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RAMAN SPECTROSCOPY: AN EXPERIMENTAL TOOL TO PROBE MOLECULAR LEVEL PROCESSES

Ms. Tanvi*
Dr. Anamika Mukhopadhyay*

ABSTRACT

Raman Spectroscopy (Marchl, Knoll, & Kiefer, 1988) is an experimental technique to study the molecular interactions and gives molecular fingerprints corresponding to different Raman Spectra. These fingerprints (unique in nature) in turn identify the vibrational and rotational levels and thus a particular molecule. In Raman Spectroscopy, the concentration of the molecule is measured from the intensity of the Raman lines emitted or absorbed which forms the basis for the quantitative analysis. Following the principle of inelastic Raman Scattering, Raman Spectroscopy works on the lines of absorption or emission of radiations by a transparent substance under consideration. The scattered radiations can have energies lower or higher than the incident beam in accordance with the laws of quantum i.e. the energy difference between two allowed rotational or vibrational levels/states. This review article illustrates the classical and quantum viewpoint of Raman Spectroscopy, its experimental design and working, its types and applications. Perturbation of hydrogen bond network of pure water upon addition of salt has described at the end.

KEYWORDS: Raman Effect, Types of Raman Spectroscopy, Salt-Water interaction, Hydrogen bond

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* Research Scholar, Department of Physics, Panjab University, Chandigarh.
* Assistant Professor, Department of Physics, Guru Gobind Singh College for Women, Sector-26, Chandigarh.

Introduction

Raman spectroscopy, which is based on the ‘Raman effect’, is a very powerful non-invasive technique to probe molecular vibration. Hence this spectroscopic method can furnish information about molecular structure and molecular interaction. Raman spectroscopy is complementary to Infrared (IR) spectroscopy. Though both the processes come under the same branch of vibrational spectroscopy, but it is worth mentioning here that the two processes probe different molecular vibrations. Raman spectroscopy is a scattering process, where photon gets scattered by the molecule and the molecular vibrations which involve change in polarizability is finally detected. On the other hand, IR is an absorption technique. A definite advantage of Raman spectroscopy over IR is in case of the measurement of aqueous solution. The later one is limited because of its severe absorption in water. In the Raman process the incident light get scattered by the molecule in different wavelengths and has numerous application in almost all the major areas like medicine (Choo-Smith et al., 2002), pharmacy (Fini, 2004), chemistry (Schlücker, 2014), electronics and many more. This technique holds its specialization in the measurement of the intensity in comparison to its wavelength shift giving unique fingerprints corresponding to different molecules. Biologists and chemists can use these fingerprints to identify the structure as well as the chemical composition of various molecules capable of both qualitative and quantitative examination. Raman Spectroscopy works on the principle of frequency change when the radiations of particular frequency are scattered by the molecules emitted or absorbed by the substrate. It is based on the molecular vibrations across the equilibrium positions of atoms inside a molecule causing change in the molecular polarizability which is directly related with the intensity of Raman Spectra. The strongest detected line on the Raman spectrum is the Rayleigh line with the wavelength equal to the incident wavelength with the Raman scattered lines on both sides of it. The line on the longer wavelength side of the Rayleigh line is the Stoke’s line while the line on the shorter wavelength side is the Anti-Stoke’s line. Due to its lower cross-section, typically of the order of 10^{-30} cm^2, linear Raman Spectroscopy is considered to have limited application especially where the fluorescence excitation takes place. But gradually with time, after the invention laser it has come with much advanced versions of it such as Surface-enhanced Raman Spectroscopy (SERS) (Brown & Milton, 2008), Tip-enhanced Raman Spectroscopy (TERS) (Kumar, Mignuzzi, Su, & Roy, 2015), Resonance Raman Spectroscopy (RR), Stimulated Raman Spectroscopy (Cotter, Hanna, Karkkainen, & Wyatt, 1975) and many more that has enabled its applications to minute imaging, nano-science technology, detection of cancerous cells, in optical fibres etc.

