Baseline Assessment for the Consistency of Raman Shifts Acquired with 26 Different Raman Systems and Necessity of a Standardized Calibration Protocol

Nobuyasu ITOH,† Katsuhiro SHIRONO, and Toshiyuki FUJIMOTO

National Metrology Institute of Japan (NMIJ), National Institute of Advanced Industrial Science and Technology (AIST), Central 3, 1-1-1 Umezono, Tsukuba, Ibaraki 305-8563, Japan

Although Raman shifts originate from molecular vibrations and in theory must be independent of analytical systems, acquired Raman shifts, in practice, are not so. Since the consistency of Raman shifts acquired with different systems has not been investigated previously, we have compared the Raman spectra of polystyrene, benzonitrile, and cyclohexane obtained with 26 different systems. The medians of 26 measurements for the characteristic peaks were found to be 1001.3, 1001.1, and 802.0 cm⁻¹ for polystyrene, benzonitrile, and cyclohexane, respectively, and were consistent with their corresponding ASTM E1840 values, which have been widely used for the calibration of Raman systems. However, only 16 measurements of the Raman shift of the polystyrene peak (1001.4 cm⁻¹) met the tolerance standard of the European Pharmacopoeia (±1.5 cm⁻¹). Thus, consistency of Raman shifts obtained using different systems is low, and this mainly originates from differences in the Raman systems rather than materials. Although correction of the offset using the Raman shift of the peaks of cyclohexane (802.0 or 2852.4 cm⁻¹) could improve the consistency of the Raman shifts acquired with different systems, the magnitude of improvement was not uniform over the range of shift values. Thus, there is a need for a standardized calibration protocol that can be used for multiple Raman shifts of common materials to improve the consistency of Raman shifts for different systems.

Keywords Raman shift, consistency, baseline assessment, offset correction, Raman shift calibration, ASTM E1840

(Received November 15, 2018; Accepted January 16, 2019; Advance Publication Released Online by J-STAGE January 25, 2019)
26 different analytical systems from nine labs after calibration according to each system’s protocol provided by the manufacturer. The distributions of the data obtained were examined and compared with the Ph. Eur. tolerance standards. The improvement of the data consistency with offset corrections was also considered. From these results, we conclude that the standardization of a calibration protocol including the use of common materials should be established to achieve consistency in the Raman shifts obtained from different analytical systems.

Experimental

Materials

A polystyrene board (364 × 257 × 1.7 mm thickness) for plastic handicrafts was obtained from TAMIYA (Shizuoka, Japan). Spectroscopy grade cyclohexane was obtained from Wako Pure Chemical Industries (Osaka, Japan) and benzonitrile was obtained from Tokyo Chemical Industry (Tokyo, Japan). The polystyrene board was cut into small pieces (50 × 20 mm) for ease of handling during measurements. Cyclohexane and benzonitrile were separately placed in cuvettes, which were closed tightly with a screw-cap made of quartz glass (S15-G-10, GL Sciences, Tokyo, Japan). The data for cyclohexane and benzonitrile were subsequently obtained using these cuvettes.

Analytical systems

The Raman spectra of polystyrene, cyclohexane, and benzonitrile were collected using 26 different analytical systems in nine labs. Out of these, the data from 11 different analytical systems were collected by our research team and the rest were provided by collaborators. The analytical systems included 11 models fabricated by eight manufacturers ranging from microscopes to portable/hand-held systems. Although we will not describe each model here, they cover most popular models in the market and the analytical systems consisted of combinations of the following: laser, 532 – 1064 nm; grating, 150 – 2400 gr/mm; spectral resolution, 1.5 – 14 cm–1; pixel resolution, 0.3 – 6.3 cm–1.

