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Far infrared spectra of solid state aliphatic amino acids in different protonation states

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Far infrared spectra of zwitterionic, cationic, and anionic forms of aliphatic amino acids in solid state have been studied experimentally. Measurements were done on glycine, L-alanine, L-valine, L-leucine, and L-isoleucine powder samples and film samples obtained from dried solutions prepared at pH ranging from 1 to 13. Solid state density functional theory calculations were also performed, and detailed potential energy distributions were obtained from normal mode results. A good correspondence between experimental and simulated spectra was achieved and this allowed us to propose an almost complete band assignment for the far infrared spectra of zwitterionic forms. In the 700–50 cm^{-1} range, three regions were identified, each corresponding to a characteristic set of normal modes. A first region between 700 and 450 cm^{-1} mainly contained the carboxylate bending, rocking, and wagging modes as well as the ammonium torsional mode. The 450–250 cm^{-1} region was representative of backbone and sidechain skeletal bending modes. At last, the low wavenumber zone, below 250 cm^{-1} , was characteristic of carboxylate and skeletal torsional modes and of lattice modes. Assignments are also proposed for glycine cationic and anionic forms, but could not be obtained for all aliphatic amino acids due to the lack of structural data. This work is intended to provide fundamental information for the understanding of peptides vibrational properties. © 2010 American Institute of Physics. [doi:10.1063/1.3356027]

I. INTRODUCTION

Amino acids are the elementary units of proteins which are essential molecules for life. Having a good knowledge of low frequency vibrations of amino acids is of critical importance for understanding protein reactivity and function. Early investigations were reported by Heintz¹ and later by Genzel *et al.*^{2–4} During the last decade, the number of studies on the vibrational properties of amino acids strongly increased, including amino acids in the solid state,^{5–13} in aqueous solution,^{14–18} gas phase,¹⁹ cryogenic matrixes,^{20–23} or in a context mimicking a protein active site.^{24,25}

Depending on their environment and physical state, the structural properties of amino acids will differ. In the gas phase, the uncharged form is predominant, while in aqueous solution and in solid state the zwitterionic form is present.^{26,27} In a charged medium or complex environments like a protein, protonated or deprotonated forms of amino acids can also be observed and changes in protonation states of amino acids are important in many enzymatic reactions.¹⁴ Whereas the amino acid conformation is well defined in solid state, it is more variable in aqueous solution and gas phase.

In addition, the solid state zwitterionic form, where the ammonium group of a molecule usually forms interactions with carboxylate groups of other molecules,²⁸ has a different origin than the zwitterionic form in aqueous solution, where hydrogen bonding occurs between amino acids and surrounding water molecules.²⁹ These chemical and structural differences influence the infrared vibrational spectrum, in particular in the far infrared region, which is the most affected by intermolecular interactions. The solid state spectrum of a molecular crystal is thus expected to include crystal lattice and hydrogen bond vibrations below 250 cm^{-1} .¹²

This study focuses on aliphatic amino acids: glycine, L-alanine, L-valine, L-leucine, and L-isoleucine. These amino acids involve two main acid-base couples, $\text{CO}_2\text{H}/\text{CO}_2^-$ and $\text{NH}_3^+/\text{NH}_2$, with corresponding pKa values around pH 2.3 and 9.8, respectively.³⁰ The 700–50 cm^{-1} range of the infrared spectrum of these aliphatic amino acids is measured from film samples obtained after drying water solutions prepared at different pH values. Infrared spectrum measurement is also done on powder samples for comparison. Solid state quantum mechanical vibrational calculations based on the density functional theory (DFT) and a plane-wave pseudopotential approach are performed to complement experimental infrared measurements and enable de-

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tailed band assignments. Modeling the zwitterionic form of amino acids either requires implicit solvation, explicit water molecules, or a solid state environment to be taken into account. In gas phase calculations, the zwitterion is not stable and reverts to the uncharged form.³¹ The choice of solid state calculations in this work is based on the expected physical state of our dried film and powder samples when measured by the ATR technique.

Theoretical studies of the vibrational spectra of zwitterionic amino acids have been previously reported. Kumar and co-workers^{16,17} performed gas phase and implicit solvation vibrational calculations on glycine and alanine species, using semiempirical, *ab initio*, and DFT methods. Tortonda *et al.*³² and Gontrani *et al.*³³ explored the geometry and vibrations of glycine and alanine zwitterions with *ab initio* and DFT methods and polarizable continuum models. Other *ab initio* or DFT studies employed a different approach by including explicit water molecules around glycine or alanine zwitterions.^{15,34,35} Some of these theoretical works were not successful in reproducing experimental geometries or had difficulties to sample all conformations accessible in liquid phase. They did not discuss the correspondence with experimental data below 400 cm⁻¹. Very few studies focused on the cationic and anionic forms of amino acids and the lowest wavenumbers were not explored.^{36,37} The plane-wave pseudopotential computational methodology employed in this work was shown by Jepsen and Clark³⁸ to give accurate predictions of terahertz vibrational modes of organic crystalline systems, provided that stringent convergence criteria are employed. Calculations using a similar approach have recently been applied to the prediction of vibrational modes of solid state glycine and L-alanine by Chowdhry *et al.*³⁹ The authors compared solid state and aqueous solution vibrational calculations with infrared and Raman experimental data. Assignments of solid state normal modes were realized by visual inspection. Williams *et al.*⁴⁰ employed an interesting scaled quantum mechanical harmonic force field approach including anharmonic corrections to the study of polycrystalline L-alanine. Good agreement with experimental spectra is obtained, however, scaling factors needed to be determined empirically.

In this study, experimental far infrared measurements of glycine, L-alanine, L-valine, L-leucine, and L-isoleucine are presented showing the spectra pH-dependence over a 1.0–13.0 pH range. DFT calculations are performed on the amino acid zwitterion crystals. We concentrate on the far infrared, lowest frequency normal modes, which require the most precision and convergence in calculations. Analysis of the computational results and potential energy distributions (PEDs) of modes allows detailed and almost complete assignments of experimental spectra bands. This study is in line with the growing use of far infrared spectroscopy (FIR) measurements applied to biomolecules.

II. EXPERIMENTAL METHODS

Glycine (purity $\geq 99\%$), L-alanine, L-valine, L-leucine, and L-isoleucine (purities $\geq 98\%$) were obtained from Sigma-Aldrich and used without further purification. Solu-

tions were prepared by dissolving amino acid powders in distilled water to a final concentration of 0.10 mol L⁻¹ (± 0.01). Solutions at pH values ranging from 1.0 to 13.0 were obtained by addition of HCl or NaOH, and control by a pH electrode.

Far infrared measurements were carried out at room temperature on a Vertex 70 (Bruker) Fourier transform spectrometer equipped with a global source, a RT-dTGS detector and a silicon beam splitter. The sample compartment was purged with dry air to reduce interference of H₂O and CO₂. Spectra were recorded from 700 to 50 cm⁻¹ with an ATR cell (Harrick) equipped with a diamond crystal (6 mm²) as reflecting element. Films of aliphatic amino acids were obtained by drying 2 μ L of water solution in a stream of warm air on the diamond surface. Resolution was 4 cm⁻¹ and 128 scans were accumulated for each spectrum. The instrument and the sample chamber were purged with dry air to avoid contributions from humidity. Residual absorption of gas water on the spectra was subtracted. All spectra were smoothed with nine points.

