Calibration of Raman Spectrometer Instrument Response Function with Luminescence Standards: An Update

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An error in a previously reported procedure for correcting Raman spectra for instrument response was corrected, and then the approach was tested against reported values of Raman cross sections. The method uses luminescent standards with known emission curves of intensity vs. Raman shift, expressed in polynomial form. After acquisition of an observed emission curve from the luminescent standard, the correction of observed Raman spectra is automatic. Agreement of corrected peak areas with those calculated from literature cross sections is 5–20%. This correspondence is approximately as good as the agreement between different reported values, and much better than that observed for uncorrected spectra. Due to variations in the luminescence of the materials, it is likely that these or similar Raman intensity standards will be calibrated by the manufacturer or an independent agency.

Index Headings: Raman; Intensity calibration; Intensity standards; Instrument response function.

INTRODUCTION

In a recent report[1], we presented an intensity calibration procedure for Raman spectrometers based on luminescent standards. The standard emits a continuum of light over the Raman shift range of 0–3500 cm^{-1} when illuminated by the laser used in the spectrometer. Provided that the emission spectrum of the standard is known, the instrument response function may be determined from a spectrum of the standard acquired under the same conditions as the Raman spectra to be calibrated. The standard and sample experience the same optical geometry, laser power, etc., and the correction is easily implemented in the spectrometer or data analysis software. In practice, corrected spectra may be acquired routinely after a single calibration spectrum of the luminescent standard. In principle, such standards could be sold commercially along with a polynomial that describes their emission intensity as a function of Raman shift relative to a given laser wavelength.

In the initial publication, a systematic error occurred in the polynomials for 514.5 and 785 nm standards, related to the transformation of the output of a tungsten emission standard from a nanometer to wavenumber axis. In this brief report, we will provide corrected polynomials, and also report some new observations about the reproducibility and stability of the standards. In addition, comparison of corrected spectra with literature values of Raman cross sections are made to assess calibration accuracy.

EXPERIMENTAL

Several samples of Kopp type 2412 filter glass were obtained from Gray Glass Co. (Jamaica, NY) to evaluate consistency of their luminescence. Figure 1 shows the uncorrected emission spectra of seven 2 × 2 in. filters obtained on a Chromex 2000 spectrometer upon illumination by a 785 nm laser. The photostability of the Kopp glass was examined by monitoring the luminescence at 1600 cm^{-1} vs. time after exposure of a pristine spot to the laser. The decay of intensity reported previously[1] occurred in two segments, a fast period upon initial exposure and a much slower decay after about 5 min. For 50 mW of laser power focused to a ∼50 μm circular spot, the fast slope was 9% min and the slow was 0.08%/min. For a ∼50 × 2000 μm line focus and 50 mW, the initial slope was 3%/min followed by 0.09%/min. Overall, the luminescence at 1600 cm^{-1} decreased by 7–13% during the first 6 min and an additional 7% over the next 100 min. As noted previously, the emission vs. wavelength curve *shape* changes only slightly with exposure time. For example, the ratio of the emission at 500 cm^{-1} to that at 2500 cm^{-1} varies by less than 5% over 2 h for a spot focus, even though the total intensity decreased by 20%.

In order to convert the observed output of the standards shown in Fig. 1 to the real output in terms of photon/s per wavenumber of Raman shift, the output of a standard tungsten source must be known in the same units. The bulb manufacturer's output curve of watts/cm^2 nm must be divided by $h\nu$, then multiplied by λ^2 (where λ is the emission wavelength) with the result expressed in units of photons/s/cm^2/(cm^{-1}). The factor of λ^2 was neglected in the previous report,[1] resulting in an error in the luminescent intensity and the polynomials used to describe its shape. With the use of this corrected emission curve for the tungsten bulb as a standard, the real emission curve of the Kopp glass as a function of Raman shift relative to 785 nm was determined by Eq. 10 of Ref. 1, then normalized to a maximum intensity of 1.0. Adequate polynomial fits to these curves were obtained by extrapolating the ends of the curves to −500 and +4000 cm^{-1}, then least-squares fitting with a sixth-order polynomial. The corrected polynomials for Kopp sample #1 and for coumarin 540a are listed in Table I. To test the reproducibility of the fitting procedure, five sets of independent polynomial coefficients were determined for five different Kopp samples (numbers 3–7 in Fig. 1). The emission curves calculated from these curves exhibited relative standard deviations of 5.3% at 1000 cm^{-1}, 3.5% at 2000 cm^{-1}, and 1.4% at 3000 cm^{-1}.

