



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 2241

Relative Intensity Correction Standard for Raman Spectroscopy: 785 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 785 nm laser excitation. SRM 2241 consists of an optical glass that emits a broadband luminescence spectrum when excited with 785 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 (cm^{-1}) expressed as the Raman shift from the excitation wavelength of 785 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 intended for use in measurements over the range of 20 °C to 25 °C and with Raman systems that employ laser excitation at 785 nm. It may also be used for Raman excitation with lasers that range from 784 nm to 786 nm in excitation wavelength.

Certification: The polynomial describing the relative luminescence spectrum of SRM 2241 is given in Table 1.

Expiration of Certification: The certification of this SRM is valid until **31 July 2007**, 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.

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 coordination of the technical measurements required for certification of this SRM were performed by G.W. Kramer, J.G. Gillen, and C. Presser 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 of the NIST Statistical Engineering Division.

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Certified Values: A NIST certified value [1] represents 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 2241, excited at 785 nm, are listed in Table 1. The spectrum and its associated expanded uncertainty [2] (95 % confidence curves (CC)) are shown in Figure 1. 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 (cm^{-1}) from the laser excitation wavelength of 785 nm. This polynomial is certified for use when the SRM is between temperatures of 20 °C to 25 °C. In addition, the polynomial is certified for Raman excitation wavelengths varying from 784 nm to 786 nm. The polynomial certifies the shape of the luminescence spectrum between 255 cm^{-1} and 3350 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 of the luminescence spectrum of SRM 2241. These upper and lower bounds, together with the certified spectrum, are shown in Figure 1. Accordingly, the uncertainty can be stated as approximately 4 % between 255 cm^{-1} to 1300 cm^{-1} , 2 % between 1300 cm^{-1} and 2900 cm^{-1} , and 8 % from 2900 cm^{-1} to 3350 cm^{-1} . This is illustrated in Figure 2, where the percent fractional differences between the confidence curves and the certified polynomial are plotted as a function of Raman shift.

The confidence curves were calculated using a point-by-point Bound on Bias (BOB) [3] error analysis. This method was used to evaluate the uncertainties of the shape of the luminescence spectrum of SRM 2241 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.

Reference Values: A NIST reference value [1] is a best estimate of the true value, where all known or suspected sources of bias have not been fully investigated. Reference values expressed as reference polynomials and the corresponding 95 % confidence curves describing the luminescence spectrum of SRM 2241 are given for the use of this SRM at temperatures other than 20 °C to 25 °C. The coefficients of these reference polynomials for temperatures of 15 °C and 30 °C are listed in Tables 2 and 3 and may be used to within ± 3 °C of the nominal temperature values, respectively.

Physical Description: SRM 2241 is a chromium-doped (0.02 mol % Cr_2O_3) sodium borosilicate matrix glass. One unit of this SRM consists of a glass slide that is approximately 10.7 mm in width \times 30.4 mm in length \times 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 0.93 μm to 1.26 μm , as determined by stylus profilometry. 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 2241 were made using three Raman spectrometers. One was a commercial Raman microscopy system, while the other two were 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 120 mm focal length collection lens. The x-axis of each spectrometer was calibrated for either wavelength or Raman shift 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 1 cm^{-1} to 3 cm^{-1} as measured by the full-width-at-half-maximum of the neon emission lines. All Raman systems used the same excitation laser system. An argon ion laser was used to pump a titanium:sapphire solid-state tuneable laser. The excitation laser wavelength (785 nm \pm 0.03 nm, 12739.0 cm^{-1} \pm 0.5 cm^{-1}) was measured daily using a wavemeter. All certification data were acquired at nominal room temperature (23.5 °C).

INSTRUCTIONS FOR USE

SRM 2241 is used to provide intensity-corrected Raman spectra. 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 often 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 2241, 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, and this 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, on a point-by-point basis.

The relative intensity of the measured Raman spectrum 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, $\Delta\nu = 255 \text{ cm}^{-1}$ to 3350 cm^{-1} , compute the elements of the certified relative spectral intensity of SRM 2241, $I_{\text{SRM}}(\Delta\nu)$, according to Equation 1

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

where $(\Delta\nu)$ is the wavenumber in units of Raman shift (cm^{-1}) and the A_n 's are the coefficients listed in Table 1. The elements of $I_{\text{SRM}}(\Delta\nu)$ are obtained by evaluating Equation 1 at the data point spacing used for the acquisition of the luminescence spectrum of the SRM and of the Raman spectrum of the sample. The data sets that are the measured glass luminescence spectrum, S_{SRM} , and the measured Raman spectrum of the sample, S_{MEAS} , must have the units of Raman shift (cm^{-1}). The elements of the correction curve, $I_{\text{CORR}}(\Delta\nu)$, defined by Equation 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

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

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 λ^2 . The certified relative spectral intensity polynomial, I_{SRM} , includes this λ^2 factor. As a result of Equations 1 through 3, the elements $S_{\text{MEAS}}(\Delta\nu)$ and $S_{\text{SRM}}(\Delta\nu)$ may or may not include this λ^2 factor as 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 4 and 5.

