8280 *J. Phys. Chem. A* **2008,** *112,* 8280–8287

# The Infrared Spectrum of Solid L-Alanine: Influence of pH-Induced Structural Changes

**Ana R. Garcia,[†,‡] Ricardo Brito de Barros,[†] João P Lourenço,[‡] and Laura M. Ilharco*,[†]**

*Centro de Química-Física Molecular, Complexo I, Instituto Superior Técnico, Av. Rovisco Pais, 1, 1049-001 Lisboa, Portugal, and CIQA, Departamento de Química, Bioquímica e Farmácia, FCT, Universidade do Algarve, Campus de Gambelas, 8000 Faro, Portugal*

*Received: March 12, 2008; Revised Manuscript Received: June 23, 2008*

The influence of the pH on the infrared spectrum of L-alanine has been analyzed by diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. The amino acid was precipitated from aqueous solutions and dried at 36.5 °C, in order to stabilize cationic L-alanine or alaninium [$CH_3CH(NH_3^+)COOH$] at pH 1, the zwitterionic form [$CH_3CH(NH_3^+)COO^-$] at pH 6, and anionic L-alanine or alaninate [$CH_3CH(NH_2)COO^-$] at pH 13. New insight on the specific inter and intramolecular interactions in the different forms of L-alanine was reached by a novel methodological approach: an infrared technique not used before to analyze solid amino acid samples (DRIFTS), in combination with a detailed analysis based on spectral deconvolution. The frequency ranges of interest include the carbonyl/carboxyl stretching and amine deformation modes and the OH/NH stretching modes. It was shown that intermolecular hydrogen bonds between the $NH_3^+$ and $COO^-$ groups are predominant in the zwitterionic form, whereas in cationic L-alanine, H bonds between the COOH groups are responsible for the formation of dimers. In anionic L-alanine, only strong electrostatic interactions between the $COO^-$ groups and $Na^+$ ions are proposed, evidencing the relevant role of the counterion.

## 1. Introduction

L-alanine is the simplest chiral amino acid present in nature and is therefore an excellent model for understanding general structural and chemical properties of amino acids. Depending on the medium, the L-alanine molecule can be stabilized in different forms, schematized in Figure 1: neutral [$CH_3CH(NH_2)$-COOH], zwitterionic [$CH_3CH(NH_3^+)COO^-$], cationic or alaninum ion [$CH_3CH(NH_3^+)COOH$], and anionic or alaninate ion [$CH_3CH(NH_2)COO^-$].

The neutral form is observed in the gas phase and in inert matrix and, as a result of its flexibility, occurs in a large number of rotational conformers, the geometries of which were assessed by ab initio quantum chemical calculations[1] and by density functional theory (DFT).[2,3] Some of the conformers are stabilized by intramolecular hydrogen bonds, namely, the most stable, where a hydrogen bond is formed between the carbonyl oxygen and one amine hydrogen.[4,5] The preferred conformational structures were also characterized by millimeter-wave spectroscopy[6] and more recently by laser-ablation molecular-beam Fourier transform microwave spectroscopy (LA-MB-FTMW).[7]

The zwitterionic form is predominant in the crystalline state and in aqueous solutions in a wide pH range.[8,9] The crystalline structure, formed by hydrogen-bonded columns of molecules, was determined by X-ray diffraction[10] and by neutron diffraction[11] as orthorhombic, with four molecules per unit cell. The influence of the interactions between zwitterionic L-alanine (among other amino acids) and hydrogen bond acceptors and donors on the conformational stability has been emphasized by ab initio methods.[12]

The stabilities of the cationic and anionic forms in aqueous solution depend on the pH, because L-alanine has two acid−base equilibria, with p$K_a$ 2.35 and 9.87, at 25 °C,[13] respectively:

$$CH_3CH(NH_3^+)COOH \rightleftarrows CH_3CH(NH_3^+)COO^- + H^+ \quad (1)$$

$$CH_3CH(NH_3^+)COO^- \rightleftarrows CH_3CH(NH_2)COO^- + H^+ \quad (2)$$

Taking into account the p$K_a$ values and overlooking the buffer effect, simple calculations show that, at 25 °C, above pH 11, more than 90% of L-alanine molecules in aqueous solution are anionic, whereas below pH 1.5, more than 90% are in the cationic form. Between pH 4.5 and 7.5, L-alanine is almost fully zwitterionic (more than 99%). In solid phase, the cationic form has been stabilized as mono-L-alaninium nitrate, by drying a solution of L-alanine in nitric acid. A network of hydrogen bonds between alaninium cations and $NO_3^-$ anions results in an orthorhombic structure.[14] The anionic form of L-alanine has also been stabilized by adsorption from the gas phase onto the clean Cu(110) surface[15–17] and onto evaporated copper films,[18] as well as by adsorption on Ag colloidal particles in an aqueous phase.[19]

Vibrational spectroscopy, among other techniques, has played a determining role in characterizing the different forms of L-alanine, mainly through the spectral features associated with the two functional groups ($NH_2$/$NH_3^+$ and COOH/$COO^-$) and through modifications induced by isotope effects.[20–22] This is a complex task because of the large overlap of frequencies characteristic of those groups and also because of overtone and combination bands. For neutral L-alanine, a successful correlation between the mode frequencies calculated by DFT for the different conformers and the values obtained in matrix-isolation infrared studies was achieved.[5] For zwitterionic L-alanine, it was shown that the three N−H bonds may become inequivalent because of intermolecular $NH_3^+$/$COO^-$ hydrogen bonds of unequal strength.[10,23] The different types of H bonds in polycrystalline L-alanine have been identified by making use of isotopic shifts and temperature dependence of the FTIR spectra.[24] Although the vibrational spectrum of zwitterionic L-alanine is not much affected by the medium polarity,[25] it was shown that, in aqueous solution, it is sensitive to the arrangement of the neighboring water molecules, shifting with respect to the

---

* Corresponding author. Tel: +351-218419220. Fax: +351-218464455. E-mail: lilharco@ist.utl.pt.

[†] Instituto Superior Técnico.

[‡] Universidade do Algarve.

