National Institute of Standards & Technology

Certificate

Standard Reference Material® 2245

Relative Intensity Correction Standard for Raman Spectroscopy: 633 nm Excitation

This Standard Reference Material (SRM) is a certified spectroscopic standard for the correction of the relative intensity of Raman spectra obtained with instruments employing 632.8 nm laser excitation. A unit of SRM 2245 consists of an optical glass that emits a broadband luminescence spectrum when excited at this laser wavelength. The relative spectral intensity of the glass luminescence has been determined through the use of a white-light, uniform-source, integrating sphere that has been calibrated for its irradiance at NIST. The shape of the mean luminescence spectrum of this glass is described by a mathematical expression that relates the relative spectral intensity to the wavenumber (cm^{-1}) expressed as the Raman shift from the excitation laser wavelength. This model, together with a measurement of the luminescence spectrum of the standard, can be used to determine the spectral intensity response correction that is unique to each Raman system. The resulting instrument intensity response correction may then be used to obtain Raman spectra that are largely free from instrument-induced spectral artifacts.

This SRM is the fifth in a series of SRMs (2241, 2242, 2243, and 2244) that provide relative intensity corrections for Raman spectrometers employing lasers commonly used for Raman spectroscopy. This SRM is intended for use in measurements in the temperature range of 20 °C to 25 °C.

Certification: The log-normal model parameters describing the mean relative luminescence spectrum of SRM 2245 and associated confidence and prediction curves are given in Table 1.

Expiration of Certification: The certification of **SRM 2245** is valid, within the measurement uncertainty specified, until **30 September 2021**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Handling, Storage, and Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Production and certification of this SRM were performed by S.J. Choquette of the NIST Office of Reference Materials; A.A. Urbas and P.C. DeRose of the NIST Biosystems and Biomaterials Division; and J.R. Anderson of the NIST Fabrication Technology Division. The SRM units were cut and polished by J. Fuller of the NIST Fabrication Technology Division.

Statistical consultation was provided by S.D. Leigh, A.L. Rukhin, A.N. Heckert, and A.M. Possolo of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

Preparation and certification of this SRM were supported in part by the Test & Evaluation and Standards Division, Science and Technology Directorate, of the Department of Homeland Security.

Anne L. Plant, Chief
Biosystems and Biomaterials Division

Gaithersburg, MD 20899
Certificate Issue Date: 23 August 2016
Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

Table 1. Coefficients of the certified (CERT), linearly shifted log-normal model[a,b] defined in Equation 1 that describes the mean luminescence spectrum of SRM 2245 for 632.8 nm excitation, and coefficients of the analytical approximations (with the same functional form as the model itself) to the upper and lower envelopes of the 95 % confidence (L_{CONF}, U_{CONF}) and 95 % prediction (L_{PRED}, U_{PRED})[c] bands depicted in Figure 1 (includes the results of the Type A and Type B evaluations of uncertainty).

	L_{PRED}	L_{CONF}	CERT	U_{CONF}	U_{PRED}
H	9.4970 E –01	9.4240 E –01	**9.5071 E –01**	9.5912 E –01	9.5172 E –01
w	1.6587 E +03	1.6631 E +03	**1.6577 E +03**	1.6526 E +03	1.6568 E +03
ρ	9.5194 E –01	9.5163 E –01	**9.5207 E –01**	9.5265 E –01	9.5220 E –01
x_0	1.9600 E +03	1.9596 E +03	**1.9600 E +03**	1.9604 E +03	1.9601 E +03
m	1.9037 E –05	1.9313 E –05	**1.8981 E –05**	1.8649 E –05	1.8924 E –05
b	−2.8091 E –02	6.2351 E –03	**1.1698 E –02**	1.7054 E –02	5.1487 E –02

(a) The consensus (CERT) curve coefficients are for an unweighted log-normal model fit to the response data from four spectrometers. The uncertainty curve coefficients (CONF, PRED) are for log-normal model fits to 95 % confidence and 95 % prediction band expanded uncertainties, combining uncertainty components evaluated by Type A and Type B methods, with a coverage factor of $k = 2$, following the ISO/JCGM Guide [2].

