Characterization of Thermal Behavior of Biodegradable Poly(hydroxyalkanoate) by Two-Dimensional Correlation Spectroscopy

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Introduction

The poly(3-hydroxybutyrate) (PHB) and its copolymers poly(hydroxyalkanoate)s (PHA)s are biologically synthesized polyester produced by the fermentation of renewable biomass and are completely biodegradable under aerobic and anaerobic conditions[1]. Recently, the Procter & Gamble Company (Cincinnati, USA) has introduced a new kind of PHA copolymers with a small amount of medium length side groups, which has been commercialized under the trade name *Nodax*™ [2,3].

Generalized two-dimensional (2D) correlation spectroscopy has attracted a high level of interest of analytical science community, as it provides considerable utility and benefit in many fields of spectroscopic studies.[4,5] Some of the most notable features of generalized 2D correlation spectra are: simplification of complex spectra consisting of many overlapped peaks; enhancement of spectral resolution by spreading peaks along the second dimension; establishment of unambiguous assignments through the correlation of bands of selectively coupled by various interaction mechanisms; and determination of the sequence of the spectral peak emergence.

We have already reported the potential of principal component analysis-based 2D (PCA2D) correlation spectroscopy to improve the data quality for 2D correlation analysis.[6] We have formulated the reconstructed data matrix, which no longer contains the residual (i.e., noise) contributions, from a few selected significant scores and loadings derived from PCA of the original set of perturbation-dependent spectra. The PCA-reconstructed data matrix instead of the original data matrix has been successfully utilized for the calculation of improved 2D correlation spectra. The 2D correlation analysis of this reconstructed data matrix accentuated the most important features of synchronicity and asynchronicity without being hampered by noise. Furthermore, a radically new idea of *eigenvalue manipulating transformation* (EMT) for the PCA2D correlation analysis was demonstrated.[7-9] By uniformly raising the power of a set of original eigenvalues, the influence of factors associated with major eigenvalues becomes more prominent, while the minor eigenvectors primarily arising from the noise component are no longer strongly represented. Thus, this transformation of the data matrix becomes a gradual noise reduction scheme with attractive flexibility of continuously fine-tuning the balance between the desired noise suppression and retention of pertinent spectral information. However, by uniformly lowering the power of a set of eigenvalues associated with the original data, the smaller eigenvalues becomes more prominent and the contributions of minor components become amplified. Thus, much more subtle difference of spectral behavior for each component is now highlighted.

In this study, we have applied PCA2D correlation spectroscopy to the temperature-dependent IR spectra of biodegradable poly(hydroxyalkanoate). To better understand the thermal behavior of biodegradable poly(hydroxyalkanoate), *eigenvalue manipulating transformation* (EMT) technique was also employed.

Experimental

Bacterially synthesized poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(HB-*co*-HHx) (HHx=10 mol %)) were obtained from the Procter and Gamble Company, Cincinnati, OH. They were dissolved in hot chloroform, re-precipitated in methanol as fine powder, and vacuum-dried at 60 °C.

The transmission IR spectra were measured at a 2 cm^{-1} resolution using a Thermo Nicolet NEXUS 470 Fourier transform IR spectrometer with a liquid-nitrogen-cooled mercury-cadmium-telluride detector. A total of 512 scans were co-added for each IR spectral measurement to ensure a high signal-to-noise ratio. The temperature of the IR cell was controlled by a thermoelectric device (CN4400, OMEGA) with an accuracy of ± 0.1 °C. The temperature was increased at a rate of ca. 2 °C/min. After changing the temperature, the cell was maintained at that temperature for 15 min to make the samples equilibrate.

Prior to PCA calculation, the mean centering operation was applied to the data matrix. To preserve the amplitude information of the variation of spectral intensities, which becomes important later for 2D correlation analysis, other steps commonly used in PCA such as normalization scaling of data according to the standard deviation, were not carried out. PCA analysis was performed in the Pirouette software (Infometrix Inc.).

Synchronous and asynchronous 2D correlation spectra were obtained using the same software as those described previously.[6-9]

Results and discussion

The temperature-dependent IR spectra of PHB obtained during heating from 120 to 260 °C are shown in Figure 1. Here, we focus on C=O stretching band. As shown in Figure 1, there are two distinct bands in C=O stretching band, crystalline at 1722 cm^{-1} and amorphous band near 1740 cm^{-1}. The intensity of crystalline band decreases with increasing temperature and changes suddenly above 180 °C. It is well known that the melting temperature of PHB is 170 °C. Thus this spectral change reflects the melting of PHB.

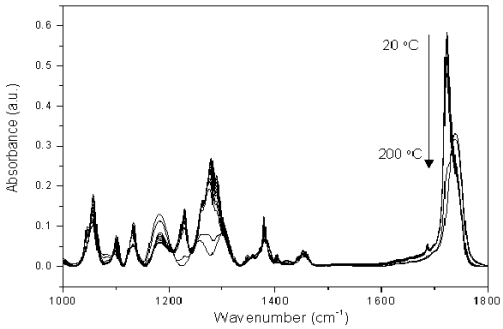

Figure 1. Temperature-dependent IR spectra of PHB obtained during heating from 120 to 260 °C

We applied PCA2D correlation spectroscopy to the temperature-dependent IR spectra of PHB shown in Figure 1. Synchronous 2D correlation spectrum shows the crystalline band at 1722 cm^{-1} and the amorphous band at 1746 cm^{-1}. However, a band at 1722 cm^{-1} in synchronous spectrum clearly resolved into two bands at 1722 and 1732 cm^{-1} in asynchronous spectrum, which is not readily detectable in the original 1D spectra. Two types of crystalline population are well ordered primary crystals observed at lower wavenumber and less ordered secondary crystals observed at higher wavenumber. From the sign of asynchronous cross peaks, the sequence of intensity changes with increasing temperature is such that the higher wavenumber band is changing at the earlier stage.

Details of thermal behavior of biodegradable poly(hydroxyalkanoate) studied by PCA2D correlation spectroscopy with EMT will be discussed.

References

[1] Satkowski, M. M.; Melik, D. H.; Autran, J.-P.; Green, P. R.; Noda, I.; Schechtman, L. A. in *Biopolymers,* Steinbüchel, A. and Doi, Y. Eds. (Wiley-VCH, Wienhiem, 2001), p. 231.
[2] Web site: www.nodax.com.
[3] Noda, I.; Green,. P. R.; Satkowski, M. M.; Schechtman, L. A. *Biomacromolecules* **2005,** *6*, 580.
[4] Noda, I. *Appl. Spectrosc.* **1993,** *47*, 1329.
[5] Noda, I.; Ozaki, Y. *Two-Dimensional Correlation Spectroscopy: Applications in Vibrational Spectroscopy* John Wiley & Sons, Inc., New York, 2004.
[6] Jung, Y. M.; Shin, S. H.; Kim, S. B.; Noda, I. *Appl. Spectrosc.* **2002,** *56*, 1562.
[7] Jung, Y. M.; Kim, S. B.; Noda, I. *Appl. Spectrosc.* **2003,** *57*, 557.
[8] Jung, Y. M.; Kim, S. B.; Noda, I. *Appl. Spectrosc.* **2003,** *57*, 564.
[9] Jung, Y. M.; Kim, S. B.; Noda, I. *Appl. Spectrosc.* **2003,** *57*, 850.