Talanta 89 (2012) 109–116

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Simple transmission Raman measurements using a single multivariate model for analysis of pharmaceutical samples contained in capsules of different colors

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ARTICLE INFO

Article history:
Received 4 August 2011
Received in revised form
23 November 2011
Accepted 23 November 2011
Available online 2 December 2011

Keywords:
Transmission Raman measurement
Pharmaceutical capsule
Capsule color
Ambroxol

ABSTRACT

Direct transmission Raman measurements for analysis of pharmaceuticals in capsules are advantageous since they can be used to determine active pharmaceutical ingredient (API) concentrations in a non-destructive manner and with much less fluorescence background interference from the capsules themselves compared to conventional back-scattering measurements. If a single calibration model such as developed from spectra simply collected in glass vials could be used to determine API concentrations of samples contained in capsules of different colors rather than constructing individual models for each capsule color, the utility of transmission measurements would be further enhanced. To evaluate the feasibility, transmission Raman spectra of binary mixtures of ambroxol and lactose were collected in a glass vial and a partial least squares (PLS) model for the determination of ambroxol concentration was developed. Then, the model was directly applied to determine ambroxol concentrations of samples contained in capsules of 4 different colors (blue, green, white and yellow). Although the prediction performance was slightly degraded when the samples were placed in blue or green capsules, due to the presence of weak fluorescence, accurate determination of ambroxol was generally achieved in all cases. The prediction accuracy was also investigated when the thickness of the capsule was varied.

1. Introduction

Transmission Raman spectroscopy was recently demonstrated as an effective and non-destructive method for the analysis of diverse pharmaceutical products [1–7]. In comparison with conventional back-scattering measurements, transmission Raman measurements of samples in capsules would be especially advantageous since the adverse influence of fluorescence from the capsule itself can be greatly reduced [2]. The reduction of the fluorescence background as well as quantitative analyses of intact multi-component pharmaceutical capsules have been presented by the Matousek group, who pioneered applications of transmission Raman spectroscopy in the pharmaceutical field [6].

To improve the practical applicability of direct transmission Raman measurements of samples in capsules, several issues still need to be addressed. First, the variation in measurement accuracy according to the degree of fluorescence emanating from capsules needs to be examined. As previously mentioned, fluorescence generated by the capsule itself can be substantially reduced by employing the transmission spectral collection scheme [2]; however, fluorescence may remain and overlap with the Raman features of a sample when it is strong. The relationship between the degree of fluorescence and the corresponding accuracy of transmission Raman measurements therefore must be evaluated. Second, it is beneficial to examine the possibility that calibration models developed using samples in transparent containers such as glass vials could be simply used to analyze the same samples in different colors of capsules, rather than building separate calibration models for individual measurements. This is a practical issue that could make transmission Raman measurements much simpler and more easily adoptable in scientific practice. Third, since the thicknesses of capsules can vary, the influence of capsule thickness on transmission Raman features of a sample must be determined. A capsule may generate fluorescence and also function as a barrier attenuating the Raman signal of a sample inside the capsule. The degrees of fluorescence and Raman signal attenuation would vary depending on the thickness as well as the color of the capsule.

These issues were investigated in the present study. Binary mixtures of ambroxol and lactose were prepared and transmission Raman spectra of samples in capsules of four different colors (blue, green, white and yellow) were collected. Transmission Raman spectra were also collected by transferring the same samples into glass vials with inner diameters identical to that of the capsules to evaluate the accuracy of the determination of ambroxol concentration

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doi:10.1016/j.talanta.2011.11.075

without influence from the capsules. Such comparisons served as criteria to compare the accuracies of capsule measurements. Using the spectral datasets collected for each capsule, the ambroxol concentration was determined by partial least squares (PLS) [8,9] and the resulting accuracies were compared according to capsule color. Next, using a PLS model developed from the glass vial dataset, ambroxol concentrations were predicted using the spectra collected in four different capsules, and the prediction accuracies were examined. Finally, transmission Raman spectra of 12.5 wt% ambroxol samples were collected by increasing the number of capsule layers up to 8. Then, the resulting transmission spectra of the samples in each capsule were examined and the influence of capsule thickness on prediction accuracy was assessed in conjunction with capsule color.

2. Experimental

2.1. Samples and Raman spectral collection

Fifteen different concentrations of ambroxol samples (range: 9.0–16.0 wt%, increments of 0.5 wt%) were prepared by mixing appropriate amounts of ambroxol and lactose powders (Sigma–Aldrich). The mixed samples (total of 350 mg) were transferred into capsules of 4 different colors (blue, green, white and yellow) and a glass vial for spectral collection. The inner diameters of capsule caps and bodies were 6.40 and 6.26 mm, respectively. The capsules were 0.10 mm thick. The inner diameter and thickness of the glass vial was 6.26 mm and 0.93 mm, respectively.

Transmission Raman spectra were collected by directly illuminating 785 nm laser radiation (Innovative Photonic Solutions, Monmouth Junction, NJ, USA). The size of the laser spot was adjusted to 1 mm in diameter, and the corresponding power was 250 mW at the surface of the capsule. The bottom part (body) of the capsule was illuminated for measurement. Raman scattering was collected at the opposite side of laser illumination using a wide area illumination (WAI) scheme (PhAT system, Kaiser Optical Inc., Ann Arbor, MI, USA), which was the same setup used to collect back-scattering Raman spectra of samples in yellow capsules in a previous study [10]. Raman spectra were collected using an exposure time of 3 s with 80 accumulations (resolution: 4 cm^{-1}). Triplicate spectra were collected for each sample. For the triplicate measurement, a capsule filled with ambroxol sample was shaken and repositioned to ensure random sample packing as well as capsule orientation.