With the corrected polynomials in hand, observed Raman spectra were corrected by using Eq. 11 of Ref. 1. The standard emission curve calculated from the polynomial was multiplied by the ratio of the observed sample intensity over the observed standard intensity. The correction was implemented automatically with Array

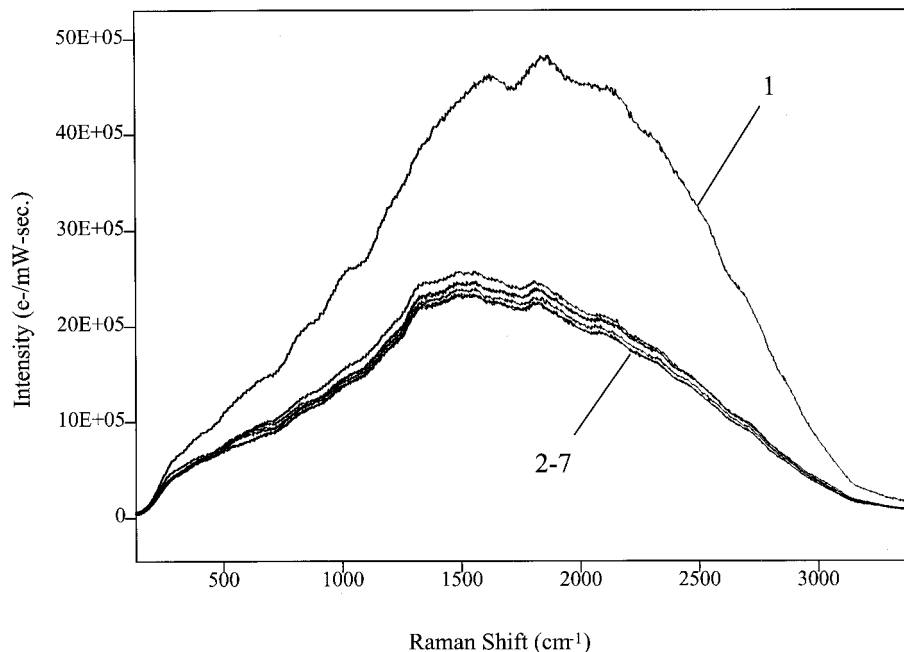* Author to whom correspondence should be sent.

APPLIED SPECTROSCOPY

FIG. 1. Raw emission curves for seven samples of Kopp 2412 filter glass, obtained on a Chromex 2050 spectrometer with 50 mW of 785 nm laser light. Samples 3–7 were from the same batch of glass, while 1 and 2 were purchased separately. Intensity axis is photoelectrons collected by a superpixel resulting from full-height binning, divided by integration time and laser power measured at the sample.

Basic in the GRAMS® software package and a disk file containing the appropriate polynomial coefficients from Table I. In practice, the user need only obtain a spectrum of the standard (Kopp glass or coumarin solution) by using the same conditions as those for the sample. Spectra corrected with Kopp glass are shown in Fig. 2 for reagent-grade liquid methylene chloride, chloroform, benzene, acetonitrile, and cyclohexane in a 1 cm cuvette. Numbers accompanying peaks are peak areas determined from the GRAMS® "curvefit" function with the use of a Voigt line profile,[2] and including peak shoulders as indicated. The areas are normalized to that of a prominent peak for each sample, and correspond to the "average" values in Table II.

