

# The Raman spectra of serine and 3,3-dideutero-serine in aqueous solution

S. Jarmelo<sup>a</sup>, P.R. Carey<sup>b</sup>, R. Fausto<sup>a,\*</sup>

<sup>a</sup> Department of Chemistry, University of Coimbra, 3004-535 Coimbra, Portugal

<sup>b</sup> Department of Biochemistry, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106-4935, USA

Received 5 May 2006; received in revised form 30 May 2006; accepted 1 June 2006

Available online 17 August 2006

## Abstract

The Raman spectra of serine [ $\alpha$ -amino- $\beta$ -hydroxypropionic acid; HO-CH<sub>2</sub>-CH(NH<sub>3</sub>)<sup>+</sup>-COO<sup>-</sup>] and 3,3-dideutero-serine [HO-CD<sub>2</sub>-CH(NH<sub>3</sub>)<sup>+</sup>-COO<sup>-</sup>] in aqueous solution were studied in the range 4000–300 cm<sup>-1</sup>. The data obtained for the deuterated compound are novel and provide compelling evidence that previously reported assignments for the undeuterated amino acid should be revised.

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**Keywords:** Serine; 3,3-Dideutero-serine; Aqueous solution; Raman spectrum

## 1. Introduction

As a characteristic feature of biological systems, chirality accounts for highly selective recognition, while chiral expression at higher-levels of complexity is thought to hold the key not only for molecular recognition, transformation and translocation, but also for the self-assembly and the enormous information content of living species [1,2]. The simple amino acid serine has been proposed as a key player in prebiotic chemical processes because of its unique tendency to form stable homochiral clusters, which in turn have been shown to be able to undergo enantioselective substitution reactions [3].

In the gaseous phase, serine exists in its neutral molecular form (HO-CH<sub>2</sub>-CHNH<sub>2</sub>-COOH). This species was recently studied in detail in our laboratories by a concerted matrix-isolation infrared spectroscopic and quantum chemical calculations approach [4,5]. In those studies, relevant serine conformers could be characterized both structurally and vibrationally [4] and their photochemistry investigated [5].

The infrared spectra of crystalline samples of both DL- and L-serine were also recently investigated in our laboratories using an isotopic-doping/low-temperature methodology [6,7]. In the

crystalline state, serine exists in the zwitterionic form and in a single conformation. The isotopic-doping/low-temperature methodology allowed the identification, in the infrared spectra of the doped crystals, of bands associated with individual (uncoupled) proton vibrational modes. From these data, estimations of the energies of the different H-bonds present in DL- and L-serine crystals and H-bond lengths were made using previously established empirical correlations [8–11], which compare well with available experimental data obtained independently. The vibrational spectra of crystalline DL-serine have also been previously studied by Machida et al. [12] and Susi et al. [13]. In those studies, the authors also presented results of normal coordinate analysis that were tentatively correlated with the experimental data.

In the present study, the Raman spectrum of serine in aqueous solution was reinvestigated. The knowledge of the conformational properties of amino acids in water, the natural medium for biological activity, is crucial in the understanding of molecular interactions in biological systems. In the case of serine, the relevance of such information is particularly useful because this amino acid possesses unique properties due to the presence of the side chain hydroxyl group, which gives it a hydrophilic character. Nevertheless, the primary alcohol group of serine does not ionize in water solutions at biological pH. Hence, it can react within the active sites of a number of enzymes. Indeed, serine residues often take an active role in

\* Corresponding author. Tel.: +351 919236971; fax: +351 239827703.

E-mail address: [rfausto@ci.uc.pt](mailto:rfausto@ci.uc.pt) (R. Fausto).

enzyme-catalyzed reactions, such as, for example, protein hydrolysis by serine proteases (*e.g.*, chymotrypsin, trypsin, elastase) [14,15]. It is then of great relevance to have a precise characterization of the vibrational properties of serine, since vibrational spectroscopy (in particular Raman and resonance Raman spectroscopies) can be of outstanding value to probe the way of action of enzymes in their active form, providing dynamical information which can expand that obtained by structural methods, like X-ray [16,17]. The vibrational properties of serine have also been used as spectroscopic probes for identification of protein secondary structures [18,19].

In the present study, the analysis of the spectroscopic data obtained for serine was supported by the simultaneous investigation of its 3,3-dideutero-serine isotopologue, whose vibrational spectrum in aqueous solution is reported here for the first time. As it will be pointed out in detail below, the data obtained for this latter species clearly revealed some inconsistencies in the previously reported [20,21] assignments for the non-deuterated amino acid.

