# Module-3 Unit-5 Principles and instrumentation: Raman spectroscopy

Raman spectroscopy was named in the honor of its inventor, C.V. Raman, who, along with K.S. Krishnan, published the first paper on this technique[1].Raman spectroscopy (RS) is a versatile method for analysis of a wide range of forensic samples. It resolves most of limitations of other spectroscopic techniques and provides both qualitative and quantitative information about the sample being examined. Qualitative analysis involves measurement of the frequency of scattered radiation, whereas measurement of the intensity of scattered radiation is the part of quantitative analysis [2,3].

# **Principle:**

Raman spectroscopy is an important technique to identify several materials which can be solids, liquids or gases. It is a simple, non-invasive system and requires no elaborate sample preparation. The sample is irradiated by a monochromatic light and a spectrometer examines the scattered light. This technique works on inelastic scattering of the monochromatic light. During the scattering process, frequency of the incident photons is changed. Photons of incident light are first absorbed and then reemitted by sample. Frequency of these re-emitted photons shifts either up or down in respect to the primary monochromatic frequency. The phenomenon is termed as Raman effect. These shifts contain valuable information on the vibrational, rotational and low frequency transitions of the sample molecules. Raman effect depends on molecular deformations in electric field E influenced by molecular polarizability ( $\alpha$ ). Laser beam may be regarded as an oscillating electromagnetic wave with electrical vector E. After interacting with sample it induces electric dipole moment  $P = \alpha E$  and deforms the molecules. Due to the periodic deformation, molecules vibrate at characteristic frequency  $v_m$ . Thus, the monochromatic laser beam having frequency  $v_0$  excites the molecules and converts them to oscillating dipoles. These oscillating dipoles produce light of dissimilar frequencies as revealed in **Figure 1**:

- 1. The molecule without any Raman active modes absorbs a photon of frequency  $v_0$ . The excited molecule comes back to its fundamental vibrational state after emitting light of same frequency  $v_0$ . This phenomenon is described as the elastic Rayleigh scattering.
- 2. A Raman active molecule (in its fundamental vibrational state) absorbs a photon. Some of the photon's energy is transmitted to molecule with frequency  $v_m$ , decreasing the frequency of scattered light is to  $v_o-v_m$ . This Raman frequency is known as Stokes frequency or simply "Stokes".
- 3. The molecule absorbing the photon is already in its excited vibrational state. Excessive energy of excited Raman active mode is released, molecule comes back to its fundamental vibrational state and the frequency of scattered light increases to  $v_0+v_m$ . This Raman frequency is called Anti Stokes frequency or just "Anti Stokes". Raman shift isindependent of the incident light frequency. It is the characteristic feature of material undergoing Raman shift. The shift,  $\Delta v$ , is positive for Stokes and negative for anti-Stoke's.



Figure 1 Vibrational levels of the material.

From Figure 1, it is seen that the shift in stokes as well as in anti-stokes linewith respect to the Rayleigh line is equal. This happens since both cases involve the gain and loss of one vibrational quantum of energy (or same amount of energy). Moreover, the intensity of anti-Stokes line is lower than that of Stokes line. This is because anti-stokes line is caused by molecules which are vibrationally excited (very less in numbers) before irradiation. Thus, in Raman investigation, more intense stokes line is usually recorded. Infrared (IR) absorption spectroscopy is the other vibrational method to study molecular structures however it diverges from Raman spectroscopy in the manner of molecular transitions. For transitions to be Raman active the polarizability of molecule should vary during vibration. Thereby necessitating change in position of molecules' electron cloud. Whereas for Infrared transitions molecule undergoes change in dipole moment during vibration. Homonuclear diatomic molecules including  $H_2$ ,  $N_2$ ,  $O_2$ , etc. that do not exhibit infrared spectra due to absence of permanent dipole moment, are Raman active because their vibration is associated with variation in molecule's polarizability. Therefore, Raman spectroscopy allows examination of vibrational spectra of compounds which cannot be studied by IR absorption spectroscopy. Raman spectroscopy is invariably employed for gases, liquids, and solids thereby lending it an extremely versatile tool for examining diverse materials. Raman spectrum is characteristic to the materials being studied, due to their distinct structural arrangements; therefore the composition of unknown substances can be easily determined. This renders Raman spectroscopy best for qualitative examination of substances.

