Dr. K. Bhavya Sri et al/J. Pharm. Sci. & Res. Vol. 14(7), 2022, 776-782

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Raman Spectroscopy in Reverse Engineering

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Abstract:

This article aims to explain about deformulation of existing drug products using Raman imaging. Raman spectroscopy in reverse engineering can be used when a generic company wants to develop an innovator product and for the purpose of submission, safety and efficacy data from the innovator can be applied. Raman spectroscopy is a branch of vibrational spectroscopy, which allows an easy interpretation and highly sensitive structural identification of trace amounts of chemicals based on their unique vibrational characteristics. The main reason for using Raman spectroscopy in deformulation (reverse engineering) is fewer samples (1-2 mg) are needed, and samples are treated to a minimum, which is not only suitable for drug powder but also for rapid and non-destructive detection and analysis of liquid and solid preparations. Raman spectroscopy can solve some of the problems in reverse engineering analysis, such as the crystal identification of active ingredients (API), the determination of ingredients in the original preparation, and the analysis of process parameters, such as API distribution, coating layer thickness, coating layer number, production process, and spatial distribution related to dissolution characteristics

Keywords: Deformulation, Detectors, Raman spectroscopy, Sources, Sample illumination system.

INTRODUCTION

Also known as deformulation. Deformulation is defined as the separation, identification, and quantitation of ingredients in a formulation. Extractions and specialized instrumental techniques are involved in deformulation to identify and quantify the components of a complex mixture. The innovator's formulation may include components such as active pharmaceutical ingredients, polymers, plasticizers, fillers, stabilizers, lubricants, antioxidants, flame retardants, etc.[1] Deformulation can be explained in a simple term as the analysis of a product to identify and quantify product components by breaking it in a lab. Deformulation can be known as "chemical reverse engineering,"

this procedure enables scientists to take a medicine and discover the contents of its core and coatings. Reverse engineering is the method to create a generic version of medication and requires various chemical processes to discover the exact contents of a formula. Pharmaceutical deformulation, which is the reverse engineering of a product's formulation, is an important process to create generic drugs. Before the innovator's patent expires, skilled chemists will perform a detailed analysis of the product to identify, quantify, and characterize the active pharmaceutical ingredients (APIs) and their excipients, whether it's a drug or supplement in the innovator's formulation.[2] Successful generics companies are the first to market after patent expiration, bringing bioequivalent products to pharmacies as quickly as possible.[3] For a generic company to get endorsement under ANDA (Abbreviated New Drug Application), it should show a bioequivalent product. The levels of the active ingredient in the blood must be highly similar in the generic composition to the original product. The FDA, however, won't give it out to any generics company because it considers the innovator's formula proprietary. Because of this the generic company lacks the guidance and creates the need for pharmaceutical reverse engineering to prove bioequivalence and discover a manufacturing method for the new medication [4]

Levels of Pharmaceutical Reverse Engineering are:

Pharmaceutical deformulation can be a complex process and will differ depending on the composition of the drug in question.

The first step begins with level 1 deformulation, in which ingredients are identified and quantified. At this level, quantitation is not performed. At this level, label claims are verified and assist a chemist with the formulation. Deformulation levels 2nd and 3rd are to quantitate the components of the formulation, including coating systems and ingredients, used to provide the taste and will ensure the FDA approves the ANDA quickly. Once the quantitative formula is decoded, the components have to be separated and individually quantified using gravimetric or other detection tools, like UV light, IR, and Raman spectroscopy**.** To ensure similar stability and dissolution profile, as well as to pass the ANDA the new generic company must use the same polymorphic form as the RLD (Reference Listed Drug). After you have the formula, it's necessary to select a suitable manufacturing method, such as dry and wet granulation, direct compression, or other methods, based on the drug properties.

