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▲⟨1858⟩ RAMAN SPECTROSCOPY—THEORY AND PRACTICE

THEORY

Raman is a vibrational spectroscopic technique; it is complementary to mid-infrared (IR) and near-infrared (NIR) spectroscopy where there is center of symmetry, because both techniques rely on interaction with the molecular structure of the material. The Raman effect itself arises as a result of a change in the polarizability of molecular bonds during a given vibrational mode and is measured as inelastically scattered radiation, whereas absorption intensity at IR or NIR wavelengths occurs due to differences in dipole moments. Raman spectroscopy is particularly sensitive to non-polar bonds (e.g., C–C, single or multiple bonds), and thus, strong dipole oscillators do not show Raman scattering because the molecular geometry does not give rise to polarizability. The appearance of a Raman spectrum is much like an IR absorbance spectrum. The intensities, or the number of Raman photons counted, are plotted against the shifted energies. The *x*-axis is generally labeled "Raman shift/cm^{-1}" or "wavenumber/cm^{-1}". The Raman shift is usually expressed in terms of wavenumber and represents the energy difference between the incident photon energy and the inelastically scattered photon energy. In addition, water, which has a strong IR absorption spectrum, only exhibits a weak Raman signature and is a useful solvent for this technique. The spectral features and intensities of Raman and IR/NIR are therefore different for most organic molecules, and thus the spectra of the different techniques may be used to complement specificity and characterization.

A Raman spectrum is generated by exciting the sample of interest to an excited state with an appropriate light source. Light elastically scattered (no change in wavelength) is known as Rayleigh scatter and is not of interest in Raman spectroscopy, except for marking the laser

wavelength. However, if the sample relaxes to a vibrational energy level that differs from the initial state, either up or down, the scattered radiation is shifted in energy. This shift is commensurate with the energy difference between the initial and final vibrational states. This "inelastically scattered" light is referred to as Raman scatter. Only about 1 of 10^6–10^8 photons incident on the sample undergoes Raman scattering. Lasers can provide high photon densities and are used in Raman spectrometers to compensate for this inherently low Raman scattering efficiency; also, the Rayleigh scatter can be filtered easily to reduce stray light interferences. Lasers can also be tuned to avoid fluorescence from the sample or matrix or to excite to resonant modes to enhance the signal. If the Raman-scattered photon is of lower energy than the excitation source, it is referred to as Stokes scattering, and if it is of higher energy, it is referred to as anti-Stokes scattering. In practice, nearly all analytically useful Raman measurements make use of Stokes-shifted Raman scatter.

APPLICATIONS

Raman spectroscopy can be used for a variety of applications ranging from verification of raw materials to process monitoring of drug production to quality control of products. These applications include optimizing polymorph (or crystalline structure) analysis; synthesis of new drugs; gaining process understanding through real-time monitoring of reactors, crystallizers, and blenders; verifying raw materials; and ensuring product quality. Once a potential new drug is identified, the method for synthesizing the drug is developed, and Raman spectroscopy is ideal for monitoring reactant, intermediate, and product concentrations, as well as determining pathways, kinetics, mechanisms, end-points, and yields for a variety of reaction types. Quality-by-design control of the product requires assurance that its final form (e.g., gel capsules or tablets) contains the correct amount of active pharmaceutical ingredient (API), its polymorph (if appropriate), excipient, and other additives such as dyes. Raman spectroscopy has been used to monitor mixing in blenders, as well as to inspect individual products before shipment, and may be a useful alternative to HPLC in stability studies. Analysis of the data using chemometrics tools is common for many applications. Proper design of experiment, calibration standard, instrument selection, and maintenance are important considerations for any chemometric model, and it is these requirements that will dictate the specification of the instrumentation required to ensure that it is fit for purpose. For further discussion of the theory and applications of chemometrics, see *Chemometrics* ⟨1039⟩.

QUALITATIVE AND QUANTITATIVE RAMAN MEASUREMENTS

There are two general classes of measurements that are commonly performed using Raman spectroscopy: qualitative and quantitative.

