Mechanism, Development and Comparison of Infrared and Raman Spectra in the Pharmaceutical Diagnosis and Living Cell Detection

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Abstract: Nowadays, applications of new detection methods for pharmaceutical diagnosis and living cell detection have attracted more and more attention. Among these detection methods, there is a large improvement in the Raman spectroscopy, which leads to a wide range of applications in different fields. The infrared spectroscopy is still act as a main and important techniques in the different areas. This research compares the similarities and differences of these two technologies. At the same time, this research will concern about the mechanism of Raman and infrared spectroscopy and their applications in some domains, especially in the various disease and pharmaceutical fields. Applications of Raman spectroscopy for detection of living cells is systematically analyzed, including detection mechanism and specific detection process. For pharmaceutical diagnosis, the advantages and disadvantages of Raman spectroscopy are present in this research. This research provides a new idea for the applications of Raman spectroscopy and infrared spectroscopy in the field of disease detection.

1 INTRODUCTION

Raman spectroscopy is a vibrational spectroscopy technique with "fingerprint" identification of molecular composition and structure. It can distinguish samples of various substances and also is one of the main analytical techniques used in optical metrology.

Raman spectroscopy is not interfered by aqueous solvents. As a result, it can be used in biomedical analysis better than traditional infrared spectroscopy. Raman spectroscopy is a promising diagnostic tool that can help uncover the molecular basis of diseases and provide objective, quantifiable molecular information for diagnosis and therapeutic evaluation. Raman scattering occurs when the polarizability changes during the interaction of light with molecular vibration/rotation and molecular motion. When light interacts with a molecule, it can be excited to a transient virtual state, which immediately returns to the vibrationally excited state of the electron's ground state. Due to this interaction, a small amount of energy is transferred or removed from the molecule, and the resulting scattered light is red-shifted or blue-shifted, which contains encoded vibrational molecular information. This causes light to be scattered at the optical frequency at which it moves on the incident light. By monitoring the intensity distribution of inelastic scattered light as a function of frequency, a unique spectral fingerprint of tissue samples was obtained. Because each sample has a unique composition, spectral profiles generated by Raman active functional groups of nucleic acids, proteins, lipids and carbohydrates. Raman scattering in tissue provides rich information about the vibrational structure of proteins, gag, lipids and DNA.

The Raman spectrum is usually recorded in the so-called fingerprint region, which contains relatively weak but highly specific Raman peaks. More recently, additional attention has been paid to the use of the high wave-number region, which contains less specific Raman bands but shows a higher degree of signal intensity. An important advantage of Raman spectroscopy is the low intensity of the water wave segment, which makes the analysis of biomaterials very difficult in infrared

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spectroscopy. However, Raman spectroscopy and infrared spectroscopy are complementary, Raman cannot replace infrared. It can complement each other to provide more comprehensive and accurate information about molecular vibration state and molecular structure. But in some routine detection, using Raman instead of infrared or using Raman as a supplement to infrared, can improve the work efficiency and detection speed. Many active components in medicinal materials have different pharmacological effects due to their different functional groups and configurations. Raman spectroscopy has certain advantages in structural analysis and isomer identification of drug active components due to its high selectivity and no need for separation in mixture analysis.

Raman spectroscopy has powerful analytical capability and can provide quantitative information about chemical composition in biological samples. It uses inelastic scattering of light to provide the spectral characteristics of the internal structure and conformation of the cell, thus reflecting the material changes of the sample. In the process of tissue and cytopathic disease, the structure, content and conformation of each component in the cell will change to varying degrees. It follows that it may have a new role in the diagnosis of disease.

Raman spectroscopy can detect substance changes in samples. And similarly, it can reflect substance changes in the body caused by early cancer. Cancer has been threatening people's life and health. In the context of an increasingly high incidence of cancer worldwide, early diagnosis of cancer is particularly important. There are numerous lives behind the huge numbers in cancer reports. Early detection, diagnosis and treatment will lead to a greater chance of survival for patients. The Raman spectrum of the tissue can be measured using a microscope or custom optical fiber. In simple terms, a single mode fiber is used to couple the laser to a microscope and illuminate the sample with a microscope objective. Confocal imaging based on Raman spectrum can be achieved by using optical fiber to collect backscattered light. A single optical fiber acts as a pinhole to couple the light to a high-throughput spectrometer, which is then dispersed to a charge-coupled device (CCD) camera.