Advantages of Reverse Engineering

Instability and bio studies, product failure risk will be less. The negative impact on the environment will be minimum. We will have information on how the competitors' products work. The design of defective products will improve. Manufacturing techniques will be optimized and the right material will be selected. After the process of de-formulation is completed, the data can be used by an expert to know how the ingredients are combined to form the pharmaceutical product. [5]

Raman Spectroscopy

Spectroscopy is the study of the interaction of electromagnetic radiation with matter. Spectroscopic methods are based on the phenomenon of emission, absorption, fluorescence, or scattering. Raman spectroscopy is an analytical technique where the vibrational energy modes of the sample are measured by using scattered light. C. V. Raman an Indian physicist,

was the first to observe Raman scattering in 1928 along with his research partner K. S. Krishna. From Raman spectroscopy, we get both chemical and structural information about substances, as well as the identification of substances through their characteristic Raman 'fingerprint'.

This spectroscopic method is versatile for the analysis of various types of forensic samples. It resolves most of the disadvantages with other spectroscopic techniques. It can be used for both qualitative as well as quantitative purposes. By measuring the frequency of scattered radiations Qualitative analysis can be performed whereas quantitative analysis can be performed by measuring the intensity of scattered radiations.

The basic principle of Raman spectroscopy is a scattering technique, which is based on the Raman Effect, i.e., the frequency of a small fraction of scattered radiation is different from the frequency of monochromatic incident radiation. It's far primarily based on the inelastic scattering of incident radiation via its interplay with vibrating molecules. It probes the molecular vibrations [6]. Figure 1 shows the principle of Raman Spectroscopy.

In Raman spectroscopy, the sample is illuminated with a monochromatic laser beam which interacts with the molecules of the sample and originates a scattered light. The scattered mild having a frequency exceptional from that of incident light (inelastic scattering) is used to construct a Raman spectrum. Raman spectra arise because of inelastic collision among incident monochromatic radiation and molecules of the sample. Whilst monochromatic radiation strikes at the pattern, it scatters in all instructions after its interplay with sample molecules. [7] Much of this scattered radiation has a frequency that's the same as the frequency of incident radiation and constitutes Rayleigh scattering. Simplest a small fraction of scattered radiation has a frequency distinct from the frequency of incident radiation and constitutes Raman scattering. While the frequency of incident radiation is higher than the frequency of scattered radiation, Stokes strains seem in the Raman spectrum. The magnitude of Raman shifts does now not depend upon the wavelength of incident radiation. [8]Raman scattering depends on the wavelength of incident radiation. An alternate in polarizability at some point of molecular vibration is a critical requirement to achieve the Raman spectrum of the sample. Because Raman scattering because of water is low, water is an ideal solvent for dissolving samples. Glass can be used for optical components (mirror, lens, sample cell) in Raman spectrophotometer however whilst the frequency of incident radiation is lower than the frequency of scattered radiation, anti-Stokes strains appear in Raman spectrum. Scattered radiation is typically measured from a proper perspective to incident radiation. Stokes shifted Raman bands contain the transitions from decrease to better strength vibrational degrees and consequently, Stokes bands are greater excessive than anti-Stokes bands and therefore are measured in traditional Raman spectroscopy even as anti-Stokes bands are measured with fluorescing samples because fluorescence reasons interference with Stokes bands. Figure 2 shows three types of scattering processes that can occur when light interacts with a molecule.

Raman spectrum is presented as an intensity-versus wavelength shift. Raman spectra can be recorded over a range of 4000–10 cm1 (10). Raman active normal modes of vibration of organic molecules occur in the range of 4000– 400 Dcm1. Depending on the spectrophotometer's design and optical components, typical Raman spectra cover the wavenumber region between 400–5 Dcm1 and 4000– 3800 Dcm1(8). A Raman spectrum is drastically less complicated than their Infrared (IR) opposite numbers because, in ordinary Raman overtones, aggregate and difference bands are uncommon.

INSTRUMENTATION

Block diagram of Raman Spectrophotometer is shown in Figure 3.Components of Raman spectroscopy are

1. Source
2. Sample
3. Illumination system
4. Detectors

Raman instrument is shown in figure 4.