There is significant variation in published Raman cross sections, due in part to uncertainties in peak integration limits and a host of experimental variables. Most of the values used here are liquid-phase differential Raman cross sections ($d\sigma/d\Omega$) from either Nestor and Lippincott[3] or Schrötter and Klöckner.[4] Nestor's values reported for 488 nm excitation were adjusted to 514.5 or

785 nm by the factor $\bar{\nu}_O(\bar{\nu}_O - \bar{\nu}_j)^3$, where $\bar{\nu}_O$ is the laser frequency and $\bar{\nu}_j$ is the Raman shift for a given peak. This factor is appropriate for photon counting detectors, rather than the more common ν^4 factor appropriate when intensity is measured in watts instead of photons/s.[5] Schrötter and Klöckner's gas-phase values in Table 4.4 of Ref. 4 were averaged, adjusted by $\bar{\nu}_O(\bar{\nu}_O - \bar{\nu}_j)^3$, then multiplied by the liquid/gas ratios of Table 4.6.

"Dilor" refers to spectra obtained with a Dilor X-Y spectrometer "macro" chamber with a 300 line/mm grating in the 250 mm focal length "second" spectrograph and an 800 × 2000 front-illuminated charge-coupled device (CCD) cooled to −140 °C. This system was normally used with a 785 nm Ti : sapphire laser, but occasionally was operated at 514.5 nm with an 1800 line/nm grating in a 600 mm spectrograph. The standard tungsten bulb was placed 1 meter from the sample position, and its light was diffused with a Kimwipe placed at the sample position. The spectrometer was carefully shielded to avoid stray light from any point other than the slit image at the sample. The Kopp standard spectrum was obtained with the same apparatus, with illumination by a Ti:sapphire laser tuned to 785 nm with the aid of a small monochromator. "Chromex" refers to a Chromex 2000/2050 multichannel spectrometer with a 1024 × 256 EEV front-illuminated deep-depletion charge-coupled device at −90 °C and an SDL 8530 laser. For both the Dilor and Chromex systems, a holographic band reject filter preceded a single spectrograph, and both CCDs were binned vertically along their full height.

RESULTS AND DISCUSSION

We reported previously that the Kopp 2412 glass showed good uniformity over 28 spots on the same sample, with a 1% variation of the intensity ratio at 526 and

TABLE I. Polynomial coefficients for intensity standards, normalized to maximum intensity.

Raman shift[a]	Coumarin 540a (514.5 nm)	Kopp 2412 #1 (785 nm)
$(\Delta\bar{\nu})^0$	0.2701196431	0.015647
$(\Delta\bar{\nu})^1$	1.5743416716e-3	6.284845e-5
$(\Delta\bar{\nu})^2$	−1.0957287534e-6	−1.515732e-8
$(\Delta\bar{\nu})^3$	2.5681273909e-10	8.564479e-11
$(\Delta\bar{\nu})^4$	−2.0594831955e-14	−9.472784e-15
$(\Delta\bar{\nu})^5$		1.594599e-18
$(\Delta\bar{\nu})^6$		−1.375663e-21

