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Nondestructive determination of the ambroxol content in tablets by Raman spectroscopy

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Abstract

We describe a method for determining the ambroxol content in tablets nondestructively. To obtain a reliable quantitative calibration, we prepared 20 pellet samples (ambroxol content: 8.30–16.25 wt.%) and acquired their Raman spectra while rotating the pellets. The spectra of the rotated samples reflected the compositional variations better than those that were recorded without rotation. To reduce both the baseline variations and the spectral noise simultaneously, the spectra were pre-processed using wavelet transformation (WT). Then, we used the normalization method before partial least-squares (PLS) regression to correct Raman intensity variation from laser power fluctuation. The achieved standard error of cross validation (SECV) was 0.30%. Two different datasets where Raman intensity was artificially changed were prepared and the corresponding spectra were quantitatively analyzed. The result was reproducible even if laser intensity was fairly changed. Additionally, two different commercial tablets were analyzed and the accuracy of measurement was better for a tablet that had the similar spectral features of the standard pellet samples. The proposed method can be utilized for the analysis of commercial tablets if standard tablets of various ambroxol concentrations that have the same chemical components including additives and the same physical shape of tablets are available.

Keywords: Ambroxol; Raman spectroscopy; Partial least-squares; Wavelet transformation

1. Introduction

Ambroxol [1] is one of the most popular medicines used to relieve the symptoms of coughs, asthma, and colds. The weight of a normal tablet is ca. 240 mg, comprising mostly ambroxol and lactose as the excipient (support); the concentration of ambroxol is 12.5wt.%. To determine the ambroxol concentration in tablets, high-performance liquid chromatography (HPLC) and ultraviolet (UV) spectroscopy are used most frequently [2–5], but these approaches are destructive, slow, and require the use of chemical reagents. Additionally, it is hard to utilize these methods practically for the fast on-line analyses required for continuous quality assurance of tablets and therefore, there is a strong demand for alternative analytical methods to replace them.

Raman spectroscopy [6–8] has strong potential for use in the fast, nondestructive, on-line analysis of ambroxol tablets because it provides rich chemical and structural information without destruction of the samples. Recently, diverse non-contact Raman optical fiber probes have been developed for nondestructive analysis. In this paper, we describe a method for determining the ambroxol content of tablets using Raman spectroscopy with the aid of partial least-squares (PLS) regression [9,10]. We prepared 20 pellets with concentrations ranging from 8.30 to 16.25% and collected their Raman spectra in two different ways, i.e., with and without rotation of the sample during spectral collection. The Raman spectra obtained upon rotating a sample during measurement represented the concentration variation more correctly. Additionally, we employed wavelet transformation (WT) [11–13] to

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reduce the baseline variation selectively and the spectral noise superimposed on the raw Raman spectra. The variation of Raman intensity was corrected by using a normalization method that each spectrum is divided by area of spectral range used. The achieved standard error of cross validation (SECV) was 0.30% using PLS regression.

To evaluate the analytical performance in a situation of laser intensity variation, two different Raman sets, where laser power was artificially changed, were prepared separately to simulate routine analysis. The resulting standard error of predictions (SEP) from both sets was 0.36% approximately. Even though the resulting SEPs were slightly increased, we have achieved reproducible quantitative analytical performance by normalizing Raman intensity variation.

We have also studied actual applicability of the proposed method to commercial tablets. For this purpose, two different kinds of commercial tablets were analyzed and the accuracy of measurement was better for the tablet that had the similar spectral features of the standard pellet samples. The spectral features of commercial tablets were slightly different owing to the presence of additives such as binder, disintegration agent, and tablet lubricant. If we can prepare the standard tablets of various ambroxol concentrations that have the same chemical components including additives and the same physical shape, the proposed Raman spectroscopy combined with normalization, baseline correction, and WT will be useful for determining the ambroxol content non-invasively in practical routine analysis.

