Polarized Raman spectra of *L*-arginine hydrochloride monohydrated single crystal

J.L.B. Faria
Departamento de Física, Universidade Federal de Mato Grosso, Cuiabá-MT, 78060-900, Brazil

P.T.C. Freire,* R.O. Gonçalves, F.E.A. Melo, and J. Mendes Filho
Departamento de Física, Universidade Federal do Ceará, Fortaleza-CE, 60455-760, Brazil

R.J.C. Lima, A.J.D. Moreno
Centro de Ciências Sociais, Saúde e Tecnologia, Universidade Federal do Maranhão, Imperatriz-MA, 65900-410, Brazil
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Polarized Raman spectra of *L*-arginine hydrochloride monohydrated single crystal in nine different scattering geometries of the two irreducible representations of factor group C_2 were studied at room temperature. The experimental wavenumber values are compared with those obtained from ab-initio calculation and the assignment of the Raman bands to the respective molecular vibrations is also given. Finally, a discussion related to a previously reported phase transition undergone by *L*-arginine hydrochloride monohydrated single crystal at low temperature is furnished.

Keywords:

1. INTRODUCTION

Amino acids ($NH_2-CH-COOH-R$, where R is a radical) are the basic units of proteins and peptides of all living beings. For an unknown reason Nature has choose 20 of these special molecules, differing in the R part, to form the impressive number of proteins found in our planet. *L*-arginine, among other amino acids, and its salts, including *L*-arginine·HCl, is known to protect protein from inactivation in frozen solutions, during freeze-drying [1], during spray-drying [2] and in the storage of lyophilized solids [3]. Beyond this, *L*-arginine and acid combinations have been extensively used in the last years to assist in the recovery of chemically unfolded proteins and recombinant proteins expressed in inclusion bodies [4].

Beyond these biological aspects related to amino acid in general, and *L*-arginine in particular, it is also possible to found interesting physical properties related to this amino acid. For example, the search for new non-linear optical materials with high non-linear coefficients and high damage threshold has lead to the discovery of *L*-arginine phosphate monohydrate (LAP) [5]. These studies also allowed the discovery of new materials having *L*-arginine as the main substance. Among these material is *L*-arginine hydrochloride monohydrate (LAHW) whose several physical properties as thermal expansion, elastic properties and dielectric characteristics were presented in Ref. [6].

Some works deal with the temperature behavior of *L*-arginine·$HCl \cdot H_2O$ monocrystals. It was observed that under high temperature conditions LAHW begins to lost water of crystallization at 70^oC and at 200^oC about two-third of it are eliminated [7]. Under low temperature conditions, on the other hand, it was observed through Raman scattering technique, evidence of a phase transition undergone by LAHW between 100 and 110 K [8]. Although in the paper of Ref. [8] some Raman spectra are presented, up to now, there is no complete investigation of the polarized Raman spectra for LAHW, as well as, an assignment of the observed modes. Such assignment is very important because it helps us to understand what vibrations are related to the crystal modifications, in particular under temperature and pressure changes.

The objective of this paper is twofold: (i) to present the Raman spectra in the spectral region $20-3700cm^{-1}$ for nine scattering geometries of the two irreducible representations of the C_2 factor group; (ii) to give a tentative assignment of the observed Raman bands based on *ab-initio* calculations.

2. EXPERIMENTAL

Single crystals of *LAHCL* were grown from aqueous solution containing *L*-arginine hydrochloride powder, $C_6H_{14}N_4O_2 \cdot HCl$, from Sigma by the slow evaporation method at controlled temperature $(293K)$. In order to characterize and to do the orientation of the crystals we used X-ray diffraction patterns obtained from a Rigaku DMAX diffractometer using $Cu\ K\alpha$ radiation monochromated with a graphite crystal. The Raman spectra were obtained with a Jobin Yvon T64000 micro-Raman system equipped with an N_2-cooled charge coupled device system. The samples were polished with diamond paste with granulations with 10, 3 and $1\mu m$. The slits were set for a $2cm^{-1}$ spectral resolution. Excitation was effected with the $514.5nm$ radiation from an argon ion laser. The incident laser had a power less than $5mW$ on the surface of the sample.

