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2D-IR correlation analysis of deteriorative process of traditional Chinese medicine 'Qing Kai Ling' injection

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Abstract

The traditional Chinese medicine 'Qing Kai Ling' injection after deterioration is distinguished from the original formulation using Fourier-transform infrared spectroscopy (FTIR) and two-dimensional correlation infrared spectroscopy (2D-IR). The IR spectra of the injections before and after deterioration are so similar, that it is very difficult to distinguish them by using the conventional approach. However, the higher spectral resolution and more structural information provided by 2D-IR analysis makes the identification possible. According to the results of 2D correlation analysis, the band at 1611 cm^{-1} in IR spectra, in fact, consists of the overlap of three bands at 1572, 1667 and 1729 cm^{-1}, which are assigned to the alkaloids (heterocyclic compounds), flavone derivatives, and carbonyl compounds in the injection, respectively. Interestingly, the autopeak at 1667 cm^{-1} disappears in synchronous spectrum of the deteriorated 'Qing Kai Ling' injection. It is suggested that the deterioration of the injection in air at room temperature be due to the oxidation of the flavone compounds mainly. As all intensities of the correlation peaks involved with the band in 1059 cm^{-1} decrease greatly, it is assigned to the thermal decomposition of the glucoside components. Hence, it can conclude that the oxidation of the flavone compounds and the decomposition of the glucoside components are some of the main causes concerning to the deterioration of 'Qing Kai Ling' injection at ambient temperature. Thus, the 2D-correlation analysis provides a powerful method for the quality control of the traditional Chinese medicine injection.

Keywords: 2D-IR correlation analysis; Traditional Chinese medicine; 'Qing Kai Ling' injection; Deterioration process

1. Introduction

The concept of 2D-correlation spectroscopy originates in 2D-NMR COSY, which is obtained by the double Fourier transformation of free induction decay signals of relaxation process of a nuclear spin system excited by a set of pulse sequences. It can tell us the correlation of the atoms or groups and the interactions within and between molecule(s). This method cannot be applied to IR spectroscopy in a straightforward manner because, the time scale of IR is much

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faster than that of NMR, as much as several orders of magnitude. Noda [1] developed a two-dimensional correlation infrared spectroscopy (2D-IR) correlation spectroscopy by applying a periodic perturbation of low frequency to a sample, measuring the series of IR dynamic spectra, processing the data by a mathematical correlation analysis technique and then a pair of 2D-IR correlation spectrum, synchronous and asynchronous spectrum, was obtained. The coordinates of two dimensions in these spectra both use frequency or wave number as unit. The peaks of 2D-IR spectrum showed us the sensitivity for each IR band or each functional group and the correlation between the functional groups, even the order of the influence when the system was subjected to a given perturbation. Noda later put forward a mathematical method to treat dynamic IR spectra obtained from a non-periodic perturbation [2], which greatly promoted the development of 2D-IR correlation technique, and made it easy to perform the 2D-IR correlation analysis by using an ordinary IR spectrometer. It has been shown that 2D-IR correlation analysis can improve the spectral resolution, obtain some dynamic information of molecular structure, as well as investigate interactions of functional groups within and between molecule(s) [3–5].

The traditional Chinese injection, originated from the traditional Chinese medical Broth, is a new sort of the traditional Chinese medicine. In recent years, it is applied widely and has been demonstrated to have excellent clinical effects, especially in the fields of circulatory system diseases and immunodeficiency. However, due to the complication of the traditional Chinese medicine system, there are still many problems in the traditional Chinese injection, such as the solubility of different effective components, absence of unified method to purify various components, fewer doses than that of the traditional medical broth, etc. In recent years, many accidents of adverse drug reactions after the use of the traditional Chinese injection are reported [6]. It is popularly viewed that the essential factor of those adverse drug reactions is the lack of an accurate quality standard in manufacture process, and the properties changed in the procedure from preparation, transportation and storage to clinic usage. Thus, the quality control of traditional Chinese injection is a very important subject of research for its farther development [8].