## 2. Materials and methods

Serine and 3,3-dideutero-serine (>98% D) { $pK_a$  (COOH) = 2.19;  $(NH_3^+) = 9.21$ ;  $(CH_2OH) \approx 13$  [22]} were obtained as spectroscopic grade from Sigma and ICON Stable Isotopes, respectively, and used without any additional purification.

The Raman spectra were acquired using the 647.1 nm laser excitation from an Innova 400 Krypton Laser System (Coherent, Inc.), a back-illuminated charge-couple device (CCD) detector (model 1024EHRB/1, Princeton Instruments, Inc.) operating at 183 K, and a HoloSpec f/1.4 axial transmission spectrometer (Kaiser Optical Systems, Inc.) employed as a single monochromator as described in ref. [23]. The samples (60  $\mu$ L; 50 mM) were held in a 2 mm  $\times$  2 mm quartz cuvette under the 90° excitation/collection geometry. For each spectrum, generally 10 exposures of 1 min were accumulated. The solvent (water) spectrum was subtracted, and the baseline correction was made by multipoint selection. Wavenumber calibration was performed by recording the Raman spectra of the 1:1 mixture of cyclohexanone and acetone-D6, providing band positions to within  $\pm 1$   $cm^{-1}$  for sharp bands.

## 3. Results and discussion

Previously obtained data on serine in aqueous solution are scarce. Its vibrational spectrum was first described by Gargaro et al. [20] and, later on, by Ramírez et al. [21]. Theoretically, there were also some attempts to predict the vibrational spectrum of serine in aqueous solution, either using *ab initio* methods applied directly to the system under study [21,24] or assuming the transferability of scale factors estimated from smaller constituents [25]. There are, however, two fundamental problems with the theoretical calculations on this system. First, the zwitterionic form of serine, which is the relevant species in solution, is not a minimum energy structure on the potential

energy surface of the isolated molecule [26]. Indeed, for the isolated molecule of serine, like for other simple amino acids, it has been shown that the zwitterion is only obtained as a minimum energy configuration when the calculations are performed at a very low level of theory, as a result of an inadequate description of the electronic structure of the system [27–29]. Second, the methods available for the indispensable consideration of the solvent have not yet proved to provide results with the desired accuracy: in this case, continuum methods do not seem to be appropriate, considering the essentially specific nature of the water/serine interactions [30–32], and severe constraints must still be applied to use solvent discrete models to treat a system of this size, strongly limiting their predictive capabilities. In the analysis of the Raman spectra of serine and 3,3-dideutero-serine in aqueous solution presented below, a strictly empirical approach is thus used. As it will be shown in the next sections, the data obtained for the first time for the deuterated compound provide strong support to revise the assignments previously made for the undeuterated amino acid. Previous data obtained for serine in the crystalline state (both for L- and DL-serine crystals [6,7]) were also taken into consideration to help interpretation of the spectra obtained in aqueous solution.

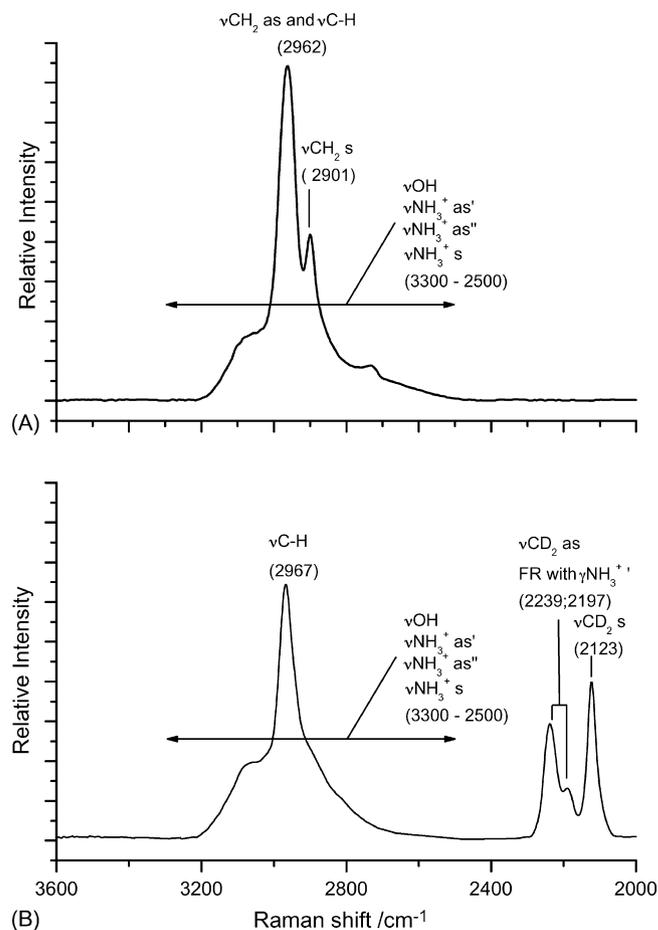


Fig. 1. Raman spectra (3600–2000  $cm^{-1}$  range) of serine (A) and 3,3-dideutero-serine (B) in aqueous solution (50 mM), at room temperature. FR: Fermi resonance.

