Robust Raman measurement of hydrogen peroxide directly through plastic containers under the change of bottle position and its long-term prediction reproducibility

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Robust Raman measurement of hydrogen peroxide directly through plastic containers under the change of bottle position and its long-term prediction reproducibility

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ABSTRACT

Hydrogen peroxide concentration in antiseptic solutions has been non-destructively measured with the use of wide area illumination (WAI) scheme by applying a laser beam directly through a plastic container. The prediction accuracy was comparable to that from the use of quartz cell instead of a plastic bottle as well as near-infrared (NIR) prediction result in the previous study. Although the position of a plastic bottle was artificially changed in three-dimensional directions up to ±10 mm to simulate real situation of practical measurement, the prediction accuracy was not degraded within ±5 mm positional variation. This robust prediction performance was powered by the merits of WAI scheme that could provide improved reproducibility by illuminating large sample areas as well as less sensitivity of sample placement by the design of long focal length. Moreover, the prediction accuracy was successfully maintained over a long period of time. Finally, the prediction was also reproducible under the situation of laser power variation. The overall results demonstrate that WAI Raman scheme can provide robust and non-destructive quantitative analysis for not only hydrogen peroxide in antiseptic solution, but also other active pharmaceutical ingredients in diverse containers.

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1. Introduction

The advantages of the wide area illumination (WAI) Raman scheme has been recently demonstrated for the quantitative analyses of active ingredients in various pharmaceutical products such as tablets, capsules, pharmaceutical suspensions and eye-wash solutions [1–4]. By using a laser that illuminates a large sample area for Raman spectral collection (6-mm laser spot with a focal length of 248 mm), the reproducibility of Raman measurements were greatly improved, especially for solid and turbid samples. Additionally, the unwanted spectral variation in Raman measurements often originating from the sensitivity of sample placement with regard to the excitation focal plane was reduced owing to the long focal length inherent in the design of the WAI probe. In these publications, we have demonstrated that Raman spectroscopy can be a viable candidate as a process analytical technology (PAT) tool in the pharmaceutical sector, along with ubiquitous, established techniques such as near-infrared (NIR) spectroscopy.

From the perspective of PAT requirements, the most competitive merit of the WAI Raman scheme is its ability to measure an active pharmaceutical ingredient directly through an unopened container, thus avoiding manual sampling. This was demonstrated by the successful measurement of povidone (active ingredient in eye-wash solution) through translucent plastic bottles [4]. It is generally known that the reproducibility of Raman measurement is inferior to that of NIR measurement; however, it can be greatly enhanced by simultaneously collecting Raman scattering over large sample area. Good reproducibility was maintained using WAI scheme although Raman spectra were collected directly through plastic bottle. Although absorption (transmission) based measurements such as NIR spectroscopy could be more accurate and reproducible, it would be difficult to employ for the same purpose since the direct transmission through large bottles results in long optical pathlength and limits to collect spectra due to total attenuation of NIR radiation. Alternatively, a sample can be measured using a normal cell with workable pathlength without peak saturation; however, the nature of non-destructive analysis should be sacrificed.

Although the feasibility of direct Raman measurements through a container has been demonstrated [4–6], there are important issues to address in order to test the performance of the technique

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in real measurement situations. These include the prediction variation from the change of bottle position (measurement presentation) and long-term prediction accuracy. The main purpose of this study is to evaluate the remaining important issues concerning the use of WAI Raman measurements directly through plastic bottles. In this paper, we describe the use of the WAI Raman scheme to measure hydrogen peroxide concentrations (1–5 wt% range) in antiseptic solutions directly through opaque plastic bottles (HDPE, high density polyethylene). The tight positional control of bottles presented for measurement would be practically difficult in a real quality control situation. Therefore, Raman prediction accuracy must be maintained even with minor variations in bottle position. To address this requirement, we have evaluated the prediction performance by artificially changing the positions of plastic bottles to simulate variations likely to occur with routine analysis. The bottle position was changed by *x*, *y* and *z* directions independently respective to the direction of laser illumination (maximum ±10 mm at each direction). We have also compared the resulting Raman accuracy with that acquired using NIR spectroscopy (simple invasive transmission measurement) in the previous publication [7]. Raman prediction accuracy was comparable although the measurement was accomplished directly through an opaque plastic container with variations in bottle position.