(b) Where $I_{SRM}(\Delta\upsilon) = H \cdot e^{\left[\frac{-\ln 2}{(\ln \rho)^2}\left(\ln\left[\frac{(\Delta\upsilon - x_0)(\rho^2-1)}{w\cdot\rho}+1\right]\right)^2\right]} + m \cdot \Delta\upsilon + b;$

for $\Delta\upsilon$ = **150 cm^{-1} to 4000 cm^{-1}** Raman Shift relative to 632.8 nm excitation.

$I_{SRM}(\Delta\upsilon)$ has units of photons per second per square centimeter per wavenumber.

(c) The prediction band is one way of expressing the measurement uncertainty associated with the certified value: a measurement of this SRM over the range of Raman shifts considered in this certificate, made by a competent laboratory using a method comparable to those used at NIST to produce the certified value, is very likely (probability approximately 95 %) to lie within the prediction band.

Certified Values: A NIST certified value [1] represents a value derived from data reported in an SRM certificate for which NIST has the highest confidence in its accuracy to the extent that all known or suspected sources of bias have been fully investigated or taken into account. The measurand is the relative luminescence measured as a function of Raman shift (cm^{-1}) from the laser excitation wavelength of 633 nm. Metrological traceability is to the NIST spectral radiance scale.

The certified values of the coefficients of the model describing the mean shape of the luminescence spectrum of SRM 2245, excited at 632.8 nm, are listed in Table 1. The spectrum and its associated expanded uncertainty (confidence) and prediction bands (for ± 95 % coverage probability) are shown in Figure 1.

The dependent variable of this model is the relative spectral intensity of the luminescence. The independent variable of this model is the wavenumber expressed in units of Raman shift (cm^{-1}) from the laser excitation wavelength of 632.8 nm. This model is certified to describe the luminescent response of the SRM when it is measured in the temperature range of 20 °C to 25 °C. This model certifies the shape of the luminescence spectrum between 150 cm^{-1} and 4000 cm^{-1} Raman shift for excitation with a 632.8 nm laser.

Certification Uncertainty: The combined standard measurement uncertainty comprises one set of components evaluated by application of statistical methods (Type A evaluation), and one set of components evaluated by other methods (Type B evaluation), combined in root sum of squares.

The Type A uncertainties include contributions attributable to differences between spectrometers used in the certification process, and contributions from uncontrolled experimental factors, all of which find expression in the dispersion of the values of luminescent intensity that were obtained experimentally.

The Type B uncertainties includes contributions from the uncertainty in the white-light, uniform-source, integrating sphere irradiance calibration, assessed at 1 % (expanded uncertainty for 95 % coverage probability) of the certified value. Careful measurements of the glass have shown it to be spatially homogeneous in spectral luminescence. No significant changes in the shape of the luminescence spectrum occur over the range of laser power densities commonly used in Raman instruments.

The certified model was obtained by unweighted nonlinear least squares fitting the log-normal model of Equation 1 to measured values of $I_{SRM}(\Delta\upsilon)$ made by four different spectrometers. The associated uncertainty curves combine components evaluated by Type A methods and Type B methods, combined according to the ISO/JCGM Guide [2]. The Type A evaluations were performed using a Monte Carlo method consistent with the Supplement 1 to that Guide [3], and by application of standard methods for uncertainty analysis for linear and nonlinear statistical models [4,5].

Physical Description: SRM 2245 is a bismuth-doped (0.11 % mole fraction) oxide in a phosphate matrix glass. Each unit of this SRM consists of a glass slide that is approximately 10 mm in width × 10 mm in length × 1.65 mm in thickness, with one surface optically polished and the opposite surface ground to a frosted finish using a 400 grit polish. Two mounts are furnished with the slide. One is a 12.5 mm square cuvette-style optical mount. This mount is designed for the typical 12.5 mm sampling accessories widely used in chemical spectroscopy, i.e., absorbance, fluorescence, etc. The glass slide is retained, frosted side out, in a slot on the front face of the holder. Two plastic springs, also retained in the slot, hold the glass slide in place while allowing for positioning within the slot to accommodate different beam heights. Grooves on the sides of the slot require that the glass slide and springs be loaded into the slot from the bottom of the holder. The other mount is a 2.5 cm × 7.6 cm × 0.3 cm microscope slide style holder. On the top face is a rectangular slot to retain the glass slide over a circular aperture in the center of the holder. Two plastic springs serve to hold the glass slide in the slot centered over the circular aperture. Removal of the SRM glass for measurements that are physically hindered by the holders do not alter the certified properties of this SRM.