Although the spectral ranges were dependent on the analytical systems, most of the data were acquired from 200 to 3200 cm–1 with and without rotation of the gratings depending on the feasibility. The smallest dataset had a size of 21 for 384.1 cm–1 of cyclohexane in ASTM E1840 due to the data being out of data range for some systems. Prior to data acquisition, all the spectrometers were calibrated according to each system’s protocol provided by the manufacturer. Most of the Raman microscopes used the peak of the Si substrate at 520 cm–1 for calibration. All the data were recorded 3 – 5 times to check reproducibility, and no change was observed between repetitions in all the cases. The peak-top value obtained as the output data (without any fitting) was used for the analysis in this paper.
Results and Discussion

Comparison of analytical results with ASTM E1840 values over Raman spectrum range

ASTM E1840 provides Raman shifts of eight materials, amongst which polystyrene, benzonitrile, and cyclohexane are used regularly for the calibration of Raman spectrometers attributed to having some peaks in a wide range of Raman spectra. Figure 1A shows the typical Raman spectra of polystyrene, benzonitrile, and cyclohexane obtained using a 532 nm laser. Although the range of the spectra and the relative intensities of the peaks were dependent on the properties of analytical systems such as laser wavelength and optical characteristics, all the data were obtained with a good S/N ratio as shown in this figure.

Figure 1B shows the relation between the values of ASTM E1840 and the analytical results for the three materials obtained using 26 different systems. Clearly there is a good correspondence between all the peaks and their ASTM values, even though the data were obtained under different analytical systems (see Experimental section). The medians of polystyrene, benzonitrile, and cyclohexane data (polystyrene: 1001.3 cm\(^{-1}\), benzonitrile: 1001.1 cm\(^{-1}\), and cyclohexane: 802.0 cm\(^{-1}\)) were comparable to their corresponding ASTM values (polystyrene: 1001.4 cm\(^{-1}\), benzonitrile: 1000.7 cm\(^{-1}\), and cyclohexane: 801.3 cm\(^{-1}\))\(^{17}\), even though the data were obtained under different analytical systems. Figures 2A – 2C present the histograms of the data distributions (Figs. 2A – 2C). Some data were outside the Ph. Eur. tolerance standards (±1.5 cm\(^{-1}\)) for all the materials and only 62% of the data (16 out of 26 datasets obtained) for the Raman shifts of polystyrene (1001.4 cm\(^{-1}\)) and cyclohexane (801.3 cm\(^{-1}\)) met the Ph. Eur. tolerance standards (±1.5 cm\(^{-1}\)). Although the Ph. Eur. does not provide information on the tolerance for benzonitrile itself, only 54% of the data (14 out of 26 datasets obtained) was within ±1.5 cm\(^{-1}\), which is the range typically given in the Ph. Eur. tolerance around the benzonitrile peak (1000.7 cm\(^{-1}\) in the ASTM value). These facts again suggest that the consistency of Raman shifts obtained under quite different systems is not high, even though individual systems were calibrated prior to data collection. Furthermore, there were no clear trends with respect to laser wavelengths, spectral/pixel resolutions, microscope/portable/hand-held types, and measurers.

However, we observe a strong relationship between the
deviations of the data and the analytical systems. Figures 3A and 3B show the relationships between the deviations to the median for the cyclohexane-polystyrene and the cyclohexane-benzonitrile data, respectively. Both plots are almost linear. The linearities can be evaluated through the correlation coefficients, for which a robust estimator of standard deviations is necessary. In a previous work, one of the authors presented a method to compute the correlation coefficient for data that can be possibly contaminated by some outliers. By applying this computation method, we have determined the correlation coefficients for the cyclohexane-polystyrene and the cyclohexane-benzonitrile data to be 0.88 and 0.91, respectively. These large correlation coefficients imply strong linearities, which support the idea that the deviations of the data depend on the analytical systems rather than the material type. Therefore, there is a strong possibility that the x-axis of the Raman spectra should be commonly shifted over the spectral range and the offset correction of the data might significantly improve the consistency of the Raman shifts.

**Offset correction with Raman shift of cyclohexane peak with median at 802.0 cm⁻¹**

As observed in Fig. 2, polystyrene, benzonitrile, and cyclohexane showed similar distributions of Raman shifts under the same analytical systems. Thus, we examined the offset correction of the Raman shifts for polystyrene (with the median of 1001.3 cm⁻¹) and benzonitrile (with the median of 1001.1 cm⁻¹) using the median value for cyclohexane (802.0 cm⁻¹). Setting \( x_{\text{cyclohexane}}(i) \) as the reported wavenumber for the cyclohexane peak using the \( i \)-th system, the offset value for the \( i \)-th system, \( \Delta(i) \), can be defined as follows: \( \Delta(i) = x_{\text{cyclohexane}}(i) - 802.0 \) cm⁻¹. Using \( \Delta(i) \), the reported values for polystyrene and benzonitrile from the \( i \)-th system, \( x_{\text{polystyrene}}(i) \) and \( x_{\text{benzonitrile}}(i) \), are modified as \( y_{\text{polystyrene}}(i) = x_{\text{polystyrene}}(i) - \Delta(i) \) and \( y_{\text{benzonitrile}}(i) = x_{\text{benzonitrile}}(i) - \Delta(i) \).

