-N_9	1.498	1.498	$C_3-C_2-H_8$	106.5	109.7	$C_1-C_2-N_9-H_{11}$	−149.3	164.3
C_3-C_4	1.539	1.520	$H_8-C_2-N_9$	105.5	106.6	$C_1-C_2-N_9-H_{12}$	89.8	76.5
C_3-H_{13}	1.083	1.110	$C_2-C_3-C_4$	121.1	114.0	$C_3-C_4-C_5-O_{17}$	19.1	−13.3
C_3-H_{14}	1.083	1.105	$C_2-C_3-H_{13}$	105.7	107.2	$C_3-C_4-C_5-N_{18}$	−162.9	167.2
C_4-C_5	1.521	1.511	$C_2-C_3-H_{14}$	107.7	108.8	$O_{17}-C_5-N_{18}-H_{19}$	5.1	−3.4
C_4-H_{15}	1.082	1.104	$C_3-C_4-C_5$	118.8	113.1	$O_{17}-C_5-N_{18}-H_{20}$	−179.9	−175.6
C_4-H_{16}	1.085	1.100	$C_3-C_4-H_{15}$	109.8	111.8			
C_5-O_{17}	1.220	1.231	$C_3-C_4-H_{16}$	106.7	110.0			
C_5-N_{18}	1.336	1.334	$C_4-C_5-O_{17}$	122.1	122.1			
N_9-H_{10}	1.008	1.027	$C_4-C_5-N_{18}$	116.1	115.2			
N_9-H_{11}	1.015	1.044	$O_{17}-C_5-N_{18}$	121.8	122.7			
N_9-H_{12}	1.016	1.048	$C_2-N_9-H_{11}$	110.4	111.2			
$N_{18}-H_{19}$	0.997	1.004	$C_2-N_9-H_{11}$	113.4	111.0			
$N_{18}-H_{20}$	1.003	1.010	$C_2-N_9-H_{12}$	109.6	108.1			
$O_7\cdots H_{10}$	2.23		$H_{10}-N_9-H_{11}$	107.6	107.3			
$O_{17}\cdots H_{12}$	1.86		$H_{10}-N_9-H_{12}$	107.5	110.2			
			$H_{11}-N_9-H_{12}$	108.1	109.0			
			$H_{10}-N_9-H_{11}$	116.3	117.7			
			$C_5-N_{18}-H_{19}$	122.5	120.8			
			$C_5-N_{18}-H_{20}$	121.0	121.1			

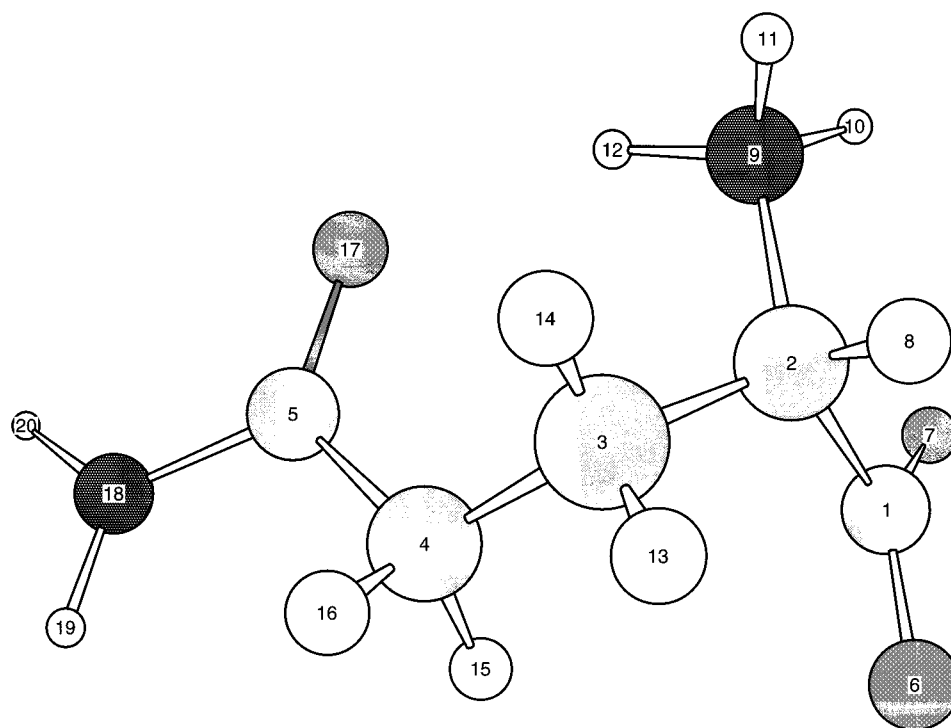[a] Data from ref 21. [b] See Figure 1 for numbering.

Figure 1. Optimized structure of glutamine molecule with the atomic numbering used in this work.

except the hydrogens, has been suggested[15] as suitable to study vibrational features of strongly polar species, and hence it has been selected for our calculations.. For consideration of continuum solvent effects an extra link[16] has been added. Infrared absorption intensities were evaluated from the atomic polar tensors.[17]

The Cartesian force constants were transformed into a set of nonredundant local symmetrical internal coordinates, defined accordingly to the Pulay methodology.[18] This allows for a more useful description of the vibrational potential energy and makes further calculations easier. The force field in internal coordinates was subsequently scaled to compensate for systematic

overestimations. Wavenumbers and normal coordinates were calculated by the Wilson FG matrix method.[19]

