FT-Raman study of (hydroxypyridin-3-yl-methyl)phosphonic acid with varying pH  
2D correlation method

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Abstract

In the current study, the (hydroxypyridin-3-yl-methyl)phosphonic acid, a model compound of risedronate, a drug that have been used recently in osteoporosis treatment, is investigated by FT-Raman spectroscopy. The main goal of this work is to examine the changes in the molecular structure of this ligand as a function of pH. The vibrational band assignments of pH dependent species of (hydroxypyridin-3-yl-methyl)phosphonic acid is very complex. Thus, in this work, 2D correlation method is applied successfully to analyze the FT-Raman spectra of the investigated forms of a title compound obtained from aqueous solution at different pH. The change of pH is used as the external perturbation in this correlation method.

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1. Introduction

Several bisphosphonate drugs have been used in the last decade in osteoitic bone disease in humans. Among others, phosphonopyridines and their derivatives have been investigated due to their potential biological importance [1–7]. The most powerful one, 2-(3-pyridinyl)-1-hydroxyethylidene bisphosphonic acid (so-called risedronate), has been found to be very active at the single intravenous dose [8]. However, even the small changes in the structure of the bisphosphonates can lead to extensive alterations in their physicochemical, biological, therapeutic, and toxicological characteristics [1–7].

In this work we investigate molecular structure of (hydroxypyridin-3-yl-methyl)phosphonic acid, called MC5, in dependence on pH. Four species are expected for MC5 to appear in aqueous solution at different pH. Their \(pK_a\) values were calculated previously [6,9]. Their structures are presented in Fig. 1. The first dissociation of the proton occurs from the phosphonic group (\(pK_{a1}\)) well below 2.0. Additionally, at this pH, pyridine nitrogen atom is protonated (MC5(0)). Then the next step is the deprotonation from the pyridine nitrogen (MC5(−1)) that is followed by the next proton dissociation from the phosphonic group. Their respective values were established at 5.13 (\(pK_{a2}\)) and 6.93 (\(pK_{a3}\)) based on potentiometric measurements [6].

FT-IR spectra of MC5 species precipitated from aqueous solution at different pH have been reported previously [10]. Assignment of the experimental absorptions was made based on quantum-chemical calculations using the B3PW91/6-31G* method. The exact interpretation of experimental data was in many cases not very accurate because of the presence of very complex hydrogen bond network. In the current study, FT-Raman spectra of four MC5 species are presented. Additionally, generalized 2D frequency correlation method is applied to analyzed these spectra with varying pH which is regarded as an external stimulus.

2. Experimental

The compound abbreviated as MC5 was synthesized as described earlier [11,12] in its zwitterionic form. The
cationic form of MC5 in the solid state was obtained by slow and controlled evaporation of MC5 aqueous solution of concentrated HCl at pH below 1.0. Both anions were prepared by titration of MC5 aqueous solutions with a simple base (for example NaOH) to the values of 6.6 (monoanion) and 8.0 (dianion) followed by solvent evaporation. In these conditions simple seeds were obtained with ionic bond cation-ligand anions (cation-MC5(+1), cation-MC5(+2)).

The FT-Raman spectra were measured on a Bio-Rad Raman Accessory spectrometer model FTS 6000 equipped with liquid nitrogen-cooled germanium detector. The samples were excited with the 1064 nm line from a Spectra-Physics cw Nd:YAG laser. Power at the sample was maintained at 200 mW. Spectra were collected with 4 cm\(^{-1}\) resolution. The accuracy of the frequency readings for all presented spectra was ±2 cm\(^{-1}\).

Four pH dependent spectra of MC5 were analyzed by 2D correlation technique as proposed by Noda [13]. The software for calculation and presentation of 2D spectra is included in the Bio-Rad software.

