Solid L- α -alanine: spectroscopic properties and theoretical calculations

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Abstract

A spectroscopic study of L- α -alanine in different environments is presented, with special emphasis on the neutral to zwitterion transformation of this amino acid. Spectra of room temperature solids as deposited from the vapour and in KBr pellets are compared and discussed revealing some discrepancies. An assignment is proposed based on theoretical calculations of the solid structure. Vapour deposits at 25 K are prepared both of pure alanine and of mixtures with polar (H_2O) and non-polar (CO_2 , CH_4) components. The spectra of the solids contain IR bands which can be individually assigned to the neutral and to the zwitterion, and whose relative intensity variation can be used to follow the neutral to zwitterion transformation. The assignment of the spectrum of the neutral species is proposed, again with help from theoretical calculations of the single neutral molecule. The neutral/zwitterion ratio in deposits at 25 K varies between ~ 60 % for pure alanine and H_2O mixtures and ~ 90 % for non-polar matrices. This ratio drops when the solid is heated until the ionic species only remains at 200 K. The conversion process depends on the environment surrounding the alanine molecules.

1. Introduction

The physico-chemical properties of alanine have attracted so much attention that it would be pointless to attempt to cite all papers dealing with them, even in the recent literature. This interest in alanine is well deserved, as this molecule plays relevant roles in several and varied fields. It is one of the simplest amino acids, the so-called building blocks for proteins, giving rise to many chemical reactions of direct effect on life. Along this line, the presence of alanine in astronomical objects, which could have transported it to the Earth, is a finding directly related to the possible origin of life in our planet [1-3]. From a purely fundamental point of view, the different structures that this molecule can adopt in solid or gas phase, and in solutions, constitute an active field of research. In this respect, vibrational spectroscopy has proved to be most useful, with early spectral data on alanine and other amino acids reported already during the thirties [4-6] followed by reviews later on (see for instance [7, 8]). We shall mention here only a selection of those papers directly related to the goals of the present investigation.

As other biological molecules, alanine adopts a neutral structure in gas phase ($\text{H}_2\text{N}-\text{CH}(\text{CH}_3)-\text{COOH}$), but an ionic structure (zwitterion, $\text{H}_3\text{N}^+-\text{CH}(\text{CH}_3)-\text{COO}^-$) in crystalline solids or in water solution (see lower panel of Figure 1). The neutral species can have several conformers, unravelled by high-sensitive molecular beam Raman spectroscopy [9] and FT microwave techniques [10,11]. In turn, the zwitterion can present slightly different configurations arising from the possible H-bonding interactions in which the NH_3^+ group can be involved [12-15], and also depending on the pH and characteristics of the solution [16, 17]. Sophisticated techniques have been devised and applied to study the solids as room temperature matrices [18]. Rosado et al. [19] published one of the key papers on the spectroscopy of alanine, recording infrared and Raman spectra of samples prepared as KBr pellets of the solid or as vapor-deposits in an Ar matrix at 17 K. In addition, theoretical calculations of the structures of both the neutral and zwitterionic forms have been carried out at various degrees of theoretical development [11,19, 20].

With such abundance of publications in this area, any further research must be focused on aspects not specifically treated before. In the present work, we prepare pure alanine samples by direct deposition of the vapour on a cold substrate, which offers the

possibility of studying together the neutral and ionic forms, without any solvent effects, and changes in the relative concentration of both species that vary with the temperature of the solid. The spectral alterations induced by KBr sampling can also be investigated by comparison to vapour-deposited samples. One point sometimes neglected in previous literature, and of special spectroscopic relevance in many fields [21-23] is the estimation of intensity values for the main vibrational features. We address this problem from the spectra of alanine in KBr pellets of known concentration. In addition, we report a theoretical spectrum of the L- α -alanine crystal, which is, as far as we know, an original calculation for this species. Finally, possible astrophysical applications of this study are envisaged by the consideration of mixtures of alanine with species present in many astrophysical media, and which could accompany this amino acid in frozen particles. Specifically, we prepare mixtures of alanine with water, carbon dioxide and methane and analyse their spectra in an attempt to predict possible effects of polar and non-polar environments on the spectra of alanine.

This paper is structured as follows. First, in section 2, the experimental part and computational methodology are briefly described, and related to our previous works [24, 25]. Next, the IR spectroscopic results for zwitterionic alanine are presented in section 3.1, followed by the results for the neutral form in section 3.2. Finally, the effect of the different environments in the spectrum and in the neutral/zwitterionic conversion is described in section 3.3. In the last section the main conclusions are summarized.