III. COMPUTATIONAL METHODS

Starting coordinates and lattice parameters were extracted from the Cambridge Structural Database⁴¹ (CSD). Entries were GLYCIN,⁴² GLYCIN02,⁴³ LALNIN,⁴⁴ LVALIN01,⁴⁵ LEUCIN01,⁴⁶ and LISLEU02⁴⁷ for β polymorph glycine, α polymorph glycine, L-alanine, L-valine, L-leucine, and L-isoleucine crystals, respectively. All these entries contained hydrogen atom positions. Because of the uncertainty on glycine polymorphic state, we performed calculations on two states, α and β . A glycinium chloride structure was also extracted from the CSD, entry GLYHCL01,⁴⁸ and the coordinates of a lithium glycinate structure were taken as published by Müller *et al.*⁴⁹ The lithium cation was replaced by a sodium cation to match experimental conditions.

Plane-wave pseudopotentials DFT calculations were done with the CASTEP program.⁵⁰ The PW91 functional⁵¹ was employed along with norm-conserving pseudopotentials and a kinetic energy cutoff of 1200 eV. Brillouin zone integration was performed using a k-point set defined by a $2 \times 2 \times 2$ Monkhorst–Pack grid.⁵² Electronic minimization was performed using the conjugate gradient method to a convergence criterion of 10^{-10} eV/atom. Geometry optimization was carried out using the Broyden–Fletcher–Goldfarb–Shanno optimization scheme until atomic forces were lower than 10^{-5} eV/Å. Cell optimization was performed until the maximum component of the stress tensor was lower than 0.1 GPa. Density functional perturbation calculations⁵³ were then performed on optimized structures. The zone center phonon modes were calculated, as well as corresponding frequencies and infrared intensities.

A set of nonredundant local symmetrical internal coordinates was defined for each molecule according to the Pulay methodology.⁵⁴ This provides a more useful description of the vibrational potential energy than working with Cartesian coordinates, making comparisons between similar molecules easier. PEDs of each mode were calculated with the MOLVIB

program.⁵⁵ Modes with similar molecular components but different phase relationships between the individual molecules were grouped together by calculating the squared dot products between all mode molecular components and maximizing it within a group of modes. Simulated infrared spectra were obtained by convolution of computed mode intensities with Gaussian functions of 10 cm^{-1} width.

IV. RESULTS AND DISCUSSION

Far infrared spectra ($700\text{--}50\text{ cm}^{-1}$) of aliphatic amino acids powder samples and film samples obtained from dried solutions prepared at different pH are presented. The respective spectra of zwitterionic, cationic, and anionic forms can be identified based on spectra pH-dependence. For pH values near the respective pKa values, spectra display absorptions of two species (anion and zwitterion or zwitterion and cation).⁵⁶ At very high and very low pH, presence of the counterion is not negligible and can explain some additional changes in the spectrum (sharpening of bands below 250 cm^{-1}).⁵⁷ Solid state quantum chemistry vibrational calculations results are reported and employed to interpret experimental spectra based on the order of modes, relative infrared intensities, PEDs, and symmetry considerations. Additional details as well as annotated comparisons of experimental and theoretical spectra in higher resolution are available in supplementary material⁵⁸ and can help the reader to follow band assignments.

In the following, amino acid heavy atom names follow IUPAC nomenclature (N, C, C α , C β , ...). Abbreviations employed for vibrations are ν (stretching), δ (bending), ρ (rocking), t (twisting), ω (wagging), and τ (torsion).

A. Glycine

Glycine is the simplest amino acid. Far infrared spectra measured for the powder sample and homogeneous films obtained from aqueous solutions at different pH are presented in Fig. 1. Based on spectra comparison as a function of pH, we attribute the 3.0–9.0 pH range to the zwitterionic form, the 1.1–2.1 pH range to the cationic form, and the 10.2–13.0 pH range to the anionic form.

Zwitterion. Glycine spectra measured from the dried solution prepared at pH 6.6 and from the powder sample presented significant differences, in particular, in the low frequency region below 250 cm^{-1} . It is known that glycine zwitterionic crystals exist in three main polymorphic states, α , β , and γ . Based on comparison in the mid-infrared region of our powder and film samples to α , β , and γ glycine spectra published by Chernobai *et al.*,⁵⁹ we propose that the α polymorph is the predominant form in glycine powder samples, and the β polymorph is predominant in glycine film samples. The α and β glycine crystals are both monoclinic but differ in their space groups, $P2_1/n$ and $P2_1$, respectively. Although β glycine is known to be a metastable form,⁵⁹ its presence in film samples would not require long time stability as the solid is formed *in situ* by drying the solution on the diamond cell and measured straight afterwards.

Glycine spectra in the $700\text{--}450\text{ cm}^{-1}$ range first showed a medium band centered at 606 cm^{-1} for the powder sample,

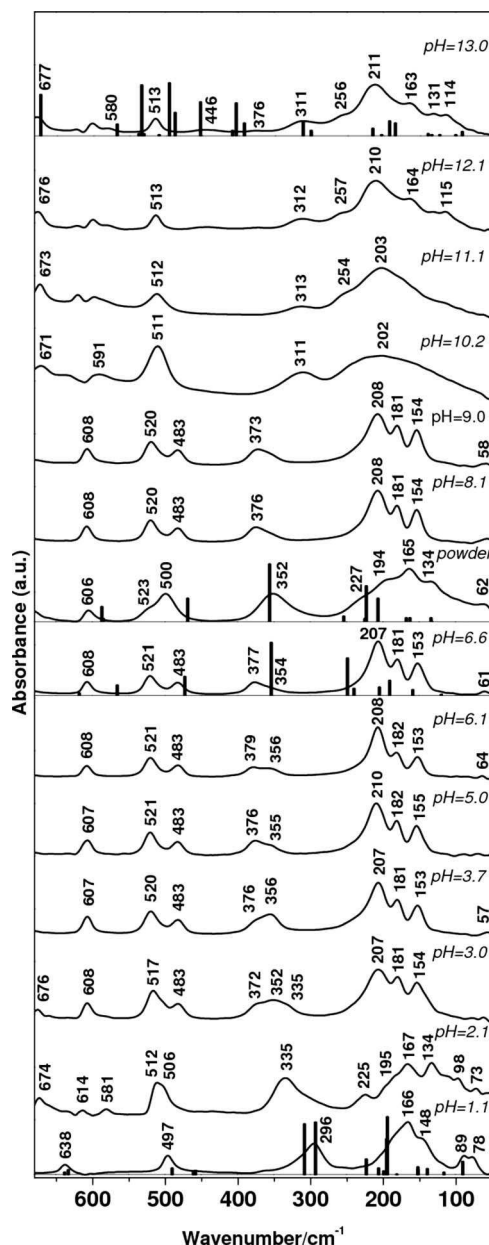


FIG. 1. Far infrared spectra of glycine powder sample and dried film samples prepared from solutions at different pH. Major experimental peaks are labeled. Calculated peaks for glycine β zwitterion, α zwitterion, cation, and anion are superposed on pH 6.6 film sample, powder sample, pH 1.1, and pH 13.0 film sample experimental spectra, respectively.