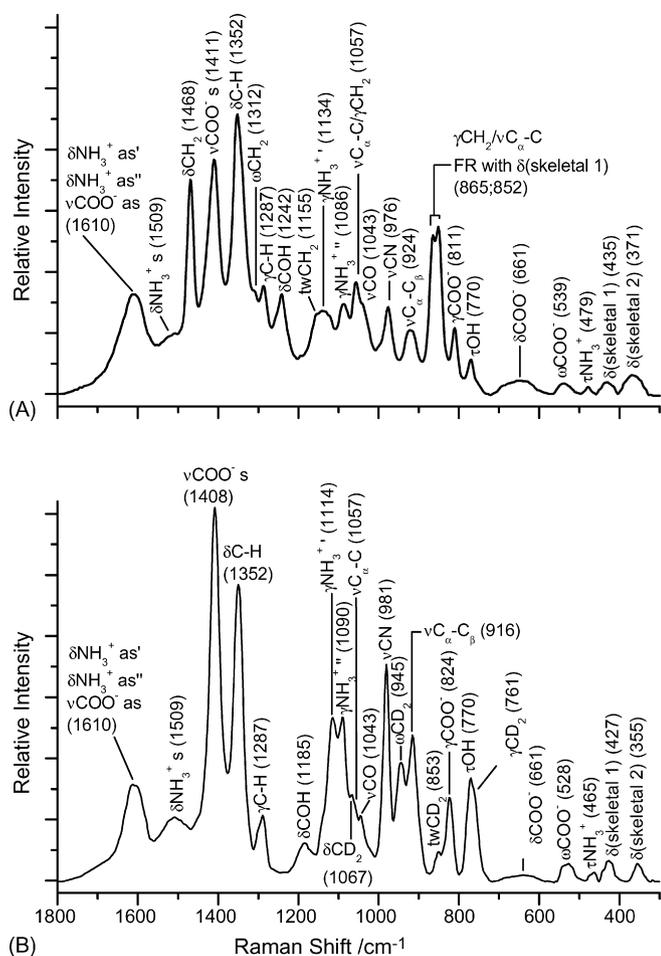


Fig. 2. Raman spectra (1800–300  $\text{cm}^{-1}$  range) of serine (A) and 3,3-dideutero-serine (B) in aqueous solution (50 mM), at room temperature. FR: Fermi resonance.

The Raman spectra of serine and 3,3-dideutero-serine in aqueous solution are presented in Figs. 1 (high frequency spectral region) and 2 (low-frequency range). The assignments are provided in Table 1, where they are compared with those previously reported in refs. [20] and [21].

### 3.1. 3600–2000 $\text{cm}^{-1}$ range

In the spectrum of serine, in this spectral region one could expect to observe features due to the stretching vibrations of hydroxyl ( $\nu\text{OH}$ ), ammonium ( $\nu\text{NH}_3^+$ ; three vibrations), methylene ( $\nu\text{CH}_2$  sym and  $\nu\text{CH}_2$  asym) and methyne ( $\nu\text{C-H}$ ) groups. All these vibrations are expected to occur above 2400  $\text{cm}^{-1}$ . For the deuterated compound, the two stretching modes due to the  $\text{CD}_2$  group give rise to bands at lower frequency and could be easily ascribed to the features at 2123  $\text{cm}^{-1}$  ( $\nu\text{CD}_2$  sym) and 2239/2197  $\text{cm}^{-1}$  ( $\nu\text{CD}_2$  asym; tentatively assigned as corresponding to a Fermi doublet due to interaction with the first overtone of the  $\gamma\text{NH}_3^+$ , whose fundamental is observed at 1114  $\text{cm}^{-1}$ ).