Back-scattering Raman spectra of samples were also collected using the WAI scheme (λ_{ex} = 785 nm), which is capable of covering large sample areas (area: 28.3 mm^2), to improve sample representation as described [10–12]. Capsules were located at the focal point of the WAI scheme for collection of back-scattering Raman spectra.

All calculations, including baseline correction, normalization, principal component analysis (PCA) and PLS regression, were conducted using MATLAB version 7.0 (The Math-Works Inc., MA, USA). All of the spectra were mean centered before performing PCA and PLS. For PLS, out of 15 total samples, 11 (33 spectra) and 4 (12 spectra) samples were assigned to calibration and validation sets for full cross-validation, respectively.

3. Results and discussion

3.1. Back-scattering Raman spectral features of pure samples and empty capsules

Fig. 1 shows the Raman spectra of pure ambroxol (black) and lactose (red) powder in the 1485–300 cm^{-1} range. Each pure sample was transferred into a quartz cuvette and the corresponding spectra were collected in back-scattering mode using the WAI scheme. Raman spectral features of ambroxol and lactose differ due to differences in molecular structure, as shown in a previous publication [13]. The intensities of ambroxol bands at 1036, 822 and 789 cm^{-1} are stronger than those of other bands, while the 822 and 789 cm^{-1} bands overlap less with the bands of lactose. These two strong Raman bands are valuable for the determination of ambroxol concentrations in the mixtures.

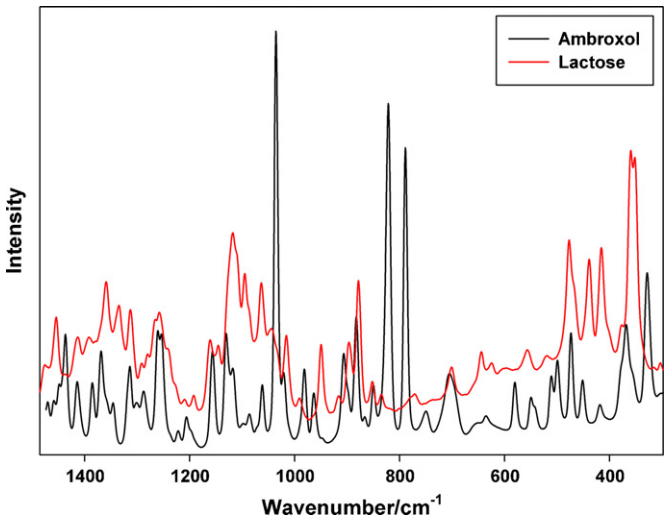

Fig. 1. Raman spectra of pure ambroxol (black) and lactose (red) powder in the 1485–300 cm^{-1} range. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 2 shows the Raman spectra of empty capsules of 4 different colors in the 1485–300 cm^{-1} range. Spectra were collected using the back-scattering scheme. As shown, at the 785 nm excitation, both blue and green capsules exhibit fluorescence, but their fluorescence patterns slightly differ. The fluorescence of a blue capsule is more curved in shape and stronger than that of a green capsule. Meanwhile, no significant fluorescence was observed for white and yellow capsules, so their intensities are much lower than those from blue and green capsules. Raman spectra of white and

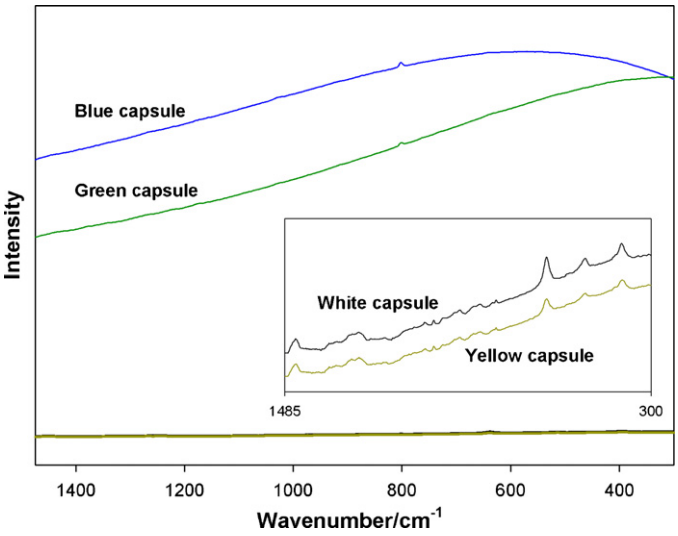

Fig. 2. Raman spectra of empty capsules with 4 different colors (blue, green, white and yellow) in the 1485–300 cm^{-1} range. The spectra of white and yellow capsules are further enlarged inside the figure for detailed examination. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