The polynomial expression, Equation 1, is certified for use between 255 cm^{-1} and 3350 cm^{-1} . This is the spectral range common to the three instruments used for the certification. In additional studies, the luminescence of SRM 2241 from 100 cm^{-1} to 3600 cm^{-1} were measured. The polynomial listed in Table 1 has been tested over this extended range and was found to describe adequately the shape of the luminescence curve outside the certified limits. This observation is provided for information purposes only. Extrapolation of the polynomial outside the certification limits of 255 cm^{-1} and 3350 cm^{-1} is not a supported use of this SRM.

Certified values of the polynomial coefficients given in Table 1 are for use of the SRM at temperatures between $20 \text{ }^\circ\text{C}$ to $25 \text{ }^\circ\text{C}$.

Reference values of the coefficients of the polynomials that can be used when the SRM is measured at $15 \text{ }^\circ\text{C}$ or $30 \text{ }^\circ\text{C}$ are given in Tables 2 and 3. The tabulated coefficients of the two reference polynomials are applicable for SRM 2241

temperatures varying by ± 3 °C from the listed temperature(s). In Figure 2, a comparison is made between the reference polynomials for 15 °C and 30 °C and the certified polynomial. The two large dashed curves show, respectively, the percent fractional differences between the reference curves and the certified polynomial.

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 2241, the glass slide should be handled only in its mount. While not in use, the SRM should be stored in the container provided or in one with similar mechanical protection.

Luminescence Spectrum on the Wavelength Scale: The equation describing the luminescence spectrum of the glass SRM is given in Equation 1, where ($\Delta\nu$) is the wavenumber in units of Raman shift (cm^{-1}). 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

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

where

$$Z = 10^7 \cdot [(1.0/\lambda_L) - (1.0/\lambda)] \quad (5)$$

and λ_L is the wavelength of the laser in nm and λ is the wavelength in nanometers. The prefactor of 10^7 in the first term of Equation 4 is needed only if it is desired to preserve the numerical value of spectral areas computed relative to the two x-axis coordinate systems. The shape of the luminescence spectrum of SRM 2241 on the Raman shift scale has less than 2 % spectral variation with laser excitation wavelength varying between 784 nm to 786 nm. This is well within the 95 % confidence curves of SRM 2241. When the certified constants are used with data obtained using laser excitations that vary between 784 nm and 786 nm, it follows that with the x-axis expressed in the Raman shift (cm^{-1}) units the certified polynomial shape is the same for these varying laser excitations. Equations (4) and (5), then imply that there will be changes in the certified polynomial shape with excitation wavelength with the x-axis expressed in wavelength (nm) units. These changes are small (approximately 2 % or less).

REFERENCES

- [1] May, W.E.; Parris, R.M.; Beck II, C.M.; Fassett, J.D.; Greenberg, R.R.; Guenther, F.R.; Kramer, G.W.; Wise, S.A.; Gills, T.E.; Gettings, R.J.; and MacDonald, B.S.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurement*; NIST Special Publication 260-136, U.S. Government Printing Office, Washington, DC (2000).
- [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 Special Publication 1297, 1994 Edition, U.S. Government Printing Office, Washington, DC (1994); available at <http://physics.nist.gov/Pubs>.
- [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).
- [4] Ray, K.G.; McCreery, R.L.; *Simplified Calibration of Instrument Response Function for Raman Spectrometers based on Luminescent Intensity Standards*; Appl. Spectrosc. Vol. 51(1), pp. 108-116 (1997).
- [5] Frost, K.J.; McCreery, R.L.; *Calibration of Raman Spectrometer Response Function with Luminescence Standards: An Update*; Appl. Spectrosc. Vol. 52(12), pp. 1614-1618 (1998).

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; or via the Internet <http://www.nist.gov/srm>.

