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Raman spectroscopy: A potential technique in analysis of pharmaceuticals

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ABSTRACT

The characterization of drug and drug intermediates is necessary in the drug development process, particularly in the pharmaceutical industry. Various analytical techniques are employed for this purpose. The selection of the technique depends upon many parameters including ease in use, reproducible and accurate results. Raman spectroscopy is one of those techniques which have immense potential in the analysis of pharmaceuticals. It has been widely utilized in many other areas like food and dyestuff industry, but has been very limitedly used in the pharmaceutical industry for drug characterization. However recent literature reflects ongoing application of Raman spectroscopy in pharmaceuticals. These studies demonstrate how transmission Raman spectroscopy can be used in the quantitative, non-invasive probing of the bulk content. It can be used in characterization of drug molecules to reveal the solid-state properties arising from polymorphism and quantitative applications in formulations. The range of applications can be expanded further if Raman spectroscopy is used in conjunction with the conventional techniques like IR. Infact, Raman spectroscopy exhibits several advantages over IR and the other physical analytical techniques. This review presents an exhaustive to Raman spectroscopy and its several applications with special focus on pharmaceuticals.

Key words: Raman Spectroscopy, applications.

INTRODUCTION

Spectroscopy was originally the study of the interaction between radiation and matter as a function of wavelength (λ). However, spectroscopy is the use of the absorption, emission, or scattering of electromagnetic radiation by matter to qualitatively or quantitatively study the matter or to study physical processes. The matter can be atoms, molecules, atomic or molecular ions, or solids. The interaction of radiation with matter can cause redirection of the radiation

and/or transitions between the energy levels of the atoms or molecules. All the spectroscopic techniques have been widely explored for the pharmaceutical applications. For years Raman spectroscopy (RS) is an established spectroscopic technique for quantitative analysis of molecular materials of all types because it is a non-contact characterization method that does not require any sample preparation. It is a versatile tool in pharmaceutics and biopharmaceutics, with a wide field of applications ranging from characterization of drug formulations to elucidation of kinetic processes in drug delivery. Data is acquired quickly and water does not interfere significantly with the vibrational bands of the drugs. Recently, the USP has officially included the Raman Spectroscopy as one of the spectroscopic technique for drug analysis [1]. The Raman measurement essentially involves directing a focused laser onto the sample and recording the energy profile of the light that is scattered. Every compound gives a unique spectrum (which arises from excitation of the vibrational modes of the molecule) and for mixed samples the spectra are a superposition of the signals from each of the constituents, which allows relative band intensities to be used for quantitative analysis.

Vibrational Spectroscopy*:*

The vibrations of a polyatomic molecule can be considered as a system of oscillators. If there are N atomic nuclei in the molecule, there will be a total of 3N degrees of freedom of motion for all the nuclear masses in the molecule [2].It is well known fact that both infrared and Raman spectroscopy falls under vibrational spectroscopy. A normal mode is called infrared-active, if there is any change in the electric dipole moment of the molecule. Otherwise, a vibrational mode is said to be Raman-active, if the polarizability of the molecule changes. Thus, as a rule of thumb, strong IR bands are related to polar functional groups, whereas non-polar functional groups give rise to strong Raman bands. IR and Raman spectra, which are complementary to each other, provide images of vibrations of the atoms of a compound. Therefore, both techniques are also referred as vibrational spectroscopy [3]. Recently, a review of the analysis of pharmaceutical substances and formulated products by vibrational spectroscopy published by Clark [4] and studies on polymorphs of drugs is reported by Threlfal [5].