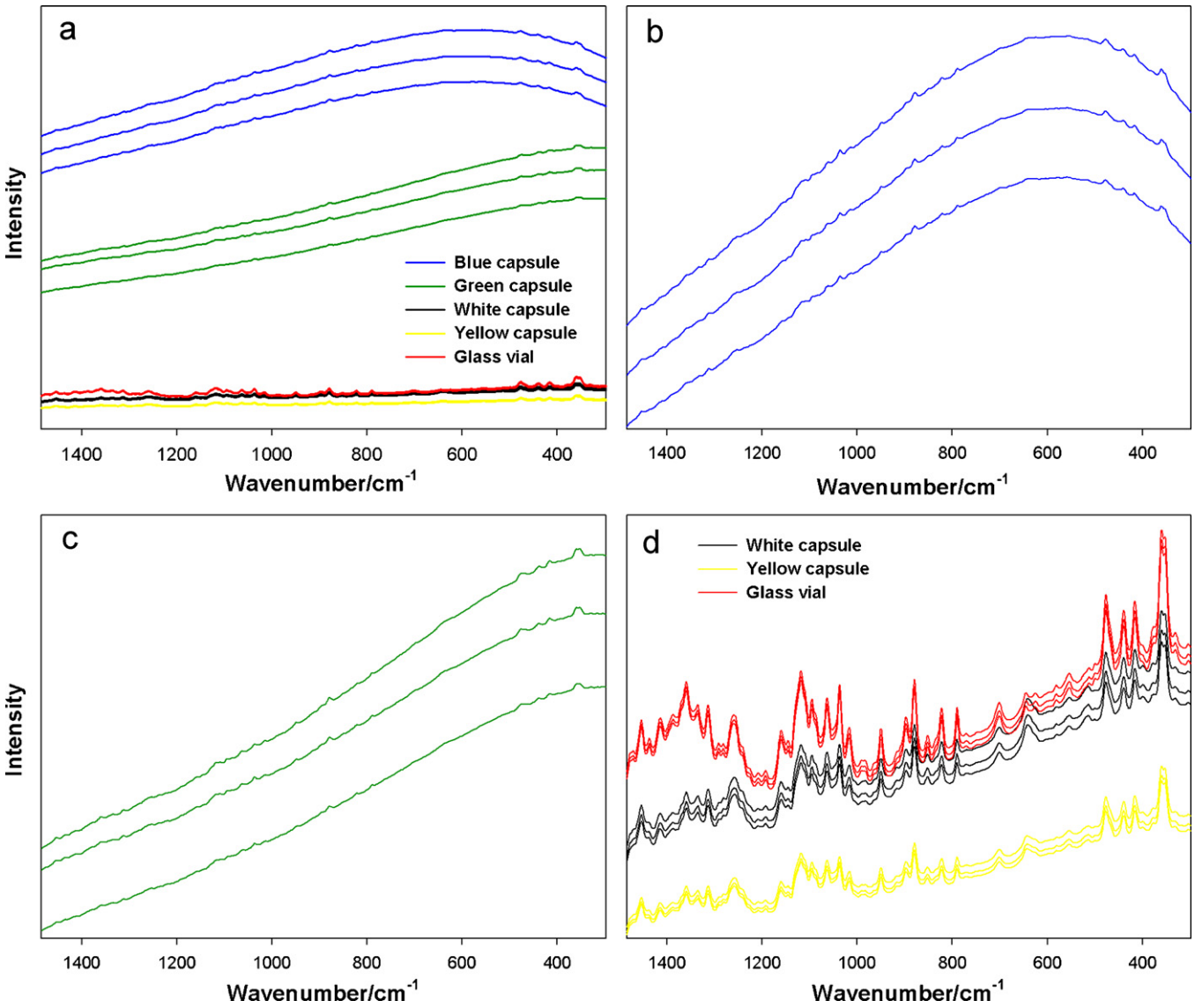

Fig. 3. Back-scattering Raman spectra of 12.5 wt% ambroxol sample filled in 4 different capsules as well as a glass vial in the 1485–300 cm^{-1} range (a). For closer examination, the spectra collected in a blue (b) and green (c) capsule are separately highlighted. Raman spectra of the same sample collected in a white capsule, a yellow capsule and a glass vial are separately shown (d). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

yellow capsules are further enlarged inside the figure for detailed examination. The features corresponding to the constituents of a capsule (gelatin, agarose, gum arabic, colorant, and preservative) are clearly present and similar for both white and yellow capsule cases, while the same features are hardly observable in the blue and green capsule spectra due to overlap with the fluorescence background. Fluorescence emanating from the blue or green capsules is expected to adversely influence accuracy for the determination of ambroxol concentrations when the samples are directly analyzed in these capsules.

3.2. Back-scattering Raman spectral features of ambroxol samples in capsules with different colors

Fig. 3(a) shows triplicate back-scattering Raman spectra of 12.5 wt% ambroxol sample for all 4 capsule colors as well as a glass vial in the 1485–300 cm^{-1} range. As expected, the fluorescence background is dominant for the spectra collected from blue and green capsules, while the intensities of spectra collected in white and yellow capsules are much lower due to the absence of fluorescence. For closer examination, the spectra collected in blue and green capsules are separately highlighted in Fig. 3(b) and (c), respectively. Although Raman bands corresponding to the sample appear at the top of the fluorescence background, their features are broad and much less distinct due to overlap with the fluorescence signal. Fig. 3(d) separately presents spectra for the same sample in a white capsule, a yellow capsule and a glass vial. Distinct Raman bands are clearly observable without influence of fluorescence, and overall spectral features from these 3 measurements are almost identical. However, the intensity of Raman bands collected for a glass vial is stronger than those obtained from white and yellow capsules. Glass is transparent, so a larger Raman signal can be collected. In addition, weak broad Raman features of glass itself in the 1485–1200 cm^{-1} range are present under the sample bands. Overall, back-scattering Raman features are directly affected when a capsule emanates fluorescence at a given excitation wavelength.

