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Structure analysis of ascorbic acid using near-infrared spectroscopy and generalized two-dimensional correlation spectroscopy

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Abstract

Ascorbic acid was investigated by FT-NIR (Fourier transform near-infrared) spectroscopy and generalized two-dimensional (2D) correlation spectroscopy. The FT-NIR spectra were measured over a temperature range of 30–130 °C. Combined near-infrared spectroscopy and generalized 2D correlation spectroscopy, the molecular structure (especially the hydrogen bond) was analyzed, four kinds of intermolecular hydrogen bond and two kinds of intramolecular hydrogen bond of ascorbic acid were elucidated, the bands of ascorbic acid were assigned. The results will be helpful to the understanding of ascorbic acid and utility of this substance.

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Keywords: Ascorbic acid; Structure analysis; NIR; 2D-correlation spectrum; Hydrogen bond

1. Introduction

Ascorbic acid (vitamin C) is an essential component in the diet of humans and a small range of other mammals. It is a six-carbon keto-lactone (Scheme 1), a strong reducing agent, serves as an antioxidant and as a cofactor in hydroxylation reactions. The hydrogen donation from ascorbic acid is thought to be primarily responsible for the anti-oxidant properties attributed to ascorbic acid. It contains four hydroxyls (two enol hydroxyls on carbon 2 and carbon 3) and a lactone, it is very easy to get oxidized and changes to dehydroascorbic acid (DHA) [1], hydroxyls play a very important role as an antioxidant.

The stability of ascorbic acid is a serious problem in the use of ascorbic acid till now, the study of the molecule structure is very important, the better understanding of the fundamental behavior of this molecule will be helpful to improve the utility of this substance.

NIR spectrum embodies abundant information of hydroxyl (O–H), amido (N–H), and C–H's vibration absorption, so it is a very powerful tool to the study of ascorbic acid containing four

hydroxyls. NIR spectra are rather complicated because they contain many overlapping bands that cannot be differentiated exactly. Thus, generalized two-dimensional (2D) correlation spectroscopy analysis [2–4] is also employed in the present study.

2D correlation spectroscopy has recently been a subject of much attention from the points of both basic science and practical applications [2], it has the ability of resolution enhancement, enables one to correlate many bands each other, makes the band assignment more feasible. Using the 2D spectroscopy, it is also possible to probe the specific order of chemical functional changes upon perturbation, perform detailed dynamics study of molecule vibration.

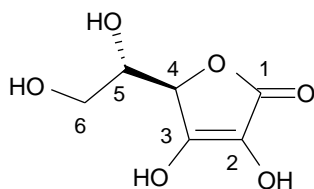
2. Two-dimensional correlation spectroscopy

The major breakthrough of 2D correlation spectroscopy occurred in 1993 when Noda published the description of the generalized 2D correlation spectroscopy concept [3–5].

The analysis of 2D correlation spectroscopy is performed through synchronous spectrum and asynchronous spectrum. As far as the specific order of chemical functional changes is concerned, when $\Phi(v_1, v_2) > 0$, in synchronous spectrum, the sign of an asynchronous cross peak becomes positive ($\psi(v_1, v_2) > 0$) if the intensity change at v_1 occurs predominantly before v_2 . It becomes negative, on the other hand; if the change at v_1 occurs after v_2 , this rule, however, is reversed if $\Phi(v_1, v_2) < 0$.

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Scheme 1. The structure of ascorbic acid.

The computations of 2D correlation spectra were performed by programming on matlab 6.5 software in the present study.

3. Experimental

3.1. Apparatus and reagents

A Bruker MPA FT-NIR (Bruker Optics Inc.) spectrometer equipped with quartz beamsplitter, an InGaAs and PbS detector. The accessories include absorption compartment (quartz cuvette cell), integrating sphere.

Ascorbic acid (purity > 99%) powder, AR grade.

3.2. Collection of the NIR spectra

The near-infrared spectra in 12,500–4000 cm^{-1} region were measured, all the spectral data were collected with a 8 cm^{-1} spectral resolution, and 64 scans were co-added to ensure an adequate signal-to-noise ratio.

The spectra of ascorbic acid on different temperature (30–130 °C) were measured by integrating sphere accessory (diffuse reflection, PbS detector) loaded in a variable-temperature quartz bottle, the interior background of MPA FT-NIR spectrometer was used which is obtained by measuring the Gold (Au) foil in the interior of instrument, and the background was collected before every measurement, the sample was kept for 15 min before every measurement.

