

Valine infrared absorbance at cryogenic temperatures

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The amino acid *L*-valine is analyzed by Fourier Transform InfraRed Spectroscopy (FTIR) in the 40–300 K temperature range. A valine sample was deposited onto a ZnSe substrate, cooled down to 40 K and warmed up back to 300 K, annealed at 120 °C for 12 h, cooled down to 40 K and warmed up again to 300 K. FTIR spectra were acquired in transmission mode during this thermal excursion. It was observed that: i) no phase transition has occurred; ii) as temperature decreases, the band absorbances increase linearly and the band widths become narrower; iii) compared to 300 K values, the integrate absorbances (peak areas) at 40 K are 40–100% higher and bandwidths are about half; iv) the FTIR spectrum behaviors of the annealed and non-annealed samples are similar. The sample is considered to be constituted only by valine zwitterions; bandwidth decrease is explained partially by Doppler effect; absorbance enhancement at low temperatures is caused by stronger Van der Waals forces. These general conclusions are expected to be similar for other amino acids.

Keywords: amino acid *L*-valine, FTIR spectra, zwitterions.

Introduction

Amino acids are organic molecules that play a fundamental role in the dynamics of all living organisms, once some species of them are proteinogenic, building-blocks of proteins, while others have metabolic functions. Four elements are always present in amino acids: carbon, hydrogen, oxygen and nitrogen; sulfur or phosphorus may also occur. All amino acids have the same molecular structure, characterized by a “central” carbon (called α -carbon) connected to four groups: the amino radical — NH_2 , the carboxyl radical — COOH , a hydrogen atom and a side chain. Therefore, only the side chain group gives the differences among them. An interesting property of amino acids is that the proton of the carboxyl radical can migrate to the amino radical, transforming them into the carboxylate and the ammonium groups, respectively, modification which changes substantially the amino acid permanent dipole moments; the first isomer is called neutral and the second one is defined as zwitterion. In the gas phase the neutral isomer dominates, while (at least for glycine and alanine) the zwitterionic isomer is the most stable configuration in the condensed phase [1–3]. As zwitterions, solid amino acid molecules have three hydrogen atoms located in the ammonium group, which are H-bond candidates for possible intermolecular interactions.

Amino acids became relevant astrophysical materials when they were discovered in comets [4] and meteorites [5];

the existence of glycine beyond the solar system is controversial [6,7]. Based on these findings, obvious questions arose: how such molecules have been formed? Do they resist travelling up to Earth? Are they partially responsible for the existence of life on Earth? The research for answers brought technical problems as, for instance, how to identify molecules in space, how to quantify their abundance and which are the local (cryogenic) temperatures of their environment?

The main objective of the current work is to contribute to the solution of these problems.

The amino acid valine (Val) is analyzed by Fourier transform infrared spectroscopy (FTIR) in the 40–300 K temperature range. Valine has molar mass $117.15 \text{ g}\cdot\text{mol}^{-1}$, being one of the lightest amino acids, has hydrophobic aliphatic (nonpolar) side chain and presents chirality properties [8]. Although these valine’s specific characteristics, we expect that the conclusions of the current work are general, in the sense that they may be extended for other amino acids.

Thermal treatment of valine before FTIR analysis is reported in the literature. Proton beam [9] and electron beam [10] irradiations on amino acids have shown that the decreasing rate of their FTIR absorbance changes during radiolysis. This fact is commonly attributed (e.g., [11]) to phase transition induced by the ionizing beam, that is, besides dissociating the sample molecules, the beam causes annealing (compaction or rearrangement) of the intact ones. For this reason, we also decided to compare the infrared

spectra of annealed and non-annealed samples at different low temperatures.

FTIR, through the Beer–Lambert law, is a common analytical technique useful to measure column density of samples. To perform calculations, it is necessary the knowledge of band strengths (A -values), quantities sensitive to crystallographic structures which, in turn, are temperature dependent. Results of the current work are expected to allow corrections in the A -values when measurements are not carried out in the same temperature as the reference data.

