Available online at www.sciencedirect.com
ScienceDirect
Analytica Chimica Acta 587 (2007) 200–207
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

A new non-invasive, quantitative Raman technique for the determination of an active ingredient in pharmaceutical liquids by direct measurement through a plastic bottle

Minjung Kim[a], Hoeil Chung[a,*], Youngah Woo[b], Mark S. Kemper[c]

[a] *Department of Chemistry, Hanyang University, Seoul 133-791, Republic of Korea*
[b] *Korea Institute of Toxicology, Daejon 305-343, Republic of Korea*
[c] *Kaiser Optical Systems, 371 Parkland Plaza, Ann Arbor, MI 48103, USA*

Received 8 November 2006; received in revised form 5 January 2007; accepted 16 January 2007

Abstract

The concentration of an active pharmaceutical ingredient (povidone) in a commercial eyewash solution has been measured directly through a plastic (low-density polyethylene: LDPE) container using a wide area illumination (WAI) Raman scheme. The WAI scheme allows excitation using a 6 mm laser spot (focal length: 248 mm) that is designed to cover a wide sample area. As a result, it has the potential to improve the reliability Raman measurements by significantly enhancing representative sample interrogation, thus improving the reproducibility of sampling. It also decreases the sensitivity of sample placement with regard to the excitation focal plane. Simultaneously, isobutyric anhydride was placed in front of the bottles to use for a synchronous external standard configuration. This helps to correct the problematic variation of Raman intensity from the inherent fluctuation in laser power. Using the WAI Raman scheme combined with the synchronous standard method, the povidone concentration was successfully measured with spectral collection that was performed through a plastic barrier. The conventional Raman scheme was difficult to employ for the same purpose because of the degraded spectral reproducibility resulting from the smaller laser illumination area and the sensitivity of such an approach to the position of the sample bottle. The result from this study suggests that the WAI scheme exhibits a strong potential for the non-destructive quantitative analysis of pharmaceuticals measured directly in plastic containers. Preliminary work also shows that similar measurements can also be made in glass bottles. If implemented, this technique could be utilized as a simple and rugged method for quality assurance of final products in a manner consistent with Process analytical technology (PAT) requirements.

Keywords: Pharmaceutical liquids; Raman spectroscopy; Process analytical technology; Non-destructive analysis

1. Introduction

Process analytical technology (PAT) is an evolving philosophy in the pharmaceutical industry that promotes strategies for the control of primary and secondary manufacturing processes [1]. The announcement of the PAT guideline by the Food & Drug Administration (FDA) in 2004 has prompted a substantial increase in the investigation of new and rapid analytical methodologies for at-line and on-line measurements [2,3]. Near-infrared (NIR) and Raman spectroscopy are both good candidates for PAT. Of course, each has advantages and disadvantages in practice. Pharmaceutical process applications such as the measurement of residual moisture in drying operations, monitoring of uniformity in blending processes, monitoring of organic reactions and the analysis of tablets in or near production have been extensively studied [4–8].

Final products stored in containers such as glass or plastic are common in the pharmaceutical industry. A method enabling the non-destructive, direct analysis of these materials would be greatly beneficial and of great interest to those responsible for production and quality assurance operations.

The composition of pharmaceutical oral liquids in polyethylene terephthalate (PETE) bottles had been directly measured using NIR transmission spectroscopy [9]. However, NIR absorption measurements are difficult to accomplish for aqueous samples in large bottles since there will be inadequate energy

---

[*] Corresponding author. Tel.: +82 2 2220 0937; fax: +82 2 2299 0762.
E-mail address: hoeil@hanyang.ac.kr (H. Chung).