Qualitative Raman Measurements

Qualitative Raman measurements yield spectral information about the molecular structure of the sample. Because the Raman spectrum is specific for a given compound, qualitative Raman measurements can be used as a compendial identification test. See *Spectroscopic Identification Tests* ⟨197⟩.

Quantitative Raman Measurements

For instruments equipped with a detector that measures optical power, such as Fourier transform (FT)–Raman spectrometers, quantitative Raman measurements use the following relationship:[1,2]

$$I(\omega_s) = I(\omega_L) N \frac{d\sigma(\omega_S)}{d\Omega} Z$$

$I(\omega_s)$ = Raman scattering intensity at wavelength ω_s

$I(\omega_L)$ = incident laser intensity at wavelength ω_L

N = number density of scatters

$d\sigma(\omega_s)/d\Omega$ = Raman cross-section at ω_s

Z = geometric factor that takes into account the spectrometer depth of field

SAMPLING FACTORS

Raman spectroscopy is a zero-background technique in that the signal at the detector is expected to be zero in the absence of a sample. This situation can be contrasted with absorption spectroscopy, where the intensity at the detector is at a maximum in the absence of a sample. Zero-background techniques are inherently sensitive because small changes in sample concentration lead to proportionate changes in the signal level. The instrument is sensitive to other sources of light that can cause sample-to-sample variations in the measured signal level. In addition, a large background signal can be caused by fluorescence. Thus, it may be very difficult to use the absolute Raman signal intensities for direct determination of an analyte. Other potential sources of variation are changes in the sample opacity and heterogeneity, changes in the laser power at the sample, and changes in optical collection geometry or sample position. These effects can be minimized by careful method design related to preparation and presentation of the sample, as well as instrument factors.

Use of an internal reference standard is the most common and robust method for eliminating variations caused by absolute intensity fluctuations. There are several options for this approach. An internal standard can be added deliberately, and isolated peak(s) from this standard can be used. Alternatively, the analyst can use a band due to a moiety such as an aromatic ring, the Raman intensity of which does not change with the way the sample is prepared. For solution spectra, an isolated solvent band can be used because the solvent will remain relatively unchanged from sample to sample. In a formulation, an excipient peak can possibly be used if it is present in a substantial excess, when compared to the analyte, in a homogeneous matrix.

A second, important sampling-based factor to consider is spectral contamination. Raman scattering is a weak effect that can be masked by a number of external sources. Common sources of contamination include sample-holder artifacts (container or substrate) and ambient light. Typically, these issues can be identified and resolved by careful experimentation.

APPARATUS

Components

Regarding the instrumentation, modern systems are easy to use, provide fast analysis times (milliseconds to several minutes), and are reliable. Conventional Raman systems are usually benchtop instruments. However, handheld devices based on solid-state electronics and detection systems are widely available and mainly used for the identification of raw materials. Raman spectra are ordinarily excited with visible or NIR radiation, and standard glass/quartz and fiber optics may be used. The signal is typically in the visible or NIR optical range, allowing samples to be analyzed directly through packaging or directly in media that are transparent at these wavelengths. In addition, process Raman analyzers used as process analytical technology (PAT) are also available. Acceptance criteria for fitness for purpose over their operational range are the same for both benchtop and handheld devices.

All commercial Raman instruments have the following components:

- Excitation source (laser)
- Sampling device
- Device to filter/reject light scattered at the laser wavelength
- Wavelength processing unit
- Detector and electronics

Excitation Source (Laser)

Table 1 identifies several common lasers used for pharmaceutical applications or Raman spectroscopy.

The danger of using high-powered lasers must be recognized, especially when their wavelengths are outside of the visible range, i.e., in either the UV or NIR regions. Fiber optic probes should be used with caution.

