Relative Intensity Correction Standards for Raman Spectroscopy for Excitation with Several Common Laser Wavelengths

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Abstract

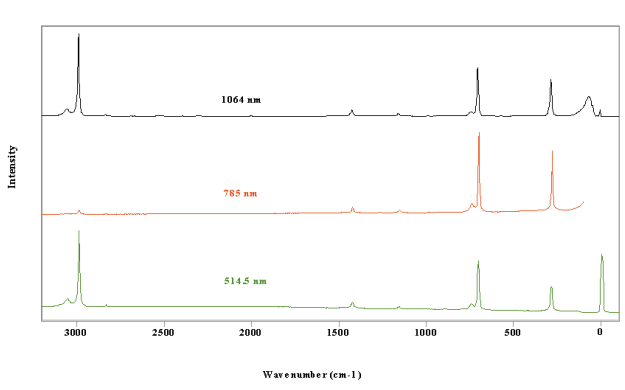
The relative intensities of spectra obtained from Raman spectrometers do not exhibit the 'true' Raman spectral shape because of wavelength-dependent instrument-response effects that are associated with all spectrometers. These effects can be a barrier to the comparison of spectra obtained with different instruments, to the application of instrument-independent and searchable libraries, and to the verification of instrument performance. Although calibrated, primary standard light sources can be used for the calibration of instrument response, they are cumbersome to use with many Raman spectrometers. NIST is currently developing luminescent glasses that can be employed as simple-to-use secondary standards to correct a Raman spectrum for instrument response. For Raman spectrometers using 785 nm excitation, Standard Reference Material (SRM) 2241 is now available. NIST is currently developing SRMs to calibrate Raman spectrometers using 532 nm, 488 nm, 514.5 nm and 1064 nm excitation wavelengths.

Key words: Raman Intensity Correction, Standard Reference Material, Luminescence, SRM 2241, Raman Spectroscopy, Calibration.

Introduction

Raman spectroscopy is a nondestructive analytical method that has many qualitative and quantitative analytical applications in the pharmaceutical industry. Its major advantage as an analytical tool is that it may be used with little or no sample preparation. However, the relative intensity of a sample's Raman spectrum is affected by the wavelength-dependent response of the spectrometer. Figure 1 illustrates that for even a very simple organic liquid such as methylene chloride, the observed Raman spectrum is significantly different for different excitation sources used with the same spectrometer. Unique differences are often observed among different spectrometers employing the same excitation laser wavelength. These effects should not to be confused with the dependence of the Raman spectral intensity on the wavelength of excitation, which exhibits a $(1/\lambda)^4$ dependence. The inability to easily correct the measured Raman intensity is part of the reason why instrument independent Raman libraries currently do not exist. The lack of intensity (y-axis) correction is also a significant issue for those industries that would employ Raman spectroscopy in their quality assurance programs and who are required to validate system performance to international quality standards or regulatory requirements such as those of the U.S. Food and Drug Administration.

FIGURE 1



Raman Spectra of Methylene Chloride (CH_2C1_2) Acquired with 1064nm (FT-Raman), 785 nm and 514 nm Excitation.

Correcting the instrument's intensity response with a light source of known relative irradiance [1,2] provides a solution. Although calibrated, standard light sources are available from national metrology institutes, such as the National Institute of Standards and Technology

(NIST), they are often cumbersome to use and difficult to adapt to the experiment, especially if daily validation and/or correction of the y-axis response of the spectrometer is required. The expense of calibrated light sources, their limited calibration lifetimes and difficulties in reproducing the Raman scattering geometry have led several researchers to consider secondary standards for intensity calibration. The McCreery Group at The Ohio State University suggested the use of fluorescent materials, whose broad-band luminescent emissions could be excited by the Raman laser [3,4], as standards. The advantage of this approach is that the standard is measured in a manner very similar to that of the sample, since the generation of the luminescent radiation closely matches the generation of the Raman-scattered radiation. Ideally, the luminescence standard should be simple to use, inexpensive, and require infrequent recalibration. The disadvantage, relative to a calibrated light source, is that a unique sample might be required for each excitation laser wavelength. This limitation is not critical as Raman systems typically utilize, at most, two unique excitation laser wavelengths.