In this review, first we have described the basic theoretical description of Raman Effect. Both the classical and quantum theoretical approaches have been described. Secondly, basic experimental design for Raman spectroscopic method has been described. Subsequently few recently developed experimental techniques have been described. At the end we have described an example explaining the use to Raman spectroscopy to probe the effect of addition of salt on the hydrogen bond network of pure water at the molecular level.

1. THEORY OF RAMAN SPECTROSCOPY:

Here we have described two theoretical approaches i) Quantum viewpoint and ii) Classical approach to describe the Raman scattering process.

i) ***Quantum Viewpoint Of Raman Spectroscopy***: Raman Spectroscopy could easily be understood by the quantum explanation of Raman Scattering. The radiation which is treated as a stream of photons having particular frequency is made to fall on the material under observation resulting in the collision between them. The collision could take two forms- either it could be elastic or inelastic. Elastic collision will let go the radiation (comprising the photons) undeflected even after the collision with the material, hence, resulting in no exchange of energies between them. This forms the case of Rayleigh scattering. The other possibility is the exchange of energies between the material and the radiation. This is known as inelastic scattering. In this case, the material after collision with the photons could gain or lose energies according to the separation between the rotational and vibrational energy levels. The process of gaining of energy from the photons will eventually lead to the scattering of photons with the energy lower than the incident radiation forming the Stokes line of radiation of Raman Spectroscopy. And if the material loses energy on collision with the radiation then the photons scatter with the energy higher than the incident radiation releasing Anti-Stokes line of radiation of Raman Spectroscopy. The energy level diagram corresponding to these three processes is shown in Figure 1. The intensity of Stokes line is more than that of Anti-Stokes line despite the lesser energies. This is because, the lower energy levels have higher population compared to higher energy levels in room temperature according to Boltzmann distribution, leading to the increased intensities.

