

# Review of Existing Standards, Guides, and Practices for Raman Spectroscopy

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## Abstract

Over the past decades Raman spectroscopy has been extensively used both on an industrial and academic level. This has resulted in the development of numerous specialized Raman techniques and Raman active products, which in turn has led to the adoption and development of standards and norms pertaining to Raman unit's calibration, performance validation, and interoperability. Purpose of the present review is to list, classify, and engage in a comprehensive analysis of the different standards, guides, and practices relating to Raman spectroscopy. Primary aim of the review is to consider the commonalities and conflicts between these standards and norms and to identify any missing aspects. Standardization in the field of Raman spectroscopy is dominated by the work of American institutions, namely, the American Society of Testing Materials (ASTM or ASTM International), with several active standards in place pertaining to terminology, calibration, multivariate analysis, and specific applications, and the National Institute of Standards and Technology (NIST), providing numerous certified reference materials, referred to as standard reference materials. The industrial application of Raman spectroscopy is dominated by the pharmaceutical industry. As such, pharmacopoeias provide not only important information in relation to pharmaceutical-related applications of Raman spectroscopy, but also invaluable insight, into the basic principles of Raman spectroscopy and important aspects that include calibration, validation, measurement, and chemometric analysis processes, usually by referring to ASTM and NIST standards. Given the fact that Raman spectroscopy is a modern and innovative field, the standardization processes are complex and constantly evolving. Despite the seemingly high number of existing standards, the standardization landscape is incomplete and has not been modernized according to the developments in Raman spectroscopy techniques in recent years. This is evident by the lack of protocols for numerous areas as well as by the fact that some of the existing standards have not been updated to reflect the advances in the technique. Therefore, it is important for the Raman community to actively engage in and contribute to a modernization process that will result in updating existing and introducing new terms, protocols, and guides. Indeed, the development of optimized common standards would be extremely beneficial and would further foster the development and application of Raman spectroscopy techniques, most notably those of surface enhanced Raman spectroscopy and low-resolution portable analyzers.

## Keywords

Harmonisation, terminology, calibration, spectrum processing, pharmacopeia, reference material, Raman spectroscopy

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## Introduction

Standardization is crucial for any analytical technique in terms of terminology, performance, validation, data formats, and model transfer. Moreover, standards are used as valuable tools to facilitate communication and trust, measurement, commerce, and manufacturing; standardization is identified in the European Framework Programme for Research and Innovation (Horizon 2020)<sup>1</sup> as one of the innovation support measures to bridge the gap between research and the market, helping the fast and easy transfer of research results to the European and international market. The use of Raman

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spectroscopy in the industry and academia and the already large number of specialized Raman techniques and Raman active products are readily increasing, which has fostered the development of standards and norms for Raman unit's calibration, performance validation, and interoperability, especially for some relevant applications. However, standardization in Raman spectroscopy has been necessary since the early stage of the development of the technique. For example, the use of the term "Raman shift" was accepted to describe the energy of the Raman bands correctly, since the wavelength of the Raman scattered light depends on the wavelength of the excitation light.<sup>2</sup> Since then, a number of standards and protocols have appeared to successfully improve the use of the technique in a broad number of fields, and all manufacturers struggle to make Raman equipment that performs according to specifications in terms of wavelength accuracy, repeatability, and scan-to-scan precision that refer to standards.<sup>3</sup>

This review aims at classifying and analyzing the existing standards, guides, and practices relevant for Raman spectroscopy, to serve as a reference catalog and to highlight any need for revision or improvement of existing Raman spectroscopy standards and to ensure that they incorporate the latest scientific research, practical experiences, and best practices from the various fields of application. It is not in the scope of this review to discuss the ease of implementation, as this depends on many factors, or to judge which are the best solutions, as some can be more accurate, but others can be viable and true as well. The objective is to compile a list of the existing standards and norms related to Raman spectroscopy and identify common, conflicting and/or missing points. The standardization activity related to Raman spectroscopy has greatly been led by American bodies, particularly the ASTM (American Society for Testing Materials, now ASTM international), with 14 active standards devoted to terminology, calibration, multivariate analysis, and specific applications, and NIST (National Institute of Standards and Technology), which provides a number of certified reference materials (CRMs), referred to as standard reference materials (SRM). Standards, guides, and practices related to Raman spectroscopy are grouped in this review in four main sections. The first section after the introduction deals with the terminology. The central sections of this review, Standards and Guidelines on Calibration and Certified (Standard) Reference Materials, focus on calibration and validation protocols from ASTM International and the pharmacopoeias (US, European, Chinese, and Japanese), and on certified reference materials and sources to be used for these purposes provided by national metrology institutes from the United States, China, and Japan. The following section covers technical documents published by the International Electrotechnical Commission (IEC), and the next one covers the International Organization for Standardization (ISO), IEC, and ASTM standards related to the use of Raman spectroscopy in specific applications, namely, microplastics, natural gas, and graphene (it is out of the scope of

this review to discuss special fields of application of Raman spectroscopy such as military applications). It must be mentioned that sampling methods and sample preparation guidelines, which, for instance, are often needed to pre-concentrate or clean-up complex samples for microscope analysis, are reported just in some standards dealing with specific Raman applications. Hopefully, this will be better covered in the standardization landscape of the near future. Finally, a part of this review discusses the existing standards related to chemometric methodologies that can be applied in Raman spectroscopy.

As a big part of the reviewed documents are not open access (and thus most of their content cannot be disclosed here due to copyright reasons). This, together with the fact that national standards or guides are not translated, makes the review of the standardization landscape difficult and probably limits a wider adoption.

## Standards and Terminology

Besides the terms included in the two ASTM guides on general standard terminology related to molecular spectroscopy and to charge-coupled device (CCD) detectors, commented in the following two subsections, [Table 1](#) summarizes relevant specific terms which definitions can be found in different standards, some described in other sections of this review for their direct relationship with Raman spectroscopy, and some not, for example, ASTM E1655, which includes terms for multivariate calibration (infrared), or the ISO standards ISO 18115 (Parts 1, 2, and 3) and ISO/TS 80004 (Part 6 and 13) that define terms used in surface chemical analysis (spectroscopy, scanning-probe microscopy, and optical interface analysis) and nanotechnologies, respectively. Unfortunately, some terms are missing while others are defined in several standards, sometimes differently. For example, Raman spectroscopy is defined in ISO 18115-2, ISO/TS 80004-6, ISO/TS 80004-13, and ASTM 7940. Interestingly, ISO 18115-3 (Surface Chemical Analysis Vocabulary, Part 3: Terms Used in Optical Interface Analysis)<sup>4</sup> is currently under development by the ISO Technical Committee 201 (Terminology) and will be a comprehensive compilation for relevant terms. This ISO standard may greatly fill the existing gap, as it will contain many relevant terms in its approximately 30 pages, especially in a dedicated section (Terms for Raman Spectroscopy); for instance, many Raman effect and spectrometer related terms will be included, such as confocal Raman microscopy, Raman scattering, electronic Raman scattering, Rayleigh scattering, notch filter, Stokes and anti-Stokes, or photobleaching, and terms related to spectrum acquisition such as Raman mapping or depth profiling. However, other terms should be also covered, such as data interval, pixel resolution, or Raman spectral resolution, terms related to data processing and analysis, for example, model-calibration transfer, definitions for raw, primary, and secondary data or outlier in Raman, different types of corrections, and the correlation factor in library search.

**Table I.** Terminology defined in ASTM and ISO standards related to Raman spectroscopy.

Standard	Main terms defined related to Raman spectroscopy	
ASTM E131-10 (2015) <sup>8</sup> (Standard Terminology Relating to Molecular Spectroscopy)	Anti-Stokes line Background Baseline Beamsplitter Certified reference material Fiber optics Filter Fluorescence Luminescence Monochromator Multiple correlation coefficient Numerical aperture (NA) Optical fiber Precision Principal component analysis Raman line (band)	Raman shift Raman spectrum Reference material (ISO Guide 30) Reference spectrum Resolution Sample pathlength Sample spectrum Scattering, 90° (or 180°) Signal-to-noise ratio Spectral slit width Spectrometer Spectroscopy Spectrum Standard error of calibration Stokes line (band) Ultraviolet Visible Wavelength Wavenumber
ASTM E2642-09 (2015) <sup>9</sup> (Standard Terminology for Scientific Charge-Coupled Device (CCD) Detectors)	Advanced inverted mode operation (AIMO) Analog-to-digital (A/D) converter Anti-blooming structure Antireflective (AR) coating Back-illuminated CCD Binning CCD bias Charge Charge-coupled device Charge transfer Charge transfer efficiency Column Complementary metal oxide semiconductor Correlated double sampling Cosmic event Dark current Dark noise Deep depletion CCD Dynamic range Electron-multiplying CCD Etaloning Exposure time Frame Frame-transfer CCD Front-illuminated CCD Full-frame CCD Full well capacity Gate structure Horizontal binning Indium tin oxide	Intensified CCD Interline transfer CCD Linear array CCD Multi-pinned phase (MPP) Noise Open electrode CCD Horizontal register Outgassing Output amplifier Output node Parallel shift Peltier cooler Pixel Pixel non-uniformity Potential well Quantum efficiency (QE) Read noise Readout rate Region of interest (ROI) Row Serial register Shot noise limit Signal-to-noise ratio (SNR) Silicon Slow-scan CCD Spectral rate Thermoelectric cooling Thinning Vertical binning
ASTM D8333 <sup>10</sup> (Standard Practice for Preparation of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers Using Raman Spectroscopy, IR Spectroscopy, or Pyrolysis-GC/MS)	Effluent Influent Microplastic Surface waters Suspended solids	

(continued)

Table I. (continued)

Standard	Main terms defined related to Raman spectroscopy	
ASTM D6122 <sup>11</sup> (Standard Practice for Validation of the Performance of Multivariate Online, At-Line, Field and Laboratory Infrared Spectrophotometer, and Raman Spectrometer Based Analyzer Systems)	Action limit Analyzer Analyzer calibration Analyzer model Analyzer repeatability Analyzer result Analyzer site precision Analyzer validation status Analyzer validation test Between-method reproducibility Chart expectations based on the multivariate model Control limits Exponentially weighted moving average control General validation Individual observation control chart Inlier Inlier detection methods In-line probe Instrument Instrument standardization Line sample Local validation Model degrees of freedom (DOF) Model variables Moving range of two control chart Multivariate calibration Multivariate model	Nearest neighbor distance inlier Optical background Optical reference filter Outlier detection limits Outlier detection methods Outlier spectrum Performance test Physical correction Post-processing Predicted primary test method result Prediction deviations Pre-processing Primary test method (PTM) Predicted primary test method result (PPTMR) Process analyzer system Process analyzer validation samples Spectrometer cell, Raman Standard error of calibration Test sample Transfer optics Uncertainty of predicted primary test method result (U(PPTMR)) Validated result Validation reference material (VRM) Validation samples Validation test
ASTM E2719 <sup>a, 12</sup> (Standard Guide for Fluorescence—Instrument Calibration and Qualification)	Calibrated detector Calibrated optical radiation source Calibration Certified reference material (E131) Certified value Diffuse scatterer Flux Grating equation Inner filter effects Lambertian reflector Limit of detection Noise level Photobleaching	Qualification Quantum counter Raman scattering Rayleigh scattering Responsivity Sensitivity Spectral bandwidth/bandpass or resolution Spectral flux Spectral responsivity Spectral slit width (E131) Traceability Transfer standard Transition dipole moment
ASTM E1655 <sup>a, 13</sup> (Standard Practices for Infrared Multivariate Quantitative Analysis)	Analysis Calibration Calibration model Calibration samples Estimate Model variation Multivariate calibration	Reference method Reference values Spectrometer/spectrophotometer Surrogate calibration Surrogate method Validation samples
ASTM E456 <sup>a, 14</sup> (Standard Terminology Relating to Quality and Statistics)	Acceptance Accepted reference value Accuracy Deviation Error of result Method of least squares Observed value Precision Repeatability Reproducibility Robustness	Sample (size) Specification/tolerance limits (within laboratory) standard deviation Statistic Systematic error of result Systematic sampling Test method/result/specimen, etc. Uncertainty Unit Variance
ASTM E2056 <sup>15</sup> (Standard Practice for Qualifying Spectrometers and Spectrophotometers for Use in Multivariate Analyses, Calibrated Using Surrogate Mixtures)	Spectrometer/spectrophotometer qualification Surrogate calibration Surrogate test method	
ASTM D7940 <sup>16</sup> (Standard Practice for Analysis of Liquefied Natural Gas (LNG) by Fiber-Coupled Raman Spectroscopy)	Accumulations Charge-coupled device Charge-coupled device binning Exposure time Incident light Raman scattering effect	Raman spectroscopy Raman spectrum Scattered light Signal strength Wavenumber

