2.26 Raman Spectrophotometry

(ラマンスペクトル測定法)

Raman spectroscopy is a vibrational spectroscopic technique, which evaluates a sample to be examined qualitatively or quantitatively by analyzing a spectrum obtained by dispersing very weak scattered light, having different wavelengths from irradiation light, generated when the sample is irradiated with the light. Raman scattering is observed when the polarizability of molecules changes with the vibration of chemical bonds of molecules in a sample.

Raman spectroscopy generally uses monochromatic laser light as excitation light. When the laser light is irradiated to the sample to be examined, the molecules in the sample is excited and the light with the same wavelength of the irradiation light, known as Rayleigh scattering, is scattered. The scattered light detected in the shorter wavelength side than the Rayleigh scattering is referred to as anti-Stokes scattering. The scattered light detected in the longer wavelength side than the Rayleigh scattering is referred to as Stokes scattering. Generally Stokes scattering with strong Raman scattering intensity is used for analysis. Raman spectra are usually indicated by Raman shift (cm^{-1}) or wave number (cm^{-1}) on the horizontal axis and Raman scattering intensity on the vertical axis.

Raman spectroscopy is capable of measuring samples (solid, semi-solid, liquid, gas, etc.) rapidly and non-destructively without pre-treatment. Application of Raman spectroscopy in the pharmaceutical field includes qualitative or quantitative evaluation of the active pharmaceutical ingredients and additives in drug substances or drug products. Raman micro-spectroscopy can be used for the evaluation of the distributions of active pharmaceutical ingredients and additives in the drug products. Moreover, Raman spectroscopy can also be used for the evaluation of the physical conditions of substances, such as crystal form and crystallinity. Furthermore, using an optical fiber probe enables it to measure the spectra of samples at a location remote from the equipment body without sampling, so that it can be used to perform pharmaceutical manufacturing process control online (or in-line).

1. Apparatus

Raman spectrometers are composed of a light source unit, a sample unit, a spectrometry unit, a detector unit, a signal processing unit, a data processing unit and a display-record-output unit. Raman spectrometers are classified into dispersive Raman spectrometers and Fourier transform Raman spectrometers according to their spectroscopic methods.

1.1. Light source

The laser which stably emits monochromatic light as excitation light to samples is used for the light source. The lasers include gas lasers such as a He-Ne laser and solid state lasers, and select a laser with wavelengths and output power according to the purpose. Pay attention to safety standards relating to a laser, when this test is performed.

1.2. Sample unit

The sample unit is composed of an optical system for collecting Raman scattering light generated by irradiation of excitation light and a sample cell. Combination of these take the form of a sample chamber, while there are apparatuses with no sample chamber, such as optical fiber probes and portable Raman spectrometers that can be carried. Representative sample chambers are macroscopic sample chambers and microscopic sample chambers. The components of these optical systems are different, respectively.

1.3. Spectroscope unit and detector

Many dispersive Raman spectrometers use an optical filter to eliminate excitation light and use a single monochromator combined with a multichannel detector, since the configuration is simple and high sensitivity can be obtained. Detectors include multiple elements detectors and single element detectors, and general dispersive Raman spectrometers use a multiple elements detector such as a CCD detector.

Fourier transform (FT) Raman spectrometers obtain spectra by Fourier transformation of interference waveforms using an interferometer. FT-Raman spectrometers are mainly used for near infrared Raman measurement.

2. Measurement methods

The Raman spectrophotometry is applicable to solid samples having a complicated shape in addition to gas/solution samples inside a glass sample cell being transparent in the visible region, using mainly light in the visible region as excitation light. In the view of the size of a measurement region and Raman scattering efficiency, an optimum optical system is selected according to the sample. The excitation wavelength, the measurement mode of the apparatus, etc. should be selected and set.

2.1. Macroscopic measurement

Since the macroscopic sample chamber has a high degree of freedom in the scattering configuration, samples can be measured irrespective of solid, liquid, gas, size and shape. It is also applicable to Raman measurement under low temperature, high temperature and high pressure which require the setting of a large sample cell. Usually, in the macroscopic sample chamber three configurations: forward scattering, 90° scattering and back scattering configurations, can be usable and an appropriate scattering configuration can be selected depending on a sample.

2.2. Microscopic measurement

The microscopic sample chamber is based on an optical microscope and applicable to local analysis. In the optical system of the microscopic sample chamber a microscope objective lens works simultaneously as an excitation light converging lens and a Raman scattering light condensing lens.

Mapping measurement repeats local measurements by moving a sample or laser light position to generate a Raman image showing the two or three dimensional distribution of Raman scattering intensity. Raman images are made by using various spectral information such as a ratio of the intensity of two bands.

2.3. Probe measurement

The optical fiber probe is the collective term of the apparatus of which sample section is separated from a Raman spectrometer body by using an optical fiber and is applicable to *in situ* measurement and on-line (or in-line) measurement.

2.4. Measurement by portable apparatus

The portable Raman spectrometer is possible to carry and perform analysis using Raman spectroscopy outside of laboratory. Main application of this apparatus is judgement on acceptance of pharmaceutical materials. It is used for rather simple measurement.

