

Low temperature FTIR spectroscopy and hydrogen bonding in cytosine polycrystals

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Abstract

The FTIR spectra of both the pure NH and isotopically substituted ND (<10% and >90% D) polycrystalline cytosine were recorded in the range 400–4000 cm⁻¹ as a function of temperature (10–300 K). For the first time, uncoupled NH(D) stretching mode bands of amine and imine groups were observed in the spectra of isotopically diluted cytosine at low temperatures. These bands correspond to the three distinct H-bonds that are present in the crystal, in agreement with the available data obtained by structural methods. At least nine bands were observed below 1000 cm⁻¹ and, in consonance with their temperature and isotopic exchange behavior, were assigned to the NH proton out-of-the-plane bending modes. Six of these bands were found to correspond to additional “disordered” H-bonds, which could not be observed by structural methods. Empirical correlations of spectral and thermodynamic parameters enabled to estimate the contribution of the H-bonds to the sublimation enthalpy of the crystal, in agreement with independent experimental data.

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1. Introduction

Cytosine is one of the constituents of nucleic acids and it has been studied intensively by infrared spectroscopy in the free monomeric state—in the gas phase [1] or in low temperature inert matrices [2–4]—and crystalline phase both at room [3,5,6] and low temperatures [7]. The vibrational spectrum of cytosine was also predicted theoretically at different levels of approximation, in order to clarify the assignment of the experimental spectra of either the monomer or the crystal [3,4,7,8].

In the crystalline state, cytosine adopts its amino–oxo tautomeric form (Fig. 1): four planar molecules are arranged into sheets exhibiting a network of H-bonds involving their –NH₂, –N₍₁₎H, –N₍₃₎= and C=O groups [9,10]. In the case of the isolated cytosine molecule, the dominant tautomer was found to be the amino-hydroxy form [1–4]. The preference for different tautomeric forms in the crystal and for the isolated molecule is a clear indication of the importance of

the intermolecular interactions, in particular H-bonding, to determine the structure of the condensed phase. However, despite the chemical and biological relevance of cytosine and all the effort already put in the study of this compound, the understanding of the dominant H-bond interactions present in the crystalline state still requires further investigation. For example, no studies have yet been reported where a detailed spectroscopic analysis of the bands due to the stretching and out-of-the-plane bending modes of imine [–N₍₁₎H] and amine [–NH₂] groups of cytosine is done, despite the well known connection of the spectral properties exhibited by these bands with the H-bond properties—e.g. H-bond energy and length [11].

In crystals with differently bound multiple NH– groups the bands due to the stretching (ν_1) and out-of-the-plane bending (ν_4) modes (NH proton modes) are usually broad and extensively overlapped. However, at low temperature, the broadening originated from the interaction of both ν_1 and ν_4 modes with thermally excited low frequency intermolecular deformational modes (librations) can be suppressed [12]. On the other hand, in isotopically doped crystals, the vibrational coupling between neighboring H-bonds in the differently organized H-bonded chains is removed.

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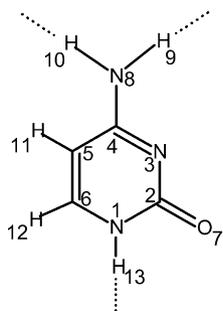


Fig. 1. The molecular structure of amino-oxo cytosine structure as it exists in crystal.

Thus, in the low temperature spectra of isotopically doped crystals the spectral resolution is in general remarkably improved, the bandwidth decreasing from 200 to less than 10 cm^{-1} in both ν_1 and ν_4 spectral ranges [13–15]. Hence, the ν_1 and ν_4 bands can afterwards be correlated with the different types of H-bonds existing in the crystal. Note that very often, the number of H-bond contacts found by infrared spectroscopy is larger than that obtained by structural methods such as X-ray diffraction, due to the sensitivity of the former method to short range interactions as compared with the long range periodic order seen by the latter techniques [16]. This fact further reinforces the relevance of using spectroscopic methods to investigate H-bonding characteristics of crystalline phase.

A very important property exhibited by ν_4 is that, in contrast to ν_1 , it gives rise to bands that do not change their molar integral intensity with the H-bond energy and thus they remain narrow and well resolved independently of the strength of the H-bond interaction [11]. Consequently, ν_4 can be used for a direct quantitative estimation of the relative abundance of different H-bonds in a given crystal [16,17]. On the other hand, the peak positions of the bands due to both ν_1 and ν_4 are sensitive to the H-bond energy (and, consequently, to the H-bond geometry) and can be used to estimate this property on the basis of simple empirical quantitative relationships [11,18–20].