Results and Discussion

(a) Molecular Geometry. The geometry optimization was performed taking as the starting point the structure of crystalline glutamine, obtained from neutron and X-ray diffraction techniques.[20,21] The results concerning bond lengths and angles for the optimized structure are presented in Table 1. Figure 1 displays this structure with the atomic numbering followed in the present work. We have included in Table 1 the geometrical
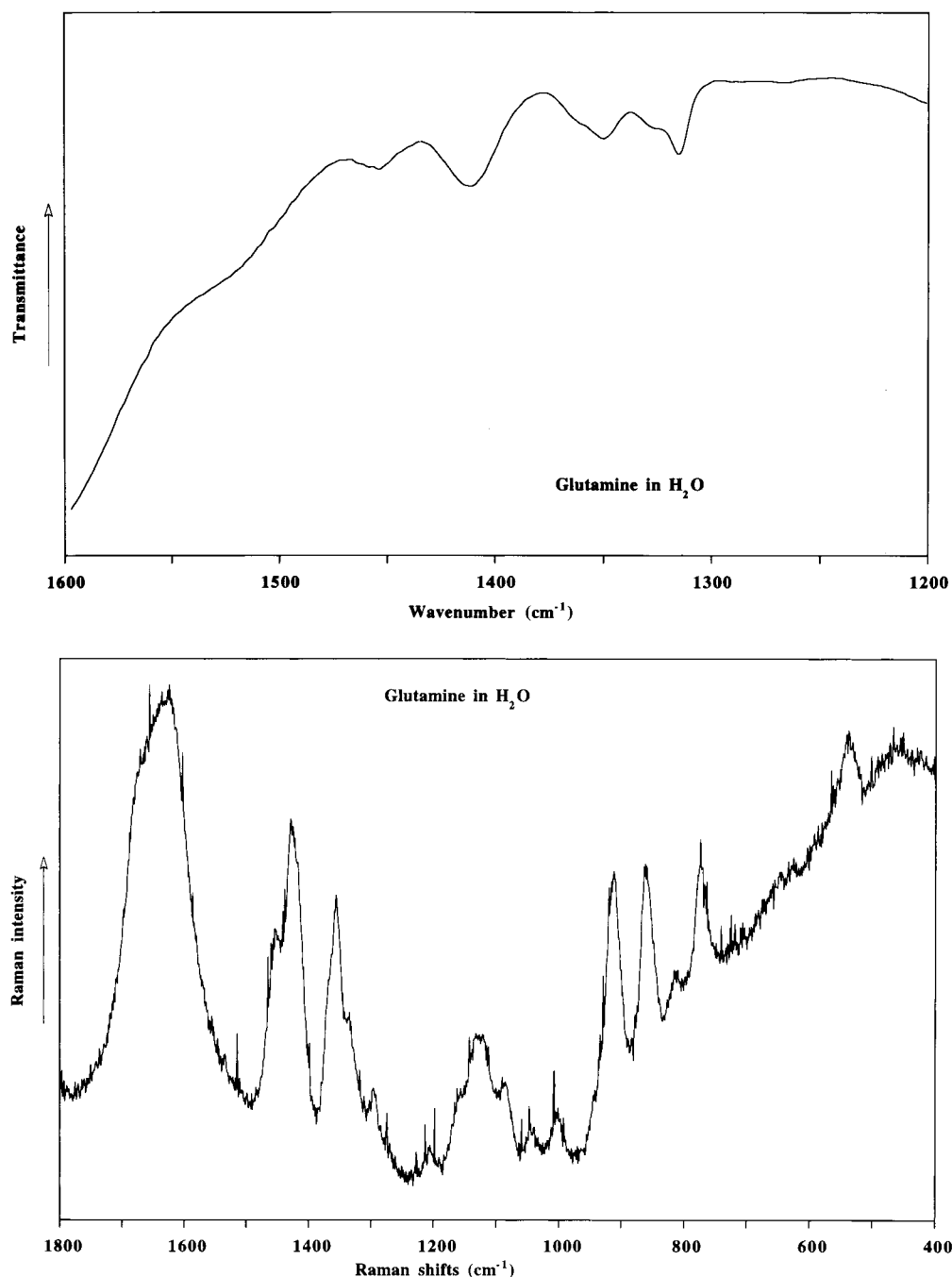
Figure 2. Vibrational spectra of glutamine in H_2O solution: (a) infrared spectrum between 1600 and 1200 cm^{-1}; (b) Raman spectrum between 1800 and 400 cm^{-1}.

parameters reported for glutamine molecule in the crystal[21] because no data about the structure of this amino acid in solution were found in the literature.

Concerning the optimized structure, Figure 1, the molecular flexibility allows for the development of an intermolecular interaction (hydrogen bond like) between the H12 and O17 atoms, which are separated by 1.86 Å, thus setting up a seven-membered cycle. This interaction makes the C5−O17 bond length longer than it could be expected by solvation. If we compare it with the same bond length in solid glutamine (see Table 1), the difference of 0.008 Å indicates that this theoretical prediction results in an environment for the O17 similar to what exists in the crystal. A second nonbonded H···O distance is within the usually accepted hydrogen bond range,[21] even though the N−H···O angle is quite far from 180°, as occurred for the aforementioned interaction. In this case the O7 and H10 atoms

are involved, but the O···H distance is longer, 2.23 Å. As a consequence, the C1−O7 bond is elongated by 0.014 Å with respect to the C1−O6 bond length, where the oxygen atom is not involved in intramolecular contacts. In fact, this interaction gives rise to a five-membered ring that usually occurs in a great number of amino acids with zwitterionic structures in condensed phases and particularly in solution. In a recent article about the glycine zwitterion,[23] density functional calculations have been employed to study solvent effects in the molecular geometry, and a similar cycle containing a weak O···H hydrogen bond is predicted.

With the exceptions discussed above, the values listed in Table 1 are within the ranges expected for the corresponding structural parameters, and they do not appreciably deviate from those reported for a glutamine molecule in the crystal.[21] There, the intermolecular interactions (that are stronger than what occur
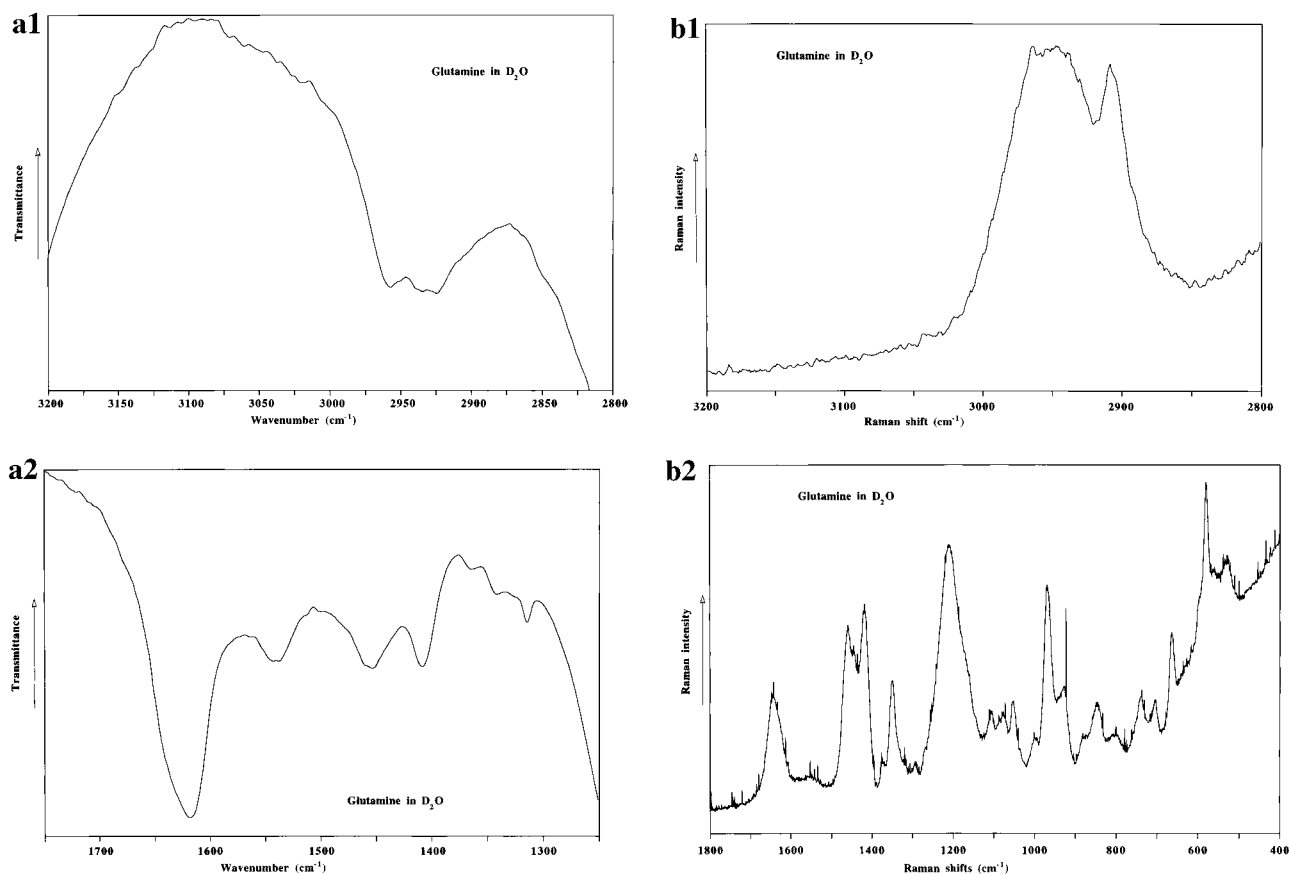
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Figure 3. Vibrational spectra of glutamine in D_2O solution: (a1) Infrared spectrum between 3200 and 2800 cm^{-1}; (a2) infrared spectrum between 1600 and 1200 cm^{-1}; (b1) Raman spectrum between 3200 and 2800 cm^{-1}; (b2) Raman spectrum between 1800 and 400 cm^{-1}.