Finally, we have tested the long-term reproducibility of prediction by collecting the spectra of new antiseptic solutions that were measured independently three times after initial spectral collection. The original prediction accuracy was successfully maintained without any bias or slope in the prediction results.

2. Experimental

2.1. Sample preparation

The concentration of hydrogen peroxide in commercial antiseptic solutions is usually 3.0 wt%. Five bottles containing a commercial antiseptic solution were purchased from a local drug store, and only plastic bottles were used for this study. Fig. 1 shows the picture of a bottle and the corresponding dimensions. The red, opaque bottles were made from HDPE.

For each bottle, nine different hydrogen peroxide solutions (concentration range: from 1.0 to 5.0 wt%) were prepared by dissolving the appropriate amount of hydrogen peroxide (Aldrich, 30 wt%) in distilled water. Each sample was prepared just prior to spectral collection in order to minimize sample preparation error due to the possible decomposition of hydrogen peroxide. In between measurement, each bottle was washed with distilled water and dried thoroughly using dried air.

2.2. Raman spectral collection

The schematic diagram and a detailed description of the wide area illumination scheme can be found in previous publications [1,2]. For this work, the center of the plastic bottle was positioned at the focal length to collect Raman spectra. Fig. 1 shows an actual picture of the top view of the Raman measurement scheme. The spectrometer was a Kaiser RamanRxn1 unit (Kaiser Optical Systems, Ann Arbor, MI, USA) that employed a 785-nm excitation laser. The laser spot was circular with an illumination diameter of 6 mm (area: 28.3 mm^2). A 2-mm thick rectangular quartz cell containing cycloheptane was placed 20 mm in front of the plastic bottle. Non-overlapping bands of cycloheptane were used to correct Raman intensity variation from random fluctuations in the laser power. The WAI probe uses an array of 50 optical fibers to collect that Raman scatter. The spectrograph employs a holographic transmission grating and uses a CCD detector.

Raman spectra of nine different concentrations of hydrogen peroxide were collected and triplicate spectra were collected for each sample. With each spectral collection, the plastic bottle was randomly turned to illuminate different spots. Each spectrum was collected with using eight exposures of 15 s (total acquisition time: 120 s). A total of 135 spectra were collected using five different plastic bottles (27 spectra per bottle). Eighty-one spectra from three bottles were used for the calibration set to build partial least squares (PLS) calibration models [8–10] and 54 spectra from two independent bottles were used for the test set (or prediction set) to validate the developed models.

All the calculations including baseline correction, intensity correction and PLS regression, were accomplished using Matlab version 7.0 (The Math-Works Inc., MA, USA).

3. Results and discussion

3.1. Raman spectral features

Fig. 2(a) presents the Raman spectrum of a 3 wt% hydrogen peroxide solution contained in a plastic (HDPE) bottle. To easily identify the peak of hydrogen peroxide (marked by circles), a spectrum of water in the same plastic bottle is also displayed for comparison (Fig. 2(b)). These two spectra were acquired using the WAI scheme (shown in Fig. 1) without the use of the cycloheptane standard. Water is a very weak Raman scatterer without significant Raman bands. Therefore, the Raman data collected from the sample of pure water exhibited mostly the spectral features of the plastic container. In the case of 3 wt% hydrogen peroxide solution, the hydrogen peroxide band at 875 cm^{-1} (O–O stretching) is clearly observed; while,
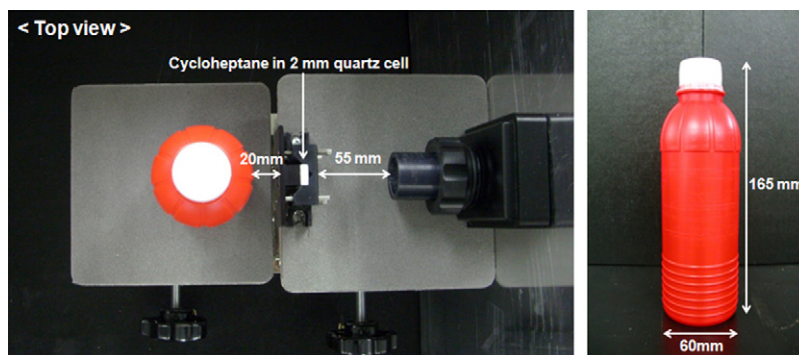