1. Source:

Modern Raman spectroscopy nearly always involves the use of lasers as excitation light sources. Because lasers were not available until more than three decades after the discovery of the effect, Raman and Krishnan used a mercury lamp and photographic plates to file spectra. Mercury arc lamp becomes used as a light source in Raman spectrophotometers in the early days. 435.8 nm line of coiled low-pressure mercury arc lamp was used as a light source until the 1960s. In the late 1960s, Laser sources became available and replaced the mercury lamp completely. [9]

1.1. Lasers:

The resources used in current Raman spectrometry are almost continually lasers due to the fact their high intensity is necessary to produce Raman scattering of high intensity to be measured with a reasonable signal-to-noise ratio. Due to the fact, that the intensity of Raman scattering varies as the fourth energy of the frequency, argon and krypton ion assets that emit within the blue and inexperienced vicinity of the spectrum have an advantage over the opposite sources. Nowadays sources used are lasers because they have a high intensity which is necessary to produce Raman scattering. These laser sources provide a stable and intense beam of radiation [10]Laser used in Raman Spectrometer is shown in figure 5. Laser sources for Raman spectroscopy are given in table 1.

2. Sample Illumination System

2.1. Liquid Samples: An advantage of sample handling in Raman spectroscopy when compared with infrared is water is a weak Raman scattered but a strong absorber of infrared radiation. This is the reason why the aqueous solutions can be studied by Raman spectroscopy but not by infrared spectroscopy. This advantage is specifically crucial for biological and inorganic systems and in studies dealing with water pollution troubles. [11]

2.2. Solid Samples: Raman spectra of solid samples are often acquired by filling a small cavity with the sample after it has been ground to a fine powder. Polymers can normally be examined immediately without a sample pre-treatment. [12]

2.3. Gas samples: Gas is normally contained in glass tubes, 1-2 cm in diameter and about 1mm thick. Gases can also be sealed in small capillary tubes

3. Filters

In Raman spectroscopy, an extreme laser beam is used to excite a pattern and the Raman "fingerprint" is measured by way of a dispersive or Fourier transform spectrometer. Optical filters are used to prevent the undesired light from reaching the spectrometer and drowning out the noticeably weak Raman signal. There are four basic types of filters to choose from: a long wave pass (LWP) edge filter, a short-wave pass (SWP) edge filter, a notch filter, and a laser line filter (each shown below). While passing through longer and shorter wavelengths, the laser line filters transmit only the laser light and all other lights are blocked, while the notch filter blocks only the laser line. With the aid of the usage of those two filters collectively, each Stokes and Anti-Stokes Raman scattering may be measured concurrently. [13]An edge filter can provide a superior alternative, as they offer the narrowest transition to see Raman signals extremely close to the laser line. Different filters in Raman spectrometer is shown in figure 6.

4. Detectors

For Fourier Transform (FT) detection methods, Raman scattered light is typically collected and either dispersed by a spectrograph or used with an interferometer. In many cases commercially available FT-IR spectrometers can be modified to become FT-Raman spectrometers.

Detectors for dispersive Raman

In most cases, modern Raman spectrometers use array detectors such as CCDs. various types of CCDs exist which are optimized for different wavelength ranges. Intensified CCDs can be used for very weak signals and/or pulsed lasers the spectral range depends on the size of the CCD and the focal length of the spectrograph used. It was once common to use monochromators coupled to photomultiplier tubes. In this case, the monochromators would need to be moved to scan through a spectral range.

Detectors for FT–Raman

FT–Raman is almost always used with NIR lasers and appropriate detectors must be used depending on the exciting wavelength. Germanium or Indium gallium arsenide (InGaAs) detectors are commonly used.