2. Experimental and computational section

2.1. Experimental part

Our experimental setup was described in detail before [24-26]. In brief, it consists on a high vacuum chamber provided with a closed cycle helium cryostat, with a 1 mm thick deposition substrate made of Si in contact with the cryostat cold finger. The substrate can be cooled between 14 K and 300 K with 1 K accuracy. The residual pressure of the chamber at room temperature is 5×10^{-8} mbar. The system is coupled to a Bruker Vertex 70 FTIR spectrometer through a purged pathway, with KBr windows for incident and transmitted radiation. Spectra were recorded from 6000 to 500 cm^{-1} with a nominal resolution of 2 cm^{-1} , co-adding 300 scans for each spectrum.

L- α -alanine was purchased from Sigma-Aldrich (purity $\geq 99.0\%$). We also carried out some experiments with D-alanine, but the IR spectra of the D- and L-enantiomers were practically identical, and consequently we will refer to L-alanine only in the rest of this paper. The amino acid was heated by means of a home-made mini-oven located inside the vacuum chamber. The mini-oven has been designed in our group and is described in detail in [25]. A simple scheme of the experimental set up is shown in the upper panel of Figure 1. Solid layers of pure alanine were prepared by deposition of the sublimated alanine vapour on the Si substrate, kept at 25 K. The effect of the sublimation temperature on the deposited samples was studied by a series of measurements, as described in section 3.2.1, leading to the choice of an oven temperature of 115 °C for the rest of this investigation. Alanine is stable up to about 300 °C [27] and therefore we do not expect contaminating decomposition products in our samples. Oven temperatures are indicated in Centigrade to keep a closer reference to the actual measuring practice and to avoid confusion with cryostat temperatures, expressed in Kelvin as customary. Mixed layers of alanine and water vapour, CO₂ or CH₄ were prepared by introducing these compounds through an independent inlet to backfill the chamber to a controlled pressure at the same time that the alanine flow was being admitted, always keeping the substrate at 25 K. The temperature evolution of the solid films was studied by heating the substrate at a 5 K/min rate, up to 200 K. At this temperature the volatile species on the mixed layers were completely evaporated and the spectra recorded correspond to the remaining pure alanine solid.

Figure 1. Above: schematic view of the experimental setup; below: schematic formulas of the normal and zwitterionic conformers of L- α -alanine, (2*S*)-2-aminopropionic acid.

The column densities of H_2O , CO_2 or CH_4 in the ice mixtures were calculated from the integrated absorbance of the main bands of these species, via the Lambert-Beer relation, using the corresponding integrated absorption coefficients, A [26]. The bands chosen for this purpose were: ν_1 of H_2O around $3.1 \mu\text{m}$ (3200 cm^{-1}), ν_3 of CO_2 at $\sim 4.3 \mu\text{m}$ (2340 cm^{-1}), and ν_4 of CH_4 at $\sim 7.7 \mu\text{m}$ (1300 cm^{-1}). We used $A=1.9 \times 10^{-16} \text{ cm/molecule}$ for H_2O , $A = 7.1 \times 10^{-17} \text{ cm/molecule}$ for CO_2 , and $A = 8.085 \times 10^{-18} \text{ cm/molecule}$ for pure CH_4 ice (corrected by assuming a CH_4 density of 0.47 g/cm^3 as described by Herrero et al. [26]), taken from Mastrapa et al. [28], Gerakines et al. [29], and Hudgins et al. [30], respectively.

The estimation of column densities for alanine is hampered by the lack of experimental information on the infrared absorption coefficients of its spectral bands. In this work we have opted by preparing dilute KBr pellets of the solid, with a known concentration (1:400) in weight, aiming to measure the integrated absorption coefficients for L-alanine from the infrared spectra at room temperature. As it is known, the main problem to obtain integrated intensities from pellets is the possible lack of homogeneity in the samples. To overcome this, we used the whole amount of weighted KBr plus alanine powder to prepare the pellets, and averaged out the spectra of all of

them. We selected the 2000 -700 cm^{-1} spectral region to calculate the integrated band strength summing all the corresponding infrared bands, yielding a value of $2.4 \pm 0.4 \times 10^{-16} \text{ cm/molecule}$. This value was used to determine the amount of alanine in vapour deposited samples.