Experimental set-up

Experiments have been carried out in the Van de Graaff Laboratory of the Physics Department at Pontifícia Universidade Católica do Rio de Janeiro. *L*-valine (99.5%, purchased from Sigma-Aldrich) was sublimated by Joule effect (current ~ 30 A, Edwards 306 coater unit) and deposited onto a ZnSe substrate. The deposition occurred during around 10 min under a residual pressure of $4 \cdot 10^{-6}$ mbar. The 737 nm film thickness was measured by perfilometry. The *L*-valine density is considered to be $1.32 \text{ g} \cdot \text{cm}^{-3}$ [8].

Inside the vacuum chamber (pressure 10^{-6} at 300 K, decreasing to $4 \cdot 10^{-8}$ mbar at low temperatures), the sample was fixed on a Cu sample-holder thermally connected to a He cryostat (JANIS CCS-UHV/204). The sample-holder temperature was regulated by a Lake Shore controller, model 325.

The infrared analysis was performed in transmission mode by a JASCO FTIR-4100 spectrometer. The $4000\text{--}500 \text{ cm}^{-1}$ spectral region was inspected by averaging 100 scans with 1.0 cm^{-1} resolution. Peak areas were measured by subtracting a linear background in between the band limits.

Results

Initially the system was cooled down by 50 K steps and, below 100 K, by 20 K steps. The next operation was to bring the sample to 300 K, using the inverse procedure. To be sure that the equilibrium temperature was reached, the infrared acquisition started 30 min after the indication of nominal temperature by the controller. When this stage ended, the sample was exposed to air at room temperature, annealed at 120 °C for 12 h, and introduced again in the UHV chamber. The second cycle of temperature excursion, identical to the previous one, was then performed.

Six of the obtained FTIR spectra over the $3200\text{--}600 \text{ cm}^{-1}$ wavenumber region are presented in Fig. 1 for the extreme temperature excursions. The chronological sequence is from top to bottom. Four spectra were acquired at 300 K: the first one corresponds to the sample as deposited; the two central ones were obtained just before and after the annealing, and the last one after the second excursion. One observes clearly the peak resolution improvement when the sample is cooling down in both excursions; the position of certain peaks is shifted mainly during the temperature variation in the 100–300 K interval. The peak shape modifica-

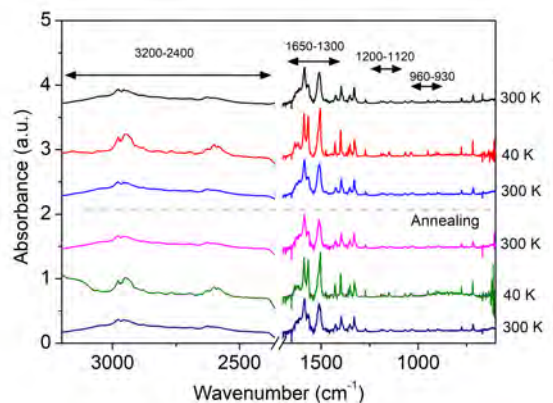


Fig. 1. (Color online) Evolution of the valine infrared spectra as the sample temperature varies. Temperature is initially 300 K, decreases down to 40 K and increases up to 300 K; the sample is annealed and the cycle is repeated.

tions and positions are not permanent since the spectrum at room temperature (300 K) is practically the same before and after each temperature cycle. In the $3300\text{--}3200 \text{ cm}^{-1}$ region and at 40 K, for the first and principally for the second excursion, absorbance increases with respect to 300 K; this is due to water condensation.