At the request of the American Society of Testing and Materials (ASTM, subCommittee E13.08 on Raman Spectroscopy) and several pharmaceutical companies, and in collaboration with the McCreery Group, NIST has begun to fabricate and certify luminescent standards to provide relative corrections for the intensity axis of Raman spectrometers. The approach was to develop luminescent standards with glass matrices and metal ion fluorophores chosen to produce broad-band, featureless luminescence around the laser wavelength of interest. The most commercially important Raman excitation wavelengths were identified as 785 nm, 532 nm, 514 nm, 488 nm, and 1064 nm. The first standard developed was for excitation lasers at 785 nm.

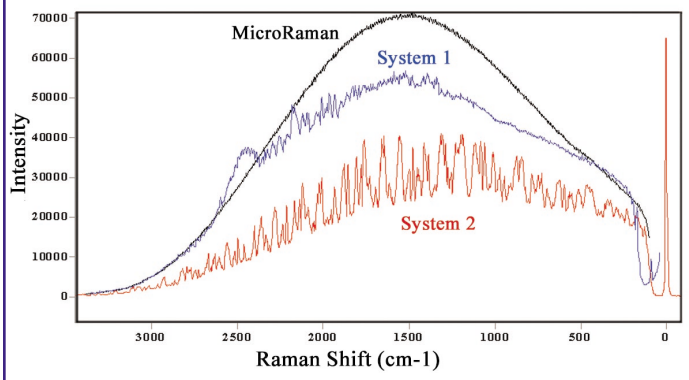
Here we will discuss the development and use of SRM 2241, the currently available SRM for the relative intensity correction of Raman spectrometers operating with 785 nm excitation [5]. NIST is currently working on other luminescent standards for use with 532 nm, 514 nm and 488 nm, and 1064 nm excitation.

SRM 2241-Relative Intensity Correction Standard for Raman Spectroscopy: 785 nm Excitation

Following the advice of the ASTM E13.08 subcommittee, we chose 785 nm laser excitation for our initial work since currently it is widely used. Unlike green excitation (at either 514.5 nm or 532 nm), this wavelength is long enough that it does not excite most unwanted fluorescence in the sample, yet it provides a higher Raman scattering efficiency than systems that operate at 1064 nm. The Raman spectral region to be corrected, for most users, lies within approximately 100 cm^{-1} (Raman shift) to 3400 cm^{-1} of the excitation. For 785 nm, this corresponds to an absolute spectral range from approximately 791 nm to 1070 nm. With 785 nm excitation, the Raman signal response in the C-H stretching region (2800 cm^{-1} to 3400 cm^{-1}) is markedly reduced, since the silicon-based CCD detectors employed have significantly lower quantum efficiencies (QE) in spectral regions beyond 1000 nm.

For use as a secondary standard, the ideal attributes of a luminescent glass standard are: resistance to laser-induced bleaching (photostability), homogeneity of the luminescent response, a smoothly varying luminescence shape, full coverage of the Raman spectral shift region, and chemical stability. Several candidate materials were brought to our attention by the ASTM Raman Subcommittee, but these materials, mostly commercially available color filter glasses, proved to be generally unsuitable. Of these, the Kopp 2412 glass suggested by the McCreery Group initially looked promising, but was eventually rejected because of photobleaching and significant batch-to-batch luminescence curve shape variations [4]. Several organic dyes that had been proposed were similarly rejected because of long-term photo/chemical

FIGURE 2A



Luminescence Spectra of SRM 2241 Obtained with each of the Three Spectrometers Used for Certification, Before Intensity Correction.