2. Experimental

2.1. Sample preparation

Twenty different sample pellets (concentration range: 8.30–16.25 wt.%) were prepared by mixing appropriate amounts of ambroxol and lactose, which were purchased from Sigma–Aldrich. The mixed powder samples were transferred into a vial and mixed thoroughly and then were pressed using a conventional infrared (IR) presser to produce final pellet samples. The diameter, thickness, and weight of each pellet were 13 mm, 0.6 mm, and 240 mg, respectively.

2.2. Raman spectral collection and data processing

Raman spectra were collected using a dispersive Raman spectrometer equipped with a diode laser (785 nm), a CCD detector, and a holographic grating (Kaiser Optical, Ann Arbor, MI, USA). A non-contact optical fiber probe was used to collect the spectra at a 4 cm^{-1} resolution. Each Raman spectrum corresponded to an accumulation of 64 scans with an exposure time of 2 s for each scan. The spectra of the pellets were collected both with and without rotation of the sample. The rate of pellet rotation was 60 revolutions per minute (rpm).

Partial least-squares regression and wavelet transformation were accomplished using Matlab Version 6.5 (The MathWorks Inc., MA, USA).

3. Results and discussion

3.1. Raman spectral features

Fig. 1 presents the Raman spectra of ambroxol and lactose, together with their molecular structures. The benzene ring in ambroxol causes the isolated and unique bands, centered in the 1670–1550 cm^{-1} range, and the intense aromatic feature at 1002 cm^{-1} (ring breathing mode). Since the spectral features of these compounds are clearly different, we expected that we would have no significant difficulty in quantifying ambroxol as long as we could collect reproducible Raman spectra. Therefore, we focused more of our efforts on finding a method of collecting Raman spectra that allows accurate quantitative representations to be made. This analysis can be accomplished by using an appropriate spectral collection configuration and spectral processing algorithms.

Fig. 2 displays a visual microscopic image (160 μm × 130 μm) of an ambroxol pellet's surface. We examined the surface to find out whether or not the collected Raman spectra were representative of the sample composition; the size of the laser illumination spot is small (1–5 μm) relative to the sample size and therefore, if the sample were inhomogeneous on the micron scale, there is a possibility of acquiring only localized chemical information rather than averaged chemical features. The picture in Fig. 2 presents a case in which the sample is inhomogeneous on the micron scale: the light- and dark-gray areas are spread irregularly. The Raman spectra of four random areas (designated A–D) were collected selectively with the aid of a microscope; the corresponding spectra are displayed in Fig. 2. Initially, we examined the range 950–650 cm^{-1} where both ambroxol and lactose have strong Raman peaks. Spectra A and D are similar to one another, but are different from spectra B and C; that is to say, although the distances

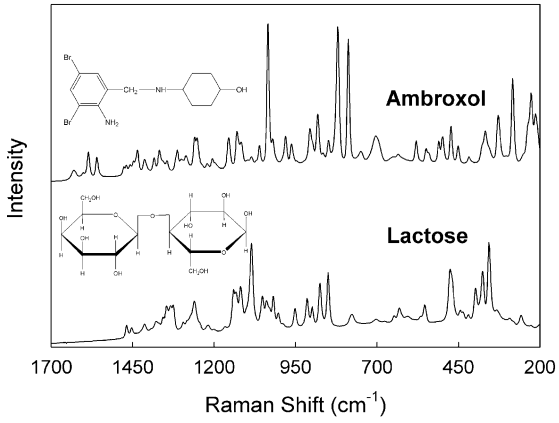

Fig. 1. Raman spectra of ambroxol and lactose.

