



# National Institute of Standards & Technology

## Certificate of Analysis

### Standard Reference Material<sup>®</sup> 2242

#### Relative Intensity Correction Standard for Raman Spectroscopy: 532 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 532 nm laser excitation. SRM 2242 consists of an optical glass that emits a broadband luminescence spectrum when excited with 532 nm laser radiation. 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 luminescence spectrum of this glass is described by a polynomial expression that relates the relative spectral intensity to the wavenumber ( $\text{cm}^{-1}$ ) expressed as the Raman shift from the excitation wavelength of 532 nm. This polynomial, 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 instrument independent.

This SRM is the second in a series of SRM's (2241, 2242) that will provide relative intensity correction for Raman spectrometers employing lasers commonly used for Raman spectroscopy. This SRM is intended for use in measurements over the range of 20 °C to 25 °C and with Raman systems that employ laser excitation at 532 nm.

**Certification:** The polynomial describing the relative luminescence spectrum of SRM 2242 is given in Table 1.

**Expiration of Certification:** The certification of this SRM is valid until **01 January 2009**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. See "Instructions for Use". The certification is nullified if the SRM is modified or physically damaged.

**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. Return of the attached registration card will facilitate notification.

The coordination of the technical activities required for certification of this SRM were performed by G.W. Kramer, S.A. Buntin, and C. Presser of the NIST Chemical Science and Technology Laboratory. Production and certification of this SRM were performed by S.J. Choquette, E.S. Etz, W.S. Hurst, and D.H. Blackburn of the NIST Chemical Science and Technology Laboratory.

The SRM units were cut and polished by J. Fuller of the NIST Fabrication Technology Division.

Statistical consultation was provided by S.D. Leigh and A.N. Heckert of the NIST Statistical Engineering Division.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by B.S. MacDonald of the NIST Measurement Services Division.

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Table 1. Coefficients of the Certified Polynomial<sup>a</sup> and of the Confidence Curves<sup>b</sup>  
 Describing the Luminescence Spectrum of SRM 2242 excited at 532 nm  
 (Valid for Temperatures of 20 °C to 25 °C)

Polynomial Coefficient	Certified Value Polynomial Coefficient*  20 °C to 25 °C	Polynomial Coefficient <sup>c</sup> of the $\pm 2\sigma$ Confidence Curves <sup>b</sup>	
		+ 95 % CC, (+ 2 $\sigma$ )	- 95 % CC, (- 2 $\sigma$ )
A <sub>0</sub>	0.037014	0.040358	0.033670
A <sub>1</sub>	1.22531E-04	1.07950E-04	1.37112E-04
A <sub>2</sub>	-4.21311E-08	-1.16688E-08	-7.25933E-08
A <sub>3</sub>	2.19705E-10	1.99942E-10	2.39468E-10
A <sub>4</sub>	-9.04836E-14	-8.55445E-14	-9.54227E-14
A <sub>5</sub>	9.76795E-18	9.36292E-18	1.01730E-17

<sup>a</sup> A NIST certified value represents data, reported in a SRM Certificate, for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been fully investigated or accounted for by NIST.

<sup>b</sup> The confidence curves were calculated point-by-point using the uncertainty analysis of [3].

<sup>c</sup> Where  $I_{SRM}(\Delta\nu) = A_0 + A_1 \times (\Delta\nu)^1 + A_2 \times (\Delta\nu)^2 + A_3 \times (\Delta\nu)^3 + A_4 \times (\Delta\nu)^4 + A_5 \times (\Delta\nu)^5$ ; for  $\Delta\nu = 150 \text{ cm}^{-1}$  to  $4000 \text{ cm}^{-1}$  and  $\Delta\nu$  is the wavenumber expressed in the units of Raman shift ( $\text{cm}^{-1}$ ).