Table 1. Lasers Used in Pharmaceutical Applications

Nominal Laser λ (nm)	Type	Typical Power at Laser	Wavelength Range (nm) (Stokes Region, 100–3000 cm^{-1} shift)	Comments
NIR Lasers				
1064	Solid state [neodymium-doped:yttrium–aluminum–garnet (Nd:YAG)]	Up to 3 W	1075–1563	Commonly used in FT instruments
830	Diode	Up to 650 mW	836–1105	Typically limited to 2000 cm^{-1} Raman shift because of charge-coupled device (CCD) spectral response; less common than the other lasers
785	Diode	Up to 500 mW	791–1027	Most widely used dispersive Raman laser
Visible Lasers				
632.8	Helium–Neon (He–Ne)	Up to 500 mW	637–781	Relatively small fluorescence risk
532	Doubled Nd:YAG	Up to 1 W	535–633	High fluorescence risk
514.5	Argon (Ar)-Ion	Up to 1 W	517–608	High fluorescence risk
488.0–514.5	Ar-Ion	Up to 1 W	490–572	High fluorescence risk

Nominal Laser λ (nm)	Type	Typical Power at Laser	Wavelength Range (nm) (Stokes Region, 100–3000 cm^{-1} shift)	Comments
NIR Lasers				
UV Lasers				
257.3	Doubled Ar-Ion	Up to 200 mW	258–279	—
248.3	Neon–Copper (Ne–Cu)	20 mW to 1.8 W peak	249–268	—
244.0	Doubled Ar-Ion	Up to 100 mW	245–263	—
229.0	Doubled Ar-Ion	Up to 10 mW	230–246	—
224.3	Helium–Silver (He–Ag)	20 mW to 1.8 W peak	225–241	—

Sampling Device

Several sampling arrangements are possible, including direct optical interfaces, microscopes, fiber optic-based probes (either non-contact or immersion optics), and sample chambers, including specialty sample holders and automated sample changers. The sampling optics can also be designed to obtain polarization-dependent Raman spectra. The choice of sampling device is often dictated by the analyte and sample. However, considerations such as sampling volume, speed of the measurement, laser safety, and reproducibility of sample presentation should be evaluated to optimize the sampling device for any given application.

Filtering Device

The intensity of scattered light at the laser wavelength (Rayleigh scatter) is many orders of magnitude more than the Raman signal and must be rejected before it reaches the detector. Notch filters are almost universally used for this purpose and provide excellent rejection and stability combined with small size. The traditional use of multi-stage monochromators for this purpose, although still viable, is now rare. In addition, various filters or physical barriers to shield the sample from external radiation sources (e.g., room lights or laser plasma lines) may be required, depending on the collection geometry of the instrument.

Wavelength Processing Unit

The wavelength scale may be encoded by either a scanning monochromator, a grating polychromator [in charge-coupled device (CCD)-Raman spectrometers], or an interferometer (in FT-Raman spectrometers). A discussion of the specific benefits and drawbacks of each of the dispersive designs compared to the FT instrument is beyond the scope of this chapter. Any properly qualified instrument should be suitable for qualitative measurements. However, care must be taken when selecting an instrument for quantitative measurements because dispersion and response linearity might not be uniform across the full spectral range.

Detector

The silicon-based CCD array is the most common detector for dispersive instruments. The cooled array detector allows measurements over the spectral range from 4500 to 100 cm^{-1} Raman shift with low noise when most visible lasers, such as frequency-doubled neodymium-doped:yttrium–aluminum–garnet (Nd:YAG; 532 nm) or helium–neon (He–Ne; 632.8 nm) lasers, are used. When a 785-nm diode laser is used, the wavelength range is reduced to about 3100 to 100 cm^{-1}. The most commonly used CCD has its peak wavelength responsivity when matched to the commonly used 632.8-nm helium–neon (He–Ne) gas laser or 785-nm diode laser. FT instruments typically use single-channel germanium or indium–gallium–arsenide (InGaAs) detectors responsive in the NIR to match the 1064-nm excitation of an Nd:YAG laser.

SPECIALIZED TECHNIQUES

In addition to “normal” Raman spectroscopy, there are several more specialized Raman-based techniques. These include Raman microscopy and confocal Raman microscopy (CRM), spatially offset Raman spectroscopy (SORS), resonance Raman (RR), deep UV resonance Raman spectroscopy (DUVRRS), surface-enhanced Raman spectroscopy (SERS), Raman optical activity (ROA), coherent anti-Stokes Raman spectroscopy (CARS), Raman gain or loss spectroscopy, and hyper-Raman spectroscopy. Some of these techniques are currently in use, and these are described below. Those that have very specialized uses in pharmaceutical laboratories are not addressed in this chapter.