10.1021/jp802170n CCC: $40.75

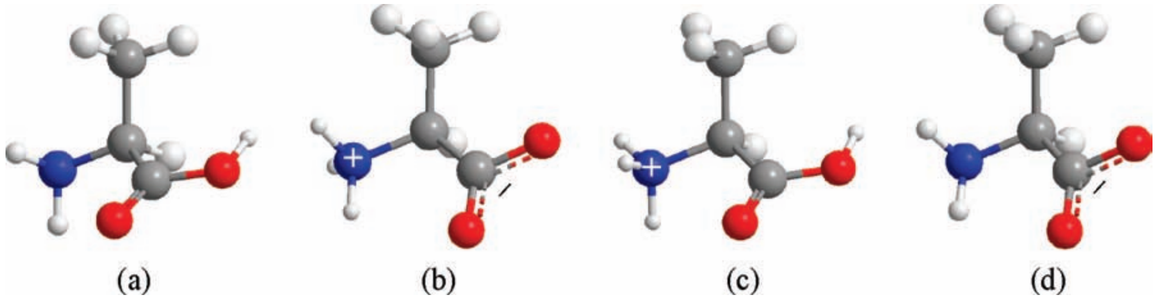

**Figure 1.** Ball and stick models of the different forms of L-alanine, optimized by energy minimization (Chem 3D Ultra 8.0.3). (a) nonionized, (b) zwitterionic, (c) cationic, (d) anionic. Red, oxygen; blue, nitrogen; dark gray, carbon; light gray, hydrogen.

neutral gas and solid phases.[26,27] This effect is stronger for groups involved in specific interactions, namely, in the N−H stretching modes that undergo a downshift of ~500 cm$^{-1}$ with respect to those of the free monomer. Additionally, theoretical data point to one amino hydrogen being involved in an intramolecular H bond, whereas the other two interact with water molecules.[28] Vibrational circular dichroism has been extremely useful to clarify the interpretation of the N−H and C−H stretching regions of solid state D and L-alanine.[20,29,30]

Most of the work published on the vibrational modes of L-alanine regards neutral and zwitteronic forms, under different approaches: (i) theoretical calculations by ab initio and DFT methods,[28] (ii) experimental studies on isolated molecules in Ar matrix at low temperature,[31] (iii) infrared and Raman spectra of L-alanine oriented single crystals,[21,32] and (iv) reflection−absorption spectra of alanine adsorbed onto clean and well-defined metal single crystal surfaces, prepared in ultrahigh-vacuum environments, such as Cu(110).[15] The vibrational spectra of the ionic forms of L-alanine have not received as much attention. Anionic L-alanine in solution was analyzed by polarized Raman spectroscopy, in comparison with other amino acids of longer alkyl chains,[33] and adsorbed on Cu(110) as a function of coverage and temperature, by RAIRS.[34] FTIR and FT Raman analysis of solid salts, such as L-alaninium nitrate[14] and $\beta$-alanine $\beta$-alaninium picrate crystals,[35] allowed the identification of an extensive network of intermolecular hydrogen bonding.

In the present work, the diffuse reflectance infrared Fourier transform (DRIFT) spectra of L-alanine in the mid-infrared region are analyzed for solid samples obtained by solvent evaporation from aqueous solutions prepared at different pH values (1, 6 and 13). These were chosen in order to stabilize different L-alanine forms in solution (cationic, zwitterionic, and anionic), which are retained in the resulting dry solid phase according to the present DRIFT data, similarly to what occurs with other amino acids, namely, glycine.[36] The spectral deconvolution in the regions corresponding to the carboxylate/carbonyl stretching modes and amine deformations and to the amine and hydroxyl stretching modes allowed concluding on the predominant interactions in the different forms of the solid amino acid.

## 2. Experimental Section

Aqueous solutions (~0.1 M) of L-alanine (Aldrich, 99%) were prepared at pH 1, 6, and 13, by using as solvent a 0.15 M solution of HCl (Aldrich Fixanal 0.5 M), bidistilled water, and a 0.15 M solution of NaOH (Aldrich, 97+%), respectively. They were kept at 36.5 °C in an incubator with continuous orbital stirring (100 rpm) until complete solvent evaporation (~96 h). The residual water was subsequently removed, at the same temperature, in a vacuum oven (at ~10$^{-4}$ mbar, for 24 h).

Because the dried L-alanine samples were visually different, ranging from needle-shaped crystals (pH 1) to a fine powder (pH 6) and a gel-like solid (pH 13), they were analyzed by X-Ray diffraction. The powder XRD patterns were recorded on a Panalytical X'Pert Pro diffractometer by using Cu K$\alpha$ radiation filtered by Ni, a X'Celerator detector, and a zero background silicon substrate as sample holder. Several diffractograms were recorded for each sample, with different grinding degrees.

The molecular structures of the different samples as well as that of untreated L-alanine were analyzed by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). The sample preparation consisted simply of grinding a mixture of KBr (Sigma-Aldrich, FTIR grade) and each sample powder, in appropriate weight proportions to obtain spectral absorbance in the range of applicability of the Kubelka−Munk transformation.[37] The amount of sample in the sample holder was consistent with infinite thickness. The DRIFT spectra were collected at 4 cm$^{-1}$ resolution, with a Mattson Research Series 1 FTIR spectrometer, by using a wide-band MCT detector (4000−400 cm$^{-1}$) and a Graseby/Specac Selector (with specular reflection blocker). The spectra were the ratio of 500 single beam scans of the sample to the same number of background scans for pure KBr. No baseline corrections were made. The diffuse reflectance spectra were transformed to Kubelka−Munk units.[37]

## 3. Results and Discussion

The DRIFT spectra of solid L-alanine, untreated and after precipitation from aqueous solutions at different pH values, are shown in Figure 2A,B for the 2000−4000 and 450−1900 cm$^{-1}$ wavenumber regions, respectively. The proposed band assignments resulting from a brief spectral analysis are summarized in Table 1. Numerous references were considered, because there is no general agreement in the literature. As mentioned above, this is a consequence of band overlapping, mode mixing, overtones, and Fermi resonance bands, which were taken into account.

The spectra of L-alanine dried from a pH 6 solution and untreated are similar, with the characteristic pattern of the zwitterionic form [$CH_3CH(NH_3^+)COO^-$]:[9,38] the strong bands associated with $NH_3^+$ fundamental modes (stretching at 3080 and 2988 cm$^{-1}$ and antisymmetric deformation at 1590 cm$^{-1}$) and with the $\nu_{as}OCO^-$ and $\nu_sOCO^-$ modes, at 1620 and 1360 cm$^{-1}$, respectively. The medium intensity modes of $NH_3^+$ ($\delta_s$, $\rho$, and $\tau$ at 1519/1507, 1237, and 486 cm$^{-1}$, respectively) are additional confirmations.[23] The downward shift and broadness of the $\nu NH_3^+$ and $\nu OCO^-$ bands with respect to those predicted by Hartree−Fock (HF/SCRF) calculations for monomeric zwitterionic L-alanine suggest significant hydrogen bonding between the amino and carboxylate groups,[5,39] with potential