We have also conducted solid state calculations of the alanine crystal as will be detailed in section 2.2. The integrated band strength given by the calculations in the 2000 - 700 cm^{-1} spectral range is $3.5 \times 10^{-16} \text{ cm/molecule}$, 1.5 times higher than the experimental value obtained from the KBr pellets.

The samples prepared by vapor deposition at 25 K contain a mixture of the neutral and zwitterionic species, but after warming to 200 K, the ionic structure only remains. The fraction of neutral form in pure alanine samples was estimated from the relative intensity of the bands in the 1486 -1676 cm^{-1} region, assigned to the zwitterion, for measurements between 200 K and 25 K. This procedure assumes that the absorption coefficient of this band does not change with temperature.

2.2. Computational methods

We have used different theoretical methods to study L- α -alanine as a solid and as an isolated molecule. For the solid state, we have utilized the CASTEP package [31], a program based on a density functional theory (DFT) plane-wave pseudo-potential method that is specifically designed to calculate different properties of solids. We have tested several correlation-exchange functionals using different parameterizations: PBE [32], PW91[33] and RPBE[34] gradient-corrected functionals (GGA), and even non local potentials as B3LYP [35]. Comparing to the experimental structure, all GGA calculations predicted considerably short hydrogen bond distances, and gave a value of the “a” unit cell parameter 15% larger than the experimental one. However, after we included dispersion corrections in the Tkatchenko-Scheffler (TS) [36] or Ortmann-Bechstedt-Schmidt [37] schemes in the PBE and PW91 functionals respectively, the results turned out to be much more accurate, with the PBE cell parameters differing by less than 2% from the experimental values, and similar for both methods. It follows that dispersion corrections are necessary to predict good geometry values of the alanine crystal. A similar conclusion was reached regarding the calculated IR spectrum for PBE,

RPBE and PW91 functionals. In the rest of this work, we will present our results for only the PBE functional, including dispersion correction in the TS [36] scheme. Convergence criteria of 1.0×10^{-5} eV/atom, 0.03 eV/Å, 0.001 Å and 0.05 GPa for energy, maximum force, maximum displacement and maximum stress, respectively, were selected. Norm-conserving pseudo-potentials with a plane wave energy cutoff of 750 eV were also used. The calculations were performed in a k-point grid of the entire Brillouin zone at a k-point spacing of 0.07 Å^{-1} .

We used the Gaussian 09 suite [38] to study the isolated neutral alanine molecule, at B3LYP/aug-cc-pvTZ level of theory, which afforded predicting the harmonic and anharmonic vibrational spectra with a reasonable level of accuracy [25].

3. Results and discussion

3.1. *Solid alanine in zwitterionic form*

Solid alanine at room temperature adopts the zwitterionic form. However, when evaporated from the oven, the molecules become neutral, and on impact on the substrate they may retain the neutral structure. When the substrate is at room temperature, though, only the ionic form is present. This is shown in the spectra represented in traces (a) and (b) of Figure 2. Trace (a) corresponds to this high-temperature substrate experiment and trace (b) to a KBr pellet with a 1:400 alanine to KBr concentration. The bands that appear in these spectra correspond to the ionic form, whereas bands of the neutral structure are not seen here (see below). The assignment of the main spectral features in the spectra of the zwitterion, supported by the theoretical calculations of the solid, is presented in Table I. These assignments, in which groups of vibrations are sometimes clustered together, agree well in general with previous literature descriptions [12, 13, 15, 19].

Figure 2. IR spectra of zwitterionic alanine. a) Thin black: Solid vapour deposited on a substrate at room temperature. Thick light grey: the same deposit after cooling the substrate to 25 K; b) (1:400) alanine/KBr pellet at room temperature; c) theoretical prediction in arbitrary units obtained following the procedure described in section 2.2. Absorption bands are represented with Lorentzians with a FWHM of 8 cm^{-1} for wavenumbers lower than 2000 cm^{-1} and of 20 cm^{-1} in the rest of the calculated spectrum. Spectra are offset in the vertical scale for clarity.

Table I. Assignment of the infrared spectrum of solid L- α -alanine at room temperature (zwitterionic form), and calculated vibrational spectrum of the crystal.