in solution) lead the molecular disposal in the unit cell. However, the values for both environments are rather comparable. The carbon–carbon distances monotonically decrease on going from the carboxylate to the amide moieties, from 1.540 to 1.511 Å for the optimized structure and from 1.586 to 1.509 Å for the molecule in the crystal. The C–N distances are almost equal in both sets of values, and the C–H and N–H distances are in general underestimated. A good correlation also exists for the bond angles, in spite of the different environments. We would like to emphasize that the bond angles related to the ammonium and amide groups have a mean deviation of 0–2°, thus confirming the availability of the theoretical model employed to simulate reliable polar environments. Finally, the data listed in Table 1 show some significant differences between the theoretical dihedral angles in solution and those corresponding to the experimental structure. This is a consequence of the intramolecular interactions, which lead the molecule to internal rotations, largely around the C3–C4 and C2–N9 bonds.

(b) Infrared and Raman Spectra. We have recorded the infrared and Raman spectra of glutamine in solutions of H_2O and D_2O. They are displayed in Figures 2 and 3, respectively. As the first step, we have analyzed these spectra in terms of characteristic group frequencies, and a general assignment was proposed based on the isotopic shifts, the previous vibrational study on solid glutamine,[4] and vibrational studies reported for other amino acids. The measured frequencies, relative intensities, and proposed assignments are summarized in Table 2 and 3.

The strong adsorptions from solvent molecules prevent the observation of the N–H and N–D stretching vibrations; the absence of any signal in the 3000–3400 cm^{-1} region in the D_2O solution proves that the hydrogen–deuterium change has

TABLE 2: Experimental Frequencies (in cm^{-1}) and Relative Intensities^a Measured in the Infrared and Raman Spectra of Glutamine–h_5 Molecule

infrared		Raman		
ν	I	$\Delta\nu$	I	assignment
1595	sh			amide II
1520	s, sh			antisym. carboxylate stretching
1453	m	1447	m	methylene bending
		1422	s	amide II
1411	s			sym. carboxylate stretching
1359	sh			methylene wagging
1350	m	1348	m	methyne bending
1323	w	1327	sh	methyne bending
1292	vw	1289	w	methylene twisting
1271	sh			methylene wagging
1200	vw	1200	w	methylene twisting
		1125	m	ammonium rocking
		1114	m	ammonium rocking
		1082	w	NH$_2$ rocking
		1037	w	skeletal stretching
		999	w	methylene rocking
		903	s	methylene rocking
		854	s	skeletal stretching
		807	w	carboxylate bending
		766	m	carboxylate bending
		717	vw	skeletal stretching
		618	vw	amide bending
		530	m	amide bending
		452	w	skeletal bending
		413	vw	skeletal bending

^{a} s = strong; m = medium; w = weak; v = very; br = broad.

been almost complete for nitrogen-attached hydrogens. Carbon-attached hydrogens are not acid enough to allow for a quick interchange at room temperature, so that they remain unchanged. Because vibrations appearing in this region are all rather local

TABLE 3: Experimental Frequencies (in cm^{-1}) and Relative Intensifies^a Measured in the Infrared and Raman Spectra of Glutamine-d_5 Molecule

infrared		Raman		
ν	I	$\Delta\nu$	I	assignments
2957	w			CH$_2$, CH stretching
		2950	vs, br	CH$_2$, CH stretching
2923	w			CH$_2$, CH stretching
2910	w	2908	vs	CH$_2$, CH stretching
		1637	m	amide I
1540	s	1546	m	antisym. carboxylate stretching
1453	s	1453	m	methylene bending
		1439	sh	methylene bending
1409	s	1411	m	sym. carboxylate stretching
1364	vw	1369	w	methylene wagging
1341	w	1342	m	methyne bending
1326	sh			methyne bending
1314	m	1312	vw	amide III
		1286	vw	methylene twisting
		1178	sh	ND$_2$, ND$_3^+$ bending
		1139	sh	ND$_2$, ND$_3^+$ bending
		1101	w	ND$_2$, ND$_3^+$ bending
		1086	w	skeletal stretching
		1047	w−m	skeletal stretching
		997	w	skeletal stretching
		963	m	methylene rocking
		928	m	methylene rocking
		875	w	skeletal-carboxylate bending
		837	m	skeletal-carboxylate bending
		792	w	ND$_2$, ND$_3^+$ rocking
		728	m	ND$_2$, ND$_3^+$ rocking
		696	w	ND$_2$, ND$_3^+$ rocking
		655	m-s	skeletal bending
		571	s	amide bending
		519	m	amide bending

^{a} s = strong; m = medium; w = weak; v = very; br = broad.