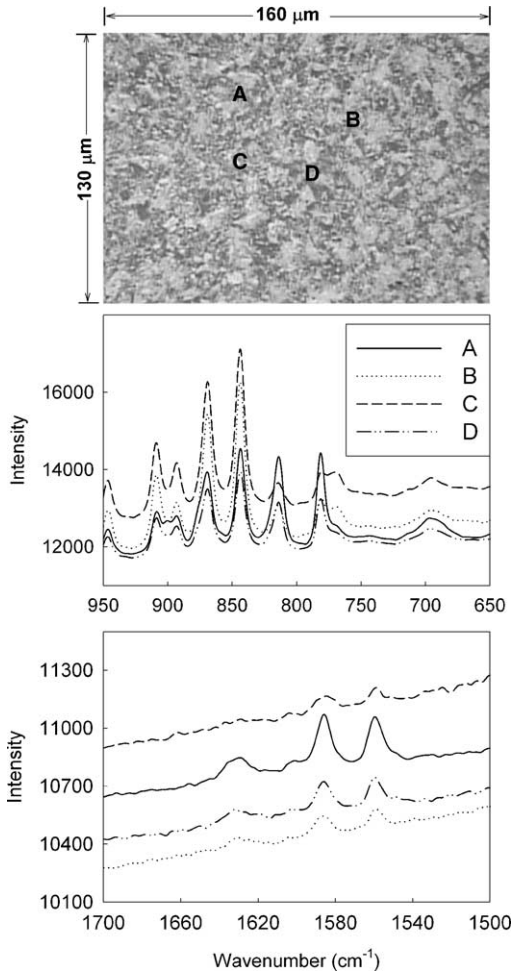

Fig. 2. Visual microscopic image of an ambroxol pellet's surface (top), Raman spectra of four different spots in the 950–650 cm^{-1} (middle) and 1700–1500 cm^{-1} (bottom).

between these four spots are very close, the resulting Raman spectra are clearly different. In addition, we examined the spectral range 1700–1500 cm^{-1} in which the unique peaks arising from the benzene ring of ambroxol appears. Again, the peak intensities at each spot obviously change as a result of variations in the local ambroxol content. These observations imply that a Raman spectrum obtained from a particular sample area, equivalent in size to the area of the laser spot, do not necessarily represent the total sample composition and, therefore, to achieve reliable quantitative calibration models we must average Raman spectra collected over a larger sample area.

To cover a larger sample area of each pellet, we collected Raman spectra while rotating the samples (60 rpm). A spectral collection period of ca. 2 min was sufficient to provide an average spectrum over a large area. Fig. 3 displays Raman spectra of 10 selected samples with and without rotation. Based on a simple visual inspection, there are no significant differences between the two sets of spectra except that baseline variations are slightly higher for the Raman spectra obtained for the rotating samples. From the results in Fig. 2, we

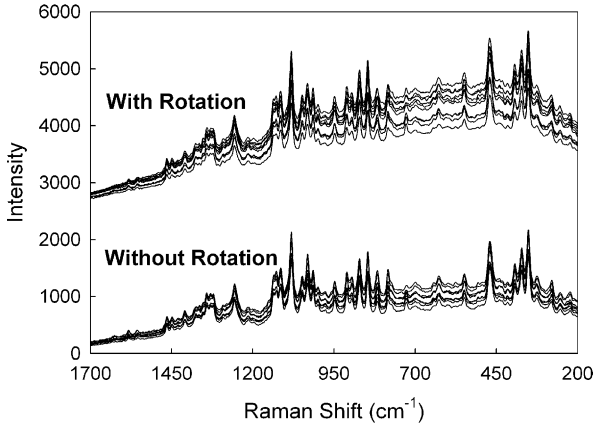

Fig. 3. Raman spectra of 10 selected ambroxol samples with and without rotation.

believe that the Raman spectra recorded with rotation should better represent the average sample composition, but it is hard to make such a conclusion merely by visual inspection of the spectra presented in Fig. 3.

To investigate the spectral variation more in detail, we examined the intensity of the two strong ambroxol bands centered at 815 and 785 cm^{-1} in the Raman spectra obtained by both rotation and non-rotation; Fig. 4 presents the Raman spectra (840–760 cm^{-1} range) of three different concentrations of ambroxol (10.42, 14.17, and 15.42%). With sample rotation, the intensities of the two bands clearly increase with an increase in concentration, but the spectral variations for the static sample do not follow such a systematic trend. Even though this examination was performed over a narrow spectral range (840–760 cm^{-1}), it seems reasonable to conclude that the spectra obtained with rotation provide greater quantitative information throughout the whole Raman spectra.