(continued)

Table I. (continued)

Standard	Main terms defined related to Raman spectroscopy
ISO/TS 80004-13:2017 <sup>a, b 17</sup> (Nanotechnologies—Vocabulary—Part 13: Graphene and related two-dimensional (2D) materials)	Raman spectroscopy
ISO/TS 80004-6:2021 <sup>a 18</sup> (Nanotechnologies—Vocabulary—Part 6: Nano-object characterization)	Fluorescence Raman effect Raman spectroscopy
ISO 18115-1:2013 <sup>a 19</sup> (Surface chemical analysis—Vocabulary—Part 1: General terms and terms used in spectroscopy)	Surface-enhanced Raman spectroscopy (SERS) Tip-enhanced Raman spectroscopy (TERS)
ISO 18115-2:2013 <sup>a 20</sup> (Surface chemical analysis—Vocabulary—Part 2: Terms used in scanning-probe microscopy)	Resolution, lateral Sample area viewed by the analyzer Signal-to-noise ratio Smoothing Samples <multivariate analysis> Variables <multivariate analysis>
ISO/DIS 18115-3 (under development) <sup>a,4</sup> (Surface chemical analysis—Vocabulary—Part 3: Terms used in optical interface analysis)	Apertureless Raman microscopy Anti-Stokes scattering Aperture Fluorescence Fluorescence quenching Image/map Near-field Raman microscopy Numerical aperture (NA) Optical resolution Polarization Raman effect Raman spectroscopy Stokes scattering Surface-enhanced Raman scattering (SERS) Surface-enhanced resonant Raman scattering (SERRS) Tip-enhanced Raman spectroscopy (TERS) Tip enhancement
	Anti-Stokes Raman scattering Background signal (ISO 18115 1: 2103) Backscattering configuration CCD detector Circular polarization Coherent anti-Stokes Raman scattering (CARS) Coherent Raman scattering microscopy (CRS) Coherent Stokes Raman scattering (CSRS) Confocal optical microscopy (ISO/TS 80004 6:2021) Confocal Raman microscopy Confocal volume (ISO 10934(en): 2020) Depolarization ratio Depth of focus (ISO 19262:2015) Edge filter Electronic Raman scattering Enhancement factor Fluorescence (ISO 18115-2:2013) Fluorophore (ISO 18115 2:2013) Hyper-Raman scattering Irradiance (ISO 29464:2017) Luminescence (IEC 60050 845: 1987) Monochromator (ISO 17861:2014) Monochromatic radiation (ISO 10934: 2020) Notch filter Objective lens (ISO 9849:2000) Peak height (ISO 7941:1988) Peak shape Peak width (ISO 18115 1:2013) Photobleaching Photodetector Photomultiplier tubes, photomultipliers (PMTs) Plasmon Polarizability Polarization Polarized Raman spectroscopy
ISO 23978:2020 <sup>21</sup> (Natural Gas—Upstream area—Determination of composition by Laser Raman spectroscopy)	Polarizer Plane/linear polarization Raman amplification Raman depth profiling Raman mapping/imaging Raman optical activity (ROA) Raman scattering/effect Raman spectroscopy (ISO 80004 13: 2017) Raman tensor Raman shift Rayleigh scattering Resonance Resonance Raman spectroscopy (RR) Resonant excitation profile Rotational Raman spectroscopy Selection rules Silicon diode detector Spontaneous Raman spectroscopy Spatially offset Raman spectroscopy (SORS) Spectral purity Stimulated Raman scattering (SRS) Stokes Raman scattering Surface-enhanced Raman spectroscopy (SERS) Surface-enhanced Raman scattering (SERS) (ISO 18115 2: 2013) Surface-enhanced resonant Raman scattering Surface-enhanced resonant Raman spectroscopy (SERRS) (ISO 18115 2:2013) Tip-enhanced Raman spectroscopy (TERS) Tip-enhanced resonance Raman spectroscopy (TERRS) Transmission Raman spectroscopy Transmission configuration Vibrational-rotational spectroscopy Wavelength, $\lambda$ (ISO 80000-3:2006) Wavenumber, $\sigma$ (ISO 19702:2015)
	Raman effect Multichannel photodetector Avalanche photodiode (APD)
	Signal intensity External cavity/interactivity <sup>c</sup> Base span calibration

(continued)

**Table I.** (continued)

Standard	Main terms defined related to Raman spectroscopy	
ISO Guide 30:2015 <sup>a, 22</sup> (Reference materials—Selected terms and definitions)	Certified reference material (CRM) Certified value Characterization Homogeneity Interlaboratory comparison/study/ test, collaborative study	Lifetime Reference material (RM) Reference method/procedure Sample Stability

<sup>a</sup>Standards not further commented on in this review.

<sup>b</sup>ISO/TS 80004-13:2017 Nanotechnologies—Vocabulary—Part 13: Graphene and related two-dimensional (2D) materials currently published is to be replaced by ISO/AVI TS 80004-13

<sup>c</sup>Measuring gas with a spectrograph outside and inside the laser, respectively.

It must be mentioned that some relevant terminology definitions can be found in the International Union of Pure and Applied Chemistry (IUPAC) Gold Book,<sup>5</sup> and that ISO<sup>6</sup> and IEC<sup>7</sup> maintain terminological databases for use in standardization. These three resources are freely available online. Ideally, terminology should be homogenized and open access.

### *ASTM E131-10 (2015) Standard Terminology Relating to Molecular Spectroscopy*

Some major terms of interest for Raman spectroscopy are defined in this standard<sup>8</sup> and are presented in alphabetical order in Table I. Some of the terms are well defined in ASTM E131-10, such as Raman spectrum, Raman line (band), Raman shift, or spectrometer. However, other definitions require further discussion. For example, those related to:

- (i) Reference material (RM): This term contains any kind of material used as a reference for whatever purpose. For standardization, more precision would be required, for example, for the accuracy of calibration. A distinction and definition of this term and the terms certified/standard reference material (CRM/SRM) is included in ISO/Guide 30:2015<sup>22</sup> and is of great importance since these terms tend to be confused/misused. An RM is a material with defined properties while a CRM is a traceable RM with accredited properties (defined as an RM certified by a company or regulated body or professional association according to traceable verifications of its properties/composition). An SRM is a synonym of CRM and is the term used by NIST. CRM should be used for standardized procedures, but the majority of the ASTM standards dealing with calibration procedures as well as the pharmacopoeias are only referring to reference materials, without any specification, except for ASTM E2911-13, where the use of NIST SRMs is indicated. A striking example is the use of eight commercial materials for spectrometer calibration in the ASTM 1840 standard, with no further specifications.
- (ii) Sample: Sampling, condition, or preparation of the material to be analyzed under a Raman spectrometer,

and related terms such as volume of analysis, in Raman microscope, fiber probe, hyphenated systems with infrared (IR), atomic force microscopy (AFM), scanning electron microscopy (SEM) and surface-enhanced Raman spectroscopy (SERS). According to the IUPAC Gold Book, sample is a portion of material selected from a larger quantity of material, the term needs to be qualified (e.g., bulk sample and representative sample) and implies the existence of a sampling error (otherwise the correct term would be test portion, aliquot, or specimen). The manner of selection of the sample should be prescribed in a sampling plan.

- (iii) Laser and optics related terms such as mode (single or multimode), power density, spot size, polarization, and optical path.
- (iv) Spectrum and spectrum data analysis related terms, such as quantitative and qualitative analysis.

Besides, some relevant terms are not even defined. Fortunately, some ASTM and ISO standards contain some more definitions (see Table I), and ISO/DIS 18115-3,<sup>4</sup> currently under development, contains a Raman spectroscopy specific section.

### *ASTM E2642-09 (2015) Standard Terminology for Scientific Charge-Coupled Device Detectors*

This standard terminology defines and discusses the basic terms related to CCD detectors, the most common in Raman spectroscopy, considering that CCD detection technology is applicable in the spectrum range of the CCDs, 200–1100 nm.<sup>9</sup> The terminology includes several types of CCDs and some terms that are of interest for Raman spectroscopy presented in alphabetical order in Table I.

## **Standards and Guidelines on Calibration**

This section covers Raman spectrometer testing, calibration, and validation procedures published by standardization bodies (only ASTM has published standards on these topics) and by the European, American, Chinese, and Japanese

**Table II.** Components of the configuration system.

Light source	Irradiation system <sup>a</sup> : dose rate, radiation energy and radiation dosimeter
Focusing/collection optics	Temperature-controlled container
Interfacing optical fiber	Collection optics into detection system
Light radiation filtering	Optical detection
Spectral activator sample: standard recommended samples are acetonitrile, benzene, and carbon tetrachloride	Recorder system
Optical interconnections	Ambient light shielding

<sup>a</sup>Dose rate, radiation energy, and radiation dosimeter.

pharmacopoeias; the following section covers the certified/standard reference materials (from NIST and the Japanese and Chinese Metrology Institutes) and light sources (from NIST and spectroscopy companies) proposed for that purpose. Calibration standards can refer to defining and verifying several different parameters, for example, the resolution of the spectrometer (ASTM E2529 and future ISO/AWI 23124), or the Raman shift *x*-axis calibration (ASTM E1840). They may also be specific to a type of instrumentation, for example, scanning Raman spectrometers (ASTM E1683). ASTM methods and external or internal NIST materials and sources are typically used by Raman equipment manufacturers for calibration and validation. Users of commercial Raman equipment and firmware may be afforded with different options in terms of calibration and validation depending on the manufacturer–model–technique and on the target user; standard practices are sometimes referred to in user's manuals, but mainly for advanced equipment and users. Qualification and validation protocols associated with chemometrics are covered in the last section on standards of the document. Chemometric models for data analysis are often implemented in the industry and are mainly application specific.