3.3. Transmission Raman spectral features of ambroxol samples in capsules of different colors

Fig. 4 shows triplicate transmission Raman spectra of the same 12.5 wt% ambroxol sample in capsules of 4 different colors and a

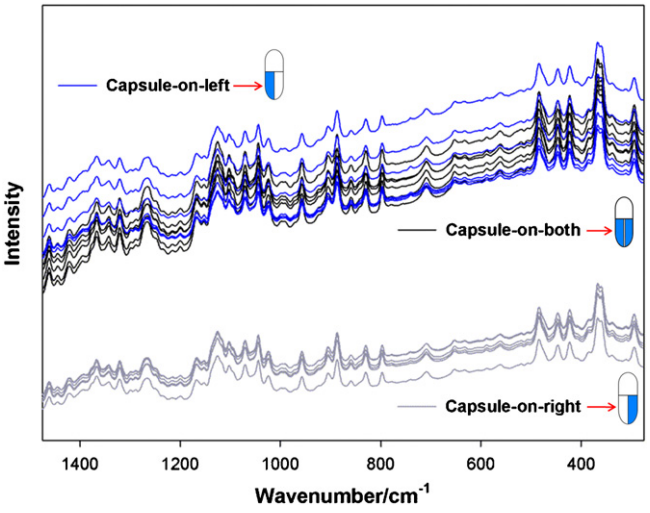

Fig. 4. Transmission Raman spectra of 12.5 wt% ambroxol sample collected in 4 different capsules and a glass vial in the 1485–300 cm^{-1} range. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

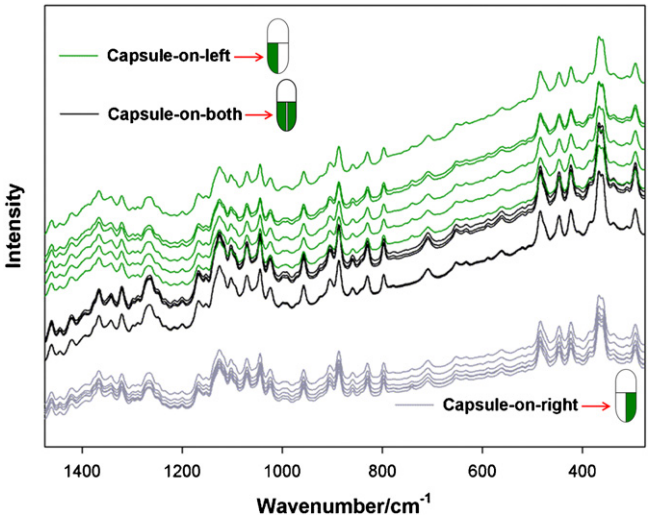

Fig. 5. Transmission Raman spectra of 12.5 wt% ambroxol sample collected by three different schemes (capsule-on-left, capsule-on-right and capsule-on-both) using a blue capsule. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

glass vial. In the transmission spectra obtained for blue and green capsules, the strong fluorescence background present in the corresponding back-scattering spectra is nearly gone. The percentage of volume occupied by each capsule out of the total sampling volume is small (approximately 1.5%), so the intensity of fluorescence should be greatly reduced, as demonstrated in previous studies [3]. The baselines of spectra obtained for a blue capsule are higher in intensity than those acquired for other capsules. This indicates that the fluorescence generated by a blue capsule remains in the spectra of samples, although transmission measurement is employed. The transmission spectral features obtained from other capsules are almost identical, with similar baseline intensities, while the baselines of spectra obtained for a glass vial are slightly higher. Weak broad glass features present in the 1485–1200 cm^{-1} range in the back-scattering spectra do not appear in the transmission spectra.

Overall, when transmission spectral collection was employed, the Raman bands of the sample are clearly apparent and their features were close regardless of capsule color. The influence of fluorescence was greatly diminished although a highly fluorescent blue capsule was used for measurement. Obviously, when samples are placed into blue or green capsules, more accurate determination of ambroxol concentration could be possible by employing transmission Raman measurements.

To more systematically understand the transmission spectral features, transmission spectra of 12.5 wt% ambroxol samples were acquired in three different ways. First, an empty capsule was cut in half lengthwise and a half-capsule was placed on the left side of a glass vial (referred to as "capsule-on-left") for transmission spectral collection. The direction of laser illumination was from left to right. Second, a half-capsule was placed on the right side of a glass vial (referred to as "capsule-on-right") to collect transmission spectra. Third, two half-capsules were placed on both sides of a glass vial for spectral acquisition (referred to as "capsule-on-both"). Only blue and green capsules exhibiting strong fluorescence were tested by these spectral collection schemes.

Fig. 5 shows the transmission Raman spectra of a 12.5 wt% ambroxol sample collected using a blue capsule by the three schemes described above. Six replicate spectra collected for each scheme are shown. The magnitude of the fluorescence background is similar for both capsule-on-left and capsule-on-both measurements, but it is slightly higher for the capsule-on-left measurement. This result indicates that the fluorescence background arises mostly from the left part of the capsule, where the laser beam initially hits for transmission spectral collection; while, the contribution of fluorescence from the right side of a capsule is minimal. Laser power that reaches the right side of the capsule will be greatly decreased due to attenuation by scattering. Due to this fact, the fluorescence intensity largely decreases in the case of capsule-on-right measurements.