The intense band observed at 2967  $\text{cm}^{-1}$  in the spectrum of 3,3-dideutero-serine is unequivocally assigned to the stretching

vibration of the methyne group. In the spectrum of serine, the band observed at the same frequency is then ascribable to both  $\nu\text{C-H}$  and the  $\nu\text{CH}_2$  asym modes, whereas the  $\nu\text{CH}_2$  sym vibration gives rise to the band at 2901  $\text{cm}^{-1}$ , which is absent in the spectrum of the deuterated molecule (see Fig. 1). The present results do not support the assignment of  $\nu\text{C-H}$  to a shoulder at 2992  $\text{cm}^{-1}$  in the spectrum of serine made in ref. [21], which in fact we were unable to observe. It is worth noticing that such a shoulder was not also observed in the Raman spectrum of serine-D4 [ $\text{DO-CH}_2\text{-CH}(\text{ND}_3^+)\text{-COO}^-$ ] in heavy water [21].

In this spectral region, crystalline serine (DL-crystal) gives rise to bands at 2976 and 2943  $\text{cm}^{-1}$ , ascribed to  $\nu\text{CH}_2$  asym and to both  $\nu\text{C-H}$  and the  $\nu\text{CH}_2$  sym modes, respectively, whereas the crystal of 3,3-dideutero-serine gives rise to only a band at 2943  $\text{cm}^{-1}$  ( $\nu\text{C-H}$ ) [6]. Though being small, the observed shifts in the frequencies of these modes, in going from the aqueous solution to the crystalline state, clearly reveal the importance of the chemical environment on the vibrations of serine, including those associated with the methylene and methyne groups, which could be expected to be less sensitive to these effects.

The assignment of the stretching vibrations of both hydroxyl and ammonium groups is not possible under the experimental conditions used. Indeed, due to the involvement of these groups in H-bonding, these modes can be expected to give rise to very broad bands and, taking into consideration that the solvent also gives rise to broad features in the same spectral range, the bands due to the hydroxyl and ammonium stretching vibrations could not be clearly separated from the solvent bands. Nevertheless, the broad shoulder at ca. 3067  $\text{cm}^{-1}$ , observed in the spectra of both serine and 3,3-dideutero-serine, is certainly due to these vibrations.

### 3.2. 1800–1150 $\text{cm}^{-1}$ range

The low-frequency range corresponds to the spectral region where the most intense bands are observed in the Raman spectra of both serine and 3,3-dideutero-serine (see Fig. 2). In keeping with the data obtained for the studied compounds in the crystalline state [6,7], in this spectral range one can expect observation of bands due to the two stretching modes of the carboxylate group ( $\nu\text{COO}^-$  asym and  $\nu\text{COO}^-$  sym) and to the bending modes of the ammonium ( $\delta\text{NH}_3^+$  asym',  $\delta\text{NH}_3^+$  asym'' and  $\delta\text{NH}_3^+$  sym), methylene (scissoring,  $\delta\text{CH}_2$ ; wagging,  $\omega\text{CH}_2$ ; twisting,  $\tau\text{wCH}_2$ ), methyne ( $\delta\text{C-H}$  and  $\gamma\text{C-H}$ ) and hydroxyl ( $\delta\text{COH}$ ) groups.

The key to the interpretation of the spectra in this range was the assignment of the bands due to the three modes of the methylene group expected to occur in this region, in particular the wagging and twisting modes, which could not be made unambiguously in both refs. [20] and [21], because of inexistence of data on the  $\text{CD}_2$  substituted isotopologue. Comparison of the spectra of serine and 3,3-dideutero-serine enabled us to easily identify the bands due to the methylene group, since they are absent in the spectrum of the deuterated isotopologue (see Fig. 2). In consonance with the previous

Table 1

Peak frequencies, qualitative intensities<sup>a</sup> and assignment of bands of the Raman spectra of serine and 3,3-dideutero-serine in aqueous solution at room-temperature, in the range 4000–300 cm<sup>-1</sup>

