

Raman Scattering Studies of Monohydrated L-Asparagine

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Polarized first-order Raman scattering studies at 300 K were carried out in L - asparagine monohydrate crystal in order to obtain the general assignment of its phonon spectra. A careful analysis of the vibrational spectra shows that the assignment of the fundamental vibrational modes can be done on the basis of amino, carboxylic and water groups vibrations, and correlations with previous data reported for other amino acids. However, some vibrations were correctly assigned from the study of its temperature - dependence behavior performed in the range 10 - 300 K. The assignment proposed confirm the zwitterionic structure of L-asparagine monohydrate crystal.

I Introduction

Amino acids are the molecular building blocks of peptides and proteins. The structure adopted by amino acids, after condensation into larger units, effectively determines their secondary structure in crystalline samples. Studies of vibrational spectra of amino acids by Raman and infrared spectroscopies are useful in obtaining information regarding the molecular conformation and the nature of hydrogen bonding in these biologically important substances.

Among other biological substances, asparagine is a very important amino acid because it plays a role in the metabolic control of some cell functions in nerve and brain tissue, and is also used by many plants as a nitrogen reserve source [1]. However,

despite its biological interest, there are few works published in the literature concerning to investigation of fundamental vibrations of asparagine compounds and its derivatives [2,3]. For instance, Casado et al. [3] presented a detailed study of the anhydrous asparagine $[\text{NH}_2\text{CO}(\text{CH}_2)\text{CH}(\text{NH}_3^+)\text{COO}^-]$, Asn-h₅, and deuterated anhydrous asparagine $[\text{ND}_2\text{CO}(\text{CH}_2)\text{CH}(\text{ND}_3^+)\text{COO}^-]$, Asn-d₅, as microcrystalline powder samples, using unpolarized Raman scattering and infrared absorption techniques. Although no data on the molecular structure of Asn-h₅ and Asn - d₅ are known, the authors have assumed the usual zwitterionic structure, supported by X-ray and neutron diffraction studies on L-asparagine monohydrate $[\text{NH}_2\text{CO}(\text{CH}_2)\text{CH}(\text{NH}_2)\text{COOH}\cdot\text{H}_2\text{O}]$, MLA [4]. A general assignment for the fundamental vibrations of these

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molecules was proposed on the basis of the isotopic shifts and correlations with similar amino acids.

Surprisingly, up to 1997 no one had investigated the fundamental vibrational modes of MLA as crystalline sample and its structural modifications under uniaxial or hydrostatic pressure. Recently, we have studied MLA by Raman spectroscopy in a diamond-anvil cell up to pressures of 2.0 Gpa at 300 K [5]. From the analysis of results we observed that MLA undergoes a series of three phase transitions. All these are characterized by drastic changes in the external modes and some internal ones of MLA, which clearly shows the transitions induced by hydrostatic pressure. As a continuation of this study, here we present for the first time a detailed investigation on the fundamental vibration spectrum of MLA at 300 K in the frequency range 50 - 3600 cm^{-1} through polarized Raman scattering technique. Data obtained from Raman spectra taken at several temperatures in the range 10 - 300 K [6] and correlations with others amino acids were used to propose a general assignment of the vibration modes of MLA at room temperature.

II Experimental

Single crystals of MLA were grown from aqueous solution containing untreated L-asparagine monohydrate powder furnished by Vetec, Co., by slow evaporation at $T = 278$ K. The crystals obtained were prisms, elongated along the a-axis and bounded by $\{011\}$ planes. The samples used in the experiments were selected using a polarizing microscope and X-ray diffraction data. They were cut into parallelepipeds with dimensions $4 \times 4 \times 6$ mm^3 and polished on Metcloth cloth using diamond paste.

The excitation source in the Raman experiments was a 514.5 nm radiation Spectra Physics argon ion laser (Model 2020) working at 80 mw, and the scattered light was analysed using a Jobin-Yvon Triplemate 64000 spectrometer equipped with N_2 - cooled CCD system. The slits were set for a 1 cm^{-1} spectral resolution.