At present, the diagnosis of cancer mainly depends on X-ray, CT examination, B ultrasound, MRI examination and tumor marker detection. And biopsy is still the best indicator of cancer confirmation. Conventional imaging results can only provide the basis for diagnosis, but they are not sensitive and economical, and will bring great pain to patients. By contrast, Raman spectroscopy has high chemical specificity and can obtain abundant molecular information without staining or labeling the specimen. Raman spectroscopy, as a non-invasive means of detection, can directly detect biological samples, which is not only more sensitive, but also relieves the pain and economic burden of cancer patients.

Raman, an Indian physicist, irradiated benzene liquid with a mercury lamp in 1928 and discovered a new radiation spectrum line: this is a new molecular radiation, called Raman scattering. Raman won the Nobel Prize in physics in 1930 for the discovery of this new molecular radiation and many light scattering research achievements. At the same time, Landsberg and Mandelstad of the former Soviet Union reported the discovery of a similar phenomenon in quartz crystals, namely Raman scattering caused by optical phonons, called merger scattering.

Roquette and Cabens in France and Wood in the US confirmed the results of Raman's observational study. Because the Raman effect is too weak, it is difficult to observe and study the weak Raman scattering signal, let alone measure and study the higher order Raman scattering effect. And the volume of the tested sample must be large enough, colorless, no dust, no fluorescence and so on. By the mid-1940s, the progress of infrared technology and commercialization of Raman spectroscopy applications declined.

After 1960, the appearance of ruby laser makes the study of Raman scattering into a new period. Because the laser has good monochromaticity, strong directivity and high-power density, using it as excitation light source greatly improves the excitation efficiency. It is an ideal light source for Raman spectroscopy. With the improvement of detection technology and the reduction of the requirements for tested samples, Raman spectroscopy has been widely used in physics, chemistry, medicine, industry and other fields.

In the mid-1970s, the appearance of laser Raman probe brought the possibility of microanalysis. Since the 1980s, Spex company of the United States and Rrinshow company of the United Kingdom have launched a confocal laser Raman spectrometer, bitman probe, because of the use of notchfilter to filter out the excitation light, so that stray light is suppressed. It is not necessary to use double or even triple monochromator, and only need to use a single monochromator. The efficiency of the light source is greatly improved, so that the power of the incident

light can be very low. And the sensitivity is greatly improved.

2 CONVENTIONAL INFRARED SPECTROSCOPY

Infrared spectrum is also called infrared absorption spectrum. It is the characteristic absorption spectrum curve generated by resonance absorption between infrared photon and molecular vibration and rotation quantized energy level.

2.1 Mechanism

In organic molecules, the atoms that form chemical bonds or functional groups are in a state of constant vibration at frequencies comparable to those of infrared light. When organic molecules are irradiated with infrared light, chemical bonds or functional groups in the molecules can occur vibration absorption. Different chemical bonds or functional groups absorption frequency is different. It will be in different positions in the infrared spectrum, so as to obtain the information of what kind of chemical bonds or functional groups in the molecule.

The infrared spectrum is usually divided into three regions: near infrared region (0.75~2.5 μm), middle infrared region (2.5~25 μm) and far infrared region (25~1000 μm). Generally speaking, the near infrared spectrum is produced by the double frequency and combination frequency of molecules. The mid-infrared spectrum belongs to the fundamental frequency vibration spectrum of molecules. Far infrared spectrum belongs to the rotational spectrum of molecules and vibration spectrum of some groups.

2.2 Development

In the 1960s, a linear relationship between the content of substances and the absorption peaks of several different wavelength points in the near infrared region is demonstrated, which made this technique widely used in the analysis of agricultural products. In the middle and late 1960s, the classical near infrared spectroscopy (NIR) was exposed to the weakness of low sensitivity and poor anti-interference. With the emergence of various new analytical techniques, people ignored the application of this technique in analytical testing.

The successful application of multiple correction technology in spectral analysis in 1970s promoted the promotion of near-infrared spectroscopy technology. In the late 1980s, with the development of computer technology, the digitization of analytical instruments and stoichiometry have been fully developed. The good results obtained by stoichiometry in solving spectral information extraction and background interference have led to the application of NIR spectroscopy in various fields.