Fig. 1. Pictures of Raman measurement scheme and actual plastic bottle.

the remaining spectral features emanate from the plastic bottle. The hydrogen peroxide band at 875 cm^{-1} exhibits no significant overlap with the bands from the plastic.

A suitable means of correcting Raman intensity variation should be employed in order to achieve optimal results in a quantitative application. Using the configuration shown in Fig. 1, the Raman spectrum of the sample solution (hydrogen peroxide), the plastic bottle, and the external standard (cycloheptane) could be collected in a synchronous fashion. As shown in Fig. 2(c), the resulting Raman spectrum from such a measurement contains mixed spectral features from all three components. Fig. 2(d) corresponds to the pure spectrum of cycloheptane. Cycloheptane was chosen as an external standard since the corresponding Raman peaks are simple and do not overlap with any of the hydrogen peroxide peaks. In this study, cycloheptane peaks both at 730 cm^{-1} (ring vibration) and 1005 cm^{-1} (C–C stretching) were used to correct the Raman intensity variation. The two peaks are indicated by asterisks in Fig. 2.

To investigate the possible negative influence on the quantitative performance resulting from making measurements through the plastic bottles, we also collected Raman spectra of the same samples using a 10-mm quartz cell as the sample container. Cycloheptane was again used as the external standard. The result for the quantitative calibration using the data without spectral features from the plastic is a reasonable comparison in order to evaluate any negative influence resulting from the HDPE container.

3.2. PLS calibration

A PLS regression was used to build calibration models using the designated calibration set (81 spectra from three bottles). PLS models were also constructed using the Raman data collected with the quartz cell (81 spectra from three separate measurements). Before performing PLS using the spectra collected directly through the plastic bottles, all of the spectra were baseline-corrected and intensity-corrected (normalized) using the area of the two cycloheptane bands indicated earlier (1005 cm^{-1} and 730 cm^{-1}). Baselines of raw spectra were corrected at 1050 cm^{-1} and 695 cm^{-1}, and then the resulting spectra were divided by the area of the two cycloheptane peaks integrated from the 1050–960 cm^{-1} and 760–695 cm^{-1} ranges, respectively. This was to correct possible Raman intensity variation from occasional change of laser power. The intensity-corrected spectra between 1050 cm^{-1} and 700 cm^{-1}
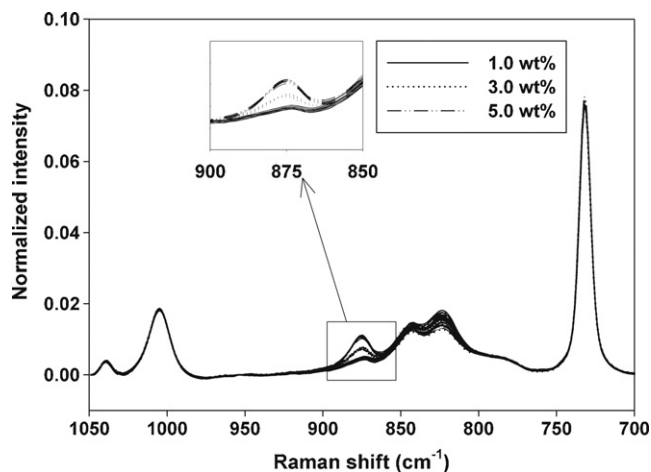
Fig. 3. Intensity-corrected spectra of 1, 3, 5 wt% hydrogen peroxide using the peaks of cycloheptane. The corresponding hydrogen peroxide peak is enlarged in the figure.