3. RESULTS AND DISCUSSION

Fig. 1 presents the zwitterion form of an arginine molecule (the number associated to the atoms will be used in the assignment of the normal modes). Single crystal data of LAHW crystal confirms that the compound grows with a monoclinic lattice belonging to the $P2_1(C_2^2)$ space group with two molecules of arginine $(C_6H_{14}O_2N_4)$, two units of HCl and two units of H_2O per unit cell. In the primitive cell

*Electronic address: tarso@fisica.ufc.br

Table 1. Wavenumbers (cm^{-1}) and tentative assignment for the bands appearing in the Raman spectra of LAHW crystal at room temperature. The notation *a(bc)d* is the conventional Porto notation, where *a* and *d* represent the directions of the incident and the scattered light and *b* and *c* represent the directions of polarizations of the incident and the scattered light.

ν (cm-1) Calculate	Base Set		scattering geometries								
	6-311++G**	6-311G	x(yy)x	z(yy)z	y(xx)y	z(xx)z	x(zz)x	y(zz)y	x(yz)z	z(yx)z	y(zx)y
44					43	43	41	43	42	43	
48	τ (N17-C19)										
57			59	59	60	59	58	59	58	59	61
61	r(NH_3^+) ip		63	63		63	61	63	62	63	
			68	68	71	71	69	70	68	70	71
78		r(H16-C15-H18)	75	75	85	75	74	75	74	75	77
86	r(H16-C15-H18)			87		83	85	87		86	
			94		96	96	95	96	95	96	90
			105	102	102	102	101	102	105	102	100
						109	106	106			106
			120	120	118	118	117	119	118	119	119
131		r(H13-C12-H14)									132
137	r(H13-C12-H14)		142	142	140	141	138	139	141	142	
							149	151			152
163	w(C15-N17-C19)	r(N1-H7-H26)	158	159	159	162	156	158	158	162	
			185	190	189	189	189	192		189	191
237	τ (NH_3^+)		244	245	243	245	243	243	243	244	
261	r(H10-C9-H11)										
262		r(H10-C9-H11)	268	273	269	274	267	270		273	
305	δ (C9-C12-C15)		307	307	306	307	303	303	305	307	303
308											
326											321
327	τ (skel.)										
334		τ (NH_3^+)			331		331	332	335		
			340	340	341	340				340	340
389			377	378	378	379	377	377	380	378	
394	δ (C3-C2-N1)										
415										415	
			419	417			419	418	422		425
446	$r_1(NH_2)$	$r_1(NH_2)$	446	446		448	445	447	446	447	
457	$r_2(NH_2)$		466	466	465	465				465	470
513		δ (N17-H20)		529	531		532	533	533	530	535
545	δ (N17-C19-N22)		539			537				539	
557	ν (C2-C3-N1)		551	553	550	551			552	552	
558											
567	δ (C2-C9-C12)										
585	δ (N17-C19-N21)		621	620			618	619	619	621	619
625									629		
670	ν (C2-C3)		678	679		679	675	678	677	678	

of LAHW, all the atoms occupy sites with symmetry C_1 and the 186 vibrations can be decomposed into the irreducible representations of the factor group C_2 as $\Gamma = 93A + 93B$, with one A and two B belonging to the acoustic branch and all the other being Raman and infra red active. In Figs. 2 – 5, Z represents an axis parallel to the [001] direction, X represents an axis parallel to the [100] direction and Y is an axis perpendicular to the X and Z axes.