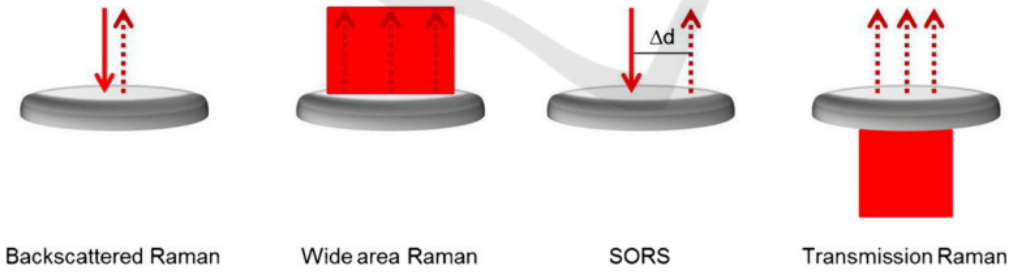

Figure 1: Schematic illustration Raman spectroscopy in measuring process.

In the 1990s, the application of NIR spectroscopy in the industrial field expanded rapidly, and the literature on the research and application of NIR spectroscopy increased almost exponentially, becoming one of the fastest developing and most eye-catching analytical techniques. Because of its good transmission characteristics in conventional optical fibers, this technology is also applied in the field of online analysis.

3 COMPARISON OF RAMAN AND INFRARED SPECTRA

3.1 Similarities

For a given bond, the infrared absorption frequency is equal to the Raman shift and represents the energy of the first vibrational level. For a given compound,

the infrared absorption wave number and Raman displacement of some peaks are exactly the same, and both of them are in the infrared region, both of which reflect the molecular structure information. Raman spectroscopy, like infrared spectroscopy, is also used to detect the vibration and rotational energy levels of matter molecules.

The vibration which has symmetry relation with the center of symmetry is invisible in infrared, but visible in Raman. The vibration with no symmetry relation to the center of symmetry is visible by infrared, but not by Raman.

3.2 Differences

As shown in Figure 1, the incident light and detection light of infrared spectrum are infrared light, while the incident light of Raman spectrum is mostly visible light, and the scattered light is also visible light. Infrared spectroscopy measures absorption of light, while Raman measures scattering of light. When photons interact with molecules, they do so through electric dipole moment transitions.

Therefore, molecules that have no polarity or symmetry, have essentially no infrared absorption effect, because there is no electric dipole moment. Raman spectrum is not absorption spectrum, but after the incident photon resonates with the molecular vibrational and rotational quantized energy level, the photon emits at another frequency. The energy difference between the incoming and outgoing photons is equal to the vibrational and rotational transition energy levels of the molecules involved in the interaction.

Unlike infrared absorption spectroscopy, Raman spectroscopy is a photon-molecular interaction of higher order, which is much weaker than infrared absorption spectroscopy. However, because the mechanism of its generation is the electric quadrupole moment or magnetic dipole moment transition, it does not require the molecule itself to have polarity, so it is particularly suitable for the detection of those symmetric molecules without polarity.

Infrared spectroscopy measures the absorption of light, expressed by wave number or wavelength, while Raman spectroscopy measures the scattering of light, and the horizontal axis is Raman displacement. Infrared spectroscopy mainly reflects the functional groups of molecules while Raman spectroscopy mainly reflects the framework of molecules and is mainly used to analyze biological macromolecules.

4 LIVING CELLS DETECTION

4.1 Mecahnism

Raman spectroscopy has powerful analytical capability and can provide quantitative information about chemical composition in biological samples. It uses inelastic scattering of light to provide the spectral characteristics of the internal structure and conformation of the cell, thus reflecting the material changes of the sample. When photons of monochromatic beam interact with molecules, elastic collision and inelastic collision can occur, as shown in Figure 3.

In the inelastic scattering process, energy exchange occurs between photon and molecule, and photon loses part of energy due to scattering by molecule, resulting in the change of photon frequency. But the difference between the frequency of the scattered light and the frequency of the incident light does not change because the frequency of the incident light changes. Among them, the change in the frequency of light is collectively called the Raman shift. Raman scattering light can carry information of substance because it is affected by its structure.

According to the Boltzmann distribution law, due to thermal equilibrium, the number of molecules in the second highest energy level is always smaller than the number of molecules in the lower energy level, so the intensity of stokes Raman scattering light is always greater than the intensity of anti-Stokes Raman scattering light.

