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RESEARCH ARTICLE

A REVIEW ON RAMAN SPECTROSCOPY FOR PHARMACEUTICAL ANALYSIS

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ABSTRACT

Raman spectroscopy has become a pivotal tool in pharmaceutical analysis due to its precision, versatility, and non-destructive capabilities. It provides critical insights into the structural, polymorphic, and electronic properties of pharmaceutical compounds, essential for all stages of drug development, from raw material testing to quality control. Advancements like Surface-Enhanced Raman Spectroscopy (SERS) and hyper spectral imaging have overcome challenges such as low sensitivity and fluorescence interference, expanding its use in complex analyses. Once underutilized in drug characterization, Raman spectroscopy is now a key technique in the pharmaceutical industry, offering non-invasive, quantitative analysis of drug content, polymorph identification, and formulation assessment. When combined with other methods like infrared spectroscopy, Raman enhances analytical power, offering faster data acquisition and higher specificity. This review explores the principles, recent innovations, and diverse applications of Raman spectroscopy in pharmaceutical sciences, highlighting its role in revolutionizing drug development and quality control to create safer, more effective therapeutic solutions.

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INTRODUCTION

Spectroscopy initially focused on examining how radiation interacts with matter based on wavelength (λ). Today, it encompasses the analysis of absorption, emission, or scattering of electromagnetic radiation by matter, enabling both qualitative and quantitative investigations of various substances, including atoms, molecules, atomic or molecular ions, and solids. The interaction between radiation and matter can lead to changes in the direction of the radiation and transitions among the energy levels of atoms or molecules. Various spectroscopic techniques have been extensively researched for their applications in the pharmaceutical field. Among these, Raman spectroscopy (RS) has long been recognized as a reliable method for the quantitative analysis of diverse molecular materials, primarily due to its non-contact nature and the absence of sample preparation requirements. This technique serves as a powerful tool in both pharmaceutics and bio pharmaceutics, with applications that range from the characterization of drug formulations to the investigation of kinetic processes in drug delivery. Data collection is rapid, and water has minimal impact on the vibrational bands of the drugs. Recently, the United States Pharmacopeia (USP) has officially recognized Raman Spectroscopy as a valid technique for drug analysis. The process of Raman measurement involves directing a focused laser beam onto the sample and capturing the energy profile of the scattered light. Each compound produces a distinct spectrum, resulting from the excitation of the molecule's vibrational modes. In the case of mixed samples, the resulting spectra represent a combination of signals from all constituents, allowing for the use of relative band intensities in quantitative analysis.[1]

Vibrational Spectroscopy: The vibrations within a polyatomic molecule can be viewed as a collection of oscillators. For a molecule containing N atomic nuclei, there are 3N degrees of freedom associated with the motion of all nuclear masses present. It is a well-established fact that both infrared and Raman spectroscopy are categorized under vibrational spectroscopy. A normal mode is classified as infrared-active if there is a variation in the molecule's electric dipole moment. Conversely, a vibrational mode is deemed Raman-active when there is a change in the molecule's polarizability. Generally, strong infrared bands are associated with polar functional groups, while non-polar functional groups tend to produce strong Raman bands. The IR and Raman spectra, which complement each other, illustrate the vibrational movements of a compound's atoms. Consequently, these techniques are collectively referred to as vibrational spectroscopy. Recently, Clark published a review on the analysis of pharmaceutical substances and formulated products using vibrational spectroscopy, and threlfa reported studies on drug polymorphs.

Theoretical Aspects of Raman Spectroscopy: Raman spectroscopy is based on the Raman Effect, which occurs when light strikes a molecule and interacts with its electron cloud

and bonds. In the case of spontaneous Raman scattering, a photon elevates the molecule from its ground state to a temporary virtual energy state. As the molecule returns to its ground state, it does so in a different rotational or vibrational state. This transition results in a change in energy, causing the emitted photon's frequency to shift from the original excitation frequency. For a molecule to display a Raman Effect there must be a change in its molecular polarization potential or a deformation of the electron cloud relative to the vibrational coordinate. The extent of this change in polarizability directly influences the intensity of the Raman scattering. A typical Raman spectrum spans a spectral range of 0–3500 cm-1. The position and intensity of vibrational bands are indicative of the molecular movements and the atoms involved in the chemical bonds, their arrangement, and their surrounding environment. Consequently, specific sub molecular groups generate bands within distinct spectral regions, forming the empirical foundation for interpreting vibrational spectra. Raman spectroscopy has a variety of conventional applications.[2]