Measurement Conditions: The certification measurements of the luminescence spectrum of SRM 2245 were made using four spectrometer systems: one commercial Raman microscopy system operated in a 180° backscatter geometry, two Raman systems based on 270 mm and 300 mm focal length spectrograph designed for array detectors operated in 90° and 180° backscatter geometries, respectively, and one photomultiplier tube-based commercial spectrofluorometer operated in a 90° geometry. The three Raman systems utilized HeNe laser sources for excitation and either edge or notch Rayleigh rejection filters. The commercial spectrofluorometer utilized both Xe lamp and HeNe laser sources for excitation with no Rayleigh rejection filters used before the emission monochromator. The absolute wavenumber axis of each spectrometer was calibrated using emission lines from low-pressure pen lamps operated with a DC power supply. The y-axis (relative spectral intensity) of each system was calibrated with a white-light, uniform-source, integrating sphere that had been calibrated for irradiance at NIST. All certification data were acquired at nominal room temperature (22 °C ± 1 °C).

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Handling and Storage: When not in use, the SRM should be stored in the container provided or in one providing comparable mechanical protection. Although not recommended, the glass standard may be removed from its mount without altering the certified properties of the glass.

SRM 2245 is used to provide Raman spectra corrected for relative intensity. This requires a measurement of its luminescence spectrum on the Raman instrument and then a mathematical treatment of both this observed luminescence spectrum and the observed Raman spectrum of the sample.

For proper use of this procedure, attention must be paid to the following experimental conditions. The spectrometer laser and x-axis should be calibrated using the manufacturer's recommended methods. Validation of the Raman shift axis may be accomplished by referring to ASTM E1840-96 [6]. It is important that the laser excitation be incident only on the frosted surface of the glass. The shape of the spectral luminescence will have some sensitivity to the placement of the glass surface relative to the collection optics of the spectrometer, which is minimized by scattering from the frosted surface. Measurement conditions should be arranged to furnish a spectrum of optimum signal-to-noise ratio of the SRM. The luminescence spectrum must be acquired over the same Raman range as that of the sample.

The relative intensity of the measured Raman spectrum of the sample can be corrected for the instrument-specific response by a computational procedure that uses a correction curve. This curve is generated using the certified model and the measured luminescence spectrum of the SRM glass. For the spectral range of certification, $\Delta\upsilon = 150\ \text{cm}^{-1}$ to $4000\ \text{cm}^{-1}$, compute the elements of the certified relative mean spectral intensity of SRM 2245, $I_{SRM}(\Delta\upsilon)$, according to

$$I_{SRM}(\Delta\upsilon) = H \cdot e^{\left[\frac{-\ln 2}{(\ln \rho)^2}\left(\ln\left[\frac{(\Delta\upsilon - x_0)(\rho^2 - 1)}{w \cdot \rho} + 1\right]\right)^2\right]} + m \cdot \Delta\upsilon + b \tag{1}$$

where ($\Delta\upsilon$) is the wavenumber in units of Raman shift (cm^{-1}), H is peak height, w is peak width, ρ is half width ratio, x_0 is a location parameter for the log-normal profile, while m and b are the slope and intercept terms, respectively, for the linear term, with values as listed in Table 1. The elements of $I_{SRM}(\Delta\upsilon)$ are obtained by evaluating Equation 1 at the same data point spacing used for the acquisition of the luminescence spectrum of the SRM and of the Raman spectrum of the sample. $I_{SRM}(\Delta\upsilon)$ has been normalized to unity and is a relative unit expressed in photons per second per square centimeter per wavenumber**.** The data sets that are the measured glass luminescence spectrum, $S_{SRM}(\Delta\upsilon)$, and the measured Raman spectrum of the sample, $S_{MEAS}(\Delta\upsilon)$, must have the units of Raman shift (cm^{-1}). The elements of the correction curve $I_{CORR}(\Delta\upsilon)$, defined by Equation 2, are obtained from $I_{SRM}(\Delta\upsilon)$ and the elements of the glass luminescence spectrum, $S_{SRM}(\Delta\upsilon)$, by

$$I_{CORR}(\Delta\upsilon) = I_{SRM}(\Delta\upsilon) / S_{SRM}(\Delta\upsilon) \tag{2}$$