3. Results and discussion

3.1. 1D spectra

Experimental FT-Raman spectra of MC5 species in the solid state obtained from aqueous solution at the pH values of: 8.0, 6.6, 3.5, and below 1.0 are presented in Fig. 2. The most important information of MC5 deprotonation is appears in the spectral range of 700–1700 cm\(^{-1}\). The deprotonation sequence and pK\(_a\) constants are known from the

Fig. 2. FT-Raman spectra of MC5 in solid state precipitated from aqueous solutions at the pH values of: 8.0, MC5(+2); 6.6, MC5(+1); 3.5, MC5(0); <1.0, MC5(+1).
previous potentiometric studies (see Fig. 1) [6]. The changes of pH influence predominantly the aromaticity of the pyridine ring and phosphonic moiety [7,10,14]. Thus, we focus our attention on changes of the characteristic frequencies of these fragments. The proposed assignment of the modes is based on previously conducted calculations based on the B3PW91/6-31G** method [10] and on characteristic group frequencies [14–16].

The most characteristic vibrations of the pyridine ring are observed at 1631 and 1048 cm$^{-1}$ for MC5(1), 1636 and 1048 cm$^{-1}$ for MC5(0), 1588 and 1044 cm$^{-1}$ for MC5(–1), and 1588 and 1044 cm$^{-1}$ for MC5(–2). The cationic and zwitteranionic forms of MC5 possess the protonated pyridine nitrogen in contrast to the both anionic forms. Thus, it is not surprising that the deprotonation at this part of molecule (nitrogen atom) influences significantly the frequency of these modes.

Phosphonic acids in ionic forms, i.e., compounds containing R-PO$_3$H$_2$, R-PO$_3$H$^-$ or R-PO$_3^{2-}$ groups, can be characterized based on their P=O stretching, $\nu$(P=O), P=O(H) stretching, $\nu$(P–O), and P–C stretching, $\nu$(P–C), vibrations. However, in the case of compounds with long alkyl chains or strong ring deformation vibrations these characteristic above-mentioned bands can be easily obscured. Additionally, in the presence of the strong hydrogen bonds that are often encountered in these compounds, $\nu$(P=O) and $\nu$(P–O) can be broadened and down-shifted below expected regions.

The successive proton dissociations shift the phosphonic group vibrations as a result of the changes in the P–O bond order. The modes associated with this group are seen in the IR spectra as medium to strong absorptions, but it is necessary to stress that their exact frequencies are often uncertain because of the complexity of the modes, that appear in these regions. Aforementioned changes are often more difficult to observe in Raman spectra, due to weak intensity of characteristic bands of the phosphonic group. However, with the help of DFT calculations and comparison with the FT-IR spectra the assignment of the observed FT-Raman bands is possible. This procedure allows to extract from the FT-Raman spectra characteristic modes associated with the –PO$_3$H$_2$, –PO$_3$H$^-$ and R-PO$_3^{2-}$ groups [10]. Thus, the P=O and P–O stretchings for cationic form MC5(1) are observed at 1257 cm$^{-1}$ and in the range of 941–760 cm$^{-1}$, respectively. Following, comparison with the FT-IR spectra allows to locate $\nu$(P=O), in case of MC5(0), at 1225, 1040, and 1012 cm$^{-1}$. On the other hand, $\nu$(P–O) is observed at 810 cm$^{-1}$. After deprotonation of the pyridine nitrogen atom, for MC5(–1), the $\nu$(P=O) stretching is observed at 1272, 1054, and 1034 cm$^{-1}$, whereas the $\nu$(P–O) stretching is assignment to a band at 963 cm$^{-1}$. MC5(–2) shows characteristic three equivalent P–O bonds. The vibrations of this group are seen as bands at 1168, 1053, and 875 cm$^{-1}$.

3.2. 2D spectra

The 700–1700 cm$^{-1}$ synchronous correlation map of four spectra, is shown in Fig. 3. The intensity of synchronous 2D correlation spectrum represents the simultaneous or coin-
cidental changes of spectral intensity variations of pH regarded as an external perturbation. Correlation peaks are seen at both diagonal and off-diagonal positions. The diagonal peaks, referred to as autopeaks, are always positive and reflect overall extend of spectral intensity variation observed during MC5 deprotonation. In the 2D spectrum of MC5 shown in Fig. 3, the strongest autopeaks appear in the range of 1055–1045 cm\(^{-1}\). This means that this region of spectral intensity changes to a great extend under a pH perturbation. In the range of 1044–1048 cm\(^{-1}\) one expects vibrations of the pyridine ring. On the other hand, \(\nu(C-N)\) should be observed in the range of 1012–1054 cm\(^{-1}\). A distinct autopeak that is observed at about 1220 cm\(^{-1}\) can be attributed to the phosphonic group vibrations. The weaker autopeaks seen at 1636 and 760 cm\(^{-1}\) are due to the vibrations of the pyridine ring and phosphonic group vibrations, respectively. The weakest diagonal peaks at 1190 and 1022 cm\(^{-1}\) are the most probably originated from the aliphatic carbon atom and the pyridine moiety vibrations, respectively.

