# Raman spectroscopy

## Introduction and underlying Theory

Raman spectroscopy is a spectroscopic technique named after and discovered by Sir Chandrasekhara Venkata Raman in 1928. Sir Raman published work in 1922 on the “ The Molecular Diffraction of Light” which was the initial investigation which ultimately led to the discovery of Raman Spectroscopy (1). Along with associates Sir Raman investigated this newly discovered spectroscopy and was the first Asian person to be awarded the Nobel Prize for Physics in 1930(2).

Raman Spectroscopy is a light scattering technique. It can be described as a process where a photon of light interacts with a sample to produce scattered radiation of different wavelengths. When monochromatic light is focused upon a sample, it will interact with the sample. The light may be deflected, absorbed or scattered in some fashion (3).

On analysis of the frequency of the scattered radiation, a small amount of radiation is seen which is scattered at different wavelengths, and also the incident radiation wavelength. This incident radiation wavelength is known as Rayleigh Scattering. The small amounts of the radiation scattered at different wavelengths is known as Stokes and Anti-Stokes Raman Scattering (3). Described by Lord Rayleigh, Rayleigh Scatter is the scattering process without any change in frequency. Depending on the vibrational state of the molecule, Raman shifted photons of light can be of higher or lower energy.

Approximately only 1 x 10-7 of the scattered light is Raman. The change in wavelength of the scattered photon provides the chemical and structural information (3).

As mentioned above, it has been established that light which is scattered from a molecule has various components – the Rayleigh scatter and the Stokes and Anti-Stokes Raman scatter. The Raman Effect being due to photons being scattered inelastically, losing or gaining energy as result of their interactions with the vibrational molecules of the sample (17). These frequencies are in the range associated with rotational, vibrational and electronic level transitions. Due to the scattered radiation occurring in all directions there may also be noticeable changes in the polarisation and wavelength of the molecule (3).

The stronger of the two processes is Stokes Scattering where photons are scattered at low energy. The population state of a molecule is generally in its ground vibrational state; this is the larger Raman scattering effect. When a small number of molecules are at a higher vibrational level, photons can be scattered at higher energy. This is the weaker Anti-Stokes Raman scattering.

Incident photons will react with the present molecule, and the energy lost or gained by a photon aid identification of the type of bond present (3). Depending on the symmetry of the molecule not all vibrations will be observed with Raman spectroscopy. However enough information is attained in most cases to establish some identity of the molecular structure.

Changes in vibrational, rotational and electronic energies of a molecule can cause Raman scattering to occur. The energy of the vibration of the scattering molecule is equal to the difference in the energy between the incident molecule and the Raman scattered photon (4).

The process of light absorption requires the energy of the incident photon to be equal to the difference in energy between two states of the molecule, and the transition between the two states being accompanied by a dipole moment change in the molecule. The molecule will now be found in an electronic excited state or a vibrational excited state (5).

Photons that interact with a molecule, but are not absorbed, will be scattered and the incident photons will not need to be between the two states of the molecule for scattering to occur. Here the photon polarizes the electronic cloud of the molecule and this causes the formation of a virtual excited state. This is an extremely short lived excited state and the energy of the photon will be re-radiated.

During re-radiation of the photon, the photon is said to be elastically scattered (5).

## Instrumentation

The Raman system typically consists of four major components:

i) Excitation Source (Laser)

ii) Sample illumination system and light collection optics

iii) Wavelength selector (Filter or Spectrophotometer)

iv) Detector (Photo diode array, Charged Coupled Devices or Photomultiplier Tubes)

The sample is illuminated with a laser beam in the Ultraviolet, Visible or near-infrared range. The cheapest light source is the He/Ne laser at 638.

2nm. A better source is the Argon ion laser at 488. 0 nm. However the best instruments now use the near IR Nd-YAG (Neodymium ions in Yttrium aluminium garnet) laser at 1064nm(6). A lens collects scattered light and sends it through an interference filter or spectrophotometer to obtain the Raman spectrum of a sample (7).

As random Raman scattering is weak, the main difficulty of Raman spectroscopy is separating the Raman scattering from intense Rayleigh scattering. A point of concern is the fact the intensity of stray light from the Rayleigh scattering may exceed the intensity of the Raman Effect. By cutting the spectral range close to the laser line where stray light has most effect, this problem can be overcome. Commercially available interference filters are used which cut off the spectral range of ± 80-120 cm-1 from the laser line (7). This method does not permit the detection of low frequency Raman modes in the range below 100 cm-1.

Upon light dispersion on gratings, stray light is generated in the spectrometer. The quality of light produced depends on the condition and quality of the grating. Holographic gratings are generally used in Raman spectrometers. These have less manufacturing defects than the ruled gratings. Stray light produced from the ruled gratings is more intense than stray light produced from the holographic grating. Reducing stray light can also be done using multiple dispersion stages. Using multiple dispersion stages without the use of notch filters, Raman frequencies as low as 3 – 5 cm-1 can be detected (7).

Over the years analysts have used single-point detectors such as photon-counting Photomultiplier Tubes (PMT). To obtain a Raman spectrum of decent quality, longer exposure times are often required. This is due to the weakness of a typical Raman signal. In recent times, there has been an increase in laboratories worldwide where researchers are using multi-channel detectors like Photo diode Arrays (PDA), or, Charge-Coupled Devices (CCD)for detecting Raman scattered light. Sensitivity and performance of modern CCD detectorsare improving and so are becoming the detectors of choice for Raman spectroscopy (7).