are shown in Fig. 3. In this figure, three different concentrations of hydrogen peroxide (1, 3 and 5 wt%) are shown (triplicate spectra for each concentration). As shown in Fig. 3, the intensity of the band at the 875 cm^{-1} band is clearly correlated to the concentration of hydrogen peroxide. Additionally, the reproducibility of the measurement is evident from the triplicate spectra collected for each hydrogen peroxide, which are superimposed on one another in the figure. Compared to NIR spectral variation of hydrogen peroxide (broadly overlapping water band) in the previous publication [7], the Raman spectral variation is much more definitive because of the lack of influence from water. The higher spectral sensitivity of Raman is the most compelling advantage over NIR. The HDPE peak at 820 cm^{-1} is quite variable due to the difference in the sites on the bottle selected for laser illumination spots. This is due to the slight differences in wall thickness as well as the variable morphology of the bottle molding. However, this band is relatively narrow and does not significantly influence the spectral features of hydrogen peroxide. The intensity-corrected spectra (Fig. 3) in the 1050–700 cm^{-1} range were ultimately used for PLS modeling. The same preprocessing scheme outlined above was applied to the spectra collected using the quartz cell and the sample spectral range was also used for PLS modeling. This enabled a meaningful direct comparison to assess the impact of the measurement through the plastic.

The optimum number of factors was identified by examining the prediction residual error sum of squares (PRESS) plot with a concomitant *F*-ratio test. The standard error of prediction (SEP) was calculated by predicting the test samples (total of 54 spectra collected from two independent bottles). The same procedure was repeated for the dataset collected using the quartz cell. The overall results are summarized in Table 1. The numbers in parentheses correspond to optimal number of PLS factors. The corresponding concentration correlation plots (spectral collection directly through plastic bottles) of the calibration and prediction data are shown in Fig. 4. The results from the different bottles are differentiated using five different symbols. No bias was observed based on the individual bottles.
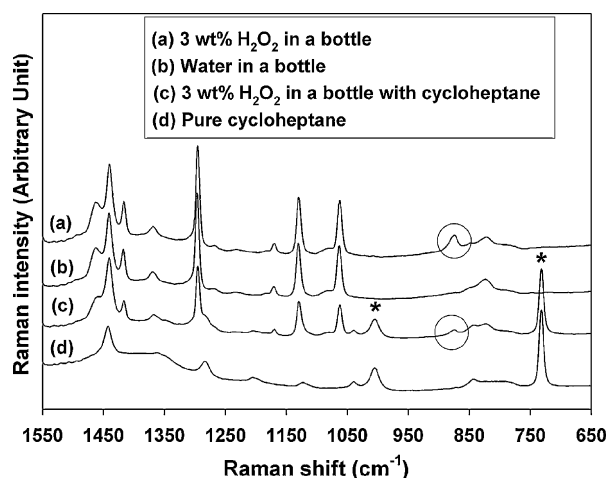

Fig. 2. Raman spectra of (a) 3.0 wt% hydrogen peroxide solution in a plastic bottle, (b) water in a plastic bottle, (c) 3.0 wt% hydrogen peroxide solution in a plastic bottle with cycloheptane standard and (d) pure cycloheptane. Spectra were arbitrarily offset for comparison.

Table 1
PLS calibration results for the spectral data collected using a quartz cell and directly through plastic bottles

Method	SEC (wt%)	SEP (wt%)
Spectral collection using a quartz cell	0.11 (2)	0.15
Spectral collection directly through plastic bottles	0.13 (3)	0.17

The numbers in parentheses correspond to optimal number of PLS factors.

Compared with the result from the measurement using the quartz cell, the prediction accuracy was not substantially affected by making the measurements through the plastic bottles. The 0.2 wt% absolute increase in the SEP would generally be considered acceptable because of the increased simplicity and non-destructive nature of the measurements made in the product packaging. One more factor was required to construct a PLS model for direct measurement through the bottles. It is likely this was due to the increased spectral variation resulting from the use of different bottles.

The SEP from the NIR method (transmission measurement using a fiber optic probe) reported in the previous publication was 0.16 wt% using six PLS factors [7]. As such, a relatively large number of factors was necessary to model the NIR spectral variation, which was much less selective and distinct compared to features in the Raman data. Comparing the Raman and NIR prediction performances, the SEP of 0.17 wt% from the three-factor model using the Raman measurements through the plastic bottles appears quite acceptable. This result was obtained in spite of the fact that these non-invasive measurements were performed through round, opaque plastic barriers.