Theoretical Aspects of Raman Spectroscopy

The Raman effect occurs when light impinges upon a molecule and interacts with the electron cloud and the bonds of that molecule. For the spontaneous Raman effect, a photon excites the molecule from the ground state to a virtual energy state. When the molecule emits a photon and returns to the ground state, it returns to a different rotational or vibrational state. The difference in energy between the original state and this new state leads to a shift in the emitted photon's frequency away from the excitation frequency. A change in the molecular polarization potential or amount of deformation of the electron cloud with respect to the vibrational coordinate is required for a molecule to exhibit a Raman effect. The amount of the polarizability change will determine the Raman scattering intensity. A standard Raman spectrum comprises the spectral range of 0–3500 cm^{-1}. The position and intensity of a vibrational bands are characteristic of the underlying molecular motion and consequently of the atoms participating in the chemical bond, their conformation, and their immediate environment. Thus, a certain submolecular group produces bands in a characteristic spectral region. These characteristic bands form the empirical basis for the interpretation of vibrational spectra. Various conventional applications of Raman spectroscopy are available [6].

Raman scattering phenomenon:

Fig. 1 schematically depicts the Raman scattering. The phenomenon of inelastic light scattering is known as Raman radiation and was first documented by Raman and Krishnan in 1928[7]. Raman scattering (also known as Raman effect) is named after Indian physicist Dr. C. V. Raman. Firstly, in Rayleigh scattering (elastic scattering) a photon interacts with a molecule, polarizing the electron cloud and raising it to a "virtual" energy state. This is extremely short lived (on the order of 10^{-14} seconds) and the molecule soon drops back down to its ground state, releasing a photon. This can be released in any direction, resulting in scattering. However since the molecule is dropping back to the same state it started in, the energy released in the photon must be the same as the energy from the initial photon. Therefore the scattered light has the same wavelength. Rayleigh scattering, therefore, bears no information regarding the vibrational energy levels of the sample. Raman scattering is different in that it is inelastic. The light photons lose or gain energy during the scattering process, and therefore increase or decrease in wavelength respectively. If the molecule is promoted from a ground to a virtual state and then drops back down to a (higher energy) vibrational state then the scattered photon has less energy than the incident photon, and therefore a longer wavelength. This is called Stokes scattering. If the molecule is in a vibrational state to begin with and after scattering is in its ground state then the scattered photon has more energy, and therefore a shorter wavelength. This is called anti-Stokes scattering (**Fig 1**).

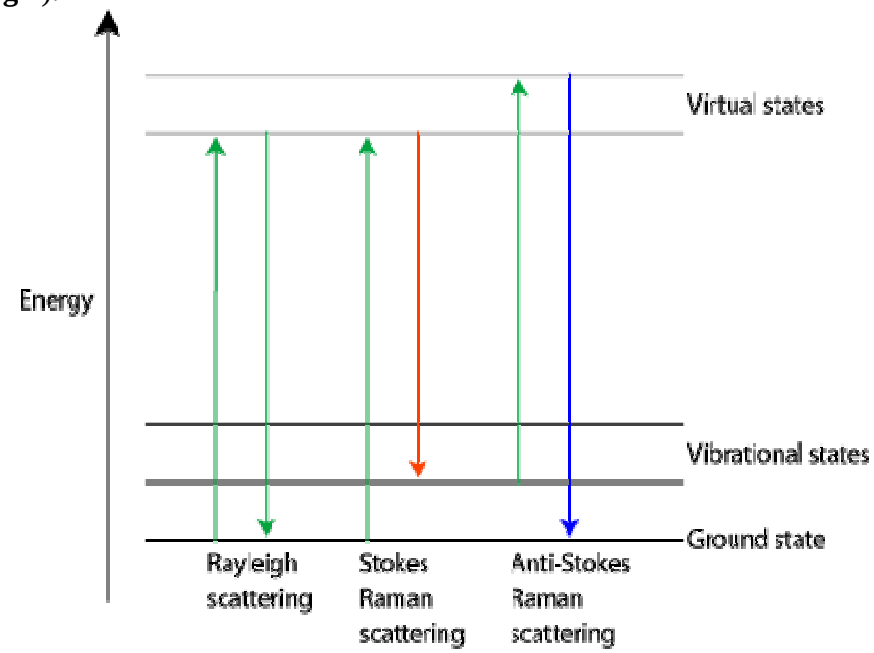

Fig 1: Different forms of scattering

Instrumentation:

Raman instruments are occupying more common place in pharmaceutical laboratories. Technological advances over the past decade have led to the production of much smaller Raman spectrometers that require only a single tabletop of laboratory space. Some of the manufacturers of Raman spectrometer includes Avalon Instruments, TechnoS Instruments, Bruker Optics Inc , Jasco Inc, Raman Systems Inc, ChemImage Corp. Schematics of Raman spectrometer is shown in **Fig 2.**

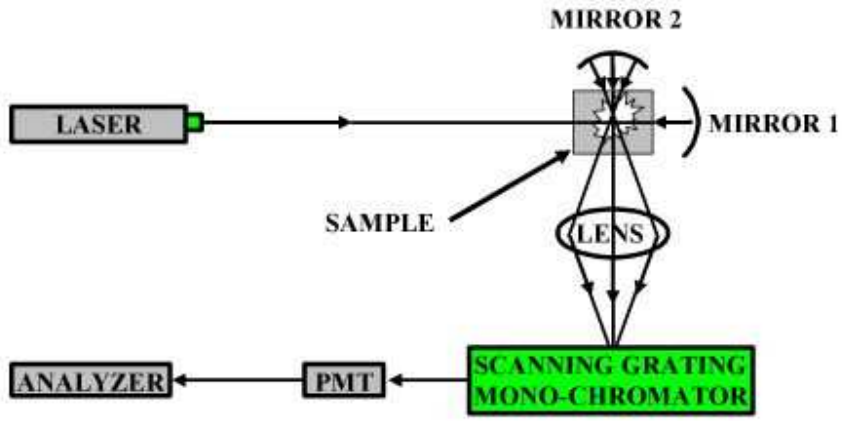

Fig 2: Instrumentation of Raman spectrometer

a) Laser excitation:

The sample is illuminated with a laser of a suitable power, wavelength and stability. Examples of some commonly employed are diode pumped solid state lasers (most often 1064 nm and 532 nm, but many other wavelengths are also available) and stabilized NIR diode lasers (typically ~780–820 nm) operating at powers of a few tens of mW to ~1 W. These lasers are generally compact, reasonably robust and reliable.

b) Delivery of excitation to sample:

The exciting laser light may be delivered to the sample either via a conventional optical system of mirrors and lenses, or via fibre optic cables, which may extend many tens of meters (note that this is not possible with mid-IR absorption spectroscopy because of absorption in the optical fibre). A lens system focuses the exciting light onto or into the sample and collects the resulting Raman scattered light.

c) Collecting and filtering the scattered light:

Efficient collection of the scattered light is essential in Raman spectroscopy because of the weakness of the effect. The collection optics (fibre optics) must, therefore, be positioned as close to the sample as possible and should have as large an aperture as possible to give a large collection angle (i.e., large numerical aperture collection optics should be used). The collected scattered light must then be spectrally filtered to remove the Rayleigh scattered component otherwise this would swamp the Raman signal. Various types of optical narrow band rejection or edge filters are available to perform this function.

d) Detectors:

A sensitive, low noise detector is required to detect the Raman scattered light. For UV-NIR excitation slow scan, cooled charged couple device (CCD) cameras are the detector of choice. Such a detector will, however, typically represent the single most costly component of a Raman system. For some applications it may be possible to employ a point detector or a simple array detector which, while providing only very limited spectral information, will be much less expensive. Array detectors suitable for Raman spectroscopy with IR excitation wavelengths have until recently been prohibitively expensive and so a single element detector coupled to an FT

spectrometer is generally used. These, however, are less sensitive than a system comprising a good dispersive spectrometer and a cooled CCD detector. Recent developments in indium phosphide/indium gallium arsenide phosphide array detectors show promise for IR excited Raman spectroscopy [8].

e) Spectral analysis:

The collected Raman light must then be spectrally analysed. This may be done using a low stray light, dispersive spectrometer, with a bandpass filter arrangement or a Fourier transform (FT) type spectrometer. A dispersive spectrometer is generally preferred for excitation wavelengths from the UV to ~820 nm while an FT system is generally required for excitation wavelengths at longer than ~820 nm .e.g., 1064 nm Nd:YAG (Neodymium–doped yttrium–aluminium–garnet) [9].