#### **Types of Raman Spectroscopy**

#### a. Surface Enhanced Raman Spectroscopy (SERS)

Surface-enhanced Raman spectroscopy (SERS) is one of the most sensitive devices which are used for highly sensitive structural detection of analytes in low concentrations. This is achieved by amplifying the electromagnetic fields produced by the excitation of localized surface plasmons (LSP) of adsorbate molecules on the roughened metal surface [4].

While the signals obtained by normal Raman scattering is usually weak, SERS can detect very low concentration of the analytes because of the large increase of the Raman cross-section of an analyte up to 15 orders of magnitude in comparison to normal Raman scattering [5,6].**Figure 2** is a sketch that presents the main parts and component of Surface enhanced Raman scattering device [7].



Figure 2 Surface enhanced Raman scattering system.

Colloidal metals and roughened metal electrodes are SERS-active surfaces [8]. Colloidal metal is easy to prepareas well as characterise because of its morphology and size. Even though it usually shows a relatively strong activity for the adsorbed molecules it has poor reproducibility of their properties because it is difficult to control its size [8]. Another type of metal surfaces was tested after that like gold and copper. All those metal surfaces increased the intensity of Raman signal up to 104 to 106-fold. In general silver and gold substrate are the most often used types because they are the most air stable materials. Copper on the other hand is more reactive with air, which will affect the success of SERS. SERS substrates become available in various shapes and coating after advance development. Different concepts have been developed to enhance the fabrication of SERS substrates using bottom up and top down approaches. The most important thing to realize to improve SERS is to provide the industry with more sensitive, cheaper components for the structure of the spectroscopy and simpler fabrication method.

# b. Tip enhanced Raman Spectroscopy (TERS)

Many research interest require Raman imaging of a small area within the sample, however conventional Raman technique can be misleading in some cases due to the masked signal contribution by the surrounding area signals [9]. Such issues encouraged further research on Raman scattering enhancement. Tip enhanced Raman spectroscopy (TERS) is a powerful solution that combines between two techniques; scanning probe microscopy and Raman spectroscopy [10]. TERS can provide a topographical and spectral/ chemical information simultaneously using SPM and Raman respectively [10,11].

TERS has similar instrumentation, material requirement and enhancement principle as SERS, but by using a metal tip or metal nanoparticle instead of the metal film [11,12]. TERS technique involves bringing a metal tip or a metal nanoparticle very closeto the sample, i.e., with a nanometer distance. An excitation laser beam will illuminate the tip apex creating an enhanced and confined electric field zone. This localized field will result in enhanced scattered light from the sample located under the tip [13,14]. A diagram of TERS concept can be seen in **Figure 3** [15]. The mechanism behind TERS ability to enhance the electromagnetic field at a tightly focused spot can be attributed to surface plasmon resonance and electrostatic lighting rod effect [14].



Figure 3 Tip enhanced Raman spectroscopy concept.

There are three main scanning probe microscopy modes that are used in TERS experiments. The most commonly used is the Atomic Force Microscopy. The other two SPM modes are Shear-Force Microscopy and Scanning Tunneling Microscopy. As can be seen in **Figure 4**, the tip can be illuminated in three ways; bottom or back reflection illumination, side illumination and top illumination [15]. The bottom illumination works for transparent or very thin film sample. While the other two are used also for opaque samples. Each of these illumination techniques has its own limitations and advantages. The selection is mainly based on the analyzed sample and the SPM modes.



Figure 4 Diagram showing optical alignments setup in TERS. (a) Bottom illumination (b) side illumination (c) bottom illumination.