Fig. 5 shows first three loadings used for PLS in the 1050–700 cm^{-1} range. Additionally, normalized spectrum of 3 wt% hydrogen peroxide is shown for comparison. It is generally known that the first loading is close to the average spectral feature. As
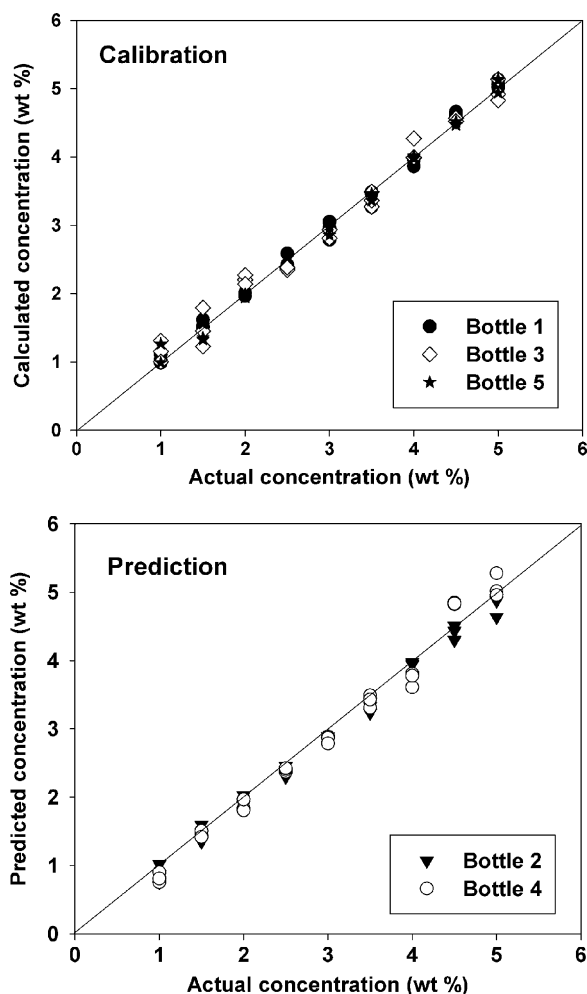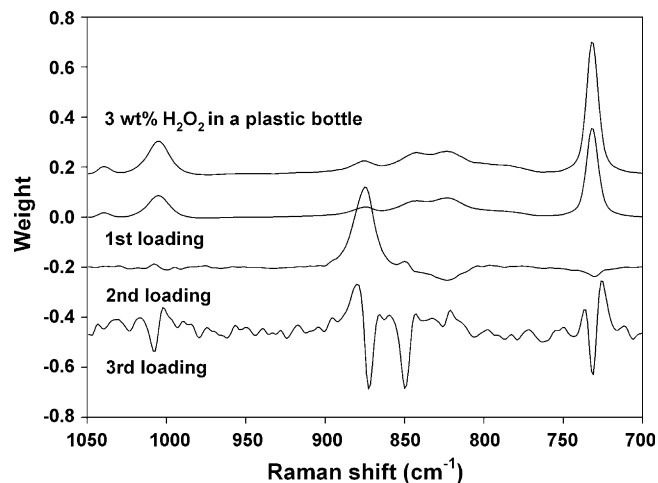

Fig. 5. First three loadings (factors) used for PLS calibration in the 1050–700 cm^{-1}. Normalized spectrum of 3 wt% hydrogen peroxide is also shown for comparison.

shown, the average spectral feature and the first loading are almost similar each other. In the second loading, the variation of hydrogen peroxide is more selectively described by higher weight on the band at 875 cm^{-1}; while, the weights of plastic bands (855–835 cm^{-1}) and cycloheptane bands (835–810 cm^{-1}) are fairly low. In the third loading, the weight on the hydrogen peroxide is apparent and also the weights on the cycloheptane and plastic bands are observable. Additionally the third loading feature starts to be noisy slightly. Based on the examination of three loadings, the hydrogen peroxide variation is effectively described using a three-factor PLS model by considerably selective weight on the hydrogen peroxide band.