### American Society of Testing Materials

Most standards related to Raman calibration currently used by manufacturers were developed in the United States under the ASTM. ASTM standards provide guidelines on obtaining correct Raman spectra through the alignment of optical elements of the spectrometers and calibration procedures using reference materials and guidelines regarding the evaluation of the performance of Raman devices. More specifically, the following subsections are dedicated to the standards providing information on procedures for relative intensity correction (ASTM E2911), Raman shift calibration (ASTM E1840), and testing and assessment of spectral resolution and calibration (ASTM E2529), as well as spectrometer performance (ASTM E1683 and ASTM E1866). Finally, ASTM E1654 covers the method for measuring ionizing radiation-induced spectral changes in optical fibers

and cables. Fales et al. compare in Table I the topics covered by the main ASTM standards.<sup>23</sup>

### ASTM E2911-13 Relative Intensity Correction of Raman Spectrometers

The relative intensity correction procedure can enable intercomparison of Raman spectra acquired with differing instruments, excitation wavelengths, and laboratories. This standard addresses the use of NIST SRMs (wavelength specific) and a calibrated irradiance source (valid for multiple excitation wavelengths).<sup>24</sup>

The determination of the intensity response function of a Raman instrument traditionally has been carried out through a white light source, often tungsten, which is recorded under the conditions used for Raman spectral acquisition. As stated in the ASTM E2911-13, intensity calibration using a white-light source can present experimental difficulties, especially for routine analytical work. Calibrated tungsten halogen lamps have a limited lifetime and require periodic recalibration.

The Raman instrument response function is determined with the use of luminescent glass reference materials, more specifically NIST-certified reference materials (see their specific Section for details): SRM 2241 (785 nm), SRM 2242 (532 nm), SRM 2243 (514.5 and 488 nm), SRM 2244 (1064 nm), SRM 2245 (633 nm), and SRM 2246 (830 nm) are proposed. These standards emit a continuum of light over the Raman shift range when excited by the laser. Their spectrum is obtained in the same manner as a sample and can be used to correct the intensities of all spectra obtained under the same experimental configuration (same optical geometry and laser power). Unlike primary calibration sources, the glasses may not require periodic recalibration (but they do require revalidation). The intensity-corrected spectrum is obtained by multiplying the intensity correction factor by the intensity of the measured luminescence of the SRM.<sup>25</sup>

This standard has proved very helpful for data management across many applications, particularly in pharmaceutical and security databases. Each Raman unit has its own, unique spectral characteristics-based on the sensor response to the spectral range in use, which affects the relative peak intensities

of the collected spectra. The standard addresses this variation by mathematically correcting the spectrum. The downside of this procedure is that the noise level in some sections of the spectrum is enhanced too (this is particularly noticeable on devices using 785 and above laser lines with Si CCD sensors). Another limitation of the standard is the extension by manufacturer of its use. The certificates for the standards cover range specific ranges (see Table III), but nowadays, more and more units have extended ranges (lower or upper limits), or the standard is used in techniques that have not been demonstrated its use for, for example, transmission Raman. Since both Raman scattering and emission of the lamp must follow the same light path through the spectrometer, the alignment with the variety of sampling arrangements that are now typical with Raman spectrometers, especially microscope-based systems, can be challenging. Optical aberrations can yield different response curves for different sample positions, leading to miscalibration when the standard and source differ in size or geometry.

### *ASTM E1840-96 (2014) Raman Shift Standards for Spectrometer Calibration*

The standard recommends checking the calibration of Raman spectrometers frequently, especially when working at high resolution.<sup>26</sup> This recommendation is particularly valid today, as many optical components in high-end or high precision Raman devices are temperature sensitive, and calibrations can be easily affected. This standard provides well established Raman shift values of eight reference solid and liquid materials, selected to cover a wide wavenumber range (from 85 to 3327  $\text{cm}^{-1}$ ), to complement the spectrometer calibration based on low-pressure arc lamp emission lines (e.g., Ne, Ar, and Hg) described in ASTM E2529. These eight materials are not certified, but readily available at high purity from commercial sources: naphthalene; 1,4-bis(2-methylstyryl)benzene; sulfur; 50/50 toluene/acetonitrile; cyclohexane; polystyrene; N-(4-hydroxyphenyl)ethanamide (IUPAC's name for 4-acetamidophenol/Tylenol/paracetamol); and benzonitrile. Various laboratories examined several batches and each one used its own calibration protocol.

Since several protocols and standards, such as the ASTM E2911 or the European Pharmacopeia directly refer to it, ASTM E1840 is fully applicable and considered a pillar in Raman spectrometer calibration. However, it has been developed mostly with FT-Raman devices (1064 nm) that have high resolution and can be very precise but are not so common nowadays, and only a minor representation of dispersive Raman (relative intensity peaks for 514.5 nm excitation are provided). It must be taken into account that a source of deviation in calibration and validation stands on using the same techniques for high-end and low-resolution Raman spectrometers. For example, the precision of Fourier transform- (FT-) Raman is difficult to achieve in

low-performance dispersive systems operating multimode diode lasers at different wavelengths. Moreover, no guidance is given to the users on the procedure for finding the peak positions, the average values provided were not standardized, and no corrections were introduced for polarizability or resonance effects. This can cause some divergencies on other devices and other excitation wavelengths. Therefore, the standard requires an update to cover well the most common new spectrometers (dispersive, modular, low resolution, and new techniques) and some aspects like resonance effects.<sup>26</sup> Furthermore, at the time of ASTM E1840 standard creation, the selection of the eight reference materials was based on high purity being available and the material having no polymorphic forms. Since then, polymorphs of some of these materials (N-(4-hydroxyphenyl)ethanamide<sup>27,28</sup> or polystyrene<sup>29,30</sup>) are known and characterized. As such, this standard does not indicate how to evaluate the traceability or uncertainties, which are important parameters for quality control, certification of reference materials or in applications such as forensic science.

### *ATSM E2529-06 (2014) Testing the Resolution of a Raman Spectrometer*

This guide describes the routine for testing and assessing the spectral resolution of dispersive Raman spectrometers and Raman shift calibration using either low-pressure arc lamp emission lines (Ar, Kr, and Xe) or calcite.<sup>31</sup> The spectral bandwidth of dispersive Raman spectrometers depends primarily on the focal length, the dispersion of the grating, and the slit width, typically fixed for portable systems and variable for laboratory stations. The spectral resolution will also depend on the pixel size of CCD detectors. The document highlights the need to assess the spectrometer resolution and instrument line shape (ILS) function of Raman spectrometers for interoperability purposes.

The procedure for pen lamp calibration is described, and the emission lines of argon, krypton, and xenon lamps are given. It is suggested that the determination of the full width at half-maximum (FWHM) should be performed in the low, middle, and long Raman shift regions of the spectra because the resolution may vary in these regions. The operator must check the symmetry of the lines in the collected spectrum and use integration times that prevent saturation of the detector, especially for the Xe source, in which the 881.9 nm line is very intense. Difficulty in lamp alignment or the dense spectra of the emission sources are some of the disadvantages of low-pressure discharge lamps identified in this guide. A stable, chemically inert compound with well distributed Raman bands is proposed as an alternative to the use of emission sources, but recognizing that there is no ideal sample. ASTM E1683 and ASTM E1840 use different Raman active materials for both resolution and Raman shift calibration (see the specific sections). This standard proposes to estimate the resolution of



Table III. NIST-certified SRMs for relative intensity correction.

Material	Composition	Laser type	Restrictions/extra features	Certification measurement conditions
SRM 2241 (out of stock, 2241a should be available in 2022) <sup>58</sup>	Cr-doped (0.02 mol % Cr <sub>2</sub> O <sub>3</sub> ) Na borosilicate matrix glass	Ar ion laser pumped Ti:sapphire solid-state tunable laser (785.00 ± 0.01 nm) Frequency-stabilized 785 nm diode laser source (784.98 ± 0.02 nm) For 784–786 nm excitation wavelength	Temperature range 20–25 °C Laser excitation incident only on the frosted surface of the glass Raman shift: range 200–3500 cm <sup>-1</sup>	Three spectrometer systems: one commercial Raman microscopy system operated in a 180° backscatter geometry, one Raman system based on 460 nm focal length spectrograph designed for array detectors operated in a 180° backscatter geometry, and one commercial near-IR spectrofluorometer operated in a 90° geometry
SRM 2242a <sup>59</sup>	Mn-doped (0.15 wt% MnO <sub>2</sub> ) borate glass matrix (69.85 wt % B <sub>2</sub> O <sub>3</sub> stabilized with 5 wt% SiO <sub>2</sub> , 5 wt% ZnO, and 5 wt% Li <sub>2</sub> O) <sup>57</sup>	532.2 nm Two diode-pumped continuous-wave lasers	The glass matrix is susceptible to water corrosion. Storage at high relative humidity or direct contact with liquid water can rapidly deteriorate the surface. A bilayer nanolaminate was deposited on the glass substrate to serve as a moisture barrier to improve stability. The coated and uncoated units' emission profile could not be distinguished on a confocal Raman microscope using a 50x objective. Raman shift range 150–4000 cm <sup>-1</sup>	Two spectrometer systems: one commercial micro-Raman spectrometer system operated in a 180° backscatter geometry and one home-built Raman system that utilized two spectrograph and detector combinations 180° backscatter geometry
SRM 2243 (discontinued since 2014) <sup>60</sup>	Mn-doped (0.15 wt% MnO <sub>2</sub> ) Borate matrix glass (same as 2242)	488 nm and 514.5 nm line-tunable Ar-ion laser Initially for 514.5 nm, updated to cover 488 nm laser lines too <sup>57</sup>	Temperature range 20–25 °C. (However, between 5–60 °C the white-light-corrected and normalized luminescence spectra do not demonstrate a temperature dependence, unlike previous SRM's in this series) Laser excitation incident only on the frosted surface of the glass. Raman shift: range 200–4800 cm <sup>-1</sup>	Three commercial spectrometer systems: a micro-Raman and two 0.5 m focal length spectrometers designed for array detectors 180° backscattering geometry
SRM 2244 <sup>61</sup>	Cr-doped (0.7 wt% Cr <sub>2</sub> O <sub>3</sub> ) Borosilicate matrix glass	1064 nm Nd:YAG laser	Laser excitation incident only on the frosted surface of the glass Raman shift: range 100–3500 cm <sup>-1</sup>	Two commercially available FT-Raman spectrometers with Nd:YAG laser, Si:CaF <sub>2</sub> beamsplitters, liquid N <sub>2</sub> cooled Ge detectors, and proprietary beamsplitters. One with a Rayleigh rejection filter (anti-Stokes spectra to 1000 cm <sup>-1</sup> relative) and the other one with a cut-off filter 180° backscattering geometry

(continued)

Table III. (continued)

Material	Composition	Laser type	Restrictions/extra features	Certification measurement conditions
SRM 2245 <sup>62</sup>	Bi-doped oxide (0.11 mol %) Phosphate matrix glass	633 nm HeNe laser sources The absolute wavenumber axis was calibrated using emission lines from low-pressure pen lamps	Some photobleaching may be observed upon exposure to high optical power densities (However, this will not affect the shape of the relative luminescence spectrum) Raman shift range 150–4000 $\text{cm}^{-1}$	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
SRM 2246 (out of stock) <sup>63</sup>	Cr-doped oxide (0.30 mol %) Borosilicate-matrix glass	830 nm Excitation laser sources included an Ar-ion-laser-pumped, Ti:sapphire, solid-state, tunable laser (830.00 ± 0.01 nm) and a frequency-stabilized diode laser source (830.0 nm)	Photostable under extended exposure to approximately 50 mW of 830 nm laser light focused through a 50X magnification microscope objective Raman shift range 110–3000 $\text{cm}^{-1}$	Three spectrometer systems: one commercial Raman microscopy system operated in a 180° backscatter geometry, one Raman system based on a 460 mm focal-length spectrograph designed for array detectors operated in a 180° backscatter geometry, and one commercial near-infrared spectrofluorometer operated in a 90° geometry