Mode	Experimental			Theoretical	
	Vapor dep. ^a	KBr Pellet ^b	Int	Crystal ^c	Int
νNH_3^+ , νCH				3060	940
νNH_3^+ , νCH	3600-2400	3600-2400	vs	2990	848
νCH_3 , νNH_3^+				2970	738
νNH_3^+				2742	2317
				---	---
	---	1648	sh	1653, 1615	150
δNH_3^+ , νCO_2^-	1634	1621	vs	1566	706
	1596	1594	vs	1548	439
	1524	1519, 1506	s, m	---	---
	---	---	---	---	---
δCH_3	1460	1455	m	1447	81
νCO_2^- , δCH_3 , δNCH	1406	1412	s	1386	123
δCH_3 , δNCH , νCCC	1371, 1350	1363	s	1336	217
δCCH	1304	1307	s	1290	189
ρNH_3^+	1220	1237	m	1238	21
ρNH_3^+ , δCCH	1152	1152	m	1157	19
ρCH_3 , νCN	1114	1114	m	1095	55
δCCH , ρCH_3 , ρNH_3^+ , $\nu\text{C-CH}_3$	1010	1014	m	1004	50
νCN , νCCC	918	919	m	897	16
νCN , νCCC	843	850	m	832	38
ωCO_2^-	773	772	m	749	16
ρCH_3 , ρNH_3^+ , δCO_2^-	663	649	m	639	18
ρNH_3	587	---	w	569	29
δCCO , $\nu\text{C-N}$	534	540	m	526	96
δCCN	---	---	---	418	111
τCH_3 , ωNH_3^+	---	---	---	321	52
δCCC	---	---	---	275	69
lattice	---	---	---	224	132
lattice	---	---	---	183	57

^a Solids generated by vapour deposition on a room temperature substrate.

^b Polycrystalline alanine in (1:400) KBr pellets at room temperature.

^c Theoretical predictions for the alanine crystal calculated as described in section 2.2.

Intensities listed are addition of the corresponding individual values, where appropriate. Wavenumber values in cm^{-1} and theoretical intensities in km/mol . Qualitative experimental intensities: vs, very strong; s, strong; sh, shoulder; m, medium; and w, weak. Greek letters stand for: ν , stretching; δ , bending; ρ , rocking; ω , wagging; τ , torsion.

We used crystallographic data on the L- α -alanine crystal lattice [39] as initial parameters for our DFT study. The optimized geometry is given in Table II, and a schematic representation of the calculated unit cell, an orthorhombic cell belonging to space group $P2_12_12_1$, is shown in Figure 3. Based on the optimized structure, the IR spectrum of the crystal was predicted, as displayed in trace (c) of Figure 2. We are not aware of any previous report on the calculated infrared spectrum of solid alanine. The comparison with the experimental spectra in Figure 2 must be made with caution, as the theoretical predictions do not include any information on bandwidths, nor on overtones or combination bands, which are present in the observed spectra. To facilitate the comparison, we have broadened the predicted spectrum with Lorentzian bands of different bandwidth. In the lower half of the spectrum, all bands have a FWHM of 8 cm^{-1} . The bands in the high-frequency range, calculated at 3060 , 2990 - 2970 and 2742 cm^{-1} (see Table I), are given widths of 25 , 50 and 150 cm^{-1} respectively. These values are somewhat arbitrary, and a reasonable comparison could also be achieved with other sets of values, but they provide a good agreement with the experimental trace. Their magnitude can be related with several physical effects, like the degree of inhomogeneity in the samples and the different response of each vibrational mode to the surrounding, or the interaction with overtones and combination bands that are expected in this region. But in particular, the large width of the 2742 cm^{-1} band is probably indicative of a large H-bonding between the NH_3^+ and COO^- groups of adjacent molecules. Hydrogen bonding is well known to induce large frequency shifts and very broad bands [40], characteristics that both affect specially this vibration.

Figure 3. The calculated orthorhombic unit cell of L- α -alanine crystal.

Table II. Calculated and experimental lattice parameters a , b , c , in Å; volume, in Å³, and bond distances, in Å, for the L- α -alanine crystal.

Parameter	Theoretical ^a	Experimental ^b
a	5.979	6.032
b	12.148	12.343
c	5.848	5.784
<i>Volume</i>	424.75	430.64
r(N-H)	0.880	1.050
r(N-H)	0.902	1.065
r(N-H)	0.951	1.045
r(N-C)	1.500	1.491
r(C-H)	1.098	0.986
r(C-CH ₃)	1.530	1.525
r(C-H)	1.096	0.973
r(C-H)	1.097	0.985
r(C-H)	1.097	0.987
r(C-COOH)	1.542	1.525
r(C-O)	1.256	1.276
r(C-O)	1.247	1.255
r(NH $\cdots$ O) _{shortest}	1.705	1.916

^a PBE gradient-corrected functional (GGA), with dispersion corrections in TS scheme.