[a] In wavenumbers relative to indicated laser. Standard emission curve (with maximum equal to 1.0 arbitrary units) is reconstructed from the coefficients, and the Raman shifts (relative to 514.5 or 785 nm) are raised to the indicated powers.
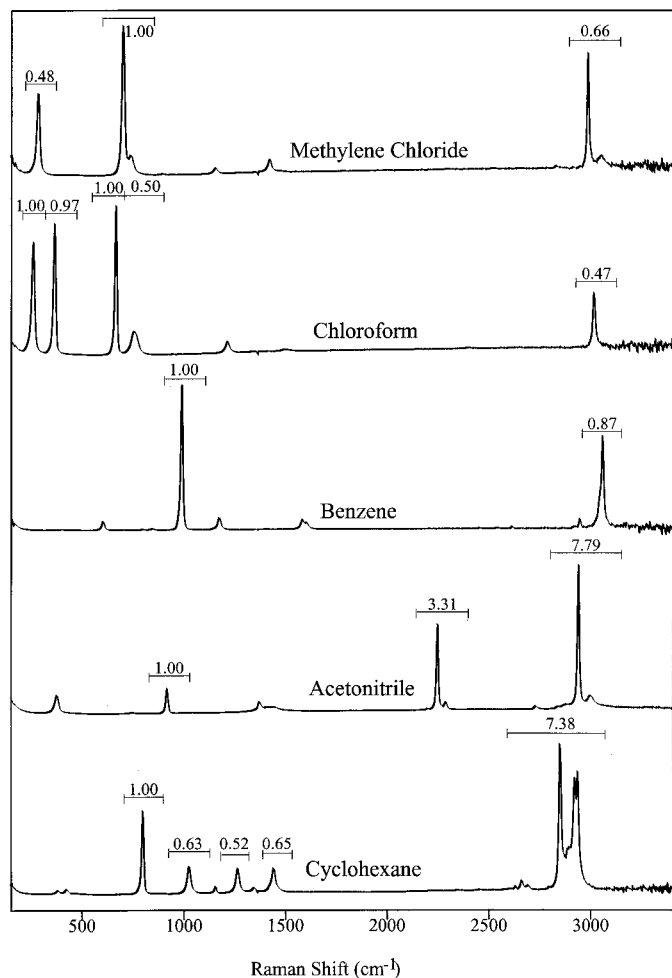
FIG. 2. Corrected spectra for five common liquids, obtained at 785 nm. The integration range for each peak is shown by the horizontal bars, and the numerical values are the peak areas relative to a prominent band. These areas are the same as those in the last column of Table II. All spectra were obtained on a Chromex 2050 spectrometer, sampling through the side of a quartz cuvette.

2412 glass is convenient and has most of the desired luminescent properties, but replacement with a more photostable material is still a desirable goal. The coumarin 540 standard for 514.5 nm excitation appears stable over months of shelf life, but its emission magnitude depends on an accurate concentration. As an indication of the reproducibility of the 2412 glass emission during daily use over an extended period, the ratio of the luminescent intensity at 2000 cm^{-1} to that at 800 cm^{-1} had a relative standard deviation (RSD) of 2.1% for 18 measurements covering a 64 day period. This period included a modification of the collection optics after two weeks. For twelve measurements over one month when the optics were unaltered, the absolute intensities at 800 and 2000 cm^{-1} varied by 7.2% and 6.7%, respectively, while the ratio had an RSD of 0.8%.

As noted in the Experimental section, the polynomial used to express intensity as a function of Raman shift was increased from fourth to sixth order for the 785 nm standard, and the ends of the raw emission curve were extrapolated before the fitting procedure. This process avoided some fitting aberrations near 0 and 3500 cm^{-1}, and better accommodated a small feature near the band gap of the CdTe in the Kopp glass.

Table II lists peak areas calculated from published cross sections, and those observed with the Dilor and Chromex spectrometers, then corrected with the luminescent standards and polynomials. For the literature values at 515 and 785 nm and both spectrometers, the peak areas were normalized to a prominent peak, so tabulated values do not indicate relative spectrometer efficiency. Each repetitive determination (indicated by $N = 6$, for example) involved acquisition of both sample and standard. The peak integration limits indicated in Table II were chosen to be wide enough to ensure that a negligible (<1%) change in area occurred if they were widened further. Relative standard deviations for a given normalized peak area are generally in the range of 1–5%, but were occasionally as high as 8–20%. The combination of all of the current determinations on both spectrometers exhibited an RSD of 5–14%.