'Qing Kai Ling' injection is one of the commonly used traditional Chinese injections and is administered via vessels or muscles to patients. It has excellence efficacy on circulation system disease, phlogistic disease, virosis and some inexplicable fever. So the research of this injection is one of the hotspot of modernizing traditional Chinese injection [7]. The effective components of 'Qing Kai Ling' injection, which is produced from Baikal skullcap root, bezoars, honeysuckle flower, isatis root, buffalo horn, etc. are extremely complex. Furthermore, it is known that, after exposure to air for certain duration, it deteriorates itself but the variation is not significant in color or other aspects. Generally it is very difficult to identity the presence of deterioration by routine analytical methods. In this study, we try to distinguish the subtle sign of deterioration of the injections by using 2D-IR correlation analysis, and then find the nature of the constituent components before and after this deterioration process. Finally, we try to investigate a new method for effectively monitoring and controlling the quality of 'Qing Kai Ling' injection.

2. Experiment

2.1. Apparatus

Spectrum GX Fourier-transform infrared spectroscopy (FTIR) spectrometer (Perkin–Elmer), equipped with a DTGS detector, in the 400–4000 cm^{-1} range with a resolution of 4 cm^{-1}. Spectra are obtained from the co-addition of a total of 32 scans.

Portable programmable temperature controller (Model 50-886, Love Control Corporation). Range: room temperature to 150 °C.

2.2. Samples

All of samples are common marketable products; produced by Shenwei Medicine Co. Ltd., Hebei Province, P.R. China; the batch number is 0005083.

2.3. Procedure

The injection was directly frozen and dried into a powder, then, blended with KBr powder, ground, pressed into a tablet. After that, the infrared spectra of all samples were collected.

Put the sample in the sample pool of the temperature controller. A pre-established program controls the whole process of increasing temperature. During temperature increasing, the spectra are collected at intervals of 20 °C.

While, other injection is opened in air for 48 h, then sample is prepared and its dynamic FTIR spectra are traced as above.

2D-IR correlation spectra are obtained by treatment of the series of dynamic spectra with homemade 2D-IR correlation analysis software.

Table 1
Principal ingredient of seven medicinal materials in 'Qing Kai Ling' injection

Original drugs	Pharmaceutical name	Principal effective components
Nacre	*Pinctada margaritifera*	Ammo acids and some micro-elements etc
Buffalo horn	*Cornu bubali*	Amino acids and some micro-elements etc
Bezoars	*Calculus bovis*	Cholic acid, deoxycholic acid etc
Isatis root	*Radix isatidis*	Indigo blue, Indigo red etc
Baikal skull-cap root	*Radix scutelluariac*	Baicalin, Scutellarin etc
Honeysuckle flower	*Flos lonicerea*	Flavone derivatives, Chlorogenic acid etc
Gardenia	*Fructus gardeniae*	Jasminoidin, Crocin, Chlorogenic acid, Quinic acid, other glucosides

3. Results and discussion

3.1. Main components analysis

'Qing Kai Ling' injection is a traditional Chinese medicine produced with seven officinal natural materials, including Baikal skullcap root, bezoars, honeysuckle flower, isatis root, buffalo horn, gardenia and nacre. According to recent researches, their main effective components are shown as Table 1, [9].

The frequencies of IR bands are considered to be the unique spectral fingerprint of the 'Qing Kai Ling' injection. This holistic assignment method is based on two reasons: first, it is consistent with 'dialectic cure', which is one of the theoretical foundations of traditional Chinese medicine; second, the information about stability and deterioration process can be obtained directly in original states. So the holistic assignment of IR bands is suitable for the practical usages. Although the components of the system are very complex, principal components in the injection can still be classified according to specific functional groups.

In Table 1 the principal effective components can be classified into several species: terpenes, flavone derivatives, glucosides, amino acids, other carboxyl acids, a few of alcohols, fats, a few of inorganic salts.

The 'Qing Kai Ling' injections before and after the exposure to air are both brown clear liquid, with no deposition and no turbidness. In other words, no noticeable difference in appearance could be detected by a simple visual inspection.

3.2. Assignment of IR spectra and real-time dynamic spectra analysis

Fig. 1 shows IR spectra of 'Qing Kai Ling' injection at room temperature before and after deterioration. By primary holistic assignment, it can be confirmed that the band at 1611 cm^{-1} is assigned to an overlap of specific bands of terpenes, flavones, and alkaloid components; 1058 cm^{-1} is assigned to a specific bands of glucosides; 3285 cm^{-1} is assigned to an overlap of specific bands of hydroxide and amine groups in all compounds; 2936 and 1407 cm^{-1} are assigned to the C–H vibration in all compounds [10].

