Recent Progress in Spectral Analysis Methods in Near-Infrared Spectroscopy
- Conventional spectral analysis method, chemometrics and generalized two-dimensional correlation spectroscopy

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In this short report I discuss how we can analyze complex NIR spectra. In general, spectra in the NIR region contain rich content of information about physical and chemical properties of molecules.[1,2] Unfortunately, however, the NIR region contains a number of overlapped bands due to overtones and combination modes, making the spectral analysis intricate. Therefore, we usually use chemometrics as well as spectroscopic analysis to extract information effectively from the NIR spectra. Recently, Noda and I showed the potential of generalized two-dimensional (2D) correlation spectra in analyzing the NIR spectra.[3-7]

In order to deepen the analysis of the NIR spectra, I think that i) spectroscopic analysis, ii) chemometrics, and iii) generalized 2D correlation spectroscopy are all very important. The usefulness of these three methods in unraveling the NIR spectra particularly in resolution enhancement will be described below.

Conventional Spectral Analysis Method

Figure 1A shows concentration dependence of NIR spectra of water-methanol mixtures. Strong features in the 7200-6300 cm^{-1} region consist of a number of overlapped bands due to the combination of OH antisymmetric and symmetric stretching modes of water and the first overtone of OH stretching modes of free and hydrogen bonded methanol, while weak features in the 6000-5800 cm^{-1} region are ascribed to the first overtones of CH_3 stretching modes of methanol.[8] I focus the discussion on the CH_3 stretching bands. They seem to show a significant shift, but the magnitude of the shift is not clear from the spectra shown in Figure 1A. Thus, we calculated the second derivative of the spectra shown in Figure 1A.

Figure 1B depicts the second derivative in the 6000-5700 cm^{-1} region. Now, it is clear from the second derivative that there are two major bands near 5950 and 5900 cm^{-1} and that they do show a band shift by about 30 cm^{-1}.[8] Why do the CH_3 bands show the shift with increasing the concentration of methanol? Probably, the CH_3 group interacts directly with OH groups of water.[8] The results in Figure 1B demonstrate the usefulness of the second derivative in the resolution enhancement as well as the potential of NIR spectroscopy in the studies of molecular interactions.
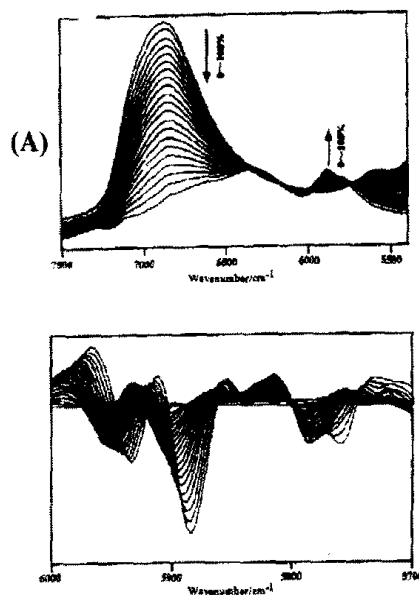
Figure 1. (A) Concentration dependence of NIR spectra of water-methanol mixtures.
(B) The second derivative in the 6000-5700 cm^{-1} region of the spectra shown in (A).

Generalized 2D Correlation Spectroscopy

Generalized 2D correlation spectroscopy applicable to various types of spectroscopy was introduced by Noda[9] in 1993 as an extension of original 2D correlation spectroscopy proposed by the same author in 1986.[10] In the new 2D correlation scheme, an external perturbation is applied to a system while being monitored by an electromagnetic probe.[9] With the application of a correlation analysis to spectral intensity fluctuations induced by the perturbation, new types of spectra defined by two independent spectral variable axes are obtained. The advantages of generalized 2D correlation spectroscopy may be summarized as follows.[6,7,9,10] i) Enhancement of spectral resolution by spreading spectral peaks over the second dimension. In other words, it can provide spectral information not readily accessible from one-dimensional spectra. ii) More detailed investigations of various inter- and intramolecular interactions through selective correlation of peaks become possible. iii) Probing the specific order of the spectral intensity changes. iv) Band assignments based upon correlations between various bands. Intriguing possibility of correlating various overtone and fundamental bands to establish unambiguous assignments in the NIR region. v) Studies of correlations between NIR bands and bands in other spectra such as Raman and mid-infrared (MIR) spectra.