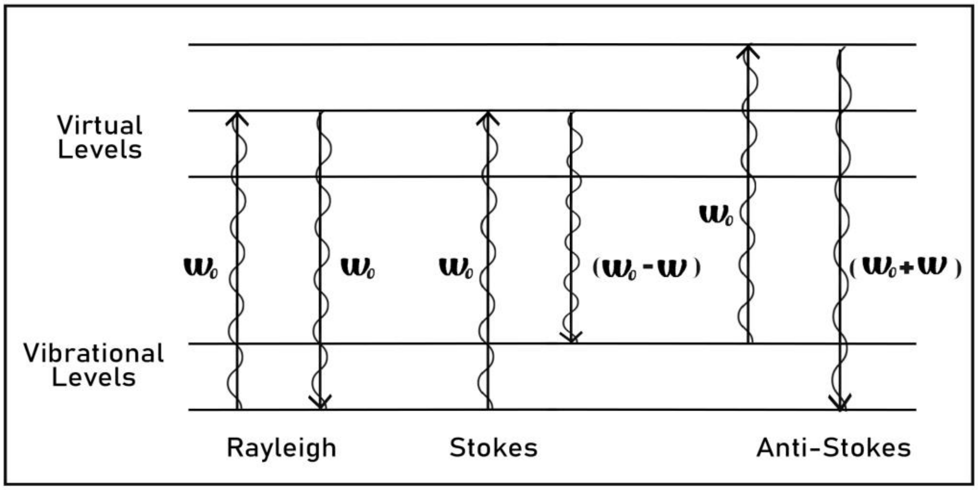

FIGURE 1: The Energy level diagram of Raman scattering process

ii) ***Classical Viewpoint Of Raman Spectroscopy***: The classical theory of Raman Scattering also known as the Polarizability Theory could to a great extent explain the basic concept of Raman Spectroscopy. It was discovered by G. Placzek in 1934. This technique is based on the polarizability of the molecule under the effect of electric field. When we place the molecule under the static electric field (laser light), some distortion or deformation of the molecule takes place. The positive charge of the nuclei is attracted towards the negative pole of the applied electric field and the negative electrons are attracted by the positive pole of the electric field, thus causing the separation between the charges which induces the dipole moment in the molecule. Such a molecule is known as polarized molecule. The size of the induced dipole moment depends on the magnitude of the electric field applied. Due to this distortion / deformation, the molecules vibrate with the particular characteristic frequency. The light after scattering forms three cases: i) scattering with same frequency as incident radiations (Rayleigh scattering), ii) scattering with lower frequency than incident radiations (Stokes line) and iii) scattering with higher frequency than incident radiations (Anti-Stokes line).

2. EXPERIMENTAL DESIGN:

Raman spectroscopy is carried out using an instrument known as Raman Spectrometer (Bumbrah & Sharma, 2016). The schematic of the experimental setup is shown in Figure 2. The design comprises of a laser source for the emission of radiation in region of the electromagnetic spectrum. Then the mirrors are placed to direct the light at the desired location which is followed by an optical lens to make the beam wider. The resultant beam is made to fall on the sample molecule to be studied. Edge filter placed blocks the radiation of original wavelength. This Raman signal is sent to the grating which let the signal to the detector for further examination. In the next section we have described different experimental techniques based on Raman scattering.

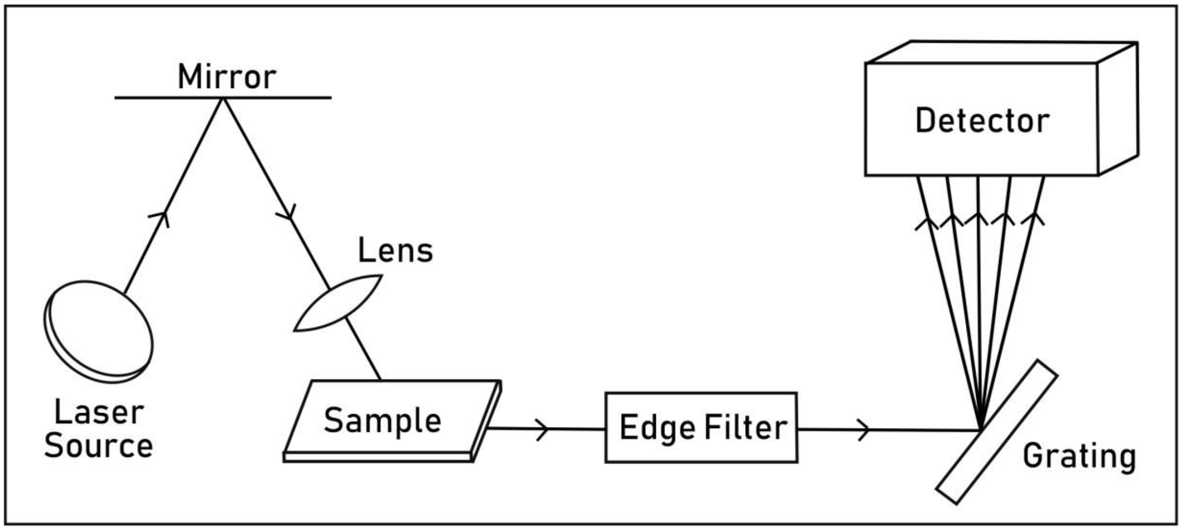

FIGURE 2: The schematic diagram for Confocal Raman Spectroscopy showing all Its major parts in the sequence of their use and function in the analysis

3. DIFFERENT TYPES OF RAMAN SPECTROSCOPIC TECHNIQUES:

i) ***Surface- Enhanced Raman Spectroscopy***: Surface-enhanced Raman spectroscopy (Stiles, Dieringer, Shah, & Van Duyne, 2008) was introduced to overcome the lower quality of the Raman signal and could make the enhancement of the response as high as four orders in the magnitude. The schematic is shown in Figure 3. Offering high sensitivity and selectivity, this technique initiates by the absorption of certain molecule on the analyte’s surface (Sharma, Frontiera, Henry, Ringe, & Van Duyne, 2012) (preferably metal surface) dealing on nanoscale which in turn is polarized for the enhancement of the surface signal.