Various advanced Raman techniques such as Fourier transform, stimulated Raman scattering, Surface enhanced Raman spectroscopy (SERS), Raman optical activity, the hyper-Raman effect, Stimulated Raman gain, inverse Raman scattering, resonance Raman spectroscopy, coherent anti-Stokes Raman spectroscopy, coherent Stokes Raman spectroscopy are available. From these the SERS, resonance Raman spectroscopy are widely used.

Advantages of Raman spectroscopy:

1. Sample preparations, such as grinding can also lead to changes in solid states (e.g. hydration state, polymorphism, hydrogen bonding), which sometimes have an impact on the final detection method. Raman requires virtually no sample preparation and thus provides significant cost savings.
2. Raman spectra can be obtained non-invasively, which means that bulk and final products can be tested directly in their packaging, such as glass bottles, plastics and blister packs. Methods can be applied to the on-line monitoring of production lines.
3. Raman analysis can be used to study small particles within inhomogeneous sample matrices. This is important because, in the early stages of development, only a few mg of material are available.
4. Raman analysis time is short, thus enabling quick feedback from the quality-control department to the production-development team.
5. Raman experiments can be carried out easily, so that the work can be done by minimally trained personnel.
6. The good reproducibility of FT-Raman spectrometric experiments has been attributed to the large entrance aperture, which permits the laser to be focused into a relatively large sample volume.
7. Since water is a weak Raman scatterer, it possible to analyze aqueous solutions by Raman spectrometer.
8. Sampling for RS has further been eased through the use of fibre optics.

Limitations of Raman spectroscopy:

1. The cost of the equipment has been the main obstacle to the widespread adoption of RS for routine analysis.

2. A major problem for Raman measurements lies in the high levels of fluorescence (intrinsic or caused by impurities) overlaying the Raman bands. However, in most cases, this can be avoided by shifting the laser wavelength to the NIR spectral region.
3. If excitation intensities are too high, they may thermally decompose the sample.
4. Both the liquid and solid samples must be free from dust particles.

Though Raman and IR spectroscopic technique are complimentary to each other, due to some aspects (as shown in **Table no. 1**) RS seems to be advantageous than IR.

The table no.1 shows the comparison between the two spectroscopy methods [10]. RS shows advantages over the IR.

Table No.1: Comparison of Raman with Infrared Spectroscopy

Sr. No.	Raman Spectroscopy	Infrared Spectroscopy
1	It is due to the scattering of light by vibrating molecules.	It is due to absorption of light by vibrating molecules.
2	Polarizability of molecule decides whether Raman spectra will be observed or not.	Permanent dipole moment of molecule is the criteria for infrared spectra to be observed or not.
3	Water can be used as a solvent.	Water cannot be used as a solvent as it is opaque to infrared radiation.
4	Optical systems are made of glass or quartz.	Optical systems are made of special crystals such as CaF_2,NaBr,etc.
5	Homonuclear diatomic molecules are often found to be active.	Homonuclear diatomic molecules are often found to be inactive.
6	It can be recorded in one exposure.	At least two separate runs with different prisms are required to cover whole region of infrared.

Applications of Raman Spectroscopy

Raman spectroscopy is gaining popularity in different areas of the pharmaceutical industry. Like IR spectroscopy, it also provides information on the fundamental vibrational bands (the fingerprint region), offering a high degree of specificity in analysis. It also forms an ideal complement for existing methods of analysis such as NMR, MS and elemental analysis. Raman spectroscopy has immense potential in the field of pharmaceuticals. The rapid identification of compounds in the analysis of drug mixtures, active ingredients and excipients, the identification of contaminants, the characterization of formulated materials and the understanding of the blending processes involved in pharmaceutical formulations are now accessible using Raman techniques. Following is the elaborative account of application of Raman in pharmaceuticals and other fields.