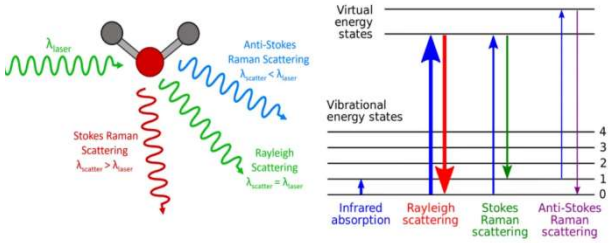

Fig 1. Raman Scattering Process

Raman scattering phenomenon

The diagram illustrates Raman scattering, an inelastic light scattering phenomenon first observed by Raman and Krishnan in 1928. Unlike Rayleigh scattering, which is elastic and does not alter photon energy, Raman scattering involves energy exchange between photons and molecular vibrations, leading to wavelength shifts. In Rayleigh scattering, incident photons temporarily polarize the electron cloud, elevating the molecule to a short-lived virtual state (~10^{-14} s). The molecule then returns to its original state, emitting a photon of the same energy, providing no vibrational energy information.

In contrast, Raman scattering is inelastic:

- **Stokes scattering**: A molecule absorbs energy, transitioning to a higher vibrational state, and emits a lower-energy photon (longer wavelength).
- **Anti-Stokes scattering**: A molecule starts in an excited vibrational state, loses energy, and emits a higher-energy photon (shorter wavelength).

These shifts provide molecular vibrational fingerprints, making Raman spectroscopy a powerful analytical tool.

INSTRUMENTATION

Raman instruments are becoming increasingly prevalent in pharmaceutical laboratories. Recent technological advancements have resulted in the development of compact Raman spectrometers that occupy minimal space, fitting comfortably on a single laboratory tabletop. Notable manufacturers of Raman spectrometers include Avalon Instruments, Techno S Instruments, Bruker Optics Inc, Jasco Inc, Raman Systems Inc, and Chem Image Corp. A schematic representation of a Raman spectrometer is illustrated in Figure.

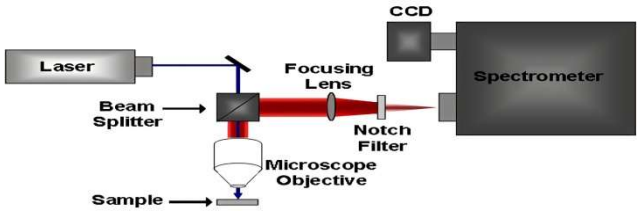

Fig.No.2: Raman spectrometer

Laser excitation: Laser excitation involves illuminating the sample with a laser that possesses appropriate power, wavelength, and stability. Commonly used lasers include diode-pumped solid-state lasers, typically operating at wavelengths of 1064 nm and 532 nm, although a variety of other wavelengths are also accessible[3]. Additionally, stabilized near-infrared (NIR) diode lasers, usually in the range of approximately 780 to 820 nm, function at power levels from several tens of milli watts up to around 1 watt. These lasers are generally compact, durable, and dependable.

Delivery of excitation to sample: The excitation laser light can be directed to the sample using either a traditional optical arrangement of mirrors and lenses or through fiber optic cables, which can extend for many meters. It is important to note that this method is not applicable for mid-infrared absorption spectroscopy due to absorption issues in the optical fibers. A lens system is employed to focus the excitation light onto or into the sample while simultaneously collecting the resulting Raman scattered light.

Collecting and filtering the scattered light: In Raman spectroscopy, effective collection of scattered light is essential due to the Raman Effect's weakness. Collection optics, typically fiber optics, should be placed close to the sample and have a large aperture for a wide collection angle, requiring a high numerical aperture. Afterward, spectral filtering is needed to remove Rayleigh scattering, often using narrow band rejection or edge filters to prevent signal interference.