Confocal Microscopy/Imaging

The distribution of the various compounds within a given material can be characterized either on the surface by microscopy or inside the sample, three dimensionally, using confocal imaging. The use of Raman scattering allows for the collection of detailed chemical information. Raman microscopy techniques have the following advantages:

- Preparation of the sample may not be required

- Excellent spatial resolution
- Clear image quality
- Outstanding chemical differentiation

It is possible to collect a signal from a single point on a sample, disperse it into a spectrum using a spectrometer, and detect the spectrum using a multi-channel detector such as a CCD or photo diode array (PDA). In this case, instead of obtaining trivial cumulative information about the spot signal intensity, we can obtain a signal spectrum, which can be transformed into detailed information about the chemical composition of the given spot on the sample. The three-dimensional array of data sets, two spatial and one spectral, which is recorded in such an imaging measurement, has become known as a "hypercube" or data cube. These microscopy systems are capable of determining a spatial resolution down to approximately 250 nm but will be influenced by the sample matrix.

The pharmaceutical industry has specific challenges for confocal Raman microscopy in characterizing the structure and distribution of the active components within surface coatings of formulations, dosage forms, and delivery devices, to name a few. For example, high-resolution chemical mapping for homogeneity testing of solid oral dosage forms, creams, and ointments may be useful in select applications.

Spatially Offset Raman Spectroscopy

SORS provides highly accurate chemical analysis of objects beneath obscuring surfaces (e.g., coatings or non-interfering packaging materials). Examples of uses include, but are not limited to, the identification of tablets inside plastic bottles or screening for counterfeit tablets inside blister packs.

A SORS measurement will make at least two Raman measurements: one at the surface and one at an offset position, typically a few millimeters away. The two spectra can be subtracted using a scalar to produce two spectra representing the surface and subsurface spectra. For a simple, two-layer system, such as powder in a plastic bottle, the powder spectrum can be measured without knowing the bottle material or its relative signal contribution. To do this without using an offset measurement would involve severe restriction by the interfering signals of the packaging and any fluorescence signals originating from the surface layer. Scaled subtraction works well for two-layer systems, but multi-variate analysis may be required for more complicated situations, such as where the overlying material contains components included in the sublayer (living tissue, for example).

Deep UV Resonance Raman Spectroscopy

DUVRRS usually uses excitation wavelengths in the 190–260 nm range. There are four major advantages of DUVRRS procedures over their near-UV, visible, or NIR counterparts. These advantages relate to:

- Sensitivity or signal enhancement
- Fluorescence background elimination
- Spectral complexity
- Ambient solar or artificial light background elimination

Current DUVRRS systems come in a large variety of configurations. These variations are primarily related to the light source (laser) and detector systems.

CALIBRATION

Raman instrument calibration involves three components: primary wavelength (*x*-axis), laser wavelength, and intensity (*y*-axis).

Primary Wavelength

In the case of FT-Raman instruments, primary wavelength (*x*-axis) calibration is maintained, at least to a first approximation, with an internal helium–neon (He–Ne) laser. Most dispersive instruments use atomic emission lamps for primary wavelength (*x*-axis) calibration. In all instruments suitable for analytical Raman measurements, the vendor offers a procedure for *x*-axis calibration that can be performed by the user. For dispersive Raman instruments, a calibration based on multiple atomic emission lines is preferred. The validity of this calibration approach can be verified after laser wavelength calibration by using a suitable Raman shift standard. For scanning dispersive instruments, calibration might need to be performed more frequently, and precision in both a scanning mode and a static operation mode may need to be verified.[3]

Laser Wavelength

Laser wavelength variation can affect both the wavelength precision and the photometric (signal) precision of a given instrument. Even the most stable current lasers can vary slightly in their measured wavelength output. The Raman shift wavelength(s) for a given material must therefore be confirmed to ensure that the wavelength scale is accurate for both FT-Raman and dispersive Raman instruments. A reference Raman shift standard material such as those outlined in American Society for Testing and Materials (ASTM) E1840[3] or other suitably verified materials, where the observed peak position(s) have been verified, can be used for this purpose. [Note—Reliable Raman shift standard values for frequently used liquid and solid reagents required for wavenumber calibration of Raman spectrometers are provided in the ASTM Standard Guide cited. These values can be used in addition to the highly accurate and precise low-pressure arc lamp emission lines that are also available for use in Raman instrument calibration.] Spectrometric-grade material can be purchased from appropriate suppliers for this use. Certain instruments may use an internal Raman standard that is separate from the primary optical path. External calibration devices reproduce exactly the optical path taken by the scattered radiation. [Note—When chemical standards are used, care must be taken to avoid contamination and to confirm standard stability.]

