

Raman spectra of dipicolinic acid in crystalline and liquid environments

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Abstract

Raman spectra of dipicolinic acid (DPA) are important for detection of bacterial spores, since DPA and its salts present one of their major components. The implementation of a deeply cooled CCD camera in combination with pulsed excitation at 532 nm allowed measuring well-resolved Raman spectra of the DPA in different forms. Powder preparations, crystals grown from saturated solutions and aqueous solutions of the DPA were studied. The spectral features in different environments and comparison with the spectra obtained by other methods are discussed.

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1. Introduction

The properties of the dipicolinic acid (DPA) and its salts as major constituents of bacterial spores [1] gained recently increased interest. In particular, optical methods promise contact free detection and identification of the spores. Several spectroscopic approaches were used including IR and spontaneous Raman spectroscopy for study of DPA powder [2] (see also our preliminary results [3]), resonance Raman [4] and surface enhanced Raman spectroscopies for study of DPA solutions [5]. The FAST CARS method (Femtosecond Adaptive Spectroscopic Techniques for Coherent Anti-Stokes Raman Spectroscopy) based on the achievement of maximal coherence was also proposed [6]. So far, Raman spectra of solid and liquid forms of the DPA were measured by different techniques, which made their direct comparison difficult.

The environment affects the vibrational modes of molecules. In particular, the theoretical analysis of the vibrational frequencies of an isolated DPA molecule [7] provided reasonable agreement with experimental data for powder [2] only after the introduction of empirical correction factors. It is

therefore of interest to investigate and compare the spectra for different environments with one method. Several factors that may affect Raman measurements were indicated previously. First, fluorescence was observed for DPA solutions with the excitation light in the UV spectral region [7]. Its interference can be mostly avoided with the excitation by light in the green spectral region [2,3]. Second, degradation, especially noticeable with solid samples, limits the energy of exciting laser pulses and consequently also the attainable signal [3].

In this paper, we study three different forms of the DPA (powder, crystalline samples and aqueous solutions) with the spontaneous Raman scattering technique. The measurements were performed using a nanosecond laser for the excitation and a spectrograph combined with a deeply cooled CCD camera for the detection. The solid samples correspond more nearly to forms of the DPA found in spores, and thus are of particular interest. Studies with aqueous solutions provide additional data on the effect of the environment on the vibrational modes.

2. Experimental setup and procedure

The experimental setup is shown in Fig. 1. The sample was illuminated by the beam of the second harmonic of a Nd:YAG

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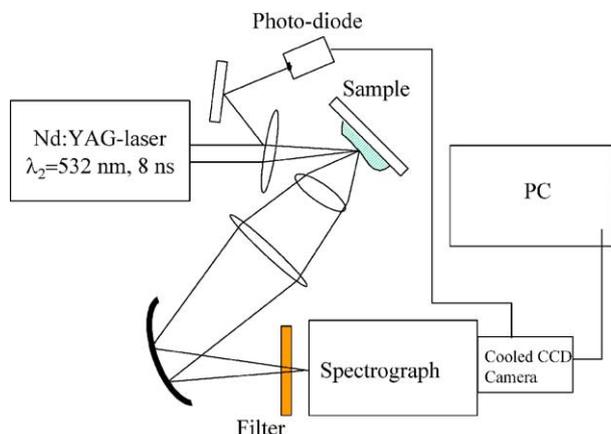


Fig. 1. Schematic of the experimental setup. The excitation was performed by a nanosecond pulse of the second harmonic of a Nd:YAG laser at 532 nm. The scattered light collected by the lenses was passed through the filter, the spectrograph and then registered by the CCD camera.

laser at 532 nm with repetition rate of 10 Hz. The scattered light was collected by a lens and focused into the entrance slit of a spectrograph (SpectraPRO 3001, Acton Research Corp., 600 grooves mm^{-1} grating) and detected by a CCD camera (Spec-10, Roper Scientific Inc.) cooled to -100°C with liquid nitrogen.

The Rayleigh component of the scattered light was suppressed by the filter (two Schott glass plates, OG550 and OG575, of 2 mm thickness each). This filter was positioned immediately before the entrance slit of the spectrograph. A portion of the excitation beam was deflected, registered by a photodiode and used for triggering of the data acquisition process. Typically, the entrance slit of the spectrograph was about $25\ \mu\text{m}$.