modes, we expect close frequencies for the C−H stretching vibrations of both the natural and deuterated derivatives of glutamine in solution. On the other hand, the oxygen−hydrogen bending vibration from the solvent molecules hides the bending vibrations from the ammonium group. In the infrared spectra of glutamine in H$_2$O we have observed two strong bands at 1595 and 1540 cm^{-1}. The infrared spectra of glutamine in D$_2$O only shows one of these, at 1540 cm^{-1} as a medium intensity band; therefore we propose a carbonyl stretching mode for the assignment of this band, while the band at 1595 cm^{-1} has to be assigned to one of these two vibrations: the symmetrical NH$_3^+$ bending or the amide II mode, which is largely a δ(NH$_2$) mode.[24]

The two methylene bending vibrations, δ(CH$_2$), appear as a single band for glutamine−H$_2$O, at 1453 cm^{-1} in the IR spectrum, being measured at 1447 cm^{-1} in the Raman spectrum. In the Raman spectra of glutamine−D$_2$O we can observe a medium-intensity band at 1453 cm^{-1}, and a shoulder at 1439 cm^{-1}, both assigned to the δ(CH$_2$) mode. Near these bands, we can measure a strong IR absorption at 1411 for glutamine−H$_2$O and at 1409 for glutamine−D$_2$O; they was assigned to the lower-frequency carbonyl stretching vibration, usually noted as the symmetrical stretching mode. It was observed at 1411 cm^{-1} in the Raman spectra of glutamine−H$_2$O, while for glutamine−D$_2$O it was hidden behind a high-intensity band, at 1422 cm^{-1}, that was not observed for the solution in H$_2$O. This is in the range of usual appearance of the amide III vibration, which has the ν(C−NH$_2$) as the major contribution and a lower δ(NH$_2$) character.[24] This secondary character causes it to shift appreciably upon deuteration, and it was assigned at 1314 cm^{-1} in the infrared spectrum of glutamine−D$_2$O.

Between 1400 and 1200 cm^{-1} a series of bands having medium or weak intensities were measured. Previous works

on related amino acids allow us to assign them to the C$_\alpha$−H bending and the methylene wagging and twisting vibrations. In the infrared spectrum of solid glutamine[4] they were measured at 1361, 1333, 1317, 1279, 1257, and 1204 cm^{-1}, in good correlation with the bands measured for the solution, namely, 1359, 1350, 1323, 1292, 1267, and 1200 cm^{-1}; most of them are also observable in the Raman spectrum. As expected, they show minimal shifts in the spectra of D$_2$O solution, which are due to small contributions from other vibrational local modes. Below 1200 cm^{-1} we can reach only the Raman of the solutions, because the calcium fluoride windows used in infrared spectroscopy have strong lattice adsorptions in this region. For glutamine−H$_2$O the bands at 1125, 1114, and 1082 cm^{-1} have been assigned to rocking vibrations of the NH$_3^+$ and NH$_2$ groups. They significantly shift upon deuteration, thus appearing at 792, 728, and 695 cm^{-1}, respectively. Between 1100 and 1200 cm^{-1} some peaks can be observed as shoulders of the very strong δ(D$_2$O) band, which correspond to the ND$_3^+$ bending and the amide II, δ(ND$_2$), vibrations.

The bands measured below 1000 cm^{-1} have to be assigned to the skeletal (C−C and C=N), methylene rocking, carboxylate bending, amide bending, and amide torsional vibrations. For molecules without any symmetry elements, as glutamine, and having a great number of degrees of freedom, all of these vibrations are extensively mixed, and as consequence, the deuterium exchange gives rise to small downshifts for the most of them. This fact makes it difficult to assign them to a single local mode; hence they have been tentatively assigned on the basis of previous work on solid glutamine[4] and asparagine solutions.[25]

(b) Force Field and Normal Coordinate Calculation. As was aforementioned, the optimized geometry was used to evaluate second derivatives of the molecular energy in cartesian coordinates. The force constants thus obtained were transformed into a set of nonredundant locally symmetrized internal coordinates; they were defined according to the Pulay methodology[26] and are listed in Table 4. As usual in such a calculation[27−29] the force field was scaled by a single factor of 0.81 to compensate for systematic overestimations of the vibrational frequencies (about 10%). The scaled frequencies and normal mode descriptions in terms of the potential energy distribution (PED) are summarized in Tables 5 and 6 for glutamine-h_5 and glutamine-d_5, respectively. Scaled diagonal force constants are listed in Table 7. Off-diagonal terms are available from the authors on request.

In spite of the fact that a low-level scaling was performed, the theoretical frequencies can be satisfactorily compared to the experimental frequencies. In addition, the PED supports the proposed assignments for the two isotopomers in most cases. This confirms the availability of the SCRF methodology in combination with high-level ab initio calculations to reproduce vibrational spectral features of such a molecule in polar solvents. This is also evidence for the zwitterion as the most stable structure of solvated amino acids. The assumption of a nonspherical cavity in our theoretical model, adapted to the molecular shape, allows us to reach good results in structural and vibrational topics without any refinement process by means of large sets of scaling factors. This fact is essential to evaluate the goodness of the methodology.

Concerning the vibrational frequencies and assignments, those proposed for the observed bands up to 1400 cm^{-1} have been unambiguously confirmed for glutamine−D$_2$O. In the case of the natural derivative, a great number of fundamentals appears between 1700 and 1300 cm^{-1}, so that we expect a high degree

TABLE 4: Locally Symmetrized Internal Coordinates Used in this Work for Glutamine Molecule