Since hydrogen peroxide band is almost isolated, we have initially used a univariate calibration method. For this purpose, hydrogen peroxide bands were baseline-corrected at 892 cm^{-1} and 867 cm^{-1}, and the corresponding areas under the 892–867 cm^{-1} range were calculated for regression. The resulting SEC and SEP were 0.22 wt% ($R^2 = 0.97$) and 0.32 wt% ($R^2 = 0.96$), respectively. Although univariate method is simple and robust when an isolated band is available, it has been shown that the use of multivariate methods such as PLS for the same situation could improve accuracy of calibration [11,12].
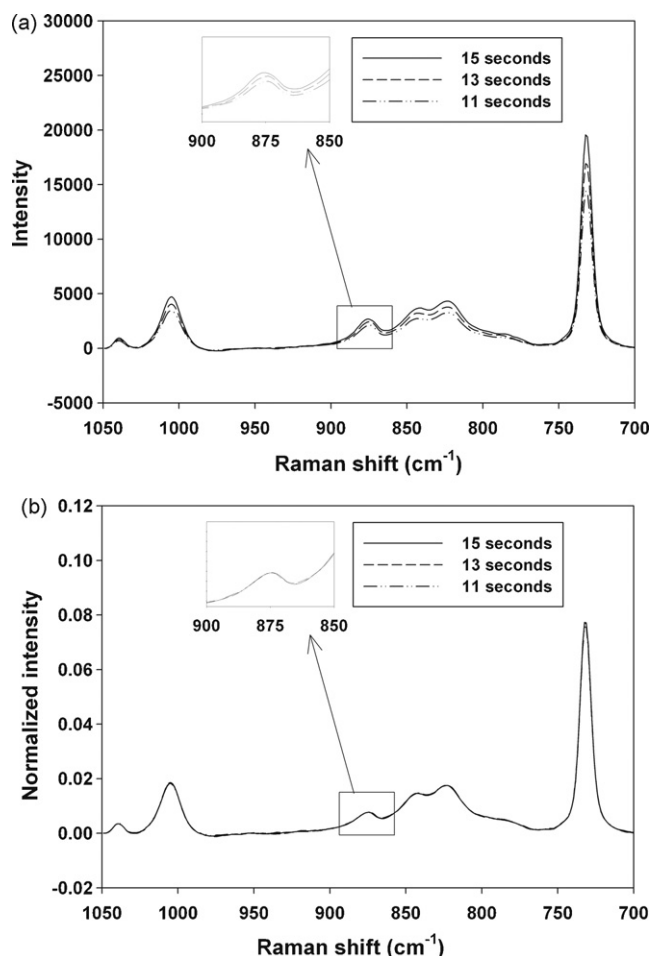
3.3. Evaluation of prediction robustness

3.3.1. Prediction performance under laser power variation

To make the proposed Raman scheme adoptable as a quality assurance method in a pharmaceutical laboratory, prediction robustness must be demonstrated by means of various practical challenges. First, the prediction accuracy should be maintained in spite of laser power variation. It is generally known that Raman laser output fluctuates and gradually degrades in the long term. Therefore, the variation of the Raman peak intensity from laser power change should be effectively compensated to prove robustness. To simulate such a situation, we intentionally decreased the exposure time in three discrete steps (15, 13 and 11 s). The original setting of the exposure time for spectral collection was 15 s.

Fig. 6(a) shows the baseline-corrected Raman spectra of a 3 wt% hydrogen peroxide solution in a plastic bottle collected with three different exposure times. As clearly shown in the enlarged plot, the intensity of the hydrogen peroxide band at 875 cm^{-1} decreases with shortening exposure times. Fig. 6(b) shows the corresponding intensity-corrected spectra using the two cycloheptane peaks as described previously. The resulting three corrected spectra are very similar to each other, visually indicating that the intensity variation

Fig. 4. Concentration correlation plots for calibration (top) and prediction sets (bottom). Each bottle is designated as different symbols.