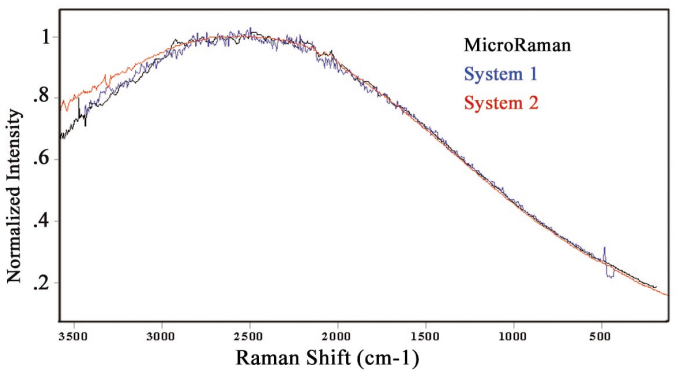
instability. Ultimately, we identified a chromium-based glass that provided all the desired characteristics of a luminescence standard and had the added benefit of being simple to fabricate. This glass contains 0.02 mole% Cr_2O_3 in a sodium-borosilicate matrix. At this concentration of Cr^{3+}, the glass provides an adequate luminescence signal for the range of laser powers encountered with commercial Raman systems (up to 100 mW), is photostable at these powers, and is chemically inert. Figure 2a shows uncorrected luminescence spectra of this glass obtained with the three different Raman systems used for the certification of SRM 2241. The observed shape of the curve is determined by the Cr-emission in this matrix and the spectrometer response. Fortunately, the profile of the curve does not reveal the Raman spectrum of the glass matrix, which would complicate its shape. The broad-band luminescence envelope adequately covers the Raman shift region.

Determination of the Relative Spectral Irradiance of SRM 2241

The determination of the relative spectral irradiance ("true" shape) of the luminescent glass standard requires that the spectrometer system be calibrated for its spectral intensity response against a source of known relative irradiance. The white light source used to correct the spectral response was a 6 inch uniform integrating sphere source fitted with a 10 Watt tungsten halogen lamp driven by a power supply operated in constant current mode. This source of white light emission was calibrated for its relative irradiance by NIST's Optical Technology Division Calibration Services program [6]. The calibration established the relative irradiance of the sphere source between 400 nm and 1800 nm.

Three Raman spectrometers were used in determining the relative irradiance of the luminescent glass: a commercial micro-Raman system employing a 500 mm focal length spectrograph (microRaman) and two macro-sampling Raman systems of approximately 0.25 meters ad 0.5 meter focal length spectrographs and custom-designed fore-optics. The macro-sampling systems (System 1 and System 2) were fitted with respectively 600 groove/mm and 1200 groove/mm gratings, with back-illuminated CCD detectors with pixel dimensions of 26 μm and 13.5 μm square. Both systems utilized NIST-developed control and analysis software. All three Raman systems utilized the same argon-ion-pumped titanium-sapphire laser optically tuned to lase at 785 nm. The holographic notch filters used for each of the three Raman systems allowed the spectrum to be collected to within 150 cm^{-1} of the excitation laser line. A depolarizer was used in the collimated portion of the collected

FIGURE 2B



Luminescence Spectra of SRM 2241 Obtained with each of the Three Spectrometers used for Certification, After Intensity Correction.

light to scramble any collection-optic-induced polarization. In all cases, the spectra were collected in a back-scattering configuration.

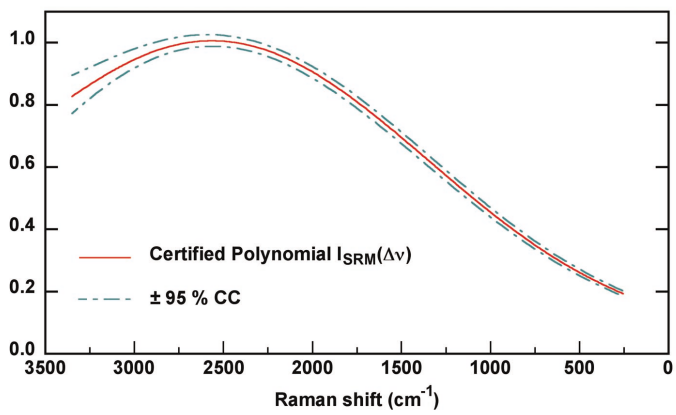
Figure 2a shows the observed (uncorrected) luminescence spectra of SRM 2241 acquired with each of the three spectrometer systems. The periodic fluctuations observed in the spectral traces acquired with the two macro-sampling spectrometers is likely due to etaloning, a well-known characteristic of back-illuminated CCD cameras. Observed spectra need to be corrected by the instrument response function unique to each instrument. This intensity correction curve is obtained from measurement of the spectrum of a white light source of known relative irradiance. The measured luminescence spectrum of SRM 2241 is corrected to its 'true' relative spectral intensity by multiplying the data points of the measured spectrum by the corresponding points of the intensity correction curve.