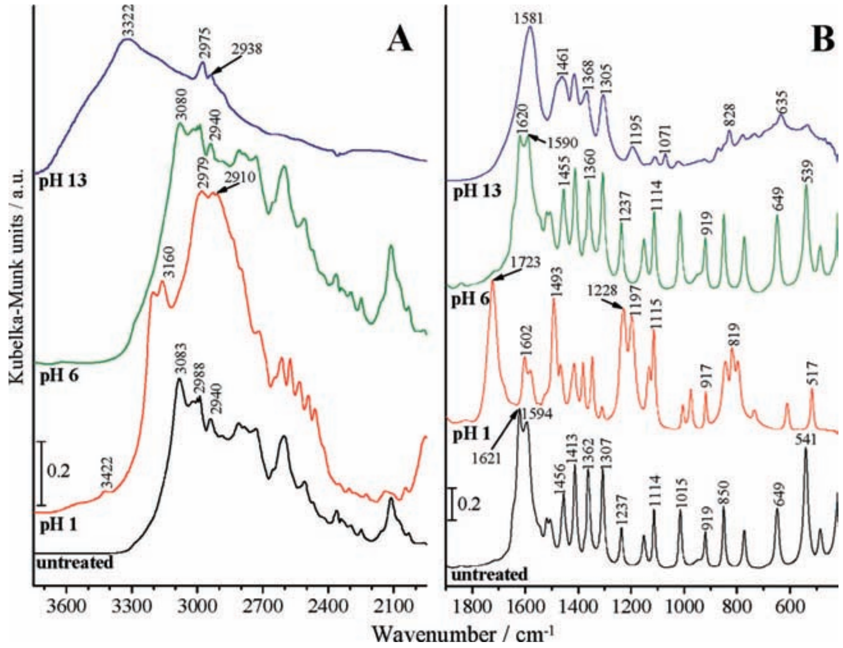

**Figure 2.** DRIFT spectra of untreated L-alanine and after precipitation from aqueous solutions at the indicated pH. The spectra were normalized to the maximum of the low-wavenumber region. The scale factors in regions A and B are different for better visualization.

**TABLE 1: Vibrational Frequencies and Proposed Band Assignments of Untreated L-Alanine and Precipitated from Aqueous Solutions at Different pH Values[a]**

| untreated | pH 1 | pH 6 | pH 13 | assignments[5,14,16,23,28,31,36,38,41,42] |
|---|---|---|---|---|
| | $3422_{(vw)}$ | | | $\nu$OH |
| | | | $3322_{(S)}$ | $\nu NH_2$ |
| | $3203_{(m)}$<br>$3160_{(m)}$ | | | $\nu$OH and$\nu_{as}NH_3^+$ |
| $3083_{(S)}$ | | $3080_{(S)}$ | | $\nu_{as}NH_3^+$ |
| $2988_{(S)}$ | $2979_{(S)}$ | $2988_{(S)}$ | | $\nu_s NH_3^+$ |
| $2940_{(m)}$ | $2910_{(S)}$ | $2940_{(m)}$ | $2975_{(S)}$ | $\nu_{as}CH_3$ |
| $2888_{(sh)}$ | | $2884_{(sh)}$ | $2938_{(m)}$ | $\nu_s CH_3$ |
| 2800−1800 | 2800−1800 | 2800−1800 | | overtones, Fermi resonances, and combination bands |
| | $1723_{(VS)}$ | | | $\nu$C═O (dimers) |
| $1621_{(VS)}$ | | $1620_{(VS)}$ | | $\nu_{as}OCO^-$ |
| $1594_{(VS)}$ | $1602_{(m)}/1582_{(m)}$ | $1590_{(VS)}$ | | $\delta_{as}NH_3^+$ |
| | | | $1581_{(VS)}$ | $\nu_{as}OCO^-/\delta_{sc}NH_2$ |
| $1519_{(w)}/1506_{(w)}$ | $1493_{(VS)}$ | $1519_{(w)}/1507_{(w)}$ | | $\delta_s NH_3^+$ |
| $1456_{(m)}$ | $1468_{(m)}$ | $1455_{(S)}$ | $1461_{(S)}$ | $\delta_{as}CH_3$ |
| $1413_{(S)}$ | $1416_{(m)}$ | $1412_{(S)}$ | $1415_{(S)}$ | $\delta_s CH_3$ |
| | $1382_{(m)}/1346_{(m)}$ | | | Comb. $\nu$C−O and $\delta$OH (dimers) |
| $1362_{(S)}$ | | $1360_{(S)}$ | $1368_{(S)}$ | $\nu_s OCO^-$ |
| $1307_{(S)}$ | $1310_{(w)}$ | $1307_{(S)}$ | $1305_{(S)}$ | $\delta$CH |
| $1237_{(m)}$ | | $1237_{(m)}$ | | $\rho NH_3^+$ |
| | $1228_{(S)}/1197_{(S)}$ | | | $\nu$C−O (dimers) |
| | | | $1195_{(m)}$ | $\omega NH_2$ |
| $1152_{(w)}$ | $1132_{(m)}$ | $1152_{(m)}$ | | $\rho NH_3^+$ |
| $1114_{(m)}$ | $1115_{(S)}$ | $1114_{(m)}$ | $1110_{(w)}/1071_{(w)}$ | $\rho NH_3^+/\nu$ CC |
| $1015_{(m)}$ | $1005_{(w)}$ | $1015_{(m)}$ | $1023_{(w)}$ | $\rho CH_3/\ \nu$CN |
| | $975_{(m)}$ | | | $\delta$OH···O o.o.p. (dimers) |
| $919_{(m)}$ | $917_{(m)}$ | $919_{(m)}$ | $923_{(w)}$ | $\rho CH_3$ |
| $850_{(m)}$ | | $849_{(m)}$ | $828_{(m)}$ | $\delta OCO^-$ i.p. |
| | $844_{(S)}/819_{(S)}/797_{(S)}$ | | | $\delta$O-CO i.p./$\gamma$COH |
| $772_{(m)}$ | | $772_{(m)}$ | $778_{(w)}$ | $\gamma OCO^-$ |
| | $734_{(w)}$ | | $734_{(w)}$ | $\tau$CC/$\omega$CH |
| $649_{(m)}$ | | $649_{(m)}$ | $635_{(w)}$ | $\delta OCO^-$ o.o.p. |
| | $611_{(m)}$ | | | $\delta$OCO o.o.p. (dimers) |
| $541_{(S)}$ | | $539_{(S)}$ | $536_{(w)}$ | $\delta$CCO/$\delta$NCC |
| | $517_{(m)}$ | | | $\omega$OCO (dimers) |
| $486_{(m)}$ | | $486_{(m)}$ | | $\tau NH_3^+$ |

[a] VS, very strong; S, strong; m, medium; w, weak; vw, very weak; sh, shoulder; sc, scissors; o.o.p., out of plane; i.p., in plane.

formation of a three-dimensional network.[11] Besides, the absence of well-defined $NH_3^+$ stretching modes in the 3100−3300 cm$^{-1}$ range is indicative that there are no significant amounts of free amine groups, that is, nonassociated L-alanine monomers.[9] The 2000−4000 cm$^{-1}$ region is not well resolved because of band overlapping and intermolecular coupling, which render the