3.2. Wavelet transformation and PLS calibration

Before performing PLS regression, we employed WT to reduce the background baseline selectively and the noise in

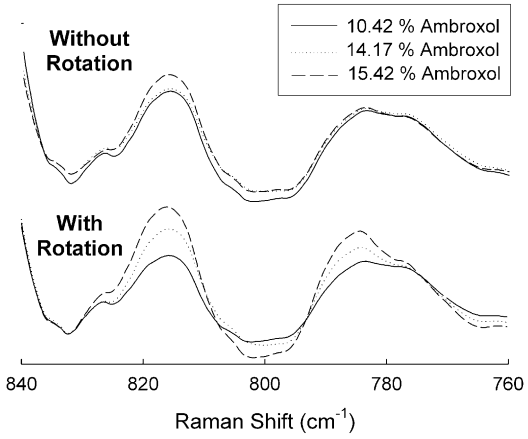

Fig. 4. Raman spectra (840–760 cm^{-1} range) of three different concentrations of ambroxol (10.42, 14.17, and 15.42%) with and without rotation.

the raw spectra displayed in Fig. 3. WT decomposes an original spectrum using a wavelet, i.e., the projection onto a wavelet basis function. Wavelet coefficients of the decomposed spectrum can be manipulated for various purposes, such as de-noising or signal compression. Generally, the procedure involves removing the specific wavelet coefficients associated with baseline variation or noise, and then inversely transforming back to the original domain. In this study, we employed the discrete wavelet transformation (DWT) using Daubechies wavelet (order: 8, level: 8). The details of the DWT process have been described in several articles [11–13]. As a result of DWT, we obtained two sets of wavelet coefficients, the detail (d^n) and approximation coefficients (a^n), where the superscript n reflects the level of resolution. The detail and approximation coefficients, which can be viewed as the result of high- and low-pass filtering, usually are regarded as noise and broad baseline variations, respectively.

Fig. 5 displays the raw and wavelet-transformed spectra; the 1700–1500 cm^{-1} range is magnified and presented in the same plot. The transformed spectrum was obtained by removing three levels of detail (d^1, d^2, d^3) and one level of approximation (a^1) coefficients that we presumed to be the spectral noise and baseline, respectively. As presented in the magnified view, the baseline variation and spectral noise are decreased effectively without distortion of the spectral features.

It is known generally that the spectral range and the number of PLS factors are important parameters when applying the PLS algorithm [14,15]. The spectral range determines the location of the necessary spectral information, and the number of PLS factors should be selected optimally to avoid an overfitting. In this study, we used the 1170–750 cm^{-1} range because both Raman peaks of ambroxol and lactose were strongest in this range. Additionally, the underlying baseline could be regarded as linear, so it was easy to correct baseline variations by using simple two-point correction. In this study, we corrected baselines and zeroed at 1170 and 750 cm^{-1} after WT.

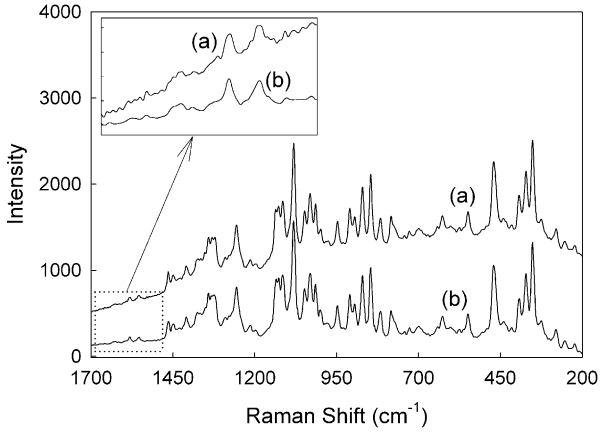

Fig. 5. Raw (a) and wavelet-transformed spectra (b) of the ambroxol sample. The range 1700–1500 cm^{-1} is magnified for the better examination.