In order to give insights about the assignment of the normal modes of the crystal we have performed *ab initio* calculations. All calculations were carried out using Gaussian98 (by computational resources in CENAPAD's facilities) and the results were viewed by MOLEKEL programme packages. Geometry optimization and frequency calculation for *L*-arginine in the gas phase were performed with Self-Consistent methods and Hartree-Fock (HF) level of theory, with $6-31+G(d,p)$ and $6-311++G(d,p)$ basis sets. The molecular framework was designed in Gchempaint (gnome chemical software packages) and the atomic coordenates of isolated *l*-arginine was used in input file. This structure was optimized using $\delta E < 10^{-8}$ as convergence parameter and the vibrational wavenumbers were then calculated. The out-

ν (cm-1) Calculate	Base Set		scattering geometries								
	6-311++G**	6-311G	x(yy)x	z(yy)z	y(xx)y	z(xx)z	x(zz)x	y(zz)y	x(yz)z	z(yx)z	y(zx)y
692			701	702		702	700	701	700	702	
717	w(N21-C19-N17-N22)										
732	w(N17-C19-N22)		751	753	751				748	753	
786	τ (skel.)		784	784	782	782			783	783	777
799											
829											
831				831	831	830				830	
846	δ (CO_2^-)		843	843	843	843	842	843	842	843	841
885											
890		δ (N21-H23)		892	897	898	890	892	890	892	891
894	r(NH_3^+) op		897	898			897	898	897	898	898
900											
902											
904											
929	w(NH_2)		930	931	931	930	929	930	930	930	930
			939	940	939	939	939	939	939	940	940
950			955				953	955			
957			960	960	959	959	958	959	958	959	957
1001	r_1'(NH_3^+) op										
1006											
			1029	1029	1027	1028	1028	1029	1029	1021	1029
			1049	1049	1049	1049	1049	1049	1049	1050	
1053	ν(C2-N1)		1052	1053	1059	1052			1052		
1073				1062		1059	1060	1060		1057	1064
1096	r_1(NH_3^+) op		1089	1092	1092	1090	1090	1090	1093	1091	
			1099	1100	1099	1099	1100	1101	1099	1099	1101
1112		δ_1(N1-H26)									
				1132	1134	1135				1136	1133
1120	r_2'(NH_3^+) op										
1144		w(N1-H5-H26)	1139				1138	1138	1140		1142
1163	ν(C19-N22)										
1172		δ 2(N1-H26)									
1180	r_2(NH_3^+) op		1179	1181	1177	1179	1176	1178	1179	1180	1179
1211	r_1''(NH_3^+) op	w (NH_3^+)									
1231							1231	1232			
1247	r_2''(NH_3^+) op		1253				1250	1250	1253		1253
1257	t_1(NH_2)										
1259		r_2(NH_2)									
1275											
1282		δ (N21-H23)									1291
1294	t_2(NH_2)				1297	1298	1297	1298	1297	1298	
				1309			1307	1307		1308	
			1312		1312	1312	1312	1313	1312	1313	1313
			1326	1326	1325	1326	1324	1325	1326	1326	1326

put file contained the optimized structure, the Raman and IR intensities and frequencies, and the atomic displacements for each mode. At the optimized structure of the molecule, no imaginary frequency was obtained, proving that a true minimum of the potential energy surface was found.

In all spectra presented in this paper we use the conventional Porto notation *a(bc)d*, where *a* and *d* represent the directions of the incident and the scattered light and *b* and *c* represent the directions of polarizations of the incident and the scattered light. The scattering geometries in all figures are related to the irreducible representations of the factor group C_2 as follows: $x(yy)x$, $z(yy)z$, $y(xx)y$, $z(xx)z$, $x(zz)x$, $y(zz)y$ and $z(yx)z$ are from *A* irreducible representation and $x(yz)x$ and $y(zx)y$ are from the *B* irreducible representation.

Figure 2 presents the Raman spectra of LAHW for nine different scattering geometries in the spectral region $25 - 225\ cm^{-1}$ at room temperature. This region is known to have the lattice modes of the crystal ($\omega < 200\ cm^{-1}$), although some internal modes can also be observed with low wavenumber, as occurs with the torsional vibration of CO_2, $\tau(CO_2)$, for *L*-asparagine monohydrated [9], *L*-valine [10] and *L*-isoleucine [11].