and a medium band at 608 cm^{-1} for the dried film sample prepared from the solution at pH 6.6. These bands are both attributed to the $\omega(\text{CO}_2^-)$ wagging mode by us and earlier works,^{39,60,61} while Matei *et al.* assigned them to the $\delta(\text{CO}_2^-)$ bending mode.⁸ The assignment of the $\tau(\text{NH}_3^+)$ torsional mode for glycine has been a source of controversy due to the difficulty to accurately predict its position with theoretical approaches.³⁹ This was the case in our DFT calculations as well. The assignment was therefore based on experimental evidence.^{8,9,60,62}

The film sample showed two well resolved bands at 521 and 483 cm^{-1} . We attribute the film sample medium band at 521 cm^{-1} to the $\tau(\text{NH}_3^+)$ torsional mode and the medium band at 483 cm^{-1} to the $\rho(\text{CO}_2^-)$ rocking mode. This assign-

ment is supported by additional hydrogen-deuterium exchange and low temperature experiments (see supplementary material).⁵⁸ A 31 cm^{-1} downward shift of the band at 521 cm^{-1} was indeed observed after deuteration. Moreover, by cooling the film sample to 14 K, a 12 cm^{-1} shift of the band at 521 cm^{-1} toward higher frequencies was observed, whereas the band at 483 cm^{-1} shifted only by 2 cm^{-1} in the same direction. This is in agreement with previous results^{2,62} showing a strong temperature dependence of the $\tau(\text{NH}_3^+)$ vibration as opposed to the carboxylate deformation, and in contradiction with Murli *et al.*⁹

The powder sample displayed a broad band at 500 cm^{-1} with a shoulder at 523 cm^{-1} . In other studies where samples were cooled to liquid nitrogen temperature, splitting of the broad band at 500 cm^{-1} occurred and two bands centered at 493 and 520 cm^{-1} became clearly visible.^{9,62} Fairheller *et al.*⁶² assigned the latter band to the $\tau(\text{NH}_3^+)$ mode while Murli *et al.*⁹ assigned it to the $\rho(\text{CO}_2^-)$ mode. Based on deuteration and temperature effects observed by us on the film sample spectrum, we assign the $\tau(\text{NH}_3^+)$ mode to the shoulder at 523 cm^{-1} and the $\rho(\text{CO}_2^-)$ mode to the band at 500 cm^{-1} .

In the $450\text{--}250\text{ cm}^{-1}$ range, only one medium broad band at 352 cm^{-1} was observed for the glycine powder sample and is assigned to the $\delta(\text{NC}\alpha\text{C})$ backbone bending mode.^{8,61} The glycine pH 6.6 film displayed two shouldered bands centered at 377 and 354 cm^{-1} . The relative intensity of these two bands had a strong pH-dependence (see Fig. 1). An additional band at 335 cm^{-1} was seen at pH 3.0 (shoulder) and pH 2.1. We note that at pH values close to the glycine $\text{CO}_2\text{H}/\text{CO}_2^-$ pKa value of 2.35,⁶³ zwitterionic and cationic forms are in equilibrium and mixed spectra are expected. We assign both bands observed for the pH 6.6 film spectrum at 377 and 354 cm^{-1} to the $\delta(\text{NC}\alpha\text{C})$ mode.⁶⁰

Spectra of powder and film samples presented significant differences below 250 cm^{-1} . In this region, we observed for the powder sample a very broad massif containing shoulders at 227 and 194 cm^{-1} and bands at 165 , 134 , and 62 cm^{-1} . Four thin and well defined bands centered at 207 , 181 , 153 , and 61 cm^{-1} were observed for the film sample. Based on our calculations, we assign all these bands to lattice modes with the exception of bands at 165 cm^{-1} (powder) and 153 cm^{-1} (film) which are attributed to the $\tau(\text{CO}_2^-)$ torsional mode. The band at 181 cm^{-1} (film) was previously assigned to the $\tau(\text{CO}_2^-)$ mode by Goryainov *et al.*,⁶⁰ while the band at 134 cm^{-1} (powder) was assigned to hydrogen bond $\text{N}\text{--}\text{H}\cdots\text{O}$ elongation vibration by Matei *et al.*⁸ and Fairheller *et al.*⁶² This latter attribution is not incompatible with our results since hydrogen bond parameters were not included in the set of nonredundant symmetry coordinates employed. Visual examination of the modes attributed to the band at 134 cm^{-1} indeed shows important contribution to hydrogen bond elongations. We note a relatively good correspondence of experimental and calculated spectra overall shape below 250 cm^{-1} for both forms. This is in favor of the attribution of powder and film samples to glycine α and β polymorphs, respectively.

Cation. The spectrum of the film sample at pH 1.1 is taken as reference for the analysis of the glycine cationic form. As mentioned above, intermediate spectra observed at

pH 2.1 and 3.0 suggest that a mixture of zwitterionic and cationic forms are present at these pH values. Surprisingly, the film sample at pH 2.1 and zwitterion powder sample spectra present some similarities below 250 cm^{-1} . It is unclear whether this resemblance is fortuitous or results from a change in polymorphic state of the zwitterionic part of the mixture. The cationic form displayed bands at 638 and 497 cm^{-1} , which are, respectively, assigned to the $\delta(\text{CO}_2\text{H})$ and $\rho(\text{CO}_2\text{H})$ modes. The $\tau(\text{NH}_3^+)$ mode is predicted to have a frequency around 460 cm^{-1} and to be coupled to the $\nu_a(\text{Cl}\text{--}\text{H}(\text{N}))$ chloride-ammonium hydrogen antisymmetric stretching mode by calculations, however, no experimental band is visible in this region. By analogy with the frequency overestimation of the $\tau(\text{NH}_3^+)$ mode noticed in glycine zwitterion calculations, we propose that this mode corresponds to the weak experimental band near 375 cm^{-1} , to the left of the broad peak centered at 296 cm^{-1} . This latter band is assigned to the $\delta(\text{NC}\alpha\text{C})$ vibration. Low frequency peaks at 166 , 148 , and 89 cm^{-1} are tentatively attributed to a rotational/ $\nu(\text{Cl}\text{--}\text{H}(\text{O}))$ chloride-carboxylic acid hydrogen stretching mode, a $\tau(\text{CO}_2\text{H})/\tau(\text{NH}_3^+)$ mode, and a rotational mode, respectively.