**TABLE 2: Summary of the Deconvolution Results (Positions and Relative Areas of the Components) Obtained for Untreated L-Alanine and Dried from a pH 6 Solution**

| | wavenumber/cm$^{-1}$ (% area) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| untreated | 3231 (2.0) | 3113 (15.8) | 3085 (5.3) | 3015 (16.3) | 2984 (1.2) | 2942 (2.1) | 2899 (15.3) | 2804 (6.9) | 2732 (22.4) | 2604 (7.7) | 2514 (4.2) | 2451(0.8) |
| pH 6 | 3258 (2.2) | 3105 (25.4) | 3086 (3.4) | 3007 (11.1) | 2985 (0.3) | 2939 (0.7) | 2889 (21.7) | 2807 (3.1) | 2735 (16.6) | 2603 (10.7) | 2511 (4.4) | 2464 (0.4) |
| assign. | $\nu_{as}NH_3^+$ (free) | $\nu$NH (H bonded) | | | $\nu_sNH_3$ (free) | $\nu_{as}CH_3$ | $\nu_sCH_3$[a] | overtones and combination bands | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| untreated | 1645 (8.9) | 1623 (24.8) | 1596 (46.2) | 1557 (12.3) | 1521 (2.4) | 1506 (5.4) | 1378 (3.9) | 1362 (96.1) |
| pH 6 | 1646 (8.8) | 1622 (24.0) | 1593 (39.1) | 1555 (17.5) | 1521 (2.0) | 1506 (8.6) | 1378 (6.8) | 1360 (93.2) |
| assign. | $\nu_{as}OCO^-$ (intramol. H bonded) | $\nu_{as}OCO^-$ (H bonded) | $\delta NH_3^+$ (H bonded) | | $\delta NH_3^+$ (intramol. H bonded and free) | | $\nu_sOCO^-$ (intramol. H bonded) | $\nu_sOCO^-$ (H bonded) |

[a] In Fermi resonance with $2\delta_{as}CH_3$.

**TABLE 3: Summary of the deconvolution results (positions and relative areas of the components) obtained for L-alanine dried from a pH 1 solution.**

| | wavenumber/cm$^{-1}$ (% area) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3209 (3.0) | 3161 (1.3) | 3109 (29.7) | 2986 (14.0) | 2906 (6.6) | 2824 (29.1) | 2701 (3.1) | 2615 (6.2) | 2573 (0.9) | 2534 (2.9) | 2493 (0.4) | 2464 (2.8) |
| assign. | $\nu_{as}NH_3^+$ (free) | $\nu$NH (intramol. H bonded | $\nu$OH (H bonded) | $\nu_sNH_3^+$ (free) | $\nu_{as}CH_3$ | $\nu_sCH_3$ | overtones and combination bands | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1724 (44.2) | 1680 (3.3) | 1647 (5.2) | 1603 (7.2) | 1580 (7.7) | 1504(8.7) | 1491 (13.5) | 1466 (4.2) | 1417 (6.0) |
| assign. | $\nu$C=O (H bonded) | $\nu$C=O (intramol. H bonded) | $\nu_{as}OCO^-$ (zwitt) | $\delta_{as}NH_3^+$ (intramol. H bonded) | $\delta_{as}NH_3^+$ (free) | $\delta_sNH_3^+$ (intramol. H bonded) | $\delta_sNH_3^+$ (free) | $\delta_{as}CH_3$ | $\delta_sCH_3$ |

**TABLE 4: Summary of the Spectral Deconvolution Results (Positions and Relative Areas of the Components) Obtained for L-Alanine Dried from a pH 13 Solution[a]**

| | wavenumber/cm$^{-1}$ (% area) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3505 (18.4) | 3319 (29.2) | 3151 (18.2) | 2978 (12.9) | 2935 (12.1) | 2737 (5.8) | 2647 (0.4) | 2552 (3.0) |
| assign. | $\nu_{as}NH_2$ (free) | $\nu_sNH_2$ (free) | $\nu_{as}CH_3$ | | $\nu_sCH_3$ | overtones and combination bands | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1630 (20.0) | 1580 (28.4) | 1526 (1.3) | 1500 (4.1) | 1468 (14.2) | 1446 (2.7) | 1414 (8.8) | 1392 (0.6) | 1370 (11.1) | 1304 (8.8) |
| assign. | $\delta_{sc}NH_2$ | $\nu_{as}OCO^-$ | $\nu_{as}OCO^{-b}$ | | $\delta_{as}CH_3$ | | $\delta_sCH_3$ | $\nu_sOCO^{-b}$ | $\nu_sOCO^-$ | $\delta$CCH |

[a] $\delta_{sc}$ - scissors mode. [b] - from sodium carbonate

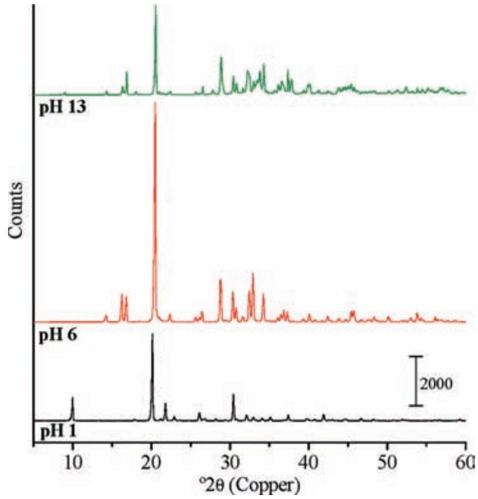

**Figure 3.** Powder X-ray diffraction patterns of the solid L-alanine samples dried from aqueous solutions at the indicated pH.

intensity comparison difficult. Nevertheless, the $\nu_{as}CH_3$ mode appears clearly at 2940 cm$^{-1}$. Below 2800 cm$^{-1}$, a number of overtones, combination, and Fermi resonance bands appear.[23] In the C−H deformation region, $\delta_{as}CH_3$ (1455 cm$^{-1}$), $\delta_sCH_3$ (1412 cm$^{-1}$), and $\delta$CH (1307 cm$^{-1}$) bands are very well defined. An independent confirmation of the predominant form of L-alanine in this sample was provided by the powder XRD pattern (Figure 3), which is typical of the zwitterionic structure.[40]