Unless the instrument is of a continuous-calibration type, the primary wavelength (*x*-axis) calibration should be performed, as per vendor procedures, just before measuring the laser wavelength. For external calibration, the Raman shift standard should be placed at the sample

location and measured using appropriate acquisition parameters. The peak center of a strong, well-resolved band in the spectral region of interest should be evaluated. The position can be assessed manually or with a suitable, valid peak-picking algorithm. The software provided by the vendor might measure the laser wavelength and adjust the laser wavelength appropriately so that this peak is at the proper position. If the vendor does not provide this functionality, the laser wavelength should be adjusted manually. Depending on the type of laser, the laser wavelength can vary with temperature, current, and voltage. Wavelength tolerances can vary depending on the specific application.

Intensity

Calibration of the photometric *y*-axis can be critical for successful quantification using certain analytical methods and for method transfer between instruments. Both FT-Raman and dispersive Raman spectrometers should undergo similar calibration procedures. The photometric precision acceptable for a given measurement should be assessed during the procedure development stage.

To calibrate the photometric response of a Raman instrument, a broadband emission source should be used. Most manufacturers will provide appropriate calibration sources and software. If the manufacturer does not provide a procedure or method, the user can accomplish the task using a source obtained from National Institute of Standards and Technology (NIST) and appropriate software. If a manufacturer's method is used, attention must be paid to the calibration procedure and source validity. The user should obtain appropriate documentation from the manufacturer to ensure a qualified approach.

There are two accepted methods for calibration; however, other appropriate procedures may be used. *Method A* uses a tungsten white light source.[4] The output power of such sources is traceable to the National Metrology Institute. In the United Kingdom, the National Physical Laboratory also provides calibrated light sources. Several other vendors provide NIST-traceable irradiance calibration standards. *Method A* is applicable to all common laser excitation wavelengths listed in *Table 1*. In *Method B*, NIST standard reference materials (SRMs) are used.[5,6] Several doped-glass fluorescence standards are currently available.

METHOD A

The source should be placed at the sample location with the laser off and the response of the detector measured (using parameters appropriate for the instrument). The output of the source used for calibration should be known. The ratio of the measured response to the true response should be determined, and a correction file should be generated. This correction should be applied to all spectra acquired with the instrument.

METHOD B

The fluorescence standard should be placed at the sample location. With the laser on, a spectrum of the SRM should be obtained (using parameters appropriate for the instrument). The output of the standard used for calibration should be known. The ratio of the measured response to the true response should be determined, and a correction file should be generated. This correction should be applied to all spectra acquired with the instrument. [Note—*Method B* is currently appropriate for systems with 785-nm (SRM 2241), 532-nm (SRM 2242), 514.5-nm and 488-nm (SRM 2243), 1064-nm (SRM 2244), 632.8-nm (SRM 2245), and 830-nm (SRM 2246) excitation.]

External Calibration

Detailed functional validation using external reference standards is recommended to demonstrate instrumental suitability of laboratory instruments, even for instruments that possess an internal calibration approach. The use of external reference standards does not obviate the need for internal quality control procedures; rather, it provides independent documentation of the fitness of the instrument to perform the specific analysis or purpose. For instruments installed in a process location or in a reactor where routine positioning of an external standard is not possible, including those instruments that use an internal calibration approach, the relative performance of an internal versus an external calibration approach should be checked periodically. The purpose of this test is to detect changes in components that might not be included in the internal calibration method (process lens, fiber optic probe, and others).