Table 1. Coefficients of the Certified Polynomial¹ and of the Confidence Curves²
 Describing the Luminescence Spectrum of SRM 2241
 (Valid for Temperatures of 20 °C to 25 °C)

Polynomial Coefficient	Certified Value Polynomial Coefficient* 20 °C to 25 °C	Polynomial Coefficient* of the ± 2σ Confidence Curves ²	
		+ 95 % CC, (+ 2σ)	- 95 % CC, (- 2σ)
A ₀	1.3535E-01	1.4221E-01	1.2916E-01
A ₁	2.1658E-04	2.2349E-04	2.1016E-04
A ₂ **	0	0	0
A ₃	1.8936E-10	1.9434E-10	1.8034E-10
A ₄	-9.837E-14	-1.0331E-13	-9.099E-14
A ₅	1.2414E-17	1.3532E-17	1.0948E-17

Table 2. Coefficients of the Reference Polynomial³ and of the Confidence Curves²
 Describing the Luminescence Spectrum of SRM 2241 at 15 °C

Polynomial Coefficient	Reference Value Polynomial Coefficient* 15 °C ± 3 °C	Polynomial Coefficient* of the ± 2σ Confidence Curves ²	
		+ 95 % CC, (+2σ)	-95 % CC, (-2σ)
A ₀	1.1898E-01	1.2800E-01	1.1495E-01
A ₁	2.1398E-04	2.1609E-04	2.0274E-04
A ₂ *	0	0	0
A ₃	1.9120E-10	2.0147E-10	1.8747E-10
A ₄	-9.798E-14	-1.0586E-13	-9.354E-14
A ₅	1.2201E-17	1.3745E-17	1.1164E-17

* where $I_{SRM}(\Delta\nu) = A_0 + A_1 \cdot (\Delta\nu)^1 + A_2 \cdot (\Delta\nu)^2 + A_3 \cdot (\Delta\nu)^3 + A_4 \cdot (\Delta\nu)^4 + A_5 \cdot (\Delta\nu)^5$; for $\Delta\nu = 225 \text{ cm}^{-1}$ to 3350 cm^{-1} and $\Delta\nu$ is the wavenumber expressed in the units of Raman shift (cm^{-1}).

** Regression analysis yields a $\Delta\nu^2$ term with a t statistic of less than 2 (coefficient statistically indistinguishable from zero).

¹ 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.

² The confidence curves were calculated point-by-point using a BOB [3] error analysis.

³ A NIST reference value is a best estimate of the true value, where all known or suspected sources of bias have not been fully investigated.

Table 3. Coefficients of the Reference Polynomial³ and of the Confidence Curves²
Describing the Luminescence Spectrum of SRM 2241 at 30 °C

Polynomial Coefficient	Certified Value Polynomial Coefficient* 30 °C ± 3 °C	Polynomial Coefficient* of the ± 2σ Confidence Curves ²	
		+ 95 % CC, (+2σ)	-95 % CC, (-2σ)
A ₀	1.4339E-01	1.5048E-01	1.3744E-01
A ₁	2.2947E-04	2.3638E-04	2.2303E-04
A ₂ **	0	0	0
A ₃	1.7596E-10	1.8114E-10	1.6714E-10
A ₄	-9.1976E-14	-9.680E-14	-8.447E-14
A ₅	1.1587E-17	1.2629E-17	1.0043E-17

* where $I_{\text{SRM}}(\Delta\nu) = A_0 + A_1 \cdot (\Delta\nu)^1 + A_2 \cdot (\Delta\nu)^2 + A_3 \cdot (\Delta\nu)^3 + A_4 \cdot (\Delta\nu)^4 + A_5 \cdot (\Delta\nu)^5$; for $\Delta\nu = 225 \text{ cm}^{-1}$ to 3350 cm^{-1} and $\Delta\nu$ is the wavenumber expressed in the units of Raman shift (cm^{-1}).

** Regression analysis yields a $\Delta\nu^2$ term with a t statistic of less than 2 (coefficient statistically indistinguishable from zero).

¹ 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.

² The confidence curves were calculated point-by-point using a BOB [3] error analysis.

³ A NIST reference value is a best estimate of the true value, where all known or suspected sources of bias have not been fully investigated.