no.	coordinate[a]	symbol	description
1	$r_{1\,2}$	$\nu(CC)$	C−C stretch
2	$r_{2\,3}$	$\nu(CC)$	C−C stretch
3	$r_{3\,4}$	$\nu(CC)$	C−C stretch
4	$r_{4\,5}$	$\nu(CC)$	C−C stretch
5	$r_{1\,6}$	$\nu(CO_2^-)$	CO_2^- stretch
6	$r_{1\,7}$	$\nu(CO_2^-)$	CO_2^- stretch
7	$r_{2\,8}$	$\nu(CH)$	C−H stretch
8	$r_{2\,9}$	$\nu(CN)$	C−N stretch
9	$3^{-1/2}(r_{9\,10} + r_{9\,11} + r_{9\,12})$	$\nu_s(NH_3^+)$	NH_3^+ sym. stretch
10	$6^{-1/2}(2r_{9\,10} - r_{9\,11} - r_{9\,12})$	$\nu_a(NH_3^+)$	NH_3^+ antisym. stretch
11	$2^{-1/2}(r_{9\,11} - r_{9\,12})$	$\nu_a(NH_3^+)$	NH_3^+ antisym .stretch
12	$2^{-1/2}(r_{3\,13} + r_{3\,14})$	$\nu_s(CH_2)$	CH_2 sym. stretch
13	$2^{-1/2}(r_{3\,13} - r_{3\,14})$	$\nu_a(CH_2)$	CH_2 antisym. stretch
14	$2^{-1/2}(r_{4\,15} + r_{4\,16})$	$\nu_s(CH_2)$	CH_2 sym. stretch
15	$2^{-1/2}(r_{4\,15} - r_{4\,16})$	$\nu_a(CH_2)$	CH_2 antisym. stretch
16	$r_{5\,17}$	$\nu(C=O)$	C=O stretch
17	$r_{5\,18}$	$\nu(C-NH_2)$	$C-NH_2$ stretch
18	$2^{-1/2}(r_{18\,19} + r_{18\,20})$	$\nu_s(NH_2)$	NH_2 sym. stretch
19	$2^{-1/2}(r_{18\,19} - r_{18\,20})$	$\nu_a(NH_2)$	NH_2 antisym. stretch
20	$6^{-1/2}(2\beta_{1\,2\,8} - \beta_{9\,2\,8} - \beta_{3\,2\,8})$	$\delta(CH)$	C−H bend
21	$2^{-1/2}(\beta_{9\,2\,8} - \beta_{3\,2\,8})$	$\delta(CH)$	C−H bend
22	$18^{-1/2}(4\beta_{1\,2\,3} + \beta_{1\,2\,9} + \beta_{3\,2\,9})$	$\delta(CCC)$	CCC bend
23	$18^{-1/2}(4\beta_{1\,2\,9} + \beta_{3\,2\,9} + \beta_{1\,2\,3})$	$\delta(CCN)$	CCN bend
24	$18^{-1/2}(4\beta_{3\,2\,9} + \beta_{1\,2\,3} + \beta_{1\,2\,9})$	$\delta(CCN)$	CCN bend
25	$26^{-1/2}(5\beta_{14\,3\,13} + \beta_{2\,3\,4})$	$8(CH_2)$	CH_2 scissor
26	$26^{-1/2}(5\beta_{2\,3\,4} + \beta_{14\,3\,13})$	$\delta(CCC)$	CCC bend
27	$1/2(\beta_{2\,3\,14} - \beta_{2\,3\,13} + \beta_{4\,3\,14} - \beta_{4\,3\,13})$	$r(CH_2)$	CH_2 rock
28	$1/2(\beta_{2\,3\,14} + \beta_{2\,3\,13} - \beta_{4\,3\,14} - \beta_{4\,3\,13})$	$\omega(CH_2)$	CH_2 wagg
29	$1/2(\beta_{2\,3\,14} - \beta_{2\,3\,13} - \beta_{4\,3\,14} + \beta_{4\,3\,13})$	$t(CH_2)$	CH_2 twist
30	$26^{-1/2}(5\beta_{16\,4\,15} + \beta_{3\,4\,5})$	$\delta(CH_2)$	CH_2 scissor
31	$26^{-1/2}(5\beta_{16\,4\,15} + \beta_{3\,4\,5})$	$\delta(CCC)$	CCC bend
32	$1/2(\beta_{3\,4\,15} - \beta_{3\,4\,16} + \beta_{5\,4\,15} - \beta_{5\,4\,16})$	$r(CH_2)$	CH_2 rock
33	$1/2(\beta_{3\,4\,15} + \beta_{3\,4\,16} - \beta_{5\,4\,15} - \beta_{5\,4\,16})$	$\omega(CH_2)$	CH_2 wagg
34	$1/2(\beta_{3\,4\,15} - \beta_{3\,4\,16} + \beta_{5\,4\,15} - \beta_{5\,4\,16})$	$\tau(CH_2)$	CH_2 twist
35	$6^{-1/2}(\beta_{6\,1\,7} - \beta_{2\,1\,6} - \beta_{2\,1\,7})$	$\delta(CO_2^-)$	CO_2^- in-pl. bend
36	$2^{-1/2}(\beta_{2\,1\,6} - \beta_{2\,1\,7})$	$\delta(CO_2^-)$	CO_2^- in-pl.bend
37	ϕ_1	$\gamma(OCO^-)$	OCO^- out-pl. bend
38	$6^{-1/2}(\beta_{11\,9\,12} + \beta_{10\,9\,12} + \beta_{10\,9\,11} - \beta_{2\,9\,10} - \beta_{2\,9\,11} - \beta_{2\,9\,12})$	$\delta_s(NH_3^+)$	NH_3^+ sym. bend
39	$6^{-1/2}(\beta_{11\,9\,12} - \beta_{10\,9\,12} - \beta_{10\,9\,11})$	$\delta_a(NH_3^+)$	NH_3^+ antisym. bend
40	$2^{-1/2}(\beta_{10\,9\,12} - \beta_{10\,9\,11})$	$\delta_a(NH_3^+)$	NH_3^+ antisym. bend
41	$6^{-1/2}(2\beta_{2\,9\,10} - \beta_{2\,9\,11} - \beta_{2\,9\,12})$	$r(NH_3^+)$	NH_3^+ rock
42	$2^{-1/2}(\beta_{2\,9\,11} - \beta_{2\,9\,12})$	$r(NH_3^+)$	NH_3^+ rock
43	$2^{-1/2}(\beta_{4\,5\,17} - \beta_{4\,5\,18})$	$\delta(CONH_2)$	$CONH_2$ in-pl. bend
44	$6^{-1/2}(2\beta_{17\,5\,18} - \beta_{4\,5\,17} - \beta_{4\,5\,18})$	$\delta(CONH_2)$	$CONH_2$ in-pl. bend
45	ϕ_5	$\gamma(NCO)$	NCO out-pl. bend
46	$6^{-1/2}(2\beta_{19\,18\,20} - \beta_{5\,18\,19} - \beta_{5\,18\,20})$	$\delta(NH_2)$	NH_2 bend
47	$2^{-1/2}(\beta_{5\,18\,19} - \beta_{5\,18\,20})$	$r(NH_2)$	NH_2 rock
48	ϕ_{18}	$\gamma(NH_2)$	NH_2 out-pl. bend
49	$\tau_{2\,3}$	$\tau(CC)$	CC torsion
50	$\tau_{3\,4}$	$\tau(CC)$	CC torsion
51	$\tau_{4\,5}$	$\tau(CC)$	CC torsion
52	$\tau_{1\,2}$	$\tau(CO_2^-)$	CO_2^- torsion
53	$\tau_{2\,9}$	$\tau(NH_3^+)$	NH_3^+ torsion
54	$\tau_{5\,18}$	$\tau(NH_2)$	NH_2 torsion

[a] See Figure 1 for atomic numbering; r_{ij} is the stretching vibrations of the bond between atoms i and j; β_{ijk} is the in-plane bending vibration of the angle between atoms i, j, and k; ϕ_i is the out-of-plane bending vibration of the atom i; τ_{ij} is the torsion vibration with respect to the bond between atoms i and j.

of mixing among them. For instance, the symmetrical carboxylate stretching mode $\nu_s(CO_2^-)$ participates in three calculated normal modes, whose frequencies are 1413, 1379, and 1362 cm^{-1}. In the infrared spectrum the $\nu_s(CO_2^-)$ was assigned, taking into account its usually high intensity, to the band at 1411 cm^{-1}. The fitting to one of the calculated frequencies is good enough, but the PED only gives for it 23% carboxylate stretching character. As usual, the coupling among local modes is enhanced for lower frequencies, thus making troublesome the comparison between calculations and experiments.