III Crystal structure and factor group analysis

Similarly to many other amino acids, MLA molecule occurs in the dipolar form ($^+\text{H}_3\text{N} \cdot \text{CHR} \cdot \text{COO}^- \text{H}_2\text{O}$).

According to Ref. [4], at 300 K MLA crystallizes in an orthorhombic system belonging to space group $\text{P}2_12_12_1(\text{D}_2^4)$ with lattice parameters $a=5.582$ Å, $b=9.812$ Å and $c=11.796$ Å, and four molecules per unit cell which occupy general sites of C_1 symmetry. No intramolecular hydrogen bonds have been found. The structure is stabilized by a complex network of seven intermolecular hydrogen bonds involving all the hydrogen atoms bound to oxygen and nitrogen ones. From the correlation diagram the 237 zone-center optical modes vibrations are seen to decompose into

$$\Lambda = 60A + 59(B_1 + B_2 + B_3) \quad (1)$$

where 162 modes are optical translatory- and rotatory-type ones, whereas 72 modes are due to internal vibrations. Phonons belonging to A symmetry are Raman active but infrared inactive, whereas phonon belonging to B symmetries are both infrared and Raman active. The polarized Raman spectra were obtained following the traditional notation of Tell et al [7]. Phonons belonging to A symmetry were observed in the a(cc)b geometry, while the B_1 , B_2 and B_3 modes correspond to b(ab)c, c(ac)b and a(bc)b configurations, respectively.

IV Results and discussion

Following the usual procedure, the assignments are discussed in four regions, namely: (i) high-wavenumber region (2800 - 3600 cm^{-1}); (ii) medium-wavenumber region (1050 - 1800 cm^{-1}); (iii) low-wavenumber region (190 - 1000 cm^{-1}); and (iv) lattice vibration region (below 190 cm^{-1}).

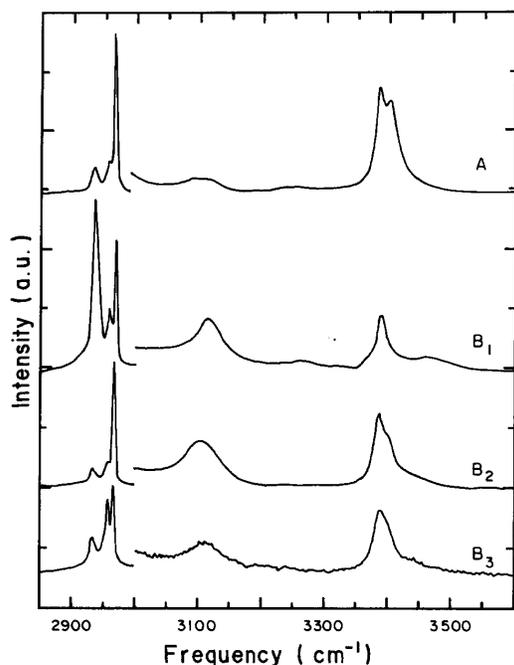
High-wavenumber region

It is observed from Fig. 1 that this spectral region contains some very broad bands. Such a situation is usually found in crystals which present vibration modes associated to hydrogen bonds. In this region we can assign the NH_2 , NH_3^+ , CH , CH_2 and H_2O stretching vibrations. The following discussion is based on B_2 symmetry modes. However, the bands located at 3264 cm^{-1} and 2921 cm^{-1} are analyzed from B_1 species. The observed wavenumbers, relative intensities and the proposed assignment for the vibrations in this spectral region are listed in Table 1.

TABLE 1. Experimental wavenumbers from the vibration spectra of MLA at 300 K in the frequency range 3600-2800 cm^{-1} .