The elements of the intensity-corrected Raman spectrum, $S_{CORR}(\Delta\upsilon)$, are derived by multiplication of the elements of the measured Raman spectrum of the sample, $S_{MEAS}(\Delta\upsilon)$, by the elements of the correction curve [7]

$$S_{CORR}(\Delta\upsilon) = S_{MEAS}(\Delta\upsilon) \bullet I_{CORR}(\Delta\upsilon) \tag{3}$$

The Table 1 coefficients are certified for use **between 150 cm^{-1} and 4000 cm^{-1}**. The certified model is intended as a simple numerical descriptor of the spectral response observed over the wavenumber range studied. It is not claimed to be physically meaningful. **The model coefficients listed in Table 1 cannot be used to extrapolate the limits of certification without incurring significant error. Extrapolation of the model outside the certification limits of 150 cm^{-1} and 4000 cm^{-1} is not a supported use of this SRM**.

Use of this SRM at temperatures other than the certification temperature is not currently supported.

This SRM is not intended for use as a standard for the determination of absolute spectral irradiance or radiance.

Some photobleaching may be observed with this SRM upon exposure to high optical power densities. However, this will not affect the shape of the relative luminescence spectrum of the SRM.

Luminescence Spectrum on the Wavelength Scale: The equation describing the mean luminescence spectrum of the glass SRM is given in Equation 1, where $\Delta\upsilon$ is the Raman shift in units of wavenumbers (cm^{-1}). For correction of spectra where the x-axis is in wavelength with units of nanometers, the same model coefficients can be used to calculate $I_{SRM}(\lambda)$ through the following transformation:

$$I_{SRM}(\lambda) = \left[\frac{10^7}{\lambda^2}\right] \cdot \left(H \cdot e^{\left[\frac{-\ln 2}{(\ln\rho)^2}\left(\ln\left[\frac{(z-x_0)(\rho^2-1)}{w\cdot\rho}+1\right]\right)^2\right]} + m \cdot z + b \right) \tag{4}$$

where

$$z = 10^7 \cdot [(1.0/\lambda_L) - (1.0/\lambda)] \tag{5}$$

and λ_L is the wavelength of the laser in nanometers and λ is the wavelength in nanometers. The prefactor of 10^7 in the first term of Equation 4 is required only if it is desired to preserve the numerical value of spectral areas computed relative to the two x-axis coordinate systems.

For user convenience, a spreadsheet (Microsoft Excel) containing the coefficients of the certified model, confidence band and prediction interval as well as an example relative spectral intensity correction calculation is available on the website and can be obtained at https://www-s.nist.gov/srmors/view_datafiles.cfm?srm=2245.