The presence of the L-alanine protonated cation [$CH_3CH(NH_3^+)COOH$] in the sample dried from a pH 1 solution is evidenced by two very strong bands in the spectrum (Figure 2): the $\nu$C=O, at 1723 cm$^{-1}$, and the $\delta_sNH_3^+$, at 1493 cm$^{-1}$. The stretching region is broad, with relative maxima at 3203, 3160, 2979, and 2910 cm$^{-1}$, the last two assigned to the $\nu_sNH_3^+$ and $\nu_{as}CH_3$ modes, respectively. The others result from the overlapping of $\nu_{as}NH_3^+$ and $\nu$OH modes and are difficult to identify at this point. Their assignment will be addressed later. Anyway, the downward shifts of the $\nu$C=O and $\nu$OH bands with respect to neutral monomeric alanine[31,39] point to the existence of intermolecular hydrogen bonds involving the carboxylic acid group. Characteristic bands of amino acid dimers are observed, such as the doublets at 1228/1197 cm$^{-1}$, assigned to the $\nu$C−O mode,[39] and at 1382/1346 cm$^{-1}$, assigned to the combination of the $\nu$C−O and $\delta$OH modes.[14] Other features detected only in this sample may also correlate with acid dimers, as indicated in Table 1. Because these bands regard only the carboxylic acid group, we are driven to propose that the hydrogen bonds responsible for this dimeric arrangement involve mostly these groups. The powder XRD pattern (Figure 3) suggests a well-crystallized structure, given the low baseline and the sharpness of the main peaks. However, the crystalline arrangement is clearly different from that of zwitterionic L-alanine. The lower number of diffraction peaks is consistent with a more constrained orientation of L-alanine in the crystal (that may be due to the presence of the above referred dimers).

The spectrum of L-alanine precipitated from the solution at pH 13 (Figure 2) shows a broadband with apparent maximum at 3322 cm$^{-1}$, tentatively assigned to a $\nu NH_2$ mode.[5] This feature, associated with the strong bands at 1581 and 1368 cm$^{-1}$ ($\nu_{as}OCO^-/\delta_{sc}NH_2$ and $\nu_sOCO^-$ modes, respectively), points to a large predominance of alaninate ion [$CH_3CH(NH_2)COO^-$].[36] The $CH_3$ related modes are more evident in the deformation region, at 1461 cm$^{-1}$ ($\delta_{as}$) and 1415 cm$^{-1}$ ($\delta_s$), but an antisymmetric stretching mode and a symmetric stretching mode may also be identified at 2975 and 2938 cm$^{-1}$, respectively.[2,15] The strong band at 1305 cm$^{-1}$ was assigned to the $\delta$CH mode.

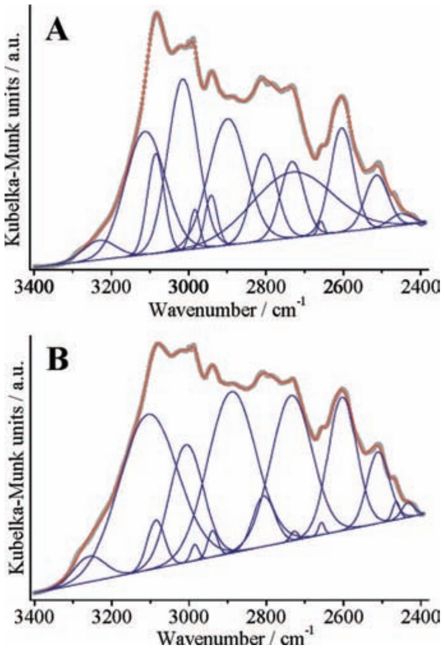

**Figure 4.** Deconvolution of the DRIFT spectra of untreated L-alanine (A) and after precipitation from an aqueous solution at pH 6 (B) in the 2400−3400 cm$^{-1}$ region.

The poor resolution of this spectrum in both wavenumber regions may be due to an agglomeration of smaller crystals, as suggested by the gel-like appearance of the sample after drying. In fact, it produced a more complex powder XRD pattern (Figure 3), pointing to the presence of a predominant crystalline phase with similarities to the zwitterionic structure, plus minor amounts of different structures of sodium carbonate.[43]

From the previous brief analysis, it becomes clear that L-alanine precipitated from aqueous solutions at pH 1 (HCl) and 6 and 13 (NaOH) crystallizes as alaninium chloride (needle-shaped crystals), zwitterionic L-alanine (powder), and sodium alaninate (with residual sodium carbonate), respectively. Therefore, as previously observed for glycine,[36] L-alanine retains the predominant form in solution (at pH 1, the cationic form of L-alanine exceeds 96%, and at pH 13, more than 99% correspond to the anionic form).

Although the identification of the L-alanine form in each solid phase was straightforward from the DRIFT spectra, the specific interactions between amino acid molecules and the eventual three-dimensional structures formed were not understood because of the partial band overlap, especially in the high-wavenumber region. The novelty introduced in this work is to clarify those interactions through a detailed spectral analysis achieved by deconvolution in two regions corresponding to the carbonyl/carboxyl stretching and amine deformation modes and to the OH and NH stretching modes. The exact wavenumber range varied, depending on the particular spectrum.

A nonlinear least-squares fitting method was used, assuming Gaussian band profiles for all the components. No baseline corrections were made, and no restrictions were imposed on the band positions and widths. The band positions were confirmed by the second derivative of the spectra. The best fits were obtained with $\chi^2 \approx 10^{-7}$ and a correlation coefficient of 0.999.

**3.1. Untreated L-Alanine and L-Alanine Dried from a pH 6 Solution.** The spectral deconvolutions in the 2400−3400 cm$^{-1}$ range for untreated L-alanine and dried from a pH 6 solution are shown in Figure 4. In the low-frequency range, two separate regions were considered, as shown in Figure 5, 1330−1390 cm$^{-1}$ and 1470−1700 cm$^{-1}$.

The deconvolution results for the two samples are very much comparable and are summarized in Table 2

The components assigned to the $\nu_{as}$ and $\nu_s$ modes of free $NH_3^+$ groups in the two samples (columns 2 and 6 of Table 2) represent a small fraction of the corresponding total band intensity, which means that only a few of these groups are not interacting. The large shift between the antisymmetric modes in the two samples (27 cm$^{-1}$) may be explained by the presence of different conformations of zwitterionic L-alanine. The broadband assigned above to the $\nu_{as}NH_3^+$ mode, with maximum at ~3080 cm$^{-1}$, includes in fact three components, compatible with non equivalent N−H bonds that result from hydrogen bonds of different strengths, either inter- or intramolecular.[24] The components retrieved at 2899 cm$^{-1}$ (untreated) and 2889 cm$^{-1}$ (pH 6) are most probably due to the symmetric $CH_3$ stretching mode, owing their intensity to Fermi resonance with the first overtone of the antisymmetric $CH_3$ deformation.[20,14]

Two components were identified for the symmetric and antisymmetric stretching modes of the carboxylate group. The lower-wavenumber components are predominant (at 1622 cm$^{-1}$ for $\nu_{as}OCO^-$ and 1360 cm$^{-1}$ for $\nu_sOCO^-$), confirming the extensive intermolecular H bonding involving this group. The minor ones, at 1646 and 1378 cm$^{-1}$, may be correlated with less interacting carboxylate groups, possibly in intramolecular H bonds. Good correlation was obtained from the deconvolution in the $NH_3^+$ deformation region, where, instead of the antisymmetric and symmetric vibrations, four components were recovered. The two minor components at lower frequencies (less affected by specific interactions) were tentatively assigned to intramolecularly bonded and/or free amine groups, at 1521 and 1506 cm$^{-1}$, respectively. Similarly to what was already known for crystalline L-alanine, the detailed assignment achieved by spectral deconvolution adds support to extensive intermolecular hydrogen bonding between the $NH_3^+$ and $COO^-$ groups of zwitterionic L-alanine dried from an aqueous solution at pH 6. Some weaker intramolecular hydrogen bonds between the same groups are also proposed.