Approximate description <sup>b</sup>	This study				Ref. [20] Serine Frequency (cm <sup>-1</sup> )	Ref. [21]							
	Serine		3,3-Dideutero-serine			Serine		Serine-D-4					
	Frequency (cm <sup>-1</sup> )	Intensity	Frequency (cm <sup>-1</sup> )	Intensity		Frequency (cm <sup>-1</sup> )	Intensity	Frequency (cm <sup>-1</sup> )	Intensity				
$\nu$ OH	} 3300–2500	} vb	} 3300–2500	} vb									
$\nu$ NH <sub>3</sub> <sup>+</sup> asym''													
$\nu$ NH <sub>3</sub> <sup>+</sup> asym'													
$\nu$ NH <sub>3</sub> <sup>+</sup> sym''													
$\nu$ C–H	} 2962	} vs	2967	vs		2992	sh						
$\nu$ CH <sub>2</sub> asym						2969	s	2965	s				
$\nu$ CH <sub>2</sub> sym	2901	s				2903	m	2900	m–s				
$\nu$ CD <sub>2</sub> asym			2239 <sup>c</sup>	s									
			2197 <sup>c</sup>	sh									
$\nu$ CD <sub>2</sub> sym			2123	s									
$\delta$ NH <sub>3</sub> <sup>+</sup> asym'	} 1610	} vb	} 1610	} vb		} 1514	} vw						
$\delta$ NH <sub>3</sub> <sup>+</sup> asym''													
$\nu$ COO <sup>-</sup> sym													1622
$\delta$ NH <sub>3</sub> <sup>+</sup> sym	1509	b	1509	b		1408	m						
$\delta$ CH <sub>2</sub>	1468	m			1460	1467	m	1467	m				
$\nu$ COO <sup>-</sup> sym	1411	m	1408	m	1412	1352	m–s	1344	sh				
$\delta$ C–H	1352	m	1352	m	1347	1313	w	1335	m–s				
$\omega$ CH <sub>2</sub>	1312	sh			1242			1416	s				
$\gamma$ C–H	1287	w	1287	w		1313	w	1298	w				
$\delta$ COH	1242	w	1185	w	1242								
twCH <sub>2</sub>	1155	w			1294	1238	w						
$\gamma$ NH <sub>3</sub> <sup>+/</sup>	1134	w	1114	w	1150								
$\gamma$ NH <sub>3</sub> <sup>+/</sup>	1086	w	1090	w	1125	1084	m						
$\delta$ CD <sub>2</sub>			1067	sh									
$\nu$ C <sub><math>\alpha</math></sub> –C/ $\gamma$ CH <sub>2</sub>	1057	w	1057	sh	1052	1056	m	1054	m				
$\nu$ CO	1043	sh	1043	sh	978								
$\nu$ CN	976	w	981	m	1083								
$\omega$ CD <sub>2</sub>			945	w									
$\nu$ C <sub><math>\alpha</math></sub> –C <sub><math>\beta</math></sub>	924	w	916	w	919	912	m						
$\gamma$ CH <sub>2</sub> / $\nu$ C <sub><math>\alpha</math></sub> –C	865 <sup>d</sup>	m			851	975	w						
	852 <sup>d</sup>	m											
twCD <sub>2</sub>			853	sh									
$\gamma$ COO <sup>-</sup>	811	w	824	w		807	s						
$\tau$ OH	770	w	770	w		433–372	w	407–326	w				
$\gamma$ CD <sub>2</sub>			761	sh									
$\delta$ COO <sup>-</sup>	661	b	661	b	620	600	m	599	m				
$\omega$ COO <sup>-</sup>	539	w	528	w		548	w	548	w				
$\tau$ NH <sub>3</sub> <sup>+</sup>	479	vw	465	vw		490	w	490	w				
Skeletal 1	435	w	427	w									
Skeletal 2	371	w	355	w									

<sup>a</sup> vs: very strong; s: strong; m: medium; w: weak; vw: very weak; sh: shoulder; vb: very broad; b: broad.

<sup>b</sup>  $\nu$ : bond stretching;  $\delta$ : bending;  $\omega$ : wagging;  $\gamma$ : rocking (out-of-plane); tw: twisting;  $\tau$ : torsion; asym: antisymmetric; sym: symmetric.

<sup>c</sup> Fermi resonance interaction involving the first overtone of the  $\gamma$ NH<sub>3</sub><sup>+/</sup>, whose fundamental is observed at 1114 cm<sup>-1</sup>.

<sup>d</sup> Fermi resonance interaction involving the first overtone of the skeletal deformational band whose fundamental is observed at 435 cm<sup>-1</sup>.

studies [20,21],  $\delta$ CH<sub>2</sub> is assigned to the band at 1468 cm<sup>-1</sup>. On the other hand, the  $\omega$ CH<sub>2</sub> and twCH<sub>2</sub> modes are now re-assigned to the bands observed at 1312 and 1155 cm<sup>-1</sup>, respectively. In ref. [20], the methylene wagging and twisting modes were assigned to the bands at 1242 and 1287 cm<sup>-1</sup>, respectively, which correspond to bands appearing in the spectra of both serine and 3,3-dideutero-serine (though the first somewhat shifted to lower frequencies in the spectrum of the deuterated compound, as discussed in detail below) and

cannot then be due to the methylene group.<sup>1</sup> In ref. [21] no band was assigned to  $\omega$ CH<sub>2</sub>, whereas twCH<sub>2</sub> was assigned to the band observed at 1242 cm<sup>-1</sup>.