For studies of powders, we directly used DPA in dry form purchased from Sigma–Aldrich. The powder with an average grain size about $20\ \mu\text{m}$ was pressed to the surface of a solid substrate (an aluminum plate) and formed a layer of about 1 mm thickness. The upper limit of the energy (2–4 mJ) used for the excitation was determined by the laser fluence that DPA could withstand without changes in its chemical composition. These changes could be noticed due to appearance of a yellow shade on the initially white sample surface.

A spot of 5 mm in diameter was illuminated by the laser beam. Thus, the average fluence on the surface was below $0.01\ \text{J cm}^{-2}$ and peak intensity was below $5 \times 10^6\ \text{W cm}^{-2}$. In this case, no changes in the appearance and the spectral characteristics of the sample could be noticed even after 20 min of exposure to the laser radiation.

To see the influence of the grain size on the spectra, we produced crystals from heated and oversaturated ($8\ \text{g l}^{-1}$ or 48 mM) DPA solutions. In this case, the typical size of a crystal was about 1 mm. Crystals were fixed between two glass slides and the scattered light from them was analyzed. It was checked that the glass without the sample did not provide a noticeable contribution to the spectra.

For liquid samples, we used cuvettes of different sizes. Typically, the scattered light was collected by a lens from a laser beam path in the DPA solution of about 8 mm.

3. Results of spectral measurements

With powder preparations, the sample surface was directly subjected to laser radiation. A typical Raman spectrum of a DPA powder is shown in Fig. 2(a). This spectrum was renormalized taking into account the transmission of the filter. The measured region of Raman shifts $630\text{--}3100\ \text{cm}^{-1}$ contains many characteristic peaks that can be used for DPA identification. Especially rich with vibrational bands is the

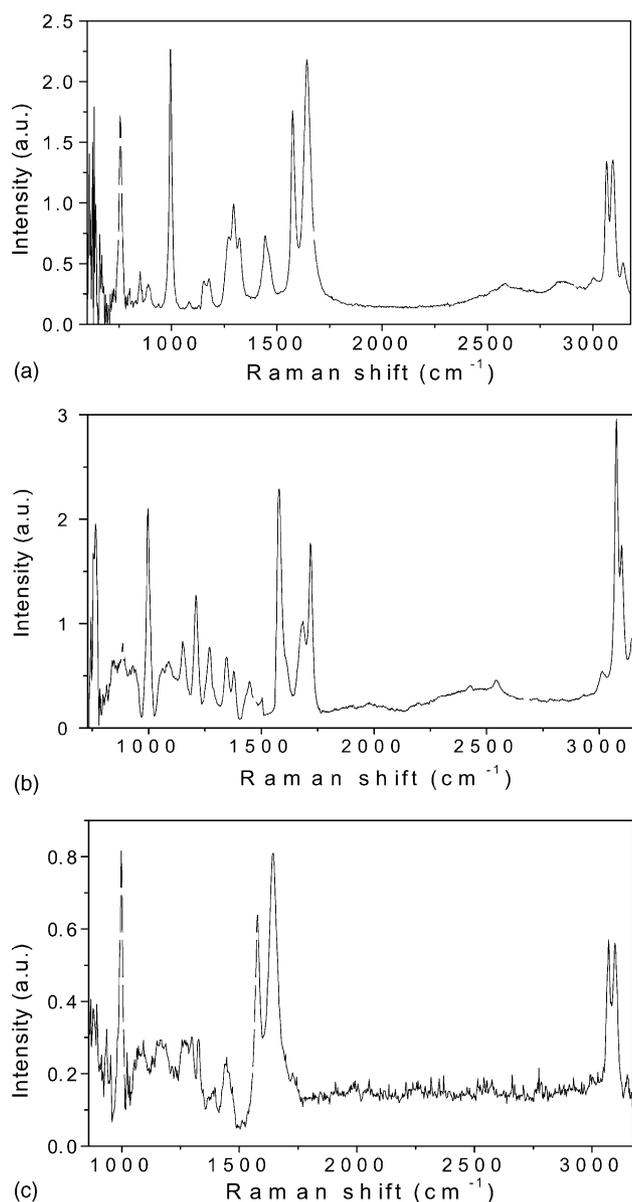


Fig. 2. The Raman spectra of the dipicolinic acid (DPA): (a) powder; (b) crystal; (c) aqueous solution.

frequency interval below 1750 cm^{-1} . The high level of the Raman signal from the powder made it possible to perform measurements with averaging only 100 laser pulses.