**3.2. L-Alanine Dried from a pH 1 Solution.** For L-alanine dried from a pH 1 solution, the spectral deconvolution was made in the 2400−3400 and 1390−1800 cm$^{-1}$ wavenumber ranges (Figure 6).

The deconvolution results and proposed assignments are summarized in Table 3

The spectral deconvolution in the stretching region allowed retrieving a major component with maximum at 3109 cm$^{-1}$ that, given its broadness and shape,[36] has been assigned to the stretching mode of OH groups involved in a diversity of hydrogen bonds. Taking into account the frequency of this mode in association with the $\nu C{=}O$ dominant component, at 1724 cm$^{-1}$, it follows that the carboxylic acid groups must be involved in hydrogen bonds, forming dimers, small chains, or clusters. As found for the acid salt of glycine,[36] dimers will most probably form by resulting from two equivalent hydrogen bonds ($C{=}O\cdots HO$) between the carboxylic groups of two acid molecules. Amino acid dimers resulting from hydrogen bonds between the $C{=}O$ and $NH_3^+$ groups of a neutral and a cationic form (proton bond dimers) were observed by electrospraying an aqueous solution of alanine.[44] Because these alanine dimers give rise to $\nu C{=}O$ bands at 1787 and 1730 cm$^{-1}$,[45] it is possible to exclude their formation in the present sample of alaninium. The minor $\nu C{=}O$ overlapping component, at 1680 cm$^{-1}$, is also assigned to hydrogen-bonded carbonyls, more strongly interact-

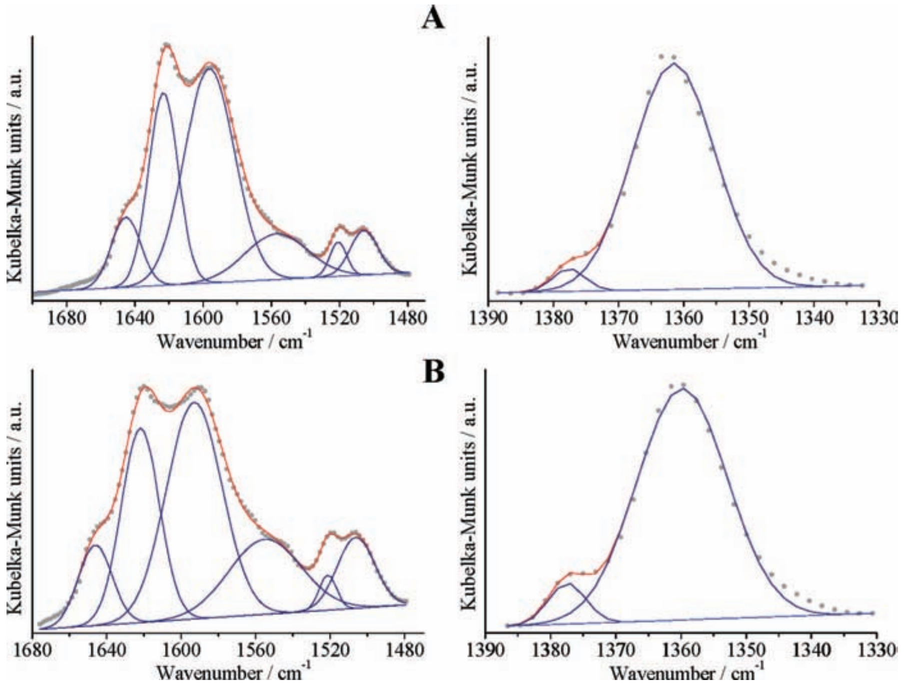

**Figure 5.** Deconvolution of the DRIFT spectra of untreated L-alanine (A) and after precipitation from an aqueous solution at pH 6 (B) in the wavenumber ranges 1330−1390 and 1470−1700 cm$^{-1}$.

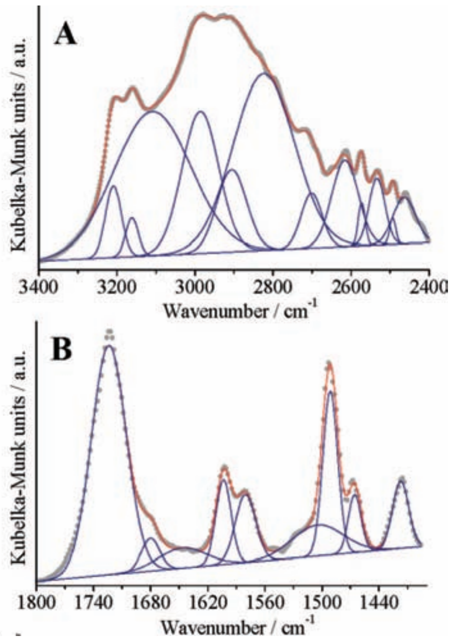

**Figure 6.** Deconvolution of the DRIFT spectrum of L-alanine after precipitation from an aqueous solution at pH 1 in the (A) 2400−3400 and (B) 1390−1800 cm$^{-1}$ regions.

ing, possibly with the $NH_3^+$ group of the same ion.[42] The existence of a small fraction of strongly hydrogen-bonded $NH_3^+$ groups was confirmed by the minor components retrieved in the $\delta_s NH_3^+$, $\delta_{as} NH_3^+$, and $\nu$NH bands at 1504, 1603 and 3161 cm$^{-1}$, respectively. The deconvolution of these bands also allowed assigning to non-hydrogen-bonded $NH_3^+$ groups the main components at 1491 cm$^{-1}$ ($\delta_s$), 1580 cm$^{-1}$ ($\delta_{as}$), 2986 cm$^{-1}$ ($\nu_s$), and 3209 cm$^{-1}$ ($\nu_{as}$). Therefore, whereas free amine groups are in majority, most of the carboxylic acid groups are engaged in the formation of dimers.

The role of the counterion ($Cl^-$) is clearly very different from that observed in mono-L-alaninium nitrate crystals, where $NO_3^-$ is directly involved in the crystalline packing by bridging two alaninum ions through hydrogen bonds with an OH and an $NH_3^+$ group.[14] In the present case, the interactions will be mostly electrostatic between $Cl^-$ and the alaninium $NH_3^+$ groups, leaving the carboxylic acid groups free to interact between them.