Four wavenumber regions were selected for detailed analysis: a) $3200\text{--}2400$, b) $1650\text{--}1300$, c) $1300\text{--}1120$ and e) $960\text{--}930 \text{ cm}^{-1}$. The corresponding infrared partial spectra are presented in Figs. 2(a)–2(e), left side for the first cycle and right side for the second one. Decreasing the sample temperature, peaks become thinner and background level lower, allowing the appearance of low intense vibrational bands. The $3200\text{--}2400 \text{ cm}^{-1}$ region (Fig. 2(a), right side) presents an anomalous behavior below 200 K which is explained by condensation of water existent in the UHV chamber residual gas particularly at the second excursion. The region shown in Fig. 2(b) has a high band density; in particular, the 1510 cm^{-1} band splits in three bands at low temperature. Figs. 2(c) and 2(d) display the 1271 cm^{-1} (C–H bend vibration mode) and 1145 cm^{-1} (C–C stretch mode) bands, respectively; a blue shift occurs for both bands when the sample temperature decreases. On the contrary, Fig. 2(e) presents the 949 cm^{-1} (C–N stretch mode) [12] for which no shift is observed.

Figures 3 and 4 present the dependence of the integrated band absorbance and the band FWHM (Full Width at Half Maximum) on the sample temperature, respectively.

Based on the variation of the integrated absorbance (peak area) as a function of the sample temperature, presented in Fig. 3, relevant findings are:

i) For all bands, the absorbance increases when the temperature decreases and vice-versa. Excluding the absorbance behavior of the $3000\text{--}2400 \text{ cm}^{-1}$ region, which envelops many bands (including the tail of the 3200 cm^{-1} water band), the integrate absorbance decreases linearly with temperature.

