Advances In Spectroscopy Techniques

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Spectroscopy is a powerful technique that investigates the unseen world of atoms and molecules. This approach reveals the underlying concepts and its significant importance in many scientific fields such as chemistry, biology, environmental science, and materials research. Complexities associated with mass spectrometry, nuclear magnetic resonance (NMR), infrared (IR), ultraviolet-visible (UV-Vis), Raman, and X-ray spectroscopies, with a focus on current developments in technology and data interpretation will be discussed in this chapter. Mass spectrometry has made significant advancements in analyzers and ionization techniques, resulting in its widespread use in proteomics, metabolomics, and drug development. NMR spectroscopy is of significant importance in the field of structural characterisation, and recent improvements have facilitated the investigation of solid-state materials. Moreover, the progress in equipment and data processing has significantly enhanced the efficacy of FTIR, UV-visible, and X-ray spectroscopy. Surface-enhanced Raman spectroscopy, characterized by its heightened sensitivity, is a noteworthy advancement that is propelling the frontiers of scientific investigation across various fields. The vast array of applications of spectroscopy in many fields serves as a driving force for researchers to consistently enhance these methodologies and address the challenges they face, so assuring their ongoing reliability and broadening their scope in scientific exploration.

1. Introduction to Spectroscopy

Spectroscopy, the study of interactions between materials and electromagnetic radiation, has become a cornerstone for characterizing Earth Science materials. By analyzing the resulting spectra, scientists can identify and quantify specific components based on unique absorption features. This technique has seen significant advancements since the early 1900s, with the development of infrared spectrometers and the creation of spectral libraries by pioneers like Hunt and Salisbury. These libraries, acting as a Rosetta Stone for geological remote sensing, allow researchers to decipher the complex signals from instruments and understand the composition of Earth's materials (Meer, 2018).

The roots of modern spectroscopy can be traced back to early alchemists' use of flame tests for elemental analysis. These early qualitative methods, alongside colorimetry (comparing solution colors), laid the groundwork for the field. The evolution of light sources, dispersion devices, optics, detectors, and modern technology has since fueled continuous advancements in spectroscopic instrumentation. While the underlying principles and engineering of modern ultraviolet, visible, and infrared spectrometers are sophisticated, user-friendly designs often enable accurate analysis with minimal training. This accessibility has made spectroscopy a cornerstone technique in chemical analysis. In spectroscopy, a spectrum depicts how a material absorbs electromagnetic radiation across a range of wavelengths or energies. This absorption is caused by quantized energy level transitions within the sample's atoms or molecules. While these transitions ideally result in sharp lines (line spectra), most observed peaks have a finite width. This broadening can be attributed to the Heisenberg uncertainty principle as shown in equation 1, where the lifetime (τ) of the excited state is inversely proportional to the energy level uncertainty (ΔE) of the transition. In simpler terms, shorter-lived excited states lead to broader spectral peaks.

$$\Delta E = h/2\pi\tau \quad \text{.....(1)}$$

In spectroscopic analysis, a factor influencing spectral line width is Doppler broadening. This arises because the transition energy (ΔE) between molecular states remains constant. However, the observed frequency, and consequently the perceived energy, of the emitted or absorbed radiation is influenced by the motion of the emitting or absorbing molecule relative to the observer. Molecules approaching the observer experience a slight "blueshift," meaning the observed frequency is higher than the rest energy. Conversely, molecules receding from the observer exhibit a "redshift," with a lower observed frequency. This distribution of velocities within a sample contributes to the

broadening of spectral lines (Jespersen, 2006).

Spectroscopy techniques, like fingerprints for materials, are crucial across science and industry for identifying substances, analyzing their structure, and monitoring processes, making them vital tools in fields from astronomy and medicine to forensics and materials science.

2. Modern Spectroscopy Techniques

2.1 Mass Spectrometry

Mass spectrometry utilizes an ion source, mass analyzer, and detector to dissect a sample into charged particles, measure their mass-to-charge ratios (m/z), and identify individual molecules. The mass analyzer plays a critical role, especially in analyzing biomolecules, by separating these lightweight, variably-charged entities for precise mass determination, ultimately fulfilling the instrument's core function of biomolecular identification (Haag, 2016).

Advancements

The growing importance of low-abundance molecules in research, from biomarkers to single-cell metabolites, necessitates ever-more sensitive analytical tools. Mass spectrometry (MS), with its unparalleled sensitivity and specificity, has emerged as a cornerstone technique across diverse disciplines. As the demand for precise analysis intensifies, advancements in MS continue to push the boundaries of detection, ensuring its continued relevance in various research fields. Mass spectrometry sensitivity can be enhanced through various approaches. Pretreatment optimization reduces matrix interferences, improving detection of low-abundance analytes. Novel ionization techniques boost signal intensity and remove matrix interferences, while advancements in ion transmission systems like funnels and lenses improve ion movement. Finally, advancements in mass analyzers enhance sensitivity through improved ion transmission, targeted ion enrichment, utilization rate, and signal-to-noise ratio.