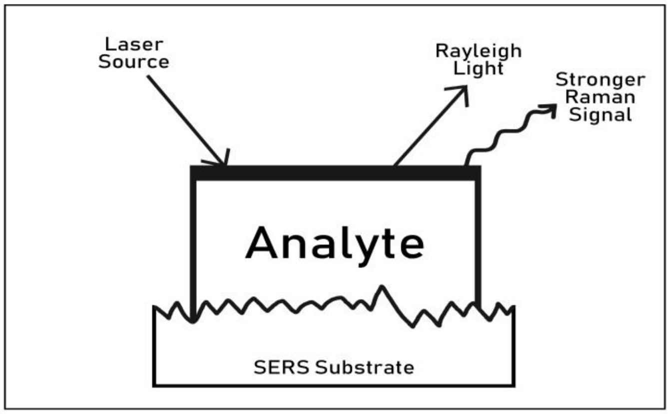

Figure 3: Diagram explaining the phenomenon of Surface-Enhanced Raman Spectroscopy

ii) ***Tip-Enhanced Raman Spectroscopy*:** Tip-enhanced Raman spectroscopy (Yeo, Stadler, Schmid, Zenobi, & Zhang, 2009) is the next step of surface-enhanced Raman spectroscopy. Due to increase in the requirement of Raman imaging, this technique came into picture to analyze the smallest portion of the sample without the interference from the neighbouring portions/areas. The chemical information of Raman spectroscopy is combined with the enhancement results of surface-enhanced Raman spectroscopy along with AFM and STM. The equipments used in TERS and SERS are similar to each other with the difference that TERS used only the metallic tips (majorly of gold) instead of the entire surface which is illuminated with the strong excitation source (laser beam) which produces the electromagnetic radiations at the tip apex, hence, forming a very confined source for the Raman Spectroscopy.

iii) ***Resonance Raman Spectroscopy*:** Resonance Raman spectroscopy enhances the intensity of the spectrum many times as compared to the traditional/spontaneous method of Raman spectroscopy. In this technique, the frequencies of the incident radiation and that of the material under examination are made to resonate (coincide) which greatly enhances the intensity in turn. The energies of the incident photons are made to match the energies needed for the electronic transitions increasing the induce dipole moment (Li, 2003). Limitation of this technique arises when the fluorescence interferes with the Raman spectrum. But this was overcome with the introduction of UV-enhanced resonance spectroscopy.

iv) ***Stimulated-Raman Spectroscopy***: This spectroscopic technique resembles to great extent to the spontaneous Raman spectroscopy apart from the number of laser beams incident on the material under examination. Stimulated Raman spectroscopy (Basiev et al., 1999) uses two laser beams to be incident on the sample. The difference between the coinciding beams is nearly matched with the frequency of rotational/vibrational levels producing enhanced beam for Raman spectroscopy by the excitation of rotational/vibrational levels.

v) ***Coherent Anti-Stoke's Raman Spectroscopy***: One of the latest kinds of techniques that is superior to combustion systems, fluorescent samples, impurities etc., Coherent Anti-Stoke's Raman Spectroscopy (Day et al., 2011) uses two non-linear high power laser beams to be focused on the sample. Generation of coherent beam of relatively higher power of resolution than spontaneous Raman spectroscopy technique takes place. The frequency of one of the laser beams is tuned to match the difference in frequency equal to the Raman active mode's frequency in the Anti-Stoke's region.

4. PRAXIS STUDY OF RAMAN SPECTROSCOPY

Below we have listed the application of Raman spectroscopy in different fields:

1. Raman Spectroscopy is commonly used in the analysis of food comprising its structural analysis, impurity content; distinguish ability and determination of carotenoids in fruits and vegetables.