Comparison of the two spectra in Fig. 1 reveals that the positions and shapes of the main specific bands are quite similar to each other, except the

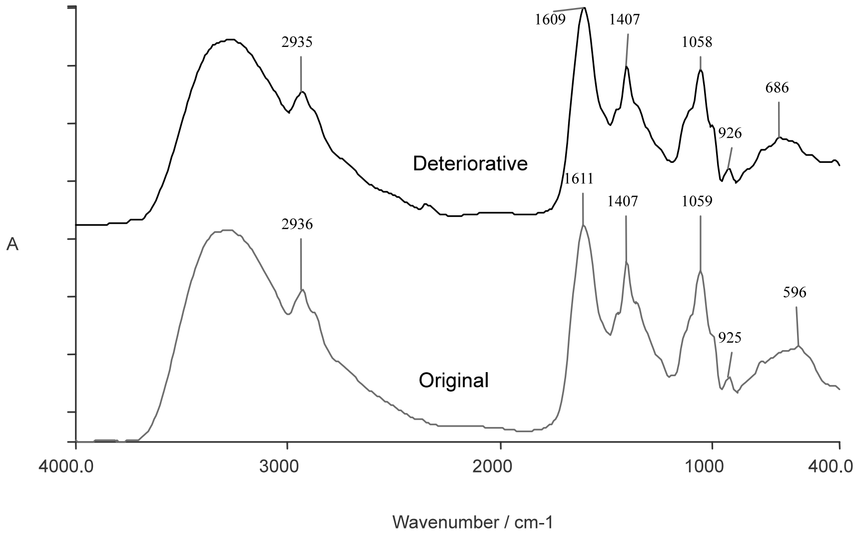

Fig. 1. FT-IR spectra of ‘Qing Kai Ling’ injection before and after slight deterioration by the exposure to air. Deteriorative sample (top); original sample (bottom).

hydroxyl group at 3285 cm^{-1} and the band of the fingerprint region at 500–800 cm^{-1}. However, this feature alone is not good enough for the purpose of monitoring and controlling the qualities of injection.

Fig. 2 is the real-time dynamic IR spectra of ‘Qing Kai Ling’ injection before (A) and after (B) deterioration scanned over a temperature region from 30 to 150 °C, respectively. Some components of the sample decompose, resulting in severe variations of the spectra at higher temperature.

It can be seen from Fig. 2(A) that the intensity of hydroxyl group band at 3285 cm^{-1} decreases rapidly. The intensities of bands at 1611 and 1059 cm^{-1} decrease together. However, their positions change a little while the temperature increased. In Fig. 2 two sets of spectra, (A) and (B), are too similar to be easily discriminated.

Hence, it can be concluded that, due to short deteriorative duration, the main components of ‘Qing Kai Ling’ injection are not changed dramatically. In addition to the complexity of the system, it is very difficult to unambiguously identify ‘Qing Kai Ling’ injection before and after deterioration utilizing conventional IR techniques only.

3.3. The results of 2D-IR correlation analysis

3.3.1. Synchronous analysis

According to the analyses and assignments of IR spectra shown in Fig. 1, the bands of the range between 900 and 1800 cm^{-1} include the specific bands of main components of ‘Qing Kai Ling’ injection. Using thermal perturbation and a home-made correlation analysis computer program, we performed 2D-IR correlation analysis for this region and obtain the 2D-IR spectra.

The 2D-IR correlation spectra of the original ‘Qing Kai Ling’ injection are shown as Fig. 3. The synchronous spectrum is on the left, and the asynchronous spectrum is on the right. The contour with blank is regarded as a positive peak, while that with shadow is regarded as a negative peak. The correlation peak in synchronous spectra represents a consistency or commonality of structural changes of the related IR vibrations with the temperature as a perturbation, while the one in asynchronous spectra represents an independency among difference structures.

In synchronous spectra, the autopeaks on the diagonal line reveal the variation of the individual band of interest with the increasing temperature,