4. Results and discussion

The NIR spectra of ascorbic acid over a temperature range of 30–130 °C at 10 °C increments by integrating sphere accessory are presented in Fig. 1.

4.1. The analysis of ascorbic acid in the 7100–6600 cm^{-1} region

Fig. 2(A) shows the NIR spectra of the ascorbic acid at different temperature (30–130 °C, every 10 °C) between 7100 and 6600 cm^{-1} . The band apparently shifts from 6850 cm^{-1} to high wave number as the temperature is increased from 30 to 130 °C, but the spectral differences are hard to visually observe.

Fig. 2(B) shows the second derivatives (Savitzky-Golay Derivative Algorithm, 17 points, the same below) of selected spectra at temperatures ranging from 30 to 130 °C, indicating peaks near 6920, 6860, 6720 cm^{-1} . They are assigned to the first overtone stretch vibration of hydroxyls (OH), all show a little shift of the peak from low wave number to high wave number with the temperature elevation, which means that the structure of ascorbic acid has some changes that are correlated with the hydroxyls (OH). It should be noted from this figure that the temperature-dependent intensity changes are greatest near at 6860 cm^{-1} , followed by the feature near 6830 cm^{-1} , and 6880, 6720 cm^{-1} .

Derivative spectra give an indication of where original spectral features cannot be found in Fig. 2(A). However, the derivative spectra are interpretable only in a visual fashion, as was also the case for the original spectra. To perform an objective and detailed analysis of the spectral dynamics, 2D correlation spectroscopy is utilized.

Fig. 3(A) and (B) shows the synchronous and asynchronous spectra, respectively, of the 2D correlation analysis in region 7100–6600 cm^{-1} . The synchronous map shows strong autopeaks at 6852 and 6925 cm^{-1} , indicating that the spectral features at these positions vary greatly (polarity changes). The appearance of negative cross-peaks (yellow color) at 6852 and

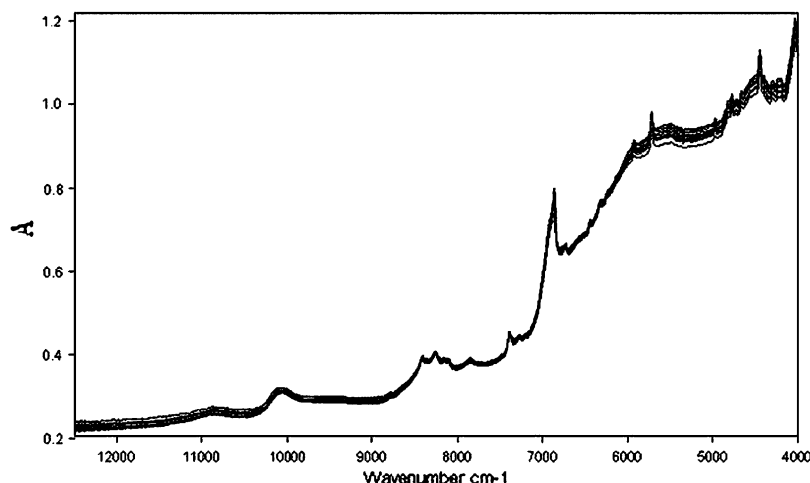


Fig. 1. The NIR spectra of ascorbic acid measured over a temperature range of 30–130 °C.

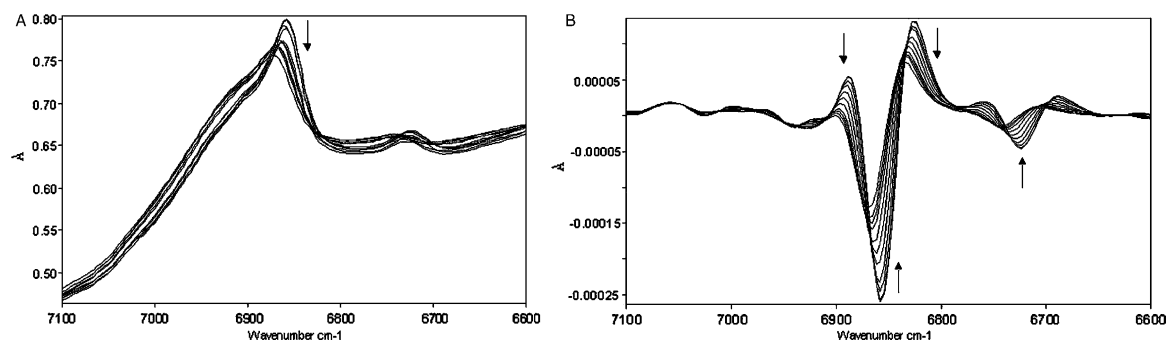


Fig. 2. NIR spectra of ascorbic acid measured over a temperature range of 30–130 °C at 10 °C increments (A) raw spectra (B) the second derivative of the same spectra, the arrows direction indicates increasing temperature.