**Table IV.** Certified values of GBW 13650 reference material from NIM.

Serial number	Name	Raman shift (cm <sup>-1</sup> )	Relative intensity	Expanded uncertainty (k = 2)
GBW 13650 <sup>64</sup>	Raman spectra relative intensity standard material with the excitation wavelength of 514.5 nm	494.5	0.05	0.01
		1006.6	0.14	0.02
		1991.3	0.52	0.06
		3015.3	0.95	0.07
		4000.0	0.91	0.07

dispersive Raman systems operating with a 785 nm laser wavelength by measuring the 1085 cm<sup>-1</sup> band of calcite. Calcite sample should be rotated to maximize the signal from this polarized band. The spectral bandwidth of the 1085 cm<sup>-1</sup> Raman band ( $BW_{1085}$ ) is given by the FWHM that can be determined by fitting to a mixed Gaussian and Lorentzian function. The calibration equation to be used for determining the spectral resolution ( $S_{resolution}$ ) of the dispersive Raman spectrometer (Eq. 1) according to this standard was obtained with a reference FT-Raman spectrometer,<sup>32</sup> which has adjustable resolution by altering the interferometer's optical path difference.

$$S_{resolution} = \frac{BW_{1085} - 0.684}{1.029} \quad (1)$$

ATSM E2529-06 (2014) provides values with 20% accuracy for dispersive Raman systems operating with a 785 nm laser wavelength, adequate for validation. It would be desirable to improve its accuracy and extend its validation to other laser wavelengths. Opposite to the emission lines of low-pressure arc lamps, which are not suitable for other wavelength lasers, calcite is suitable for use with all laser wavelengths; however, many samples have been observed to fluoresce with excitation wavelengths below 532 nm excitation. Surprisingly, few studies have dealt with testing the spectral resolution of Raman instruments, the ability to separate adjacent peaks, and/or how to transfer models for Raman instruments with different spectral resolution.<sup>32,33,34</sup>

#### ASTM E1683-02 (2014) Testing the Performance of Scanning Raman Spectrometers

ASTM E1683-02 outlines the main components of Raman apparatus, proposes guidelines for obtaining and reporting Raman spectra, as well as procedures for evaluating Raman instruments' parameters and performance.<sup>35</sup> These tests apply for single, double, or triple monochromator scanning Raman instruments commercially available, but not for multichannel or Fourier transform instruments or gated integrator systems requiring a pulsed laser source, for which performance tests must be developed according to ASTM E1866.

As in ASTM E2529, this standard proposes to check the spectral bandwidth by measuring the FWHM intensity of a

sharp plasma line emitted from a low-pressure atomic source or laser emission lines and test the scanning accuracy with samples of known band frequencies: carbon tetrachloride (no longer recommended due to safety reasons<sup>36,37</sup>), indene, cyclohexane, and L-cystine. Besides, ASTM E1683-02 indicates that dark signal level and sensitivity are relevant to the detector's performance and must be measured routinely in order to remove undesired signal contributions. The standard refers to a photomultiplier tube, while nowadays most units have CCD detectors, but the same applies to the latter. The standard also recommends an empirical approach for the evaluation of stray light rejection, without citing the ASTM E387-04 (2014).<sup>38</sup>

#### ASTM E1866-97 (2021) Spectrophotometer Performance Tests

This standard provides guidelines to develop practical performance tests when the specific ASTM E1683 for Raman type of spectrophotometers is not applicable, for instance, due to sampling or safety concerns, and can be used for online process spectrophotometers.<sup>39</sup> These tests evaluate the performance of a spectrophotometer and uncover malfunctions like misalignment of the optical components, which affect measurement stability or other changes in instrument operation, for example, contamination induced variations. The test conditions, samples to be used, type of measures, and action limits are established. Performance tests should be conducted under the same operating conditions and optical configuration as the normal operation of the spectrophotometer. Check and test samples used, which can be pure compounds or mixtures, should be in the same physical state as the samples to be analyzed. Both univariate and multivariate measures are contemplated. Univariate tests include tests of the energy level, photometric noise, short-term baseline stability, optical contamination, purge contamination, wavelength stability, resolution stability, and photometric linearity. Multivariate measures include pass/fail spectrophotometer performance tests: Level A tests using a least squares method and principal component regression (PCR) or partial least squares (PLS) method and level B tests. Finally, performance test results should be evaluated for trends and action limits determination based on performance tests historical data and by simulating instrument response changes.

**Table V.** Certified Raman shift values of GBW13651-13654 reference materials from NIM

GBW13651 <sup>65</sup> Sulfur	GBW13652 <sup>66</sup> Naphthalene	GBW13653 <sup>67</sup> Cyclohexane	GBW13654 <sup>68</sup>	
			N-(4-hydroxyphenyl)ethanamide	
83.2 ± 2.2	513.7 ± 2.3	384.1 ± 2.3	214.1 ± 2.4	968.5 ± 2.3
153.2 ± 2.2	763 ± 2.2	426.5 ± 2.4	328.8 ± 2.3	1104.6 ± 2.7
219.2 ± 2.1	1019.8 ± 2.2	801.9 ± 2.4	391.8 ± 2.2	1168.2 ± 2.3
473.2 ± 2.1	1146.3 ± 2.3	1028.1 ± 2.2	465.3 ± 2.2	1236.5 ± 2.3
	1381.3 ± 2.2	1157.6 ± 2.4	504.3 ± 2.4	1371.2 ± 2.4
	1463.5 ± 2.3	1266.4 ± 2.3	651.8 ± 2.4	1515.2 ± 2.3
	1576.3 ± 2.2	1444.2 ± 2.2	710.9 ± 2.2	1561.4 ± 2.3
	3055.1 ± 2.3	2664.2 ± 2.2	797.1 ± 2.4	1648.5 ± 2.6
		2852.4 ± 2.2	834 ± 2.3	2930.4 ± 2.2
		2923.4 ± 2.2	857.5 ± 2.2	3064.6 ± 2.2
		2937.5 ± 2.2		

### ASTM E1654-94 (2013) Measuring Ionizing Radiation-Induced Spectral Changes in Optical Fibers and Cables

This guide covers the method for measuring the real-time, in situ radiation-induced alterations to the Raman spectral signal transmitted by a multimode, step-index, silica optical fiber for use in remote Raman fiber optic spectroscopy.<sup>40</sup> Ionizing radiation affects the optical fiber sensor system's performance to transmit spectroscopic information from a remote location. The standard describes the test specimens (reference and sample), the main components of the radiation testing setup, summarized in Table II, and the information that should be reported, which includes information about the measurement setup, the procedure, sample details, and the results.<sup>41</sup>

### Pharmacopoeias

Pharmacopoeias provide general texts, standards, recommended procedures for analysis, and specifications to determine pharmaceutical substances and dosage. There are four predominant pharmacopoeias in the world, the European Pharmacopoeia (EP), the Japanese Pharmacopoeia (JP), the Chinese Pharmacopoeia (CP), and the United States Pharmacopoeia (USP), all of which share the goal of publishing and producing quality standards for pharmaceuticals. All of them have dedicated chapters on Raman spectroscopy describing the equipment and basic principles, calibration and verification procedures, and the application to pharmaceuticals.

#### European Pharmacopoeia

In chapter 2.2.48 of the European pharmacopoeia, basic principles of Raman spectroscopy and its major pharmaceutical-related applications are reported.<sup>42</sup> A general description of the equipment used in Raman spectroscopy

(benchtop and handheld) and the spectrometer's performance evaluation in terms of wavenumber, relative intensity, and resolution are mentioned, referring to existing procedures, standards, or reference materials like ASTM 1840 and NIST SRMs. Information and recommendation on qualitative and quantitative determination methods and sample preparation are also available. EP suggests cyclohexane, polystyrene as a 76 μm film, rod, or pellet (NIST 706a), and N-(4-hydroxyphenyl)ethanamide (the IUPAC name for paracetamol/acetaminophen) as reference materials for wavenumber calibration. Raman shifts and tolerances for polystyrene, paracetamol, and cyclohexane are available for benchtop and handheld Raman instruments. A revised Raman spectroscopy chapter 2.2.48 was approved in 2021 by the European Pharmacopoeia Commission, with updates on the response-intensity scale as well as new procedures for spectral resolution assessment with calcite, according to ATSM E2529, and for spectra comparison.

#### United States Pharmacopoeia

The USP Raman chapters 858 (Raman Spectroscopy) and 1858 (Raman Spectroscopy—Theory and Practice) of the 43rd edition released in 2020 replaced the general chapter 1120 (Raman Spectroscopy) of the previous edition.<sup>43,44</sup>

According to USP chapter 858, the qualification of Raman spectrometers is divided into three components: installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). This chapter provides acceptance criteria for wavelength accuracy and photometric precision for OQ. Polystyrene, cyclohexane, and N-(4-hydroxyphenyl)ethanamide are proposed for the determination of the wavelength accuracy of the Raman instrument. Contrary to other pharmacopoeias, the Raman shifts and tolerances of the proposed materials differ for quantitative and qualitative applications. The acceptance criteria for qualitative applications are broader than the values set by the European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP). The

**Table VI.** Raman shift values for the prominent peaks of cyclohexane and polystyrene reference materials according to standards' technical specifications and materials' certificates.

RM	Raman shift (cm <sup>-1</sup> )							
	ASTM		NMIJ RM8158-a	NIM		Pharmacopoeia		
	E1840	E1683		GBW13664	GBW13653	United States <sup>a</sup>	European <sup>b</sup>	Japanese <sup>c</sup>
Cyclohexane	384.1 ± 0.78	384.1		384.1 ± 2.3				–
	426.3 ± 0.41			426.5 ± 2.4				
	801.3 ± 0.96	801.3		801.9 ± 2.4		801.3 ± 2.5		
	1028.3 ± 0.45			1028.1 ± 2.2		1028.3 ± 2.0		
	1157.6 ± 0.94			1157.6 ± 2.4				
	1266.4 ± 0.58			1266.4 ± 2.3		1266.4 ± 2.0		
	1444.4 ± 0.30	1444.4		1444.2 ± 2.2		1444.4 ± 2.5		
	2664.4 ± 0.42			2664.2 ± 2.2				
	2852.9 ± 0.32	2852.9		2852.4 ± 2.2		2852.9 ± 3.0		
	2923.8 ± 0.36			2923.4 ± 2.2				
			2937.5 ± 2.2					
Polystyrene	620.9 ± 0.69	–	620.7 ± 1.2	621.2 ± 2.1		620.9 ± 3	620.9 ± 2.5	620.9 ± 1.5
	795.8 ± 0.78		795.1 ± 1.2	795.5 ± 2.2				
	1001.4 ± 0.54		1001.2 ± 1.2	1001.0 ± 2.1		1001.4 ± 3	1001.4 ± 2.0	1001.4 ± 1.5
	1031.8 ± 0.43		1031.5 ± 1.2	1031.2 ± 2.1		1031.8 ± 3	1031.8 ± 2.0	1031.8 ± 1.5
	1155.3 ± 0.56		1154.9 ± 1.2	1154.6 ± 2.3				
	1450.5 ± 0.56		1448.4 ± 1.2	1449.0 ± 2.1				
	1583.1 ± 0.86		1582.7 ± 1.2	1583.2 ± 2.2				
	1602.3 ± 0.73		1602.1 ± 1.2	1602.4 ± 2.1		1602.3 ± 1.5	1602.3 ± 3.0	1602.3 ± 1.5
	2852.4 ± 0.89		2851.0 ± 1.1	2851.3 ± 2.3				
	2904.5 ± 1.22		2906.2 ± 1.2	2907.5 ± 2.5				
	3054.3 ± 1.36		3055.1 ± 1.1	3055.7 ± 2.4		3054.3 ± 3.0	3054.3	3054.3 ± 3.0

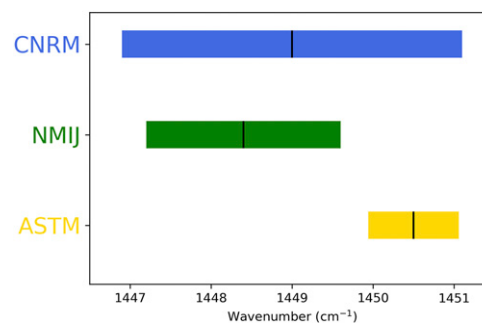
<sup>a</sup>Tolerances for quantitative measurement. For qualitative measurements are the same as JP.