In this study, the FTIR spectra of both the pure NH and isotopically substituted ND (<10 and >90% D) polycrystalline cytosine were recorded in the range $400\text{--}4000\text{ cm}^{-1}$ as a function of temperature (10–300 K). This approach enabled to obtain new relevant information on the hydrogen bonding in the crystal as well as to estimate the contribution of H-bonds to the sublimation enthalpy of the crystal.

2. Experimental

The FTIR spectra of polycrystalline pure NH- (Sigma), 5–10% ND- and 90–95% ND-cytosine in a KBr (1:200) disc, attached to the cold finger of an APD Cryogenics closed-cycle helium refrigeration system with a DE-202A

expander, were recorded with a Mattson Infinity 60AR series FTIR spectrometer, with spectral resolution of 1 cm^{-1} . The temperature (10–300 K) was measured directly at the sample holder by a silicon diode temperature sensor connected to a Lake Shore Cryogenics temperature controller (model DRC-91CA). The sample temperature during registration of spectra was stabilized to within 0.2 K. The temperature-induced spectral changes observed for all substances were found to be reversible and highly reproducible. Deuterated samples were obtained from commercial cytosine by exchange with D_2O (Aldrich) in recirculating cyclohexane at 81°C , as described in [21].

3. Results and discussion

3.1. NH and CH stretching vibrations spectral region ($3500\text{--}1800\text{ cm}^{-1}$)

Interpretation of the vibrational spectra of crystalline cytosine is strongly facilitated by the fact that in this phase cytosine exists in the amino-oxo form [9,10], which exists in the unique conformation shown in Fig. 1.

The spectrum of ND-cytosine (>90% ND)¹, in the range $3000\text{--}1800\text{ cm}^{-1}$, is shown in Fig. 2 together with the spectrum of ND-cytosine doped NH-cytosine crystal (ND-cytosine <10%) and that of commercial pure NH-cytosine. The peak positions of the bands are given in Table 1, together with their isotopic ratios.

The spectra now obtained for cytosine closely match those previously reported [7]. However, the isotopic effects and temperature dependence observed for several bands deserve to be analyzed in detail here.

The assignment of the intense narrow bands observed at 3368 and 3167 cm^{-1} in both the spectra of the pure NH-cytosine and ND-cytosine doped NH-crystals (traces 1–3 in Fig. 2) to the amine stretching asymmetric and symmetric modes ($\nu\text{NH}_2\text{ asym}$ and $\nu\text{NH}_2\text{ sym}$) is straightforward [3,5–7]. These bands have their ND₂ counterparts in the spectrum of ND-cytosine at 2532 and 2332 cm^{-1} (trace 3 in Fig. 2). The assignment of the broad band with maximum at ca. 2686 cm^{-1} , which is also observed for both NH-cytosine and ND-cytosine doped NH-crystals (which shifts to ca. 2080 cm^{-1} in ND-cytosine), to the $\text{N}_{(1)}\text{H}$ ($\text{N}_{(1)}\text{D}$) stretching mode is also doubtless [3,7]. It should be noted that this last band was not assigned in [5,6], where the imine $\text{N}_{(1)}\text{H}$ ($\text{N}_{(1)}\text{D}$) stretching mode was assigned either to the 3169 (2337) [5] or 3356sh cm^{-1} [6] band. The considerably low frequency of the band now ascribed to the imine stretching mode as well as its peculiar profile show the extensive involvement of the $\text{N}_{(1)}\text{H}$ group in the H-bonding network of the crystal.

¹ In fact, this sample corresponds to a NH-cytosine doped ND-crystal where a few (less than 10%) NH-cytosine molecules are dispersed into a ND-cytosine crystal.