Applications in pharmaceuticals:

1) Several pharmaceutical forms have already been studied by RS like Acebutolol [11], Alprenolol [11], Acetaminophen [12,13], Amiloride [14], Amoxycillin [15], Amphetamine and/or related compounds [16-19], Amphotericin A/B[20], Arterenol [21], Aspirin [22,23], Bucindolol [24,25], Calcium carbonate and glycine [26], Cimetidine [27], Ciprofloxacin [28], Cocaine [19,29-30], Diclofenac[31], Fluconazole[32], Fluocortolone [33,34], Ibuprofen[35],

Isosorbide [36], Nicotinamide [37], Spironolactone [38], Strychnine [39], Sulfamerazine[40], Sulfadiazine [40], Triamterene [41], Trifluoperazine[42] .

2) Reliable control of pharmaceutical manufacturing operations requires knowledge concerning physical as well as chemical characteristics of a drug formulation over the course of all its unit operations. For this reason, pharmaceutical researchers have recognized the utility of non-destructive Raman methodologies as a potential tool for application in advanced process analysis schemes aimed at determining tablet, drug content [43] and monitoring polymorphism transitions [44, 45]. Eliasson et al. showed that it can be used in the quantitative, non-invasive probing of the bulk content of production line relevant pharmaceutical products contained within capsules [46]. Niemczyk et al. showed that Raman spectroscopy using NIR excitation has significant potential as a rapid quality control method for pharmaceutical samples. They obtained useful spectral data directly from drug formulations in gel capsules and from gel capsules inside blister packs. The Raman scattering pass through the curved wall of the blister pack. Saly Romero-Torres et al. reported a novel approach to the measurement of colored tablet coating thickness, where they employed RS with univariate and multivariate data analysis. The results suggested that Raman sensing can serve as a viable non-invasive means to quantify tablet coating thickness in the presence of a fluorescent ingredient in the coating formulation.

3) Various experimental FT-Raman imaging procedures and their ability to both obtain and spatially resolve chemical information in the analysis of formulated tablets of pharmaceutical interest are available. The activity, stability and texture of emulsions are strongly affected by their microstructure, so an effective method for chemically imaging these microstructures is needed. Andrew et al. [47] exploited Raman imaging in distinguishing complex multi-component, multi-phase emulsion systems of inherently low contrast. They described how a confocal Raman microscope with an automated stage can be used to produce high-resolution, three-dimensional maps of the chemical composition of heterogeneous and multi-phase materials. They have characterized several commercial product systems ranging from pharmaceutical and skin creams to toothpaste. In solid dispersions, the drug is suspended in the polymer carrier in an amorphous state.

Under stress conditions recrystallisation can influence the therapeutic performance of the carrier. Breitenbach et al. [48] used confocal Raman spectroscopy to examine solid dispersions of the anti-inflammatory agent ibuprofen. The group investigated the physicochemical stability of the formulation under stress conditions (from -90 ^{0}C to 90 ^{0}C), together with the content and the homogeneity of the drug distribution in the formulation matrix. The authors state that confocal Raman spectroscopy can investigate different layers (e.g. coatings on a tablet), areas (e.g. phase separation) or simply the quality of mixing in a manufacturing process, which is of great industrial importance. Generalized 2D correlation spectroscopy was utilized for the Raman image analysis of pharmaceutical tablets to reveal molecular interactions between chemical components. By using a spatial distance as a perturbation variable in 2D correlation scheme, synchronous and asynchronous correlation analysis was possible.