<sup>b</sup>Tolerances for hand-held instruments. Tolerances for benchtop instruments are the same as JP.

<sup>c</sup>Macroscopic, microscopic, and probe measurements.

tolerance for the photometric precision of the measurements of reference materials is set to 10%. The performance validation is estimated by relative intensity precision calculated for the polystyrene peaks using the peak at 1001.4 cm<sup>-1</sup> as the reference. The peak intensity ratios for the other three polystyrene peaks (620.9 cm<sup>-1</sup>, 1031.8 cm<sup>-1</sup>, and 1602.3 cm<sup>-1</sup>) are calculated against the reference peak.

After a short introduction to Raman spectroscopy theory and sampling factors, informational chapter 1858 of USP addresses the qualitative and quantitative measurements in Raman. Description of Raman apparatus/equipment is more detailed than in EP. A section describing specialized Raman-based techniques is included, and the lasers used in pharmaceutical applications are summarized in a table. Information and guidelines on calibration are provided, specifically on primary wavelength (x-axis), laser wavelength, and intensity (y-axis). The chapter also indicates sample-based factors that affect measurement performance (fluorescence, sample heating, and effect of oriented samples in laser polarized radiation) and provides information on Raman spectrometers verification as well as procedure for ongoing performance validation, highlighting possible factors that would require the qualitative model process revalidation and improvement activities (outliers and method transfer).



**Figure 1.** Variation of the position and tolerance values given for the 1450 cm<sup>-1</sup> peak of polystyrene in the NIM GBW13664 and NMIJ RM8158a certificates and in ASTM E1840.

### Japanese Pharmacopoeia

In 2016, the Pharmaceuticals and Medical Devices Agency published the Japanese Pharmacopoeia 17th edition (JP17), which has been recently replaced by the 18th edition (JP18), published in June 2021 and not translated into English yet.<sup>45</sup> The note 2.26 included in Supplement II to JP17 New General Tests provides a general description of Raman spectroscopy and Raman apparatus's main components (light source, sample

**Table VII.** Stability of reference materials certified by NIST (U.S.A.), NIM (China) and NMIJ (Japan).

CRM type	Certificate validity	Storage
NIST SRM 2241	1 year	in the container provided
NIST SRM 2242a	5 years	in dry conditions
NIST SRM 2243	5 years	in the container provided
NIST SRM 2244	10 years	in the container provided stored in a desiccator
NIST SRM 2245	5 years	in the container provided
NIST SRM 2246	7 years	in the container provided
NIM GBW13650	1 year	in the dark at room temperature
NIST SRM706a	5 years	in the container provided
NIM GBW13651	1 year	in the dark at room temperature (20–25 °C)
NIM GBW13652	1 year	in the dark at room temperature (20–25 °C)
NIM GBW13653	1 year	in the dark at room temperature (20–25 °C)
NIM GBW13654	1 year	in the dark at room temperature (20–25 °C)
NIM GBW13664	1 year	in the dark at room temperature (20–25 °C)
NMIJ RM8158-a	1 year	in the dark at 15–25 C. aluminum-laminated container provided
NMIJ CRM 5606-a	1 year	at temperature between 15–35 °C, away from radiation

unit, spectroscopy unit, and detector). The note also proposes polystyrene as a reference material for evaluating the instrument's performance. The permissible deviation depends on the type of measurement method. Three methods are reported: macroscopic, microscopic, and in situ measurements with probe. The parameters affecting the Raman spectra are identified such as sample fluorescence and color. To avoid sample alteration by laser irradiation, it is suggested to reduce laser output, irradiate without focusing, and cooling the sample. Furthermore, in the case of measuring samples in containers, these materials' spectral characteristics should be considered. Information on qualitative and quantitative analysis is also provided. Qualitative analysis of a sample can be performed using a reference material if both spectra exhibit similar scattering intensities at the same Raman shifts, while quantitative analysis can be performed following chemometric methodologies for obtaining a calibration model.

### Chinese Pharmacopoeia

Chinese Pharmacopoeia editions of 2020 and 2015 include information on Raman spectroscopy.<sup>46</sup> Those chapters provide information on the Raman instrument, the calibration, verification, and measurement methods, as well as the qualitative identification and content determination (and factors affecting quantitative determination). The Chinese Pharmacopoeia also provides common lasers used for pharmaceutical applications or Raman spectrometry. This information is identical to the one reported by USP.

Regarding the calibration of Raman instruments, wavelength (*x*-axis), laser wavelength, and intensity (*y*-axis) are mentioned. It is advised that users should set out the specific standard operating procedure (SOP) based on the calibration methods provided by the supplier and verify the parameters following the SOP. It is recommended to use an external

reference standard for regular wavelength calibration. The wavenumber accuracy should be adapted to the spectral resolution that is required for the sample collection. Desktop, portable, and handheld instruments have different requirements. Furthermore, due to the fluctuation of the absolute intensity of the Raman signal, internal standards should be used as much as possible. An internal standard can be added purposefully. The internal standard method is suggested to be used for quantification. Under laser irradiation, the internal standard generates Raman bands, one can be selected as a reference to compare the Raman band intensities of the sample. The reference substance and the tested material are required to be measured under the same laser intensity and frequency, under the same physical state (like liquid or solid), and at the same concentration range. Since the internal standard and the sample are measured under the same experimental conditions, some influencing factors can be counteracted. The requirements of the internal standard to be used are described: chemically stable to not react with the measured components or other components in the sample, non-interfering Raman bands, high purity, and without tested components or other interfering components. For aqueous solutions, nitrate ion is referred to as a commonly used internal standard. Perchlorate was also mentioned in 2015 edition of the CP, but not in 2020. Similarly, for non-aqueous solutions, carbon tetrachloride was proposed in 2015 CP, as in ASTM E1683, but eco-friendly and non-hazardous materials are requested in 2020 CP. For solid samples, a Raman band from the sample can also be used as internal standard band.

The CP identifies spectral contamination as an important factor in the sample determination, as Raman is a rather weak effect that can be masked by many external influences. Common sources of contamination include the sample support, container or matrix, and ambient light. These problems can be generally identified and solved through meticulous

experimental methods. Furthermore, factors that affect quantitative determination such as fluorescence, thermal effects, and self-absorption of sample and matrix are also described.

### Certified (Standard) Reference Materials

The certified reference materials that are available for Raman spectroscopy are reviewed in this section. The first certified reference materials for relative intensity correction were developed by NIST. These materials are six metal-doped borate or borosilicate glasses used for different laser wavelengths varying from 488 nm to 1064 nm and are presented in Table III. Additional CRMs for relative intensity correction have been developed by the National Institute of Metrology of China (NIM) applicable for 514 nm laser excitation (Table IV). Furthermore, four Raman-shifted CRMs developed by NIST, NIM, and the National Metrology Institute of Japan (NMIJ) are currently available in the market. The characteristics of all these certified reference materials are described and compared in the following sections.

Metrologically valid procedures for the production and certification of reference materials are given in, among others, ISO 17034:2016,<sup>47</sup> ISO Guides 35:2017,<sup>48</sup> and JJF 1343-2012<sup>49</sup> (the Chinese equivalent to ISO Guide 35). A number of companies supply reference materials for Raman spectroscopy and follow the relevant standards, mostly ASTM, to validate them. The most common materials are pen lamps (Ne, Ar, Hg, etc.), polystyrene, calcite, Si, and solvent mixtures. For instance, Ne lamps are available from Ocean Insight,<sup>50</sup> Avantes,<sup>51</sup> B&W Tek,<sup>52</sup> ELODIZ,<sup>53</sup> among others; Si is offered as an internal reference in devices from manufacturers such as Renishaw or Horiba or as an external standard by ELODIZ; white light source for intensity correction is available by several manufacturers. This review does not include a comprehensive list of these commercial samples.

The calibration pen lamps emit discrete spectral lines at wavelengths which are traceable in the NIST atomic spectra database.<sup>54</sup> This database provides information (such as absolute wavelength, uncertainty, and relative intensity) on the spectral lines generated by a selected element, which includes lines which are not detected by Raman spectrometers either due to resolution or sensitivity constraints. This can make line attribution difficult and subject to user error. Lines for argon, krypton, and xenon are reported in ASTM E2529 for a 785 nm Raman instrument; however, their purpose is not x-axis calibration, they are used for spectrometer resolution assessment.

#### NIST SRMs 2241-2246 for Relative Intensity Correction (Optical Glasses)

Included in Table III for relative intensity correction of Raman spectra, ASTM E2911-13 refers to the NIST-certified reference materials (termed by NIST as SRMs). The

development of the CRMs for intensity correction referred to in this ASTM standard resulted from an extensive work led by McCreery's and Choquette's research groups. The former considered the use of fluorophores that produce broadband emission when excited by 514.5 or 785 nm light, coumarin 540a and Kopp 2412 standard luminescent glasses, respectively.<sup>55</sup> The latter also worked on the development and certification of NIST reference materials.<sup>56,57</sup> The CRMs are designed for Raman spectrometers utilizing a 180° backscatter sampling geometry. However, Schloesser et al. investigated if and how SRM 2242 (532 nm) can be employed in a 90° scattering geometry.<sup>25</sup> Relative intensity correction of the measured Raman spectra of the samples is achieved by a computational procedure that uses a correction curve. This curve is generated using the model included in the material certificate and the measured luminescence spectrum of the glass.

#### NIM GBW13650 for Relative Intensity Correction (Zinc Borosilicate Optical Glass)

A relative intensity correction standard for 514.5 nm for Raman spectroscopy developed by the National Institute of Metrology of China to guarantee the accuracy and comparability of relative intensity between 400 cm<sup>-1</sup> and 4000 cm<sup>-1</sup> is GBW13650.<sup>64</sup> This CRM is a zinc borosilicate optical glass that was prepared by a melting process. The sample is cut to size 30 mm × 10 mm × 1 mm in a sample holder. The specific characteristic standard values of Raman spectra and uncertainty (Table IV) are provided in the certificate of the standard.

The sample is suitable for the calibration of the relative intensity of the Raman spectrometer with an excitation wavelength of 514.5 nm, using an 1800 g/mm grating and a 100× objective lens (numeric aperture = 0.9), no further configuration is reported. Moreover, it is difficult to buy from Europe. Consequently, we cannot verify if this standard can be used with deviation of these conditions. The background noise of the detector needs to be deducted when the sample is tested. The sample can be reused without damage. This standard material is a glass product that should be handled with care during use and transportation to avoid scratches caused by broken glass. It should be protected from light and preserved at room temperature (20–25 °C). The certification limits of GBW 13650 are between 400 cm<sup>-1</sup> and 4000 cm<sup>-1</sup>, while the polynomial expression of the relevant NIST SRM 2243 is certified for use between 200 and 4800 cm<sup>-1</sup>.