ν (cm-1) Calculate	Base Set		scattering geometries								
	6-311++G**	6-311G	x(yy)x	z(yy)z	y(xx)y	z(xx)z	x(zz)x	y(zz)y	x(yz)z	z(yx)z	y(zx)y
1337	t (CH_2)		1341	1342	1341	1342	1340	1341	1340	1342	
1344		t (CH_2)	1349	1349	1349	1349	1348	1349	1348	1349	
											1358
			1371	1371	1370	1371			1370	1371	1389
1388		ν (C2–C3)	1388	1389	1389		1390	1391		1389	
1404	δ (CH_2)		1406		1406	1406	1405	1406	1405	1406	1409
			1439	1440	1440	1440	1438	1440	1439	1440	1440
			1450	1450	1449	1450	1449	1450	1449	1450	
			1456	1456	1456	1456	1455	1456	1455	1456	1457
1474	r(CH_2)		1472	1474		1472	1471		1472	1473	
1534		w(CH_2)	1535	1535							1535
1572		δ (NH_3)op		1567	1565	1568	1564			1564	1566
1588	δ (H-N-C)	δ (H-N-C)			1591						
				1605		1605				1604	1607
1613	δ (H10-C9-H11)						1614	1615			
1623	δ (H14-C12-H13)		1619		1622	1624			1621	1625	1621
1650	δ (H18-C15-H16)	δ (H14-C12-H13)	1654	1655		1655	1655	1657	1652	1655	
			2853	2854	2855	2854	2854	2854	2854	2854	2855
			2879	2879	2879	2879	2879	2878	2879	2879	2878
			2890	2891	2891	2891	2890	2890	2890	2891	2891
			2904	2904	2904	2904	2905	2904	2904	2904	2904
			2917	2918	2917	2917	2918	2917	2918	2918	2917
			2944	2947	2943	2945	2940	2942	2944	2944	2944
			2953	2954	2952		2953		2953	2953	
			2962	2962	2962	2962	2962	2962	2962	2962	2960
			2970	2970	2970	2971	2970	2970	2970	2970	2971
			2991	2991	2991	2991	2991	2991	2991	2991	2991
3169		ν (C12–H14)				3176					
			3179				3183	3183	3181	3183	3183
3194		ν (H16–C15-H18)		3188	3193						
			3335	3332			3335	3334	3336	3335	3332
					3347	3344					
				3392	3391					3390	3398
				3482	3482	3481				3483	3483

Legend: δ = bending, ν = stretching, τ = torsion, w = wagging, t = twist, r = rocking, ip = in plane, op = out of plane, skel. = framework vibration; subscripts indicate degenerence; superscript indicate same vibration for diferent atoms in same cluster. Atom numbering is shown in Figure 1.

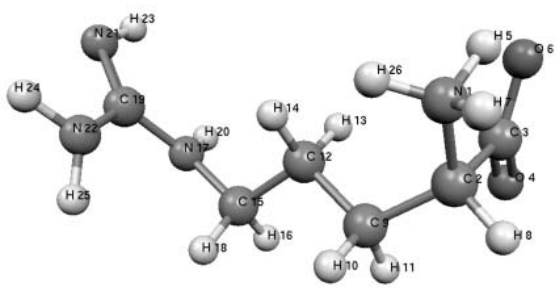

FIG. 1: Molecule of arginine in the zwitterion form.

In fact, the ab initio calculations performed in the present work show the occurrence of vibrations with $\omega < 200 cm^{-1}$ that can be associated to internal modes. As an example, using the $6-311++G**$ base set, it is possible to assign the in-plane torsion of NH_3^+, $\tau(NH3^+)ip$, as the mode appearing with wavenumber of 63 cm^{-1}.

Related to the torsional vibration of CO_2^-, $\tau(CO_2)$, our calculations were not able to identify it precisely. However, it is possible to assign the band observed at $\sim$ 189 cm^{-1} as the $\tau(CO_2)$. This is based on the studies performed on other hydrogenated amino acid crystals [9 – 11] as well as in deuterated one [12]. Such an assignment is important because under extreme conditions torsional vibrations of CO_2 can present particular behavior and furnish insights about the hydrogen bonds, as those presented by *L*-alanine-d7 under pressure variation [13].