0003-2670/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2007.01.062

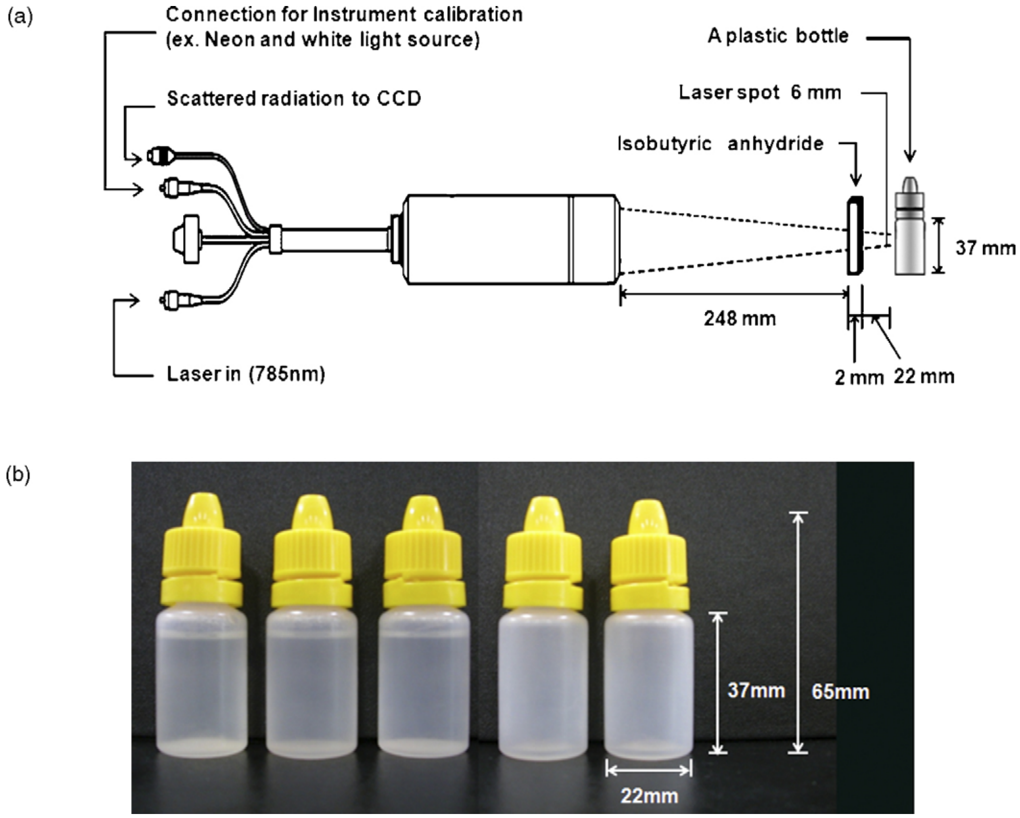

Fig. 1. Schematic diagram of the wide area illumination (WAI) scheme (a) and the picture of five different plastic bottles (b). The three bottles to the left contain eyewash solution while the other two are empty.

transmission due to the long optical pathlength [10]. Additionally, the reproducibility of such a measurement would be questionable in the case of practical on-line measurements due to the variation of pathlength resulting from the lack of reproducibility of bottle positioning. Also, simple NIR transmission measurements have been accomplished using disposable glass vials [11]. However, this approach requires manual sampling and cannot be used for continuous on-line measurements.

For the measurement of an active pharmaceutical ingredient directly through glass or plastic, Raman spectroscopy offers many advantages because it can provide a simpler optical configuration that can easily be interfaced for on-line measurements. Compared to NIR spectroscopy, the resolved details from the richer spectral features can be used to relate Raman data to molecular structure and composition. McCreery et al. successfully demonstrated the non-invasive Raman identification of United States Pharmacopoeia (USP) pharmaceutical materials in amber glass vials [12]. However, this report represented a qualitative identification and did not require consideration of the issues surrounding quantitative Raman spectroscopy.

To make reliable quantitative Raman measurements of pharmaceutical liquids directly through plastic bottles, two major issues should be addressed. The first is the impairment of spectral reproducibility from two major sources. One is the inherent lack of physical and chemical homogeneity of the containers. For example, the walls of plastic bottles will vary slightly from one to the next. The other source of variation is the sensitivity of the Raman response to the sample placement with respect to the focal plane. The second issue is laser fluctuation, which is a tractable consideration in quantitative analysis with Raman. One way to solve this is through continuous and synchronous external standardization to compensate for Raman intensity fluctuations from the variation of incident laser power [13–15].

For the *in situ* analysis of liquid pharmaceuticals in packaging materials, we propose a novel Raman collection strategy using a wide area illumination (WAI) scheme to collect reproducible Raman spectra of pharmaceutical liquid samples directly through a plastic bottle, as shown in Fig. 1(a). By using an incident laser with a large surface area (28.3 mm^2) and a long focal length (248 mm), resulting spectra are much less sensitive to the morphological variations that occur from one plastic bottle to another. The reproducibility enhancement due to the reduced sensitivity to sample placement with respect to the focal plane provides a significant advantage compared to conventional Raman measurements with a small incident laser spot. In addition, we have initiated further improvements by installing a layer of isobutyric anhydride as an external standard in front of the plastic bottles to correct for Raman intensity in a synchronous fashion, as in the previous study [16].

To evaluate the validity of the proposed Raman measurement scheme, the quantitative analysis of an active pharmaceutical ingredient (povidone) in eyewash solutions was performed. These measurements were accomplished directly and non-destructively in low-density polyethylene (LDPE) bottles.

Povidone is a widely used active ingredient in marketed eyewash solutions. The Raman data were modeled using a partial least squares (PLS) regression [17–19] to quantify the povidone content. By acquiring reproducible Raman spectra of samples in plastic bottles using the WAI scheme along with effective Raman intensity correction by external standardization, the concentrations of povidone in LDPE bottles were accurately determined.

2. Experimental

2.1. Sample preparation in plastic bottles

Commercial eyewash solutions are usually composed primarily of two components, povidone (polyvinyl pyrrolidone, 20 mg/mL (2.0 wt.%)) and benzalkonium chloride (0.1 mg/mL, (0.01 wt.%)) used as an active ingredient and disinfectant, respectively. We obtained cylindrical plastic bottles by purchasing five bottles of commercial eyewashes at a retail pharmacy. Only theses plastic bottles were used to acquire data for this study. Fig. 1(b) shows the pictures of these bottles. The three bottles to the left in the picture contain eyewash solution while the other two are empty. The average transmittance of bottles in visible range was 78%. It was calculated by collecting the visible spectrum of plastic wall cut into 2 cm × 2 cm. The corresponding dimensions are shown in the Fig. 1(b). The bodies of the plastic bottles were composed of low-density polyethylene (LDPE), which was soft and pliable commensurate with requirements for the clinical application of eyewash solutions. The average thickness of the walls of the bottles was found to be 0.98 ± 0.03 mm.