Detectors: A sensitive, low-noise detector is crucial for capturing Raman scattered light. Cooled CCD cameras are typically preferred for UV-NIR excitation, though they are often the most expensive component. For certain applications, point or basic array detectors are more cost-effective but provide limited spectral information[4]. IR-excitation Raman spectroscopy traditionally uses a single-element detector with a FT spectrometer, offering lower sensitivity than systems with cooled CCDs and dispersive spectrometers. Recent advancements in Indium Phosphide (InP) /Indium Gallium Arsenide Phosphide (InGaAsP) array detectors may improve IR-excited Raman spectroscopy.

Spectral analysis: Raman light requires spectral analysis, typically using a dispersive spectrometer with low stray light and a band pass filter, or a Fourier transform (FT) spectrometer. Dispersive spectrometers are preferred for excitation wavelengths up to about 820 nm, while FT systems are needed for longer wavelengths, such as 1064 nm. Advanced Raman techniques include Fourier transform, SERS, resonance Raman, and others, with SERS and resonance Raman being particularly common. To maintain high levels of

innovation and productivity, pharmaceutical research laboratories and manufacturing plants require robust and reliable analytical techniques at many stages in the product development pipeline, including drug discovery, process monitoring and quality control. One such technique is Raman spectroscopy, which is becoming increasingly popular because of its high level of molecular specificity, ability to perform rapid chemical analysis in real-time, and versatility in instrumentation for studying various sample types at different stages in the pipeline.

In this Application Note, we show how the Edinburgh Instruments RM5 and RMS1000 Raman Microscopes can be used to perform chemical analysis of various pharmaceutical product types, including solid tablets, nasal sprays, emulsions, Trans dermal drug delivery systems, and drug polymorphs.[5]

RS v/s IR: Ensuring dust-free liquid and solid samples is crucial for accurate Raman spectroscopy. While RS and IR are complementary, RS offers distinct advantages, as shown in Table 1.[6]

Table 1. Comparison of Raman Spectroscopy and Infrared Spectroscopy

RAMAN SPECTROSCOPY	INFRARED SPECTROSCOPY
• Arises from the inelastic scattering of light by molecular vibrations.	• Arises from the absorption of infrared light by molecular vibrations.
• The ability of a molecule to undergo changes in polarizability determines its Raman activity.	• A molecule must possess a permanent dipole moment to exhibit an infrared spectrum.
• Water serves as a viable solvent due to its weak Raman scattering.	• Water is unsuitable as a solvent due to its strong infrared absorption, making it opaque to IR radiation.
• Optical components typically consist of glass or quartz materials.	• Optical components are typically composed of specialized crystals such as CaF_2, NaBr, and others.
• Homo nuclear diatomic molecules frequently exhibit Raman activity despite lacking a permanent dipole moment.	• Homo nuclear diatomic molecules generally remain IR-inactive due to the absence of a changing dipole moment.

ADVANTAGES

- **Non-destructive Analysis:** Raman spectroscopy allows for the analysis of samples without altering or damaging them, making it ideal for sensitive materials like pharmaceuticals, artworks, or biological samples.
- **Minimal Sample Preparation:** Unlike other techniques, Raman spectroscopy often requires little to no sample preparation, saving time and reducing the risk of introducing errors.
- **High Spatial Resolution:** Raman microscopy enables the study of materials at the micron scale, providing detailed spatial information about the chemical composition and structure of heterogeneous samples.
- **Wide Applicability:** Raman spectroscopy can be applied to a broad range of materials, including solids, liquids, powders, and gases, in a variety of environments, from high-pressure systems to biological tissues.
- **Chemical Fingerprinting:** It provides unique molecular "fingerprints" based on vibrational modes, which allows for the identification of substances with high specificity and sensitivity.

LIMITATIONS

- Fluorescence interference from some organic or biological materials can overwhelm the Raman signal, complicating accurate analysis.
- Raman scattering is a weak effect, resulting in low signal intensity for samples with low concentration or small volumes, requiring sensitive detectors and extended acquisition times.[7]
- High laser power can cause damage to delicate or heat-sensitive samples, potentially altering their properties or causing degradation.
- Raman spectroscopy has limited depth penetration compared to techniques like X-ray or infrared spectroscopy, limiting its use in analyzing thick samples.