One of the most important steps for reliable quantitative calibration using Raman spectroscopy is the acquisition of reproducible spectra, since its intensity varies with laser intensity fluctuation that is always occurring in an actual measurement. The Raman intensity can be corrected using an internal reference that has separate Raman bands to keep track of intensity variation. This is a reliable method; however, it cannot be adopted for this study, since quantitative inclusion of an internal standard into a tablet is unrealistic. The other approach is to treat mathematically spectra for obtaining correct spectral variation. The reasonable approach is the normalization of spectrum by dividing the area under specific Raman peaks. The tablet is a binary mixture, so the area of spectral region containing both ambroxol and lactose information will be nearly constant if Raman scattering coefficients of both components are not significantly different from each other as shown in Fig. 1. Therefore, the normalization of acquired spectra will help to produce reproducible spectra for quantitative analysis. For PLS regression, we used the normalized spectra (1170–750 cm^{-1} range) followed by baseline correction and WT.

For the sake of comparison, we performed PLS using the Raman spectra obtained both with and without rotation. We evaluated simultaneously both the raw and wavelet-transformed spectra for each case. All the spectra were normalized to compensate for the slight Raman intensity variations from sample to sample, as discussed.

We identified the optimum number of factors as the value that gave a minimum standard error of cross validation. The cross validation method was applied by dividing the data set into five segments and examined the pattern of decreasing SECV as a function of the number of PLS factors. The SECV decreased sharply for the first factor and then decreased gradually for the following factors. At a certain factor, the value of the SECV began to increase. These trends are fairly typical in factor-based analyses. We chose the factor before the increase in the value of the SECV to be the optimum number of factors.

Table 1 summarizes the overall calibration results; the numbers in parentheses correspond to the number of PLS factors used. It is clear that rotation of the sample during Raman collection helps to represent the sample composition correctly and leads to much lower calibration errors. With correct sample representation, the subsequent WT helps additionally to improve the calibration performance further by selectively decreasing the spectral noise and baseline variation. The best SECV we obtained was 0.30% for data collection with sample rotation.

Table 1
Overall PLS calibration results (unit: wt.%)

	Raw	Wavelet transformation
Without rotation	1.24 (2)	1.04 (2)
With rotation	0.46 (3)	0.30 (3)

3.3. Raman reproducibility under laser intensity variation

To evaluate the performance of normalization in a situation of laser intensity variation, two different Raman sets were collected separately to simulate routine analysis. The A and B sets were collected with maximum and 30% decreased laser power, respectively. Each data set is composed of 15 spectra from 15 samples. All the spectra were collected with sample rotation. Fig. 6 shows all raw (top) and normalized (bottom) spectra from both sets. Before normalization, all the spectra were filtered using WT and their baselines were corrected as described. In the raw spectra, the intensities of Raman peaks are significantly different from each other owing to large laser intensity variation; on the other hand, the Raman intensity variations are effectively corrected in the comparable scale by using the normalization.

The spectra in sets A and B were analyzed using the calibration model described in Table 1. The resulting standard error of predictions (SEP) from sets A and B were 0.35 and 0.36%, respectively. Even though the resulting SEPs are slightly increased compared to the SECV, the result clearly demonstrates that the normalization helps to compensate Raman intensity variation and leads to the reproducible quantitative performance.

Additionally, we have evaluated the repeatability of the Raman measurement by using 10 spectra collected from a 12.5% ambroxol pellet sample. The spectra were collected at five different periods and two spectra were acquired at each period. The average and standard deviation of 10 predicted values were 12.42 and 0.17%, respectively. We have achieved the reasonably repeatable result by using normalization method.