**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 accounted for by NIST. The certified values of the coefficients of the fifth-order polynomial describing the shape of the luminescence spectrum of SRM 2242, excited at  $\sim 532.2 \text{ nm}$  ( $18\,789.93 \text{ cm}^{-1}$ ), are listed in Table 1. The spectrum and its associated expanded uncertainty [2] (95 % confidence curves (CC)) are shown in Figure 1 and Figure 2. The dependent variable of this polynomial expression is the relative spectral intensity of the luminescence. The independent variable of this polynomial is the wavenumber expressed in units of Raman shift ( $\text{cm}^{-1}$ ) from the laser excitation wavelength of  $18\,789.96 \text{ cm}^{-1}$ . This polynomial is certified to describe the luminescent response of the SRM when it is measured between the temperatures of 20 °C to 25 °C. In addition, the polynomial is certified to describe the luminescent response of the SRM when it is illuminated with excitation wavelengths varying between  $532.2 \text{ nm} \pm 0.15 \text{ nm}$  ( $18\,789.93 \text{ cm}^{-1} \pm 5 \text{ cm}^{-1}$ ). The polynomial certifies the shape of the luminescence spectrum between  $150 \text{ cm}^{-1}$  and  $4000 \text{ cm}^{-1}$ .

**Certification Uncertainty:** The coefficients of the polynomials that express the expanded uncertainty (95 % confidence curves) of the certified polynomial are listed in Table 1. These polynomial expressions are used to calculate the upper and lower 95 % confidence curves for the luminescence spectrum of SRM 2242. These upper and lower bounds are shown in Figure 2.

The confidence curves were calculated by applying the point-by-point uncertainty analysis of [3] to the fitted polynomial. This method was used to evaluate the uncertainties of the shape of the luminescence spectrum of SRM 2242 based on measurements using three different instruments. Components of the uncertainty include the uncertainty in the white-light, uniform-source, integrating sphere irradiance calibration and various systematic errors from the operation of the Raman instruments used in the measurements. 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.

**Physical Description:** SRM 2242 is a manganese-doped (0.15 wt %  $\text{MnO}_2$ ) borate matrix glass. Each unit of this SRM consists of a glass slide that is approximately 10.7 mm in width x 30.4 mm in length x 2.0 mm in thickness, with one surface optically polished and the opposite surface ground to a frosted finish using a 400 grit polish. The frosted surface of the slide is characterized by a surface average roughness (root-mean-square) in the range of  $1.30 \mu\text{m}$  to  $1.49 \mu\text{m}$ , as determined by stylus profilometry [4]. The slide is held in 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. This mount can easily be placed on its side for use on a

microscope stage. The mount holds the glass slide, frosted side up, in place with a clip. The glass slide extends approximately 0.3 mm above the sides of the mount to allow its use with close focus objectives.

**Measurement Conditions:** The certification measurements of the luminescence spectrum of SRM 2242 were made using three Raman spectrometers. One was a commercial Raman microscopy system, while the other two used commercial 0.5 m focal length spectrometers designed for array detectors. All were operated in a 180° backscattering geometry. The collection optics ranged from short focus objective lenses (microscope system) to an achromatic 200 mm focal length collection lens. The x-axis of each spectrometer was calibrated for wavelength accuracy using a neon emission pen lamp. 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. The spectral resolution of the systems ranged from approximately 4 cm<sup>-1</sup> to 6 cm<sup>-1</sup> as measured by the full-width-at-half-maximum of the neon emission lines. All Raman systems used the same 532.2 nm excitation laser system. The excitation laser wavelength (532.199 nm ± 0.030 nm, 18 789.96 cm<sup>-1</sup>) was measured daily using a wavemeter. All certification data were acquired at nominal room temperature (21°C ± 1°C).