Figure 2b shows the intensity-corrected spectra obtained by applying this correction to the uncorrected spectra of SRM 2241 in Figure 2a. In Figure 2b, it can be seen that the corrected luminescence curves are now equivalent, within experimental uncertainties. In addition, the procedure has effectively removed most of the periodic fluctuations in the spectra of System 1 and System 2, ascribed to etaloning effects in back-illuminated CCD cameras. While this has been successful, particularly for System 2, we should note that in most cases the minimization of etaloning effects requires a level of experimental effort that most users would like to avoid and that results such as those illustrated may be better than the general case.

The certified luminescence spectrum of SRM 2241 was determined by a statistical analysis of spectra derived from over 100 measurements utilizing different collection optics, spectrometer, and detector combinations. Figure 3 shows the Certified Polynomial representing the 'best' fit of the luminescence spectrum of the standard derived from this set of measurements along with the associated 95% confidence curves. This shape of the luminescence spectrum can be described quantitatively by a fifth-order polynomial. The coefficients and their associated uncertainties for this polynomial are listed in the certificate of analysis, available on-line at: http://patapsco.nist.gov/srmcatalog/certificates/2241.pdf

To use the SRM glass, the luminescence spectrum of the standard is measured using the same instrument settings as used for the analytical sample. The intensity correction curve to be applied to the measured analyte spectrum should be generated numerically by evaluating the Certified Polynomial for the corrected luminescence spectrum of the standard and dividing by the measured luminescence spectrum of the

FIGURE 3



Curve Defined by the Fifth-Order Certified Polynomial and its 2σ Error Bounds Describing the Luminescence Spectrum of SRM 2241 Over the Temperature range of 20 °C to 25 °C. The X-axis is in Raman Shift (Relative to 785 nm Laser Excitation).

SRM, for each data point:

$$IntensityCorrectionCurve = \frac{CertifiedPolynomialforSRM\,2241Spectrum}{MeasuredSpectrumofSRM\,2241}$$

A subsequent analyte Raman spectrum may then be corrected to its 'true' relative intensity by multiplying the spectrum by the intensity correction curve, point-by-point. We anticipate that system manufacturers will implement this calibration process in software, so that the details of this calibration become transparent to the user.

SRM 2241 is a 10.7 mm(w) x 30. 4 (l) mm x 2.0 (t) mm slide that is mounted in a 12.5 mm square cuvette-shaped holder. This style holder can be used in traditional cuvette mounts or placed on its side on a microscope stage. The mount holds the glass slide in place with a clip, frosted side up. The surface is frosted to mitigate the effects of sampling geometry, perturbations due to the glass refractive index and focussing

FIGURE 4

SRM 2241 Shown in its Cuvette-Style Mount, and Storage Container.

TABLE 1

Peak	Range	LAB1*	LAB2	LAB3	NIST
802	700 - 900	**1.00**	**1.00**	**1.00**	**1.00**
1028	925 - 1125	**0.40**	**0.56**	**0.58**	**0.60**
1267	1180 - 1315	**0.33**	**0.46**	**0.48**	**0.49**
1444	1380 - 1525	**0.40**	**0.53**	**0.57**	**0.58**
all CH	2587 - 3068	**7.54**	**8.14**	**8.44**	**8.60**

* no depolarizer and no background subtraction.

Integrated Band Ratios for Intensity Corrected Cyclohexane. Results from an evaluation of a prototype SRM 2241 glass and intensity correction protocol. All Bands are integrated and ratioed to the 802 cm^{-1} ring-breathing mode of cyclohexane. Band Area Ratio = (I_x/I_{802}).