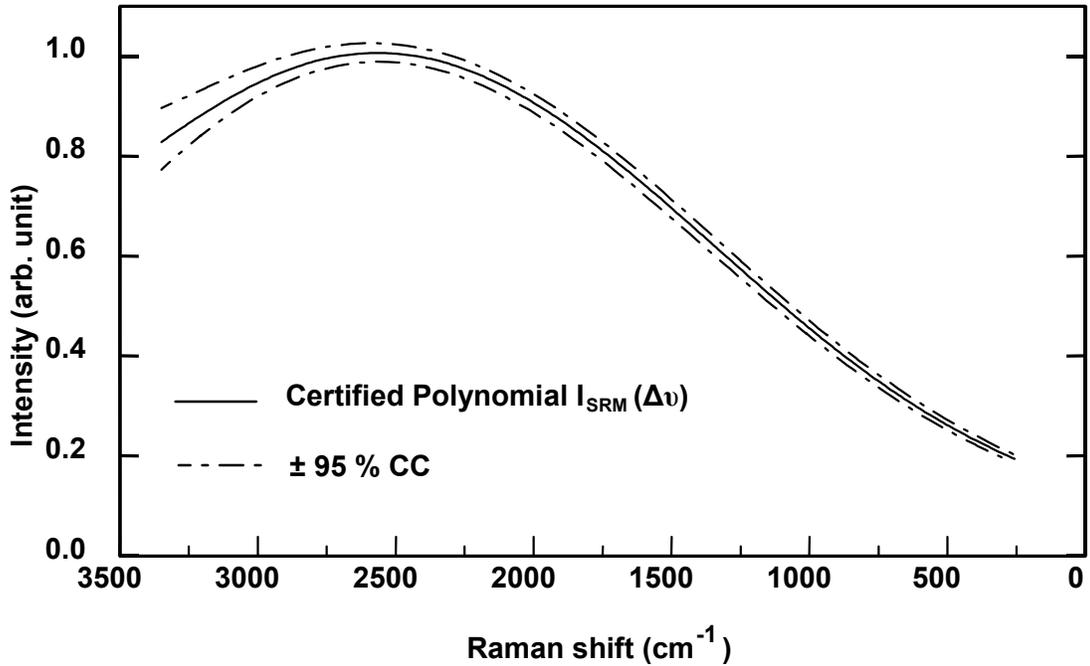


Figure 1. Fifth-order certified polynomial and $\pm 2\sigma$ confidence curves describing the luminescence spectrum of SRM 2241 valid over the temperature range of 20 °C to 25 °C. The x-axis is Raman shift (relative to 785 nm laser excitation).

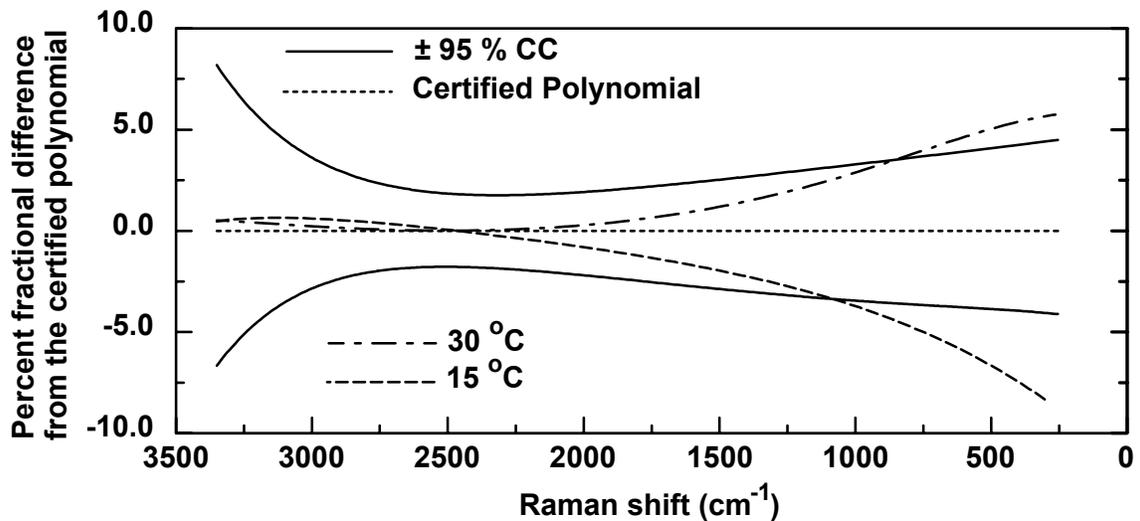


Figure 2. Comparison of the reference polynomials for 15 °C and for 30 °C to the certified polynomial. The two large-dashed curves show, respectively, the percent fractional difference between the curve describing the reference polynomial calculated for either 15 °C or 30 °C and the curve describing the certified polynomial. The two solid curves show for the SRM 2241 at room temperature, respectively, the percent fractional difference between the curve describing the + 95 % CC or the - 95 % CC of the certified polynomial and the curve describing the certified polynomial. The x-axis is Raman shift (relative to 785 nm laser excitation).