Two kinds of pharmaceutical tablets, pentoxifylline (PTX) as an active substance and palmitic acid (PA) as an insoluble excipient, were prepared with different grinding times, 0.5 and 45 min. The 2D correlation analysis of Raman images of the tablets clearly revealed both physical and

chemical effects of grinding process on the properties of the tablets [49]. It is impossible to identify and visualize every ingredient within the formulation using a single spectroscopic technique. Clarke et al. [50] combined Raman and NIR spectroscopies in thoroughly describing heterogeneous mixtures of solid dosage forms.

Raman directly probes the molecular structure of the compound, while NIR displays only overtones and combinations of fundamental molecular vibrations. In their new approach of chemical image fusion (CIF), Clarke's group acquired data from exactly the same area of the sample using both Raman and FT-NIR mapping. The best images for the components were then overlaid to produce a combined chemical image that visually describes the entire formulation.

4) RS can be utilized as an on-line method to monitor drug hydration state during drying [51-52], hydrate formation during high shear wet granulation [53], blending API with wax beads [54], polymorph turnover kinetics [55]. McCreery et al. [56] have reported on the use of NIR Raman spectroscopy in identifying pharmaceuticals inside amber USP vials. Identification was performed using a library of spectra and accuracy was in the range 88-96%.

5) Polymorphism describes different packing arrangements of the same molecular species. The sensitivity of RS to polymorphic form has been demonstrated for a number of pharmaceutical compounds. FT-Raman spectroscopy in particular, is well-suited for the identification and quantitative analysis of crystal polymorphs [57], since it requires very little sample preparation, thus minimizing the risk of solid-state transformations of metastable polymorphs, while the particle size and shape of the sample have little effect. The potential of Raman techniques in the quantitative analysis of polymorphs has been demonstrated in the case of powder mixtures as well as drug products [58-60].Some of the data explaining the analysis of polymorhs using Raman is presented here. A quantitative method employing FT-Raman spectroscopy has been utilised to successfully analyse mixtures of varying proportions of the beta and delta mannitol polymorphs.

Mannitol is a polymorphic excipient which is widely used in pharmaceutical products as the beta form, although other polymorphs (alpha and delta) are common contaminants. Binary mixtures containing beta and delta mannitol were prepared to quantify the concentration of the beta form using FT-Raman spectroscopy. Spectral regions characteristic of each form were selected and peak intensity ratios of beta peaks to delta peaks were calculated. Using these ratios, a correlation curve was established which was then validated by analysing further samples of known composition. The differences exhibited by the FT-Raman spectra of the beta and delta mannitol were used to successfully identify and quantify polymorphic mixtures [61]. **(Fig 3)**