The spectrum for the crystalline sample is shown in Fig. 2(b). This spectrum also shows strong bands around 1000 , 1600 and 3090 cm^{-1} , as the spectrum of the powder, however, a more detailed comparison reveals also differences discussed in Section 4.

We have also measured Raman spectra for aqueous solutions of the DPA. To obtain a higher concentration, the solution was heated during the measurement to about $90\text{ }^{\circ}\text{C}$. In this way, concentrations up to 40 mM could be used. To exclude the contribution of water, separate measurements of pure water were performed. The spectrum of water was normalized so that the strong Raman peaks of water around 3450 cm^{-1} had equal heights for pure water and the solution, and then the spectrum of water was subtracted. The resulting spectrum is shown in Fig. 2(c). In this case, the level of the signal was lower than for solid samples, so that very weak Raman bands could not be detected. We observed that at high energy of the excitation laser pulses (above 50 mJ) in a DPA solution two bands at 1380 and 1720 cm^{-1} appeared in the spectra. The peak at 1380 cm^{-1} corresponds to $\text{O}-\text{C}-\text{O}^-$ stretching mode characteristic for the DPA ion [2,7]. The band at 1720 cm^{-1} is characteristic for carbonyl group ($\text{C}=\text{O}$), when polymerization of DPA takes place. Thus, the appearance of these two bands may indicate that at higher laser intensity dipicolinate ions are formed, which also results

in such dynamic processes as the formation and dissociation of DPA dimers and polymers.

The frequencies for the observed Raman bands are summarized in Table 1. It should be noted that the signal-to-noise ratio of the observed Raman scattering decreased in the sequence powder–crystal–solution, therefore weak bands with close frequencies could not be well resolved for solution.

4. Assignment of the peaks in the Raman spectra and discussion

4.1. Characterization of spectral peaks

The structure of the DPA molecule is shown in Fig. 3. It consists of a pyridine ring with two carboxyl groups. The peaks observed in the Raman spectra correspond to characteristic vibrational modes of the DPA molecule. The identification of the peaks in the Raman spectrum of the DPA can be made based on the typical frequency ranges for certain vibrational modes [8] and on previously made assignments for the DPA spectra [2,7]. The assignments are also presented in Table 1. First, we will consider the observed spectral features and their relation to the functional groups of the DPA molecule.

The pyridine ring is characterized by the very strong symmetric ring “breathing” band around 1000 cm^{-1} [9], that was observed for all investigated forms of the DPA. Other ring

Table 1

Observed Raman spectral bands (the relative strength^a is indicated in parenthesis and Stokes shifts are in cm^{-1}) and assignments for different forms of dipicolinic acid

Powder	Crystal	Solution	Assignment	Powder at 100 K [2]
(4) 639 ^b			C–C ring bend	(4) 647
(4) 748	(5) 765		OCO in-plane deformation	(5) 762
(1) 796			C–H out-of-plane	(3) 802
(2) 844	(2) 843		C–CO ₂ bend	(3) 855
(3) 885	(2) 880	(2) 891	C–H out-of-plane	(3) 892
(1) 933	(1) 929	(2) 938	C–H out-of-plane	
(5) 987	(5) 998	(5) 996	Symmetric ring “breathing”	(5) 998
(1) 1080	(2) 1090	(2) 1060–1095	Trigonal ring “breathing”	(1) 1090
(2) 1146	(3) 1150	(2) 1150–1190	C–H bend	(2) 1156
(2) 1170			C–H bend	(3) 1183
(3) 1262	(4) 1211		C–C ring mode	(2) 1262
(3) 1289	(3) 1273	(2) 1271	C–H bend	(3) 1279
(3) 1317	(3) 1347	(2) 1326	C–O stretch	(3) 1304
	(3) 1376	(1) 1398	C–O stretch	(2) 1332
(3) 1437	(2) 1448	(2) 1443	C–C ring stretch	(3) 1447
(4) 1569	(5) 1581	(4) 1576	Ring stretch	(4) 1580
(5) 1638	(4) 1684	(5) 1644	C=O stretch	(4) 1640
	(4) 1717		C=O stretch	
	(1) 2426		O–H stretch	
(1) 2574	(2) 2547		O–H stretch	(1) 2650
(1) 2995	(2) 3017		C–H stretch	
(4) 3062	(5) 3089	(3) 3066	C–H stretch	(3) 3070
(4) 3093	(3) 3105	(3) 3094	C–H stretch	(2) 3105
	(2) 3147		C–H stretch	(2) 3113