Many detectors are available some of them are explained below:

4.1. Photomultiplier Tube

Photomultiplier tube diagram is shown in figure 6. Photomultiplier tubes (photomultipliers or PMTs for short), members of the class of vacuum tubes, and more specifically vacuum phototubes, are extremely sensitive detectors of light in the ultraviolet, visible, and near-infrared ranges of the electromagnetic spectrum. These detectors multiply the current produced by incident light by as much as 100 million times or 10^8 (i.e., 160 dB),[1] in multiple dynode stages, enabling (for example) individual photons to be detected when the incident flux of light is low.

4.2. Charged Coupled Detector

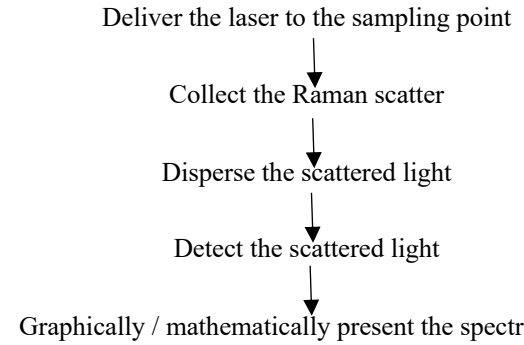
Charge-coupled device (CCD) is shown in Figure 7. A charge-coupled device (CCD) is an integrated circuit containing an array of linked, or coupled, capacitors. Under the control of an external circuit, each capacitor can transfer its electric charge to a neighboring capacitor. CCD sensors are a major technology used in digital imaging.

In a CCD for capturing images, there is a photoactive region (an epitaxial layer of silicon), and a transmission region made out of a shift register (the CCD, properly speaking).

An image is projected through a lens onto the capacitor array (the photoactive region), causing each capacitor to accumulate an electric charge proportional to the light intensity at that location. A one-dimensional array, used in line-scan cameras, captures a single slice of the image, whereas a two-dimensional array, used in video and still cameras, captures a two-dimensional picture corresponding to the scene projected onto the focal plane of the sensor. Once the array has been exposed to the image, a control circuit causes each capacitor to transfer its contents to its neighbor (operating as a shift register). The last capacitor in the array dumps its charge into a charge amplifier, which converts the charge into a voltage. By repeating this process, the controlling circuit converts the entire contents of the array in the semiconductor to a sequence of voltages. In a digital device, these voltages are then sampled, digitized, and usually stored in memory; in an analog device (such as an analog video camera), they are processed into a continuous analog signal (e.g. by feeding the output of the charge amplifier into a low-pass filter), which is then processed and fed out to other circuits for transmission, recording, or other processing

WORKING:

Working of Raman spectrometer is shown in figure 9.



DIFFERENT TYPES OF RAMAN SPECTROSCOPY

There are now more than 25 different types of known Raman spectroscopy techniques.

A variety of Raman instruments and special techniques are used for the analysis of cultural heritage materials. The choice of the instrument determines the sensitivity,

spectral range and resolution, spatial resolution, availability of different excitation sources, and convenience of operation.

1. Spontaneous Raman
2. Hyper-Raman scattering
3. Fourier transforms Raman scattering
4. Raman-induced Kerr effect spectroscopy
5. Stimulated/coherent Raman scattering
6. Resonance Raman spectroscopy (RRS)
7. Surface-enhanced Raman spectroscopy (SERS)
8. Micro-Raman spectroscopy
9. Nonlinear Raman spectroscopic techniques

Some of the types are discussed below:

1. Micro-Raman spectrometer (or Raman microscope) is the most common bench-top Raman instrument. A high-resolution spectrometer (either dispersive or FT) and one or numerous laser assets are coupled via an optical microscope. The excitation beam is focused and the secondary emission is accumulated concurrently using the microscope goal in backscattering geometry. An excessive-numerical aperture (NA) objective yields both a high spatial decision and a high collection efficiency.

2. Surface-enhanced Raman spectroscopy (SERS) involves inelastic light scattering by molecules placed close to Nano metal surfaces, which amplify the scattering by plasmonic resonance. One approach is to study molecules adsorbed onto corrugated metal surfaces such as silver or gold nanoparticles. Another approach is to stimulate the molecules with a sharp metal tip. Such tip-enhanced Raman spectroscopy is typically implemented by combining a confocal microscope and a scanning probe microscope.