## INSTRUCTIONS FOR USE

SRM 2242 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. Due to polarization effects that are present in Raman instrumentation, a polarization scrambler should be employed in the Raman light-collection optics, most preferably in a region of collimated light. Raman spectral bands that exhibit various degrees of polarization will not be properly intensity-corrected without the use of a scrambler. To acquire the luminescence spectrum of SRM 2242, the surface of the glass should be placed at the same position from which the Raman spectrum of the sample is collected. 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. The luminescence spectrum should 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 polynomial and the measured luminescence spectrum of the SRM glass. For the spectral range of certification,  $\Delta\nu = 150 \text{ cm}^{-1}$  to  $4000 \text{ cm}^{-1}$ , compute the elements of the certified relative spectral intensity of SRM 2242,  $I_{\text{SRM}}(\Delta\nu)$ , according to eq 1

$$I_{\text{SRM}}(\Delta\nu) = A_0 + A_1 \times (\Delta\nu)^1 + A_2 \times (\Delta\nu)^2 + A_3 \times (\Delta\nu)^3 + A_4 \times (\Delta\nu)^4 + A_5 \times (\Delta\nu)^5 \quad (1)$$

where  $(\Delta\nu)$  is the wavenumber in units of Raman shift (cm<sup>-1</sup>) and the  $A_n$ 's are the coefficients listed in Table 1. The elements of  $I_{\text{SRM}}(\Delta\nu)$  are obtained by evaluating eq 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.  $I_{\text{SRM}}(\Delta\nu)$  has been normalized to unity and is a relative unit expressed in terms of photons sec<sup>-1</sup>cm<sup>-2</sup>(cm<sup>-1</sup>)<sup>-1</sup>. The data sets that are the measured glass luminescence spectrum,  $S_{\text{SRM}}$ , and the measured Raman spectrum of the sample,  $S_{\text{MEAS}}$ , must have the units of Raman shift (cm<sup>-1</sup>). The elements of the correction curve  $I_{\text{CORR}}(\Delta\nu)$ , defined by eq 2, are obtained from  $I_{\text{SRM}}(\Delta\nu)$  and the elements of the glass luminescence spectrum,  $S_{\text{SRM}}(\Delta\nu)$ , by

$$I_{\text{CORR}}(\Delta\nu) = I_{\text{SRM}}(\Delta\nu) / S_{\text{SRM}}(\Delta\nu) \quad (2)$$

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

$$S_{\text{CORR}}(\Delta\nu) = S_{\text{MEAS}}(\Delta\nu) \times I_{\text{CORR}}(\Delta\nu) \quad (3)$$

In measurements with a dispersive spectrometer, the x-axis is directly related to the wavelength (nm) of the measured spectrum. The transformation from the wavelength scale to the Raman shift scale requires a multiplicative factor of  $\lambda^2$  be applied to the relative intensity. The certified relative spectral intensity polynomial,  $I_{\text{SRM}}(\Delta\nu)$ , includes this  $\lambda^2$  factor. As a result of eqs 1 through 3, the elements  $S_{\text{MEAS}}(\Delta\nu)$  and  $S_{\text{SRM}}(\Delta\nu)$  may either

include this  $\lambda^2$  factor or not, so long as it is consistently applied, since it will cancel out if it is included. A detailed description of this procedure for the intensity correction of Raman spectra can be found in references 5 and 6.

The polynomial expression, eq 1, is certified for use **between 150 cm<sup>-1</sup> and 4000 cm<sup>-1</sup>**. This is the spectral range common to the three instruments used for the certification. The 5th degree polynomial fits are intended as simple numerical descriptors of the spectral response observed over the wavenumber range studied. They are **NOT** intended for use in extrapolation; in fact, such use is strongly discouraged. Nor are these fits intended as physically meaningful models. Additional studies have measured the luminescence of SRM 2242 from -2000 cm<sup>-1</sup> to 7000 cm<sup>-1</sup>. **The polynomial listed in Table 1 can not be used to extrapolate the certification to this extended range without incurring significant error. Extrapolation of the polynomial outside the certification limits of 150 cm<sup>-1</sup> and 4000 cm<sup>-1</sup> is not a supported use of this SRM.** Certified values of the polynomial coefficients given in Table 1 are for use at temperatures between 20 °C to 25 °C.