Fig. 3 presents the Raman spectra of LAHW for nine different scattering geometries in the spectral region 200 – 650 cm^{-1}. In this region it is found the bands associated to torsional vibration of NH_3^+, $\tau(NH_3^+)$. In general, the band associated to this vibration has a low intensity, at least as

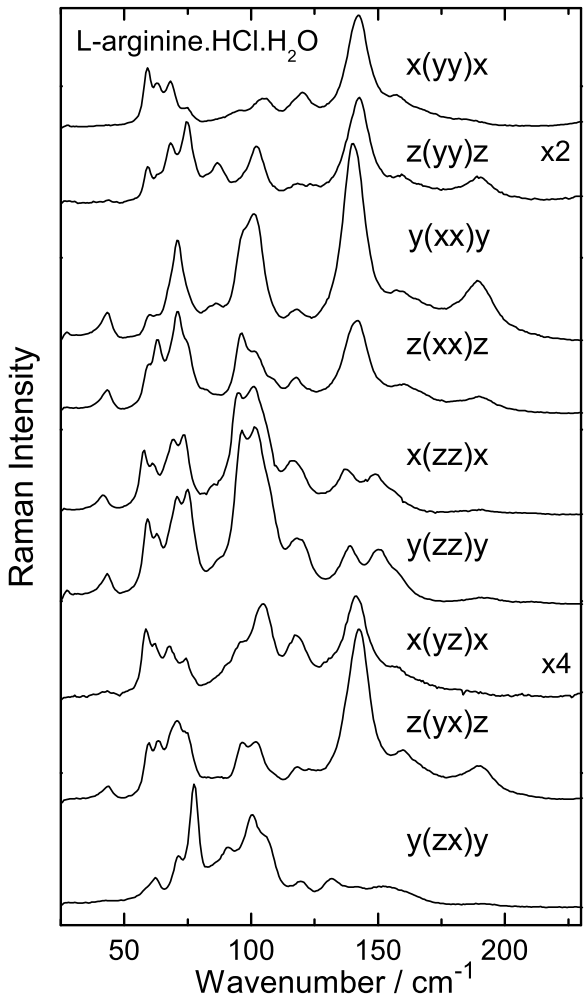

FIG. 2: Raman spectra of *L*-Arginine hydrochloride monohydrate crystal in several scattering geometries in the $25-225\ cm^{-1}$ spectral region.

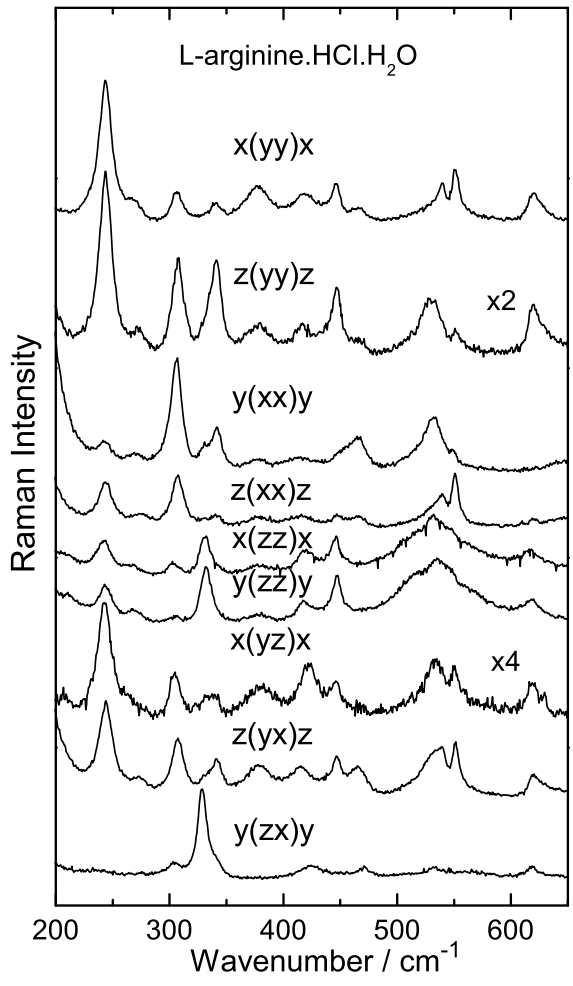

FIG. 3: Raman spectra of *L*-Arginine hydrochloride monohydrate crystal in several scattering geometries in the $200-650\ cm^{-1}$ spectral region.