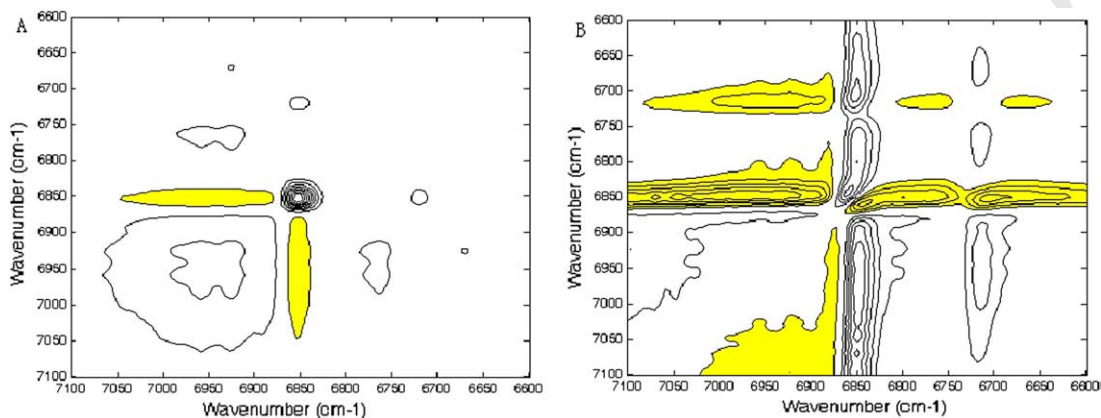


Fig. 3. Synchronous (A) and asynchronous (B) 2D correlation spectra, generated from the spectra shown in Fig. 2(A); the shaded parts denoted the negative sign.

6925 cm^{-1} between the autopeaks reveals that their changes are correlated and occur in opposite directions (one is increasing while the other is decreasing), which denotes that the intensity of the hydrogen bond OH at 6852 cm^{-1} decreased while that at 6925 cm^{-1} band increased with increasing temperature. And because the crosspeak of 6852 and 6925 cm^{-1} in asynchronous map is positive ($\psi(v_1, v_2) > 0$), so

the variance at 6852 cm^{-1} occurs after variance at 6925 cm^{-1} (the hydroxyls has higher vibration frequency with the temperature, then the hydrogen bonds were broken out) according to the principle of 2D correlation spectra.

In fact, the peak near at 6925 cm^{-1} is not a single peak, it corresponds with several vibration mode located at 6990, 6970, 6925 and 6880 cm^{-1} , respectively, which can be seen from Fig. 4 (profile map, obtained from the 3D-map of Synchronous 2D correlation spectrum).

Ascorbic acid can form four kinds of intermolecular hydrogen bond (OH and C=O), as a binary acid, the OH connected with C3 and C2 have lower vibrational frequency because the conjugate action of double bond and carbonyl. The hydrogen bond forms are exemplified in Scheme 2.

Because of the formation of hydrogen bonds, the absorption of ascorbic acid near 6900 cm^{-1} is a broad peak that can not be assigned to certain structure exactly. The apparent peak shift is attributed to the fact that the intermolecular Hydrogen bonds are generally weakened with temperature, which strengthens the covalent O–H bonds and consequently causes them to vibrate at