Use of this SRM at temperatures other than the certification temperature is not currently supported. However, the temperature response of this SRM has been studied in the range 5 °C to 60 °C. The white light corrected and normalized luminescence spectra demonstrate an overall shift of the luminescence to lower Raman shifts with increasing temperature. The shape of the luminescence spectrum is unaffected but the peak center shifts with temperature by approximately -2 cm<sup>-1</sup>/°C.

If it is desired to correct for the shift of the luminescence with temperature, the following linear relationship can be applied to the wavenumber axis of  $I_{\text{SRM}}(\Delta\nu)$ :

$$(\Delta\nu, T)_{\text{SRM}} = (T_{\text{measured}} - 21 \text{ °C}) \times (-1.9 \text{ cm}^{-1}/\text{°C}) + (\Delta\nu)_{\text{SRM}} \quad (4)$$

After applying this shift to  $I_{\text{SRM}}(\Delta\nu)$  (i.e., equation 1), an interpolation of the x-axis spacing of  $I_{\text{SRM}}(\Delta\nu)$  to the x-axis spacing of the Raman data must be done before using eq 2. However, the 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.

**Handling and Storage:** To maintain the certified properties of SRM 2242, the glass slide should be handled only in its mount. When not in use, the SRM should be stored in the container provided or in one providing comparable mechanical protection.

**Luminescence Spectrum on the Wavelength Scale:** The equation describing the luminescence spectrum of the glass SRM is given in eq 1, where  $(\Delta\nu)$  is the wavenumber in units of Raman shift (cm<sup>-1</sup>). For correction of spectra where the x-axis is in wavelength with units of nanometers, the same polynomial coefficients can be used to calculate  $I_{\text{SRM}}(\lambda)$  through the following coordinate transformation:

$$I_{\text{SRM}}(\lambda) = [10^7/\lambda^2] \times [A_0 + A_1 \times Z^1 + A_2 \times Z^2 + A_3 \times Z^3 + A_4 \times Z^4 + A_5 \times Z^5] \quad (5)$$

where

$$Z = 10^7 \times [(1.0/\lambda_L) - (1.0/\lambda)] \quad (6)$$

and  $\lambda_L$  is the wavelength of the laser in nm and  $\lambda$  is the wavelength in nanometers. The prefactor of  $10^7$  in the first term of eq 6 is needed only if it is desired to preserve the numerical value of spectral areas computed relative to the two x-axis coordinate systems.