Anion. FIR spectra of the glycine anionic form were measured at pH 10.2, 11.1, 12.1, and 13.0. Principal changes between these spectra were localized below 250 cm^{-1} . The very broad band centered at 202 cm^{-1} at pH 10.2 became refined at pH 13.0 and other maxima appeared. The spectrum of the film sample at pH 13.0 is taken as reference for the analysis of the glycine anionic form. Compared to zwitterionic and cationic forms, glycine anionic form spectrum contained more peaks in the $700\text{--}50\text{ cm}^{-1}$ range, probably due to more coupling of modes involving the alkaline cation in this region. Bands at 677 and 580 cm^{-1} are assigned to the $\delta(\text{CO}_2^-)$ and $\omega(\text{CO}_2^-)$ modes, respectively. A medium band at 513 cm^{-1} and two weak bands at 446 and 376 cm^{-1} are then observed. We tentatively attribute them to modes implicating the alkaline cation, namely, the $\nu(\text{ONa}^+)$ carboxylate oxygen-sodium ion stretching mode, the rotational/ $\delta(\text{CONa}^+)$ carboxylate-sodium ion bending mode, and the rotational/ $\tau(\text{CONa}^+)$ carboxylate-sodium ion torsional mode, respectively. The band at 311 cm^{-1} is assigned to a $\delta(\text{NC}\alpha\text{C})/\rho(\text{CO}_2^-)$ mode. Anticipating a frequency shift for the $\tau(\text{NH}_2)$ amine torsional mode, by analogy with the frequency overestimation observed for glycine cation and zwitterion ammonium torsional modes, we propose that the $\tau(\text{NH}_2)$ mode corresponds to the 256 cm^{-1} shoulder band. The band at 211 cm^{-1} has a very strong infrared intensity, which appears significantly underestimated in the simulated spectrum. We assign this band to a translational/ $\delta(\text{CONa}^+)$ mode, based on cation replacement experiment. We indeed observed a 68 cm^{-1} shift of the 211 cm^{-1} band toward lower wavenumbers when KOH was employed instead of NaOH (see supplementary material).⁵⁸ This frequency shift can be explained by the mass increase in the cation coordinated to the carboxylate group when switching from Na^+ to K^+ . Remaining bands at 163 , 131 , and 114 cm^{-1} are tentatively assigned to the $\tau(\text{CONa}^+)$ mode, a translational/rotational mode, and a $\tau(\text{CO}_2^-)$ /rotational mode.

It is interesting to note that calculated spectra of glycine

in different protonation states managed to identify several changes observed in experimental spectra as a function of pH. For example, going from the low pH of 1.1 to neutral pH and then to the high pH of 13.0, calculations reproduced well the shift of the peak attributed to the $\delta(\text{NC}\alpha\text{C})$ vibration, from 296 to 352 and then to 311 cm^{-1} . In the low pH spectra, no peaks were attributed in the range of 400 cm^{-1} and no calculated peaks were identified. In the high pH spectra, both experimental and theoretical peaks were present at 376 and 446 cm^{-1} . Finally, the low pH peak at 638 cm^{-1} was identified by the calculations. At high pH, this peak is not observed, however a peak at high frequency (677 cm^{-1}) appears, which is well reproduced by the calculations. This coherence with experimental data as a function of pH supports the utility of these calculations for peak attribution and suggests that calculations at this level can be used for predictive purposes.

B. L-alanine

L-alanine is the simplest chiral amino acid. Far infrared spectra measured for the powder sample and homogeneous films obtained from aqueous solutions at different pH are presented in Fig. 2. Based on spectra comparison as a function of pH, we attribute the 2.9–10.3 pH range to the zwitterionic form, the 1.1–2.0 pH range to the cationic form, and the 10.9–12.9 range to the anionic form.

Zwitterion. L-alanine spectra measured from the dried solution prepared at pH 5 and from the powder sample presented some differences, in particular below 200 cm^{-1} . There is only one known polymorphic state for the L-alanine zwitterionic crystal and limited variations in available crystallographic structures. Differences in the lowest frequency spectrum between powder and film samples could arise from changes in the size and quality of crystals at the microscopic level between the two forms. In the study of Rungsawang *et al.*⁶⁴ which applied angle-dependent terahertz time-domain spectroscopy to L-cysteine and L-histidine, such differences are explained by additional hydrogen bond peaks in the film spectrum as a result of a larger hydrogen-bond network in comparison to powder sample.

In the 700–450 cm^{-1} range we observed a medium band at 648 cm^{-1} , which is attributed by us to a $\delta(\text{CO}_2^-)/\delta(\text{NC}\alpha\text{C})$ coupled mode, but a $\rho(\text{CO}_2^-)$ rocking mode by Matei *et al.*⁸ and Chowdhry *et al.*,³⁹ or to a $\omega(\text{CO}_2^-)$ wagging mode by Herlinger *et al.*⁶¹ and Wang *et al.*⁶⁵ A strong band at 539 cm^{-1} is attributed to the $\rho(\text{CO}_2^-)$ vibration. As for glycine, the frequency of the $\tau(\text{NH}_3^+)$ mode was overestimated by our calculations, around 570 cm^{-1} , while a weak experimental band centered at 486 cm^{-1} did not fit with calculations. In previous reports the $\tau(\text{NH}_3^+)$ vibrational mode was attributed to the latter band.^{8,39,61,65,66} The 450–250 cm^{-1} range showed a strong band at 409 cm^{-1} , which is attributed to the $\delta(\text{NC}\alpha\text{C}\beta)$ bending mode,^{61,65} medium bands at 324 and 292 cm^{-1} are both assigned to a $\delta(\text{NC}\alpha\text{C})/\rho(\text{CO}_2^-)$ coupled mode, the medium band at 278 cm^{-1} is attributed to the $\delta(\text{CC}\alpha\text{C}\beta)$ bending mode, and the weak band at 259 cm^{-1} to the $\tau(\text{C}\beta\text{H}_3)$ torsional mode. This latter mode was assigned to the band at 292 cm^{-1} ⁶⁶ or

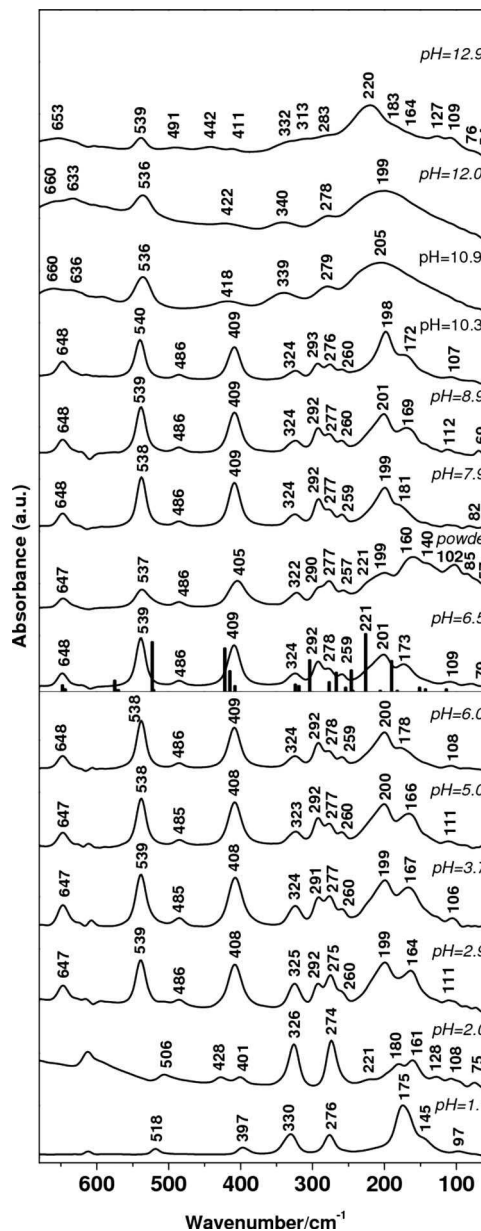


FIG. 2. Far infrared spectra of L-alanine powder sample and dried film samples prepared from solutions at different pH. Major experimental peaks are labeled. Calculated peaks for the L-alanine zwitterion are superposed on the pH 6.5 experimental spectrum.