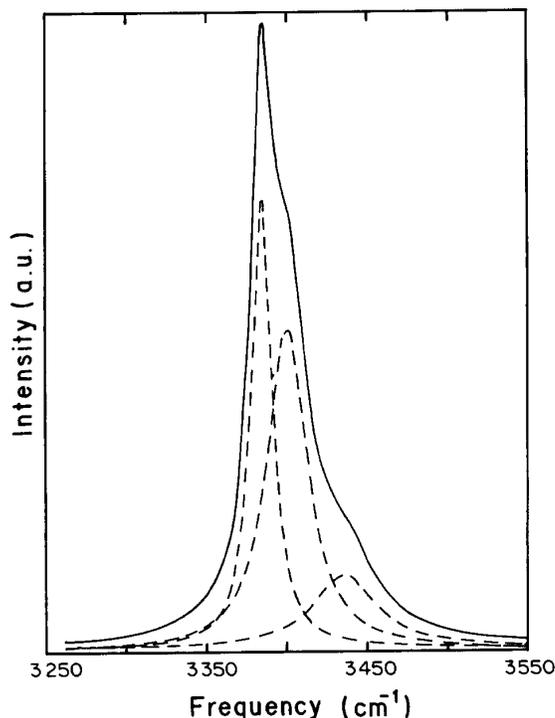
z(xx)y	x(yy)z	y(zz)x	y(xy)x	y(xz)x	z(yz)x	assignment
3423 vw	3423 vw	3423 vw	3461 w	3447 w	3441 w	$\nu_a(\text{H}_2\text{O})$
3401 s	3401 s	3401 s	-	3407 s	3404 w	$\nu_s(\text{H}_2\text{O})$
3385 s	3385 s	3385 s	3387 s	3388 vs	-	$\nu_a(\text{NH}_2)$
3252 vw	3252 vw	3252 vw	3264 w	3248 vw	-	$\nu_s(\text{NH}_2)$
-	-	-	3115 s	3117 m	3109 s	$\nu_a(\text{NK}_3^+)$
3099 w	3099 w	3099 w	-	3093 s	-	$\nu_a(\text{NK}_3^+)$
2966 vs	2966 vs	2966 vs	2966 s	2965 vw	2965 s	$\nu_a(\text{CH}_2)$
2958 s	2958 s	2958 s	2957 s	2956 vw	2956 vw	$\nu(\text{CH})$
2935 vs	2935 vs	2935 vs	2933 vs	2933 vw	2934 w	$\nu_s(\text{CH}_2)$
-	-	-	2921 sh	-	-	$\nu_s(\text{NK}_3^+)$

s = strong; w = weak; v = very and sh = shoulder

Figure 1. Polarized Raman spectra of MLA in the frequency range 3600 - 2800 cm^{-1} .

It is well known that the free water molecule (H_2O) has characteristic stretching frequencies at 3652 and 3756 cm^{-1} [8]. Due to the hydrogen bonds the effective force between O and H atoms in the H_2O molecule is reduced, and then the H_2O stretching wavenumbers in MLA crystal should present lower value than in the free molecule. In accordance with Casado et al. [3], the Raman spectrum of Asn-h₅ shows two characteristic bands corresponding to the asymmetric (ν_a) and symmetric (ν_s) NH_2 stretching vibrations located at 3388 cm^{-1} and 3248 cm^{-1} , respectively. Therefore, the band centered about 3390 cm^{-1} should contain three peaks

corresponding to $\nu_1(\text{H}_2\text{O})$, $\nu_3(\text{H}_2\text{O})$ and $\nu_a(\text{NH}_2)$ vibrations. In order to verify this assumption we have performed a lorentzian fitting as shown in Fig. 2. The fitting yields $\nu_1(\text{H}_2\text{O}) = 3447 \text{ cm}^{-1}$, $\nu_3(\text{H}_2\text{O}) = 3407 \text{ cm}^{-1}$ and $\nu_a(\text{NH}_2) = 3388 \text{ cm}^{-1}$. The $\nu_s(\text{NH}_2)$ vibration is observed at 3248 cm^{-1} . This band is very weak for all investigated scattering geometries. Its classification was done based on the temperature dependent Raman spectra, where for temperatures below 200 K this band can be clearly seen [6].

Figure 2. Lorentzian fittings of the bands appearing in the frequency range 3450 - 3380 cm^{-1} for B₂ symmetry modes.