2.5. Points to note in measurement

Note the following points for solid, liquid and suspended samples.

(i) Measurement of solid sample: There is a possibility that the filling status, the difference in the particle diameter and the roughness of the surface of the sample could affect the scattering intensity. When measuring a crystalline sample, be careful about the effect of crystal shape. There is also a possibility that the light transparency of the sample affect the spectrum intensity. When a sample is physically and chemically inhomogeneous, it might be recommended to enlarge the spot size of laser irradiation, measure plural samples, measure the plural points of the same sample or crush the sample to homogenize.

(ii) Measurement of liquid sample: It is possible to subtract the spectrum of the solvent if there is no interaction between the solvent and the sample. When there are insoluble matters in solution, remove the matters using a filter before measurement not to obtain the Raman scattering of the matters. When a sample shows high reactivity by laser irradiation in solution, measure the sample by stirring carefully not to irradiate the same place.

(iii) Measurement of suspended sample: A suspended sample may settle, so be careful about the positioning of laser irradiation. For samples that are prone to settle, devising measurement such as optimizing the irradiation time and stirring might be helpful. When the Raman scattering of a suspended sample is weak, it is also possible to subtract the spectrum of the solvent likewise the case of measurement of a liquid sample.

3. Factors that affect spectrum

When Raman spectroscopy is applied, note the following items as factors affecting spectra.

3.1. Temperature of sample

Sample heating by laser irradiation can cause a variety of effects, such as physical form change (melting and burning) and polymorph transform. Since the chance of sample heating is increased when the spot size of laser irradiation at the sample is squeezed, be careful not to damage sample when microscopic measurement is carried out. To prevent the sample overheating, a variety of methods can be employed such as suppressing laser output, irradiating a laser without focusing and cooling a sample.

3.2. Sample characteristics

Since Raman signals are very weak, the fluorescence of a sample itself and minute impurities may interfere with Raman scattering light. Fluorescence can be reduced by choosing an excitation light source with a longer wavelength, however be careful that it generally decreases the intensity of the Raman scattering. Photobleaching resulted by laser irradiation before measurement, appropriate irradiation time and accumulation count may mitigate the fluorescence.

When measuring a colored sample, select the wavelength of an excitation laser depending on the absorption characteristics of the sample. When measuring a sample in a container such as, a cell for measurement, a bag or a bottle, take careful note of the spectral characteristics derived from the container in addition to the sample.

4. Control of apparatus performance

Estimate the accuracy of Raman shift wave numbers after adjusting a Raman spectrometer. Measure Raman spectra using an excitation laser utilized for actual measurement and a standard substance such as calibrated polystyrene.

In the cases of 2.1., 2.2. and 2.3., make correction using at least three wave numbers among the below peak wave numbers (cm^{-1}) obtained from the spectrum of polystyrene. The number in parentheses indicates the permissible range.

620.9 (±1.5)
1001.4 (±1.5)
1031.8 (±1.5)
1602.3 (±1.5)
3054.3 (±3.0) (Note: 3054.3 cm^{-1} cannot be measured depending on an excitation wavelength.)

In the case of 2.4., make correction in the same manner using at least three wave numbers (cm^{-1}) among the below peak wave numbers.

620.9 (±2.5)
1001.4 (±2.0)

1031.8 (±2.0)
1602.3 (±3.0)

Other substances such as cyclohexane can be used as a standard substance, if it is validated.

5. Qualitative and quantitative analysis

5.1. Qualitative analysis

As Raman spectroscopy observe the vibrational energy of a molecule and can obtain a characteristic spectrum depending on the structure of a substance to be analyzed, qualitative analysis based on chemical structural information can be performed.

When the Raman spectra of a sample and the Reference Standard of a substance to be identified are compared and both spectra exhibit similar scattering intensities at the same Raman shifts, the identity of those can be confirmed.

When a sample treatment method for a solid sample is indicated in the monograph in the case of nonconformity of the scattering spectrum with that of the Reference Standard, treat the sample and the Reference Standard under the condition as directed in the monograph, then repeat the measurement.

When the characteristic scattering wave numbers of a substance to be identified are specified in the monograph, the clear appearance of the scattering of a sample at all the specified scattering wave numbers can confirm the identity of the sample with the substance to be identified.

Raman spectroscopy is also applicable to the process control of drug substances or drug products by using a score obtained from a Raman spectrum by a chemometric methodology such as principal component analysis, and characteristic peak wave numbers of the substance to be examined, as indices.

5.2. Quantitative analysis

The quantitative analysis can be performed by developing a calibration model about a spectrum measured using an existing standard sample by a chemometric methodology, applying the model to the spectrum of the sample to be examined and calculating the concentration of each component in the sample. Chemometric methodologies for obtaining a calibration model include multiple regression analysis method, principal component analysis method and PLS (Partial least squares) regression analysis method.

In the case where the composition of a sample is simple, the concentrations of components to be analyzed in the sample can be calculated by a calibration curve plotting the relationship between scattering intensity at a specified wave number and concentration.

The variation of peak intensity at around the reference values of wave numbers using a standard sample, for example,polystyrene etc. used in 4., is preferable to be within ±10% compared to that obtained in the last measurement.