at 278 cm^{-1} ^{8,67} by previous works. Below 250 cm^{-1} , some changes appeared between zwitterionic samples obtained from powder and dried film prepared at pH 6.5. Bands at 221 and 201 cm^{-1} were common to both samples and are assigned to lattice rotational modes. The film band at 173 cm^{-1} is attributed to the $\tau(\text{CO}_2^-)$ mode and probably corresponds to the powder band at 160 cm^{-1} . The powder sample further displayed absorptions at 140, 102, 85, and 57 cm^{-1} , whereas the film sample showed signals at 109 and 79 cm^{-1} . These latter absorptions are assigned to lattice modes.

Cation and anion. FIR spectra of L-alanine cationic form could be observed at pH 2.0 and 1.1. The spectrum of the film sample at pH 1.1 is taken as reference for the analysis of the L-alanine cationic form. The main difference at pH

1.1 was the formation of a thin and very strong band at 175 cm^{-1} . We tentatively assign this strong band at 175 cm^{-1} to the $\tau(\text{CO}_2\text{H})$ mode and the signal at 518 cm^{-1} to the $\rho(\text{CO}_2\text{H})$ vibrational mode.

We observed the spectra of the L-alanine anionic form at $\text{pH} \geq 10.9$. Changes in the spectrum between $\text{pH} 10.9$ and 12.9 were moderate and mostly significant below 250 cm^{-1} . In particular, the strong and broad band around 200 cm^{-1} observed at $\text{pH} 10.9$ was sharpened at $\text{pH} 12.9$. The spectrum of the film sample at $\text{pH} 12.9$ is taken as reference for the analysis of the L-alanine anionic form. The medium band at 539 cm^{-1} is tentatively assigned to the $\rho(\text{CO}_2^-)$ mode and the strong band at 220 cm^{-1} to the $\tau(\text{CO}_2^-)$ mode.

C. L-valine

L-valine far infrared spectra measured for the powder sample and homogeneous films obtained from aqueous solutions at different pH are presented in Fig. 3. Based on spectra comparison as a function of pH, we attribute the 2.9–10.1 pH range to the zwitterionic form, the 1.0–2.0 pH range to the cationic form, and the 10.9–12.9 range to the anionic form.

Zwitterion. L-valine spectra measured from the dried solution prepared at $\text{pH} 6.3$ and from the powder sample are very similar. While for the glycine and L-alanine spectra discussed before, where film and powder samples showed some differences, the L-valine data were identical even at low wavenumbers. Only some changes on relative intensities have been observed. The presence of two L-valine molecules in the crystal asymmetric unit, each having a distinct conformation of the $\text{C}\alpha\text{-C}\beta$ torsion angle, complicates the analysis of modes at the single molecule level and gives rise to a more complicated spectrum with duplication of peaks.

As it was seen for glycine and L-alanine, the $700\text{--}450\text{ cm}^{-1}$ region is typical of CO_2^- bending, rocking, and wagging vibrations, and NH_3^+ torsion vibration. In the case of L-valine, contributions of an additional vibrational mode appeared. We assign the medium band at 663 cm^{-1} to a $\delta(\text{NC}\alpha\text{C})/\delta(\text{CO}_2^-)/\omega(\text{CO}_2^-)$ mode. Vibrational coupling is strong here and respective contributions of the $\delta(\text{NC}\alpha\text{C})$, $\delta(\text{CO}_2^-)$, and $\omega(\text{CO}_2^-)$ vibrations are only 18%, 16%, and 16%, respectively. The strong band at 540 cm^{-1} is assigned to the $\rho(\text{CO}_2^-)$ mode. This band was also attributed to the $\rho(\text{CO}_2^-)$ mode in previous works.^{7,61,68} The position of the $\tau(\text{NH}_3^+)$ mode of L-valine, as for smaller aliphatic amino acids, is not correctly predicted by calculations. We propose that the weak band observed experimentally at 495 cm^{-1} and not predicted by the calculations corresponds to the $\tau(\text{NH}_3^+)$ vibration. This latter vibration was assigned to the band at 472 cm^{-1} by Lima *et al.*,⁷ while we attribute the weak band at 472 cm^{-1} to the $\delta(\text{C}\alpha\text{C}\beta\text{C}\gamma)$ sidechain bending mode. In the $450\text{--}250\text{ cm}^{-1}$ range, major contributions were skeletal bending modes. This result is in agreement with other works.^{7,61,68} Bands at 440 and 427 cm^{-1} are attributed to the $\delta(\text{NC}\alpha\text{C}\beta)$ mode, the medium peak at 399 cm^{-1} to the $\delta(\text{C}\gamma\text{C}\beta\text{C}\gamma)$ mode, bands at 374 and 333 cm^{-1} to the $\delta(\text{C}\alpha\text{C}\beta\text{C}\gamma)$ mode, the medium peak at 292 cm^{-1} to the $\delta(\text{NC}\alpha\text{C})$ vibration coupled to the $\rho(\text{CO}_2^-)$ mode, and the medium peak at 279 cm^{-1} to the $\delta(\text{CC}\alpha\text{C}\beta)$ mode. In the

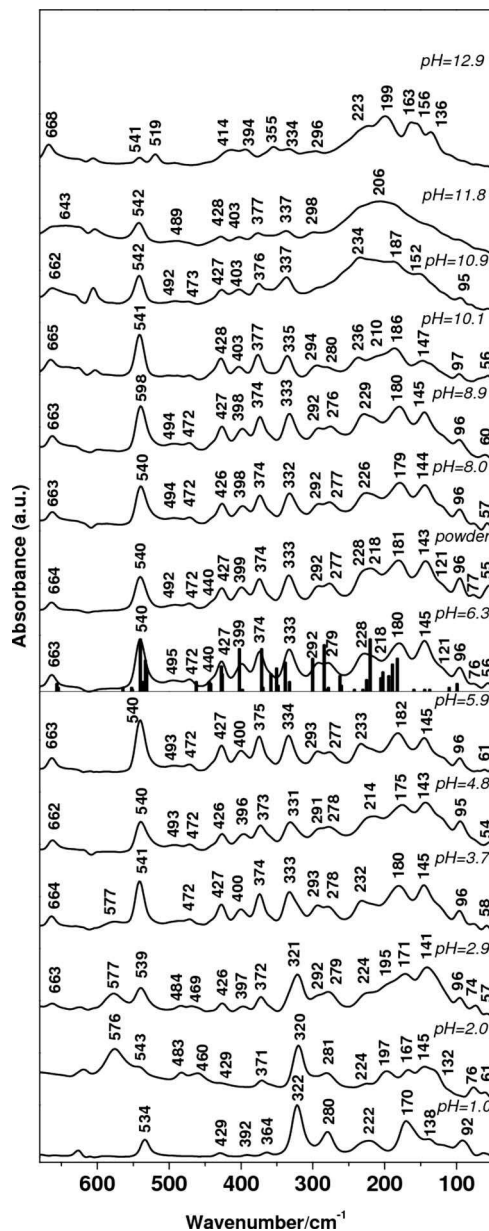


FIG. 3. Far infrared spectra of L-valine powder sample and dried film samples prepared from solutions at different pH. Major experimental peaks are labeled. Calculated peaks for the L-valine zwitterion are superposed on the $\text{pH} 6.3$ experimental spectrum.