3. In Resonance Raman spectroscopy (RRS) the incident photon strength is near electricity to a digital transition of compound or material under examination

In a portable Raman spectrometer, a miniature dispersive spectrometer and a small laser source are integrated into a portable, hand-held device. Consequently, the device can be used to carry out in situ analysis in museums, archives, also outdoors on archaeological websites for the analysis of mural or cave artwork. Such portable gadgets frequently employ a fiber-optic probes. [14]. Information from Raman Spectroscopy is shown in figure 10.

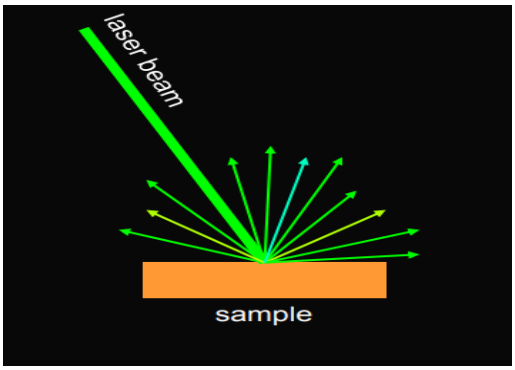


Figure 1: Principle of Raman Spectroscopy

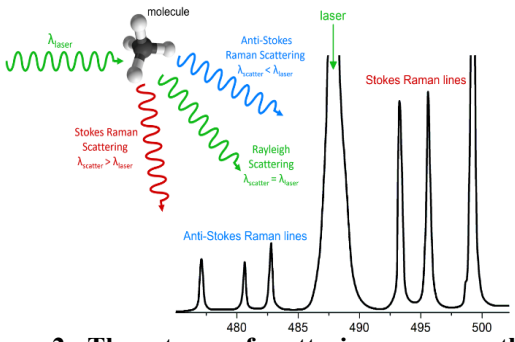


Figure 2 : Three types of scattering processes that can occur when light interacts with a molecule.

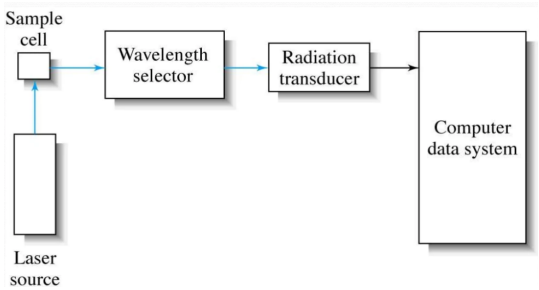


Figure 3: Block diagram of Raman Spectrophotometer

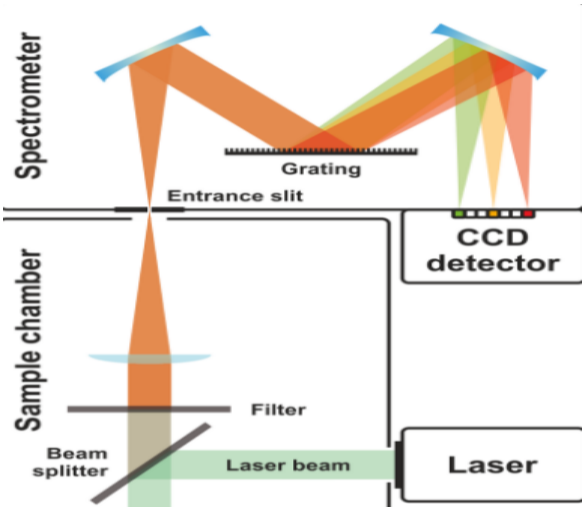


Figure 4: Raman Instrument

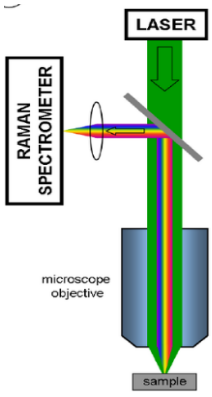


Figure: 5 Laser used in Raman Spectrometer

Table: 1 Laser sources for Raman spectroscopy

Laser Type	Available wavelengths (nm)
Argon ion (Ar^+)	364, 457, 488, 514.5 (VIS)
Nd-YAG	1064 (Near-IR) or 532 (frequency-doubled) (VIS)
He-Ne	632.8 (VIS)
Laser diodes	785 830 (Near-IR)