Figure 4 shows the distributions of the data with offset correction and their histograms. The ranges of the data distributions in Fig. 2 (994 – 1043 cm⁻¹ for polystyrene at 1001.3 cm⁻¹ and 996 – 1040 cm⁻¹ for benzonitrile at 1001.1 cm⁻¹) became much smaller than those without offset correction (999 – 1009 cm⁻¹ for polystyrene at 1001.8 cm⁻¹ and 998 – 1012 cm⁻¹ for benzonitrile at 1001.1 cm⁻¹). As much as 82% of the data (21 out of 26 datasets obtained) met the requirement for the Ph. Eur. tolerance for polystyrene at 1001.4 cm⁻¹ in the ASTM E1840. Data for benzonitrile also showed improved consistency and 82% (21 out of 26 datasets obtained) fell within the range typically given in the Ph. Eur. tolerance standards (±1.5 cm⁻¹).

---

Fig. 3 Relationships of Raman shifts from medians between cyclohexane at 802.0 cm⁻¹ and polystyrene at 1001.3 cm⁻¹ (A) as well as between cyclohexane at 802.0 cm⁻¹ and benzonitrile at 1000.7 cm⁻¹ (B), together with the estimated correlation coefficients, \( R \).

Fig. 4 Distributions of the corrected Raman shifts with cyclohexane at 802.0 cm⁻¹ in median for polystyrene at 1001.3 cm⁻¹ (A) and benzonitrile at 1000.7 cm⁻¹ (B) both in ASTM E1840, as well as their histograms (C and D). Dashed lines in (A – B) indicate the medians of the reported values.
from the ASTM value (1000.7 cm$^{-1}$). Since all the data were obtained after calibration of the systems, this improvement in consistency can most likely be attributed to the use of common material for calibration, suppression of thermal drift during analysis, or both. The application of the common material and the protocol significantly improved the consistency of Raman shifts in this study. Thus, the standardization of a calibration protocol stipulating the appropriate choice of materials and other essential information is important to achieve consistency of Raman shifts.

Comparison of the Ph. Eur. tolerance standards to our data distribution

The consistency of Raman shifts was improved significantly by offset correction as mentioned above. However, some of the data were still unable to meet the requirements of the Ph. Eur. tolerance standard. Since there is no detailed explanation available on setting the range of the Ph. Eur. tolerance standards,$^{18}$ we also examined the appropriateness of these tolerance levels using our data.

For this purpose, a robust estimator for the standard deviation was introduced. The normalized interquartile range (nIQR) has been widely used to estimate the standard deviation of a distribution.$^{20,21}$ Specifically, the nIQR is 0.7143 times the interquartile range, which is the difference between the 25th and 75th percentiles of the observed data. This estimation is based on the normality of the data. Although the small number of the data and existence of some possible outliers prevent us from rigorously discussing the normality, the nIQR is often employed in practice for proficiency tests in cases similar to this study. The nIQR, hence, seems meaningful to be compared with the Ph. Eur. tolerance standards.

Figure 5 shows a summary of our results, the Ph. Eur. tolerance standards, and the standard deviation (SD) of ASTM E1840 for the Raman shifts of polystyrene around 1001 cm$^{-1}$. The medians of the original and corrected data were comparable with the ASTM values, but the error bars were different. The nIQR of the original data (1.8 cm$^{-1}$) was smaller than that of the Ph. Eur. tolerance for the hand-held type (±2.0 cm$^{-1}$) and larger than that of the benchtop (±1.5 cm$^{-1}$).

On the other hand, the nIQR of the corrected data (0.74 cm$^{-1}$) were even smaller than of the Ph. Eur. tolerance for benchtop systems and only 40% larger than the standard deviation (SD) of ASTM E1840 (±0.54 cm$^{-1}$). These results suggest that the Ph. Eur. tolerance for benchtop systems is reasonable and is not difficult to attain, when analytical results are calibrated correctly.