Five sets of 10 different povidone concentrations ranging from 1.0 to 4.2 wt.% were prepared by dissolving appropriate amount of povidone in distilled water. For Raman measurements, each bottle was used for only one data set. Each data set was composed of 10 different concentrations. Triplicate spectra were collected for each sample. For each of the triplicate collections, the sample was randomly rotated to illuminate different spots on the bottle.

A total of 150 spectra were collected using five different plastic bottles. Ninety spectra collected from three bottles were used as the calibration set. Partial least squares (PLS) calibration models were constructed from these data. Sixty spectra from two independent bottles were used as the prediction set to validate the PLS models. Both the calibration and prediction sets included the full range of povidone concentrations used in the study (1.0–4.2 wt.%). Raman spectra were collected using an exposure time of 15 s and 8 scans (total 2 min).

2.2. Wide area WAI (WAI) Raman scheme

The schematic diagram of the wide area illumination (WAI) measurement scheme (Kaiser Optical Inc., Ann Arbor, MI, USA) is shown in Fig. 1(a). The excitation laser (785 nm, diode laser) was introduced and magnified to form a circular illumination area with a diameter of 6 mm (area: 28.3 mm^2). This allows interrogation of a representative portion of each sample. A long focal length of 248 mm is also built into the probe design. The Raman scatter was collected by an array of 50 optical fibers. The radiation from the optical fibers was dispersed via a holographic transmission grating and a combined signal from all 50 fibers was integrated over the total area illuminated using a CCD detector. An extra optical fiber is present for the connection of neon and white light sources when wavelength and intensity calibration are necessary. For each measurement, the center of the plastic bottle was positioned at the focal length to collect Raman spectra.

To correct Raman intensity variation from the normal fluctuation of laser power, the isobutyric anhydride external standard, sealed in a 2 mm thick rectangular quartz cell, was positioned at 22 mm in front of a plastic bottle. By doing this, the resulting Raman spectra contain spectral features from the povidone solution, the LDPE and the isobutyric anhydride. A non-overlapping band of isobutyric anhydride was used to correct laser power variation.

All the calculations, including baseline correction, intensity correction and partial least-squares (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 presents the Raman spectrum of a 2 wt.% povidone solution contained in an LDPE bottle. For the sake of comparison, the spectrum of povidone powder and that obtained from a plastic bottle containing only water are also displayed. All spectra were acquired using the WAI scheme without isobutyric anhydride standard. Water, a weak Raman scatter, shows no significant band; therefore, the Raman features from the data collected from the plastic bottle containing water contain the bands only from the LDPE. In the case of the 2 wt.% povidone solution, the povidone band at 936 cm^{-1} (marked by a circle) clearly matches a similar feature from povidone powder. Additionally, weak povidone bands are observed in the 900–700 cm^{-1} range.

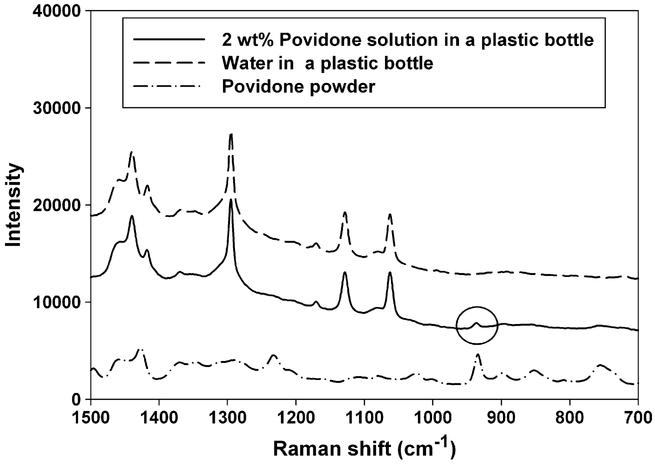

Fig. 2. Raman spectrum (1500–700 cm^{-1}) of 2 wt.% povidone solution in a plastic bottle. For the sake of comparison, the spectra of povidone powder and that obtained from a water-filled plastic bottle are also displayed.

The Raman spectrum of the plastic bottle shows the characteristic spectral features of LDPE such as CH_2 twisting bands at 1295 cm^{-1}. The povidone band at 936 cm^{-1} does not overlap with the bands from the LDPE. Consequently, although the intensity of the povidone band is relatively low due to its low concentration, this band can be used for quantitative calibration.

3.2. Comparison of WAI and conventional Raman scheme

In a routine measurement, the position of the plastic bottle will inevitably vary slightly from measurement to measurement. This will introduce minute variations in the distance between the Raman probe and a plastic bottle. This would eventually result in variations of the intensity ratio between the plastic bottle and povidone peak, and adversely affect the quantitative performance of the Raman measurement in the long term. The WAI scheme has a long focal length (248 mm). Therefore, minor changes of the bottle position should not significantly affect the intensity ratio between the plastic bottle and povidone bands.

Additionally, the WAI Raman scheme should improve the reliability of Raman measurements by significantly increasing surface coverage area. The location illuminated on each plastic bottle was not controlled in the experiments reported in this paper and would not be expected to be easily controlled in a routine measurement situation. In the case of using the conventional scheme with a small laser spot (50–500 microns), such lack of control could result in variations of the intensity ratio between the plastic bottle and povidone peak. The laser illumination spot is significantly smaller than a plastic bottle. Therefore, the Raman signal could be quite sensitive because the surface morphology and the chemical and physical properties of each plastic bottle where laser illumination takes place would not be constant. Since the WAI scheme interrogates a much larger surface area of the sample, the resulting Raman signal will be much less sensitive to the variation of the morphology of the bottle surface and the plastic properties.