#### NIM GBWs 13651-1364 for Raman Shift Calibration (Sulfur, Naphthalene, Cyclohexane, and N-(4-hydroxyphenyl)ethanamide)

The Raman shift values of these certified reference materials supplied by the National Institute of Metrology of China in small, brown glass bottles are displayed in Table V.

GBWI3651<sup>65</sup> contains 0.5 g of commercial trace metal grade (99.998%) sulfur. This reference material is used for the Raman shift calibration in the range between 83  $\text{cm}^{-1}$  and 473  $\text{cm}^{-1}$ . GBWI3652<sup>66</sup> is prepared from commercial fluorescence quenching level (99%) naphthalene (0.25 g). It is complementary to sulfur, as it covers the Raman shift range between 513  $\text{cm}^{-1}$  and 3055  $\text{cm}^{-1}$ . GBWI3653<sup>67</sup> is prepared in ampoules with 1.5 g of commercial high-performance liquid chromatography grade (99.9%) cyclohexane. It can be used for the Raman shift calibration in the range from 384  $\text{cm}^{-1}$  to 2937  $\text{cm}^{-1}$ . GBWI3654<sup>68</sup> bottles contain 0.15 commercial biological grade (99.0%) N-(4-hydroxyphenyl)ethanamide (IUPAC's name for 4-acetamidothiophenol) and can be used between 214  $\text{cm}^{-1}$  and 3324  $\text{cm}^{-1}$ . It presents the highest amount of peaks among the NIM CRMs for Raman shift calibration. As cyclohexane, its use is proposed in the EP.

### NMIJ CRM 5606-a (Silicon)

Despite the widespread use of Si for calibration in Raman, as external and even internal reference sample, no Si reference material is certified for Raman. NMIJ CRM 5606-a is a non-doped single-crystal silicon substrate with crystal orientation of  $[100] \pm 1$  developed by the National Metrology Institute of Japan for positron defect measurements and certified as reference material.<sup>69</sup> As it has not been developed for Raman spectroscopy, the certificate does not include the Raman shift values. These have been recently evaluated by Itoh and Shirono,<sup>70</sup> who applied the bracket calibration method to study the uncertainty and validate the Raman shift measurements of NMIJ CRM 5606-a using the wavenumber of the closest Ne emission lines of an unstabilized He–Ne laser. They estimated the Raman shift of this Si substrate to be  $520.45 \pm 0.28 \text{ cm}^{-1}$ . The Raman measurements are performed on the mirror-finished side of the 1 mm thick 15 mm  $\times$  15 mm Si crystal.

### Certified Reference Materials for Wavenumber Calibration (Polystyrene)

There are three certified polystyrene reference materials, developed by NIST, NIM, and NMJI. Their bands are compared in Table VI.

A polystyrene CRM of broad molecular mass distribution consisting of pellets with approximately 80 mg weight, NIST SRM 706a,<sup>71</sup> is usually used in calibration and performance evaluation of instruments to determine the molar mass and molar mass distribution. This material was not developed for use as a Raman shift standard but might be used for wavenumber calibration in Raman spectrometers, as suggested by the European pharmacopoeia (Table 2.2.48-I note B). However, NIST has not evaluated nor endorsed it for this purpose, and Raman shift values are not provided in the certificate. The values provided by the EP are those of ASTM I840 (see Table VI).

NIM GBWI3664<sup>72</sup> is a polystyrene CRM developed by the National Institute of Metrology of China. It is a standard used for spectrometer Raman shift calibration in the wavenumber range of 621  $\text{cm}^{-1}$  to 3056  $\text{cm}^{-1}$ . Expanded uncertainty of each Raman shift value is also provided for coverage factor  $k=2$ , defining an interval with a confidence level of approximately 95%. Polystyrene CRM pellets are produced via monomer polymerization and have a 10 mm diameter. Information on pellet thickness is not provided.

NMIJ RM8158-a<sup>73</sup> is a polystyrene reference material intended for Raman spectrometer validation recently developed by the National Metrology Institute of Japan.<sup>74</sup> It is a colorless disk with a diameter of 25 mm and a thickness of 4 mm. The disk was prepared by injection-molding of polystyrene pellets and was further surface-treated. One side of the disk has a mirror finish, while the other side has a rough surface. The measurements are performed on the mirror-finished side. The Raman shift values of the polystyrene material were determined by a Raman spectrometer calibrated using Ne and Ar emission lines. The indicative Raman shift values (11 peaks) and the uncertainty values of the material are provided in the RM report. The uncertainty of the indicative values was calculated using a coverage factor ( $k$ ) of two (level of confidence 95%) from the half width of the expanded uncertainty interval.

### Comparison of Cyclohexane and Polystyrene Raman Shift Values

Table VI shows the Raman shift values and their tolerances for the most prominent peaks of cyclohexane and polystyrene according to the ASTM standards and the available reference materials certified by metrology institutes. The table also includes the values provided in the pharmacopoeias, which are essentially the Raman shift values from the corresponding ASTM standards but with higher tolerances. For polystyrene, EP reports different tolerances for benchtop and handheld instruments, while USP distinguishes between qualitative and quantitative measurements instead. The tolerances of JP are the same as those for USP-quantitative and EP-benchtop. The certified Raman shift values of the Chinese NIM GBWI3664 and the Japanese NMIJ RM8158a polystyrene reference materials have some deviations and higher tolerances with respect to the ASTM E1840, being the highest tolerances those reported for the Chinese standard. As seen in Fig. 1 for the 1450  $\text{cm}^{-1}$  peak of polystyrene, there is a clear difference between the NMIJ and the ASTM tolerances. This is possibly due to the different methods of peak fitting performed for this asymmetric peak. The ASTM does not explain the peak fitting procedure employed, whereas the NMIJ certificate specifically notes that the peaks were all fit to symmetric peaks. For cyclohexane, Raman shift values reported by EP follow ASTM E1683 and ASTM E1840. Tolerance values provided by ASTM E1840 for cyclohexane, however, differ from the values



**Table VIII.** Documents of the IEC technical specification 62607 (Nanomanufacturing – Key Control Characteristics) part 6 (graphene-based materials) related to Raman spectroscopy.

Standard	Title/description
IEC TS 62607-6-6 <sup>77</sup>	Part 6-6: Graphene-based materials - Strain uniformity: spatially-resolved Raman spectroscopy
IEC TS 62607-6-11 <sup>78</sup>	Part 6-11: Graphene-based materials - Defect density: Raman spectroscopy
PNW TS 113-570 ED1 <sup>79</sup>	Part 6-12: Graphene-based materials - Number of layers: Raman spectroscopy, optical reflection
IEC TS 62607-6-14 <sup>80</sup>	Part 6-14: Graphene-based materials - Defect level: Raman spectroscopy
PWI 113-131 IEC TS 62607-6-28	Part 6-28: Graphene-based materials - Number of layers: Raman spectroscopy
PNW TS 113-580 ED1 <sup>81</sup>	Part 6-29: Graphene-based materials - Defectiveness: Raman spectroscopy

provided by EP, which are higher and in line with GBW13653. The certificate of this CRM provides similar Raman shift values to those reported by ASTM but includes an additional peak.

### Availability, Durability, and Stability of Certified Reference Materials

The CRMs availability is variable and not guaranteed and their cost is generally high. Besides, the certificates of the CRMs are only in the language of the issuing national metrology institute. All this hinders their widespread application.

The long-term stability and durability of CRM are of great importance as these materials should not be exposed to high temperature, light, oxygen, humidity, etc, because this can lead to their degradation. Information on controlled storage conditions and short or long-term stability of the materials is necessary, and thus is supplied by the certificates issued by the provider. Table VII compiles the information about the stability and storage conditions of the CRMs according to their certificates. All of them must be stored in the dark or a container.

Regarding the certified reference materials for intensity calibration, the homogeneity and 12-month stability of the zinc borosilicate GBW13650 was studied using 20 random samples and three positions per sample according to the technical specification JJF1343-2012.<sup>49</sup> The available online certificate was issued in April 2018 and expired in April 2019. Regarding the certified reference materials for Raman shift calibration, the certificate of the SRMs developed by NIST does not include specific indication about homogeneity or stability tests; 2241 is out of stock, but 2241a should be available in 2022 with an anticipated stability of five years; 2242a is certified until December 2024; 2243 is discontinued since 2014; 2245 is certified until September 2021; and 2246, although certified until September 2022, is out of stock. The certificate of GWBs provided by NIM indicates the homogeneity and stability was studied following JJF1343-2012.

Regarding polystyrene CRMs that may be used for wavenumber calibration, they should be stored in the dark at room temperatures. NIM GBW13664 polystyrene has a valid certificate until January 2021. Homogeneity and stability

assessment is performed according to JJF1343-2012. NMIJ RM8158-a has a valid certificate for a year from the day of shipping. The homogeneity and stability of the CRM were determined by analyzing 10 disks selected out of 1900 by the stratified random sampling method. Storage of the NMIJ RM8158-a is recommended in temperature between 15 and 25 °C protected from light in the aluminum-laminated plastic bag that it has been shipped. NIST 706a polystyrene recommended storage is in the original bottle with the lid tightly closed under normal laboratory conditions and its homogeneity was tested using size exclusion chromatography analysis. Although certification of this polystyrene material is valid for five years (issued in January 2021, valid until January 2026)<sup>75</sup> it does not include the Raman shift values.

The same happens for NMIJ CRM 5606-a, a non-doped crystalline Si certified for a year from the day of shipping, but not including the Raman shift values. Its homogeneity and stability were determined by analyzing 32 pieces sampled from 120 plates cut from five single crystal silicon wafers. Storage of the NMIJ CRM 5606-a is recommended at temperatures between 15 and 35 °C and away from any radiation source.

### Standards, Technical Specifications (TS), and Technical Reports (TR) by the International Electrotechnical Commission

The IEC is an organization for preparing and publishing international standards for all electrical, electronic, and related technologies. The majority of the existing IEC technical reports and specifications relevant here have been prepared by SC 86C “Fiber Optic Systems and Active Devices” of IEC/TC 86 “Fiber Optics” and are addressing topics associated with processes (IEC TS 61290-6), performance testing (IEC TS 61290-10:5, IEC TR 62324), and safe use (IEC TS 61292-4) of optical components in Raman spectroscopy. These topics are related to telecommunication systems and are out of the scope of this review, as the focus is on existing standards dealing with calibration procedures, instrument performance, validation, data formats, and model transfer related to Raman spectroscopy characterization. However, a description of IEC

60825-2:2021 on safety of laser products such as Raman amplifiers is given below.

### *IEC 60825-2:2021 Safety of Optical Fiber Communication Systems*

Technical Committee 76 (Optical Radiation Safety and Laser Equipment) has prepared this technical standard, the second part of IEC 60825 (safety of laser products).<sup>76</sup> It provides requirements and specific guidance for the safe operation and maintenance of optical fiber communication systems (OFCs), including information on proper labeling and marking of the hazard levels in the locations of the system components and subassemblies (lasers and amplifiers) intended to permit access to radiation when opened. For connectors of optical transmitters and optical amplifiers such as input ports of Raman amplifiers, the wavelength range should also be marked. Raman amplifiers should comply with the class IM hazard level. Information on the automatic power reduction (APR) function, applicable in the case of a fiber discontinuity, and the disabling of this function is provided. Especially for Class 3B laser products, common in Raman spectrometers, specific conditions should meet for disabling APR. Safety precautions in locations with hazard levels IM and 3B relevant to Raman spectrometers are also described.<sup>76</sup>

### **Standards for Specific Raman Applications**

In this section, standards and practices describing the use of Raman spectroscopy in several applications are presented. Some standards are currently available: two by ASTM regarding microplastics in water and natural gas and one IEC technical specification on graphene. Two more standards are developed by ISO regarding the use of Raman spectroscopy to characterize graphene-based materials, nano-objects, and the vocabulary related to it. Several more standards that are currently under development to assess graphene's defectiveness, number of layers, and structural characteristics. They are currently available as working documents of the ISO/IEC TC 113 (Nanotechnology for electrotechnical products and systems).