In amino acids [9] and amino sulphones [10], the stretching vibrations of the NH_3^+ group are expected in the frequency region $3150 - 3000 \text{ cm}^{-1}$. These modes are usually observed as weak bands and it is somewhat difficult to assign them when other NH or CH intense bands are present in the vibrational spectra. For MLA we assigned the $\nu_a(\text{NH}_3^+)$ degenerated vibration to the band centered at 3110 cm^{-1} . In fact, if we use a lorentzian fitting, we can observe that there are two nondegenerated $\nu_a(\text{NH}_3^+)$ at 3117 cm^{-1} and 3093 cm^{-1} . These assignments were mainly based on previous work on the L-alanine crystal [9]. The splitting of two-fold degenerated $\nu_a(\text{NH}_3^+)$ mode can be explained taking into account the lowering from C_{3v} symmetry of the free NH_3^+ group to C_1 symmetry in the crystal environment of MLA. In order to correctly assign the $\nu_s(\text{NH}_3^+)$ we analyze its temperature-dependent behavior in the range $10 - 300 \text{ K}$ [6]. At 10 K , we observed, besides three CH stretching vibrations, the appearance of a vibration around 2925 cm^{-1} . Its integrated scattered intensity remains constant with increasing or decreasing temperature. In this way, it can not be attributed to a Fermi resonance enhanced overtone or an overlapping between fundamental modes. Hence, this vibration should be assigned to $\nu_s(\text{NH}_3^+)$. At 300 K , we have assigned the $\nu_s(\text{NH}_3^+)$ to the shoulder measured at 2921 cm^{-1} . For Asn-h₅, Casado et al. [3] have assigned the $\nu_s(\text{NH}_3^+)$ vibration to the shoulder measured at 3025 cm^{-1} from the infrared spectrum. No assignment of this mode is proposed from Raman spectrum. However, from Fig. 3 of Ref. [3], we observe that there is a vibration mode at 2860 cm^{-1} . When Asn-h₅ is deuterated (Asn-d₅), this vibration disappears (cf. Fig. 4 of Ref. [3]). This result suggests that the vibration at 2860 cm^{-1} observed by Casado et al. [3] should be related to $\nu_s(\text{NH}_3^+)$, which agrees with our observations.

The three C-H stretching modes observed in the frequency range $2900 - 3000 \text{ cm}^{-1}$ are assigned following the same order as for aspartic acid [11], that is, $\nu(\text{CH})$ is between the $\nu_a(\text{CH}_2)$ at 2965 cm^{-1} and the $\nu_s(\text{CH}_2)$ at 2934 cm^{-1} . The wavenumber for the methylene stretching vibration, 2956 cm^{-1} , compares well with those reported for other amino acids such as l-alanine, 2962 cm^{-1} [9], cysteine, 2968 cm^{-1} [12], and also with Asn-h₅, 2977 cm^{-1} [3].

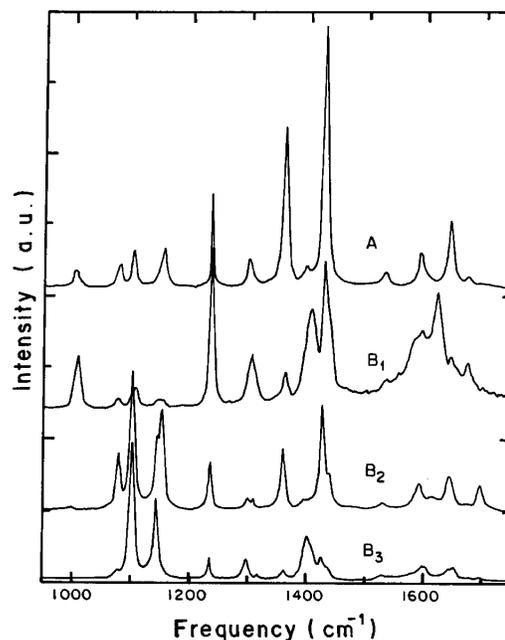


Figure 3. Polarized Raman spectra of MLA in the frequency range $1800 - 900 \text{ cm}^{-1}$.