3.4. Evaluation of commercial tablets

We have studied actual applicability of the proposed method to commercial tablets. For this purpose, two different kinds of tablets were obtained. The ambroxol contents of two tablets were different from each other (tablets A and B for 12.50 and 17.25% ambroxol, respectively). The corresponding Raman spectra of two different tablets and a 12.50% pellet used in this study are shown in Fig. 7. As shown, the spectral features of the pellet and tablet A are generally similar; however, there are subtle differences. Actual tablets contain low concentrations of starch, colloidal silicon, and magnesium stearate as binder, disintegration agent, and tablet lubricant, respectively. It is expected that the inclusion of necessary additives produce slightly different Raman spectral features. Tablet B shows the more different spectral features especially in the 980–870 cm^{-1} range, since its composition is presumably more different from that of tablet A and the pellet. It was practically difficult to identify the actual composition of tablet B.

Six Raman spectra (three spectra from one tablet) were collected from tablets A and B with rotation, and the corresponding ambroxol contents were calculated using the model described in Table 1. The average and standard deviation calculated from six predicted values are given in Table 2. As expected, the accuracy is much better for tablet A, since its spectral features are similar to those of the calibration set. The more different spectral features of tablet B lead to the larger deviation in analysis.

The Raman method developed using laboratory-prepared pellet samples of binary components cannot be directly used

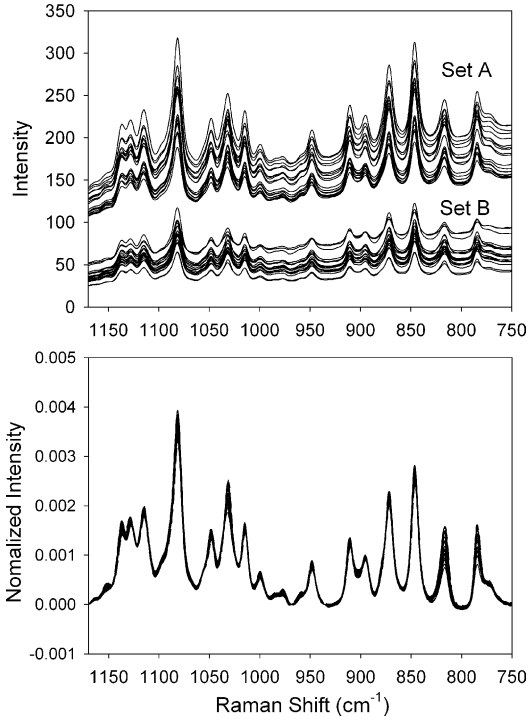

Fig. 6. All raw (top) and normalized (bottom) spectra from sets A and B. The sets A and B were collected with maximum and 30% decreased laser power, respectively.

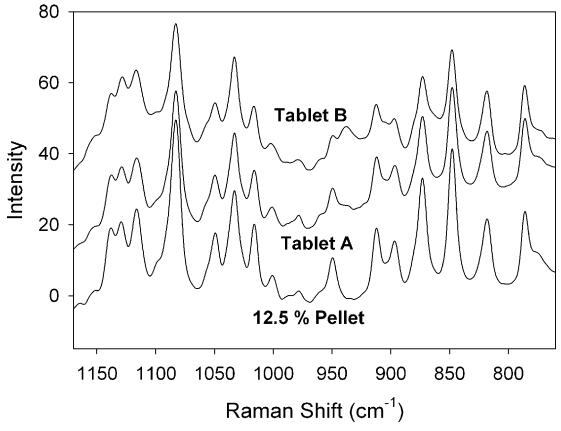

Fig. 7. Raman spectra of two different commercial tablets and the 12.50% pellet used in this study. The tablets A and B have ambroxol contents of 12.50 and 17.25%, respectively.

Table 2
The analyzed results using two different commercial tablets

	Tablet A	Tablet B
Ambroxol content (%)	12.50	17.25
Average	12.82	13.89
S.D.	0.11	0.15

for some commercial tablets, since the pellet samples used in this research do not have the same chemical composition, physical shape, and packing density as commercial tablets. If we can prepare the standard tablets of various ambroxol concentrations that have the same chemical composition including additives and the same physical shape of tablets, the proposed Raman spectroscopy combined with normalization, baseline correction and WT will be useful for the non-invasive determination of ambroxol in commercial tablets.

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