The Raman displacement of material is independent of the incident light frequency, but related to the vibration and rotational energy level structure of the molecule, which are inherent characteristics of the molecule. Therefore, any substance with Raman activity has its own specific Raman shift. If it wants to identify a substance, it just measures it and find out its characteristic Raman spectrum.

4.2 Detection of Living Cells

When the sample is irradiated by a certain frequency of laser beam, it will emit Raman scattering of light, which provides a lot of molecular information. Through the interpretation of Raman spectrum, the molecular type, spatial structure, chemical bond and other information can be obtained. Microscopic Raman spectroscopy technology can provide Raman spectra of complete living cells, from which the structure and changes of several major biological

macromolecules in complete living cells, such as proteins, DNA, lipids and carbohydrates. The traditional measurement of Raman spectrum is divided into point scanning and line scanning.

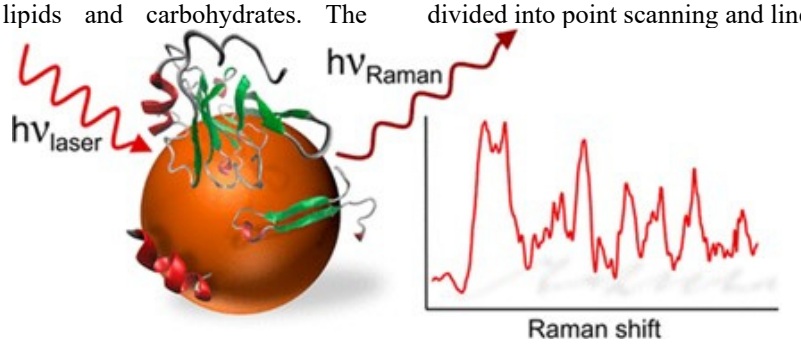

Figure 2: The Raman shift under different experimental conditions.

Point-to-point scanning imaging involves focusing a laser into a point, moving the sample under the laser with an automatic sample stand, and sequentially collecting Raman spectra through an array of points across the designated area of interest on the sample. Linear focusing scanning imaging is similar to point-to-point scanning imaging. The difference is to shine a laser on a line rather than a point on the sample. This method can collect spectra simultaneously from multiple locations on the sample, and can use more laser power, but reduces the exposure time and does not damage the sample.

Proteins are the main components of cells and the material basis of many cell functions, such as cell catalytic reaction, material transport, immune function, genetics and metabolism. Raman spectroscopy can not only provide the structural characteristics of amino acids, but also be used for quantitative analysis of secondary structure of proteins. As shown Figure 2, an obvious Raman shift can be observed when some chemical reactions occur.

Lipids are an important part of cell membranes. Raman spectroscopy can be used to study the composition of cell membrane, the location of lipids in various states and their interactions with various ions. Elucidating the composition and spatial structure of lipid membranes in living cells can be applied in many fields such as membrane pathophysiology and membrane pathophysiology.

The following is a microscopic Raman analysis for the lipidomes of individual organelles. There are hundreds of thousands of chemically different lipids. Although they usually show only minor differences in chemical structure, they tend to block chemically selective probes from labeling specific lipids.

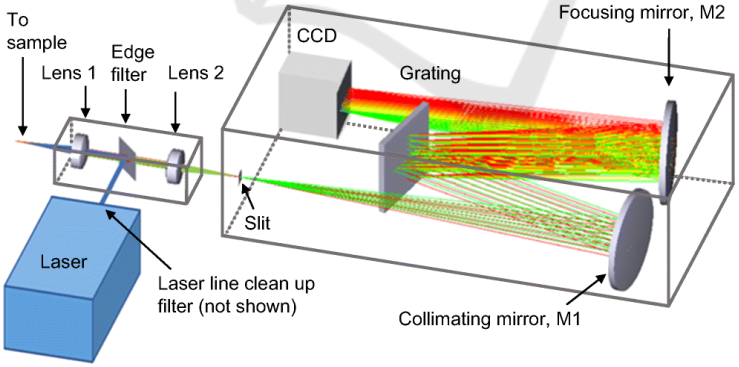

Figure 3: The used Raman spectrometer in laboratory.