2. Raman Spectroscopy finds great application in the field of agricultural products in its structural analysis and differentiation of crops as transgenic and normal.

3. In pharmaceutical industry (de Oliveira Penido, Pacheco, Lednev, & Silveira, 2016), Raman Spectroscopy is used for

-Quantitative determination of active substances.

-Identification of the minor components/raw materials.

-Biocompatibility of a material/drug.

4. Raman Spectroscopy has numerous biological applications

-Differentiation of cancerous tissues from normal tissues (Gniadecka et al., 2004).

-Analysis of enzymes (chromophoric samples).

-Detection of single molecules.

-Study of bone structure (Mandair & Morris, 2015).

-Changes related to age (Gamsjaeger et al., 2010).

-Analysis of substrates with large number of active areas.

-Determination of blood oxygenation.

5. In the field of Geology (Liu et al., 2013), Raman Spectroscopy has contributed in the

-Phase transition analysis.

-Determination of nature of minerals under different climatic changes.

-Identification of rare rocks/stones.

-Checking the distribution of minerals in rocks.

6. Chemistry has used Raman Spectroscopy in its varied forms for the

-Identification of flaws and defects in carbon derivatives.

-Study of nanotubes (electrical properties, purity check etc.) (Dresselhaus, Dresselhaus, Jorio, Filho, & Saito, 2002).

-Quality and purity of diamond structures (Sumiya, Toda, Nishibayashi, & Satoh, 1997)

5. EFFECT OF DIFFERENT SALT CONCENTRATIONS ON THE HYDROGEN BONDING NETWORK OF WATER:

Here we will take an example (Mukhopadhyay & Dubey, 2018) to explain the change in hydrogen bond network in water on addition of different salt concentrations through Raman Spectroscopy. Different Raman spectra were obtained by dissolving two different salts (Ammonium Chloride NH_4Cl (salt (1)) and Ammonium Sulphate $(NH4)_2SO_4$ (salt (2)) of varying concentrations in water. Renishaw Invia Raman Microscope was used to record Raman Spectra of pure water (Walrafen, 1964) and different salt solutions. External calibration of the instrument was done using silicon wafer prior to recording sample spectra. A plane glass slide was used to get Raman Spectra for solutions of different salt concentrations. The scattered light was detected in a back scattering geometry. A thermo-coupled device helped detect the light dispersed. The processed signal sent via Wire3.1 software was finally analysed.

The OH stretch region was recorded in the range of 2700-4000 cm^{-1}. The broad feature can be assigned to four peaks as shown in Figure 4.

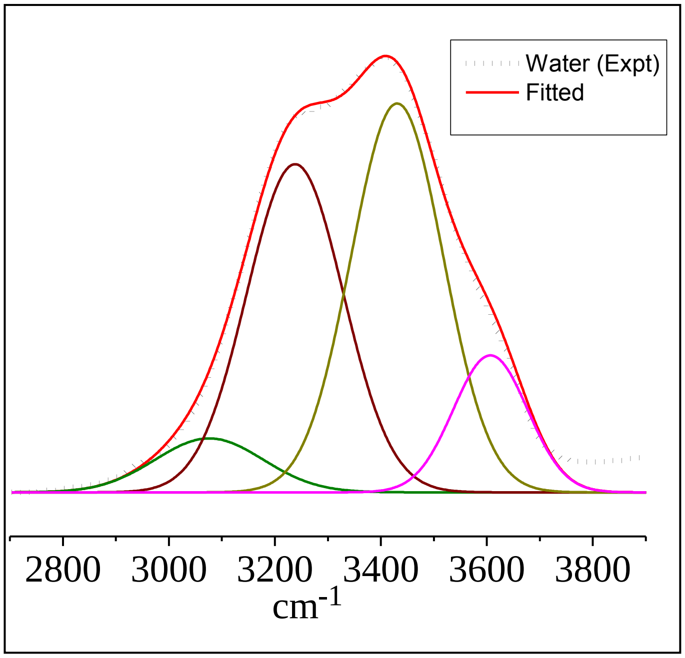

Figure 4: Raman spectrum of pure water in the frequency range 2700-3900 cm^{-1}. Both the experimental (black dot) and the fitted (red line) spectra are shown here. The experimental spectrum is fitted with four peaks.