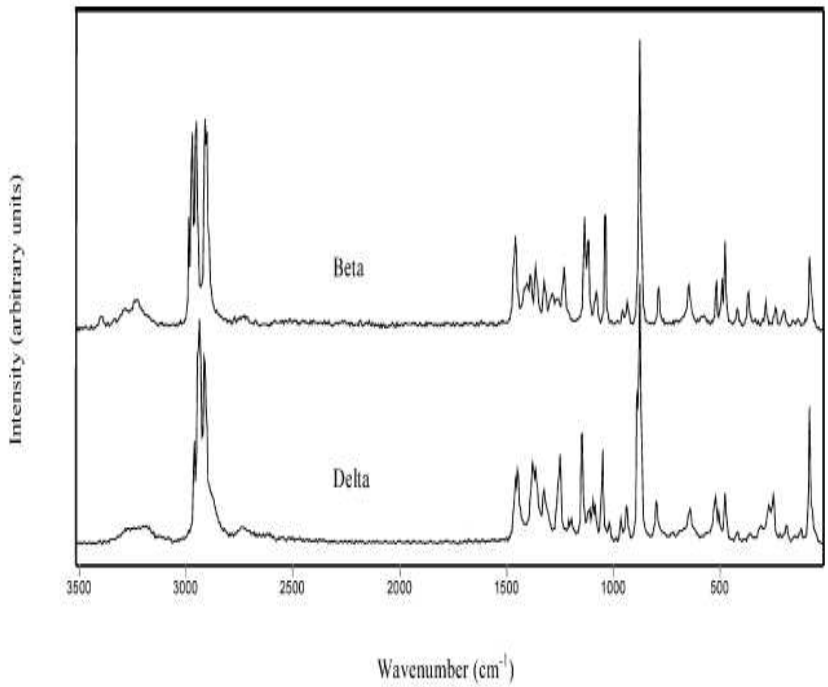

Fig 3: FT-Raman spectra of the beta and delta forms of mannitol

A fast and simple method for the quantitative analysis of monoclinic (form I) and orthorhombic (form II) paracetamol was developed, based on FT-Raman spectroscopy and PLS regression. The proposed multivariate calibration presented a significant improvement over existing methods for the quantification of paracetamol polymorphs [62]. RS, when coupled with an appropriate mathematical/ statistical data-processing and modeling proved to be an useful to identify and also to quantify the drug polymorphic forms [63].

The crystallinity of lactose (a commonly used carrier in dry powder inhaler formulations) was measured using RS. Samples of α-lactose monohydrate and amorphous lactose were prepared using ethanol precipitation and lyophilisation respectively. The Raman spectra of both α-lactose monohydrate and amorphous lactose were obtained and the differences in peak areas and intensities were obtained. Depolarisation ratios (ρ) of each form were then determined to identify the crystallinity of the lactose carrier samples. At the prominent Raman bands 865 and 1082 cm^{-1}, significant differences in ρ values were observed for crystalline (0.80±0.07, 0.89±0.06 respectively) and amorphous samples (0.44±0.07, 0.51±0.10) [64].

5) Armstrong et al. [65] employed FT-Raman microscopy to construct a profile of estradiol distribution of a transdermal drug-delivery patch. In 1994 and later, Spencer et al. [66,67] used scattered circularly polarized Raman optical activity to quantify the chiral purity of pharmaceuticals. S. Cıinta Pinzaru [68] reviewed on different Raman techniques (dispersive, Fourier transform, resonance Raman, SERS, SERRS, FT-SERS) employed in pharmaceutical investigations mainly focusing on SERS. Using Raman and SERS techniques, compounds showing different pharmacological activity were analyzed, including the antihypertensive 1,4-dihydrazinophtalazine sulfate [69], the antimicrobial agent rivanol [70], antiretrovirals [71],

narcotics [72-74], antitumour agents (6-mercaptopurinem [75], 9-phenyl- and 9- aminoacridine [76-77], camptothecin [78], and its water-soluble derivative topotecan) [79] and HIV inhibitors (betulinic acid) [80]. 6) Swatantra K. et al. [81] used RS for the characterization of ketoprofen cyclodextrin complex.

CONCLUSION

Vibrational spectroscopy is an excellent method for identifying substances because it provides fingerprint spectra that are unique to each specific compound. Of the various vibrational spectroscopies available, Raman spectroscopy can be the method of first choice because the spectra it produces are rich in information and because it needs virtually no sample preparation. This makes it ideal for the analysis of tablets, powders and liquids, thus avoiding mechanical changes during sample preparation, which could alter the physicochemical properties of the formulation. It has been proved that Raman spectroscopy is a versatile tool in pharmaceutics and biopharmaceutics. Raman spectroscopy is also used in the pharmaceutical analytical laboratory in a variety of ways. At the most basic level the technique measures particle density via the intensity of the Raman spectra. Traditional drug substance characterization is enhanced with the additional information provided by Raman spectroscopy and quantitative polymorph assays can be developed. Raman can also be used qualitatively and semi-quantitatively to support pharmaceutical development.

However, although Raman spectroscopy shows advantages over the more traditional IR spectroscopic techniques, it should not be considered as the only analytical technique to solve most problems, but as one more powerful, albeit expensive, technique that is part of a multidisciplinary approach to analysis.

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