Differences in nIQR improvements using Raman shifts of different peaks

Raman shifts are calculated from the differences in the wavenumbers of the excitation and Raman scattering. Therefore, the spectral range is dependent on the wavelength of the excitation source. Furthermore, not only is the relationship between the CCD channels and the Raman shifts non-linear for dispersive spectrometers,$^{1,3}$ the spectral and pixel resolutions vary between systems as well (see Experimental section). Thus, there is a possibility that the effectiveness of offset correction may not be constant over the spectral range.

Figure 6A shows the relationship between the Raman shift and the nIQRs for polystyrene for the original and corrected data over the spectral range with the Ph. Eur. tolerance. The offset correction for the Raman shift of the cyclohexane peak with a median at 802.0 cm$^{-1}$ obviously improved the Raman shifts of polystyrene over the range (200 – 3200 cm$^{-1}$). The nIQR of most Raman shifts decreased by about 1 cm$^{-1}$, and can either exceed or is comparable to the Ph. Eur. tolerance for benchtop systems (typically ±1.5 cm$^{-1}$). On the other hand, the magnitude of the improvement is not uniform over the range and is larger for Raman shifts in the lower range. Since both Raman shifts of the cyclohexane peak with the median of 802.0 cm$^{-1}$ and the polystyrene peak with the median of 1001.3 cm$^{-1}$ are in the lower part of the spectral range (200 – 3200 cm$^{-1}$, Fig. 1), it is unclear whether improvements are effective in a lower spectral range or in a range closer to the Raman shift used for offset correction. To understand the main reason for the improvement, the offset correction using the Raman shift of the cyclohexane peak with the median value of 2852.4 cm$^{-1}$ was also performed.
Figure 6B shows the nIQR of the original and the corrected data for Raman shifts of polystyrene peaks corrected with the cyclohexane peak of 2852.4 cm$^{-1}$. The nIQR of the Raman shifts for values greater than 1155.3 cm$^{-1}$ in ASTM E1840 decreased by about 1 cm$^{-1}$ and can be smaller than the Ph. Eur. tolerance standard for a benchtop, although no significant improvement was observed for peaks less than 1031.8 cm$^{-1}$ in ASTM E1840. This suggests that the improvement is greater for peaks in a higher spectral range. Thus, offset correction with closer Raman shifts is more effective. In this study, we calibrated most of the Raman microscopes with the Raman shift obtained for Si substrates at 520 cm$^{-1}$. This may be responsible for the poor consistency of Raman shifts for the original data because a Raman shift at 520 cm$^{-1}$ is lower than the Raman shift of cyclohexane at 802.0 cm$^{-1}$.

It should be noted that the vendors for all the systems used have already performed the correction using polynomial fitting to compensate the non-linear difference between Raman shifts and the corresponding CCD channels. Therefore, only constant offset correction was applied in this study. In addition, most users usually calibrate their systems using offset correction based on Si substrates or other materials. However, in some cases, this offset correction is insufficient to satisfy the Ph. Eur. tolerance standards. The Ph. Eur. describes the requirement for the application of Raman spectrometers such that, “A minimum of 3 wavenumber shifts covering the working range of the instrument intended for measurements should be chosen.” Thus, for an application to the pharmaceutical field, the range of the instrument intended for measurements should be established to achieve good consistency of Raman shifts.

Conclusions

We recorded the Raman spectra of polystyrene, benzonitrile, and cyclohexane under different systems to determine the consistency of the acquired Raman shifts. The Raman shifts were not very consistent, and some data did not meet the requirements of the Ph. Eur. tolerance standards, even though these results were obtained after daily calibration based on the recommendations of the individual manufacturers. Nevertheless, the consistency of the Raman shifts improved significantly after offset correction using the Raman shift of the common material, obtained under an identical condition. Since the magnitude of the improvement was not uniform over the range of shift values, offset correction based on Si substrate or other materials should not be considered enough for calibration over a wide spectral range. To achieve more consistency of results acquired with different systems, the establishment of a standardized calibration protocol based on polynomial fitting with Raman shifts of the same material having several peaks such as polystyrene, benzonitrile, and cyclohexane might be necessary.

Acknowledgements

This work was partly performed as an activity of the VAMAS TWA42 corresponding committee in Japan.

References