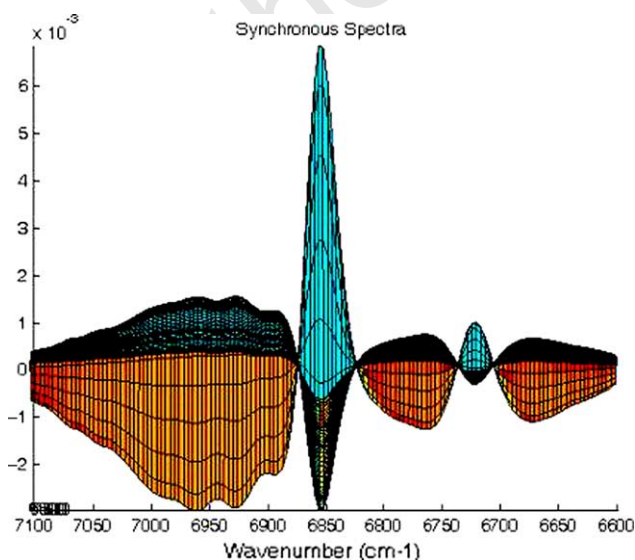
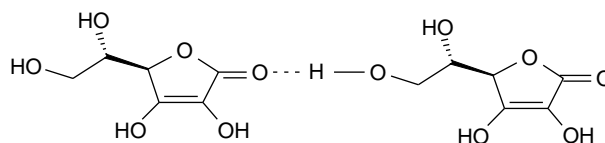
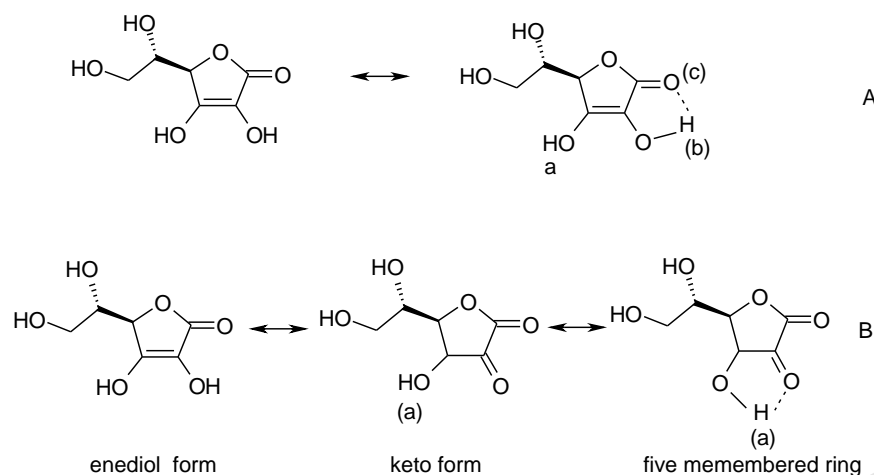


Fig. 4. The profile map of synchronous 2D correlation spectrum.



Scheme 2. The hydrogen bond form between ascorbic acid molecular.



Scheme 3. The possible intramolecular hydrogen bonds forms of ascorbic acid molecular.

higher frequencies, so it can be sure that the absorption variance at 6925 and 6852 cm^{-1} are the absorption peaks of free OH and OH in Hydrogen bond, respectively.

Combining with Mora, et al. ab initio study of ascorbic acid's minimum energy conformation [6], the bond distances of O–H in position 2, 3, 5, 6 (Scheme 1) are approximately 0.971, 0.969, 0.966, 0.964 Å, respectively. So peaks at 6990, 6970, 6925 and 6880 cm^{-1} can be assigned to the first overtone of O–H in position 6, 5, 3, 2 (Scheme 1), respectively.

There is a weak peak located at 6720 cm^{-1} in Fig. 4, but there is no a autopeak in Fig. 3, the reason is that the autopeak intensity at 6720 cm^{-1} is small compared with the autopeak at 6852 cm^{-1} , we can find the autopeak from Fig. 4 (profile map, obtained from the 3D-map of Synchronous 2D correlation spectrum). This region is very similar with the region 6852 and 6925 cm^{-1} , except that it has low absorption intensity and variance, then it implies other kind of hydrogen bonds which have low vibration frequency, then two kinds of

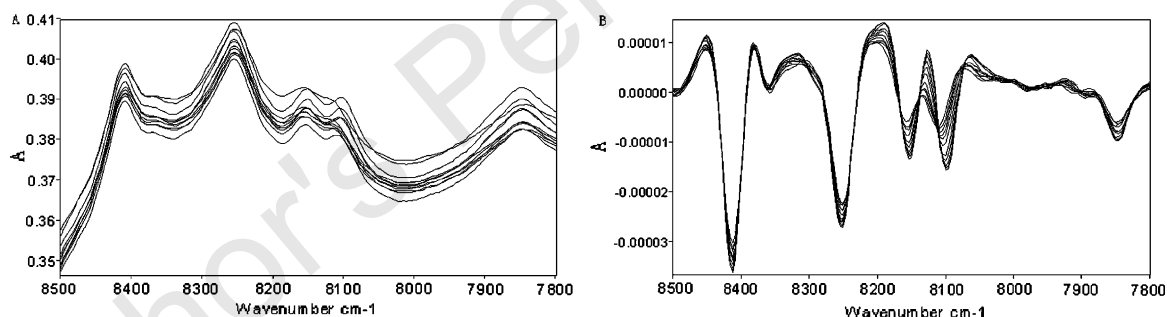


Fig. 5. NIR spectra of ascorbic acid measured over a temperature range of 30–130 °C at 10 °C increments (A) raw spectra (B) the second derivative of the same spectra.