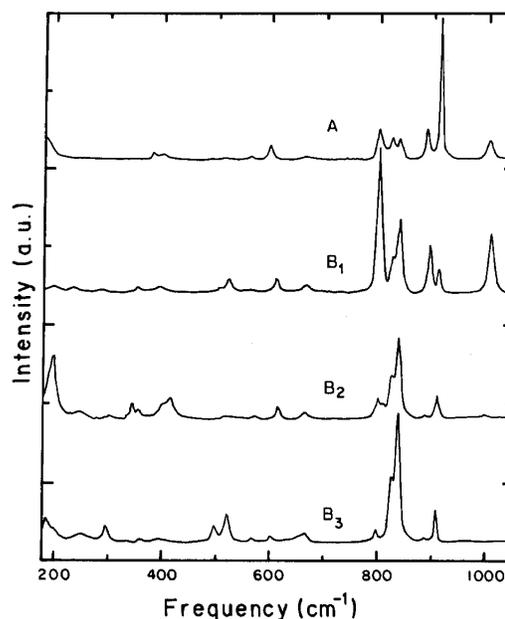


Figure 4. Polarized Raman spectra of MLA in the frequency range $900 - 190 \text{ cm}^{-1}$.

Medium-wavenumber region

From Fig. 3, we observe that this spectral region presents a large number of normal modes. The bands

are very broad for all scattering geometries. Due to this fact, the assignment of the normal modes was performed with the help of lorentzian fittings. The discussion of this frequency region is based mainly on the B_1 representation, excepting the modes at 1694 cm^{-1} and

1594 cm^{-1} which are observed from B_2 and B_3 geometries. The measured wavenumbers, relative intensities and proposed assignments for the bands observed in this region are summarized in Table 2.

TABLE 2. Experimental wavenumbers from the vibration spectra of MLA at 300 K in the frequency range $1800\text{-}900\text{ cm}^{-1}$.

z(xx)y	x(yy)z	y(zz)x	y(xy)x	y(xz)x	z(yz)x	assignment
-	-	-	-	1696 s	1694 vw	$\delta_a(\text{NK}_3^+)$
1670 vw	1670 vw	1670 vw	1673 s	1672 vw	1669 vw	$\delta_a(\text{NK}_3^+)$
1644 vw	1644 vw	1644 vw	1647 s	1644 vw	1645 w	$\nu(\text{C=O})$
1630 s	1631 s	1631 s	1621 vw	1617 w	-	$\delta(\text{NH}_2)$
1594 vw	1594 vw	1594 vw	-	1594 s	1600 vw	$\delta(\text{H}_2\text{O})$
-	-	-	1580 s	-	1582 vw	$\nu_a(\text{CO}_2^-)$
-	-	-	1534 vw	1531 w	1534 vw	$\delta_s(\text{NK}_3^+)$
1435 s	1435 s	1436 s	1435 w	1438 w	1436 vw	$\delta_a(\text{CH}_2)$
-	-	-	1428 s	1426 w	1426 w	$\nu_s(\text{CO}_2^-)$
1409 w	1409 w	1409 w	1407 vw	-	1409 w	$\delta(\text{CN})$
1396 vw	1396 vw	1396 vw	1399 s	1400 vw	1400 vw	$\delta_s(\text{CH}_2)$
1363 s	1363 s	1362 s	1362 vw	1359 vw	1361 vw	$\delta(\text{CH})$
-	-	-	1304 w	1310 vw	1318 vw	$\delta(\text{CH})$
1301 vw	1302 vw	1301 vw	1299 vw	1300 vw	1299 vw	$\omega(\text{NH}_2)$
1238 s	1237 s	1237 s	1237 s	1236 s	1237 vw	$\tau(\text{CH}_2)$
-	-	-	-	1153 s	1154 vw	$r(\text{NH}_2)$
1147 vw	1147 vw	1146 vw	1148 w	1145 vw	1141 vw	$r(\text{NK}_3^+)$
1109 s	1109 s	1109 s	1104 vw	1102 s	1102 w	$r(\text{NK}_3^+)$
1075 s	1074 s	1075 s	1076 w	1078 s	1077 w	$\nu(\text{CN})$

s = strong; w = weak; v = very and sh = shoulder

It is well known that ionized carboxylic group (COO^-) have two characteristic bands in this frequency range, namely: (i) ν_a at 1600 cm^{-1} , and (ii) ν_s at 1400 cm^{-1} . Since MLA presents COO^- and CONH_2 groups, a band related to $\nu(\text{C=O})$ stretching vibration should also appear in this wavenumber region. The bands due to vibrations of NH_3^+ , CO_2^- and NH_2 groups obey some hierarchical wavenumber relationship and then we can do a tentative assignment for these modes.