Fig. 6 shows transmission Raman spectra of a 12.5 wt% ambroxol sample (1485–300 cm^{-1} range) collected by the same schemes using a green capsule. The intensity of the fluorescence background is strongest for the capsule-on-left measurement and slightly decreased for the capsule-on-both measurement, as for blue capsules. It was also largely diminished for the capsule-on-right measurement. It is clear again that fluorescence superimposed with sample peaks comes primarily from the left side of the capsule.

In addition, the right side of a capsule would be a barrier attenuating the Raman signal of a sample. When spectra from both the capsule-on-left and capsule-on-both measurements are compared,

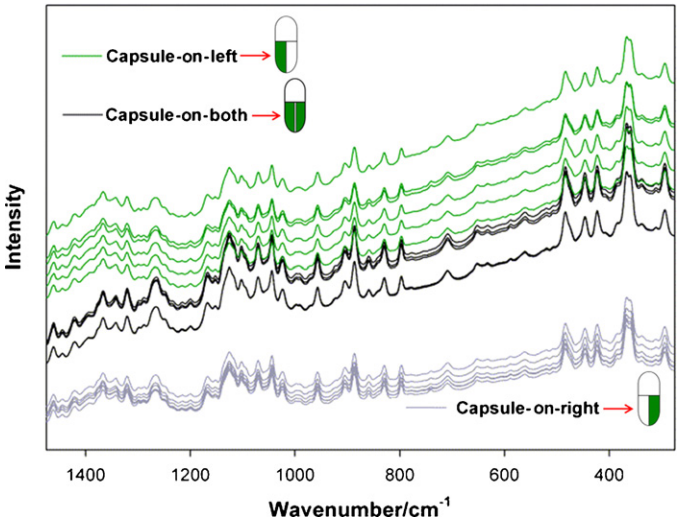

Fig. 6. Transmission Raman spectra of 12.5 wt% ambroxol sample collected by three different schemes (capsule-on-left, capsule-on-right and capsule-on-both) using a green capsule. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

it is found that the right side of the green capsule attenuates the overall Raman signal of the sample more than the blue capsule.

3.4. Reproducibility of transmission capsule measurement

Although the influence of fluorescence can be greatly reduced by employing transmission Raman spectral collection, it did not completely disappear when blue or green capsules were used. Even weakly overlapping fluorescence backgrounds could adversely affect the accuracy of Raman measurement. Therefore, it is worthwhile to investigate the reproducibility of transmission measurements when different capsules are used. The 1485–300 cm^{-1} range was used again to evaluate reproducibility. The baselines of raw spectra were corrected at six different wavenumbers (1485, 1181, 806, 680,536 and 300 cm^{-1}) and the peak area under the 1485–300 cm^{-1} range was calculated. Then, each baseline corrected spectrum was divided by the corresponding peak area for normalization. Variation in the ambroxol band at 822 cm^{-1} was evaluated. To highlight minute spectral differences in the 822 cm^{-1} band, second derivative spectra obtained from the corresponding normalized spectra were used.

Fig. 7 shows triplicate second derivative spectra of 12.5 wt% ambroxol collected in different capsules and a glass vial, where the main ambroxol band at 822 cm^{-1} is highlighted. Triplicate spectra from each measurement are generally similar in terms of band shape and position. For the detail investigation of the spectral variation among the triplicate spectra, the band was magnified as shown in the inset. The spectra in the inset were arbitrarily offset. The band position changes minutely among triplicate spectra collected in blue and green capsules, while these are consistent in the other cases. Fluorescence causes slight degradation in reproducibility of spectral features such as tiny shifts in band position.

To quantitatively determine the reproducibility of collected transmission spectra, PCA [8,9] was performed using the normalized spectra in the 1485–300 cm^{-1} range. The standard deviation of three first score values from each sample was initially calculated. Then, for each capsule measurement, a total of 15 standard deviation values calculated from 15 samples were averaged. Averaged standard deviations with the corresponding uncertainties acquired for each capsule measurement are shown in Table 1. The standard deviations are larger for the transmission measurements in blue and green capsules, indicating less reproducible spectral features among triplicate spectra. The degraded reproducibility could adversely influence accuracy in the determination of ambroxol concentration.

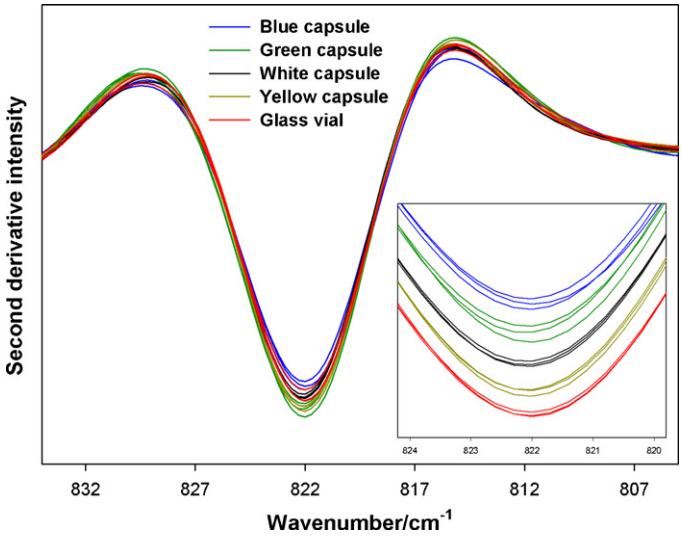

Fig. 7. Triplicate second derivative spectra of 12.5 wt% ambroxol collected in 4 different capsules and a glass vial, where the 822 cm^{-1} band is highlighted. For the detailed examination, the band was magnified as shown in the inset. The spectra in the inset are arbitrarily offset for the clear comparison. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1
Averaged standard deviations of first score values obtained from triplicate spectra of samples for each capsule measurement.