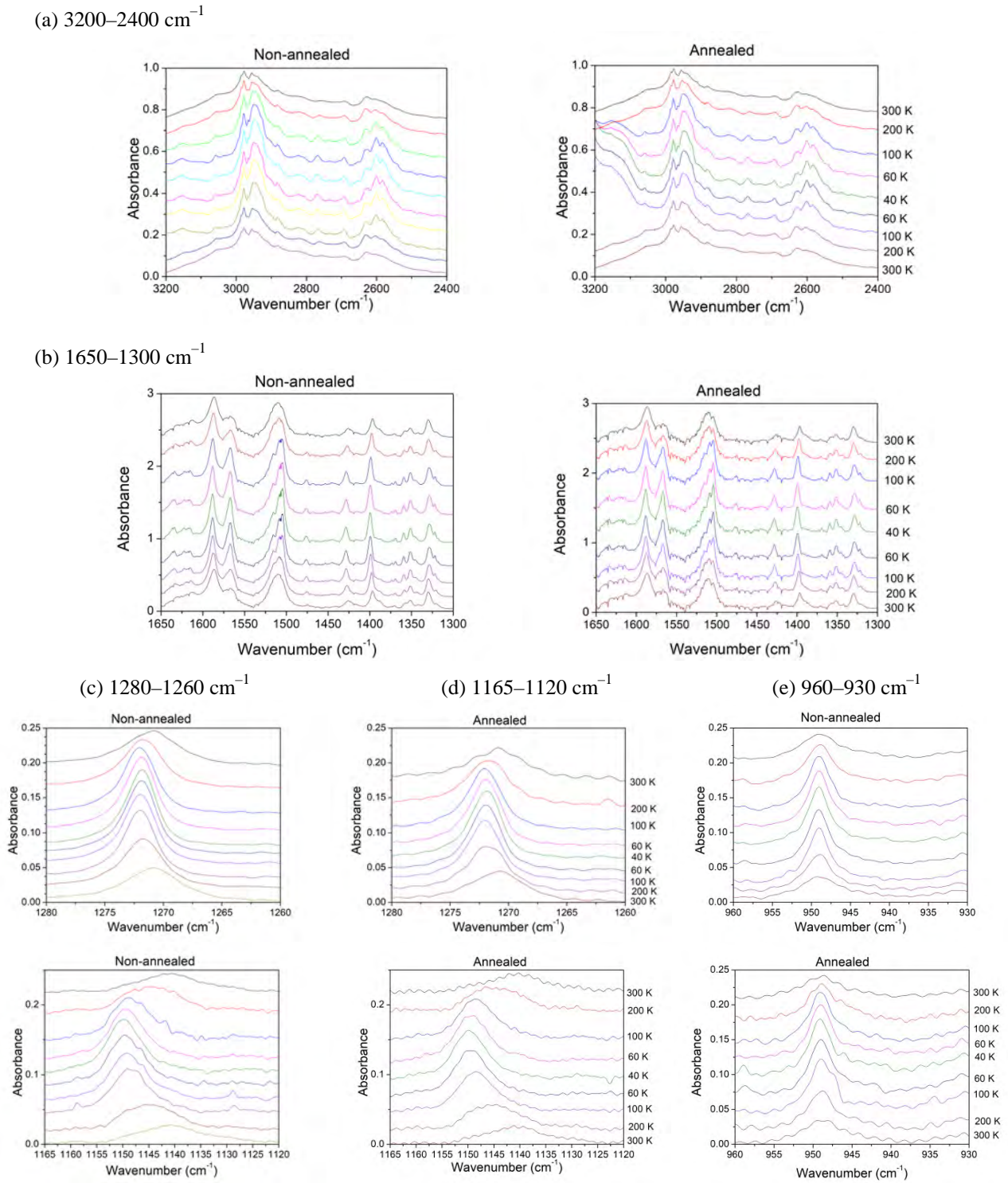


Fig. 2. (Color online) Details of the evolution presented in Fig. 1. Data is presented for sample at extreme and intermediate temperatures, for five selected regions of the FTIR spectrum. (a) and (b) left side for the non-annealed sample and right side for the same sample after annealing; (c), (d) and (e) upper side for the non-annealed sample and bottom side for the same sample after annealing.

ii) Assuming this linear dependence on with temperature, the integrate absorbance may be parametrized by $S = S_0(1 - T/T_{tr}) \equiv S_0 - \alpha\Delta vT$. The quantity S_0 represents the integrate absorbance at zero Kelvin; T_{tr} is the “transparency” temperature for which S voids (the material would be transparent for that specific band); v_1 and v_2 being the band limits, $\Delta v = v_1 - v_2$ is the total bandwidth;

α is the absorbance thermal rate coefficient equal to $S_0/(\Delta vT_{tr})$.

iii) Table 1 summarizes the parameters obtained by linear fitting of Fig. 3 data. A major remark is that the absorbance rate coefficient (given per wavenumber unit) is the same (~ 0.25) for all single absorbance peaks. This quantifies the parallelism observed in the evolution of