To confirm the improved spectral reproducibility that results from measurements using the WAI scheme compared to the conventional approach (laser spot size of about 80 μm and a focal length of 10 mm), we collected 20 Raman spectra of a 2 wt.% povidone solution using both schemes. The plastic bottle was removed and re-positioned with each measurement to simulate a routine measurement. Because of this, the position of the plastic bottle was changed at least slightly with each measurement, even though reproducible placement of the bottle was attempted. Fig. 3(a) shows the resulting spectra in the 1520–925 cm^{-1} range where spectral features from both povidone and LDPE can be observed. The spectra shown were baseline corrected between 1520 and 925 cm^{-1}. It is clear that greater spectral variation occurred when the conventional scheme was used, even though the same povidone concentration was measured. More reproducible spectra were collected using the WAI scheme. For reliable quantitative calibration, the intensity of the LDPE and povidone peaks should be constant with each spectral collection. Because of the small area of interrogation and the short focal length of the conventional scheme compared to WAI scheme, the intensities of LDPE and povidone peaks are more sensitive to slight variations in the distance between the Raman probe and a plastic bottle. Variations in the bottle also have a greater affect on the reproducibility of the conventional scheme.

Table 1
The calculations (average, standard deviation and relative standard deviation (R.S.D.)) of the peak ratio between the plastic bottle and the povidone solution peaks from 20 Raman measurements

Scheme	Average	Standard deviation	R.S.D. (%)
Conventional	0.103	0.033	32.5
WAI	0.078	0.003	3.2

In order to make the comparison quantitative, the peak ratio between the LDPE peak at 1063 cm^{-1} and the povidone peak at 936 cm^{-1} was calculated. The corresponding average, standard deviation and relative standard deviation (R.S.D.) of this ratio were calculated and are summarized in Table 1. The results are shown graphically in Fig. 3(b). The two horizontal lines in the figure correspond to the average of the peak ratio from the conventional (bottom line) and the WAI (top line) schemes, respectively. For reliable Raman measurements, the peak ratio between LDPE and povidone should ideally be constant. The R.S.D. of the peak ratio using the WAI scheme was 3.2% but was 32.5% for the conventional scheme. This significant difference suggests that the WAI scheme can substantially improve quantitative analysis. The difference in reproducibility is likely made more dramatic in this case because data collection was done through the plastic bottle.

3.3. Isobutyric anhydride as an external standard

Raman spectroscopy is a single beam technique and there is, therefore, no compensation for source fluctuations. This stands in contrast to absorption methods such as UV, NIR and FT-IR. It is commonly known that compensation for the variation in the laser power is essential in order to achieve optimal quantitative Raman measurements. Although the WAI scheme compensates for variation in sample representation and positioning, the fluctuation in the laser power is still an issue that must be addressed. Even the best lasers can exhibit a 5% fluctuation in power which translates directly to intensity variation in the spectral data. Therefore, a suitable scheme for incorporating standardization would be beneficial to obtain optimal quantitative analysis. With this in mind, we employed a synchronous external standard configuration in this study. This was achieved by positioning the standard material in front of the plastic bottles as shown in Fig. 1(a). Using this configuration, the Raman spectrum of the sample (povidone), the LDPE plastic bottle and the external standard could be collected in a synchronous fashion. Raman spectra of raw isobutyric anhydride, 2% povidone in a plastic bottle (no standard present) and the same sample with the standard positioned in front are shown in Fig. 4. To exploit an external standard method to correct Raman intensity, it is ideal to have at least one band of the standard that is not overlapped by the sample bands. The intensity changes of such an isolated band can be indicative of laser power variation. The selection of isobutyric anhydride was motivated by the fact that a carbonyl stretching band is found