low frequency region, signals at 228 , 218 , 180 , and 145 cm^{-1} are tentatively attributed to $\tau(\text{CO}_2^-)$, $\tau(\text{C}\alpha\text{C}\beta)/\tau(\text{C}\gamma\text{H}_3)$, $\tau(\text{C}\gamma\text{H}_3)/\text{rot}$, and $\tau(\text{C}\alpha\text{C}\beta)$ torsional modes, respectively. Remaining low frequency modes are attributed to lattice modes.

Cation and anion. FIR spectra of the L-valine cationic form were measured for $\text{pH} \leq 2.9$. The spectrum obtained from the solution at $\text{pH} 2.9$ showed some overlap of the bands of cationic and zwitterionic forms. Significant changes in the absorptions are observed at lower pH. The spectrum of the film sample at $\text{pH} 1.0$ is taken as reference for the analysis of the L-valine cationic form. We tentatively attribute the band at 534 cm^{-1} to the $\rho(\text{CO}_2\text{H})$ mode and the band at 170 cm^{-1} to the $\tau(\text{CO}_2\text{H})$ vibrational mode.

Spectra of the L-valine anionic form were observed at

pH ≥ 10.9 . Comparing spectra of the anionic form at pH 10.9, 11.8, and 12.9, we noticed several differences including the formation of a band centered at 519 cm^{-1} at higher pH. While we observed a strong and broad band at pH 10.9 and 11.8, a fine structure appeared at pH=12.9 below 280 cm^{-1} . The spectrum of the film sample at pH 12.9 is taken as a reference for the analysis of the L-valine anionic form. The most intense band of this spectrum was centered at 199 cm^{-1} and is tentatively assigned to the $\tau(\text{CO}_2^-)$ vibrational mode. We tentatively assign the band at 541 cm^{-1} to the $\rho(\text{CO}_2^-)$ mode.

D. L-leucine

L-leucine far infrared spectra measured for the powder and film samples obtained from aqueous solutions at different pH are presented in Fig. 4. Based on spectra comparison as a function of pH, we attribute the 3.0–10.2 pH range to the zwitterionic form, the 1.0–2.0 pH range to the cationic form, and the 10.9–13.0 range to the anionic form.

Zwitterion. Without considering the relative intensity of bands, the spectra of L-leucine powder sample and film sample prepared at pH 6.7 were very similar. As for L-valine, the presence of two molecules in the asymmetric unit complicates the calculated spectrum and the analysis.

In the $700\text{--}480\text{ cm}^{-1}$ range we observed two intense bands at 668 and 534 cm^{-1} , which we assign to $\delta(\text{CO}_2^-)$ and $\rho(\text{CO}_2^-)$ vibrational modes, respectively. Whereas the same attribution has been proposed by Façanha *et al.*⁵ and Herlinger *et al.*⁶¹ for the band at 534 cm^{-1} , they assigned the band at 668 cm^{-1} to the $\omega(\text{CO}_2^-)$ mode. For smaller aliphatic amino acids, this domain also contained the $\tau(\text{NH}_3^+)$ mode, whose calculated frequency was generally too high. Although no other major bands could be observed in the FIR spectra of the L-leucine zwitterion, a careful examination shows that a very weak band/shoulder is present around $507\text{--}502\text{ cm}^{-1}$, on the low wavenumbers side of the band at 534 cm^{-1} . We assign this very weak band to the $\tau(\text{NH}_3^+)$ torsional mode. The $480\text{--}280\text{ cm}^{-1}$ range displayed bands mostly corresponding to skeletal deformation modes, in agreement with previous studies.^{5,61} The shoulder at 456 cm^{-1} is attributed to the $\delta(\text{C}\delta\text{C}\gamma\text{C}\delta)$ mode, the medium band at 442 cm^{-1} and the strong band at 402 cm^{-1} to the $\delta(\text{C}\beta\text{C}\gamma\text{C}\delta)$ vibration, the medium band at 364 cm^{-1} to a $\delta(\text{NC}\alpha\text{C})/\rho(\text{CO}_2^-)$ coupled mode, and the strong bands at 343 and 330 cm^{-1} to $\delta(\text{C}\delta\text{C}\gamma\text{C}\delta)/\rho(\text{CO}_2^-)/\delta(\text{NC}\alpha\text{C}\beta)$ and $\delta(\text{C}\delta\text{C}\gamma\text{C}\delta)/\delta(\text{NC}\alpha\text{C}\beta)/\delta(\text{C}\beta\text{C}\gamma\text{C}\delta)$ coupled modes, respectively. Based on the hypothesis of a frequency shift between calculated and experimental low frequency bands below 280 cm^{-1} , we tentatively assign the medium band at 223 cm^{-1} to a $\delta(\text{C}\alpha\text{C}\beta\text{C}\gamma)$ /rotational mode, the strong band at 203 cm^{-1} to the $\tau(\text{C}\delta\text{H}_3)$ torsional mode, the medium peak at 170 cm^{-1} to the $\tau(\text{CO}_2^-)$ mode, the shoulder at 138 cm^{-1} to a $\tau(\text{CO}_2^-)/\tau(\text{C}\alpha\text{C}\beta)$ coupled mode, the strong band at 122 cm^{-1} to a rotational/ $\tau(\text{CO}_2^-)/\tau(\text{C}\beta\text{C}\gamma)$ coupled mode, and the shoulder at 107 cm^{-1} to the $\tau(\text{C}\beta\text{C}\gamma)$ torsional mode. We note that the strong intensity of the band at