We have already noticed that bands related to the nitrogen−hydrogen stretching vibrations were not observed because of the solvent absorption. In the case of the bending and rocking modes involving N−H or N−D bonds, several experimental frequencies can be well compared to calculated frequencies, as is shown in Tables 5 and 6. This proves that the structure of glutamine in aqueous solution has to be near that depicted in Figure 1, this is to say, an open structure in which the polar outer groups, CO_2^-, NH_3^+, and $CONH_2$, can be highly solvated, without discarding specific strong interactions with a discrete number of water molecules.

The calculated infrared spectrum of glutamine in solution between 1600 and 1200 cm^{-1} (the region that is free from solvent adsorptions) is compared with the experimental spectrum in Figure 4. Scaled frequencies have been used to display the theoretical infrared spectrum in combination with pure ab initio

TABLE 5: Scaled[a] 6-31+G*/SCRF and Experimental Frequencies (cm^{-1}) for Glutamine-h_5

vibration[b]	calc	expt	PED (greater than 10%)[c]
ν_1	3500		$88\nu_1(NH_2), 11\nu_s(NH_2)$
ν_2	3374		$87\nu_s(NH_2), 12\nu_a(NH_2)$
ν_3	3361		$83\nu_a(NH_3^+), 12\nu_s(NH_3^+)$
ν_4	3270		$82\nu_a(NH_3^+), 17\nu_s(NH_3^+)$
ν_5	3182		$71\nu_s(NH_3^+), 33\nu_a(NH_3^+)$
ν_6	2967		$88\nu_a(CH_2), 10\nu_s(CH_2)$
ν_7	2957	2957	$73\nu_a(CH_2), 17\nu(CH)$
ν_8	2950	2950	$79\nu(CH), 20\nu_a(CH_2)$
ν_9	2923	2923	$95\nu_s(CH_2)$
ν_{10}	2911	2910	$84\nu_s(CH_2), 17\nu_a(CH_2)$
ν_{11}	1664		$85\delta_a(NH_3^+)$
ν_{12}	1654		$69\nu(C=O), 18\delta_a(NH_3^+)$
ν_{13}	1609		$80\delta_a(NH_3^+), 12\nu(C=O)$
ν_{14}	1597	1595	$95\delta(NH_2), 10\nu(C-NH_2)$
ν_{15}	1536	1520	$116\nu(CO_2^-)$
ν_{16}	1508		$92\delta_s(NH_3^+)$
ν_{17}	1451	1453	$96\delta(CH_2)$
ν_{18}	1445	1447	$102\delta(CH_2)$
ν_{19}	1428	1422	$45\omega(CH_2), 23\nu(C-C), 16\nu(C-NH_2)$,
ν_{20}	1413	1411	$35\omega(CH_2), 23\nu(CO_2^-), 13\delta(CH_2),$ $11\delta(CH)$
ν_{21}	1379	1359	$41\omega(CH_2), 37\delta(CH), 24\nu(CO_2^-)$
ν_{22}	1362	1350	$40\delta(CH), 12\nu(CO_2^-)$
ν_{23}	1321	1323	$25\delta(CH), 23t(CH_2), 15\nu(C-NH_2)$
ν_{24}	1306	1292	$44t(CH_2), 33\delta(CH)$
ν_{25}	1265	1271	$32\omega(CH_2), 25\nu(C-NH_2), 19t(CH_2)$
ν_{26}	1206	1200	$73t(CH_2), 13\delta(CH)$
ν_{27}	1128	1125	$38r(NH_3^+), 20\delta(CH)$
ν_{28}	1104	1114	$45r(NH_3^+), 12\delta(CH), 13\nu(C-NH_3^+),$ $14\nu(C-C)$
ν_{29}	1076	1082	$26\nu(C-C), 23r(CH_2), 15r(NH_3^+)$
ν_{30}	1060	1037	$45r(NH_2), 13\nu(C-NH_2)$
ν_{31}	986	999	$39r(CH_2), 25\nu(C-C), 23r(NH_3^+)$
ν_{32}	962		$33\nu(C-NH_3^+), 21\nu(C-C), 14r(CH_2),$ $12r(NH_3^+)$
ν_{33}	879	903	$34r(CH_2), 25\nu(C-C), 24\delta(skel),$ $12\nu(C-NH_3^+)$
ν_{34}	871	854	$37\nu(C-C), 26r(CH_2), 15r(NH_3^+)$
ν_{35}	829	807	$32\gamma(OCO^-), 21\nu(C-C), 10\nu(C-NH_3^+)$
ν_{36}	780	766	$34\delta(CO_2^-), 17\gamma(OCO^-)$
ν_{37}	725	717	$45\nu(C-C), 21r(CH_2)$
ν_{38}	655		$39\gamma(NCO), 19\tau(NH_2), 16\delta(CCC)$
ν_{39}	605	618	$43\tau(NH_2), 29\delta(CONH_2), 14\delta(CCC),$ $13r(CH_2)$
ν_{40}	562		$31\nu(C-C), 22\delta(CO_2^-), 17\delta(CCN),$ $14\gamma(OCO^-)$
ν_{41}	527	530	$28\delta(CONH_2), 23\tau(NH_2), 14\delta(CCN),$ $15\gamma(NCO)$
ν_{42}	483		$41\delta(CO_2^-), 10\nu(C-NH_3^+)$
ν_{43}	464	452	$48r(CH_2), 32\delta(CCC), 21\gamma(NCO),$ $21\delta(CONH_2)$
ν_{44}	423		$108\gamma(NH_2)$
ν_{45}	401	413	$52\delta(CONH_2), 24\delta(CCN)$
ν_{46}	327		$67\tau(skel), 42\delta(CCN), 35\delta(CCC)$
ν_{47}	279		$94\tau(NH_3^+)$
ν_{48}	231		$40\tau(skel), 34\delta(CO_2^-), 25\delta(CCC)$
ν_{49}	224		$46\delta(CCC), 33\tau(skel), 32\gamma(NCO),$ $22\delta(CCN)$
ν_{50}	192		$49\tau(skel)$
ν_{51}	142		$63\tau(skel), 37\tau(CCC)$
ν_{52}	87		$82\tau(CO_2^-)$
ν_{53}	82		$91\tau(skel)$
ν_{54}	51		$102\tau(skel)$