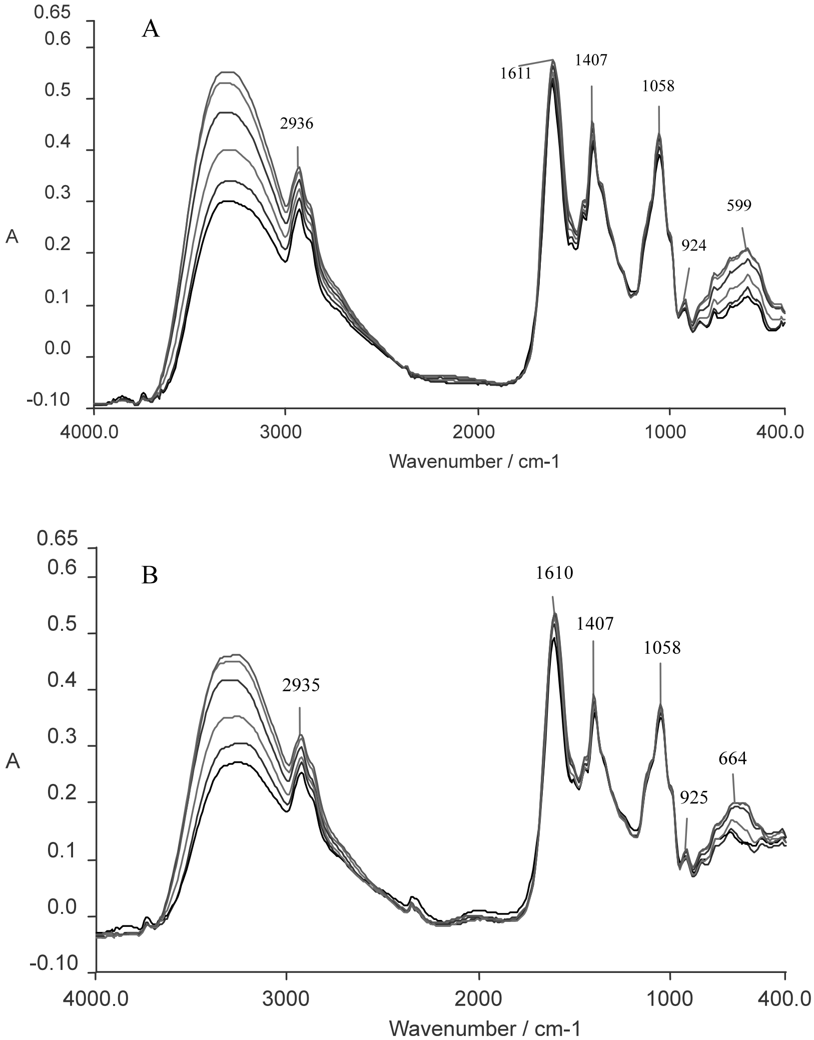

Fig. 2. FT-IR dynamic spectra of ‘Qing Kai Ling’ injection during variable temperature process. (A) Original sample; (B) deteriorative sample.

and the cross peaks located at the off-diagonal position $(\acute{\imath}_i, \acute{\imath}_j)$ reveal the coordinated variations of intensities of a pair of group vibrations corresponding their frequency $\acute{\imath}_i$ and $\acute{\imath}_j$. In asynchronous spectra, the cross-peaks reveal that the independent intensity variation of a pair of group vibrations corresponding their frequencies at different temperatures, which can provide the information about the order of microstructure or compositional variations.

Base on 2D-IR correlation principle [11], it can be seen from Fig. 3 that the broad bands in IR spectra may be generated by two overlapping peaks, and can be differentiated clearly due to their different thermo-sensibility. In synchronous spectra, two more intense autopeaks located at 1572 and 1667 cm^{-1} vary obviously with increasing temperature. Another autopeak at 1417 cm^{-1} is relatively weak. In off-diagonal area appear many cross-peaks, in which the peaks at (1667, 1417), (1667, 1572), (1572, 1059), (1572, 1417) are positive, and the positions at (1667, 975), (1667, 1217), (1667,1729), (1572, 975), (1572, 1217), (1572, 1729) are negative.