Sample container	Averaged standard deviation
Blue capsule	0.00083 ± 0.00010
Green capsule	0.00074 ± 0.00007
White capsule	0.00060 ± 0.00007
Yellow capsule	0.00060 ± 0.00008
Glass vial	0.00068 ± 0.00007

Table 2
The resulting PLS accuracy for the determination of ambroxol concentration for each capsule measurement.

Container	MSEC (wt%)	MSEP (wt%)
Blue capsule	0.34 ± 0.02	0.42 ± 0.05
Green capsule	0.29 ± 0.03	0.37 ± 0.04
White capsule	0.22 ± 0.02	0.29 ± 0.04
Yellow capsule	0.27 ± 0.02	0.33 ± 0.04
Glass vial	0.23 ± 0.03	0.32 ± 0.05

3.5. Determination of ambroxol concentration

Using PLS, the accuracy for the determination of ambroxol concentration was evaluated for each transmission measurement. In all cases, normalized spectra in the 1485–300 cm^{-1} range were used for PLS. The resulting errors were obtained by fully cross-validating all samples in each dataset. Out of 15 samples, the number of samples for the calibration and validation sets was assigned to 11 and 4, respectively. At all possible combinations of selecting 4 samples into the validation set, the corresponding standard error of calibrations (SECs) and standard error of predictions (SEPs) were calculated. Then, the mean standard error of calibration (MSEC) and the mean standard error of prediction (MSEP) were obtained by averaging these SECs and SEPs, respectively.

The resulting MSECs and MSEPs at each capsule measurement are summarized in Table 2. Two factors were used for all cases. The

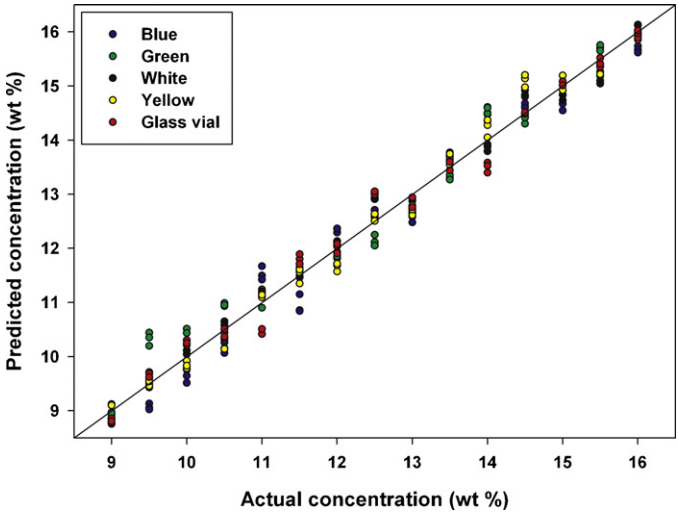

Fig. 8. The prediction correlations (actual vs. predicted concentrations) obtained from each measurement. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