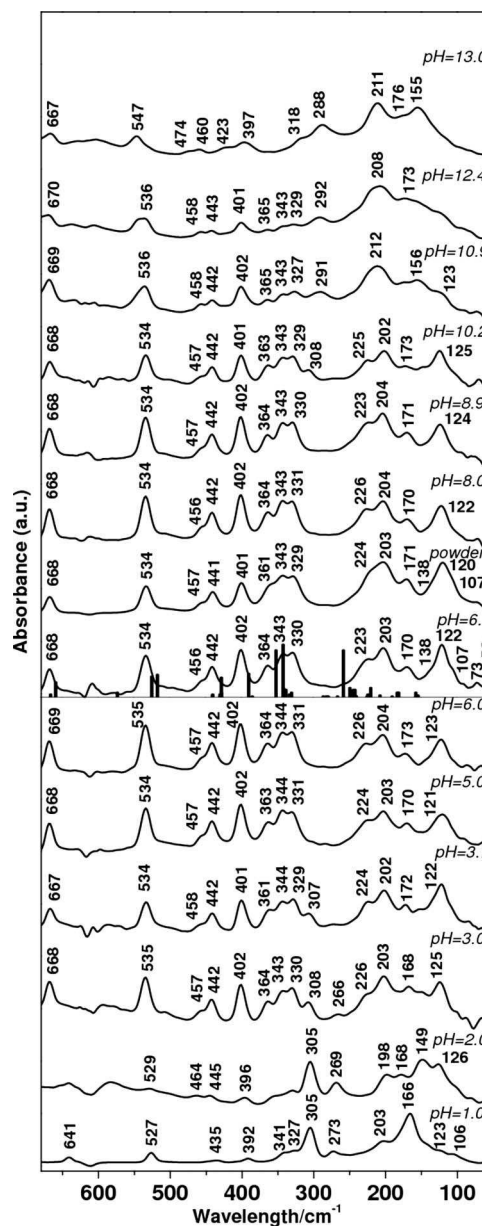


FIG. 4. Far infrared spectra of L-leucine powder sample and dried film samples prepared from solutions at different pH. Major experimental peaks are labeled. Calculated peaks for the L-leucine zwitterion are superposed on the pH 6.7 experimental spectrum.

122 cm^{-1} relative to its neighbors is not well reflected in calculations. Remaining bands are attributed to lattice modes.

Cation and anion. Spectra of the L-leucine cationic form were observed at $\text{pH} \leq 2.0$. At pH 3.0 and 3.7 the main form was zwitterionic, although a band centered around 305 cm^{-1} seems to arise from the cationic form. The spectrum of the film sample at pH 1.0 is taken as reference for the analysis of the L-leucine cationic form. We tentatively assign the bands at 527 and 166 cm^{-1} to the $\rho(\text{CO}_2\text{H})$ and $\tau(\text{CO}_2\text{H})$ vibrational modes, respectively.

FIR spectra of the L-leucine anionic form were observed at $\text{pH} \geq 10.9$. A band observed at 308 cm^{-1} in the pH 10.2 spectrum could also reveal the presence of the anion at this pH. This band shifted at higher pH. We consider the spec-

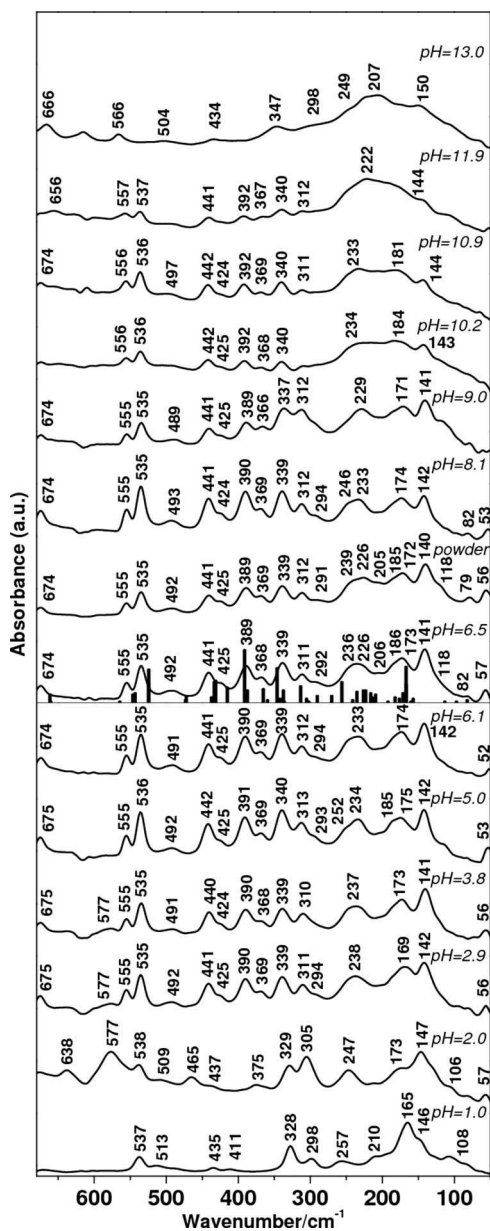


FIG. 5. Far infrared spectra of L-isoleucine powder sample and dried film samples prepared from solutions at different pH. Major experimental peaks are labeled. Calculated peaks for the L-isoleucine zwitterion are superposed on the pH 6.5 experimental spectrum.

trum of the film sample at pH 13.0 for the analysis of the L-leucine anionic form. Similarly to attributions proposed for smaller aliphatic amino acids, we tentatively assign the band at 547 cm^{-1} to the $\rho(\text{CO}_2^-)$ mode and the strong band at 211 cm^{-1} to the $\tau(\text{CO}_2^-)$ vibrational mode.

E. L-isoleucine

FIR spectra of L-isoleucine measured for the powder and film samples obtained from aqueous solutions at different pH are presented in Fig. 5. Based on spectra comparison as a function of pH, we attribute the 2.9–9.0 pH range to the zwitterionic form, the spectrum recorded at pH 1.0 to the cationic form, and the 10.2–13.0 range to the anionic form.

Zwitterion. Sample preparation did not influence peak position in the FIR spectra of the zwitterionic form except

for relative intensity. The spectra of L-isoleucine powder and film prepared at pH 6.5 were similar. As for L-valine and L-leucine, the presence of two molecules in the asymmetric unit leads to a duplication of peaks and complicates the analysis of modes.