SAMPLE-BASED FACTORS THAT AFFECT MEASUREMENT PERFORMANCE

The most important sample-based factor that deleteriously affects quantitative Raman spectroscopy is fluorescence. Other factors, such as local sample heating by the light source, photobleaching, absorption by the matrix or the sample itself, and the effect of polarization, can also be problematic and must be addressed during procedure development. If the sample matrix includes fluorescent compounds, the measured signal will usually contain a contribution from fluorescence. Fluorescence will be observed only if the laser excitation wavelength overlaps with an absorption band of a fluorescent compound. Fluorescence is typically observed as a broad, sloping background underlying the Raman spectrum. Fluorescence can cause both a baseline offset and a reduced signal-to-noise ratio. The wavelength range and intensity of the fluorescence depend on the chemical composition of the fluorescent material. Because fluorescence is generally a much more efficient process than Raman scattering, even minor amounts of fluorescent impurities can lead to significant degradation of the Raman signal. Fluorescence can be reduced by using longer wavelength excitation sources such as 785 or 1064 nm. As the intensity of Raman scattering is proportional to the fourth power of the absolute wavenumber of scattered light, a significant improvement in Raman scattering efficiency can be expected when higher exciting wavenumbers are used. However, the advantage of using a long-wavelength excitation laser to minimize fluorescence is at least partially offset by the reduced strength of the Raman signal. The greatest signal-to-noise ratio will be obtained by balancing fluorescence rejection, signal strength, and detector response.

Fluorescence in solids can sometimes be mitigated by photobleaching, where the sample is exposed to the laser radiation for a period of time before measurement, and operates by degrading the highly absorbing species. Although this is typically a factor to avoid, photobleaching may be used in exceptional circumstances to mitigate the effect of fluorescence, if no other preprocessing of the sample is possible. Photobleaching is less effective in liquids, where the sample is mobile, or if the amount of fluorescent material is more than a trace.

Sample heating by the laser source can cause a variety of effects, such as physical form change (melting), polymorph conversion, or sample burning. The likelihood of sample heating is greatest when the spot size at the sample is the smallest, i.e., when a microprobe or microscope is being used. This is usually an issue for colored, highly absorbing species, or very small particles that have low heat transfer. The effects of sample heating are usually observable, either as changes in the Raman spectrum over time or by visual inspection of the sample. Besides decreasing the laser flux, a variety of methods can be used to diminish laser-induced heating, such as moving the sample or laser during the measurement or improving the heat transfer from the sample with thermal contact or liquid immersion.

Absorption of the Raman signal by the matrix or the sample itself can also occur. This problem is more prevalent with long-wavelength FT-Raman systems where the Raman signal can overlap with an NIR overtone absorption. This effect will be dependent on the optics of the system, as well as on the sample presentation. Associated with this effect is variability from scattering in solids as a result of packing and particle-size differences. The magnitude of all of these effects is typically smaller than in NIR spectroscopy because of the limited depth of penetration and the relatively narrower wavelength region sampled in Raman spectroscopy.

Finally, it should be recognized that laser radiation is polarized, and the Raman spectra of crystalline materials and other oriented samples can differ significantly, depending on the way that the sample is mounted. If the Raman spectrometer is capable of producing linearly polarized radiation at the sample, then a polarization scrambler is recommended for routine sample analysis.

PROCEDURE VALIDATION

Validation of Raman procedures will follow the protocols described in *Validation of Compendial Procedures* ⟨1225⟩. Several of these criteria are affected by variables specific to Raman spectroscopy. Fluorescence is the primary variable that can affect the suitability of a procedure. The presence of fluorescent impurities in samples can be quite variable and can have little effect on the acceptability of a material. The procedure must be flexible enough to accommodate different sampling regimens that may be necessary to minimize the effects of these impurities.

Detector linearity must be confirmed over the range of possible signal levels. Fluorescence might drive both the signal baseline and the noise higher than the levels used in the validation, in which case the fluorescence must be decreased, or the procedure must be modified to accommodate the higher fluorescence levels. This is also true for the precision, limit of detection, and limit of quantification of the procedure, as increased baseline noise will adversely affect all of these values. Because fluorescence can also affect quantification caused by baseline shifts, acceptable quantification at different levels of photobleaching, when used, should be confirmed.