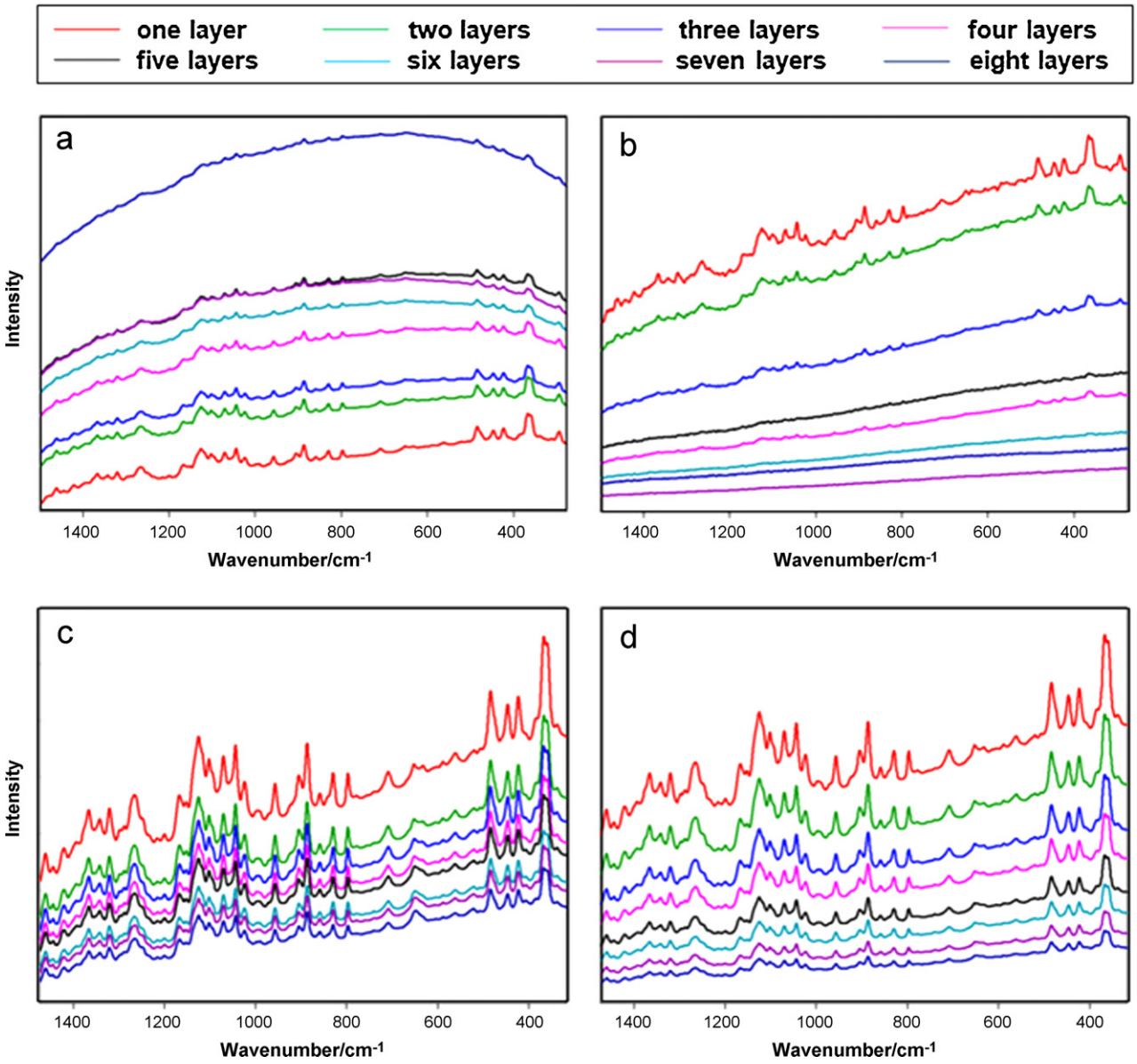

Fig. 9. Transmission Raman spectra of 12.5 wt% ambroxol sample collected in blue (a), green (b), white (c) and yellow (d) capsules with different numbers of layers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

percentages of spectral description by these two factors are 96.8, 96.7, 98.0, 97.8 and 95.6% for the blue capsule, green capsule, white capsule, yellow capsule and glass vial measurements, respectively. Since the samples used in this study are binary mixtures, the use of only one factor would be necessary to account for the variation of ambroxol concentration. However, the use of one additional factor would be effective to model uncontrollable spectral variations, such as scattering and refraction, occurring in a medium of dense solid powder.

The prediction correlations (actual vs. predicted concentrations) obtained from each measurement is shown in Fig. 8. As seen, the concentration correlations are relative more scattered at the blue and green capsule measurement; while, these are closer to the ideal line at the other measurements. Based on The Korea Pharmacopoeia [14], the acceptable limit for quantitative analysis of ambroxol using HPLC is necessary to be within 95–105%, when a sample containing 43.75 mg ambroxol out of total 350 mg (12.5 wt%) is designated as 100% ambroxol. This is equivalent to the range from 11.88 to 13.13 wt% in case of analyzing 12.5 wt% ambroxol sample. As shown in Table 2, the accuracies (MSEPs) obtained from each measurement are comparable to that of HPLC.

The most accurate determination of ambroxol concentration was achieved for the white capsule, while the accuracy was degraded for blue and green capsules. Strong fluorescence emanating from a blue capsule further degrades the accuracy of ambroxol determination. If back-scattering measurements were performed for samples in blue or green capsules, the resulting accuracy is expected to be unsatisfactory due to direct influence of fluorescence, as shown in Fig. 3(a). The MSEPs from measurements in a yellow capsule and glass vial are nearly identical and close to that for a white capsule. The degradation of accuracy for the blue and green capsule measurements was statistically significant based on a *t*-test at the 95% confidence level in comparison with that from the glass vial measurement.

From a practical viewpoint, diverse colors of capsules may be used to contain the same pharmaceutical sample. Therefore, if a single PLS model developed using ambroxol samples in a simple container such as a glass vial could accurately predict ambroxol

concentrations of samples collected in different colors of capsules, the utility of transmission measurement would be greatly enhanced. Since the transmission spectral features of ambroxol samples obtained in 4 different capsules and a glass vial are similar, as shown in Fig. 4, the use of a single PLS model is feasible.

To assess feasibility, a PLS model was initially developed using the spectra of all 15 samples collected in a glass vial. Again, two factors were used. Then, using the developed model, all 15 samples collected in 4 different capsules were predicted. The resulting SEPs were 0.58, 0.36, 0.26 and 0.30 wt%, when the spectra collected in blue, green, white and yellow capsules were separately predicted, respectively. Except for the prediction of samples in a blue capsule, the SEPs are similar to those achieved using each PLS model for each capsule color individually, as shown in Table 2. Since the transmission spectral features obtained for these capsules are nearly identical to that acquired in a glass vial, prediction accuracy is maintained. However, the SEP deteriorated further in the case of predicting samples in a blue capsule. Since the PLS model was developed using fluorescence-free spectra, the prediction accuracy could be more sensitively degraded when spectra superimposed with even minor fluorescence are predicted. For transmission Raman measurement of pharmaceuticals in highly fluorescent capsules, the use of spectra collected in the same color capsule would be a better choice because it would reflect the presence of fluorescence in a PLS model. However, the use of a single calibration model for transmission measurements using different colors of capsules would be practically feasible when a sophisticated strategy that can selectively eliminate or minimize superimposed fluorescence background is employed.