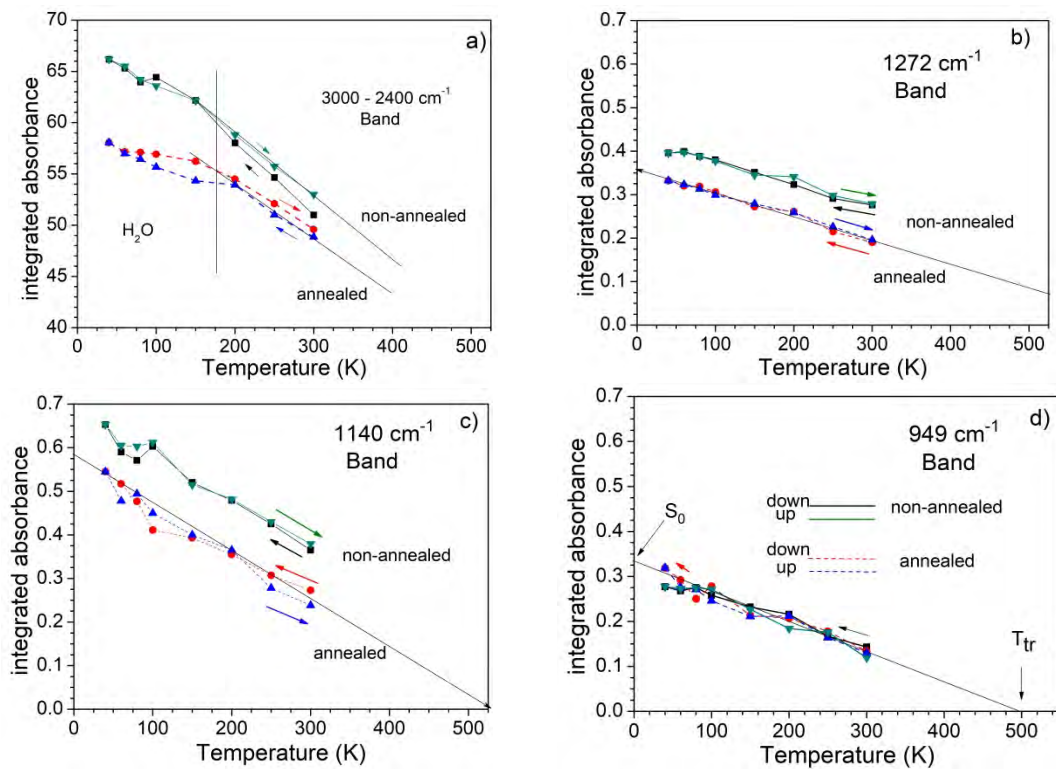


Fig. 3. (Color online) Dependence of the band integrated absorption on sample temperature. First excursion: solid line linking points; second excursion (annealed sample): dash line. The arrows indicate the sense of the temperature variation (cooling down and warming up). The lower absorbance of the second excursion is attributed to partial valine sublimation during the annealing at 120 °C. Stretch lines are fittings to determine S_0 and T_{tr} . Linear fittings of Fig. 3(a) data were performed only for temperatures above 180 K.

absorbances for distinct bands, shown in Fig. 3, which is directly related to the fact that the whole spectrum evolves proportionally when temperature varies. Inversely, the integrated absorbances depend on $\Delta\nu$, causing 40–100% variation between 300 K and 40 K, according to the band.

iv) The absorbance evolution 3000–2400 cm^{-1} region presents a slope decrease for $T < 150$ K. The origin of this behavior is not clear, but probably the explanation is the influence of the wide 3200 cm^{-1} water band. Although its contribution be relatively small for wavenumbers below 3000 cm^{-1} , the background line over a large region is al-

tered (e.g., [13]). Also, note that the water condensation on the sample surface would increase (instead of decrease) the slope in the 40–150 K range, but no significant physical-chemical interference on the valine absorbance (a bulk phenomenon) is expected. For circumstantial reasons, the second excursion was longer and got a larger amount of water contamination.

v) Peak shapes are restored after each temperature excursion: therefore, crystalline modifications due to temperature variation are not permanent. The evolutions of non-annealed and annealed samples are about the same.

Table 1. Fitting parameters of data displayed at Fig. 3. Equation: $S = S_0(1 - T/T_{tr}) = S_0 - \alpha\Delta\nu T$

Band, cm^{-1}	$\Delta\nu = \nu_1 - \nu_2$	3000–2400		1272		1140		949	
		3000–2400*		1280–1260		1165–1120		960–935	
Annealing:		non-ann.	ann.	non-ann.	ann.	non-ann.	ann.	non-ann.	ann.
S_0	cooling	73	63	0.43	0.36	0.68	0.56	0.31	0.33
	warming	71	65	0.42	0.36	0.69	0.58	0.32	0.33
T_{tr} , K	cooling	1000	1200	833	667	667	555	588	500
	warming	1100	1200	909	714	714	526	500	500
α , $10^{-4} \text{ cm}^{-1} \cdot \text{K}^{-1}$	cooling	1.2	0.87	0.25	0.27	0.22	0.22	0.21	0.26
	warming	1.1	0.90	0.24	0.26	0.22	0.24	0.25	0.26

Notes: (*) Only temperatures above 180 K have been considered for the 3000–2400 cm^{-1} band fitting.