[a] Single scale factor of 0.81 applied to the force constants. [b] Arbitrary numbering. [c] Coordinates were defined accordingly with Table 3. Contributions from coordinates with the same character were added to clarify the normal mode descriptions.

infrared intensites, because a single scaling factor uniformly applied to the force field maintains the intensities unchanged. A very strong band in the calculated spectra corresponds to the symmetrical carboxylate stretching vibration; it correlates well

TABLE 6: Scaled[a] 6-31+G*/SCRF and Experimental Frequencies (cm^{-1}) for Glutamine-d_5

vibration[b]	calc	expt	PED (greater than 10%)[c]
ν_1	2967		$88\nu_a(CH_2), 10\nu_s(CH_2)$
ν_2	2957	2957	$73\nu_a(CH_2), 17\nu(CH)$
ν_3	2950	2950	$79\nu(CH), 20\nu_a(CH_2)$
ν_4	2923	2923	$95\nu_s(CH_2)$
ν_5	2911	2910	$84\nu_s(CH_2), 17\nu_a(CH_2)$
ν_6	2594		$88\nu_a(ND_2), 11\nu_s(ND_2)$
ν_7	2475		$87\nu_s(ND_2), 12\nu_a(ND_2)$
ν_8	2447		$83\nu_a(ND_3^+), 12\nu_s(ND_3^+)$
ν_9	2411		$82\nu_a(ND_3^+), 17\nu_s(ND_3^+)$
ν_{10}	2298		$71\nu_s(ND_3^+), 33\nu_a(ND_3^+)$
ν_{11}	1641	1637	$91\nu(C=O)$
ν_{12}	1535	1546	$121\nu(CO_2^-)$
ν_{13}	1451	1453	$90\delta(CH_2)$
ν_{14}	1444	1439	$100\delta(CH_2)$
ν_{15}	1433		$35\omega(CH_2), 24\delta(CH_2), 22\nu(C-ND_2),$ $20\nu(CC)$
ν_{16}	1411	1409	$40\omega(CH_2) 22\nu(CO_2^-)$
ν_{17}	1376	1364	$40\omega(CH_2), 23\delta(CH), 20\nu(CO_2^-)$
ν_{18}	1349	1342	$63\delta(CH), 18\nu(CO_2^-)$
ν_{19}	1331	1326	$30\nu(C-ND_2), 23t(CH_2), 18\omega(CH_2),$ $12\delta(ND_2)$
ν_{20}	1303	1312	$52\delta(CH), 29t(CH_2)$
ν_{21}	1272	1286	$38t(CH_2), 25\omega(CH_2), 10\nu(C-ND_2)$
ν_{22}	1208		$67t(CH_2)$
ν_{23}	1196		$95\delta_a(ND_3^+)$
ν_{24}	1171	1178	$68\delta_s(ND_3^+), 14\nu(CN)$
ν_{25}	1150	1139	$77\delta_a(ND_3^+)$
ν_{26}	1136	1101	$63\delta(ND_2)$
ν_{27}	1083	1086	$60\nu(CC)$
ν_{28}	1054	1047	$55r(CH_2)$
ν_{29}	1003	997	$24\delta(CCN), 20\delta(CCC), 13\nu(CN), r(ND_3^+)$
ν_{30}	927	963	$20r(CH_2), 14\gamma(ND_2), 13\nu(CC)$
ν_{31}	923	928	$24\nu(CC), 11\nu(C-ND_2)$
ν_{32}	905	875	$30\nu(CC), 14\nu(CN), 13r(ND_3^+)$
ν_{33}	832	837	$46r(CH_2), 14\delta(CCC), 13r(ND_2)$
ν_{34}	786	792	$37\delta(CO_2^-), 24r(ND_3^+), 12\gamma(OCO^-)$
ν_{35}	762		$44r(ND_3^+), 33\gamma(OCO^-)$
ν_{36}	754	728	$31r(ND_3^+), 28\nu(CC), 12r(CH_2)$
ν_{37}	686	696	$45\nu(CC), 20r(CH_2), 13r(ND_2)$
ν_{38}	628	655	$41\gamma(NCO), 21\delta(CCC)$
ν_{39}	552	571	$21\delta(COND_2), 17\nu(CC), 14\gamma(NCO)$
ν_{40}	528	519	$28\delta(COND_2), 20\nu(CC), 19r(CH_2), 11r(ND_2)$
ν_{41}	468		$40\delta(CO_2^-), 10\nu(CN)$
ν_{42}	466		$41\delta(CCC), 30\delta(CCN)$
ν_{43}	398		$74\tau(ND_2), 22\delta(CH_2), 14\gamma(NCO)$
ν_{44}	370		$48\delta(COND_2), 22\delta(CCN), 14r(CH_2),$ $14r(ND_2)$
ν_{45}	323		$114\omega(ND_2)$
ν_{46}	308		$60\tau(skel), 26\delta(CCN), 21\delta(CCC)$
ν_{47}	227		$44\tau(skel), 33\delta(CCN), 16\delta(CCC), 16\delta(CO_2^-)$
ν_{48}	216		$75\delta(CCC), 24\tau(skel), 14\delta(COND_2)$
ν_{49}	195		$83\tau(ND_3^+), 31\tau(skel)$
ν_{50}	179		$30\tau(skel), 11\tau(ND_3^+)$
ν_{51}	140		$64\tau(skel), 35\delta(CCC)$
ν_{52}	85		$84\tau(CO_2^-)$
ν_{53}	78		$100\tau(skel)$
ν_{54}	50		$80\tau(skel), 21\tau(ND_3^+)$

[a] Single scale factor of 0.81 applied to the force constants. [b] Arbitrary numbering. [c] Coordinates were defined accordingly with Table 2. Contributions from coordinates with the same character were added to clarify the normal mode descriptions.

with the observed band at 1595 cm^{-1}, which clearly appears in the difference spectrum. Although Figure 4 displays a short region of the whole spectrum, it shows a reliable prediction of the experimental relative intensies, improving the correlation between both sets of frequencies.