As in the previously discussed samples, the high intensity of the $CH_3$ symmetric stretch is certainly due to Fermi resonance with $2\delta_s CH_3$. Finally, the small component at 1647 cm$^{-1}$ (~5% of the spectral area between 1400 and 1800 cm$^{-1}$) may correlate to a minor fraction of zwitterionic L-alanine.

**3.3. L-Alanine Dried from a pH 13 Solution.** The spectral deconvolution of the L-alanine sample obtained by drying a pH 13 solution was performed between 2400 and 3800 cm$^{-1}$ and between 1250 and 1750 cm$^{-1}$ (Figure 7 and Table 4).

The broad high-wavenumber region was resolved into a number of components that led to a more accurate assignment than that above. The components retrieved at 3505 and 3319 cm$^{-1}$ have been assigned to the $\nu_{as} NH_2$ and $\nu_s NH_2$ modes of free groups. In fact, these modes are expected to shift to lower wavenumbers when the $NH_2$ groups are metal coordinated or intramolecularly H bonded.[2,18] This assignment is confirmed by the spectral deconvolution in the low-wavenumber region, where the $NH_2$ scissors mode was retrieved at 1630 cm$^{-1}$, shifted to lower wavenumbers with respect to neutral L-alanine,[5] and coincident with that obtained for alaninate ion adsorbed on copper films.[18] Although it is difficult to accept that $NH_2$ does not interact by hydrogen bonds, the deconvolution results do not detect aggregation involving these groups in this sample of solid L-alanine.

The second strong component of the band at ~1581 cm$^{-1}$ (at 1580 cm$^{-1}$) was assigned to the $\nu_{as} OCO^-$ mode, despite the large shift when compared to zwitterionic L-alanine (1646 and 1622 cm$^{-1}$, depending on the type of H bond, see Table 2). It is known that strong electrostatic interactions between the carboxylate group of a zwitterionic or anionic amino acid and a metal cation, such as $Na^+$, may lead to the formation of a salt bridge so stable that it induces the transition from neutral to zwitterionic amino acid in the gas phase.[46] In concentrated aqueous lithium alaninate solutions, it was proved that the first

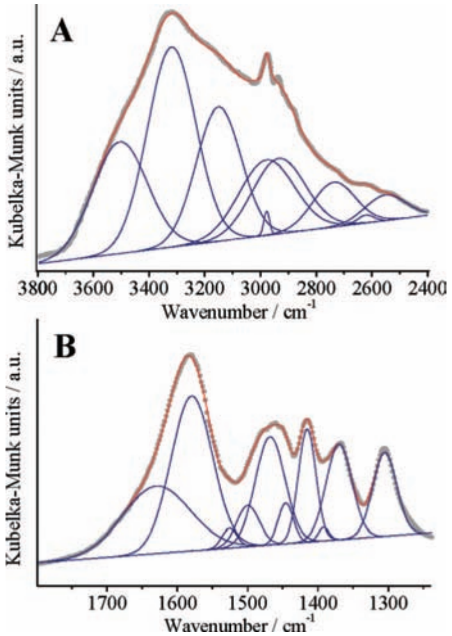

**Figure 7.** Deconvolution of the DRIFT spectrum of L-alanine after precipitation from an aqueous solution at pH 13 in the (A) 2400−3800 and (B) 1250−1750 cm$^{-1}$ regions.

coordination shell of the cation Li$^+$ includes two alaninate ions, coordinated to Li$^+$ by one carboxyl oxygen atom.[47] Therefore, in the present sample of sodium alaninate, the large frequency shift in the $\nu_{as}$OCO$^-$ mode would be expected.

In what concerns the CH$_3$ groups, the components associated with the stretching modes ($\nu_{as}$CH$_3$ at 3151/2978 cm$^{-1}$ and $\nu_s$CH$_3$ at 2935 cm$^{-1}$) are shifted to higher wavenumbers with respect to the other alanine solid samples discussed above. Because these values are in good agreement with those obtained for alaninate ion in aqueous solution[48] and adsorbed at high coverage on Cu(110),[15,16] it is possible that the substitute NH$_2$ in the chiral carbon (instead of NH$_3^+$) may induce this shift by a charge density effect. On the other hand, given the frequency of the $\nu_s$CH$_3$, the resonance with the overtone of the methyl deformation is no longer responsible for the intensity enhancement of this mode.

Accounting for the presence of sodium carbonates traces, suggested by the XRD pattern, very small components were found at 1526, 1500, and 1392 cm$^{-1}$, assigned to the antisymmetric and symmetric OCO stretching modes of those species.

## 4. Conclusions

The DRIFT spectra of solid L-alanine dried from aqueous solutions at different pH values allow gaining insight on the specific intra- and intermolecular interactions in the amino acid form predominant upon drying (cationic at pH 1, zwitterionic at pH 6, and anionic at pH 13). This is achieved by a detailed band assignment, obtained by spectral deconvolution in two frequency regions (∼2400−3500 and ∼1400−1700 cm$^{-1}$). The results on alaninium chloride and sodium alaninate are particularly interesting because they were never reported. The spectral profiles are consistent with well-ordered structures of zwitterionic and cationic L-alanine, confirmed by powder XRD patterns. The spectra of zwitterionic L-alanine (untreated and dried from a pH 6 solution) suggest extensive intermolecular hydrogen bonding between the NH$_3^+$ and COO$^-$ groups, plus some weaker intramolecular hydrogen bonds between the same groups. In L-alaninium, most of the carboxylic acid groups are involved in two equivalent (C=O···HO) hydrogen bonds, with formation of dimers, whereas most of the NH$_3^+$ groups are free or interacting electrostatically with the counterion Cl$^-$. Evidence of some (NH···O=C) intramolecular H bonds is also obtained. In the anionic form of L-alanine, there is no evidence of interactions by H bond: the NH$_2$ groups are mostly free, and the carboxylate groups interact strongly with the counterion Na$^+$.

**Acknowledgment.** This work was supported by Fundação para a Ciência e a Tecnologia (FCT), Project POCI/QUI/60918/2004. The authors wish to acknowledge IN (Instituto de Nanotecnologia e Nanociência) and Project CONC-REEQ/700/2001.

## References and Notes

(1) Upadhyay, D. M.; Rai, A. K.; Rai, D. K.; Singh, A. N.; Kumar, A. *Spectrochim. Acta A* **2007**, *66*, 909–918.

(2) Lambie, B.; Ramaekers, R.; Maes, G. *Spectrochim. Acta A* **2003**, *59*, 1387–1397.

(3) Zhang, H.; Zhou, Z.; Shi, Y. *J. Phys. Chem. A* **2004**, *108*, 6735–6743.

(4) Iijima, K.; Nakano, M. *J. Mol. Struct.* **1999**, *485*−*486*, 255–260.