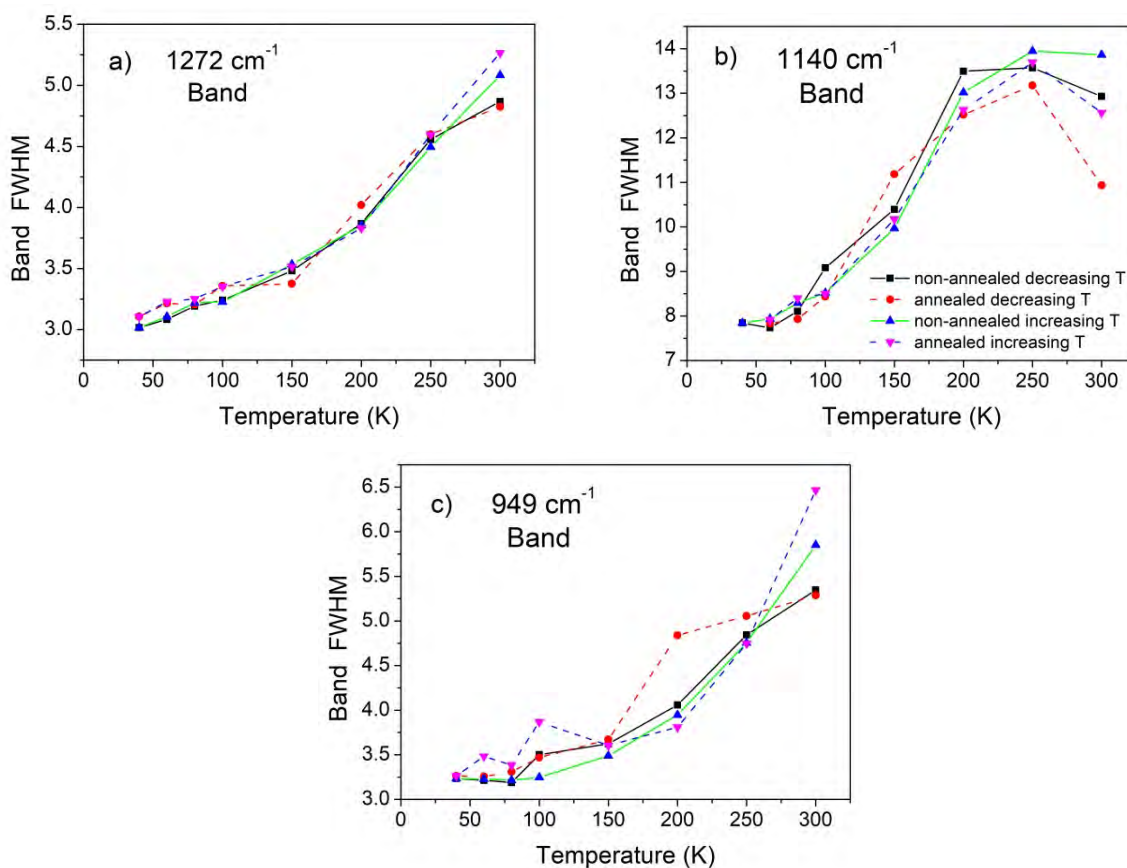


Fig. 4. (Color online) Dependence of the band FWHM on sample temperature. First excursion: solid line; second excursion (annealed sample): dash line. Bands: 1272 cm^{-1} (a); 1140 cm^{-1} (b); 949 cm^{-1} (c).

From Fig. 4 data, comments are:

i) In general, the absorbance bandwidths decrease when the temperature decreases and vice-versa. The bandwidths at 300 K are roughly twice larger than at 40 K.

ii) Each band FWHM is practically the same for a given temperature, regardless of the sense of temperature variation or whether the sample has been annealed or not.

iii) The bandwidth decrease rate is greater at high temperatures and tends to level off below 40 K.

iv) The 1140 cm^{-1} band peak width exhibits particular behavior at high temperature. It seems that a second peak at 1150 cm^{-1} is growing up during the sample cooling down, while the 1140 cm^{-1} band decreases. Although the wavenumber of each peak stays constant, the envelope of both peaks exhibits a shift if the relative population of the two bands changes.

Concerning the last point, it is worth to remark that the 1 cm^{-1} wavenumber step used as FTIR resolution is adequate for analyzing $\sim 3\text{ cm}^{-1}$ FWHM absorbance bands. The observed $\sim 1\text{ cm}^{-1}$ shifts, as for instance in the Fig. 2(d) data, can be easily followed as temperature changes. Differences in band shapes due to distinct sample preparation procedures may be detected as well.