^b Ref. [39].

Figure 2 affords also the possibility to compare the vapour deposited solid spectrum with that of the room temperature KBr pellet. The bands in the former trace appear broader than those in the latter, with a small frequency shift in some cases. The larger width probably reflects a higher degree of amorphicity in the vapour deposited solid, whereas in the KBr matrix small alanine single crystals must be present. Further support to this hypothesis comes from the comparison with previous studies on the temperature evolution of the spectrum of polycrystalline alanine in KBr pellets by Ivanov et al. [15], Barthes et al. [13] and Rozenberg et al. [12]. These authors observed significant spectral changes when the samples were cooled. In particular, several bands showed splitting at low temperature, while an anomalous intensity and frequency variation were noticed in the deformation vibration of NH_3^+ and in the torsional mode and its overtone. Those variations indicate that a reorganization of the crystal structure takes place at low temperatures, and it was suggested that this reorganization was probably caused by changes in the proton positions. In our experiment, some variations were observed upon cooling in the peaks at 1596 and 1524 cm^{-1} , attributed to the deformation mode of NH_3^+ , but these variations, barely appreciable in Figure 2, were much smaller than those reported by Ivanov et al. [15]. In general, the bands in the IR spectra do not narrow appreciably and no significant changes are seen when the temperature of the solid generated from the vapour phase is lowered (compare black and grey traces of Figure 2 (a)). Furthermore, an absorption band was observed at 972 cm^{-1} in the low temperature spectrum of the alanine pellets [12,13,15], which is assigned to an overtone of NH_3^+ coupled to one or more phonons of the crystal lattice. This band is not detected in the spectra recorded from vapour deposited films, providing evidence for a lack of a proper crystal structure that would allow the propagation of phonons, and its subsequent spectral activity.

3.2. *Neutral solid alanine*

3.2.1. *Generation conditions. Sublimation temperature effect*

Previous spectroscopic investigations on neutral alanine were carried out in gas phase [9-11] or in low-temperature [19] or alkali halide matrices [18]. To study the solid free from interferences from the matrix, alanine vapour, which contains only neutral

molecules, can be condensed onto a low temperature substrate in contact with a cryostat. We applied this technique to glycine [25], and found that the solid deposited as a mixture of neutral and ionic species, and that the composition of the mixture depended on the sublimation temperature of the solid. Our results for glycine served as a guide for the present work on alanine.

Figure 4. Spectra of a) alanine and b) glycine layers vapour-deposited at 25 K using different sublimation oven temperatures, indicated in the figure. The evolution of the neutral/zwitterionic ratio in the sample can be followed by changes on the main bands of the neutral amino acids, marked by dashed vertical lines. Spectra are offset in the vertical scale for clarity. Glycine spectra from Ref. [25]

The upper panel of Figure 4 presents spectra of alanine solids deposited on a substrate kept at 25 K, from sublimated vapours at different oven temperatures. Spectra at higher temperatures have been scaled down with the indicated factors to ease the comparison. Among the observed bands, some can be attributed to neutral alanine, other bands to the ion and others to vibrations common to both species. We want to focus here on two bands of the neutral species, indicated by dashed vertical lines, whose intensity can be easily traced down along the series of spectra. They correspond to the C=O stretch at 1721 cm^{-1} and to the band at 1256 cm^{-1} , whose controversial assignment

will be discussed in section 3.2.2. For increasing sublimation temperatures, the proportion of the neutral component, as indicated by the intensity of these bands relative to the other spectral features, is kept practically constant, until 175° C, when there is a sudden intensity drop, indicating the absence of this species in the deposited solid. The lower panel in Figure 4 collects the results of our investigation on glycine, where the trend was the same, but the neutral form was in very low proportion at a lower temperature, 150° C. Gómez-Zavaglia and Fausto [41] had noticed the same effect on their study on glycine, and put forward a possible mechanism of proton transfer from the neutral to the ionic form by taking the required energy from the impact on the substrate surface. Following this hypothesis, the structural reorganization would be less favoured in alanine, where the redistribution of collisional energy among a larger number of vibrational modes may restrict the amount of energy available for ionization, so that a higher kinetic temperature, with $T_{\text{oven}} = 175^{\circ}\text{C}$, is required for that purpose.