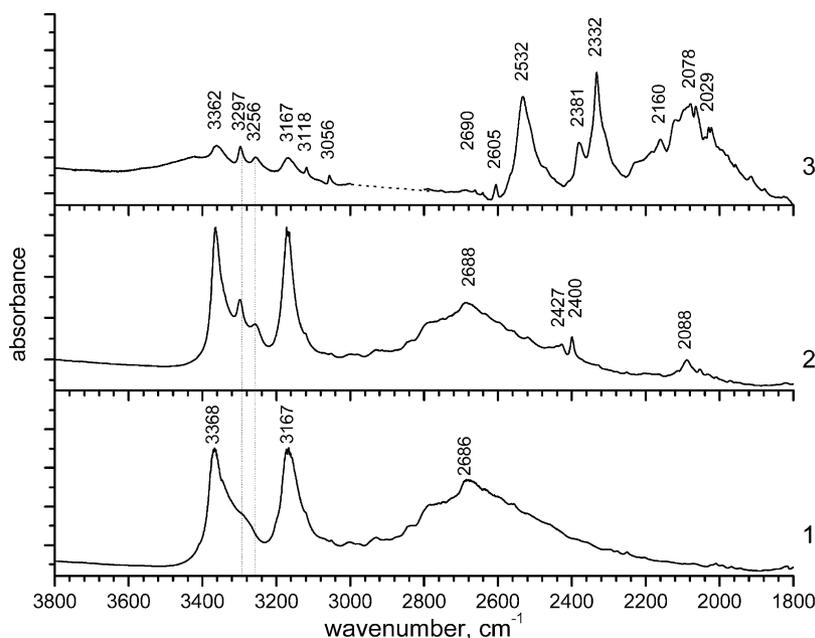


Fig. 2. FTIR spectra of crystalline cytosine and deuterated cytosine, at 10 K, in the NH/ND stretching spectral regions: (1) commercial pure NH-cytosine; (2) NH-cytosine crystal, containing less than 10% ND-cytosine molecules; (3) ND-cytosine crystal, containing less than 10% of NH-cytosine molecules; the dotted line—the range 3000–2800 cm^{-1} , which is obscured by cyclohexane contamination resulting from deuterium exchange.

On the other hand, the spectra now obtained for the doped crystals at low temperature reveal several new bands that were observed for the first time and are ascribable to cytosine molecules with the amino group exhibiting a mixed isotopic composition—NHD. The presence of the deuterium atom in the randomly H→D mono-substituted amine group breaks the local symmetry of the NH_2 group, suppressing the resonance interaction between the stretching vibrations associated with the two individual NH bonds, and the uncoupled NH-mode bands can then be observed for each proton of the amino group. These bands are shown in Fig. 2

(traces 2 and 3) at 3297 and 3256 cm^{-1} , between the two intense bands due to the NH_2 stretching vibrations, and are more clearly shown in Fig. 3, where their temperature dependence is also presented. The two corresponding decoupled individual ND-bands can be observed in the spectrum of ND-cytosine doped NH-crystal (see Fig. 2, trace 2) at 2427 and 2400 cm^{-1} . The presence of two bands due to the uncoupled stretching vibrations of the NHD group in both the NH and ND stretching spectral regions correlates with the two non-equivalent positions occupied by the amine group protons [9,10]. Taking into consideration the lengths of the

Table 1

Peak frequencies, band widths (in parentheses) and temperature shifts on cooling from 300 to 10 K {in figure brackets} (in cm^{-1}) of the stretching $\nu(\text{NH})$ and $\nu'(\text{ND})$ bands in pure and isotopically diluted cytosine crystals

ν (100%H)	ν, ν' (<10%D)	ν, ν' (>80% D)	Assignment	Isotopic ratio $\nu_{\text{NH}}/\nu'_{\text{ND}}$
3368 (60)	3364 (27) {15}	3362 (34) {15}	ν_{as} (NH_2)	1.330
	3298 (12) {12}	3297 (11) {13}	$\nu_{\text{decoupled}}$ ($\text{NH}_{(9)}\text{D}$)	1.358
	3258 (25) {10}	3256 (24) {10}	$\nu_{\text{decoupled}}$ ($\text{NH}_{(10)}\text{D}$)	1.358
3166 (50)	3172 (38) {−1}	3167 (40) {−1}	ν_{s} (NH_2)	1.330
		3118 (10) {−3}	ν (CH)	
		3056 (7) {0}	ν (CH)	
2690 (250)	2700 (250) {70}	2690 (50)	ν (N_1H)	1.294
		2605 (10) {−6}	$\nu_{\text{decoupled}}$ (N_1H)	1.290
		2532 (52) {11}	2δ (ND)?	
	2427 (34) {27}	–	ν'_{as} (ND_2)	
	2400 (10) {0}	–	$\nu'_{\text{decoupled}}$ ($\text{NH}_{(9)}\text{D}$)	
		–	$\nu'_{\text{decoupled}}$ ($\text{NH}_{(10)}\text{D}$)	
		2381 (27) {0}	ν'_{s} (ND_2)	
		2332 (26) {3}, 2307 (47)	2δ (ND_2)	
	2088 (35) {46}	–	$\nu'_{\text{decoupled}}$ (N_1D)	
		2078 (150) {60}	ν' (N_1D)	

Italic font—results from band deconvolution.