The effect of the laser on the sample must be determined. Visual inspection of the sample and qualitative inspection of the Raman spectrum will confirm that the sample is not being altered (other than by photobleaching). Specific variables to confirm in the spectrum are shifts in peak position, changes in peak height and band width, and unexpected changes in background intensity.

Precision must also encompass sample position. The sample presentation is a critical factor for both solids and liquids and must be either tightly controlled or accounted for in the calibration model. Sample-position sensitivity can often be minimized by appropriate sample preparation or sample-holder geometry but will vary from instrument to instrument, based on excitation and collection optical configuration.

In addition, many suitable chemometric algorithms for data pretreatment and calibration exist. The selection of these algorithms should be based on sound scientific judgment and suitability for the intended application.

Ongoing Procedure Evaluation

Validated Raman procedures should be subject to ongoing performance evaluation, which may include monitoring of accuracy, precision, and other suitable procedure parameters. If the performance is unacceptable, corrective action is necessary. This involves conducting an investigation to identify the cause of the change in the performance of the procedure and may indicate that the Raman procedure is not suitable for continued use. Improving the Raman procedure so that it meets measurement suitability criteria may require additional procedure development as well as validation experiments, with documentation, demonstrating that the improved procedure is suitable for the intended application. The extent of the revalidation required depends on the cause of the change in performance of the procedure and the nature of corrective action required to establish suitable performance. Appropriate change controls should be implemented to document ongoing continuous-improvement activities.

Revalidation of a qualitative model may be necessary as a result of the following:

- Addition of a new material to the spectral reference library
- Changes in the source of material supply
- Identification of previously unknown critical attribute(s) of material(s)

Revalidation of a quantitative model may be necessary as a result of the following:

- Changes in the composition of the test sample or finished product
- Changes in the manufacturing process
- Changes in the sources or grades of raw materials
- Changes in the reference analytical procedure
- Major changes in instrument hardware

OUTLIERS

Sample spectra that produce a Raman response that differs from the qualitative or quantitative calibration model may produce an outlier. This does not necessarily indicate an out-of-specification result, but rather an outlier indicates that further testing of the sample may be required. If subsequent testing of the sample by an appropriate procedure indicates that the property of interest is within specifications,

then the sample meets its specifications. Outlier samples may be incorporated into an updated calibration model after execution and documentation of suitable validation studies. For further discussion on model lifecycle, see ⟨1039⟩.

Method Transfer

Controls and measures for demonstrating the suitability of performance after method transfer are similar to those required for any analytical procedure. Exceptions to general principles for conducting method transfer for Raman procedures should be justified on a case-by-case basis. The transfer of a Raman procedure is often performed by using a Raman calibration model on a second instrument that is similar to the primary instrument used to develop and validate the procedure. When a calibration model is transferred to another instrument, procedures and criteria must be applied to demonstrate that the calibration model meets suitable measurement criteria on the second instrument. The selection of an appropriate calibration model transfer procedure should be based on sound scientific judgment.▲ (USP 1-Aug-2020)

1 Biggs KB, Camden JP, Anker JN, Van Duyne RP. *J Phys Chem A.* 2009;113(16):4581–4586.

2 McCreery RL. Raman Spectroscopy for Chemical Analysis. New York, NY: John Wiley and Sons, Inc.; 2000.

3 ASTM International. ASTM E1840. Standard Guide for Raman Shift Standards for Spectrometer Calibration. West Conshohocken, PA: ASTM International.

4 NIST traceable tungsten white light source statement: While the calibration of the Raman frequency (or Raman shift, cm^{-1}) axis using pure materials and an existing ASTM standard is well accepted, techniques for calibration of the Raman intensity axis are not. Intensity calibrations of Raman spectra can be accomplished with certified white light sources.

5 National Institute of Standards and Technology. NIST SRM 2241.

6 Ray KG, McCreery RL. *Appl Spectrosc.* 1997;51:108–116. Raman intensity correction standard for systems operating with 785-nm excitation.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
<1858> RAMAN SPECTROSCOPY - THEORY AND PRACTICE	Yang Liu Manager, Product Quality and Analytical Methods	GCCA2020 General Chapters - Chemical Analysis 2020

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