In the $700\text{--}480\text{ cm}^{-1}$ range, we assign the medium bands at 674 and 555, and the strong band at 535 cm^{-1} to $\delta(\text{CO}_2^-)/\delta(\text{NC}\alpha\text{C})$, $\rho(\text{CO}_2^-)/\delta(\text{NC}\alpha\text{C}\beta)$, and $\rho(\text{CO}_2^-)/\nu(\text{C}\alpha\text{C})$ coupled modes, respectively. The weak and broad band observed at 492 cm^{-1} was generally assigned in previous studies to a $\delta(\text{CCC})$ skeletal mode,^{6,61,69} but to the $\tau(\text{NH}_3^+)$ torsional mode by Matei *et al.*⁸ Our calculations predicted the position of the $\tau(\text{NH}_3^+)$ mode around 560 cm^{-1} but we have seen for other aliphatic amino acids that the frequency of this mode was systematically overestimated. On the other hand, a peak corresponding to the $\delta(\text{C}\alpha\text{C}\beta\text{C}\gamma_2)$ is predicted by our calculations around 473 cm^{-1} , close to 492 cm^{-1} . Additional data would be needed here to propose a definitive assignment between the $\tau(\text{NH}_3^+)$ mode and the $\delta(\text{C}\alpha\text{C}\beta\text{C}\gamma_2)$ mode for the weak band at 492 cm^{-1} experimental value. The $480\text{--}280\text{ cm}^{-1}$ region of the spectrum showed a particular pattern with three strong bands each flanked by a medium band on the low wavenumber side. These absorptions mostly correspond to skeletal bending modes, in agreement with previous studies.^{6,61,69} The strong band at 441 cm^{-1} is assigned to the $\delta(\text{C}\gamma\text{C}\beta\text{C}\gamma)$ mode, the medium band at 425 cm^{-1} to the $\delta(\text{C}\alpha\text{C}\beta\text{C}\gamma_2)$ mode, the strong band at 389 cm^{-1} to a $\delta(\text{C}\gamma\text{C}\beta\text{C}\gamma)/\delta(\text{NC}\alpha\text{C}\beta)$ coupled mode, the medium band at 368 cm^{-1} to a $\delta(\text{C}\beta\text{C}\gamma_1\text{C}\delta)/\delta(\text{NC}\alpha\text{C}\beta)$ coupled mode, the strong band at 339 cm^{-1} to a $\rho(\text{CO}_2^-)/\delta(\text{NC}\alpha\text{C})$ coupled mode, and the medium peak at 311 cm^{-1} to a $\delta(\text{NC}\alpha\text{C})/\delta(\text{C}\gamma\text{C}\beta\text{C}\gamma)/\delta(\text{NC}\alpha\text{C}\beta)$ coupled mode. Finally, a weak additional shoulder at 292 cm^{-1} is assigned to the $\delta(\text{C}\alpha\text{C}\beta\text{C}\gamma_1)$ mode. Below 280 cm^{-1} , based on the hypothesis of an overestimation of the frequency of rotational modes in the calculations, we attribute the strong band at 236 cm^{-1} to the $\tau(\text{C}\gamma_2\text{H}_3)$ mode, bands at 226 and 206 cm^{-1} to the $\delta(\text{CC}\alpha\text{C}\beta)$ mode, the shoulder at 186 cm^{-1} to the $\tau(\text{CO}_2^-)$ mode, the strong band at 173 cm^{-1} to the $\tau(\text{C}\delta_2\text{H}_3)$, the strong band at 141 cm^{-1} to a rotational mode, and the shoulder at 118 cm^{-1} to the $\tau(\text{C}\beta\text{C}\gamma_1)$ mode. The band at 236 cm^{-1} was also assigned to a $\tau(\text{CH})$ mode by Pawlukoje *et al.*⁶⁹ but the band at 173 cm^{-1} was attributed to $\tau(\text{CO}_2^-)$.^{6,69} Remaining bands at 82 and 57 cm^{-1} are attributed to lattice modes.

Cation and anion. FIR spectra of the L-isoleucine cationic form are found at $\text{pH} \leq 2.0$. We tentatively assign bands of the pH 1.0 cationic spectrum centered at 537 and 165 cm^{-1} to $\rho(\text{CO}_2\text{H})$ and $\tau(\text{CO}_2\text{H})$ vibrational modes, respectively.

Spectra of the L-isoleucine anionic form were observed at $\text{pH} \geq 10.2$. Anionic form FIR spectra of other aliphatic amino acids showed a sharpening of the strong band situated at low wavenumbers when increasing pH. This sharpening was less pronounced for the L-isoleucine anion. We tentatively assign the pH 13.0 anionic spectrum band at 566 cm^{-1} to the $\rho(\text{CO}_2^-)$ mode and the band at 207 cm^{-1} to the $\tau(\text{CO}_2^-)$ vibrational mode.

V. CONCLUSIONS

By means of a combined experimental and theoretical approach, involving far infrared measurements and quantum calculations, an almost complete assignment of low frequency vibrational modes of aliphatic amino acid zwitterions in solid state was presented. Good correspondence between simulated and experimental spectra confirmed that the film samples are in a well organized solid state and, at the same time, validated the theoretical approach. Deviations between theoretical and experimental spectra can arise from uncertainties on the exact microscopic state of experimental samples (polymorphic state, crystallinity, presence of residual water molecules, etc.) and limitations of the theoretical approach (normal modes calculations are performed at 0 K, DFT method limitations, lack of anharmonic effects, etc.). The methodology employed in this work can be employed to study other amino acids, in different physical states and environments. We insist here on the importance of a consistent definition of internal coordinates for the description of normal modes. For example, the definition of the carboxylate wagging mode in the study of Tortonda *et al.*³² corresponds to the carboxylate rocking mode of other works.^{39,54} Such inconsistencies can lead to confusion when comparing results between different authors.

From the complete analysis of far infrared amino acid zwitterion spectra, we could identify three characteristic domains. A first region between 700 and 450 cm^{-1} mostly contained rocking, bending, and wagging vibrations of the carboxylate group as well as the torsional vibration of the ammonium group. The 450–250 cm^{-1} region essentially contained skeletal backbone and sidechain bending deformation modes. Finally, the lowest frequency region, below 250 cm^{-1} , was characteristic of torsional vibrations (carboxylate, methyl groups, and sidechain skeletal torsions) as well as lattice modes.

Difficulties occurred for the correct prediction of the $\tau(\text{NH}_3^+)$ torsional mode, the frequency of this mode being systematically overestimated. It can be noted that the NH_3^+ group of amino acid zwitterions in solid state is particularly involved in intermolecular hydrogen bonds, generally by the three hydrogen atoms. Hydrogen bonds are structural parameters sensible to temperature effects and to the variations in unit cell dimensions of the crystal. Overestimation of the $\tau(\text{NH}_3^+)$ frequency in calculations could be related to a slight underestimation of hydrogen bond distances in optimized amino acid crystals. Chowdhry *et al.*³⁹ proposed that excessive rigidity of the ammonium group in calculations was at the origin of the frequency overestimation.³⁹

Below 250 cm^{-1} , prediction of bands was difficult. Frequency of modes was generally overestimated around 200 cm^{-1} and intensity was significantly underestimated. Correct predictions of modes with translational and rotational components are likely to highly depend on the intermolecular arrangement of molecules and crystal cell dimensions. As for the $\tau(\text{NH}_3^+)$ mode, possible reasons for frequency overestimation include temperature effects and theoretical model limitations. A possible explanation for the important underestimation of low frequency mode intensity

could be the presence of residual water molecules in crystals, which would cause high intensity bands in this region of the spectrum. Another explanation could be the lack of phonon dispersion contributions to lattice modes, as the calculations were done only at the gamma point.⁷⁰ In addition, the analysis of the lowest frequency modes in terms of individual contributions was complicated by important vibrational-rotational couplings. The main origin of such couplings is the network of intermolecular hydrogen bond and ionic interactions, which can couple individual molecule rigid-body degrees of freedom to the vibrational motion of other amino acids in the crystal.

It was more difficult to propose a detailed analysis of cation and anion FIR spectra, due to the lack of available structural data that would be necessary to perform calculations on all of these forms. We nevertheless identified some characteristic features of aliphatic amino acids FIR spectra at extreme pH. At pH values close to 1.0 we typically observed a strong and thin band around 170 cm^{-1} while for pH close to 13.0 we observed a strong and broad band around 210 cm^{-1} .

Additional experimental and theoretical studies are still needed to improve our understanding of amino acid structures and dynamics. Although a certain number of amino acid crystallographic structures is available in the zwitterionic form, systematic studies investigating the pH-dependence of amino acid structural properties, as well as the influence of coordinating ions and environment, would be beneficial. Such fundamental studies on individual amino acids are basic pieces needed to later address the complex vibrational properties of proteins.

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