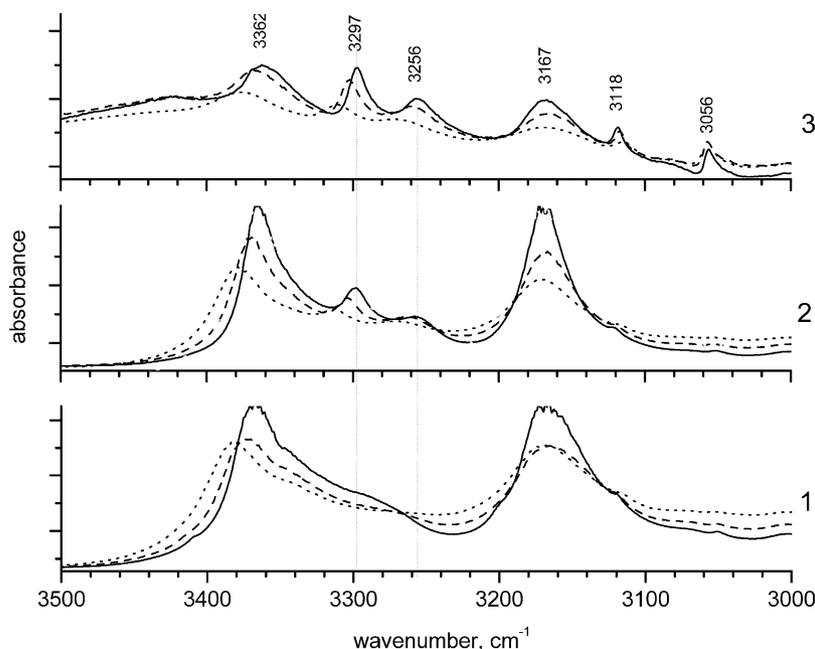


Fig. 3. FTIR spectra in the $-\text{NH}_2$ stretching spectral region of crystalline cytosine at different temperatures. (1) Commercial pure NH-cytosine; (2) NH-cytosine crystal, containing less than 10% ND-cytosine molecules; (3) ND-cytosine crystal, containing less than 10% of NH-cytosine molecules; Dotted line—300 K, dashed line—150 K, solid line—10 K.

two hydrogen bonds established by the amino group [10] these bands can be ascribed to the stretching vibration of $\text{N}_{(8)}\text{H}_{(9)}$ and $\text{N}_{(8)}\text{H}_{(10)}$, respectively (the lower frequency of the $\text{N}_{(8)}\text{H}_{(9)}$ stretching correlating with the shorter H-bond involving this proton). It should also be noticed that the isotopic frequency ratios of the uncoupled bands (1.358) are closer to the harmonic value than those corresponding to the νNH_2 *asym* and νNH_2 *sym* bands (1.330, see Table 1). Furthermore, in the studied molecules the resonance interaction in the amine group conceals the H-bond and temperature effects on the bands and considerably “pushes apart” the vibrational levels. Indeed, the differences between ν_{as} and ν_s in both NH_2 and ND_2 groups are ca. 150–200 cm^{-1} , while the distance between the two bands due to the uncoupled vibrations of the NHD group, which result from the non-equivalence of the two N–H bonds, is only 30–40 cm^{-1} (see Table 1).

Regarding their temperature dependence, the bands due to the uncoupled vibrations show the usual narrowing and red shift on cooling, which have been found to be characteristic of NH vibrations in H-bonded groups [11,14]. It should then be noted that our experiments do not support the idea that “... the variation of the high wavenumber region spectrum (above 2000 cm^{-1}) of cytosine, in going from 20 K to room temperature, is negligible” [7].

In the case of the ND-cytosine crystal, the $\text{N}_{(1)}\text{H}$ band due to the minor non-deuterated species could be clearly observed only at the lowest temperatures attained in this study (ca. 10 K), corresponding to the very weak band observed at 2690 cm^{-1} . The corresponding $\text{N}_{(1)}\text{D}$ vibration of the ND-cytosine molecules in the NH-crystal can be observed

at 2088 cm^{-1} (Fig. 2, trace 2); when compared with the equivalent band in the spectrum of the ND-cytosine crystal (Fig. 2, trace 3) this band is considerably narrower and its peak position can then be measured more accurately.

The two CH stretching infrared bands of cytosine were now clearly observed for the first time in the spectrum of ND-cytosine at 3118 and 3056 cm^{-1} (Figs. 2 and 3 (trace 3); see also Table 1), in agreement with Raman data [7]; in the infrared spectra of NH-cytosine these bands are hidden behind the most intense bands due to the NH vibrations.

It is also interesting to compare the results now obtained with previously obtained theoretical vibrational calculations (MP2/6-31G* calculations scaled to fit the low temperature condensed phase data collected for cytosine and ND-cytosine [7]). In the case of the CH stretching modes, the agreement between the experimental and calculated data is very good, with the calculated CH peak positions being 3113 ($\nu\text{C}_{(5)}\text{--H}$) and 3087 cm^{-1} ($\nu\text{C}_{(6)}\text{--H}$). For the νNH_2 *asym* mode, the calculated frequency (3358 cm^{-1} [7]) is almost coincident with the experimental value (3364 cm^{-1}), while the theoretical estimation for the νNH_2 *sym* mode (3240 cm^{-1} [7]) strongly deviates from the observed frequency (3172 cm^{-1}). This discrepancy can be ascribed to the general difficulty the theoretical calculations show to account properly for significantly strong effects of H-bonding interactions on the vibrational frequencies. Accordingly, in cytosine this weakness is more important to the prediction of the frequency of the νNH_2 *sym* mode when compared with νNH_2 *asym*, in consonance with the stronger sensitivity of the former vibration to intramolecular H-bonding interactions.