The complete set of scaled diagonal force constants is listed in Table 7. Carbon−carbon and carbon−nitrogen stretching force constants have similar values, all around 4.00 mdyn/Å, except for the C1−C2 bond, for which a force constant of 3.3
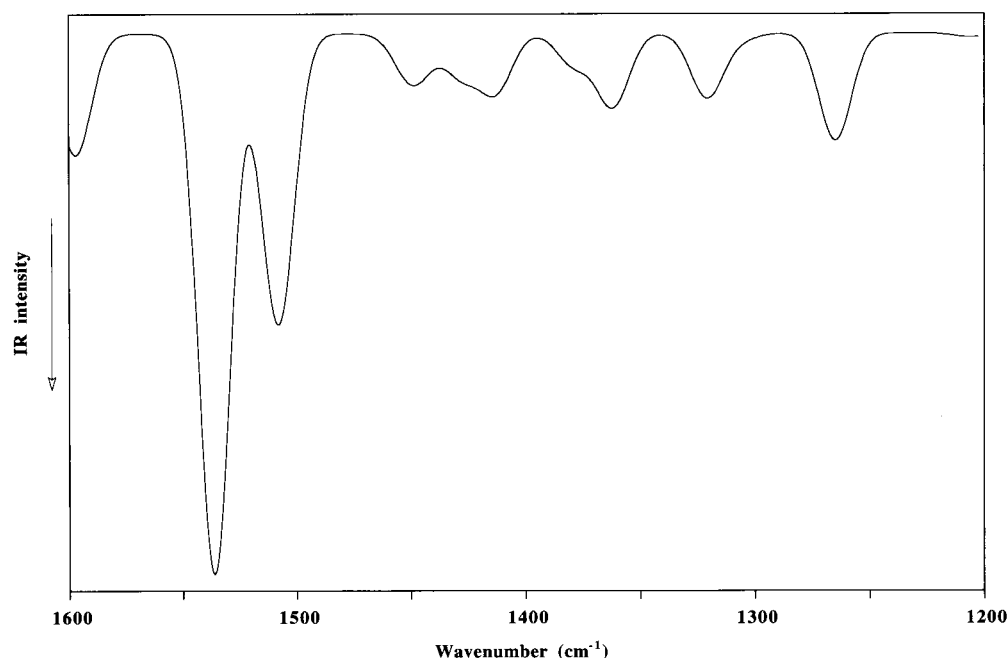
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Figure 4. Theoretical SCRF/6-31+G* infrared spectrum of glutamine in the 1600−1200 cm^{-1} region.

TABLE 7: SCRF Diagonal Force Constants^a (in mdyn/Å or Equivalent Unit) Obtained for Glutamine in an Ellipsoidal Cavity

coord.^{b}	symbol	force constant	coord.^{b}	symbol	force constant
1	$\nu(CC)$	3.334	28	$\omega(CH_2)$	0.638
2	$\nu(CC)$	3.870	29	$t(CH_2)$	0.657
3	$\nu(CC)$	4.033	30	$\delta(CH_2)$	0.811
4	$\nu(CC)$	3.929	31	$\delta(CCC)$	2.493
5	$\nu(CO_2^-)$	9.429	32	$r(CH_2)$	0.891
6	$\nu(CO_2^-)$	8.571	33	$\omega(CH_2)$	0.614
7	$\nu(CH)$	4.785	34	$t(CH_2)$	0.620
8	$\nu(CN)$	3.920	35	$\delta(CO_2^-)$	0.913
9	$\nu_s(NH_3^+)$	6.007	36	$\delta(CO_2^-)$	1.235
10	$\nu_a(NH_3^+)$	6.063	37	$\gamma(CO_2^-)$	0.528
11	$\nu_a(NH_3^+)$	5.742	38	$\delta_s(NH_3)$	0.573
12	$\nu_s(CH_2)$	4.798	39	$\delta_a(NH_3^+)$	0.633
13	$\nu_a(CH_2)$	4.731	40	$\delta_a(NH_3^+)$	0.596
14	$\nu_s(CH_2)$	4.796	41	$r(NH_3^+)$	0.628
15	$\nu_a(CH_2)$	4.711	42	$r(NH_3^+)$	0.693
16	$\nu(C=O)$	10.548	43	$\delta(CONH_2)$	1.081
17	$\nu(C\text{-}NH_2)$	6.892	44	$\delta(CONH_2)$	1.210
18	$\nu_s(NH_2)$	6.550	45	$\gamma(NCO)$	0.598
19	$\nu_a(NH_2)$	6.528	46	$\delta(NH_2)$	0.475
20	$\delta(CH)$	0.672	47	$r(NH_2)$	0.455
21	$\delta(CH)$	0.768	48	$\gamma(NH_2)$	0.054
22	$8(CCC)$	1.567	49	$\tau(CC)$	0.639
23	$\delta(CCN)$	1.835	50	$\tau(CC)$	0.416
24	$\delta(CCN)$	2.514	51	$\tau(CC)$	0.181
25	$\delta(CH_2)$	0.796	52	$\tau(CO_2^-)$	0.167
26	$\delta(CCC)$	2.004	53	$\tau(NH_3^+)$	0.141
27	$r(CH_2)$	1.386	54	$\tau(NH_2)$	0.284

^{a} Scaled by 0.9. ^{b} See Table 3 for coordinate descriptions.

mdyn/Å is obtained. The strong electronegativity of the neighborhood oxygen atoms withdraws electronic density between C1 and C2, weaking the bond. A difference of about 1 mdyn/Å is obtained for the force constants of the two C−O carboxylate stretching coordinates, as a result of the interatomic contacts inside the molecule. In addition, the related interaction off-diagonal force constant is 2.14 mdyn/Å. The agreement between the experimental and calculated frequencies and infrared intensities assigned to the carboxylate group supports this value. A similar result has been obtained for glycine and

alanine amino acids from ab initio SCRF calculations using density functionals.[23] The nitrogen−hydrogen stretching coordinates of the ammonium group were locally symmetrized, so that the three related force constants have contribution from all of the N−H bonds, and the same for the CH$_2$ and NH$_2$ moieties. For the bending force constants, the greater values correspond to the skeletal modes. Torsional force constants are calculated at the lowest values, between 0.1 and 0.3 mdyn/Å, for the outer bonds. However, the continuum solvent model applied in the present work results in these values being significantly higher than usual torsional force constants calculated for isolated gas-phase molecules by quantum-chemical methods (down to 0.1 mdyn/Å).[30]

Conclusions

The infrared and Raman spectra of glutamine in solutions of H$_2$O and D$_2$O have been recorded, and a general assignment of its fundamental vibrations has been proposed on the basis of the experimental results and previous works on solid glutamine. The spectra are fully compatible with a solvated zwitterion, and similar frequencies to those reported for solid glutamine were measured. To support the assignments, a force field and normal coordinate calculation was computed using ab initio methodology at the 6-31+G*, where the solvent was simulated by a continuum model and the solute is placed in an ellipsoidal cavity suitably adapted to the solute dimensions. Prior optimization of the molecular structure was performed to reach the absolute minimum of the potential energy surface. Pure ab initio force constants were uniformly scaled by a single factor of 0.81 (10% of systematic frequency correction). Structural parameters and vibrational frequencies were good compared to the experimental data. Infrared intensities were also evaluated from the dipole moment at the same level of calculation, and the comparison with the observed infrared spectra was satisfactory.

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