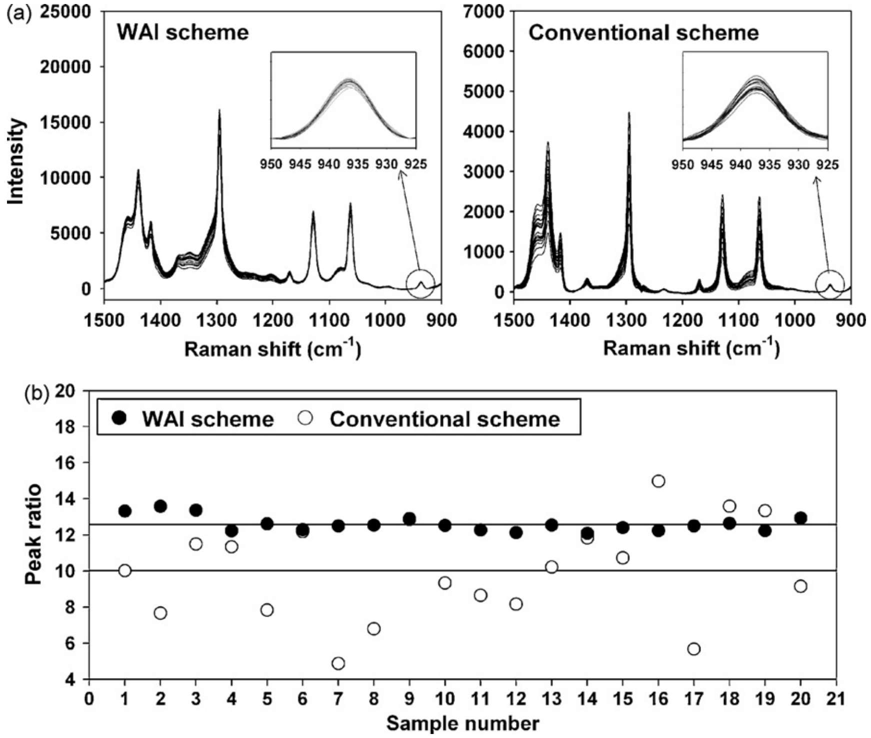

Fig. 3. Twenty baseline-corrected spectra of 2 wt.% povidone solution, that were obtained using both the WAI and conventional scheme (a) and the plot showing the peak ratio between the bottle and povidone bands using both schemes (b).

at 1810 cm^{-1}. This is a convenient standard peak because most organic compounds do not exhibit Raman bands with shifts in that area. In addition to the band at 1810 cm^{-1}, three other isolated bands at 966, 885 and 855 cm^{-1} (marked with asterisks) were also found in this case. These non-overlapping bands were close to peaks originating from the LDPE as well as a povidone band at 936 cm^{-1} (indicated by a cirlcle). In this study, we used these three isobutyric anhydride peaks at 966, 885 and 855 cm^{-1} to correct for Raman intensity variations since they were near the povidone peak of interest.

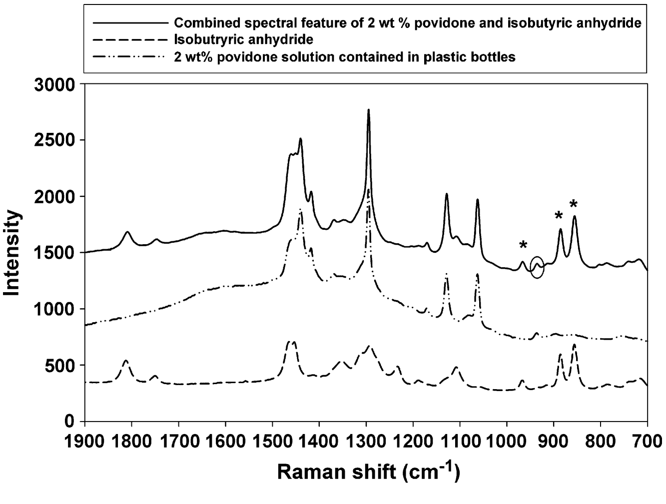

Fig. 4. Raman spectra of raw isobutyric anhydride, 2 wt.% povidone in a plastic bottle (no standard present) and the same sample with the standard positioned in front.

3.4. Investigation of bottle-to-bottle variation

Before the construction of a calibration model, we investigated the spectral variation from bottle to bottle that might influence the calibration performance. To this end, five different plastic bottles containing water were prepared. Six spectra were collected for each bottle by rotating 60° among each measurement. A total of 30 spectra (6 spectra per bottle) were collected using the WAI Raman scheme using the isobutyric anhydride standard. Fig. 5(a) shows all 30 untreated spectra collected from the five water-filled bottles. The most obvious variation is the vertical baseline offset. This is not unusual since the degree of elastic scatter (non-Raman scattering) and laser reflection can vary from bottle to bottle. To compensate for this variation, a 6-point baseline correction at 1535, 981, 951, 923, 816 and 682 cm^{-1} was performed. The band area of one of the peaks representing isobutyric anhydride (966 cm^{-1}) was integrated from 981 to 951 cm^{-1}. Two more bands (885 and 855 cm^{-1}) were integrated between 923 and 816 cm^{-1}. Finally, each spectrum was divided by the total calculated area to compensate for any Raman intensity variation from occasional laser power fluctuation. The results are shown in Fig. 5(b). By visual inspection, the most significant variation can be observed in the 1500–1050 cm^{-1} region, which mainly exhibit peaks representative of the LDPE. As shown in Fig. 3, the variation of the LDPE peaks is significantly decreased using the WAI scheme. However, it is difficult to completely remove the variation in the plastic bands. Fig. 5(c) also shows the average spectrum and standard deviation spectrum of the 30 spectra. Once again, the standard deviation spectrum shows the greatest variation to be in