suggested by former works on *L*-alanine [12] and *L*-leucine [14]. Other important vibration is associated to the rocking of NH_2, $r(NH_2)$, which ab initio calculations show to be between 445 and 465 cm^{-1}. Bending vibration of NH are also found in this spectral region, at about 530 cm^{-1}, while the bending of $N-C-N$, $\delta(NCN)$, was identified as the peak at $\sim 551\ cm^{-1}$, a band with small linewidth as can be observed in Fig. 3 for some scattering geometries.

Fig. 4 presents the Raman spectra of LAHW in the spectral region $650-1750\ cm^{-1}$ for nine different scattering geometries. This region presents a series of bands and the ab initio calculation was fundamental to assign in a most precise manner. The peak observed at about 678 cm^{-1} was associated to a $C2-C3$ stretching vibration, $\nu(C2-C3)$. Here we need to state the following observation: both, $C2$ and $C3$ carbon atoms are hold with other atoms (NH_3 for $C2$ and O_2 for $C3$) in such a way that we do not have a $C-C$ "pure" vibration, as occurs with diamond; this explains why for diamond the $\nu(C-C)$ vibration is observed at 1332 cm^{-1}. A wagging vibration related to the $N-C-N$ unit was associated to the band observed at 751 cm^{-1}. The bending of CO_2^- unit, $\delta(CO_2^-)$, was observed at 843 cm^{-1}, as pointed out by our calculations. The bands observed at about 930 and 940 cm^{-1} were associated to the wagging vibration of NH_2, $w(NH_2)$. The stretching vibration of $C2-N1$, $\nu(C2-N1)$, was associated to the band observed at $\sim 1052\ cm^{-1}$, and an out-of-plane NH_3^+ rocking vibration, $r(NH_3^+)$, was assigned as the band observed at about 1090 cm^{-1}. An out-of-plane NH_3^+ vibration was assigned as the band observed at $\sim 1250\ cm^{-1}$.

Many bands are observed between 1300 and 1400 cm^{-1}, among them the twist vibration of CH_2, $t(CH_2)$. It is worth to note that the 6-311G basis of calculation suggests that the $C2-C3$ stretching vibration should be observed at 1388 cm^{-1}. However, because the reason given previously (carbon atoms bonded to other atoms) we believe that the suggestion is not correct. The band observed at 1406 cm^{-1} was associated with the bending vibration of CO_2^-, $\delta(CO_2^-)$, and a rocking vibration of the same unit, $r(CO_2^-)$, was associated to the band observed at $\sim 1472\ cm^{-1}$. Finally, in this spectral region we also observed bending vibrations of the $H-N-C$ unit, between 1600 and 1650 cm^{-1}.

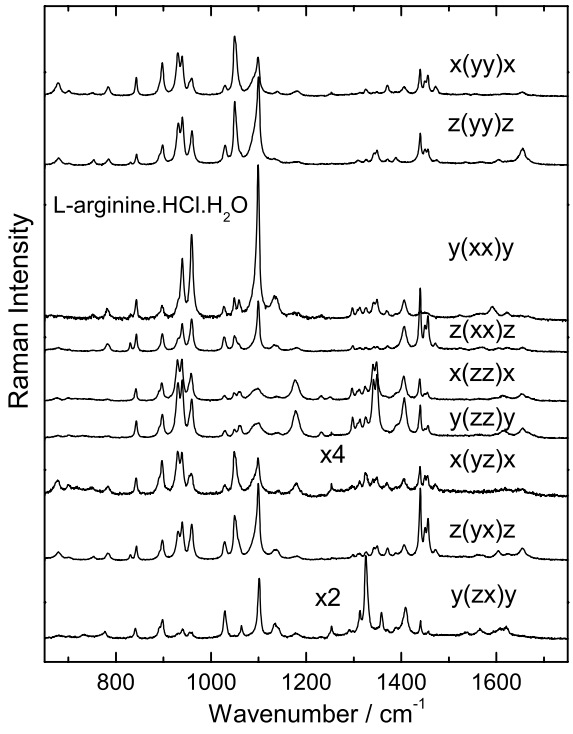

FIG. 4: Raman spectra of *L*-Arginine hydrochloride monohydrate crystal in several scattering geometries in the $650-1750\ cm^{-1}$ spectral region.