Fig. 6. Baseline corrected Raman spectra of 3 wt% hydrogen peroxide solution in a plastic bottle collected with three different exposure times (a) and the corresponding intensity (b).

Table 2
Averages and standard deviations of prediction values of 3 wt% hydrogen peroxide at each positional variation

Positional variation	Variation magnitude	Average (wt%)	S.D. (wt%)
x-Direction (right/left)	±5 mm	2.93	0.12
	±10 mm	2.75	0.17
y-Direction (up/down)	±5 mm	2.98	0.12
	±10 mm	2.94	0.17
z-Direction (forward/backward)	±5 mm	2.97	0.10
	±10 mm	2.87	0.20

from different exposure times can be effectively rectified using the non-overlapping cycloheptane bands.

At three different exposure times (15, 13 and 11 s), Raman spectra of nine different concentrations were collected and the corresponding intensity-corrected spectra were predicted using the PLS model described in Table 1. The resulting SEP was 0.12 wt%; while, the prediction results without intensity correction showed a clear pattern of bias for data collected using 13- and 11-s exposure times (actual prediction plots are shown). With optimal intensity correction using cycloheptane peaks, the prediction accuracy was not degraded by laser power variation.

3.3.2. Prediction performance with variation of bottle positions

Since a plastic bottle is round, there is a chance of variation in the Raman spectral features when the position of the bottle is changed. If the Raman prediction accuracy is sensitive to variation in bottle position, such a method will not be useful in a practical application. Consequently, it was deemed desirable to investigate the effects of the position of the plastic bottle on prediction accuracy. To address this issue, we have artificially changed the bottle position in three different directions and collected Raman spectra using the cycloheptane standard. With respect to laser illumination direction, the presentation of the bottle was changed horizontally using five different positions for measurement (original position, 5 and 10 mm to left, 5 and 10 mm right). This variation is subsequently referred to as "x-directional variation". The position was also changed ver-

tically with respective to laser illumination also employing five different positions for this directional alteration (original position, 5 and 10 mm to upward, 5 and 10 mm downward). This variation is subsequently referred to as "y-directional variation". Finally, the bottle position was changed along with laser illumination direction by 5 and 10 mm backward, 5 and 10 mm forward and the original position. This is referred to as "z-directional variation". It is expected that x-directional variation (left or right relative to laser illumination) could lead to the most significant impact on prediction accuracy since the net sample volume of laser illumination could be altered due to the round surface of the bottle. Conversely, y-directional (vertical) variation would be expected to exert relatively less influence on prediction accuracy since the net sample volume of laser illumination would not be dramatically changed. z-Directional variation corresponds to the collection of spectra at different focal points and could also result in some spectral variation. However, this variation is generally not as pronounced with the WAI scheme because of the increased depth of field.

With each directional change, Raman spectra of a 3 wt% hydrogen peroxide solution in the bottle were collected at the five different positions specified. Triplicate spectra were collected at each position by turning the face of bottle randomly (total 15 spectra at each directional change). The resulting spectra were intensity-corrected using the peaks of cycloheptane prior to prediction by the PLS model described in Table 1. The overall results are summarized in Table 2. In the table, averages and standard deviations of the prediction values of 3 wt% hydrogen peroxide at different positions are presented. In the case of x-directional variation, there is little degradation of accuracy when the position is changed by ±5 mm (left and right); while, the average predicted value resulting from changing the position by ±10 mm exhibits a large shift to a lower prediction value. When the bottle is moved to left or right from the center, the net sample volume illuminated by the laser is decreased due to round surface of a bottle (refer to Fig. 1) and this results in the lower prediction values as anticipated.

In the case of y-directional variation, the prediction accuracy is not significantly varied for both positional changes by ±5 and 10 mm (up and down) since the vertical position change does not significantly influence the net sample volume illuminated by the laser. Only different spots along the center line of a bottle are vertically illuminated. In the case of z-directional variation, the prediction accuracy is maintained for the positional change by ±5 mm (forward and backward) and slightly degraded when the position is changed by ±10 mm.