The cross peaks located at off-diagonal positions represent simultaneous or coincidental changes of spectral intensities observed at two different frequencies (Fig. 3). The most intensive off-diagonal peaks are seen for band pairs at 1220, 1053 cm\(^{-1}\) and 1053, 760 cm\(^{-1}\). These bands are associated with the vibrations of the pyridine ring. On the other hand, two peaks at 1636 and 1048 cm\(^{-1}\) are due to the vibrations of the pyridine ring. Analysis of the above-mentioned data indicates that the clear changes of spectral intensities are associated with the pyridine ring and the phosphonic group vibrations, as expected. The spectral intensity changes do not couple and they are observed separately. The other cross peaks that appear at 1220, 760 cm\(^{-1}\) and 1048, 1020 cm\(^{-1}\) have the same assignment and meaning as it was discussed above. However, a few off-diagonal peaks are also seen, for example, at 1636, 1220 cm\(^{-1}\) and 1190, 1053 cm\(^{-1}\). This probably is due to the presence of significant coupled changes of spectral intensities in all MC5 forms. All discussed synchronous cross peaks are positive. Thus, the spectral intensities at corresponding frequencies are changing simultaneously, i.e. decreasing or increasing in phase of their intensities is observed.

Fig. 4 shows the asynchronous 2D FT-Raman frequency correlation spectra of the MC5 species in the range of 700–1700 cm\(^{-1}\). The intensity of asynchronous peaks indicates the sequential or successive changes of spectral intensities measured at correlated frequencies. The strongest and the most interesting features are asynchronous cross peaks originate from the pyridine ring that appear as a positive band at 1631 cm\(^{-1}\) (characteristic for MC5(0)) and a negative band at 1048 cm\(^{-1}\) (characteristic for MC5(0) and MC5(-1)), and also as a positive band at 1048 cm\(^{-1}\) and a negative band at 1588 cm\(^{-1}\) (characteristic for MC5(-1) and MC5(-2)) as well as a positive band at 1636 cm\(^{-1}\) (characteristic for MC5(0)) a negative band at 1588 cm\(^{-1}\). The analysis of the signs of discussed asynchronous cross peaks shows the chronology of successive changes in spectral intensities. Thus, the intensity change at 1631 cm\(^{-1}\) occurs predominantly before that at 1048 cm\(^{-1}\). Next the intensity

![Fig. 4. Asynchronous 2D FT-Raman frequency correlation spectrum in the wavenumber range of 700–1700 cm\(^{-1}\) (contour 2D plotting).](attachment:image.png)
change at 1048 and 1636 cm$^{-1}$ occurs before that at 1588 cm$^{-1}$. In consequence, the simple dependence between pH increase and the changes of spectral intensities of the bands originated from the pyridine ring of different forms of MC5 is observed. The other pairs of cross peaks, all associated with the phosphonic group, appear at 1220 (positive) and 1040 cm$^{-1}$ (negative) as well as 1054 (positive) and 1040 cm$^{-1}$ (negative). In conclusion it is shown that spectral intensities originated from different forms of phosphonic group correlate asynchronously.

The comparison between 1D and 2D spectroscopy shows that correlation spectroscopy is a method that can be utilized to extract useful information that cannot be often obtained from a standard 1D spectrum. In this work, the use of 2D correlation spectroscopy allowed to extract from the FT-Raman spectra characteristic vibrations of the phosphonic group. The information about the exact values of the phosphonic group frequencies are hidden in the 1D Raman spectra and they are available using quantumchemistry calculations, only. Additionally, the synchronous correlation map shows that spectral intensities at corresponding frequencies change simultaneously. In detail, 2D asynchronous map indicates precisely the sequence of the spectral changes, confirming proposed earlier the deprotonation sequence of MC5. Presented here information is not possible to be extracted from 1D spectroscopy alone.

**References**