4.3 Use of Raman Spectra

Raman spectrometers have evolved from ordinary academic laboratory instruments to powerful commercial solution-based systems. Update instruments are easy to use because they do not require the user to constantly adjust or have complex optical knowledge, and interact with the Raman spectrum library.

At present, there are two main methods to analyze Raman data: univariate and multivariate. The first approach uses the area, intensity, or center of gravity characteristics of the Raman spectrum to understand sample chemistry. Figure 4 shows the

basic composition of Raman spectrometer. Although univariate data analysis can be used directly, it requires that the components have sufficient Raman bands to distinguish and be unique. Overlapping bands in biological and pharmaceutical area make multivariate data analysis techniques necessary. High resolution system that enables rapid measurement and is suitable for in vivo clinical applications, and it is often used in detecting living tissue such as tumor.

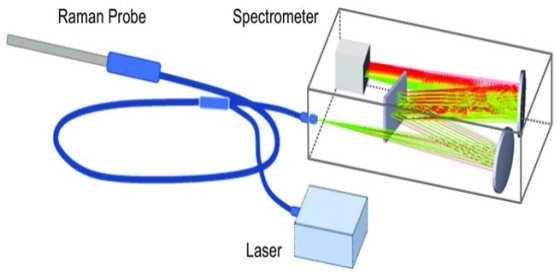

Figure 4: The typical Raman spectrometer.

5 RAMN SPECTRA IN PHARMACEUTICAL DIAGNOSIS

The wide origin, variety and complexity of Chinese medicinal materials bring many difficulties to the inspection and management of Chinese medicinal materials. In recent years, rapid and nondestructive identification of Chinese medicinal materials by Raman spectroscopy has attracted more attention. By using Raman spectroscopy, 12 different habitats and different planting modes and different collecting time of traditional Chinese medicine radix scutellariae are analyzed. The results show that the characteristic peak frequency and intensity of Raman spectroscopy to identify different ways of planting of radix scutellariae samples than traditional method. This is more direct, fast, and do not destroy samples, more accurate science. Many active components in Chinese medicinal materials have different pharmacological effects due to their different functional groups and configurations. Raman spectroscopy has been widely used in the structural analysis and isomer identification of traditional Chinese medicine (TCM) due to its strong selectivity and the absence of separation in mixture analysis. With the appearance of portable Raman spectrometer, drug regulatory departments have considered it as an important tool for drug counterfeiting. According to the existing literature, Raman spectroscopy is quick and can significantly improve the efficiency of drug market regulation. Raman spectrum can give fingerprint information about compound structure, and can distinguish some pharmaceutical excipients by Raman band, which has certain advantages over infrared spectrum in some aspects. It can be used for quality control of pharmaceutical excipients. The characterization and structure of these probes is shown in the Figure 5.

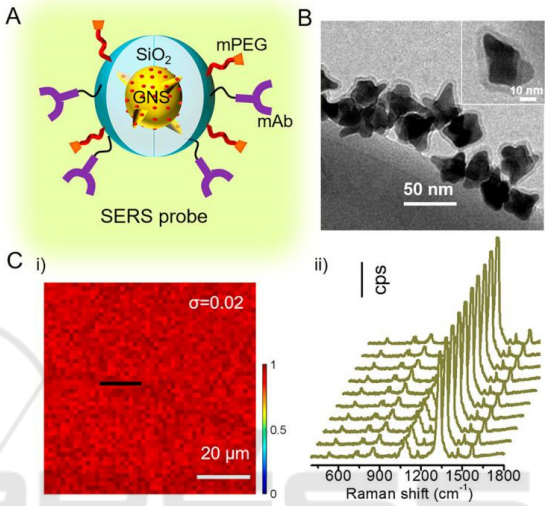

Figure 5: Structure and characterization of SERS probes

6 CONCLUSIONS

This paper shows the comparison between infrared and Raman spectra. And application of Raman spectroscopy for the pharmaceutical diagnosis and living cell detection is also analyzed. Raman spectroscopy has broad prospects in cell sorting and nondestructive detection of cancer detection. However, Raman spectroscopy usually has weak signal. If the general fluorescence signal is stronger than Raman signal, the fluorescence summit is superimposed on Raman peak, and its interference to signal judgment is relatively large. Fortunately, methods to remove strong fluorescence background to solve the influence of fluorescence background on the extraction of Raman spectral components have been developed.

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