3.6. Variation of transmission spectral features with different capsule thicknesses

The thickness of capsules used in this study was 0.10 mm. As expected, transmission Raman spectral features of a sample vary with the thickness of the capsule. To illustrate this variation, transmission Raman spectra of 12.5 wt% ambroxol were collected by increasing the number of capsule layers up to 8, equivalent to a thickness of 0.8 mm. Fig. 9 shows transmission Raman spectra of the sample collected in blue (a), green (b), white (c) and yellow (d) capsules with different numbers of layers. In the case of the blue capsule (Fig. 9(a)), as the number of layers increases, the intensity of the fluorescence background increases accordingly and the shape of the background becomes more curved. Increased thickness obviously results in stronger fluorescence intensity. Raman peaks corresponding to the sample are present on the fluorescence backgrounds; however, these gradually become less distinct with increased thickness. The stronger fluorescence background makes the spectral features of the ambroxol sample more obscure.

In the case of a green capsule (Fig. 9(b)), overall background intensity as well as Raman intensity of sample bands decrease quite dramatically with increased capsule layers. Raman bands of the sample can only be observed up to 6 layers, indicating that the attenuation of generated Raman signals by the right side of the green capsule is dominant. When spectra were collected in white and yellow capsules, the intensities of baselines and Raman peaks decrease with increased capsule thickness. However, the degree of Raman signal attenuation by these capsules is much lower than that by a green capsule, so Raman bands corresponding to the ambroxol sample are still observable even at 0.8 mm thickness (8 layers), although their intensity is relatively decreased. Overall, in the case of blue and green capsules, Raman features of a sample will sensitively vary when the thickness changes, and the use of thinner capsules is more advantageous to keep the features of a sample distinct and minimize the presence of fluorescence. Meanwhile, slight variation in thickness for white and yellow capsules would not seriously alter the spectral features of a sample.

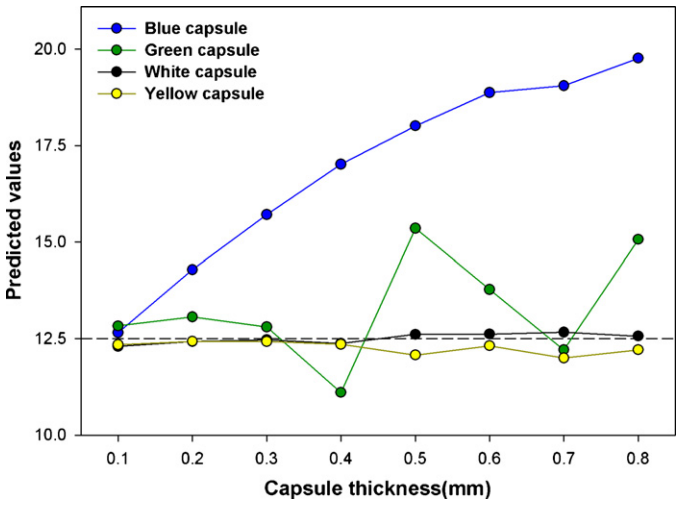

Fig. 10. Variation of the predicted ambroxol concentrations when the thickness of capsules increases. The dashed line indicates the reference concentration of ambroxol (12.5 wt%). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Using the PLS model developed from the dataset obtained in a glass vial, the spectra of 12.5 wt% ambroxol samples collected for different thicknesses of capsules were directly predicted. Before prediction, the spectra were baseline corrected and normalized as described above. Fig. 10 shows the variation of predicted ambroxol concentrations when capsule thickness increases. In the case of a white capsule, the predicted ambroxol concentrations are accurate and consistent regardless of capsule thickness. The ambroxol prediction of samples in yellow capsules is accurate up to 0.4 mm thickness and then degraded afterward. In the cases of blue and green capsules, the prediction performance starts to deteriorate even at 0.2 mm thickness, while the degradation is more substantial in the case of a blue capsule.

Our results clearly demonstrate that variation in capsule thickness directly influences the accuracy of transmission Raman measurements, especially when a sample is contained in highly fluorescent capsules and/or the sample-generated Raman signal is greatly attenuated. When capsules are non-fluorescent and/or less attenuating Raman signal, minor changes in capsule thickness do not seriously affect the accuracy of transmission measurement.

4. Conclusion

In this study, we demonstrated the feasibility of applying a calibration model developed using spectra of pharmaceutical samples collected in a glass vial to directly analyze the same samples filled in different colored capsules, rather than building separate calibration models for each capsule color. This is highly advantageous because the effort required to develop and manage multiple calibration models for routine analysis can be substantially reduced. A sophisticated strategy that can suppress the influence of fluorescence emanating from a capsule itself should enhance the utility and robustness of direct transmission measurement through capsules by allowing the determination of active pharmaceutical ingredient (API) concentrations regardless of capsule color. Future research will address the development of effective mathematical methods dedicated to eliminating superimposed fluorescence generated by capsules in transmission Raman spectra.

Acknowledgement

This research was supported by Basic Science Research Program through the National Research Foundation (NRF) funded by Ministry of Education, Science and Technology (No. 2009-0075648).

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