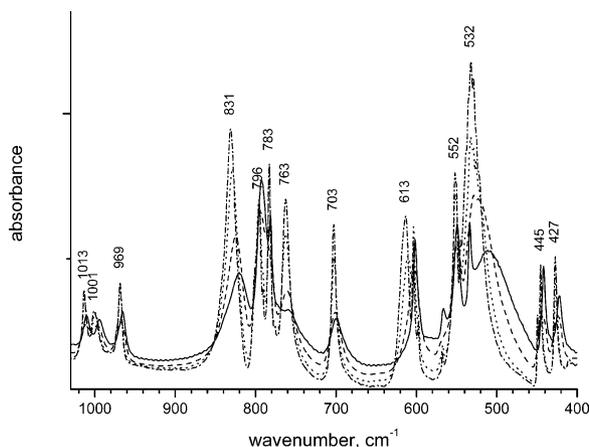


Fig. 4. Temperature dependence of commercial pure NH-cytosine in the 400–1000 cm^{-1} spectral region: 300 K, solid; 200 K, dashed; 100 K, dotted and 10 K, dash-dotted lines.

3.2. NH out-of-the-plane bending vibrations spectral region ($<1000 \text{ cm}^{-1}$)

The temperature dependence of the spectrum of pure NH-cytosine in the ν_4 mode spectral region, on cooling from 300 to 10 K, is shown in Fig. 4. There are three relatively intense bands (appearing at 831 cm^{-1} , 613 and 532 cm^{-1} in the spectrum collected at 10 K) that are affected upon temperature decrease and disappear in the spectrum of the deuterated sample. There are also six bands (1001, 969, 763, 703, 613 and 532 cm^{-1} at 10 K) that undergo considerable narrowing on cooling. Thus, all these bands should be assigned to proton modes of either imine or amine groups. Red-shifted counterparts of these bands, with isotopic frequency ratios within the range 1.33–1.40 (see Table 2), can be found in the spectra of ND-cytosine, which

Table 2

Infrared absorption bands (peak frequencies, cm^{-1}) of pure and isotopically diluted cytosine polycrystals at 10 K, assigned to the out-of-the-plane proton (ν_4) and deuterium (ν_4^D) modes on the basis of their temperature and isotopic behavior; their isotopic ratios (ν/ν'), relative abundances (%) and estimated H-bond energies ($-\Delta H$, kJ mol^{-1})

ν	ν'	ν/ν'	Abundance	$-\Delta H$ (from $\Delta\nu_1$) ^a
1013	–	–	4	50
1001	719	1.391	3	48
969	698	1.388	3	44
831	616	1.350	18	(36.0)
796	598	1.331	8	24.0
763	544	1.401	7	20.0
703	509	1.382	7	14.0
613	454	1.352	10	(20.0)
532	<400	>1.33	40	(16.0)

^a For absorption bands at 831, 613 and 532 cm^{-1} values of $-\Delta H$ were calculated using red shift of ν_1 . For the remaining bands the values of $-\Delta H$ are estimated from the peak positions of ν_4^H (Eq. (2)). Since it is unknown from which protons (amine or imine) the extra H-bonds originate, the value of ν_4^0 in this case accepted as the mean value of amine and imine -530 cm^{-1} .

are shown in Fig. 5 (trace 3). The intense band at 831 cm^{-1} undoubtedly belongs to the out-of-plane proton mode of the imine group [3,5] and upon deuteration shifts to 616 cm^{-1} . This shift masks disappearance of the band of NH-cytosine at 613 cm^{-1} upon deuteration, which shifts to 454 cm^{-1} in the deuterated species. The intense band at 532 cm^{-1} shifts upon deuteration to a position centered below 400 cm^{-1} , the wing of this band is clearly visible in Fig. 5, trace 3. Note that the band at 532 cm^{-1} in non-deuterated cytosine partially overlaps with skeletal bands, in particular with the skeletal bands lying between 532 and 552 cm^{-1} (see Fig. 5, trace 1), which results in the high apparent intensity of the whole group. We propose new assignments for the bands discussed above. The bands appearing at 613 and 532 cm^{-1} should be assigned to the two differently H-bonded protons of the amine group. It should be noticed that while the band at 532 cm^{-1} (500 cm^{-1} at 300 K) was previously [3,5] assigned to the mixed NH_2 torsion and wagging modes (without considering H-bonding effects), no assignment was previously reported for the band at 613 cm^{-1} .