## . Coherent anti-Stokes Raman Spectroscopy (CARS)

Coherent anti-stoke Raman scattering spectroscopy, also known as CARS is a nonlinear Raman technique that is used to enhance the Raman signal [16]. As the name indicates, it uses coherent laser beams to generate a signal with frequency higher than the excitation frequency. Thus, it is regarded as anti-stoke frequency technique. The main principle of this technique is based on using multiple excitation laser sources [17]. It involves pump coherent monochromatic field at frequency of  $\omega_p$ , and stoke excitation field at frequency of  $\omega_s$ , overlapping spatially and temporally. When the incident light is focused on the sample at small focal volume and probed by a third laser beam, a strong anti-stoke Raman signal is created at blue shifted frequency away from fluorescence effects and in the face matching direction [18,16,19]. A strong signal is created with frequency of  $\omega_{as} = 2 \omega_p - \omega_s$ , when the difference in the frequency of the incident light,  $\Delta \omega = \omega_p - \omega_s$ , is tuned to the Raman active molecular vibration of the sample  $\Omega_{vib}$  [16]. Hence, CARS provides information about the intrinsic vibrational bond of the sample with three dimensional resolutions. Since CARs is a four wave mixing process, the signal has a quadratic dependence on the incident field intensity [20].**Figure 5** indicates the diagram of CARS energy levels [17].



Figure 5 Diagram of the CARS process.

#### d. Stimulated Raman Scattering (SRS)

SRS is another nonlinear process stands for simulated Raman Spectroscopy [21] .The basic principle of this coherent phenomena is based on the incident of two photons (pump and stoke) like CARS principle [22]. The two laser beams coincide on the sample. When the frequency difference matches the molecular vibration frequency of a bond in the target molecule, this will induce simulated excitation in the vibrational transition. Consequently, this will result in stoke field amplification where the intensity of the stoke experiences a gain and pump- field attenuation where the pump intensity experiences a loss termed as stimulated Raman gain (SRG) and stimulated Raman loss (SRL), respectively [22,23]. It should be mentioned that SRS are not identical to CARS. Although both are coherent phenomena that based on two incident photons. The SRS does not have a signal at frequencies other than the excitation wavelength. Another difference is that SRS has a linear dependence on concentration and the resulting spectra match with spontaneous Raman spectroscopy, making interpretation much easier than CARS. Also, the SRL and SRG only occur if the frequency difference matches a molecular resonance.

Simulated Raman spectroscopy as a coherent Raman technique, allows an enhanced real time imaging, overcome the low signal intensity of spontaneous Raman spectroscopy and improve signal to noise ratio [24]. Additionally, the photon flux is directed collinearly with the probe laser, that means that SRS are not sensitive to the background fluorescence, since only a small fraction of this fluorescence is emitted collinearly with the probe [25].

## e. Resonance Raman Spectroscopy (RRS)

Resonance Raman spectroscopy is an instrument which measure the shift in the frequency of the photon when the energy of photons from the incident light is approximately similar to the energy needed for electronic transition [18]. The resonance excitation can increase the oscillation charge displacement of electrons. As a result, it will increase the induced dipole moment, which will directly enhance the efficiency of Raman scattering [26]. The enhancement factor of resonance Raman scattering comparing with normal Raman can be high as 108. It is important to note that fluorescence is more likely to interfere with resonance Raman more than non- resonance scattering.

Fluorescence is one of the challenges that limit the applicability of Raman technique. It was found that using UV resonance Raman spectroscopy for characterization of catalyst reactions and synthesis can eliminate the fluorescence effect. Most of the catalyst showed fluorescence spectra within the visible region so shifting the excitation laser to the UV region ( $\lambda < 300$  nm) will help to avoid interferences. Resonance Raman spectroscopy can be used to study the structure of hemi protein and the function of human body cell. It can help to determine the shapes of potential surfaces or molecular geometries in excited states.

## f. Confocal Raman microscopy

The probe head in Confocal Raman Microscopy work on focusing laser light onto the sample by using the microscope objective. And the pinhole will refocus the backscattered Raman signal which makes it to behave as spatial filter. After that the signal will be collected on a CCDdetector to create a spectrum [18].