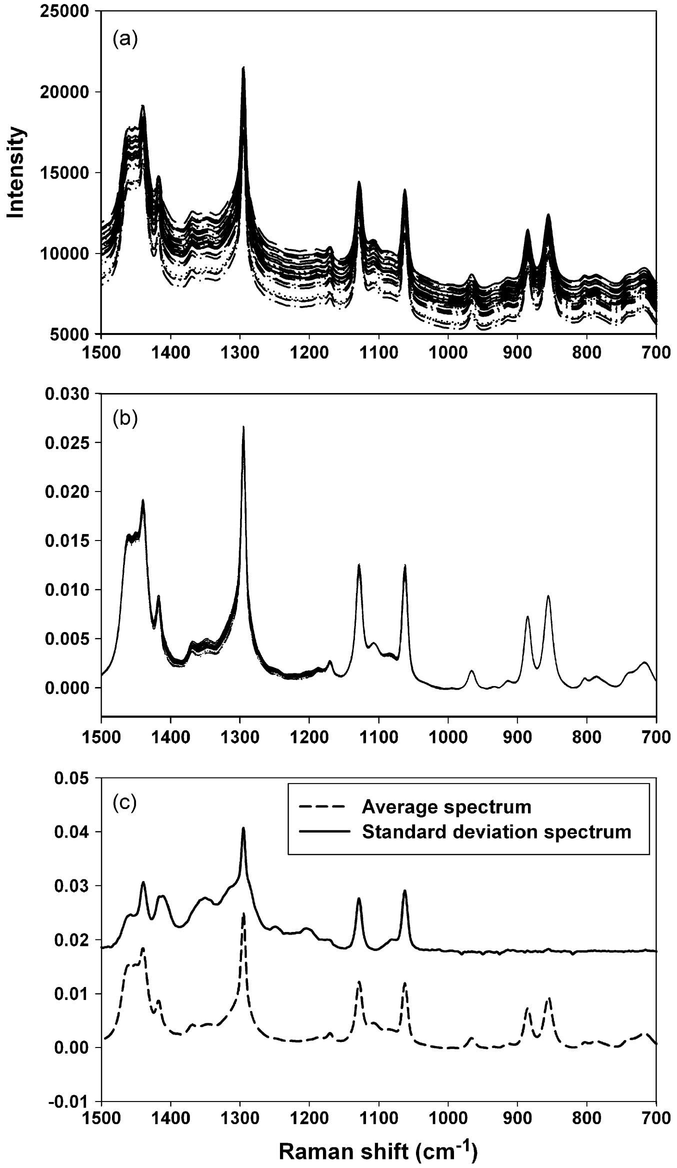

Fig. 5. Untreated Raman spectra (30 spectra) collected from five bottles by rotating bottles 60° (a), corresponding intensity-corrected spectra (b) and resulting average and standard deviation plot (c).

the spectral range of the plastic bottle bands. There is no significant spectral variation in the 1000–682 cm^{-1} range where the povidone and the three isobutyric anhydride bands are located. Therefore, this spectral range can be effectively used to build a calibration model with minimal influence from the variation of the plastic peaks.

3.5. PLS calibration

A partial least squares (PLS) calibration model was developed using the calibration spectra collected from three plastic bottles (10 samples per bottle and triplicate spectra per sample for a total of 90 spectra). Fig. 6(a) shows all 90 raw spectra used for calibration. The most dominant feature is the baseline variation, as shown previously. Before constructing a PLS model, all of the spectra were baseline corrected and normalized (intensity-corrected) using the area of isobutyric anhydride band in the same fashion as shown in Fig. 5(b). Fig. 6(b) shows the corresponding intensity-corrected spectra after baseline correction. In this figure, only the 982–685 cm^{-1} range is displayed, where the most apparent spectral variation of povidone can be observed. The systematic spectral variation corresponding to the povidone concentration change is clearly observed with the 936 cm^{-1} band (magnified in the figure). Even with the variation in the bottles, the spectra of the same povidone concentration are well superimposed on each other, which can be ascertained by simple visual inspection. Additionally, spectral variation is also observed around 760 cm^{-1} povidone band. It is somewhat difficult to observe this peak in the 2% povidone spectrum (see Fig. 2) due to the fact that it is a broad band.

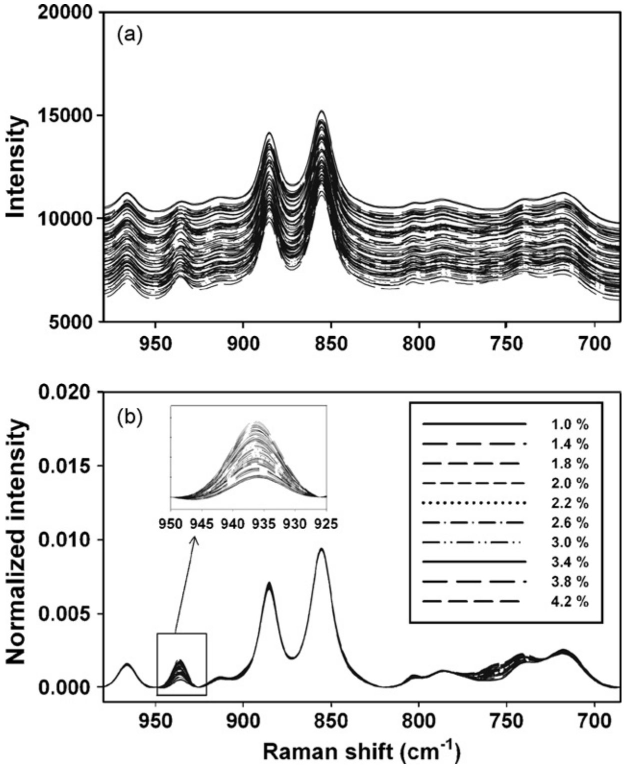

Fig. 6. Untreated Raman spectra (90 spectra) of 10 different concentrations of povidone from three bottle sets (a) and the corresponding intensity-corrected spectra to build PLS calibration models (b).