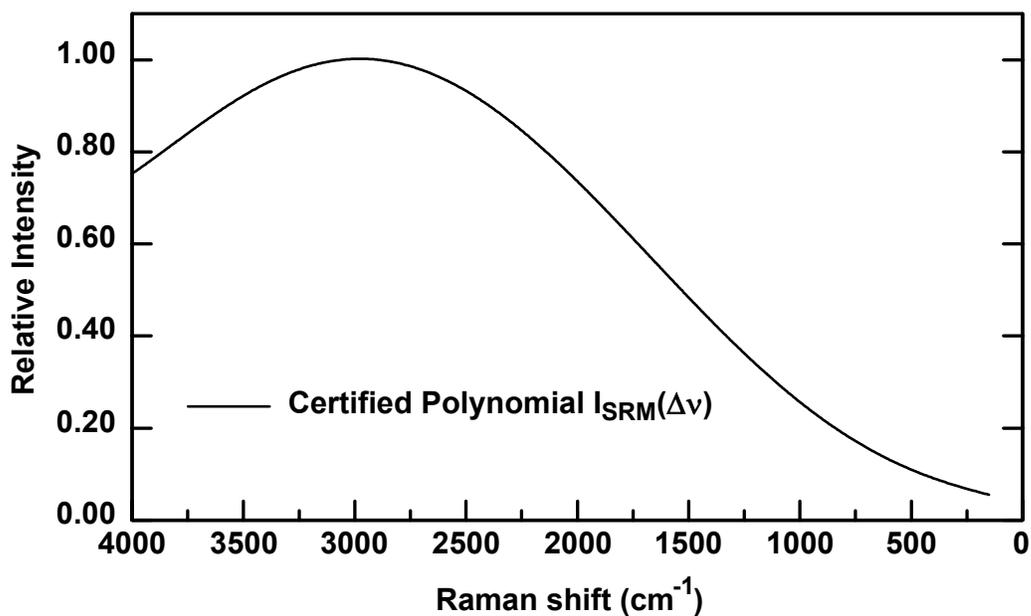


Figure 1. Fifth-order certified polynomial for SRM 2242. The x-axis is expressed in Raman shift ( $\text{cm}^{-1}$ ) relative to 532.2 nm ( $18\,789.93\ \text{cm}^{-1}$ ). The y-axis is on a relative scale and normalized to unity with the dimensions of photons  $\text{sec}^{-1}\text{cm}^{-2}(\text{cm}^{-1})^{-1}$ .

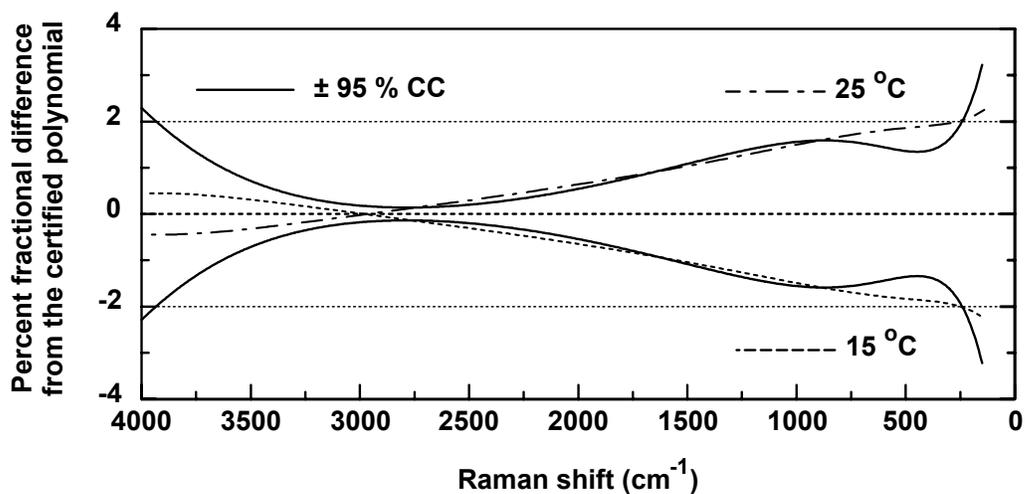


Figure 2.  $\pm 2\sigma$  confidence curves describing the luminescence spectrum of SRM 2242. The two solid black curves represent for SRM 2242 at room temperature, respectively, the percent fractional difference between the  $+95\%$  CC or the  $-95\%$  CC of the certified polynomial and the curve describing the certified polynomial. The certified polynomial is valid over the temperature range of  $20\ ^\circ\text{C}$  to  $25\ ^\circ\text{C}$ . The dashed curves (see legend) show the percent fractional difference between the curves describing the reference polynomial and the luminescence spectra at  $15\ ^\circ\text{C}$  and  $25\ ^\circ\text{C}$  operation. The x-axis is Raman shift (relative to 532.2 nm laser excitation).

## REFERENCES

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- [2] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st ed. ISO, Geneva, Switzerland (1993); 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).
- [3] Levenson, M. S.; Banks, D. L.; Eberhardt, K.R.; Liu, H.K.; Vangel, M.G.; Yen, J.H.; Gill, L.M.; Guthrie, W.F.; Zhang, N.F.; *An Approach to Combining Results From Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105(4), pp. 571-579 (2000).
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*Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.*