<sup>1</sup> In refs. [20] and [21], the reported peak frequency of some of the bands are slightly different from ours (see Table 1). However, the correspondence between the bands in all three studies is unequivocal; our measured values are used in the text throughout this paper.

Once the bands due to the methylene vibrations have been identified, the assignment of the remaining bands in this spectral range is straightforward. The broad band with maximum at  $1610\text{ cm}^{-1}$  results from the overlap of the bands due to the  $\nu\text{COO}^-$  asym mode and the two antisymmetric bending vibrations of the  $\text{NH}_3^+$  group. These modes were observed within  $1650$  and  $1550\text{ cm}^{-1}$  in the spectra of serine in the crystalline state [6,7]. It is worth mentioning that the relative intensity of this band was found to diminish considerably in the spectrum of serine in heavy water solution [21], in consonance with the present assignments, since in that case only the  $\nu\text{COO}^-$  asym mode contributes to the intensity of the band. The  $\delta\text{NH}_3^+$  sym mode gives rise to the band at  $1509\text{ cm}^{-1}$ , which is better seen in the spectrum of 3,3-dideutero-serine, because in this case the intense band due to the  $\delta\text{CH}_2$  mode at  $1468\text{ cm}^{-1}$  that partially overlaps the  $1509\text{ cm}^{-1}$  band is absent from the spectrum (see Fig. 2). In the crystalline state, the bands due to  $\delta\text{NH}_3^+$  sym were also observed around  $1500\text{ cm}^{-1}$  [6,7]. In ref. [21], however, this mode was assigned to the band observed at  $1411\text{ cm}^{-1}$ , which is without any doubt due to the  $\nu\text{COO}^-$  sym mode [6,7,20]. Note also that regarding the assignment of the  $\nu\text{COO}^-$  sym mode to the band at  $1411\text{ cm}^{-1}$  (close to the values found for serine in the crystalline state:  $1434/1414\text{ cm}^{-1}$ , for L-serine, and between  $1465$  and  $1400\text{ cm}^{-1}$ , for DL-serine [6,7]), the present assignment is in consonance with that proposed in ref. [20]. It is naturally in disagreement with the assignment made in ref. [21], since in that study the  $1411\text{ cm}^{-1}$  band was considered to be due to  $\delta\text{NH}_3^+$  sym.

The three remaining bands still to assign in the  $1800$ – $1150\text{ cm}^{-1}$  range must be mainly due to the two bending modes of the methyne group and to the  $\delta\text{COH}$  mode. The two first are easily assigned to the bands at  $1352\text{ cm}^{-1}$  ( $\delta\text{C-H}$ ) and  $1287\text{ cm}^{-1}$  ( $\gamma\text{C-H}$ ), which appear close to the bands due to these modes in serine crystals (*ca.*  $1375$ – $1345$  and  $1300\text{ cm}^{-1}$ , respectively [6,7]). In ref. [21], both methyne bending modes were assigned to the  $1312\text{ cm}^{-1}$  band, which is without any doubt due to the methylene group ( $\omega\text{CH}_2$ ), since it is absent in the spectrum of 3,3-dideutero-serine (see Fig. 2). On the other hand, the assignment of the  $\delta\text{C-H}$  mode made in ref. [20] agrees with ours, but the  $\gamma\text{C-H}$  vibration was not assigned in that study. Finally,  $\delta\text{COH}$  is now ascribed to the band at  $1242\text{ cm}^{-1}$ , also in agreement with the assignment made in ref. [20]. In 3,3-dideutero-serine, this band appears red shifted, being observed at  $1185\text{ cm}^{-1}$ , in all probability as a result of vibrational coupling between the  $\delta\text{COH}$  coordinate and vibrations associated with the neighbour  $\text{CD}_2$  group. These frequencies are similar to those found in serine and 3,3-dideutero-serine crystals (*ca.*  $1249$  and  $1213\text{ cm}^{-1}$ , respectively [6,7]).

### 3.3. Region below $1150\text{ cm}^{-1}$

This region is the most complex of the spectra of the studied species, because vibrations appearing below  $1150\text{ cm}^{-1}$  are, in most cases, considerably mixed, not strictly corresponding to well localized vibrations. Moreover, below *ca.*  $600\text{ cm}^{-1}$  the analysis of the spectra was also complicated due to the

superposition of the weak Raman features with the strong Rayleigh line. The proposed assignments must then, in some cases, be considered as indicative. Nevertheless, the assignments discussed below are in good agreement with those reported for the crystalline state [6,7], and likely represent a marked improvement over those previously proposed.