(5) Stepanian, S. G.; Reva, I. D.; Radchenko, E. D.; Adamowicz, L. *J. Phys. Chem. A* **1998**, *102*, 4623–4629.

(6) Godfrey, P. D.; Firth, S.; Hatherley, L. D.; Brown, R. D.; Pierlot, A. P. *J. Am. Chem. Soc.* **1993**, *115*, 9687–9691.

(7) Blanco, S.; Lesarri, A.; López, J. C.; Alonso, J. L. *J. Am. Chem. Soc.* **2004**, *126*, 11675–11683.

(8) Wang, C. H.; Storms, R. D. *J. Chem. Phys.* **1971**, *55*, 3291–3299.

(9) Cao, X.; Fischer, G. *Chem. Phys.* **2000**, *255*, 195–204.

(10) Destro, R.; Marsh, R. E.; Bianchi, R. *J. Phys. Chem.* **1988**, *92*, 966–973.

(11) Lehman, M. S.; Koetzle, T. F.; Hamilton, W. C. *J. Am. Chem. Soc.* **1972**, *94*, 2657–2660.

(12) Görbitz, C. H. *J. Mol. Struct. (Theochem)* **2006**, *775*, 9–17.

(13) Smith, P. K.; Taylor, A. C.; Smith, E. R. B. *J. Biol. Chem.* **1937/38**, *122*, 109–123.

(14) Nĕmec, I.; Císaøová, I.; Mièka, Z. *J. Mol. Struct.* **1999**, *476*, 243–253.

(15) Williams, J.; Haq, S.; Raval, R. *Surf. Sci.* **1996**, *368*, 303–309.

(16) Barlow, S. M.; Louafi, S.; Le Roux, D.; Williams, J.; Muryn, C.; Haq, S.; Raval, R. *Langmuir* **2004**, *20*, 7171–7176.

(17) Jones, G.; Jones, L. B.; Thibault-Starzyk, F.; Seddon, E. A.; Raval, R.; Jenkins, S. J.; Held, G. *Surf. Sci.* **2006**, *600*, 1924–1935.

(18) Ihs, A.; Liedberg, B.; Uvdal, K.; Tornkvist, C.; Bodo, P.; Lundstrom, I. *J. Colloid Interface Sci.* **1990**, *140*, 192–206.

(19) Suh, J. S.; Moskovits, M. *J. Am. Chem. Soc.* **1986**, *108*, 4711–4718.

(20) Diem, M.; Photos, E.; Khouri, H.; Nafie, L. *J. Am. Chem. Soc.* **1979**, *101*, 6829–6837.

(21) Machida, K.; Kagayama, A.; Saito, Y.; Uno, T. *Spectrochim. Acta A* **1978**, *34*, 909–914.

(22) Byler, D. M.; Susi, H. *Spectrochim. Acta A* **1979**, *35*, 1365–1368.

(23) Barthes, M.; Vik, A. F.; Spire, A.; Bordallo, H. N.; Eckert, J. *J. Phys. Chem. A* **2002**, *106*, 5230–5241.

(24) Rozenberg, M.; Shoham, G.; Reva, I.; Fausto, R. *Phys. Chem. Chem. Phys.* **2005**, *7*, 2376–2383.

(25) Nóbrega, G. F.; Sambrano, J. R.; De Souza, A. R.; Queralt, J. J.; Longo, E. *J. Mol. Struct. (Theochem)* **2001**, *544*, 151–157.

(26) Tajkhorshid, E.; Jalkanen, K. J.; Suhai, S. *J. Phys. Chem. B* **1998**, *102*, 5899–5913.

(27) Ellzy, M. W.; Jensen, J. O.; Hameka, H. F.; Kay, J. G. *Spectrochim. Acta A* **2000**, *59*, 2619–2633.

(28) Gontrani, L.; Mennucci, B.; Tomasi, J. *J. Mol. Struct. (Theochem)* **2000**, *500*, 113–127.

(29) Freedman, T.; Diem, M.; Polavarapu, P.; Nafie, L. *J. Am. Chem. Soc.* **1982**, *104*, 3343–3349.

(30) Koçak, A.; Luque, R.; Diem, M. *Biopolymers* **1998**, *46*, 455–463.

(31) Rosado, M. T.S.; Duarte, M. L. R. S.; Fausto, R. *J. Mol. Struct.* **1997**, *410*−*411*, 343–348.

(32) Berezhinsky, L. I.; Dovbeshko, G. I.; Lisitsa, M. P.; Litvinov, G. S. *Spectrochim. Acta A* **1998**, *54*, 349–358.

(33) Himmler, H. J.; Eysel, H. H. *Spectrochim. Acta A* **1989**, *45*, 1077–1083.

(34) Barlow, S. M.; Louafi, S.; Le Roux, D.; Williams, J.; Muryn, C.; Haq, S.; Raval, R. *Surf. Sci.* **2005**, *590*, 243–263.

(35) Briget Mary, M.; Sasirekha, V.; Ramakrishnan, V. *Spectrochim. Acta A* **2006**, *65*, 414–420.

(36) Rosado, M. T.; Duarte, M. L. T. S.; Fausto, R. *Vib. Spectrosc.* **1998**, *16*, 35–54.

(37) Kubelka, P.; Munk, F. *Z. Tech. Phys.* **1931**, *12*, 593–601.

(38) Rozenberg, M.; Shoham, G.; Reva, I.; Fausto, R. *Spectrochim. Acta A* **2003**, *58*, 3253–3266.

(39) Cao, X.; Fischer, G. *Spectrochim. Acta A* **1999**, *55*, 2329–2342.

(40) Reference code 00−028−1508, PDF2 ICDD database, 2006.

(41) Bellamy, L. J. *The Infrared Spectra of Complex Molecules*, 3rd ed.; Chapman and Hall: New York, 1986; Vols. *I* and *II*.

(42) Socrates, G. *Infrared and Raman Characteristic Group Frequencies*, 3rd ed.; J. Wiley & Sons: New York, 2001.

(43) Reference code 01−070−2148, PDF2 ICDD database, 2006.

(44) Oh, H. B.; Lin, C.; Hwang, H. Y.; Zhai, H.; Breuker, K.; Zabrouskov, V.; Carpenter, B. K.; McLafferty, F. W. *J. Am. Chem. Soc.* **2005**, *127*, 4076–4083.

(45) Rajabi, K.; Fridgen, T. D. *J. Phys. Chem. A* **2008**, *112*, 23–30.

(46) Kapota, C.; Lemaire, J.; Maître, P.; Ohanessian, G. *J. Am. Chem. Soc.* **2004**, *126*, 1836–1842.

(47) Kameda, Y.; Sasaki, M.; Amo, Y.; Usuki, T. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 228–236.

(48) Weir, A. F.; Lowrey, A. H.; Williams, R. W. *J. Mol. Struct. (Theochem)* **1996**, *362*, 339–354.

JP802170N