Depth profiling with confocal Raman microscopy can be applied in two ways. The first approach uses plotting of intensity of a specific band as a function of the distance from the sample surface. This way provides information about the composition and structure of gradients of the sample. The second way is to acquire a pure spectrum of buried structures and used to identify determinations. This type of the Raman microscope has proven to be a useful analytical technique. It showed a set of advantages such as high spatial resolution, clear image quality in addition the sample need no preparation.

## Instrumentation:

Either dispersive or non-dispersive spectrophotometers can be used. A prism or a grating is employed in the dispersive spectrophotometer, whereas, non-dispersive employs interferometer, analogous to Michelson interferometer in FTIR.

# Fundamental components of Raman spectrophotometer:

**1. Excitation source (Laser):**-Early Raman spectrometers employed mercury arc lamps, where a 435.8 nm line of coiled low-pressure mercury arc lamp was used as light source until 1960's.Laser sources became available in late 1960's and completely replaced the mercury lamp.These laser sources provide stable and intense beam of radiation. There are a variety of lasers which can be used, e.g., Argon ion (488 and 514.5 nm), Krypton ion (530.9 and 647.1 nm), Helium–Neon (He–Ne) (632.8 nm), Near Infrared (IR) diode lasers (785 and 830 nm), Neodymium–Yttrium Aluminum Garnet (Nd:YAG) and Neodymium–Yttrium Ortho-Vanadate (Nd:YVO<sub>4</sub>) (1064 nm), etc.Short wavelength sources, e.g. argon ion and krypton ion lasers generateconsiderable fluorescence and result in photodecomposition of the specimen. While, long wavelength sources, e.g., diode or Nd:YAG lasers can operate at very high power without decomposingthe sample and also eliminate or reduce fluorescence.

**2. Optics for sample illumination and light collection:-** Light from the irradiated/illuminated spot is collimated by a lens and guided to interference filter or spectrometer to get Raman spectrum.

**3. Wavelength selector (Filter or Spectrometer):-** Band pass filters are used to isolate a single laser beam. A combination of notch filter and high quality grating monochromator is mostly employed in dispersive instruments. Double or even triple grating monochromators, super notch filters, rejection filters, holographic notch or edge filters and holographic filters are used to separate relatively weak Raman lines from intense Rayleigh scattered radiations.

**4. Detector (Photodiode array, CCD or PMT):-**Thermoelectrically cooled photomultiplier tubes and photodiode array detectors were used in early models of dispersive Raman spectrophotometers. Advances in instrumentation and technology replace these detectors with more sensitive charge transfer devices (CTDs) such as charge-coupled devices (CCDs) and charge-injection devices (CIDs). These devices act as a detector and used in the form of arrays. In CTD's arrays, photosite converts the incoming optical signal into charge which is integrated and transferred to readout devices. Multichannel CCD detectors are used with laser wavelengths of less than 1 µm while single element low band-gap semiconductor such as Germanium (Ge) or Indium–Gallium–Arsenic (InGaAs) detectors are used with laser wavelengths of greater than 1 µm.



Figure 6 Schematics of the set of Raman spectroscope.

**Figure 6** shows the schematics of Raman spectrometer. The sample is irradiated with a laser beam. Light from the irradiated/illuminated spot is collimated by a lens and guided to interference filter or spectrometer to get Raman spectrum. Wavelengths near laser line, corresponding to elastic Rayleigh scattering, are filtered out and remaining collected light is dispersed on a detector. Spontaneous Raman signal is generally very weak since majority of incident photons suffer elastic Rayleigh scattering. Thus special methods are undertaken to separate it from the dominant Rayleigh scattering. Instruments like tunable and notch filters are employed to minimize Rayleigh scattering and acquire good Raman spectra.