Discussion

The two facts, i) absorbances and bandwidths vary smoothly during the cooling down and warming up, and ii) peak shapes are restored after the excursions, strongly suggest that phase change is not occurring. In other words, the absence of permanent modification on the crystalline structure as a function of temperature is evidence that no major molecular rearrangement happens. Nevertheless, when the sample temperature decreases, three relevant questions remain: why bandwidths decrease, why absorbances increase, and why the wavenumber of some bands shifts?

Lowering the temperature, two distinct thermal effects should be recognized:

i) Velocity effects: the mean velocities of molecules are reduced, turning narrower the absorption bandwidths. This should answer, at least partially, the first question.

ii) Geometric effects: the mean distances of atoms and molecules are reduced, changing the strengths of the multiple intermolecular interactions; van der Waals forces become stronger. The absorbance variation is related to these phenomena.

Analyzing, in detail, the two effects:

i) The absorption bands analyzed by FTIR are located in the mid-wavelength infrared, which corresponds to

60–500 meV photon energies; bandwidths are typically 30 cm^{-1} , that is, 4 meV. Sample temperatures in the current experiment varied from 40 to 300 K, corresponding to kT variations in the 3.4–26 meV range. The fact that bandwidth energies and sample thermal energies are similar indicates that the latter should be responsible for a significant fraction of the observed bandwidths. An approach to understand how this energy matching operates is reminding that photon absorption is a resonant process (as in the Mossbauer effect) and that thermal velocity distribution depends on temperature; Doppler effect may be then invoked to explain why the energy matching is less strict for absorption at high temperatures. Another approach is considering that apolar molecules free to rotate have zero time-average polarizability; cooling down the sample imposes constraints on the molecular rotation, increasing the molecular polarizability.

ii) Infrared absorbance depends on the dipole moment, which in turn depends on temperature. Lowering the temperature, the intermolecular distances decrease, external fields due to neighbor molecules increase and molecular polarizability may also increase.

The wavenumber shift may be caused by transitions between isomers, more specifically between conformers. Results reported on glycine and alanine spectroscopy at different temperatures enlighten this question. The phenomenon of the deprotonation of the carboxylate group, producing the protonation of the amino radical, and vice-versa has been studied for glycine by Gomez-Zavaglia and Fausto [2]. These authors proposed that the kinetic energy of the molecules in the gas phase, delivered in the condensation process, is used to transform irreversibly a fraction of the deposited neutral glycine molecules into their zwitterionic isomers. By heating the sample, the conversion is completed and all the molecules become zwitterions. Rodríguez-Lazcano *et al.* [3] conducted similar measurements with alanine and basically obtained the same conclusions: alanine in the gas phase is neutral but as a solid at room temperature is zwitterionic; when deposited at 25 K, the molecules in the sample are constituted by a mixture of neutral and zwitterionic forms; the neutral isomers migrate to zwitterions as the sample is heated. In order to study spectra of amino acids in which lines due to different conformers are separated by few wavenumbers, UV irradiation or annealing can be used to modify the conformational ratios of sample molecules. Wong *et al.* [14] analyzed theoretical and experimentally several conformers of β -alanine isolated in parahydrogen matrix; they did confirm that conformational transitions when the material was exposed to UV radiation. Tamulienè *et al.* [15] studied the valine fragmentation in the gas phase by low energy electrons and by DFT calculations; they determined the total energies for four valine conformers, neutral and ionized.

Assuming that a similar process happens for valine, and once in the current work the deposition was performed

at room temperature, the analyzed sample should be formed only by zwitterionic valine molecules. Warming up to 400 K or cooling down to 40 K did not alter the isomeric structure, a situation consistent with the statement that no phase transition had occurred in these thermal excursions. The band shifts observed in Figs. 2(c) and 2(d) may be interpreted by conformational transitions connected with the variation of the sample temperature.