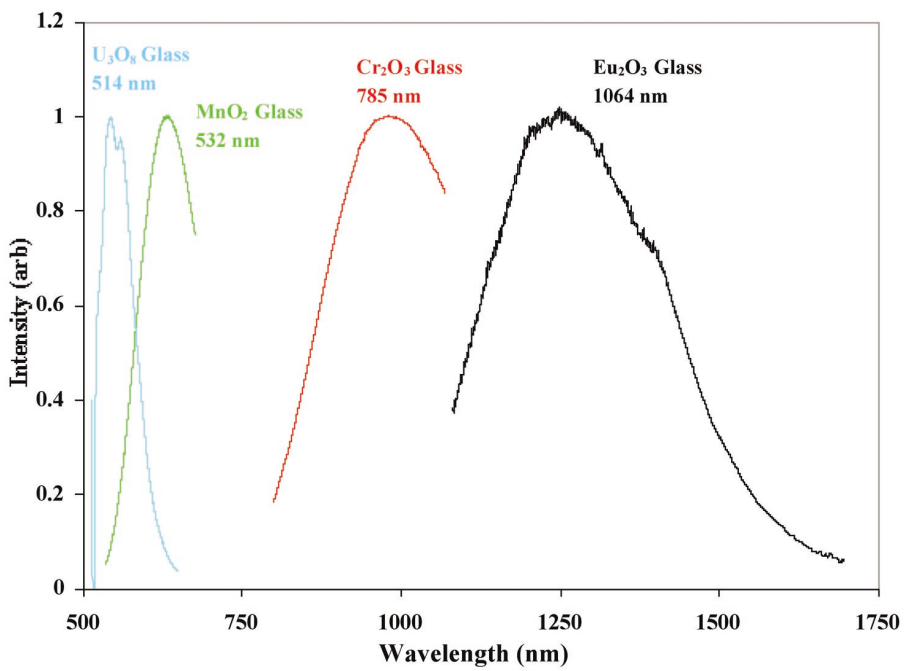
issues. The glass surface is accessible to most kinds of sampling arrangements, including short focal length objectives. The SRM and its storage box are shown in Figure 4.

Measurement of Peak Area Ratios as a Calibration Check

To verify the accuracy of the Raman intensity correction, one may compare peak area ratios of the corrected Raman spectrum to those values obtained from an established reference spectrum. McCreery and others [3,4,7] have published integrated band intensities for several common materials that have been adopted by the ASTM for use as Raman shift standards [8]. These are convenient materials, as they can be used to validate both the Raman shift axis (frequency-axis) calibration and also the intensity calibration. However, in the verification of the intensity calibration, significant variation in the peak area ratios can arise from systematic effects, particularly with 785 nm laser excitation. It is necessary to attend carefully to the effects of polarization, Raman signal collection, laser focussing, and sample positioning. Raman spectra of cyclohexane, widely used as a de facto standard for relative Raman intensity ratios, is a case in point. Literature values for the major band area ratios (C-H stretch at 2950 cm^{-1} ratioed to the ring breathing at 802 cm^{-1}) vary more than 36% [4]. Table 1 illustrates that during a recent evaluation of a prototype of SRM 2241, the variability of the intensity corrected band area ratios of cyclohexane was 20%. In our laboratory, even between very dissimilar spectrometers, the bias is reduced to less than 5% - 8% using SRM 2241. This variation between the systems borders on the lower limits imposed by the signal-to-noise of the measurements. Although further work is needed to establish the variability of the peak area ratios before this approach can be useful to the Raman community as a quantitative Raman intensity calibration check, it nevertheless serves as a very useful procedure to establish the *reproducibility* of the intensity calibration.

NIST is working to develop Raman intensity correction standards to provide relative intensity correction for excitation at 532 nm, 488 nm and 514 nm, and 1064 nm. A MnO_2-doped borosilicate glass that fluoresces when excited at 532 nm is currently being certified. A U_3O_8-doped glass designed for argon-ion laser excitation (488/514 nm) is currently under study, as is a Eu_20_3-doped glass designed for FT Raman systems operating at 1064 nm. Figure 5 demonstrates the spectral coverage of each fluorescing glass in the suite of SRM's, either currently available or projected.

FIGURE 5



Intensity-Corrected Luminescence Spectra of the Candidate Glasses, each Excited at the Indicated Wavelength.

Conclusions

A series of luminescent glasses are being developed as standards for the relative intensity correction of Raman spectra at the most commonly used excitation laser wavelengths. The standard for Raman spectroscopy at 785 nm has been issued, and work on three other standards for use at 532 nm, 514/488 nm, and 1064 nm is in progress. We may provide additional glasses for other laser wavelengths, as the need arises. These standards are easy to use and will permit daily validation of the intensity correction of analytical Raman systems. This, in turn, is expected to further the creation of instrument-independent libraries: a development that will garner Raman spectroscopy a more widespread acceptance as a practical and user-friendly analytical technique. ■

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Pictured: Edgar Etz, Steven Choquette, Douglas Blackburn, & Wilbur Hurst

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