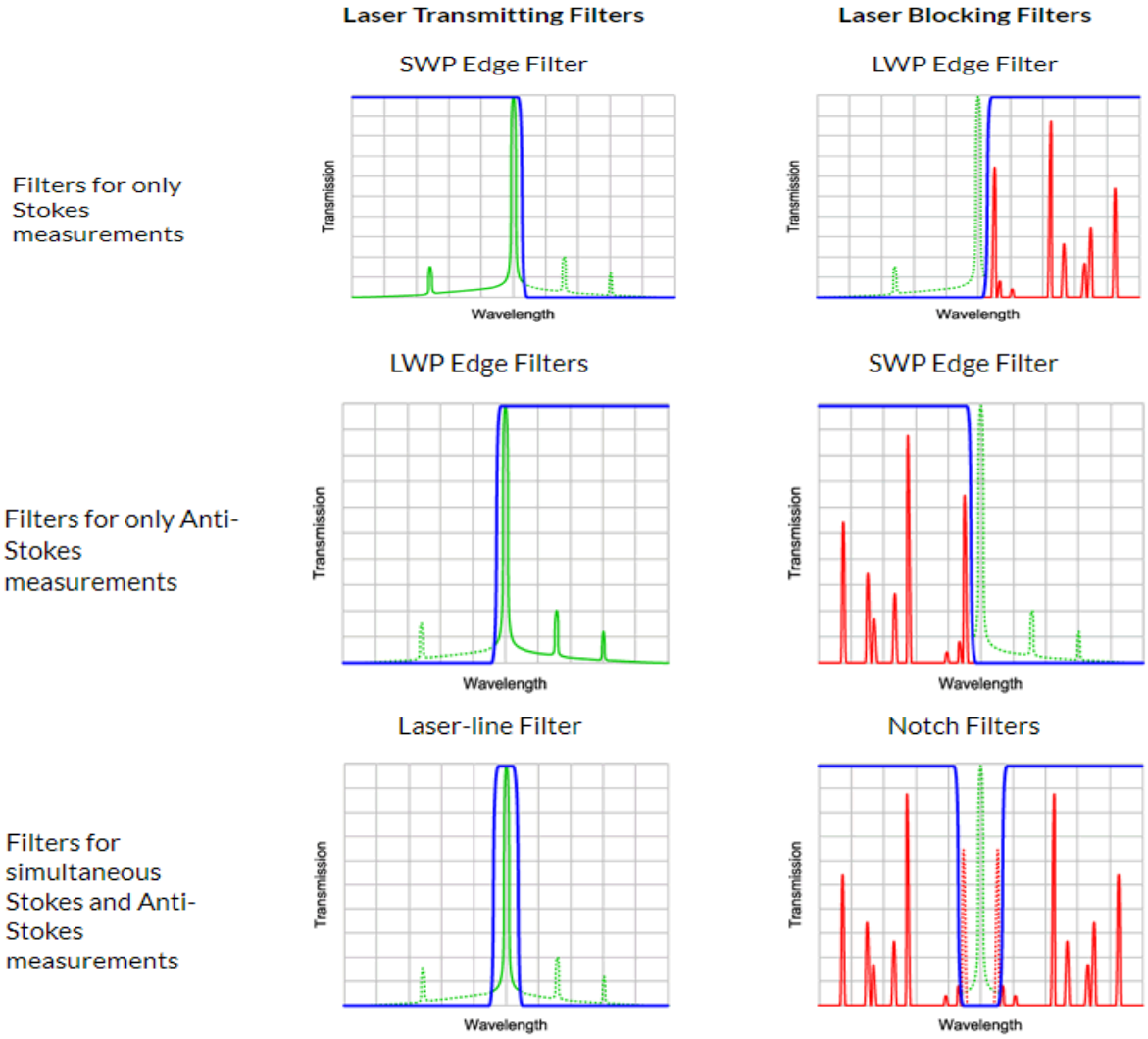


Figure 6: Different filters in Raman spectrometer

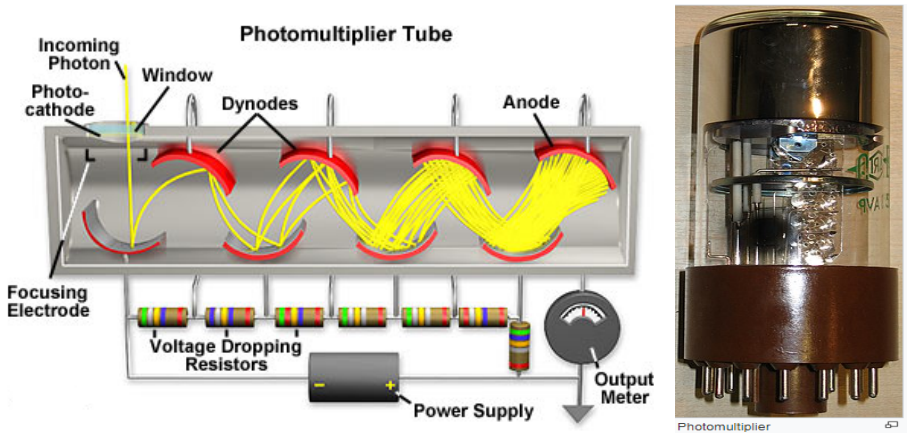


Figure 7: Photomultiplier tube

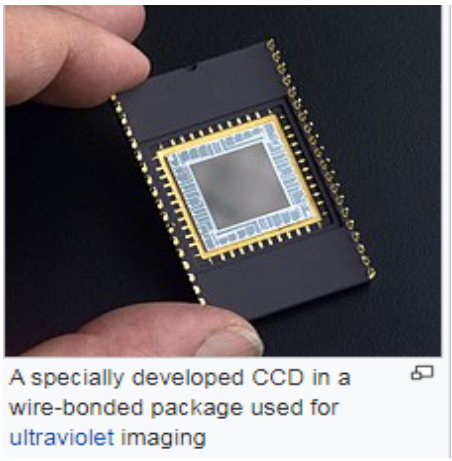


Figure 8: Charged couple detector

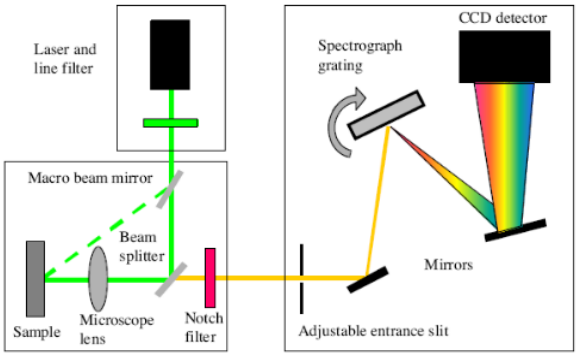


Figure 9: working of Raman spectrometer

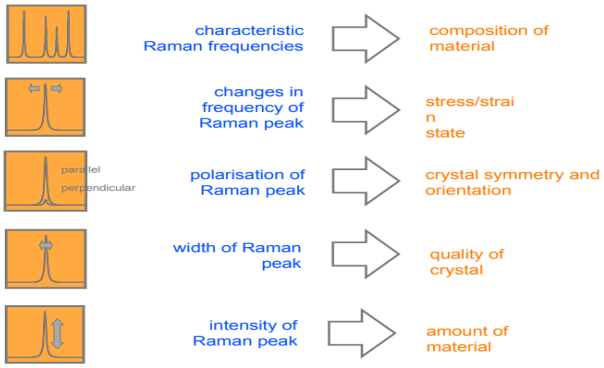


Figure 10: Information from Raman Spectroscopy

CASE STUDIES

1. Using Raman Imaging for Deformulating Drugs

This article examines the reverse engineering of existing drug products using Raman imaging. There are a number of regulatory routes with the FDA through which a company can seek approval for a generic drug. The Abbreviated New Drug Application (ANDA) route is the most notable. This is when an exact copy of the innovator product is developed by a generic company and the safety and efficacy data from the innovator can be applied for the purpose of their submission.

A drug must exhibit Q1, Q2 and Q3 sameness in order to be considered for a biowaiver

Q1	Qualitative similarity: the test and reference products contain the same active and inactive ingredients.
Q2	Quantitative similarity of composition: the same amounts of active and inactive ingredients.
Q3	Structural sameness (currently reserved for topical medications): equivalent microstructure

Conclusion from this case study:

Innovator and generic products can be successfully characterized using RA802. It is also able to facilitate deformulation activities.

The components, concentrations, particle sizes and distributions of the innovator formulation can be characterized by RA802, which allows generic followers to alter their processes in order to develop equivalent products, or products which are strategically different [15]

2. Reverse Engineering Applied to Red Human Hair Pheomelanin Reveals Redox-Buffering as a Pro-Oxidant Mechanism

Ref:Eunkyoung Kim1,2, Lucia Panzella3, Raffaella Micillo3,4, William E. Bentley1,2, Alessandra Napolitano3 & Gregory F. Payne1[15]

3. Reverse engineering the ancient ceramic technology based on X-ray fluorescence spectromicroscopy

Ref:Philippe Sciau , Yoanna Leon Philippe Goudeau Sirine C. Fakra, Sam Webb and Apurva Mehta[16]

4. Raman spectroscopy for human cancer tissue diagnosis: A pattern recognition approach

Ref:Journal of Biomedical Science and EngineeringVol. 5 No. 12A (2012), Article ID: 26283, 9 pages DOI:10.4236/jbise.2012.512A113[17]

Maher Rizkalla, Parvin Ghane, Mangilal Agarwal, Sudhir Shrestha, Kody Varahramyan

5.Redox Activities of Melanins Investigated by Electrochemical Reverse Engineering: Implications for their Roles in Oxidative Stress

Ref:Journal of Investigative Dermatology (2020)140,537e543;Eunkyoung Kim1 , Lucia Panzella2 , Alessandra Napolitano2 and Gregory F. Payne1[18]

CONCLUSION

A rapid Raman imaging system designed to help scientists tackle formulation challenges and expedite drug development. Innovator and generic products can be successfully characterized using Raman spectroscopy with revere engineering. It is also able to facilitate deformulation activities. The main reason for using Raman spectroscopy in drug detection are as follows: less samples (1-2 mg) are needed, and samples are treated to a minimum, which is not only suitable for drug powder, but also for rapid and non-destructive detection and analysis of liquid and solid preparations

Some difficulties in reverse engineering analysis can be solved by Raman spectroscopy, such as the crystal identification of active ingredients (API), the determination of ingredients in the original preparation, and the analysis of process parameters, such as API distribution, coating layer thickness, coating layer

number, production process, and spatial distribution related to dissolution characteristics

Acknowledgement

I want to acknowledge our beloved Principal Prof.M.Sumakanth and faculty of the Department of Pharmaceutical Analysis for giving the opportunity.

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21. Redox Activities of Melanins Investigated by Electrochemical Reverse Engineering: Implications for their Roles in Oxidative Stress Journal of Investigative Dermatology (2020)140,537e543;Eunkyoung Kim , Lucia Panzella , Alessandra Napolitano and Gregory F. Payne