To build a PLS calibration model, we used the intensity-corrected spectra followed by baseline correction in the 982–680 cm^{-1} range, that included two povidone peaks and the standard peaks. The optimum number of factors was identified by examining the decrease in the pattern of the standard error of cross validation (SECV) plot, which is a function of the error versus the number of PLS factors with a proper F-ratio test. The standard error of prediction (SEP) was calculated by applying calibration model to the sample spectra collected from the two remaining bottles not represented in the calibration set (total 60 spectra). Using three factors, the resulting SECV and SEP were 0.09% and 0.12%, respectively. The concentrations of the povidone solutions were accurately calibrated. The corresponding concentration correlation plots of the calibration and prediction

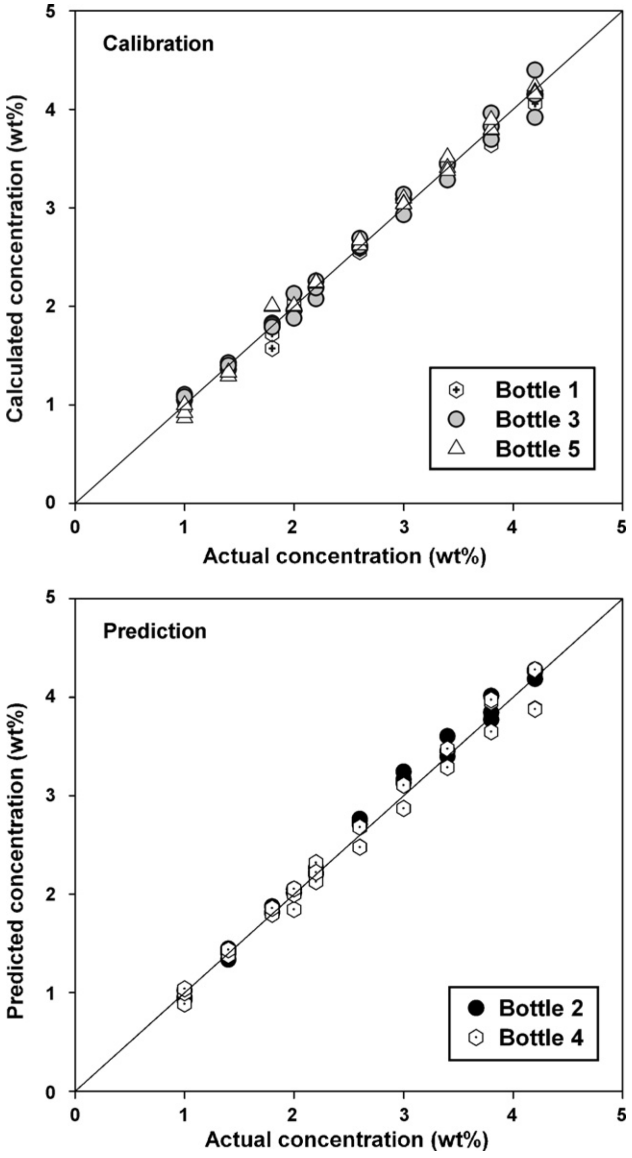

Fig. 7. Concentration correlation plots resulted from PLS calibration and prediction.

data resulting from the application of the PLS model are shown in Fig. 7. We have used five different symbols to show the results from each bottle to examine any possible prediction dependency on the individual bottles. As shown, there is no indication of a systematic tendency in the calibration and prediction results that correlate to the individual plastic bottles.

3.6. Prediction performance under the laser power variation

It is widely known that lasers experience fluctuation and a gradual decrease of power output over time. Because this is a common occurrence, the prediction accuracy of any routine method should be maintained under such circumstances. To simulate this situation, we intentionally decreased the exposure time to 13 and 11 s (104 and 88 s total collection time, respectively). These were compared to the spectra collected with an exposure time of 15 s (the initial instrumental setting). A total of 30 spectra (10 spectra per each exposure time) were collected from the three different exposure times using only one bottle. The calibration model was applied to the resulting spectra. Fig. 8(a) shows the baseline-corrected Raman spectra of the 2 wt.% povidone solution collected at three different exposure times. As shown in the enlarged plot, the intensity of the povidone band at 936 cm^{-1} and the isobutyric anhydride bands at 885 and 855 cm^{-1} both decrease with shorter exposure time. Fig. 8(b) shows the corresponding spectra that have been intensity-corrected based on the area of the external standard peaks. Inspection of the enlarged plot in the same figure reveals that the resulting povidone bands are markedly similar to each other. The intensity variation from different exposure times is effectively corrected using the isolated isobutyric anhydride bands. The prediction results with and without the use of intensity correction are compared. With the intensity correction, the concentrations of the povidone solutions were accurately predicted even with the variation in the Raman intensity (SEP: 0.06%). Without intensity correction, the prediction results were not satisfactory (SEP: 0.46%) and showed a bias pattern as expected.