It has been shown before for complexes with linear H-bonds that the red shift of the NH stretching mode, ν_1 , and the blue shift of the corresponding out-of-the-plane bending mode, ν_4 , may be empirically correlated, because both parameters correlate with the H-bond energy [20]. The empirical correlation has the form:

$$\Delta\nu_4^2 \text{ cm}^{-2} = 2.5(\Delta\nu_1 \text{ cm}^{-1})^{1/2} - 18, \quad (1)$$

where $\Delta\nu_4^2 \equiv (10^{-2}\nu_4^H)^2 - (10^{-2}\nu_4^0)^2$ and $\Delta\nu_1 = \nu_1^H - \nu_1^0$, the superscripts “H” and “0” pertaining to H-bonded and free molecules, respectively. Thus, it is possible to estimate the position of the ν_4 bands that correspond to the vibrations observed in the ν_1 region and are associated with a given H-bond.

For cytosine, the reference frequencies of the free molecules, ν_1^0 , were taken from the spectra of the amino–oxo conformer isolated in a low temperature argon matrix: for the imine proton, $\nu_1^0 = 3472 \text{ cm}^{-1}$, and for incompletely deuterated amine group ($\text{H}_{(10)}$) or ($\text{H}_{(9)}$) the decoupled values are 3527 and 3492 cm^{-1} [4]. The reference value here adopted for the ν_4^0 mode of the imine proton is 614 cm^{-1} , which was also taken from the matrix isolation spectrum [8]. In the case of the amine protons the choice of a ν_4^0 reference value was slightly more complicated, since (i) the absorption bands of amino–oxo conformer in this region are very weak and as a result (ii) their assignment in matrix spectra is controversial [2–4,8]. For the monomer there are three bands in the matrix isolation spectrum of cytosine that were reported to correspond to vibrations with significant contributions from the amine out-of-the-plane bending modes, namely those observed at 568, 535 and 235 cm^{-1} [8]. The average frequency of these three bands (446 cm^{-1}) was used here as the ν_4^0 reference value for the NH_2 group.

Using the empirical relationship (1) with the chosen reference values, the predicted frequency of the imine

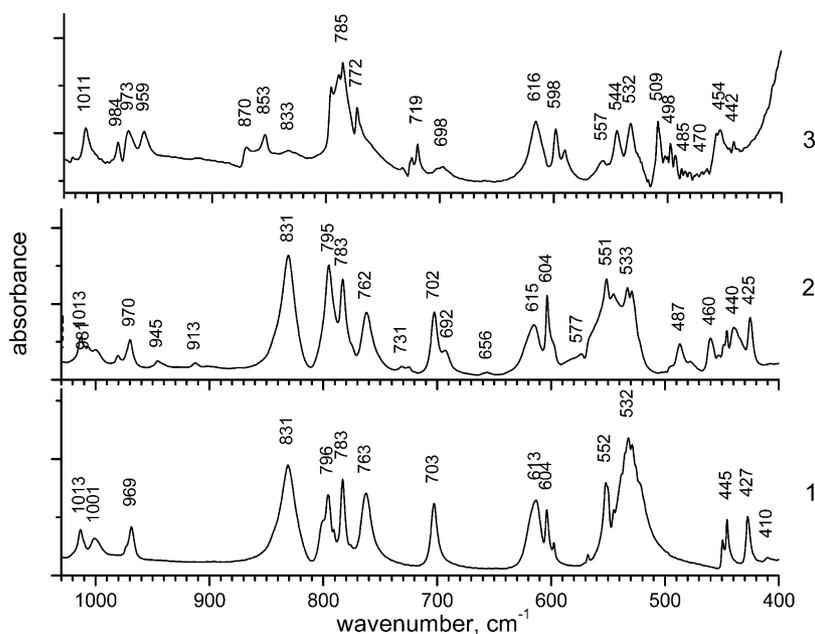


Fig. 5. FTIR spectra of crystalline cytosine and deuterated cytosine, at 10 K, in the 400–1000 cm^{-1} spectral region: (1) commercial pure NH-cytosine; (2) NH-cytosine crystal, containing less than 10% ND-cytosine molecules; (3) ND-cytosine crystal, containing less than 10% of NH-cytosine molecules.

proton ν_4 mode in the crystal is 930 cm^{-1} , while those corresponding to the two ν_4 vibrations related to the uncoupled amine stretching bands are 640 ($\text{H}_{(10)}$) and 593 ($\text{H}_{(9)}$) cm^{-1} . Despite the estimated imine proton band is higher than the experimental value (831 cm^{-1} ; see Table 2 and Fig. 4), the empirical correlation clearly indicates that this band should be observed at a considerably higher frequency than those assigned to the amine out-of-the-plane vibrations.