The peak positions of the four peaks fitted to pure water are listed in the following table.

(3600+40) cm^{-1}	OH stretch of partially H-bonded network of water
(3430+20) cm^{-1}	OH stretch of partially H-bonded network of water
(3240+30) cm^{-1}	OH stretch of fully H-bonded network of water
(3080+20) cm^{-1}	OH stretch of fully H-bonded network of water

It was noticed from the spectra that, on increasing the concentration of salt (2), the intensity of low frequency side increase, which further indicates the increase in hydrogen bonded network in the solution compared to pure water whereas, decrease in this network was observed on increasing the concentration of salt (1). The spectra for two salt solutions are shown in Figure 5. Both the salts (1) and (2) showed entirely opposite behavior.

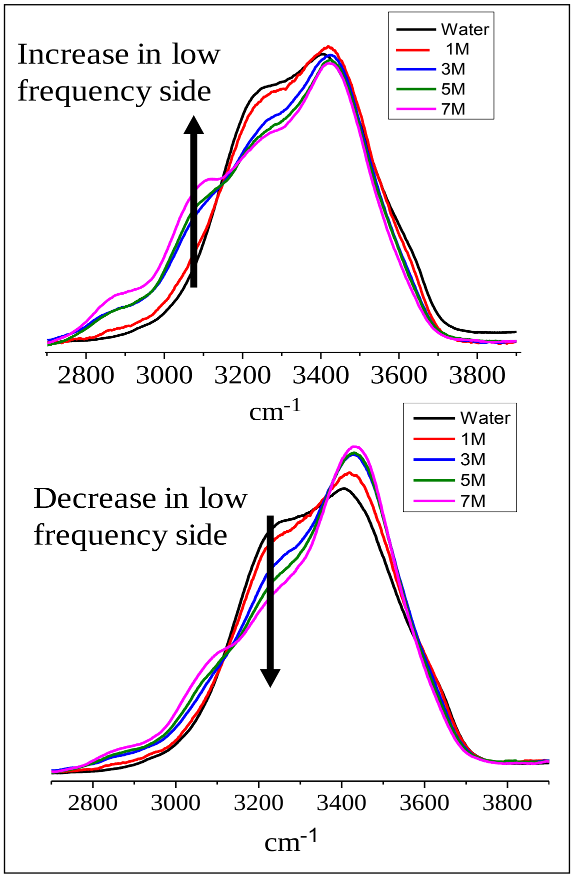

Figure 5: The effect of variation of salt concentrations on the O–H stretching region of bulk water. The top and the bottom panel represent the ammonium sulphate solutions and the ammonium chloride solutions, respectively. With increasing salt concentrations, the low frequency side of the spectrum increase in case of ammonium sulphate salt and decrease in case of ammonium chloride salt as indicated by the black arrow.

5. SUMMARY:

Raman Spectroscopy is one of the most versatile tools used in recent times. This review gives an overview of this technique in a simple and comprehensive way. The principle, various theories and instrumentation of Raman Spectroscopy has been presented in a brief explanatory manner. Thereafter, discussed are the various types and branches of Raman Spectroscopy providing different approaches to different problems. Demonstration of a wide range of applications of Raman Spectroscopy from biological and medical to forensic studies proved its great potential for its continual use in future. Lastly, this review explains a nice example to describe in detail the Raman technique through various Raman Spectra (of water and added salt solutions at various concentrations).

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