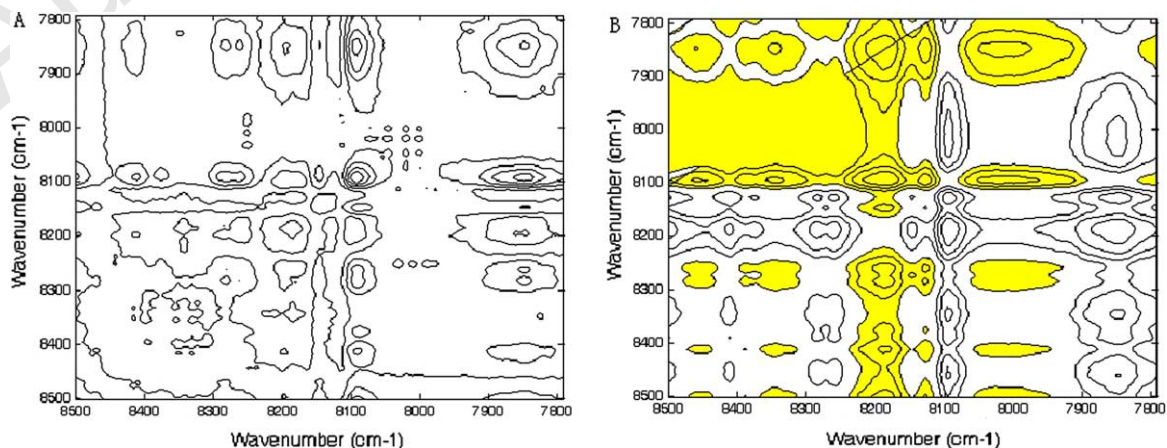
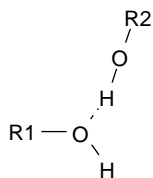


Fig. 6. Synchronous (A) and asynchronous (B) 2D correlation spectrum, generated from the spectra shown in Fig. 1(A).



Scheme 4. The hydrogen bond form.

intramolecular hydrogen bond (Scheme 3) were tentatively suggested.

The form A is agreement with Mora, et al. *ab initio* study [6] of ascorbic acid's conformation, which indicates that the H(b) and O(c) have a high value of total overlap population. Form B is agreement with John C. Deutsch's semidehydroascorbic acid free radical model [7]. Compared with normal hydrogen bond, peak of this kind of intramolecular hydrogen bond has lower frequency because of the formation of five-membered ring.

Similarly, the crosspeaks between 6720 and 6852 cm^{-1} in synchronous (positive sign) and asynchronous (negative sign) have opposite signs, so the change at 6720 cm^{-1} (intramolecular hydrogen) takes place after the change at 6852 cm^{-1} , which implies that the intermolecular hydrogen bond is more sensitive to temperature than the intramolecular hydrogen bond.

4.2. The analysis of ascorbic acid in the 8500–7800 cm^{-1} region

In order to clarify the structural changes of the intermolecular hydrogen bonding in ascorbic acid more efficiently, 2D correlation analysis is applied to the temperature-dependent NIR spectra in the spectral region of 8500–7800 cm^{-1} which involved second overtone mode of C–H in the adjacent position of OH [8]. Fig. 5 shows the raw and second derivatives spectra between 8500 and 7800 cm^{-1} at temperatures ranging from 30 to 130 $^{\circ}\text{C}$, indicating peaks near at 8410, 8251, 8152, 8095 and 7848 cm^{-1} .

Fig. 6 shows synchronous (A) and asynchronous (B) 2D correlation spectra, respectively in 8500–7800 cm^{-1} region constructed from the NIR spectra in the temperature range from

30 to 130 $^{\circ}\text{C}$. The synchronous map shows obvious autopeaks at 8095 cm^{-1} (it is assigned to C–H second overtone stretch vibration in adjacent position of C with strong O–H vibration), and 7848 cm^{-1} (assigned to C–H second overtone stretch vibration in adjacent position of C with weaker O–H vibration). The appearance of positive cross-peaks located at 7848 and 8095, 7853 and 8120 cm^{-1} , reveals that the changes are correlated and occur in the same directions. The asynchronous map also shows cross peaks at 8095 and 8185, 8095 and 8023, 8095 and 8345, 8095 and 8450 cm^{-1} ; 7848 and 8925, 7848 and 8187, 7848 and 8345, 8450 and 7848 cm^{-1} , indicating that out-of-phase spectral changes occur at these wave numbers.