3.2.2. *Infrared spectrum of neutral alanine. Temperature evolution*

In view of the spectra in Figure 4, we chose to work with a sublimation temperature of 115° C, which yields a high proportion of neutral species deposited in the 25 K substrate without saturating the spectra. The corresponding trace in Figure 4 is reproduced at the bottom of Figure 5, where we present a study of the changes taking place in the spectrum when the substrate temperature is raised from the deposition value.

As commented on above, many bands have contributions from the neutral and the zwitterionic species, but some of them can be attributed exclusively to the neutral form. The clearest of these are indicated by dashed vertical lines in the figure, and the corresponding assignments are listed in Table III, where we have also included the values measured by Rosado et al. [19] in their Ar matrix experiments at 17 K. Some discrepancies both in frequency and intensity are found between the results of the two experimental techniques, and also in some of the assignments. The alanine molecules are surrounded by a different environment in both cases, which can cause these effects, but the formation of dimers in our samples may be a further reason for disagreements. Table III collects also the results of our theoretical calculations for the isolated molecule, at the harmonic and anharmonic levels, the latter yielding in general better

agreement with the observations. These calculations have aided in the assignment of the spectra as presented in the Table. One specific case of interest is the band at 1256 cm^{-1} , fairly intense in the vapour deposited spectrum but observed as a weak feature in the Ar matrix [19], and also predicted with small intensity in the calculations; the above mentioned effects could account for the larger infrared absorption observed in the vapour-deposited spectra.

The main effect of the temperature increase depicted in Figure 5 is a depletion of the bands assigned to the neutral species, which gradually transforms into the zwitterion. At 150 K there is still a small fraction of the former, but no trace of it can be seen at 200 K. The process is not reversible: cooling of the sample does not induce the formation of the neutral species. Based on the relative intensities of the purely zwitterionic bands compared to those of the neutral species, the fractional composition of the sample can be estimated. We chose for this purpose the strongest ionic bands, in the $1676\text{--}1486\text{ cm}^{-1}$ frequency interval, and assumed that there is no contribution in this spectral range from the neutral structure, and, further, that their absorption coefficient does not change with temperature. Both of these assumptions are probably not fully correct, and it is difficult to estimate the degree of uncertainty they introduce in the evaluation of the composition of the sample. Our results indicate a fraction of neutral species of $\sim 60\%$ at the lowest temperature, and we suggest that a 20% uncertainty may cover the error induced by the above mentioned assumptions. This subject is discussed again in Section 3.3.2, in reference to the same problem for mixtures with external species.

Figure 5. Spectra of alanine layers generated with an oven temperature of 115 °C by vapour deposition on a substrate at 25 K, and warmed to the indicated substrate temperatures. The evolution of the neutral/zwitterionic ratio in the sample can be followed by changes on the main neutral alanine bands, marked by dashed vertical lines. Spectra are offset in the vertical scale for clarity.

Table III. Assignment^a of the most intense bands of neutral alanine and calculated predictions of the isolated molecule. Wavenumber values in cm⁻¹ and theoretical intensities in km mol⁻¹.

Assignment	Experimental		Theoretical ^d		
	Solid ^b	Ar matrix ^c	Harm	Anharm	Int
$\nu\text{C=O}$	1721	1774	1803	1774	295
δCOH	1256	--- ^e	1294	1249	3
$\nu\text{CN}, \nu\text{CO}$	1084	1110	1123	1087	286
ρCH_3	978	1037	1084	1054	24
ωNH_2	821	852	883	830	134

^a The normal modes with a higher relative weight in terms of potential energy distribution are shown in table, although mixtures of several modes are usually predicted for each absorption.

^b This work. Solids generated by vapour deposition on a cold substrate at 25 K.

^c Ref. [19]. (1:750) alanine in an Ar matrix at 17 K.

^d This work. Ab initio calculations for a gas phase neutral alanine molecule (harmonic and anharmonic frequencies in cm⁻¹).

^e This mode is assigned to a peak at 1206 cm⁻¹ in [19]. However, that wavenumber would imply a very large red shift from the observation, contrary to all other assignments in this table.