The results obtained from the empirical correlation give also further support to our assignments of the bands observed at 831, 613 and 532 cm^{-1} to the ν_4 mode, based on their typical temperature and isotopic behavior characteristic of predominantly H-bonded proton modes [11,13–17,19,20,22,23]. Our assignments strongly differ from those made previously. For example, the band at 831 cm^{-1} (827 cm^{-1} in [7]) was attributed mainly to the CH wagging mode [7].

As mentioned before, the bands observed at 796, 763 and 703 cm^{-1} are also affected by deuteration and sensitive to temperature (see Fig. 4). The data shown in Fig. 5 (traces 1 and 3) clearly demonstrate that all these bands disappear upon deuteration and must be associated predominantly with proton modes. We assign these bands in cytosine spectra to the out-of-the-plane mode vibrations of protons that are essentially non-ordered in the crystal, as it was found previously for other systems [16,17].

Compared with the spectrum of the commercial pure NH-cytosine, in the ν_4 spectral region the spectrum of the ND-cytosine doped NH-crystal shows nine new weak bands at 981, 945, 913, 731, 692, 656, 487, 460, 440 cm^{-1} , that can only be clearly seen at low temperature (Fig. 5, com-

pare traces 1 and 2). These bands could be, in principle, assigned to decoupled ν_4 modes of either the proton or deuteron of the $-\text{NDH}$ group or to the $-\text{N}_{(1)}\text{D}$ group. However, comparing the spectrum of the ND-cytosine doped NH-crystal with that of the 90% deuterated sample (traces 2 and 3 in Fig. 5), one can conclude that all these bands should be mainly related with proton modes, since they do not become more intense in the spectrum of the (almost) ND-sample. The corresponding deuteron bands could not be seen under the experimental conditions used because of the intrinsic low intensity of the deuteron vibrations, which are twice weaker than that of the corresponding proton modes.

The frequencies of the observed ν_4 bands, which are blue shifted when compared with the amine and imine out-of-the-plane bands observed for monomer of the matrix-isolated cytosine (vide supra), show that all these bands are yielded by protons involved in hydrogen bonds of different strength (bands below 600 cm^{-1} are related to protons of the amine group, which participates in weaker H-bonds than the imine moiety). The number of different H-bonds revealed spectroscopically is considerably larger than that expected from the data obtained by conventional structural methods (such as X-ray diffraction), which yield only three different H-bond types [9,10].

As mentioned in Section 1, because the molar integral intensities of the ν_4 bands do not depend from their energy (peak position) ([11], v. II, p. 595] they can be used directly to estimate the relative abundance of the different H-bonds present in a given crystal. From the data shown in Table 2 it can then be concluded that the three main (periodically “ordered”) H-bonds previously detected by structural

methods correspond only to 68% of the H-bond total population. The “disordered” H-bonds have relative abundances ranging from 3 to 10%.

Note that ν_4 bands may have no visible counterpart in the ν_1 mode spectral region, because of the fact that the intensity and width of ν_1 bands depend upon the H-bond energy [18] in such a way that its observation may be impossible (the bands are either narrow but too weak-for weak H-bonds, or intense but too broad-for strong H-bonds).

3.3. Estimation of hydrogen bond distances from the observed ν_1 red shifts

It was previously found [24] that the observed red shifts, $\Delta\nu$, of the ν_1 bands (cm^{-1}) correlate with the H-bond distances (nm) as: $\Delta\nu = 0.011r^{-6.1}$. Using the ν_1 red shifts observed for crystalline cytosine (and corrected to room temperature), the H \cdots O(N) H-bond distances were estimated from this relationship as: $r_{\text{NH}(10)\cdots\text{O}} = 0.193$ nm, $r_{\text{NH}(9)\cdots\text{O}} = 0.204$ nm, $r_{\text{N}(1)\text{H}\cdots\text{N}(3)} = 0.163$ nm. The corresponding crystallographic data [9,10] are $r_{\text{NH}(10)\cdots\text{O}} = 0.212$ nm, $r_{\text{NH}(9)\cdots\text{O}} = 0.214$ nm, $r_{\text{N}(1)\text{H}\cdots\text{N}(3)} = 0.186$ nm. The agreement between the two sets of data can be considered as reasonable in the case of the H-bonds established by the two amine protons, while the empirical correlation considerably underestimates the H-bond distance associated with the imine group.