If the results from the previous paper[1] are recalculated with the correct conversion of the tungsten source output, the values are close to those reported in Table II. The 2988/702 area ratio for CH_2Cl_2 determined previously at 785 nm with Kopp glass becomes 0.63, and the same value for 514.5 nm and the coumarin standard becomes 0.84. These compare well with the current observed ratios of 0.66 and 0.83. For the 2254/919 ratio of acetonitrile, the previous values for 785 and 514.5 nm correct to 3.39 and 4.10, compared to the current values of 3.31 and 3.86. So, for three spectrometers and two laser wavelengths, the corrected experimental peak area ratios agree to 10% or less.

The agreement between peak area ratios calculated from literature values and those observed here varies from a few percent (e.g., cyclohexane 1267, air 2331, $CHCl_3$ 260 and 364) to ∼20%. In some cases, such as the benzene 3060 band, the literature values are in significant disagreement, exhibiting >50% variation. It is not our purpose to critically evaluate reported cross sections, but currently available techniques and standards reported here appear to limit the accuracy of relative Ra-

1553 cm^{-1}.[1] However, Fig. 1 reveals that different batches of 2412 glass have different emission curves. Five separate 2 × 2 in. samples bought at once, and presumably from the same fabrication batch, have quite similar emission curves, but differences between batches were sometimes large. At least for the 2412 material, a new polynomial will need to be determined for each sample or at least each batch. If standards such as the Kopp glass become widely used, it is likely that each standard sample will be calibrated by the manufacturer, as is currently the practice for reflectance and fluorescence standards.

The photolability of the 2412 glass was noted earlier and has minor effects on emission curve shape and subsequently determined peak area ratios. It will cause errors when one is comparing the relative scattering of different samples, however, or when determining absolute rather than relative cross sections. As noted earlier, degradation of the 2412 glass emission depends on laser power and exposure time, but was less than 10% for the few minutes of exposure in a typical calibration. We were not able to locate a material with stable luminescence covering all 3500 cm^{-1} of Raman shift relative to 785 nm. The Kopp

TABLE II. Predicted and corrected peak areas, 785 nm excitation (brackets indicate 514.5 excitation).