^a Relative strength was ascribed using the following qualitative scale: (5) very strong; (4) strong; (3) medium; (2) weak; (1) very weak.

^b The measurement of this band was done separately with one of the filtering glass plates (OG575) removed.

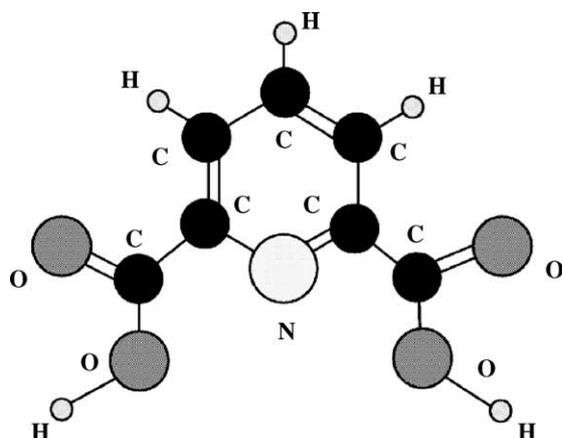


Fig. 3. The structure of the dipicolinic acid molecule.

modes include the in-plane ring deformation at 639 cm^{-1} , the trigonal ring “breathing” band around 1080 cm^{-1} and ring stretch modes at 1270 , 1443 and $1570\text{--}1580\text{ cm}^{-1}$. The presence of C–H bonds is indicated by the presence of bending vibrations at 1150 , 1170 and $1270\text{--}1280\text{ cm}^{-1}$ and three stretching modes in the region $2290\text{--}3150\text{ cm}^{-1}$. The out of plane C–H deformation mode is observed at about 885 cm^{-1} .

The carboxyl group can be identified by the OCO in-plane deformation mode at 757 cm^{-1} and C=O stretch bands around 1644 cm^{-1} and also 1717 cm^{-1} .

As was noted [7], environment can strongly affect O–H vibrational frequencies due to hydrogen bonding. It was experimentally established that the O–H stretching vibration frequencies strongly decrease in the sequence vapor–liquid–crystal [9]. A broad band around 2500 cm^{-1} was observed in powder and crystal, but it was absent in solution.

4.2. Comparison of spectra for different forms of the DPA

The comparison of characteristic frequency bands shows that the observed spectra have certain differences for different forms of the DPA (see Table 1).

Although the powder is also a crystalline form of the DPA, the scattering of light from it is different than from a large crystalline sample. This difference is quite obvious: the powder looks white, while the crystalline sample appears transparent. This means that the scattering from the powder takes place mostly at the surface of microcrystallites, while the scattering from the large crystalline sample comes mainly from the bulk. Some differences may also result from the re-growth of the crystal in the aqueous solution.

Consequently, in crystal, where scattering comes mostly from molecules of the DPA associated via hydrogen bonding [2], we observe a shift to higher frequencies of the band related to carboxyl vibrational mode (1684 cm^{-1} compared to 1638 cm^{-1} in powder). The peak of the C–H band at 3089 cm^{-1} strongly increases in the crystal, which can be also attributed to the influence of the surrounding molecules with

hydrogen bonding that strongly affects vibrational modes of hydrogen.

The comparison of the observed Raman spectra at room temperature with previous measurements of spontaneous Raman scattering spectra at 100 K [2] presented in the last column of Table 1 shows a close correspondence of the observed peaks.