Deuteration of the methylene group led to several vibrations from the high-frequency region shifting to this low-frequency region (*e.g.*, for  $\delta\text{CD}_2$ ,  $\omega\text{CD}_2$ ,  $\text{twCD}_2$  and  $\gamma\text{CD}_2$  modes), increasing the complexity of the spectrum of 3,3-dideutero-serine in this spectral region. Fortunately, with a single exception all bands due to the  $\text{CD}_2$  group give rise to well separated bands, which are absent from the spectrum of the undeuterated compound. Hence,  $\delta\text{CD}_2$ ,  $\omega\text{CD}_2$  and  $\text{twCD}_2$  can be easily ascribed to the bands at  $1067$ ,  $945$  and  $853\text{ cm}^{-1}$  (see Fig. 2). In turn, the  $\gamma\text{CD}_2$  mode is assigned to the feature at  $761\text{ cm}^{-1}$ , appearing strongly overlapped with that at  $770\text{ cm}^{-1}$ , which is observed in the spectra of both serine and 3,3-dideutero-serine and is here assigned to the hydroxyl torsion ( $\tau\text{OH}$ ), as discussed in detail below.

The two  $\text{NH}_3^+$  rocking modes are assigned to the bands at  $1134$  and  $1086\text{ cm}^{-1}$  ( $1114$  and  $1090\text{ cm}^{-1}$  for the deuterated compound), nearly at the same frequencies as in the crystalline state [6,7]. The observed shifts upon deuteration of the methylene group indicate that the  $\gamma\text{NH}_3^+$  coordinates are somewhat coupled with the vibrations of the  $\text{CD}_2$  fragment, as we could indeed anticipate. In ref. [20], the  $\gamma\text{NH}_3^+$  modes were assigned to the bands at  $1134$  and  $1155\text{ cm}^{-1}$ , *i.e.*, though the first band was correctly assigned, the last one was not, since the  $1155\text{ cm}^{-1}$  band is absent in the spectrum of 3,3-dideutero-serine and is in fact due to the  $\text{twCH}_2$  mode (as discussed in detail above). On the other hand, in ref. [21] only one  $\gamma\text{NH}_3^+$  mode was assigned ( $1086\text{ cm}^{-1}$ ). The only vibration not yet referred to that relates to the ammonium group is the  $\text{NH}_3^+$  torsion, which is now tentatively assigned to the band at *ca.*  $470\text{ cm}^{-1}$ . Though tentative, this assignment seems, however, to be quite plausible, considering that in solution the  $\text{NH}_3^+$  torsion can be expected to give rise to a band somewhat downshifted relatively to the crystalline state (*ca.*  $520\text{ cm}^{-1}$  [6,7]) because in solution the  $\text{NH}_3^+$  rotor is not under the steric constraints imposed by the rigidity of the crystalline environment.

The single vibration from the  $\text{CH}_2$  group expected to be observed in this spectral region corresponds to the  $\gamma\text{CH}_2$  (rocking) mode. It is then ascribed to the strong doublet of bands at  $865$  and  $852\text{ cm}^{-1}$ , which has no counterpart in the spectrum of the deuterated isotopologue. The splitting of the band is tentatively assigned to a Fermi resonance interaction involving the first overtone of the skeletal deformational band whose fundamental is observed at  $435\text{ cm}^{-1}$ . It is worth mentioning that in the crystalline state, the bands appearing near  $850\text{ cm}^{-1}$  were assigned to the  $\nu\text{C}_\alpha\text{-C}$  stretching vibration (these bands were observed in the spectra of both serine and 3,3-dideutero-serine crystals), whereas the  $\gamma\text{CH}_2$  mode was assigned to bands in the  $1065$ – $1010\text{ cm}^{-1}$  (absent from the spectrum of the deuterated crystal) [6,7]. In view of the present results it seems that these two coordinates are in effect

considerably coupled. Thus, in the crystals the highest frequency band has a dominant contribution from the  $\gamma\text{CH}_2$  coordinate and the lowest frequency band has a dominant contribution from  $\nu\text{C}_\alpha\text{-C}$ , while the opposite situation occurs in solution, where the highest frequency band ( $1057\text{ cm}^{-1}$ ) is observed in the spectra of both deuterated and non-deuterated samples.