#### *ASTM D8333–20 Water samples preparation for microplastics analysis*

The "Practice for Preparation of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers Using Raman Spectroscopy, IR Spectroscopy, or Pyrolysis-GC/MS" provides information on sample preparation of collected water samples with suspended solids to determine the presence, count, polymer type, and physical characteristics of microplastic particles and fibers are reported.<sup>10</sup> The experimental procedure to be followed can be summarized in two basic processes: oxidation and digestion to remove inorganic

and organic background material. After proper treatment and centrifugation, the sediment of microplastic material is obtained and can be analyzed. The sample is transferred in a microscope glass slide. Raman spectroscopy is used for quantitative analysis.

#### *Natural Gas*

*ASTM D7940–14.* This standard Practice for Analysis of Liquefied Natural Gas (LNG) by Fiber-Coupled Raman Spectroscopy is for both online and laboratory determination of liquefied natural gas composition, but it can also be applied to other light hydrocarbon mixtures in either liquid or gaseous phase.<sup>16</sup>

Terms defined in this standard related to Raman spectroscopy are described in the Standards and Terminology section of this paper. Regarding the number of Raman-scattered photons reaching the CCD (signal strength), it is highlighted that some scaled combination of natural areas of peaks from the compound is expected for LNG.

The standard indicates that Raman spectroscopy can be directly applied to measure LNG and determine the volume fractions of individual molecular species in a liquid stream of LNG. Information is provided regarding sampling, test specimens, and test units. The main components of the Raman apparatus are described; the specifications and optimal settings are also included. Unlike the ASTM standards described in the previous chapters, ASTM D7940 has a separate section dedicated to the preparation of Raman apparatus before measuring. This section includes indications on laser operation settings (temperature and warm-up time), spectral acquisition time, or optimal temperature of the CCD detectors, as well as an outline for calibration of Raman spectrometers and ongoing performance and field validation of Raman instruments using reference materials in gravimetrically established mixtures. This standard does not directly refer to the guides for x- and y-axis calibration commented in the Standards and Guidelines on Calibration section. The standard highlights the need of periodical wavelength and intensity axis calibration and suggests checking the detection module for changes in intensity calibration by comparing neon peak heights ratios.

The obtained Raman spectrum represents a histogram charting the number of photons detected at each wavelength, corresponding to the number of molecules with particular vibration frequencies. The spectra can be mathematically processed to yield the molecular composition of the liquid. Cosmic ray, thermally generated electrons, and gas-phase presence in LNG are factors that interfere and can alternate the Raman analysis/spectra of LNG.

Contamination of the probe window or fiber connections, or physical damage of the fiber or probe, can lead to a drop in signal or a large rise in the spectra's baseline background. Because of the sample's nature (for LNG it is not practical to create and ship identical liquid cryogenic reference samples to multiple locations for testing), an interlaboratory study is not feasible. Precision (repeatability and reproducibility), accuracy, and bias of the method are discussed.

ISO 23978. While ASTM D7940 deals with the determination of natural gas composition both in liquid and gaseous states, ISO 23978 (Natural gas - Upstream Area—Determination of Composition by Laser Raman Spectroscopy) provides guidelines for the determination of just gas fractions, indicating that it is a simpler and more direct method than gas chromatography.<sup>21</sup> Raman spectroscopy related definitions used in this standard are included in Table I.

The Raman analyzer and its working principle as well as laser specifications and measuring conditions are described. The detection module for measuring the gas sample can be external cavity or intracavity type (detector with focusing lens, optical filter, and APD). The standard indicates that the Raman analyzer's zero level calibration should be performed under specific conditions using reference standard gas mixtures according to ISO 6142-1, ISO 6144, or ISO 6145 and the procedure is described in detail. The equation for calculating the gas composition and concentration of natural gas is provided. The gas components that can be detected are propane (C<sub>3</sub>H<sub>8</sub>), ethane (C<sub>2</sub>H<sub>6</sub>), methane (CH<sub>4</sub>), hydrogen sulphide (H<sub>2</sub>S), carbon dioxide (CO<sub>2</sub>), and nitrogen (N<sub>2</sub>). Information on sample identification and composition, calibration, stability, and response of the measuring system, as well as repeatability and uncertainty of the results must be included in the test report. The statistical procedure for estimating the repeatability is available in the Annex of this standard.<sup>21</sup>

## Graphene

IEC TS 62607-6. The part 6 of the IEC TS 62607 series regarding Nanomanufacturing-Key control characteristics, prepared by IEC technical committee 113: Nanotechnology for electrotechnical products and systems, is devoted to graphene-based materials. The list of the technical specifications specific to Raman is listed in Table VIII. Number 6<sup>77</sup>, 11<sup>78</sup> and 14<sup>81</sup> are published while number 12<sup>79</sup>, 28 and 29<sup>82</sup> are under development.

IEC TS 62607-6-6:2021 (E) specifies how to determine the strain uniformity parameter for single-layer graphene using the width of the 2D Raman peak for structural control.

IEC TS 62607-6-11:2022 specifies how to determine the defect density of films grown by chemical vapour deposition (CVD) and of exfoliated flakes.

IEC TS 62607-6-14:2020 specifies how to evaluate the defect level. Film and powder samples exhibit different Raman spectra.

- (i) For films, graphene defects are estimated via D bands ( $I_D/I_G$ ).
- (ii) For powdered samples, the intensity ratio of D+D' and 2D bands ( $I_{D+D'}/I_{2D}$ ) is more suitable for estimating the defect level of graphene.

Graphene powder must be placed on metallic, glass, or silicon wafer substrate and pressed with a glass slide to obtain

a compact graphene tablet. Information on the sampling method to be applied is provided. The documentation reporting the measurements must contain information about the calibration status of the device, the spectral resolution of the spectrometer, the wavelength, spot size, and power of the laser used, and the signal-to-noise ratio for the Raman spectra, together with the intensity ratios of the of D+D' band and 2D band should be provided. If the sampling plan contains several measurement spots, then the measured area must be defined, while the histogram of the 2D band widths in the scan area must be included and the (2D-FWHM; 80%) values reported. Following the removal of the signals from the cosmic rays, mean values of  $I_{D+D'}/I_{2D}$  are calculated as the sample's defect level. The lower the ratio  $I_{D+D'}/I_{2D}$ , the lower the defect level.

ISO/TS 21356. The first part, ISO/TS 21356-1 Nanotechnologies—Structural characterization of graphene, published in 2021, deals with graphene from powders and dispersions.<sup>82</sup> It establishes methods for structural characterization of graphene, bilayer graphene, graphene nanoplatelets, and graphite particles isolated from powders and/or liquid dispersions using optical microscopy, SEM, transmission electron microscopy (TEM), AFM, and Raman spectroscopy. For example, Raman spectra of highly oriented pyrolytic graphite (HOPG), graphene, graphene oxide (GO), reduced graphene oxide with lower oxygen content [rGO(L)], and higher oxygen content [rGO(H)] are reported in this standard. The second part, ISO/PWI TS 21356-2, is currently under development and focuses on chemical vapor deposition (CVD) grown graphene.

## Nano-object characterisation: ISO/TR 18196:2016

The ISO/TR 18196:2016 Nanotechnologies—Measurement Technique Matrix for the Characterization of Nano-Objects, published in 2016, provides a matrix that guides users to commercially available techniques relevant to the measurements of common physiochemical parameters for nano-objects. According to this standard, the techniques listed in this document have not been validated through round-robin testing or any other means for measuring nano-objects. The clause dedicated to Raman spectroscopy and Raman imaging describes the technique and provides information on the advantages, limitations, and relevant standards on Raman spectroscopy.<sup>83</sup>

## Sampling Factors Affecting Raman Measurements

Raman spectroscopy is considered as a vibrational technique with advantages such as little or no sample preparation and direct and nondestructive analysis. Nevertheless, those advantages do not apply to some Raman spectroscopy techniques or even in some samples. Therefore, sample

characteristics and sampling factors that could possibly affect the Raman measurements should be taken in consideration for the development of standards in specific Raman applications. The purpose of this section is to point to these factors.

Sampling methods and sample preparation guidelines are reported in some of the ASTM and IEC standards dealing with specific Raman applications (e.g., ASTM D8333–20), as well as in the chapter 2.2.48 of the EP described in the above sections. Sample characteristics and sampling factors that could possibly affect the Raman measurements should be taken in consideration for the development of standards in specific Raman applications. Furthermore, chapter 858 of the US pharmacopeia points out that sample preparation is critical for both solids and liquids; this must be controlled or accounted for in the calibration model. Hopefully, all this will be better covered in the standardization landscape of the near future. Sample-position sensitivity can often be minimized by appropriate sample preparation or sample holder geometry but will depend on the instrument as well as the excitation and collection optical configuration.

Some parameters are not included in any standard but have been described to have some level of impact in the measurements, calibrations, and output obtained with the Raman units. Particle size is a parameter to be considered in Raman spectroscopy. Small particles scatter the laser excitation line preventing the deep penetration of the light into the sample and hence reducing the scattering volume. The influence of particle size on the intensity and reproducibility of Raman spectra of compacted samples was studied by Gomez et al. using macro- and micro-Raman systems.<sup>84</sup> Compacted samples of potassium hydrogen phthalate of different particle size imitating pharmaceutical samples were examined. Raman intensity was affected by both the particle size and the tablet width up to a certain value. Several groups have investigated the crystal size effect on Raman spectra in powdered samples. The results of these studies do not appear consistent. For micron-sized crystals, Raman intensity tends to increase when grain size decreases. However, Kristova et al.<sup>85</sup> reported the increase of the calcite Raman band intensity with the particle size up to 20  $\mu\text{m}$ , then the intensity decreased, while Chio et al.<sup>86</sup> reported the increase of the spectral intensity with the grain size up to a plateau for alpha quartz grains and aggregates. In the case of  $\text{TiO}_2$  nanopowders of small crystal grain sizes, broadening of the Raman band was also observed.<sup>87</sup>

Laser-induced heating of samples can lead to sample degradation (oxidation, decomposition, etc.). Boettger et al. studied the laser-induced effects on silicon and rock forming minerals, reporting local temperature increase and relevant changes in Raman spectra (broadening and shifting of characteristic Raman lines in the Stokes and anti-Stokes spectral regions) for elementary crystalline silicon particles with sizes below 250  $\mu\text{m}$ .<sup>88</sup> The effect of local laser heating in the Raman spectrum of Si nanocrystals was also confirmed by Nikolenko.<sup>89</sup>

Confocal micro-Raman spectroscopy is used because of its increased optical and depth resolution. Sampling depth, especially in transparent materials, has a direct effect in lateral resolution. In their study, Presser et al.<sup>90</sup> report that the sampling depth of Raman microscopy in optically transparent materials, such as zirconia ceramics, depends on several factors related to the sample, such as optical density, Raman cross-section, porosity, or grain boundaries, and to instrumental parameters like spectrometer efficiency and magnification, wavelength, and laser power. The numerical aperture of the objective and the wavelength of the laser significantly influence the sampled material volume; by increasing the numerical aperture at a low wavelength, however, the sampling depth decreases only to a certain degree.