## **SUGGESTED READINGS:**

- 1. www.spectroscopynow.com/coi/cda/landing.cda?chId=6&type=Education (many links including, An Introduction to Raman Spectroscopy: Introduction and Basic Principles, by J. Javier Laserna)
- 2. http://wwwobs.univ-bpclermont.fr/sfmc/ramandb2/index.html
- 3. http://www.renishaw.com http://www.optics.bruker.com/ http://www.nicolet.com/ http://www.nicolet.com/

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#### TIME LINE:

1923: Inelastic scattering of light proposed by Adolf Smekal.

1928: First observation of inelastic scattering (or Raman scattering) of light by C.V. Raman. He observed this effect using sunlight.

1930: Nobel Prize in Physics awarded to C.V. Raman. He used sunlight, a narrow band photographic filter to generate monochromatic light, and a 'crossed' filter to block the passage of monochromatic light through it. C.V. Raman observed that a small amount of light possessed altered ('shifted') frequency and passed through the 'crossed' filter.

1930-1934: Raman effect was systematically studied by George Placzek. Light source was changed to mercury arc lamp.

The following years witnessed the evolution of a catalog of molecular vibrational frequencies using Raman spectroscopy. Earliest systems had poor sensitivity and it was very difficult to observe Raman shifts. The sample was placed in a long tube and irradiated along its length with a beam of filtered monochromatic light produced by a gas discharge lamp. The photons that were scattered by the sample were collected through an optical flat at the end of the tube. To increase the sensitivity, highly concentrated and large quantity of samples were used. Due to these difficulties and the advent of IR spectrometers in 1940s, caused a decline in the use of Raman spectroscopy. Nonetheless, the advances in laser technology extremely simplified the Raman instrument, besides increasing their sensitivity.

In late 1970s, Raman spectroscopy along with optical microscope was used for microanalysis in many fields (micro-Raman spectroscopy). Presently, Raman spectroscopy is an extensively used tool for cation Course investigation of specimens in a wide variety of applications. Depending on different applications, several variants to the original Raman instrument have been developed.

# **SELF ASSESSMENT:**

#### **Objective Questions:**

- 1) A laser is typically used for Raman spectroscopy because:
- a. low intensity of the stokes and anti-stokes transitions
- b. requires a visible light source
- c. vibrational transitions have a large  $\Delta E$
- d. monochromators used for Raman spectroscopy absorb IR
- 2) Raman Spectroscopy:
- a. is directly complimentary to Atomic Absorbance Spectroscopy.
- b. provides information about the functional groups present in a molecule
- c. requires a change in dipole-moment of a bond vibration for an observed transition.
- d. frequency shifts are related to electronic changes in the molecule
- 3) Which type of scattering results in a longer wavelength than the incident light?
- a. Rayleigh
- b. Stokes
- c. Anti-Stokes
- 4) Which type of scattering is the strongest?
- Rayleigh a.
- b. Stokes
- c. Anti-Stokes
- 5) Which of these properties must change for a mode to be Raman active?
- a. Volume

- b. Dipole moment
- c. Polarizability
- 6) What are the names of the 3 normal modes of vibration of CO<sub>2</sub>?
- 7) Which modes is/are Raman active?

#### **Subjective Questions:**

- 8) What is the difference between fluorescence and phosphorescence?
- **9)** Can you use the wavelength dependence of Rayleigh scattering to explain why the daytime sky is blue? Exemplify your argument in a semi-quantitative way by comparing two wavelengths close to the extremes of the visible spectrum, e.g., 400nm and 680 nm. Note: for simplicity, assume an equal flux of photons reaches the top of the atmosphere at both wavelengths.
- **10**) Can you provide a similar argument to explain why sunsets are typically red? Note: provide a semi-quantitative argument for the same wavelengths as in question 4c; assume a path length through the atmosphere of 300 km.
- 11) Define the polarizability phenomena and discuss the factors that influence the extent of polarizability in Raman spectroscopy.
- 12) Compare between the advantages and disadvantages of Raman and IR spectroscopy.
- 13) Discuss the fiber-optic sampling in Raman spectroscopy.