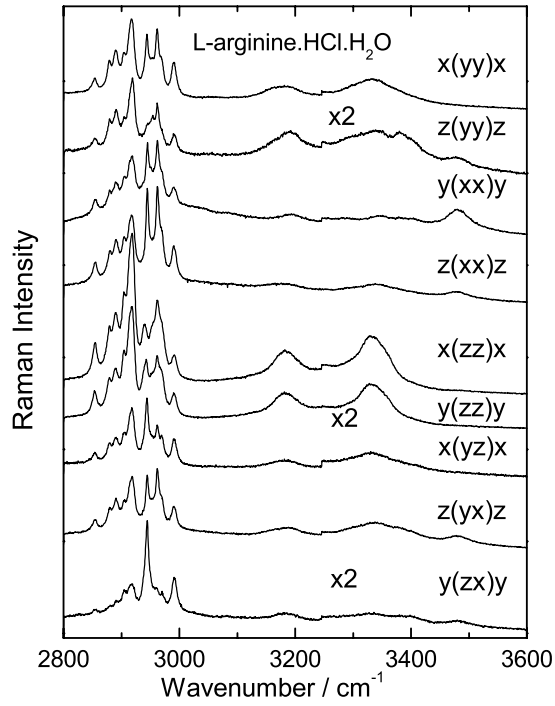

FIG. 5: Raman spectra of *L*-Arginine hydrochloride monohydrate crystal in several scattering geometries in the $2800-3600\ cm^{-1}$ spectral region.

Fig. 5 presents the Raman spectra of LAHW crystal in the spectral region $2800-3600\ cm^{-1}$ for nine different scattering geometries. The profile between 2800 and 3000 cm^{-1} is very rich, originated mainly from stretching vibrations of CH. However, our calculations were not able to assign separately all bands in this region. Finally, the bands with high linewidths centered at $\sim 3200\ cm^{-1}$ and $3400\ cm^{-1}$ can be associated to the stretching vibration of water, $\nu(H_2O)$, as it is expected from crystals with structural water [15].

With this assignment we can both (i) through light on the phase transition undergone by LAHW at low-temperatures and (ii) to give insights on future works on vibrational properties of the crystal under high pressure conditions. Related to the first point we remember that Raman spectroscopy study have suggested the occurrence of a phase transition undergone by LAHW crystal at about 110 K [8]. This phase transition was inferred mainly by the change of band profiles in the low-wavenumber region of the spectra. Obviously, as stated previously, these modifications are associated with lattice mode vibrations. Interesting enough is that we have also observed modifications in bands observed at $1088-1100\ cm^{-1}$. The assignment of the present study confirms the identification of such bands as rocking vibrations of NH_3 units. In other words, our study reinforces the interpretation that the low temperature phase transition undergone by LAHW can be understood mainly as conformational change of the *L*-arginine molecules, mainly consequence of modifications on hydrogen bonds $N-H\ldots O$, with the oxygen atom belong to a water molecule or to the $COO-$ group of another arginine molecule. This is in good accordance with experimental facts already presented by *L*-cisteine [16] and *L*-serine [17] crystals when submitted to high pressure conditions – the change of molecular conformation, due to variations in the dimensions of hydrogen bonds, modifies the symmetry of the unit cell of the crystal.

Conclusions

In this paper we presented the polarized Raman spectra of LAHW in the spectral region between 25 and 3600 cm^{-1}. Ab initio calculations were used to assign most of the bands observed in all nine scattering geometries. With such identification we gave insights about a previously observed phase transition undergone by LAHW at low temperatures. Finally, the work will be useful to understand eventual modifications in the Raman spectra of LAHW, for example, when submitted to high pressure conditions in future works.

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