Vibrations with dominant contribution from the CO, CN and  $\text{C}_\alpha\text{-C}_\beta$  stretching coordinates are now assigned to the bands at  $1043$ ,  $976$  and  $924\text{ cm}^{-1}$  in the spectrum of serine in aqueous solution. The frequencies of the two last bands shift slightly upon deuteration of the methylene group to higher and lower frequency, respectively (see Table 1), while their relative intensity also changes appreciably (Fig. 2). These results are in consonance with a somewhat different vibrational coupling in these two coordinates in serine and 3,3-dideutero-serine. The assignments of these modes closely follow those made for the crystalline state [6,7]. In the case of the  $\nu\text{C}_\alpha\text{-C}_\beta$  vibration, the proposed assignment also agrees with those made in refs. [20] and [21]. Both  $\nu\text{CO}$  and  $\nu\text{CN}$  were not assigned in ref. [21], while in ref. [20] the different description of the skeletal stretching coordinates precludes a strict direct comparison of the assignments there made with the present ones. Nevertheless, the band at  $976\text{ cm}^{-1}$  was also defined in ref. [20] as a stretching skeletal vibration (though proposed to be mainly localized in the  $\text{C-C-O}$  fragment); the  $\nu\text{CN}$  mode was assigned to the band at  $1086\text{ cm}^{-1}$ , now reassigned to one of the  $\text{NH}_3^+$  rocking modes, as explained above.

The remaining bands observed in the spectra correspond to bending vibrations of the carboxylate moiety ( $\gamma\text{COO}^-$ ,  $\delta\text{COO}^-$  and  $\omega\text{COO}^-$ ), the  $\tau\text{OH}$  torsional mode and skeletal bending modes involving mainly coupled deformations of the CCC, CCN and CCO angles. By comparison with the spectra obtained in the crystalline state,  $\gamma\text{COO}^-$ ,  $\delta\text{COO}^-$  and  $\omega\text{COO}^-$  are assigned to the bands at  $811$ ,  $661$  and  $539\text{ cm}^{-1}$ , respectively (in L-serine crystal [7], these modes give rise to bands at  $806/796$ ,  $616$  and  $577\text{ cm}^{-1}$ , respectively). In turn, the hydroxyl torsional mode is assigned to the band at  $770\text{ cm}^{-1}$  (superimposed with that due to  $\gamma\text{CD}_2$  in the case of the deuterated isotopologue), which does also closely match the observed frequency for this vibration in DL-serine crystal [6]. It is worth mentioning that in the L-serine crystal, the  $\tau\text{OH}$  vibration gives rise to a band appearing at a much lower frequency (*ca.*  $450\text{ cm}^{-1}$  [7]). As pointed out before [7], this result is a consequence of the very interesting (and somewhat unexpected) circumstance of the OH group in the L-serine crystal being practically not involved in hydrogen bonding [7,33,34]. Since the frequency of the torsional mode correlates with the strength of the hydrogen bond in which the OH group acts as a donor – the higher the frequency, the stronger the hydrogen bond [6–11] –, the practical absence of hydrogen bond in the L-serine crystal markedly lowers the  $\tau\text{OH}$  frequency. On the other hand, the present results indicate that the strengths of the hydrogen bonds involving the hydroxyl moiety in DL-serine crystal (*ca.*  $30\text{ kJ mol}^{-1}$  [6]) and in aqueous serine should not differ very much.

Finally, the two lowest observed bands ( $435$  and  $371\text{ cm}^{-1}$ ) are generically assigned to skeletal bendings and involve mostly deformations of the CCC, CCN and CCO angles. As expected, they shift to slightly lower frequencies upon deuteration of the methylene group (see Table 1).

#### 4. Conclusion

In this study, the Raman spectra of serine and 3,3-dideutero-serine in aqueous solution were investigated in the spectral range  $4000\text{--}300\text{ cm}^{-1}$ , at room-temperature. A complete assignment of the spectra was undertaken, bringing about in many cases revision of previously proposed assignments for the non-deuterated compound. The spectra of the deuterated isotopologue in aqueous solution was described here for the first time. The deuteration of the methylene group of serine proved to be particularly useful to unambiguously reassign several bands of the Raman spectra of the parent undeuterated amino acid.

#### Acknowledgments

This work was financially supported by “Fundação para a Ciência e a Tecnologia” (FCT—Projects POCTI/QUI/59019/2004 and POCTI/QUI/58937/2004, also supported by FEDER), “Instituto de Investigação Interdisciplinar” of the University of Coimbra (Project III/BIO/40/2005) and GRICES/SECyT (Project 000813//PO-PA04-EVI/001). SJ also acknowledges FCT for the Grant SFRH/BD/6696/2001.

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