3.3. Solid alanine in different environments

To explore possible astrophysical applications of this work we have investigated binary mixtures of alanine with H_2O , CO_2 , and CH_4 , species abundant in astrophysical ices. Using the same experimental conditions as for pure alanine, we have generated mixed solid layers of alanine/ H_2O , alanine/ CO_2 , and alanine/ CH_4 with amino acid concentrations of 6%, 6% and 11%, respectively. The interest of this study lies on the possible spectral differences arising when the alanine molecules are surrounded by polar species, like water or other alanine molecules in the zwitterionic structure, or by non-polar molecules, like carbon dioxide and methane, and whether such differences might provide clues to the eventual observation of alanine in astrophysical media.

3.3.1. Polar and non-polar environments

We present in Figure 6 the spectra of pure alanine and of the three mixtures mentioned above, obtained by vapour deposition on a substrate at 25 K of alanine sublimated at 115° C. The amount of alanine in each mixture is similar, although the relative concentrations are different, as indicated above. The spectrum of the water mixture is distorted with reference to that of pure alanine by the presence of strong water absorptions below 1000 cm^{-1} , and at $\sim 1650 \text{ cm}^{-1}$, where the ν_2 bending band of H_2O is located. The main alanine bands between 1085 and 1480 cm^{-1} can be seen in this spectrum, with relative intensities similar to those of the pure sample spectrum, indicating that the alanine structure and the neutral/zwitterion ratio do not vary when alanine is placed within a polar environment, like that provided by water, and in the configuration adopted in the unmixed deposits. These bands are highlighted by dashed vertical lines in the top two spectra of Figure 6.

The spectra corresponding to alanine in non-polar environments, in the c) and d) panels of Figure 6, look different to those in a) and b), hinting to fairly strong interactions between the alanine molecule and the surroundings. To begin with, the zwitterionic structure seems to be in much lower proportion than in the pure sample, judging by the weakness of the band of the ion in the 1600 cm^{-1} region. If the ionic conformer is created by a proton transfer mechanism, the present evidence suggests that this mechanism is impeded in the non-polar environment. The 1720 cm^{-1} C=O band of

the neutral form is split in two components in the non-polar mixtures, with different intensity distribution of the two peaks. The spectrum of the CO₂ mixture in this region reveals two very strong features, one being the higher component of the doublet, at 1752 cm⁻¹, and the other, at 1128 cm⁻¹, near the observed CN stretching band. On the contrary, the spectrum of the CH₄-based mixture displays less striking features, apart from the intensity enhancement of the low frequency component of the C=O doublet.

A similar situation was found in the study of glycine mixtures with non-polar species. In fact, Figure 6 looks remarkably similar to Figure 5 of Ref. [25]. In both cases we provide evidence of a slightly larger interaction between the amino acids and CO₂ than with methane. As pointed out also in [25], a proper interpretation of these observations would require some theoretical calculations of the possible interactions between the amino acids and a number of surrounding molecules. We are just at the very early stages of such project.

Figure 6. Infrared spectra of solid layers generated by vapor deposition at 25 K of a) pure alanine; b) alanine/H₂O; c) alanine/CO₂; d) alanine/CH₄. Each sample contains roughly the same amount of alanine. The H₂O bands in panel b) and the CH₄ band in panel (d) are marked with asterisks (*).

3.3.2. Neutral to zwitterionic conversion

We have also followed the transformation from neutral to ionic alanine with increasing temperature when surrounded by other species. Such process could take place in some astronomical objects, like comets, when approaching the Sun on their path. In our case we have applied the same procedure described above for pure alanine, i.e. alanine sublimation at oven temperature of 115° C and deposition on substrate at 25 K, simultaneous with admission of the other species, H₂O, CO₂ or CH₄, followed by substrate warming at 5 K/min rate up to 200 K.

The proportion of neutral to zwitterionic species was monitored using the intensity variation of the most suitable spectral features. For water mixtures, we selected the band at $\sim 1415\text{ cm}^{-1}$, while for the other two mixtures we worked with the whole band structure in the region between 1486 and 1676 cm^{-1} . The evolution of the conversion is displayed in the graph of Figure 7. For polar environments, like in the pure alanine case and the water matrix, the trend is smooth, with a gradual increase in the zwitterionic component, reaching total conversion at 200 K. At this temperature, water will be evaporated, and only the ionic alanine is left on the substrate. For the non-polar environments studied here, the conversion follows a different scheme, marked by the sublimation temperature of the gases. Methane sublimates at $\sim 50\text{ K}$ and CO₂ at $\sim 90\text{ K}$, and at those temperatures there is a sudden increase in the zwitterionic proportion in the sample, which from that point on behaves similarly to pure alanine.