APPLICATIONS

Raman spectroscopy is increasingly being utilized across various sectors of the pharmaceutical industry. Similar to infrared (IR) spectroscopy, it delivers insights into fundamental vibrational bands, particularly within the fingerprint region, which ensures a high level of specificity in analytical processes[8]. This technique serves as an excellent complement to established analytical methods such as nuclear magnetic resonance (NMR), mass spectrometry (MS), and elemental analysis. The potential of Raman spectroscopy in pharmaceuticals is substantial. It enables the swift identification of compounds within drug mixtures, active ingredients, and excipients, facilitates the detection of contaminants, aids in the characterization of formulated products, and enhances the understanding of blending processes in pharmaceutical formulations. The following section provides a detailed overview of the applications of Raman spectroscopy in pharmaceuticals and other domains.

Applications in pharmaceuticals

- RS has been studied for various pharmaceutical application s[9], including acebutolol, alprazolam, acetaminophen, amilo ride, amoxycillin, amphetamine and related compounds, am photericin A/B, arterenol, aspirin, bucindolol, calcium carb onate and glycine, cimetidine, and ciprofloxacin.
- Reliable pharmaceutical manufacturing requires understanding both the physical and chemical properties of drug formulations throughout processing. Non-destructive Raman spectroscopy has emerged as a valuable tool for advanced process analysis, enabling drug content determination and polymorphism monitoring.
- **Eliasson** demonstrated its use for quantitative, non-invasive analysis of pharmaceutical products within capsules on production lines.

Niemczyk highlighted its potential for rapid quality control using NIR excitation, successfully obtaining spectral data from gel capsules, even within blister packs.

Saly Romero Torres introduced a novel method for measuring colour tablet coating thickness using Raman spectroscopy with univariate and multivariate analysis, proving its effectiveness in quantifying coating thickness despite fluorescent ingredients.

- Various FT-Raman imaging techniques are used to analyze pharmaceutical tablets and resolve chemical information. Emulsion activity, stability, and texture are influenced by microstructure, requiring effective imaging methods. **Andrew** used Raman imaging with a con focal Raman microscope to create high-resolution 3D maps of chemical composition in complex multi-phase emulsion systems, such as pharmaceuticals and skin creams. In solid dispersions, drugs suspended in polymer-carriers can recrystallize under stress, affecting performance. **Breitenbach** used Raman spectroscopy to study ibuprofen dispersions, assessing stability and drug content under stress conditions. Raman also evaluates tablet coatings, phase separations, and mixing quality in manufacturing.
- 2D correlation spectroscopy was used to analyze Raman images of tablets, revealing molecular interactions between components[10]. For example, 2D correlation analysis of pentoxifylline and palmitic acid tablets showed the effects of grinding on properties. Clarke combined Raman and NIR spectroscopy to analyze heterogeneous mixtures, using chemical image fusion (CIF) to create comprehensive chemical images of solid dosage forms.
- **Drug Quality:** Quality-by-design in drug manufacturing ensures raw material purity and the final product's quality. This includes verifying the correct amounts of active ingredients, polymorphs (if applicable), excipients, and additives like dyes. Raman spectroscopy is used for monitoring mixing in blenders and inspecting individual products. For example, an Excedrin® tablet contains 44% aspirin, 44% acetaminophen, and 12% caffeine. Raman spectra of the pure APIs can be used to determine the composition, but single-point measurements can be misleading due to tablet non-uniformity. To improve accuracy, techniques like sample mapping, spinning, using larger spot sizes, or transmission Raman are employed. Mapping multiple points, as shown in Figure 3, helps stabilize the concentration results, achieving nearly 43% aspirin, 45% acetaminophen, and 12% caffeine after around 20 measurements.

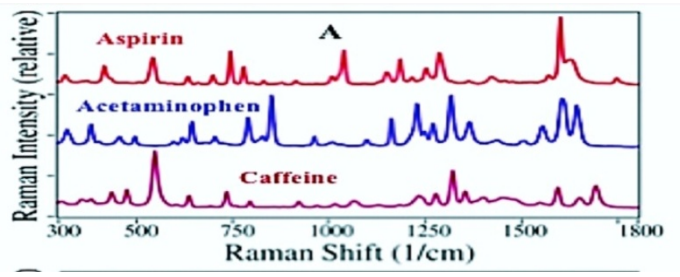

Fig.No.3. A) Raman spectra of Excedrin components: Aspirin, Acetaminophen, and Caffeine