We have identified a lack of standardization in this area, there are few existing studies that correlate laser spot size with power density and Raman signature. These studies are focused on carbon nanostructures which can be prone to heating due to laser irradiation.<sup>91</sup> More study is required into the effect the power density, both by area and volume for certain materials, has on the Raman shift peak positions in materials and if that effect is only due to thermal deformation or another effect.

## Multivariate Analysis Standards

Due to the ability for standoff analysis and its nondestructive nature, Raman spectroscopy is being adopted more and more as a process analytical technology. In this role, Raman spectra are predicted or classified by multivariate data analysis (MVDA) techniques as a quality control. Moreover, multivariate models can be used to validate the performance of a Raman instrument. These validation models ensure that any degradation over time is corrected so that the results obtained from an instrument are easily reproducible.<sup>92</sup> The need for multivariate qualification and validation protocols to be established is clearly reflected by the growing number of standards and guidelines developed over the last years. Information on validation procedures, test methods, and instrument performance are already available for Raman spectroscopy in some of the technical reports and practices described in this document. More specifically, the following subsections analyze ASTMs E2056 (qualification), D6122 (MVDA for liquid petroleum products and fuels), E2891 (MVDA for pharmaceuticals), and E2617 (empirical calibration), which deal with the use of multivariate analysis for the qualification of spectrometers and the validation of calibration. The multivariate techniques within the standards are limited to partial least squares (specifically the PLS-I algorithm), PCR, or multilinear regression (MLR). Besides, EP (chapter 5.21) and USP (chapter 1039) have both a specific chapter dedicated to chemometric methods applied in data analysis. ASTM 1866 covers multivariate measurements for spectrophotometer performance test.\*

### *ASTM E2056-04 (2016) Practice for Qualifying Spectrometers and Spectrophotometers for Use in Multivariate Analyses, Calibrated Using Surrogate Mixtures*

This practice specifies calibration and qualification data set requirements for interlaboratory studies (ILSs), or round robins, of standard test methods specifically employing surrogate calibration techniques for mid-and near-infrared, ultraviolet–visible, fluorescence, and Raman spectroscopies.<sup>15</sup> Surrogate mixtures are “gravimetrically or volumetrically prepared mixtures which contain significantly fewer components than the samples that will ultimately be analysed.”<sup>15</sup> The error calculations for surrogate mixtures are slightly different than those used on models trained using actual samples, and thus have no relationship. Definitions on spectrometer/spectrophotometer qualification, surrogate calibration, and surrogate test method are reported, as they are specific to this standard. Specifications and requirements for surrogate test methods in ILSs are provided, as well as Raman spectrometer characteristics which should be specified, for example, allowable spectral bandwidth. It is also noted that data collection parameters and computational methods used should be specified.

### *ASTM E2617-10 for Empirically Derived Multivariate Calibrations*

ASTM E2617-10<sup>93</sup> indicates that the empirically derived multivariate calibration relies on a validation set. This validation set must contain sufficient examples of all the combinations of variable expected to be tested on the calibrated instrument. Furthermore, the range of variation in the validation set must also be equal to the range of variation tested on the calibrated instrument. These variables must be uniformly and mutually independent, and the number of samples must be statistically sufficient to test the between measured and modelled variables (recommended minimum of 20 validation samples in the set).

Equations are given for the standard error of validation (SEV) (aka standard error of prediction) and the standard deviation of validation residuals (SDV). These equations are altered based on the number of reference values and the number of estimate values (i.e., single reference value and estimate, multiple reference and single estimate, multiple of both). To determine if the SEV or SDV should be used a t-value must be calculated to determine the significance of validation bias.

Positive and negative fractions identified are defined (fraction of sample correct identified as either having or lacking the characteristic, respectively), but it is noted that their use is only applicable when the identification criteria is binary and is not applicable when the test has multiple outcomes.

The need for qualification of each measurement prior to application of the validated calibration can be performed using

several different techniques. Only two are explored in depth: nearest neighbour Mahalanobis distance (NNMD) and standard residual variance of the independent variables (SRVIV). The use of these two tests is described in conjunction with principal component analysis and partial least squares MVDA techniques.

Appendixed to this guide is an example of the protocols for validating pharmaceutical analytical methods, which gives the user an understanding of the actual use of the guide.

### *ASTM D6122-20a Validation of the Performance of Multivariate IR and Raman Spectrometer-Based Analyzer Systems*

This resource covers practices for the validation of the performance of multivariate online, at-line, field, and laboratory infrared spectrophotometer, and Raman spectrometer-based analyzer systems that covers requirements for the validation of measurements made by infrared (near- or mid-infrared analyzers, or both) and Raman analyzers used in the calculation of physical, chemical, or quality parameters of liquid petroleum products and fuels.<sup>11</sup> The requirements include verification of (i) instrument performance, (ii) applicability of the calibration model to the sample, and (iii) the uncertainties associated with the degree of agreement between the results calculated from the measurements and the primary test method (PTM) used for the development of the calibration model, which meet user requirements. The terminology used is defined or included in ASTM E131 (for IR and Raman spectroscopy), E1655 (for multivariate calibration), and D6299 and E456 (statistical quality control).

According to ASTM D6122, the validation operation of a laboratory or process stream analyzer system for prediction of a single physical, chemical, or quality property over the long term and their significance typically involve certain validation activities such as correlation, analyzer qualification, local, general, and continual validation. Apparatus and considerations for quantitative IR and Raman measurements are described. An analytical description of the validation procedure is presented: analyzer qualification (at initial startup, at restart, after maintenance, and after model update), process analyzer validation practice normal operations, validation tests, probationary local validation, continual local validation, general validation, and continual general validation. Performance tests for determining the instrument’s performance (spectrophotometer, optical cell, and all transfer optics in between) and adequacy to produce spectra of the quality sufficient for valid analyses are reported. These tests include the timing of analyzer performance tests and reference materials (single, pure, liquid hydrocarbon compounds or mixtures of liquid hydrocarbon compounds, or optical filters) for instrument performance tests. More information on instrument performance tests, especially reference materials, is given in this

standard's annexes. Annexes also include information on considerations for quantitative online process spectral measurements and outlier detection methods.

### *ASTM E2891-20 for Multivariate Data Analysis in Pharmaceutical Manufacturing Applications*

This guide covers the logic and considerations taken when creating an MVDA method for use in the pharmaceutical industry.<sup>93</sup> Within this method is the MVDA model, which takes the acquired data and turns it into an output, such as a validation report. ASTM E2891 does not provide guidelines on application-specific analysis procedures.

The concepts of MVDA model and method are presented. It is proposed that an assessment of the suitability for the use of data analysis, which is divided into three parts, should be made. First, the criteria for acceptable data analysis should be defined, though these will be user- and project-specific. Second, the data source should be appropriate and only relevant data should be used in the MVDA. Third, the data analysis practice (technique and procedure) should be outlined, as the data analysis will be iterative.

The data collection and diagnostic section are more specific to the pharmaceutical industry when it comes to the data collection aspect, though it does state that the data should be of acceptable quality (i.e., free of obvious errors), and the data should be reviewed with that in mind. The broad notions set out in the sections in pre-processing and outliers should be considered. Regarding pre-processing, the design intention is to enhance key features within the data and should not be designed to transform data that was originally unfit for purpose into usable data. An entire practice exists in ASTM (E178)<sup>94</sup> for dealing with outlying observations, though E178 was developed for simple univariate data.<sup>†</sup> For MVDA, this should be done in the model building phase to ensure the model is not distorted by non-representative data as well as the model prediction phase to ensure comparability with the model built. Model validation is used to confirm that the model results are representative of the real world. This is defined by the intended use. A fundamental step in this is to calculate the error outlined in another ASTM guide (E1355). While internal validation is important (i.e., via cross-validation), external validation of the MVDA model is recommended (E2617). This guide also outlines the need for subject matter experts (an individual or a team) and life-cycle management of the MVDA. These will help to outline, upon review, when adjustments to the model are required.

### **Conclusion**

The standardization landscape in Raman spectroscopy is incomplete, complex, and evolving, with some aspects covered by various documents and others still to be addressed or covered in standards that are under preparation. ASTM is the

institution with most publications about Raman standardization, while only a couple of standards from ISO have been found to be relevant for Raman spectroscopy. National metrology institutes, mainly from the US and China, have some CRM for calibration, but they are expensive, with limited availability, and the list is incomplete. For instance, there are no CRMs for some excitation wavelengths, such as 405 nm, and no certified Raman shift values for the most used reference material in Raman spectroscopy, Si. Even if the number of standards for Raman spectroscopy is high, the advance of the technique in the past years has not been accompanied by the modernization of the standardization landscape, which shows a lack of protocols in many areas, while some of the standards are outdated; areas that require particularly close attention are techniques like SERS, or low-resolution portable analyzers. Ultimately, the desirable harmonization of Raman spectroscopy remains difficult. Consequently, the Raman community can and should contribute to update and/or introduce new terms, protocols, and guides. A joint public/private effort to develop optimized common and accessible standards for Raman spectroscopy would greatly benefit the development and application of the technique.

The pharmaceutical industry leads the industrial use of Raman spectroscopy, and therefore the pharmacopoeias not only report on the major pharmaceutical-related applications of Raman, but also describe the basic principles of Raman spectroscopy and guide through measurement, calibration, and validation processes, often referring to ASTM and NIST standards. Nevertheless, in general, chemometric models implemented in the industry for data analysis are often application-specific and lack transferability. The academia has made good efforts to understand,<sup>96</sup> with basic research and interlaboratory studies, and remove,<sup>97,23</sup> though RMs as well as chemometrics or protocols development, the setup-induced spectral variations, but manufacturers and standardization bodies engagement is key. Raman equipment and firmware offer different options for calibration and validation depending on the manufacturer/model/technique and on the target user, but they should provide complete calibration modules as well as open access to the real raw data and technical details of the applied corrections.

By creating new standards for qualitative and quantitative analysis in Raman spectroscopy and improved practices for Raman calibration and intensity correction, as well as exploring the use of new reference materials, the development of harmonization protocols could be achieved. In this line, the Versailles Project on Advanced Materials and Standards (VAMAS), devoted to support world trade in products dependent on advanced materials technologies through international collaboration aimed at providing the technical basis for harmonized measurements, testing, specifications, and standards, may shed some light through the Technical Working Area (TWA) 42,<sup>98</sup> which focuses on Raman spectroscopy and microscopy. Besides, EU H2020 funded projects

as CHARISMA (GA 9521)<sup>99</sup> are working toward contributing to this standardization landscape. Finally, it must be mentioned that the consolidation of the theoretical framework of terminology continues to be carried out due to industrial and technical development as well as globalization. The need for systematic terminological work (including standardization) to eliminate misunderstandings and trade barriers is crucial and inevitable.<sup>1</sup>

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### Notes

\* ASTM E1655 is a guide for the multivariate calibration of infrared spectrometers used in determining the physical or chemical characteristics of materials. The first note within the scope informs the reader that while the standard is specifically for infrared, the mathematical and procedural details may be applicable to other spectroscopies. However, these may differ from the best practices for that spectroscopy. Due to the relationship between infrared and Raman, there is likely to be a heavy overlap in their best practices. ASTM E2056, D1622, E2891, and E2617 are directly referring to ASTM E1655 for more detailed descriptions of multivariate analysis and the related terminology.

† ASTM E178 covers outlying observations in samples and how to test the statistical significance of outliers. The procedures in this practice were developed primarily to apply to the simplest kind of experimental data, that is, replicate measurements of some property of a given material or observations in a supposedly random sample.

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