Overall, it is clear that the prediction accuracy is insensitive to the positional change in all three directions by ±5 mm and this level of positional control is comfortably accomplished in a real-world application. The sustainable prediction performance with the change of bottle positions are mainly attributed to the combined benefits of WAI scheme which offers improved reproducibility by illuminating a large sample area. This makes this approach relatively less immune to the change of sample illumination volume. It is also less sensitive to sample placement with respect to the excitation focal plane due to the design that features a long depth of

Table 3
The results of evaluation for long-term prediction performance

Trial	SEP (wt%)
1	0.18
2	0.14
3	0.18

field. When a conventional Raman scheme with a small laser spot (50–500 μm) is used in a similar analysis situation, the resulting accuracy would be significantly degraded and nearly impossible to employ routinely based on our previous studies [4].

3.3.3. Long-term prediction reproducibility

To be accepted as a robust analytical method for quality assurance, the prediction accuracy of this analysis should be maintained over a long period. To evaluate long-term prediction reproducibility, we prepared new nine samples with the same concentration range of the original dataset. Raman spectra of these samples were collected in plastic bottles. This procedure was repeated three times over 150 days (approximately once per 50 days). These spectra were intensity-corrected and predicted using the original calibration model. The results are summarized in Table 3. The prediction accuracy was maintained in each case without indication of the degradation of prediction parameters such as bias or slope. Since the hydrogen peroxide band is distinct in Raman spectra, there is no reason for accuracy degradation as long as reproducible Raman spectra are collected using the WAI scheme and the proper scheme is employed for Raman intensity variation to ensure laser power change corrected.

4. Conclusion

We have evaluated the performance of the WAI Raman scheme for the determination of hydrogen peroxide directly through plastic containers under conditions that could be practically employed in a routine pharmaceutical analysis. This was done to demonstrate the capability of the WAI Raman scheme as a robust and practical analytical system that can fulfill the diverse requirements for PAT in the pharmaceutical industry. The accuracy of Raman measurement was successfully maintained even with variation of the bottle position and with laser power variation. The prediction accuracy was also shown to be robust over a long period time. As a competing analytical method to NIR spectroscopy in pharmaceutical field, the proposed Raman scheme has advantages with respect to the difficult demands of non-destructive analysis of samples in various containers. We have studied the influence of Raman measurement by varying only one experimental condition at each time. Future investigation will be direct to combinatorial study varying all possible variables (e.g. laser power, bottle position and long-term reproducibility) simultaneously and evaluating resulting Raman accuracies. This could demonstrate practical capability of the proposed Raman scheme for real industrial application.

References

[1] M. Kim, H. Chung, Y. Woo, M. Kemper, Anal. Chim. Acta 579 (2006) 209–216.
[2] J. Kim, J. Noh, H. Chung, Y. Woo, M.S. Kemper, Y. Lee, Anal. Chim. Acta 598 (2007) 280–285.
[3] S.C. Park, M. Kim, J. Noh, H. Chung, Y. Woo, J. Lee, M.S. Kemper, Anal. Chim. Acta 593 (2007) 46–53.
[4] M. Kim, H. Chung, Y. Woo, M.S. Kemper, Anal. Chim. Acta 587 (2007) 200–207.
[5] R.L. McCreery, A.J. Horn, J. Spencer, E. Jefferson, Pharm. Sci. 87 (1998) 1–8.
[6] M.G. Orkoula, C.G. Kontoyannis, C.K. Markopoulou, J.E. Koundourellis, Talanta 73 (2007) 258–261.
[7] Y. Woo, H. Lim, H. Kim, H. Chung, J. Pharm. Biomed. Anal. 33 (2003) 1049–1057.
[8] H. Martens, T.M. Naes, Multivariate Calibration, Wiley and Sons, New York, 1989.
[9] K.R. Beebe, R.J. Pell, M.B. Seasholtz, Chemometrics: A Practical Guide, John Wiley and Sons., New York, 1998.
[10] K.R. Beebe, B.R. Kowalski, Anal. Chem. 59 (1987) 1007A–1017A.
[11] H. Chung, M. Ku, Vib. Spectrosc. 31 (2003) 251–256.
[12] H. Chung, M.A. Arnold, Appl. Spectrosc. 54 (2000) 277–283.