In the frequency range $1500\text{-}1700\text{ cm}^{-1}$ we observe from all scattering geometries, seven vibrational modes. The highest wavenumber bands in this region 1694 and 1673 cm^{-1} , were assigned to asymmetric bendings of NH_3^+ (δ_a), while the related symmetric bending of NH_3^+ (δ_s) was observed at 1534 cm^{-1} . The asymmetric stretching vibrations of the carboxylate group are

is observed at $\nu_a(\text{COO}^-) = 1580\text{ cm}^{-1}$. The $\nu(\text{C=O})$ mode appears as the most intense band at 1647 cm^{-1} for B_1 species. Close to this band we have assigned the following vibrations: (i) $\delta(\text{NH}_2)$ at 1621 cm^{-1} ; and (ii) $\delta(\text{H}_2\text{O})$ at 1594 cm^{-1} .

In the frequency range $1050\text{-}1500\text{ cm}^{-1}$ we observe twelve bands. From these, there are six bands which depend weakly on the temperature [6]. They are due to vibrations of groups not linked by hydrogen bonds. These bands are: (i) $\delta_a(\text{CH}_2)$ at 1435 cm^{-1} ; (ii) one due to wagging-type vibration (ω) of CH_2 group at 1299 cm^{-1} ; (iii) one due to torsion-type vibration (τ) of CH_2 group at 1237 cm^{-1} ; (iv) $\delta_s(\text{CH}_2)$ at 1399 cm^{-1} ; and (v) two $\delta(\text{CH})$ at 1362 and 1304 cm^{-1} . These assignments compare well with those reported for glutamic acid [12] and Asn-h₅ [3].

The remainder six bands which depend on temperature are: (i) one due to symmetric stretching of the carboxylate group observed at $\nu_s(\text{COO}^-) = 1428 \text{ cm}^{-1}$; (ii) two due to rocking-type (r) vibrations of NH_3^+ group at 1142 and 1102 cm^{-1} ; (iii) one $r(\text{NH}_2)$ at 1154 cm^{-1} ; and finally (iv) two $\nu(\text{CN})$ at 1409 and 1077 cm^{-1} . For the sake of comparison, the $r(\text{NH}_3^+)$ modes were observed at 1152 and 1120 cm^{-1} for aspartic acid [11] and at 1138 and 1119 cm^{-1} for glycine [13], whereas $r(\text{NH}_2)$ was reported at 1162 cm^{-1} for glutamine [14] and at 1149 cm^{-1} for acetamide [15]. Also, Diem et al [9] observed that $\nu(\text{CC})$ stretching vibration of L-alanine takes place around 1000 cm^{-1} , which is in good

agreement with our result.

Low-wavenumber region

In this region we expect to be able to identify besides the C-C stretching modes, the rest of the bending vibrations along with the torsional and skeletal stretching modes. The Raman spectra for the four scattering geometries are shown in Fig. 4. The discussion of the mode assignment is performed mainly on B_3 representation, excepting the 807 cm^{-1} mode which is observed only in the B_2 representation. The results are reported in Table 3.

TABLE 3. Experimental wavenumbers from the vibration spectra of MLA at 300 K in the frequency range 900-190 cm^{-1} .