From the synchronous and asynchronous maps, there are three important bands located at 8195 (the autopeak is weak), 8095 and 7848 cm^{-1} generally, and all the changes are in the same direction from synchronous map. Combining with the sign of asynchronous, the change at 8195 cm^{-1} is slower than other bands, while changes at 8095 and 7848 cm^{-1} are faster. At the same time, the two bands at 8095 and 7848 cm^{-1} have no obvious sequence order, they changes at the same phase, indicating that the two bands share an identical temperature-dependent pattern. So the peaks at 8095, 7848 cm^{-1} were assigned to the C–H connected with intermolecular hydrogen bond, peak at 8195 cm^{-1} to the absorption of C–H at position C3 in Scheme. 1 (keto form, has weak autopeak). This also indicates a sequence order of intensity changes that the intermolecular hydrogen is faster than intramolecular hydrogen.

4.3. The analysis of ascorbic acid at 7387 cm^{-1}

It is very strange that there is a absorption bands located at 7387 cm^{-1} which cannot be assigned to certain group or structure, because the wave number is a little higher than the first overtone of OH, but lower than the second overtone of C–H. But, compared with the NIR spectra of water, it can be seen that the 7387 cm^{-1} belongs to the first overtone absorption range of OH too. Because the absorption peak of water's free OH located near 7090, so the 7387 cm^{-1} is not the absorption of free OH, and the peak shape is very similar with the peaks at 6852, so the 7387 cm^{-1} peak was tentatively

Table 1
The assignments of NIR bands

Hydroxyls (O–H)				C–H	
Wave number (cm^{-1})	Bond and vibration	Structure	Wave number (cm^{-1})	Bond and vibration	Structure
10,870	O–H 2nd overtone str	CH_2OH	8410	C–H astr 2nd overtone	CH_2
10,050	O–H 2nd overtone str	CHOH	8251	C–H str 2nd overtone	CH_2
7387	O–H 1st overtone astr	Hydrogen bond	8152 and 8113	C–H str 2nd overtone	CH
6925	O–H 1st overtone str	Free OH	7845	C–H str 2nd overtone	CH
6857–6868	O–H 1st overtone str	OH (hydrogen bond)	5920	C–H str 1st overtone	CH_2
6722	O–H 1st overtone str	Five membered ring	5713	C–H str 1st overtone	CH
4967	C=O 2nd overtone str	C=O	4030	C–H str and C–C str combination	C–CH
				Or: CH_2 combination	CH_2
4444	OH str and C–O str combination	C–C–OH			

The wave numbers of NIR peaks are not exactly same with autopeaks and crosspeaks; str, stretch vibration; astr, asymmetric stretch vibration.

assigned to the first asymmetric stretch vibration overtone of hydrogen bond just like the hydrogen bond existed in water (Scheme 4).

Because the O connected with two H in Scheme 4, so it is possible to have asymmetric stretch vibration mode, and then has higher vibration frequency. This kind of hydrogen bond has low formation probability in ascorbic acid, so the peak intensity at 7387 cm^{-1} is weak.

4.4. The assignments of ascorbic acid's NIR bands

Summing up above analysis results and referring to corresponding literature [9–12], the assignments of NIR bands are presented at Table 1.

5. Conclusion

Generalized 2D correlation spectrum has advantages for analyzing the structural changes with external perturbation to the molecule with hydrogen bond. The overlapped bands can be elucidated by the synchronous spectrum, and the sequential order of various bands can also be revealed by the asynchronous spectrum. The 2D NIR spectra are particularly useful for making band assignments and showing the sequence of spectral intensity changes.

Following conclusions can be achieved in the present study.

(1) Four kinds of intermolecular hydrogen bond and two kinds

of intramolecular hydrogen bond of ascorbic acid were suggested. (2) 2D correlation spectra constructed reveal that the intermolecular hydrogen bond is more sensitive than intramolecular hydrogen bond. (3) The band assignments of ascorbic were performed combining 2D correlation spectra. The results gain more understanding of ascorbic acid, and will be helpful to its further investigation and utility.

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