From data presented in Fig. 4, it is clear that linewidths remain finite for all bands even at zero Kelvin. Such bandwidth levelling off at cryogenic temperatures should be attributed to the non-uniform chemical environment experienced by the valine molecules: the sample is not monocrystalline, being probably a mixture of amorphous and polycrystalline structures.

Conclusions

A thin film of the organic molecule valine is analyzed by FTIR technique in the 40–300 K temperature range. Typically, the absorbance peak resolution is a factor 2 better at 40 K as compared with that at 300 K. The bandwidth reduction at low temperatures is mostly due to the kinetic energy reduction of the sample atoms.

The dependence of the absorbance peak shape (intensity and/or width) on temperature allows the use of infrared spectroscopy as a rough thermometer. This procedure may be useful for measuring the temperature of astrophysical grains with amino acid content.

Infrared spectroscopy uses the Beer–Lambert Law to extract column densities, which requires that A -values (band strengths) are known. Data presented in the current article show that A -values are temperature dependent; however, the absorbance thermal rate coefficient does not depend on the vibrational band. For instance, the absorbances at 40 K compared to that at 300 K, are $\sim 40\%$ and 70% higher for the 1272 and 1140 cm^{-1} bands, respectively.

The valine sample analyzed in the current experiments should be completely in the zwitterion form if physical-chemical properties of valine are similar to those of glycine and alanine. The zwitterion form was kept during the 300–40–400–40–300 K thermal excursion. No phase change has been observed.

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Інфрачервоне поглинання валіну при криогенних температурах

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Амінокислоту *L*-валін було досліджено за допомогою інфрачервоної фур'є-спектроскопії (FTIR) в інтервалі температур 40–300 К. Зразок валіну осаджували на підкладку з ZnSe, охолоджували до 40 К та нагрівали до 300 К, далі відпалювали при 120 °С протягом 12 годин, охолоджували до 40 К і знову нагрівали до 300 К. FTIR спектри було отримано в режимі пропускання під час цієї теплової еволюції. Було відзначено наступне: фазовий перехід не спостерігається;

з пониженням температури інтенсивність смуг поглинання лінійно зростає, а смуги звужуються; інтегральна оптична густина (площа піка) при 40 К більше на 40–100% у порівнянні зі значеннями при 300 К, а ширина смуги становить близько половини; FTIR спектри відпалених та невідпалених зразків поведуться аналогічно. Було встановлено, що зразок складається тільки з цвіттер-іонів валіну; зменшення ширини смуги частково пояснюється ефектом Доплера; збільшення поглинання при низьких температурах викликано посиленням взаємодії Ван-дер-Ваальса. Очікується, що для інших амінокислот ці загальні висновки будуть аналогічними.

Ключові слова: амінокислота *L*-валін, FTIR спектри, цвіттер-іони.

Инфракрасное поглощение валина при криогенных температурах

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Аминокислота *L*-валин была исследована с помощью инфракрасной фурье-спектроскопии (FTIR) в интервале температур 40–300 К. Образец валина осаждали на подложку из ZnSe, охлаждали до 40 К и нагревали до 300 К, далее отжигали при 120 °С в течение 12 ч, охлаждали до 40 К и снова нагревали до 300 К; FTIR спектры были получены в режиме пропускания во время этой тепловой эволюции. Было отмечено следующее: фазовый переход не наблюдается; с понижением температуры интенсивность полос поглощения линейно растёт, а полосы сужаются; интегральная оптическая плотность (площадь пика) при 40 К больше на 40–100% по сравнению со значениями при 300 К, а ширина полосы составляет около половины; FTIR спектры отожженных и неотожженных образцов ведут себя аналогично. Было установлено, что образец состоит только из цвиттер-ионов валина; уменьшение ширины полосы частично объясняется эффектом Доплера; увеличение поглощения при низких температурах вызвано усилением взаимодействия Ван-дер-Ваальса. Ожидается, что для других аминокислот эти общие выводы будут аналогичными.

Ключевые слова: аминокислота *L*-валин, FTIR спектры, цвиттер-ионы.