z(xx)y	x(yy)z	y(zz)x	y(xy)x	y(xz)x	z(yz)x	assignment
-	-	-	1008 s	-	1004 w	$\nu(\text{CC})$
910 vw	911 vw	911 vw	911 w	909 w	913 s	$\nu(\text{CC})$
890 w	890 w	889 w	894 w	886 w	888 w	$\nu(\text{CC})$
841 s	841 s	841 s	839 s	838 s	838 w	$\gamma(\text{NH}_2)$
825 s	825 s	825 s	826 w	825 w	823 w	$\gamma(\text{CO}_2^-)$
-	-	-	-	807 w	-	$\gamma(\text{H}_2\text{O})$
800 s	800 s	800 s	800 s	799 w	799 w	$r(\text{CH}_2)$
669 w	668 w	669 w	665 s	667 w	664 s	$\delta_a(\text{CONH}_2)$
-	-	-	-	-	648 w	$\delta_a(\text{CO}_2^-)$
602 w	602 w	602 w	602 s	609 s	603 w	$\delta_s(\text{CONH}_2)$
564 s	564 s	565 s	568 w	565 w	569 w	$\delta_s(\text{CO}_2^-)$
530 s	531 s	530 s	522 s	-	521 s	$\tau(\text{NH}_2)$
495 w	494 w	495 w	504 w	507 w	498 s	$\delta(\text{struct})$
401 w	401 w	402 w	-	407 s	402 w	$\delta(\text{struct})$
390 w	389 w	389 w	394 w	390 s	386 w	$\tau(\text{NK}_3^+)$
339 s	340 s	340 s	353 w	351 w	360 w	$\delta(\text{struct})$
-	-	-	-	295 w	290 w	$\delta(\text{struct})$
-	-	244 w	238 w	242 w	250 w	$\delta(\text{struct})$

s = strong; w = weak; v = very and sh = shoulder

According to Ref. [16], the symmetric stretchings of C-C and/or C-C-N structures appear in the frequency range 750 - 1000 cm^{-1} of the vibrational spectra of several amino acids. The modes observed at 1004, 913 and 888 cm^{-1} were assigned as the $\nu(\text{CC})$ vibrations. Near them we also expect to observe one methylene rocking vibration ($r(\text{CH}_2)$), one out-of-plane vibration of amide group ($\gamma(\text{NH}_2)$), one $\gamma(\text{COO}^-)$, and finally one $\gamma(\text{H}_2\text{O})$. From lorentzian fitting of the bands observed

in the frequency range 820 - 920 cm^{-1} we have assigned the following vibrations: (i) two $\nu_s(\text{CC})$ at 913 and 888 cm^{-1} , (ii) one $\gamma(\text{NH}_2)$ at 838 cm^{-1} , and (iii) one $\gamma(\text{COO}^-)$ at 823 cm^{-1} . The vibration of 807 cm^{-1} is assigned to $\gamma(\text{H}_2\text{O})$, since it is not present in the spectrum of Asn-h₅ [3]. The methylene rocking vibration was assigned at frequencies around 780 cm^{-1} for glutamine [14] and DL - serine [17]. Here, we assigned the $r(\text{CH}_2)$ at 799 cm^{-1} . However, for Asn-h₅, Casado et

al. [3], have assigned the ν (CH_2) at 874 cm^{-1} . It seems to be a contradiction between our assignment and that proposed in Ref.[3]. In order to verify our assignment, we observe its temperature-dependent behavior in the range 10 - 300 K [6]. We observed no modifications in the frequency and linewidth of this mode, which indicates that it is not affected by hydrogen bonds, and indeed it must be assigned to ν (CH_2). On the basis of correlations with related molecules [13-17] the bands at 648 and 569 cm^{-1} were assigned as two $\delta(\text{COO}^-)$, while the bands at 664 cm^{-1} and 603 cm^{-1} as two $\delta(\text{CONH}_2)$.

There are still 8 bands in the frequency range $180 - 570\text{ cm}^{-1}$. Five of them are related to torsional (τ) and bending modes of skeletal structure. The remainder three are assigned to τ (COO^-) at 200 cm^{-1} , τ (NH_3^+) at 386 cm^{-1} and τ (NH_2) at 521 cm^{-1} . These results are in good accordance with those reported for other amino acid compounds [3,9,13-17].

Lattice vibration region

Unfortunately, there are only few reports concerning the investigation on the assignment of lattice vibrations in amino-acids [18-20]. Some bands observed below 190 cm^{-1} are tentatively assigned to stretching, torsional and skeletal bending vibrations, whereas other spectral lines should be assigned to intermolecular hydrogen bonding between the NH_3^+ and COO^- groups in the neighboring molecules. The other types of intermolecular interactions presumably do not contribute significantly to the spectrum as the force acting between hydrocarbon molecules is generally weak, and furthermore the atoms in different MLA molecules are separated greatly from each other.