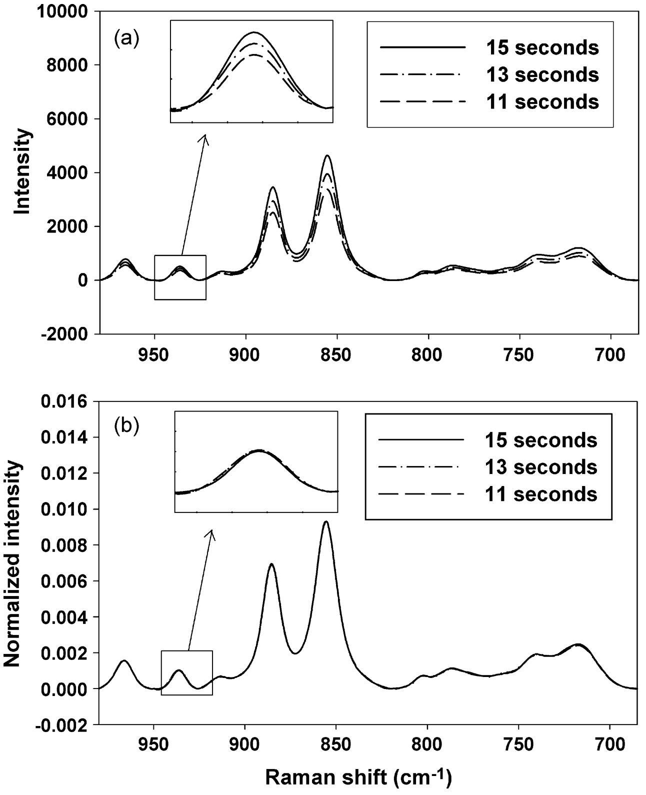

Fig. 8. Baseline corrected Raman spectra of 4.2 wt.% povidone solution collected with three different exposure times (a) and corresponding intensity corrected spectra (b).

4. Conclusion

Direct Raman measurement through a plastic (LDPE) bottle has been successfully accomplished for the measurement of an active pharmaceutical ingredient (povidone) in an eyewash solution. Non-overlapping peaks from an external standard (isobutyric anhydride) were effectively used to correct for the variations of the Raman intensity resulting from the fluctuation in laser power. The WAI scheme significantly improves representative sampling including collection through a plastic barrier. The use of the conventional Raman scheme presents difficulties for quantitative analysis directly through a plastic bottle. The WAI scheme with the use of a synchronous external standard has the potential to allow Raman to be used for QC analysis for diverse pharmaceutical liquids contained in glass (normal or amber) or plastic containers. Additionally, the proposed scheme has wide applicability for other sectors such as food and chemical industrial fields. Further, this could allow quantitative Raman measurements to be performed directly through diverse containers without manual sampling, such as would be the case for on-line measurements. This is the future direction for pharmaceutical analyses in order to comply with FDA guidelines for PAT. The direct Raman measurement using the proposed scheme in this paper through glass or amber bottles will undoubtedly be easier since there is no strong Raman signal from the glass itself. No fluorescence is observed from amber bottles based on a preliminary study performed in our laboratory.

Acknowledgements

The authors greatly acknowledged the Brain Korea 21 Project.

References

[1] J. Workman, M. Koch, D.J. Veltkamp, Anal. Chem. 75 (2003) 2859.

[2] D.C. Hinz, PAT-J. Process Anal. Technol. 1 (2004) 16.

[3] D.C. Hinz, Anal. Bioanal. Chem. 384 (2006) 1036.

[4] W. Plugge, C. Van der vlies, J. Pharm. Biomed. Anal. 11 (1993) 435.

[5] S.S. Sekulic, H.W. Ward, D.R. Brannegan, E.D. Stanley, C.L. Evans, S.T. Sciavolino, P.A. Hailey, P.K. Aldridge, Anal. Chem. 68 (1996) 509.

[6] P.A. Hailey, P. Doherty, P. Tapsell, T. Oliver, P.K. Aldridge, J. Pharm. Biomed. Anal. 14 (1996) 551.

[7] W. Cao, C. Mao, W. Chen, H. Lin, S. Krishnan, N. Cauchon, J. Pharm. Sci. 95 (2006) 2077.

[8] M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, C. De la Pezuela, Analyst 123 (1998) 135R.

[9] N.W. Broad, R.D. Jee, A.C. Moffat, M.J. Eaves, W.C. Mann, W. Dziki, Analyst 125 (2000) 2054.

[10] A. Nordon, A. Mills, R.T. Burn, F.M. Cusick, D. Littlejohn, Anal. Chim. Acta 548 (2005) 148.

[11] S. Cho, H. Chung, Y. Lee, Microchem. J. 80 (2005) 189.

[12] R.L. McCreery, A.J. Horn, J. Spencer, E. Jefferson, Pharm. Sci. 87 (1998) 1.

[13] R.N. Favors, Y. Jiang, Y.L. Loethen, D. Ben-Amotz, Rev. Sci. Intrum. 76 (2005) 033108/1.

[14] X. Zheng, W. Fu, S. Albin, K.L. Wise, A. Javey, J.B. Cooper, Appl. Spectros. 55 (2001) 382.

[15] S.G. Skoulika, C.A. Georgiou, Appl. Spectrosc. 55 (2001) 1259.

[16] M. Kim, H. Chung, Y. Woo, M. Kemper, Anal. Chim. Acta 579 (2006) 209.

[17] H. Martens, T. Naes, Multivariate Calibration, Wiley and Sons, New York, 1989.

[18] K.R. Beebe, R.J. Pell, M.B. Seasholtz, Chemometrics: A Practical Guide, John Wiley and Sons, New York, 1998.

[19] K.R. Beebe, B.R. Kowalski, Anal. Chem. 59 (1987) 1007A.