4.3. Comparison with other Raman spectroscopic techniques

The comparison of the spectra measured via spontaneous Raman with those obtained by other methods, such as resonance Raman spectroscopy [4] and surface enhanced Raman spectroscopy (SERS) [5] shows that they are not identical.

The UV electronic resonance Raman spectra [4] were measured at several wavelengths in the region $222\text{--}251\text{ nm}$. These measurements show that some bands are more pronounced at certain excitation wavelengths. For instance, the bands at 1060 and 1272 cm^{-1} produce strong peaks in the scattered light for the excitation wavelength 231 nm , while they are only slightly noticeable or absent at other excitation wavelengths. This reflects a general feature of the UV resonance Raman spectra that the relative intensities of the spectral peaks may change significantly with relatively small changes of the excitation wavelength [10].

The SERS spectra of the DPA on silver nanoparticles were measured recently [5]. These spectra differ from those measured via spontaneous Raman scattering without the surface enhancement effect. One of the reasons is that, as the authors concluded, a DPA molecule adsorbs on the Ag surface as a dipicolinate ion. The vibrational frequencies of the dipicolinate ion were measured [2] and also calculated [7] and the most striking difference is the appearance of a strong stretching OCO mode around 1380 cm^{-1} . The presence of this strong carboxylate band in the SERS data was explained by the suggestion that dipicolinate ions adsorb through the carboxylate moiety of the molecule.

5. Conclusion

Spontaneous Raman scattering spectra were measured for different forms of the DPA at room temperature with pulsed laser excitation at 532 nm . These spectra exhibit many characteristic bands with frequencies mainly below 1750 cm^{-1} useful for the identification of DPA molecules. The strength of the observed Raman scattering decreased in the sequence powder–crystal–solution. Crystalline forms of the DPA provided several strong bands, including two ring modes at around 1000 and 1570 cm^{-1} , carboxyl mode at about 1660 cm^{-1} and two C–H stretch modes in the region $3060\text{--}3100\text{ cm}^{-1}$. These strong bands were observed for all studied forms of the DPA (powder, crystal and solution). The sample structure and surrounding molecules induce certain changes in the spectra. In particular, in a large crystalline

sample grown from oversaturated solution a shift of carbonyl vibrational mode was observed. A strong influence of the environment and structure was also detected for the hydrogen vibrational modes.

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References

- [1] G.W. Gould, A. Hurst (Eds.), *The Bacterial Spore*, Academic Press, London, 1969.
- [2] P. Carmona, *Spectrochim. Acta* 36A (1980) 705.
- [3] M. Scully, G.W. Kattawar, R.P. Lucht, T. Opatrny, H. Pilloff, A. Rebane, A.V. Sokolov, M.S. Zubairy, *PNAS* 99 (2002) 10994.
- [4] A.A. Kolomenskii, S.N. Jerebtsov, T. Opatrny, H.A. Schuessler, M.O. Scully, *J. Mod. Phys.* 50 (2003) 2369.
- [5] E. Ghiamati, R. Manoharan, W.H. Nelson, J.F. Sperry, *Appl. Spectrosc.* 46 (1992) 357.
- [6] T. Phillips, J.L. Sample, P.F. Scholl, J. Mirragliotta, *Mater. Res. Soc. Symp. Proc.* 738 (2003) G8.7.1–G8.7.6.
- [7] H.F. Hameka, J.O. Jensen, J.L. Jensen, C.N. Merrow, C.P. Vlahacos, *J. Mol. Struct. (Theochem)* 365 (1996) 131.
- [8] A. Fadini, F.-M. Schnepel, *Vibrational Spectroscopy*, Wiley, New York, 1989 (pp. 40–62).
- [9] F.R. Dollish, W.G. Fateley, F.F. Bentley, *Characteristic Raman Frequencies of Organic Compounds*, Wiley, New York, 1974 (pp. 284–291).
- [10] D.L. Rousseau, J.M. Friedman, P.F. Williams, in: A. Weber (Ed.), *Raman Spectroscopy of Gases and Liquids*, Springer, Heidelberg, 1979, pp. 203–252.