Sample (all pure liquids)	Literature	Observed, then corrected with observed intensity standard		Average[a]
		Chromex	Dilor	
CH$_2$Cl$_2$				
288 cm^{-1}				
(225–360 cm^{-1})[b]	0.58[c]	0.47 ± 0.02 (N = 12)[d]	0.49 ± 0.04 (N = 4)	0.48 ± 0.03 (N = 16)
702 cm^{-1}				
(600–850)	1.00	1.00	1.00	
2988 cm^{-1}	0.58[c]	0.68 ± 0.05 (N = 13)	0.59 ± 0.04 (N = 4)	0.66 ± 0.06 (N = 17)
(2900—3150)	[0.74][c]	[0.99 ± 0.19 (N = 3)]	[0.80 ± 0.09 (N = 15)]	[0.83 ± 0.12 (N = 18)]
CHCl$_3$				
260 cm^{-1}				
(205–470)[b]	1.12[c], 1.02[e]	0.98 ± 0.01 (N = 6)	1.03 ± 0.03 (N = 5)	1.00 ± 0.03 (N = 11)
364				
(205–470)[b]	1.13[c], 1.03[e]	0.95 ± 0.01 (N = 6)	0.99 ± 0.03 (N = 5)	0.97 ± 0.03 (N = 11)
666				
(550–900)	1.00	1.00	1.00	
758				
(550–900)	0.56[c], 0.62[e]	0.45 ± 0.02 (N = 6)	0.56 ± 0.05 (N = 5)	0.50 ± 0.07 (N = 11)
3021				
(2920–3120)	0.42[c], 0.48[e]	0.51 ± 0.02 (N = 6)	0.41 ± 0.06 (N = 5)	0.47 ± 0.06 (N = 11)
Benzene				
992				
(900–1100)	1.00	1.00	1.00	
3060	0.80[c], 0.92[e], 1.26[f]	0.91 ± 0.03 (N = 6)	0.83 ± 0.03 (N = 8)	0.87 ± 0.05 (N = 14)
(2960–3160)	[0.99[c], 1.17[e], 1.48[h]]	[1.12 ± 0.04 (N = 4)]	[1.02 ± 0.18 (N = 8)]	[1.05 ± 0.16 (N = 12)]
Acetonitrile				
919 cm^{-1}				
(825–1025)	1.00	1.00		
2254	3.19[g], 2.88[c]	3.31 (N = 2)		
(2150–2400)	[4.0[f], 3.3[b]]	[3.86 (N = 2)]		
2943	5.83[c]	7.79 (N = 2)		
(2800–3150)	[7.23][c]	[9.71 (N = 2)]		
Cyclohexane				
801				
(700–900)	1.00	1.00	1.00	
1028				
(925–1125)	0.58[c]	0.62 ± 0.03 (N = 7)	0.64 ± 0.01 (N = 4)	0.63 ± 0.03 (N = 11)
1267				
(1180–1315)	0.49[c]	0.51 ± 0.02 (N = 7)	0.53 ± 0.02 (N = 4)	0.52 ± 0.02 (N = 11)
1444				
(1380–1525)	0.64[c]	0.63 ± 0.03 (N = 7)	0.67 ± 0.04 (N = 4)	0.65 ± 0.04 (N = 11)
All CH				
(2587–3068)	6.50[c]	7.17 ± 0.77 (N = 7)	7.74 ± 0.68 (N = 4)	7.38 ± 0.76 (N = 11)
Air				
1555 (O$_2$)				
(1450–1650)	[1.00]			
2331 (N$_2$)				
(2225–2425)	[3.26][i]	[3.22 ± 0.04 (N = 3)][j]		

[a] Average of all determinations on Dilor and Chromex spectrometers. Does not include literature values.

[b] Integration range for peak area. The CHCl$_3$ 260 and 364 cm^{-1} bands were integrated as two bands within the 205–470 cm^{-1} range, and the 666 and 758 cm^{-1} bands were integrated as two bands within the 550–900 cm^{-1} range.

[c] Calculated from Ref. 3 as described in Experimental section.

[d] Stated as mean ± standard deviation. N = number of spectra analyzed.

[e] Calculated from Ref. 4, as described in Experimental section.

[f] Calculated from Ref. 6 by adjusting reported 514.5 nm cross sections by $\bar{\nu}_o(\bar{\nu}_o - \bar{\nu}_j)^3$.

[g] Calculated by using ''A'' term parameters and Eq. 11 of Ref. 5.

[h] Reported in Ref. 6.

[i] Calculated from Ref. 4 for 21% O$_2$, 78% N$_2$.

[j] For Chromex 250 spectrograph with custom 180° collection optics.

man intensities to approximately 10–20%. The National Institute of Standards and Technology is currently developing luminescence standards for Raman intensity calibration that should substantially improve accuracy.[7]

Nevertheless, reliable 10–20% variation is far less than that observed in uncorrected spectra. Particularly with red lasers, the CCD response curve varies rapidly with wavelength over the common Raman shift range, and very large distortions of relative peak intensities are likely for uncorrected spectra. For example, the ratio of the 3060/992 peak area for uncorrected spectra of benzene at 785 nm differs by a factor of 4 between the Dilor and Chromex spectrometers, and differs from the corrected (and literature) values by a factor of about 50.

Overall, the main advantage of luminescent standards for correcting Raman spectra is the ease and accuracy of their use, plus the precise duplication of excitation conditions and geometry for both standard and sample. With suitable luminescent standards available, the correction of Raman spectra for instrument response is sufficiently straightforward to be used on a routine basis, in a wide range of applications.

ACKNOWLEDGMENTS

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