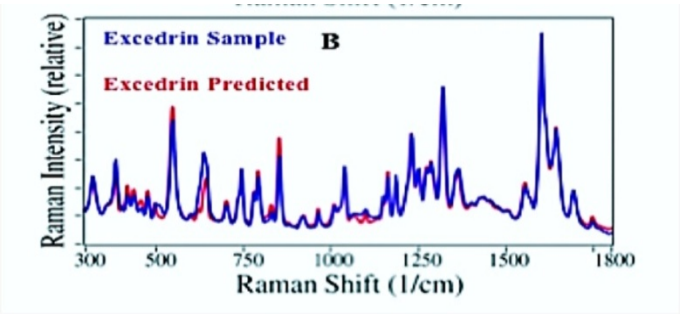

Fig.No.4. B) Overlay of a measured Raman spectrum from an Excedrin® tablet (500 mW, 1064 nm laser, 5-min acquisition) with a reconstructed spectrum from its components.

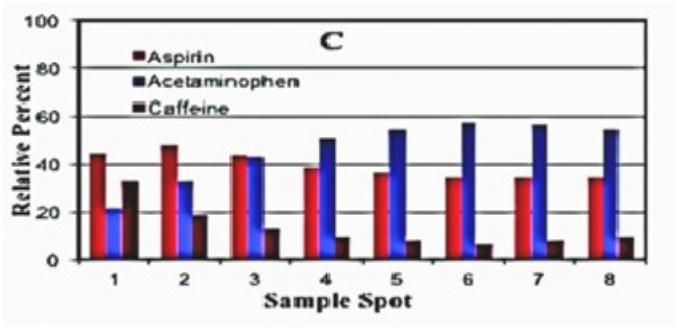

Fig.No.7. C) Normalized intensities of Aspirin, Acetaminophen, and Caffeine at eight spots on an Excedrin® tablet, summing to 100%

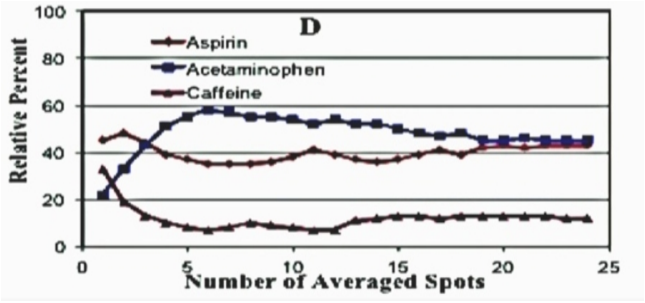

Fig.No.6: D) Running average of normalized intensities for 24 spots, starting with the most inaccurate spot to highlight the method's effectiveness

Product Self Life: Drug formulations include additives and coatings to protect active ingredients from degradation, maximizing shelf life, typically defined as the period a drug maintains over 90% potency. While most degradation products are harmless, acetaminophen degrades into p-aminophenol, a toxic compound that can cause liver damage and contribute to accidental overdose deaths, especially when expired. High-performance liquid chromatography (HPLC) is the main method for detecting drug degradation, but Raman spectroscopy offers advantages such as minimal sample preparation, non-destructive analysis, and speed. Raman spectra of acetaminophen and p-aminophenol are distinct, enabling accurate degradation detection. For low-concentration drugs, like injectables, Raman spectra may not detect the active ingredient, but surface-enhanced Raman spectroscopy (SERS) can amplify signals, enabling detection of degradation products. SERS effectively identifies and quantifies degradation products, as seen with epinephrine and its degradation product, nor-epinephrine.

CONCLUSION

Vibrational spectroscopy is an effective technique for identifying substances, providing unique spectra that serve as a chemical fingerprint for each compound. Among various vibrational methods, Raman spectroscopy is particularly advantageous due to its detailed spectral information and minimal sample preparation. This makes it ideal for analyzing tablets, powders, and liquids, as it avoids potential changes to the sample's physicochemical properties during preparation. Raman spectroscopy has proven versatile in pharmaceutics and bio pharmaceutics, being used for applications ranging from particle density measurement to improving drug substance characterization and developing polymorph assays. It can also

support pharmaceutical development through qualitative and semi-quantitative analysis. While Raman spectroscopy offers significant benefits over traditional infrared techniques, it should not be considered the only analytical tool. Instead, it should be regarded as a valuable, though expensive, component in a comprehensive, multidisciplinary analytical strategy.

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