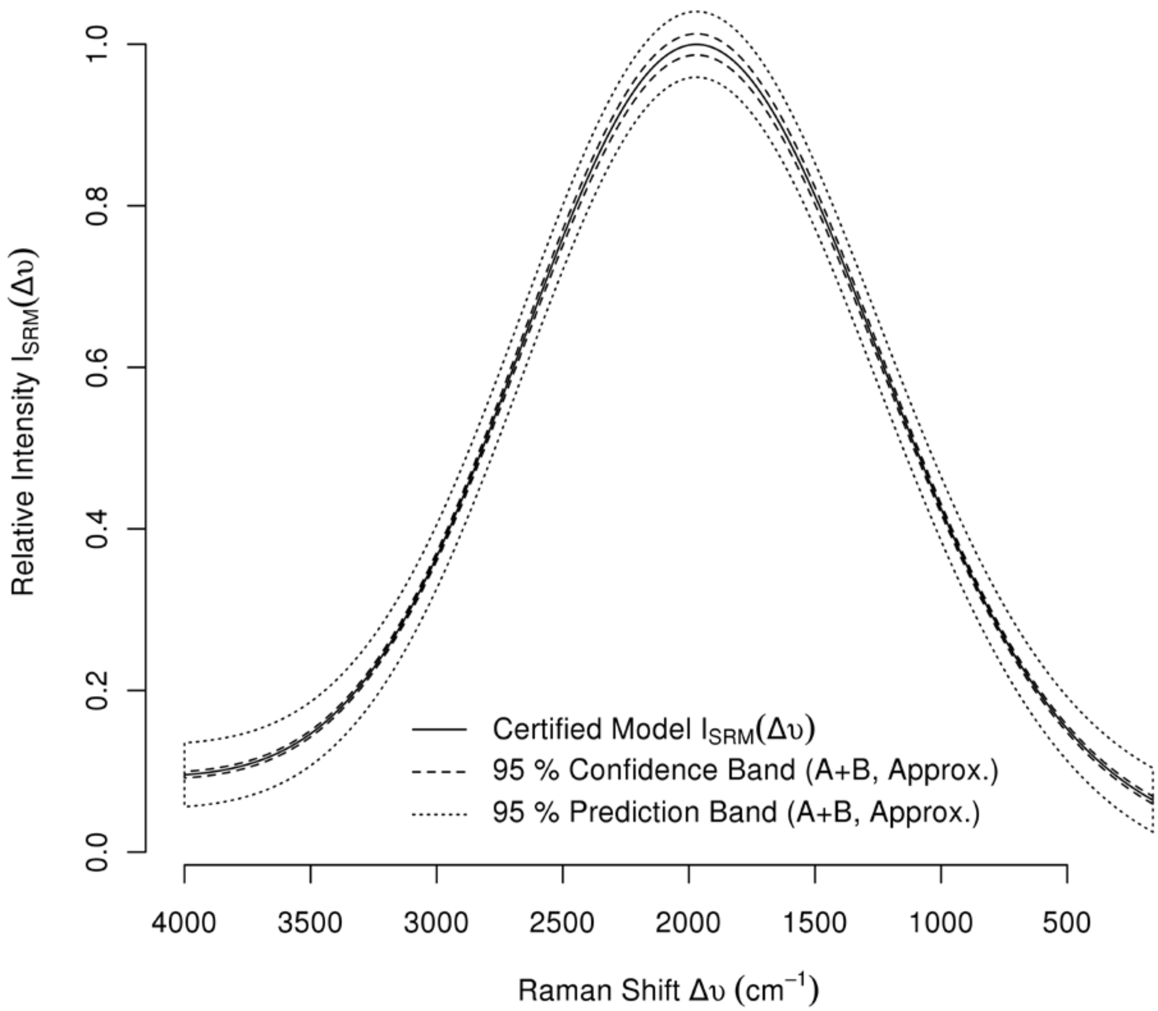

Figure 1. Certified, linearly shifted log-normal model describing the luminescence spectrum of SRM 2245 when excited at 632.8 nm. The horizontal axis has dimensions of Raman shift (cm^{-1}). The vertical axis is on a relative scale and normalized to unity with the dimensions of number of photons per second per square centimeter per wavenumber. The dashed lines (---) represent an approximate 95 % confidence band for the spectrum as a whole, reflecting components of uncertainty that have been evaluated by statistical methods (Type A) and by other methods (Type B). The wider, dotted lines (···), are the corresponding 95 % prediction band for individual spectra measured using methods and instruments comparable to those that were used in the certification.

REFERENCES

[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); available at http://www.nist.gov/srm/publications.cfm (accessed Aug 2016).

[2] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (JCGM) (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Aug 2016); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at http://www.nist.gov/pml/pubs/index.cfm (accessed Aug 2016).

[3] JCGM 101:2008; *Evaluation of Measurement Data — Supplement 1 to the "Guide to the Expression of Uncertainty in Measurement" — Propagation of distributions using a Monte Carlo method*; JCGM (2008); available at http://www.bipm.org/en/publications/guides/gum.html (accessed Aug 2016).

[4] Bates, D.M.; Watts, D.G.; *Nonlinear Regression Analysis and its Applications;* John Wiley & Sons: New York, NY (1988).

[5] Davison, A.C.; Hinkley, D.; *Bootstrap Methods and their Applications;* Cambridge University Press: New York, NY (1997).

[6] ASTM E1840-96; *Standard Guide for Raman Shift Standards for Spectrometer Calibration*; ASTM International, West Conshohocken, PA (2007).

[7] Frost, K.J.; McCreery, R.L.; *Calibration of Raman Spectrometer Response Function with Luminescence Standards: An Update*; Appl. Spectrosc., Vol. 52, pp. 1614–1618 (1998).

Certificate Revision History: 23 August 2016 (Change of expiration date; editorial changes); 16 December 2015 (Addition of parenthesis to equation four, alternate wavelength models; editorial changes); 27 September 2011 (Original certificate date).

Users of this SRM should ensure that the Certificate in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730, email srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.