Since generalized 2D correlation spectroscopy was first applied to the NIR region in 1995,[3] it has been employed to analyze temperature-dependent spectral variations of various compounds such as N-methylacetamide (NMA)[4] and Nylon-12[5]. Quite recently, generalized 2D NIR correlation analysis of concentration-dependent spectral changes of albumin solutions at various temperatures11 and 2D NIR-MIR heterospectral correlation analysis of Nylon-12[12] were also

reported.

I explain here how generalized 2D correlation spectroscopy is powerful in resolution enhancement. Figure 2A (a), (b), (c), and (d) shows NIR spectra in the 4900-4550 cm^{-1} region of ovalbumin solutions with the concentration of 0, 2, 5, and 8 wt%, respectively, measured at 60 ℃. It is impossible to identify any band in the spectra. The corresponding second derivatives of the spectra in Figure 2A are depicted in Figure 2B. Note that the calculation of the second derivative makes some bands observable. Two bands at 4850 and 4600 cm^{-1} may be due to combinations of free NH stretching? amide II(amide A/11) and intramolecular hydrogen-bonded NH stretching amide II(amide B/11) of ovalbumin, respectively.[11]

Figure 2C shows a synchronous 2D correlation spectrum, which was constructed from the concentration-perturbed NIR spectra of ovalbumin. Two autopeaks are clearly observed at 4850 and 4800 cm^{-1} in the synchronous spectrum. The band at 4800 cm^{-1} can not be identified even in the second derivative spectra. This band probably arises from the second overtone of an OH bending (3 ν_2) of water, The appearances of the autopeaks suggest that the intensities of these two bands vary most significantly with increasing the concentration. In a synchronous spectrum for 45 ℃ positive cross peaks (4850 vs.4800cm^{-1}) are also observed, indicating that their band intensities increase simultaneously.[11] In this way, 2D correlation spectroscopy emphasizes spectral features not readily observable in conventional one-dimensional spectra.
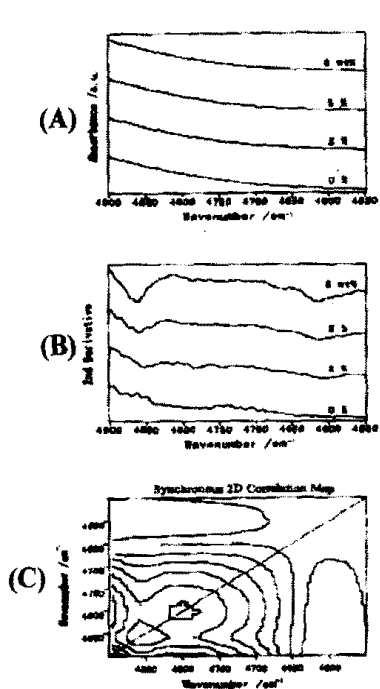

Figure 2. (A) NIR spectra in the 4900-4550 cm-1 region of ovalbumin solutions with a concentration of 0, 2, 5, and 8 wt%, respectively at 60 ℃.
(B) The corresponding second derivative spectra.
(C) A synchronous 2D NIR correlation spectrum, constructed from concentration-perturbed spectra of ovalbumin solutions at 60 ℃.