At 25 K the fraction of neutral form in CO₂ and CH₄ mixtures is $\sim 90\%$, while in the pure alanine deposit and in water mixtures it is of $\sim 60\%$. The higher amount of neutral species in the non polar environments at low temperature is easily understood if we accept that initially the CH₄ (or CO₂) molecules occupy a zone surrounding alanine and hinder the auto-transformation process, capturing some of the collision energy liberated at the impact of the gas phase neutral molecules on the substrate while the solid is formed. When methane or carbon dioxide sublimate, alanine alone remains and follows the same transformation process as the pure species. Similar results were obtained in the glycine study (see Figure 8, bottom panel, of Ref. [25]).

As explained in Section 3.2.2 we estimate a large uncertainty of about 20 % for all the quoted values shown in Figure 7.

Figure 7. Percentage of neutral alanine as a function of substrate temperature (see text for details). The estimated error for all depicted values is $\sim 20\%$.

4. Summary and conclusions

We present here an investigation on some spectral characteristics of alanine which had not received enough consideration in the previous literature, aiming to deepen the understanding of the processes in which this amino acid is involved. We have recorded spectra of the zwitterionic species in KBr pellets and as prepared by vapor deposition on a room-temperature substrate. In addition, we have recorded spectra of samples containing the neutral and the ionic species at low temperature, and also of mixed samples of alanine with water, carbon dioxide and methane, trying to simulate solids that may exist in astronomical or astrophysical media. We have also carried out theoretical calculations of the structure and spectra of crystal zwitterionic alanine, and of the isolated neutral molecule. The main conclusions are outlined below.

From the spectra of alanine crystals in KBr pellets, recorded from 2000 to 700 cm^{-1} , the integrated band strength is estimated to be $2.4 \pm 0.4 \times 10^{-16} \text{ cm/molecule}$, in reasonable agreement with our calculated theoretical value of $3.5 \times 10^{-16} \text{ cm/molecule}$.

The spectra of the zwitterion present some discrepancies when the sample is prepared as KBr pellet and when it is obtained by vapor deposition at room temperature.

Whereas very small crystals of alanine are dispersed in the halide matrix, the solid formed by sublimation in an oven and deposition on a substrate, has a structure in which some of the crystal characteristics are less evident. In particular, the band at 972 cm^{-1} , attributed to an overtone of the NH_3^+ rocking mode coupled to lattice phonon modes, cannot be seen in the latter spectrum, hinting to a certain disorder or lack of long-range crystal structure. The theoretical prediction of the spectra of the crystal, performed for the first time, provides valuable information for the proposed assignment of the spectrum.

When the vapor phase neutral molecules reach a low-temperature substrate, a solid is formed with a certain proportion of the neutral and ionic species that depends on the sublimation temperature of the oven and on the substrate temperature. For pure alanine deposited on a 25 K substrate from $T_{\text{oven}} = 115\text{ }^\circ\text{C}$, the sample contains about 60% of the neutral species. Some IR bands can be assigned to this structure, and their depletion is used to follow the neutral to zwitterionic transformation that takes place on heating the substrate. One specific case of interest is a fairly intense band measured at 1256 cm^{-1} , which is predicted with small intensity in the calculations for the neutral species, but is stronger than predicted when surrounded by polar environments, which is the case in our samples.

The neutral to zwitterion transformation that takes place on a substrate at room temperature when the gas phase neutral molecules impact on the surface, requires higher collision energy than the similar process for glycine. This can be interpreted in terms of a higher degree of internal modes redistribution of the collision energy in the larger alanine molecule, which restricts the neutral to ionic conversion.

When alanine is co-deposited with other species, the nature of this foreign component influences the neutral/zwitterion ratio. With water, as a paradigm of polar species, this ratio behaves similarly to that in pure alanine deposits. On the other hand, with CO_2 or CH_4 , a larger proportion of neutral molecules is found in the deposit at 25 K. Only when the foreign substances sublime at their specific temperatures, 50 K for CH_4 and 90 K for CO_2 , the remaining sample in the substrate follows the same pattern as the pure alanine deposits. Thus, if either of these two species is present in a given

astronomical sample at low temperature, like an asteroid for instance, a fairly large value of the neutral/zwitterionic alanine ratio could be expected.

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