The following discussion is based on B_3 symmetry modes showed in Fig. 5. The measured wavenumbers, relative intensities and proposed assignments for the bands observed in this frequency region are summarized in Table 4. The vibrations of 185 cm^{-1} and 146 cm^{-1} are assigned to bending vibrations of skeletal structure, whereas, according to Ref.[19], the bands at 138 cm^{-1} , 131 cm^{-1} , 118 cm^{-1} , 96 cm^{-1} , 90 cm^{-1} , 82 cm^{-1} and 54 cm^{-1} can be tentatively assigned to stretching or bending vibrations of ($\text{A}^+ - \text{H}^+ \dots \text{O}^-$) hydrogen bonds.

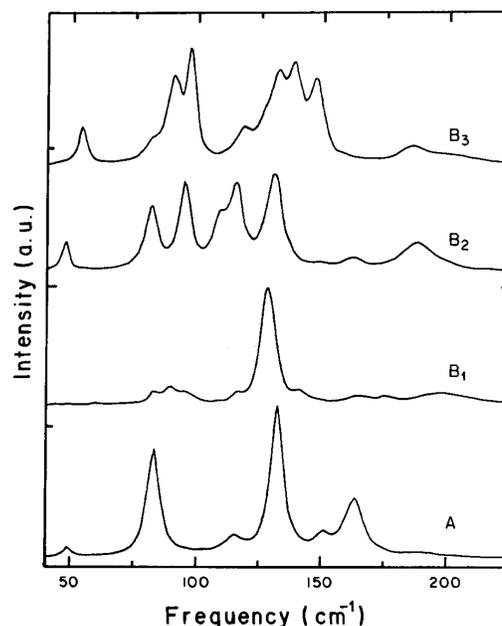


Figure 5. Polarized Raman spectra of MLA for frequencies below 190 cm^{-1} .

V Conclusions

In conclusion we have presented a polarized Raman scattering study of MLA crystals at room temperature. We observed most of the fundamental vibrations expected from the group theory analysis. A general assignment for these fundamental vibrations was proposed on the basis of the detailed analysis of Raman spectra taken at several temperatures in the range 10 - 300 K and correlation with similar amino acids. The results confirmed the zwitterionic structure of MLA, since no hydroxyl stretching vibrations were found. The assignments proposed in this work are in good agreement with those previously reported for Asn-h₅ by Casado et al. [3]. There are few but important differences between these assignments, namely: (i) the observation of fundamental modes related to the water molecule vibrations; (ii) the correct assignment for ν (CH_2); (iii) the assignment for $\nu_s(\text{NH}_3^+)$ vibration; and (iv) the tentative assignment for low-frequency vibrations. This agreement was expected since all amino acids (hydrate or not) already studied, present common structures which are subject to similar environments and bonding distances. Further works for the anharmonic effects induced by temperature changing in some fundamental vibrations associated to hydrogen bonds is in progress.

TABLE 4. Experimental wavenumbers from the vibration spectra of MLA at 300 K for frequencies below 190 cm^{-1} .

$z(xx)y$	$x(yy)z$	$y(zz)x$	$y(xy)x$	$y(xz)x$	$z(yz)x$	assignment
191 w	191 w	191 w	197 w	198 w	200 w	$\tau(\text{CO}_2^-)$
-	-	-	175 w	190 s	185 s	$\delta(\text{struct})$
163 w	163 w	163 w	165 w	160 w	-	$\delta(\text{struct})$
150 w	150 w	150 w	141 w	153 s	146 s	$2\delta(\text{struct})$
132 vs	132 vs	132 vs	130 vs	135 s	138 s	
-	-	-	127 vs	131 s	131 s	
115w	115 w	115 w	116 s	121 s	118 w	
-	-	-	96 w	102 s	96 vs	
-	-	-	90 w	87 vs	90 vs	
83 s	83 s	83 s	83 w	82 s	82 w	
48 w	48 w	48 w	-	48 vw	54 s	

s = strong; w = weak; v = very and sh = shoulder

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