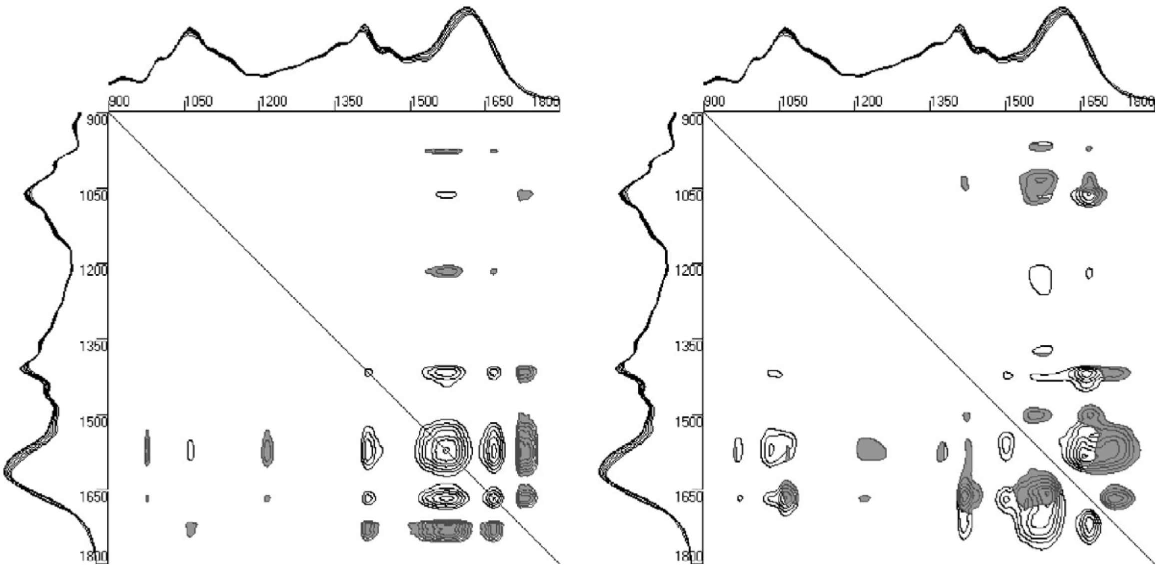

Fig. 3. 2D-IR correlation spectra of 'Qing Kai Ling' injection for original sample. Left, synchronous spectrum; right, asynchronous spectrum (unit, cm^{-1}).

Furthermore, it can be concluded that the position at 1611 cm^{-1} in spectra in fact consists of three absorption peaks: 1572, 1667 and 1729 cm^{-1}. These groups correlate to each other, and the thermo sensibility of IR band at 1729 cm^{-1} is reversed to that of the others. These bands are tentatively assigned to the alkaloids (heterocyclic compounds), flavone derivatives, and other carbonyl compounds in the injection, respectively.

3.3.2. Asynchronous analysis

By combining the information from asynchronous and synchronous spectra, we can estimate the variation sequence of different groups daring the heating. When the sign of cross peaks in synchronous spectrum, φ (v_i, v_j) is greater than zero, the sign of an asynchronous cross peak, ψ (v_i, v_j) (when $v_i < v_j$), becomes positive if the intensity change at v_j occurs predominantly before (i.e. at a lower temperature compared with) v_j; it becomes negative, on the other hand, if the change occurs after (i.e. at a higher temperature compared with) v_j. This rule is reversed if φ $(v_j,\ v_j) < 0$. For example, the cross-peaks (1572, 1667) appear both in the synchronous and asynchronous spectrum, in the synchronous correlation spectrum its sign of intensity φ (1572, 1667) > 0, and in the right-up region of the asynchronous correlation spectrum its sign of intensity ψ (1667, 1572) > 0 also, so the variation of the group corresponding the band at higher wave number 1667 cm^{-1} is earlier (i.e. lower temperature) than the one at 1572 cm^{-1}. Similarly, the band at 1729 cm^{-1} is prior to 1667 cm^{-1}, the 1417 cm^{-1} lags the others of three and 1059, 1217 cm^{-1} are prior to 1572, 1667 cm^{-1}.

Fig. 4 shows 2D-IR correlation spectra of 'Qing Kai Ling' injection after exposed to air, which differs obviously from Fig. 3. The autopeak at 1667 cm^{-1} and the cross peak at (1417, 1667) disappeared. It can be deduced that the deterioration of the injection at room temperature mainly ascribes to oxidation of the flavones. Simultaneously, the cross-peaks around 1059 cm^{-1} greatly decrease. It suggests that main reasons of the deterioration are due to the oxidation of the flavones and the thermal decomposition of the glucosides.

Cursory comparison of the asynchronous correlation spectra of deteriorated and original injection reveals that they are apparently quite dissimilar to each other. However, from the asynchronous spectra, it is found that the variation pattern and