Chemometrics

Is chemometrics useful for the resolution enhancement of NIR spectroscopy? Yes, it is. Loadings plots or regression coefficients play important roles in it. Figure 3 shows NIR spectra of sec-butanol in a CC[14] solution of 0.1M measured over a temperature range of 10-60°C.[13] An intense band at 7081 cm^{-1} is assigned to the first overtone of the OH stretching mode of the monomeric species. In general, alcohols form various kinds of self-associated species such as linear and cyclic dimers and oligomers in CCl$_4$ and also form weak hydrogen bond with CCl$_4$. In addition, alcohols have rotational isomers which may give a splitting of the OH stretching band. Thus, the OH stretching band regions are usually very complicated. The band at 7081 cm^{-1} should consist at least of four OH stretching features; two bands arising from rotational isomerism of the alcohol, band due to the terminal free OH group of the hydrogen-bonded species, and that attributed to the OH group hydrogen bonding with CCl$_4$.

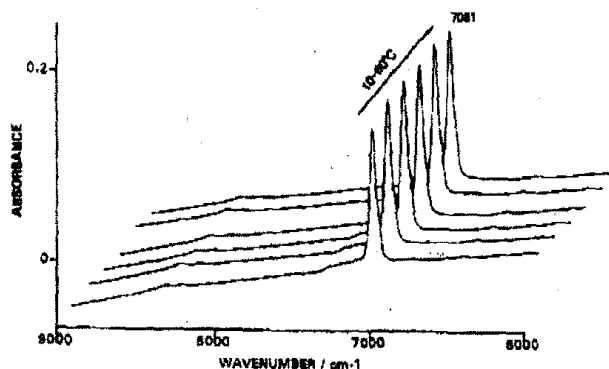Figure 3. NIR spectra of sec-butanol in a CCl4 solution of 0.1M measured over a temperature range of 10-60°C.13

In order to make these component bands observable, we calculated difference spectra by subtracting the spectrum measured at 10°C from the spectra obtained at higher temperatures. In Figure 4A is shown the difference spectrum of sec-butanol in CCl$_4$ at 40°C.[13] Based upon the temperature dependent behavior we assign the upward peaks at 7093 and 7046 cm^{-1} to the free OH groups of the monomer.[13] The splitting arises from the rotational isomerism. The bands at 7062 and 7031 cm^{-1} are due to the OH group engaging in the hydrogen bond with CCl$_4$ and to the terminal OH groups.

Figure 4B shows regression coefficients (RC$_s$) for the model predicting temperature of sec-butanol based upon the NIR spectra measured over a temperature ranges of 10-60°C.[13] The model was developed by use of partial least squares (PLS) regression.13 Interesting enough, the RC$_s$ are so close to the difference spectrum. Not only the difference spectrum but also RC$_s$ can clearly show the existence of many bands.

In conclusion, although the spectra in the NIR region are, in general, complicated, we do

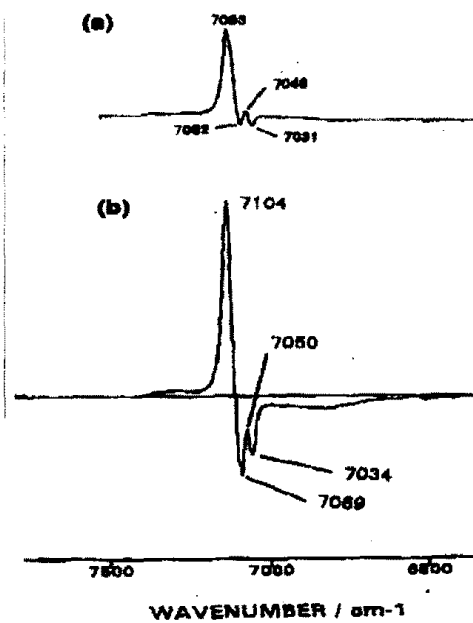have some powerful techniques which may be able to unravel them.

Figure 4A. A difference spectrum of sec-butanol in the CCl4 solution of 40°C. (B) Regression coefficients for the model predicting temperature of sec-butanol based upon the NIR spectra measured over a temperature range of 10-60°C.13

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