3.4. Estimation of the H-bond energies from the red shift of ν_1 and blue shift of ν_4

It is known [18] that the H-bond energy (in kJ mol^{-1}) and the red shift ($\Delta\nu_1$ in cm^{-1} , see Eq. (1)) of ν_1 band correlate as $(\Delta H)^2 = 1.92[(\Delta\nu) - 40]$, at room temperature. Using this relationship, the energies of the three “ordered” H-bonds present in crystalline cytosine were estimated as equal to ca. -16.0 , -20.0 and -36.0 kJ mol^{-1} , respectively, for the two amine NH \cdots O ($\Delta\nu = 176$ (H9) and 244 (H10) cm^{-1}) and for imine NH \cdots N bonds ($\Delta\nu = 700$ cm^{-1}). To account for the temperature correction all these shifts were reduced by 10% [16]. The energies of the H-bonds established by the amine protons are then reasonably stronger than those associated with the dimerization process of cytidine [25], obtained from the temperature dependence of association constants in solution: -13.3 ± 2.5 kJ mol^{-1} per one bond².

An approximate estimation of the energy of the “disordered” H-bonds in cytosine crystal can be done with the empirical relationship between the H-bond energy (kJ mol^{-1}) and the blue shift of the ν_4 mode relatively to the

frequency of the same mode in the non hydrogen bonded group [19]:

$$-\Delta H = 0.67 \times 10^4 \Delta\nu_4^2 \quad (2)$$

where $\Delta\nu_4^2$ is defined as in equation (1). Using the above correlation, values ranging from -50 to -20 kJ mol^{-1} were obtained (see Table 2) for the H-bond energies. Using equation (2) we estimated the contribution of H-bonding to the sublimation heat of crystalline cytosine. Uncertainty of this estimation amounts to ca. 10%.

The standard sublimation enthalpy was measured for cytosine as -167 ± 10 [1] and -150 kJ mol^{-1} [26]. If the crystals were “ideal” and contained only three different types of H-bonds, the sum of these three H-bond energies—their contribution in the sublimation heat—would amount to -72 kJ mol^{-1} . However, accounting for the abundance of all H-bonds now detected spectroscopically and taking into consideration their proportional contribution the total H-bonding energy (see Table 2), the total H-bonding contribution to the sublimation heat is equal to only -24.0 kJ mol^{-1} , or ca. 30% of the estimation assuming the “ideal” crystal. The experimentally measured [26] sublimation heats of cytosine, N₍₁₎-methyl cytosine and N_{(1),N(8)}-dimethyl cytosine are -150 , -142 and -132 kJ mol^{-1} , respectively. From these values one can roughly estimate energy of each individual imine and amine hydrogen bond. To do this, it must be assumed that the differences in the heats of sublimation of N₍₁₎-methyl cytosine and N_{(1),N(8)}-dimethyl cytosine, when compared with that of cytosine, are essentially determined by the absence of the H-bond involving the $-\text{N}_{(1)}\text{H}$ group (in N₍₁₎-methyl cytosine) and of this group and one of the H-bonds established by the $-\text{NH}_2$ group (in N_{(1),N(8)}-dimethyl cytosine). Thus, the total energy stored in H-bonds in cytosine crystal estimated from [26] is ca. -28 kJ mol^{-1} . This number, obtained from an independent thermodynamic experiment, coincides with our estimation (-24 kJ mol^{-1}) and then supports our suggestion pointing to the existence, in the crystal, of additional “unordered” H-bonds, in addition to those revealed from structural data.

4. Conclusion

This study demonstrates the utility of combining isotopic exchange techniques and cooling to low temperature to extract detailed information on H-bonding properties from infrared analysis of both ν_1 and ν_4 proton spectral regions of crystals with complex H-bonding networks. For the first time, observation and assignment of the infrared bands due to different types of H-bonds present in the cytosine crystal was undertaken. The bands of both amine and imine proton decoupled stretching modes were identified for the first time in isotopically diluted samples at 10 K. The energies of the H-bonds formed by imine and amine protons were estimated from empirical correlations. The number of observed uncoupled ν_1 bands corresponds to the number of ordered H-bond

² The estimation of the H-bond energy for this system from the observed ν_1 red shift in solution upon dimerization (150 cm^{-1} [25]) gives practically the same H-bond energy as obtained from the dissociation constants: -14.5 kJ mol^{-1} .

contacts indicated by crystal structure analysis studies [8,9]. However, our spectroscopic study reveals that the ordered contacts represent no more than 70% of the total amount of H-bonds in the crystal, as follows from the analysis of the ν_4 -mode spectral range. The remaining H-bonds correspond to “disordered” hydrogen bonds (in the sense of absence of the long range periodicity) that are not accessible to conventional structural methods such as X-ray diffraction. The total contribution of all H-bond types to the sublimation enthalpy of crystalline cytosine estimated from the present infrared results amounts to -24 kJ mol^{-1} , in agreement with independent experimental thermodynamic data.

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