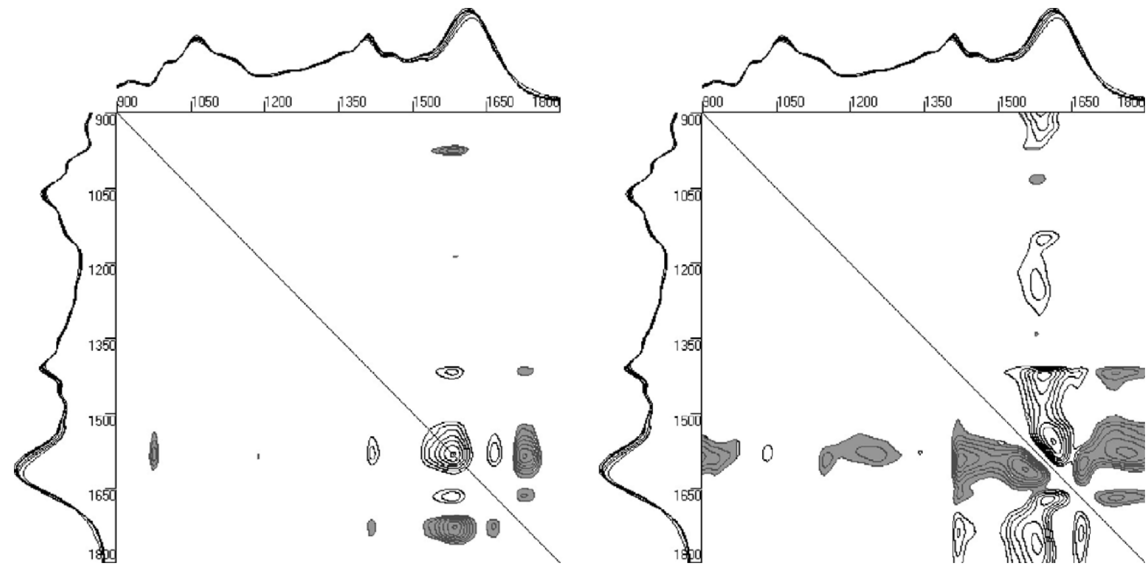

Fig. 4. 2D-IR correlation spectra of ‘Qing Kai Ling’ injection for slightly deteriorated sample. Left, synchronous spectrum; right, asynchronous spectrum (unit, cm^{-1}).

mutual relation of the main four IR bands at 1729, 1667, 1572 and 1417 cm^{-1} are actually very similar to that of the original injection. Combined with IR spectra, it may be suggested that the four groups corresponding to these IR bands are more susceptible to temperature than other components, and hold larger proportion in the complex system. Although in the dynamics process the variation of other groups is not the same between the samples before and after deterioration, the main variation is still surprisingly similar. It is difficult to obtain this information by the sample observation of IR spectra.

4. Conclusion

Using 2D-correlation analysis technique to treat a series of dynamic IR spectra, the spectral resolution can be increased, and the overlapping band in a complex spectrum can be simplified. The analyses show that the band at 1611 cm^{-1} in IR spectra consists of three overlapping bands which at 1572, 1667 and 1729 cm^{-1}. These bands are assigned to the contributions from alkaloids (heterocyclic compounds), flavone derivatives, and some other carbonyl compounds in the injection, respectively. Hence, it can be concluded that the main causes about the deterioration of ‘Qing Kai Ling’ injection at ambient temperature are the oxidation of flavone compounds and decomposition of glucoside components. Further experiments along the similar line of the 2D-correlation analysis of thermally induced spectral variations should provide powerful evidence to the research in clinic application.

References

[1] I. Noda, J. Am. Chem. Soc. 111 (21) (1989) 8116.
[2] I. Noda, Appl. Spectrosc. 47 (9) (1993) 1329.
[3] P. Wu, H.W. Siesler, J. Mol. Struct. 521 (2000) 37.
[4] G. Tian, Q. Wu, S.Q. Sun, Appl. Spectrosc. 55 (7) (2002) 888.
[5] Q. Wu, G. Tian, S.Q. Sun, J. Appl. Polym. Sci. 82 (4) (2001) 934.
[6] J. Chen, Z.Z. Ding, Q.C. Chen, X.N. Li, Chin. Traditional Patent Med. 9 (1996) 52.
[7] X.C. Liao, L.J. Wei, S.Y. Chen, Shizhen Traditional Chin. Med. 4 (1995) 38.
[8] B.Q. Wang, Research of Quality Standard and Normal Substance of Chinese Medicine, Chinese Medicine Science and Technology Press, Beijing, 1994.

[9] Y.B. Ji, Pharmacological Action and Application of Available Composition of Chinese Traditional Medicine, Heilingjiang Science and Technology Press, Haerbin, 1995.
[10] L.J. Bellamy, The Infrared Spectra of Complex Molecules, Chapman & Hall, London, 1975.
[11] I. Noda, Appl. Spectrosc. 44 (4) (1990) 550.