## Comparison to Infra-red Spectroscopy

Raman spectroscopy combines the advantages of Near-IR spectroscopy with the advantages of Infrared spectroscopy. Both Raman and Infrared spectroscopy excite fundamental molecular vibrations even though their fingerprinting techniques are based on different physical processes (8). In infrared a vibrational mode of the molecule is required to have a change in dipole moment. Radiation of the same frequency can now interact with the molecule and elevate it to an excited vibrational state. In Raman spectroscopy, scattering involves the distortion of electrons around a bond, with remission of the radiation as the bond returns to its normal state (16). Water does not interfere with Raman spectroscopy and so Raman is more useful than infrared when working with aqueous samples. Raman is also quite useful for symmetrical molecules which have zero dipole moment. These molecules are not suitable for Infrared (8).

Raman spectroscopy also requires little sample preparation in comparison to Infrared spectroscopy. In line process control and remote analysis is also

possible with Raman. Infrared is only used for qualitative analysis, whereas with Raman quantitative and qualitative analysis is possible. Glass containers are also used in Raman. Infrared Spectroscopy doesn't have problems with background fluorescence, where in Raman; fluorescence is notorious and can even mask the spectra. The sample can also be damaged by laser light in Raman (5).

## Advantages & Disadvantages

With the increasing technological advances in computer and laser design, Raman spectroscopy has becoming a more viable option for routine analysis in laboratories worldwide (11).

Raman spectroscopy has a number of advantages over other analysis techniques. Because Raman spectroscopy is a scattering process, samples of any size or shape can be used.

Very small amounts of material can be studied down to microscopic levels in the range of 10 microns. The region from 80-500 cm-1 can be studied with no changes on the same instrument (9). Raman spectroscopy can be used with solids, liquids or gasses. As mentioned above no sample preparation is needed and it is a non destructive technique. There is no concern with sample thickness, size or shape.

Furthermore samples can be analysed directly in bottles, bags or blisters (11).

Also no vacuum is needed which saves on various expensive pieces of vacuum equipment. In terms of time, the spectra are produced relatively

quickly. Further advantages include use of aqueous solutions, use of glass and the use of down fibre optic cables for remote sampling (10).

Disadvantages include that metals or alloys cannot be used. The Raman Effect is very weak, which leads to low sensitivity, making it difficult to measure low concentrations of a substance (10). Sample heating through the intense laser radiation can destroy the sample or mask the Raman spectrum. Serious problems in Raman occur when large background signals from fluorescence from impurities or the sample itself arise (8).

Other disadvantages of Raman spectroscopy include equipment cost and the sensitivity of the technique. Raman spectrophotometers can be quite costly, depending on their applications, and the technique generally cannot compete with chromatography for analytical sensitivity in quantitative analyses (11).

## Applications & the Future of Raman Spectroscopy

Raman spectroscopy is an important analytical and research tool, being used for applications as wide ranging as pharmaceutical, forensic science, thin films, polymers, geology and planetary science, arts, and semiconductors.

Currently there are many applications of Raman Spectroscopy. Some examples of the many in practice include, Surface Enhanced Raman Spectroscopy, Resonance Raman Spectroscopy, Surface Enhanced Resonance Raman spectroscopy, Hyper Raman, Spontaneous Raman, Coherent Anti-Stokes Raman, and Transmission Raman.

Surface Enhanced Raman spectroscopy is a technique in which the sample is adsorbed onto a colloidal metal particle surface. Silver or gold is usually used. The adsorbed molecule produces Raman lines on spectra which are enhanced by 103 to 106(17).

Currently a few uses of Raman spectrometry are on the edge of being groundbreaking in the fact Raman hadn’t been used in this way previously. An example is Raman’s use in Urology. Here it has been used to detect changes at the molecular level during the pathological transformation of biological tissue. Raman spectrometry has shown some encouraging results in the in vitro diagnosing of cancers of the bladder and prostrate. Raman showed itself to be an exciting tool for real time diagnosis and in-vivo evaluation of living tissue (12).

Another similar study showed that Raman can be used to accurately identify benign prostatic hyperplasia at three different grades of prostatic adenocarcinoma in vitro (13).

Raman spectroscopy has been useful in dentistry to study dental hard tissue and calculus. The interest here is the mineral components in enamel, dentin and calculus, and to calcium fluoride formed on/in enamel (14). Raman spectroscopy recently has been used in coronary artery disease with pleasing results which encourage its use further in that particular field. A 1. 5mm Raman catheter capable of collecting Raman spectra in the fingerprint and the high-wave number regions is used to measure the chemical and molecular composition of coronary atherosclerotic lesions. Results showed

that distinct spectral differences can be identified by intracoronary Raman spectroscopy in vivo (15).

Overall it is fair to say that Raman spectroscopy is not always regarded as the first choice for analysis. However, it is undoubtedly a vital analytical tool available, which has some clear advantages over other methods of analysis. If these advantages and Raman's other beneficial attributes are exposed more, Raman spectroscopy could once again be back in the minds of analytical chemists worldwide.

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