Due to their simple design and affordability, quadrupole mass analyzers are prevalent tools for applications like atmospheric monitoring and aerospace exploration. The Curiosity rover on Mars, for instance, utilizes a quadrupole analyzer to detect light isotopes and volatile compounds, confirming the presence of organic molecules like chlorobenzene on the red planet. However, their limitations in mass range and resolution render them less suitable for analyzing complex macromolecules. Pioneered by Wolfgang Paul and colleagues, the 3D ion trap mass analyzer revolutionized mass spectrometry. Its simple design, combining hyperboloid end-cap electrodes with a ring electrode, facilitates miniaturization while enabling ion focusing, selective ion isolation (m/z), and MSn analysis. These features, along with its modest vacuum needs and high sensitivity, make it ideal for qualitative proteomic studies and have significantly contributed to the advancement of this field. Time-of-flight (TOF) mass spectrometry offers a powerful combination of rapid analysis and high mass accuracy, making it an ideal choice for high-throughput applications and the characterization of large biomolecules. For high-resolution mass spectrometry, the Orbitrap analyzer reigns supreme, often working in tandem with other analyzers. Similarly, the C-trap can be incorporated as a collision cell to generate fragment ions, mirroring the function of a quadrupole analyzer (Li et al., 2021).

2.2 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) offers a powerful technique for studying materials at the atomic level. It relies on the interaction between nuclei and magnetic fields. When placed in a strong, constant magnetic field, certain atomic nuclei with non-zero spin angular momentum will align themselves. The application of a weak, oscillating magnetic field can then perturb this alignment. If the frequency of the oscillating field matches a specific resonant frequency of the nuclei, an electromagnetic signal is emitted. This resonant frequency is influenced by the surrounding

chemical environment of the nucleus, providing valuable information about the structure and dynamics of molecules.

Biomacromolecules' functions arise from a delicate interplay between their intricate 3D structures and their inherent dynamic nature. These flexible molecules exhibit a wide range of motions, from picoseconds to seconds. NMR spectroscopy has become the gold standard for investigating both protein structure and dynamics in solution, as it offers sensitivity to both aspects simultaneously. However, despite advancements in experimental techniques and computational analysis, reconciling structural and dynamic information from NMR data remains a challenge. Thankfully, molecular dynamics simulations have become an invaluable tool to bridge this gap and provide a more comprehensive understanding of these biomolecules. NMR spectroscopy offers a powerful technique for determining biomolecule structure. By studying various "relaxation channels" sensitive to both structure and dynamics, it provides a set of distance and angle restraints. These restraints are then used within a molecular modeling algorithm to generate a three-dimensional representation of the biomolecule (Markwick et al., 2008).

Advancements

Advancements in instrumentation and software have propelled NMR spectroscopy to the forefront of analytical methods in biology. While multidimensional pulse sequences continue to be developed, a small set of 2D experiments can rapidly determine the structure of small metabolites. Even more complex 3D and 4D techniques enable the elucidation of protein structures up to 100 kDa, with over 100 plant protein structures established using NMR. Additionally, NMR finds application in the nascent field of metabolomics, offering unbiased metabolite profiling of plant extracts. High-resolution NMR has become a cornerstone technology for deciphering biosynthetic pathways and metabolite flux through the quantification of multiple isotopologues (Eisenreich & Bacher, 2007).

Solid state NMR

The development of metal-organic frameworks (MOFs) represents a significant leap in materials science. However, despite their exciting potential applications, a gap exists in understanding the local structures, molecular details, and guest interactions that govern their functionalities. Solid-state nuclear magnetic resonance (SSNMR) emerges as a complementary technique to X-ray diffraction (XRD), offering crucial insights into these critical aspects of MOFs. Solid-state NMR (SSNMR) spectroscopy has undergone a remarkable transformation in recent decades, becoming a powerful tool for investigating microporous materials. Multinuclear and multidimensional techniques offer a wealth of information on the framework structure of these materials, including the dynamics of guest molecules within their pores. Solid-state nuclear magnetic resonance (SSNMR) studies often utilize deuterium (^{2}H or D) nuclei instead of protons (^{1}H) due to the significant broadening of signals caused by strong homonuclear dipolar interactions between protons. Deuterium possesses a quadrupolar moment, which allows researchers to extract information about the mobility of hydrogen molecules (H_2) within Metal-Organic Frameworks (MOFs) by analyzing the lineshape of the ^{2}H NMR signal. This approach provides valuable insights into the dynamic behavior of H_2 within these porous materials.

Pulsed field gradient (PFG) NMR allows researchers to probe the self-diffusion coefficient and pathway of guest molecules navigating the channels. Hyperpolarized 129Xe NMR provides detailed insights into the cage and channel structure, as well as communication pathways within the material. Variable temperature (VT) SSNMR is a well-established method for studying the flexibility and dynamic behavior of porous functional materials at the molecular level. Furthermore, SSNMR offers significant potential for elucidating host-guest interactions between small molecules and these porous materials. In situ SSNMR techniques are emerging as a powerful tool for unraveling catalytic reaction mechanisms over microporous materials by enabling the observation

of reactive intermediates trapped within the material's voids or channels. Recent advancements in instrumentation and techniques are continuously improving the sensitivity of SSNMR for materials analysis, allowing detection of nuclei with low natural abundance, weak magnetic properties (γ), or significant quadrupole interactions (Li et al., 2020; Wong et al., 2019).

2.3 Infrared (IR) Spectroscopy

Infrared (IR) spectroscopy is a cornerstone technique in analytical pharmacy and chemistry. It provides a window into the vibrational motions of atoms within a molecule, hence the term vibrational spectroscopy. An IR spectrum is generated by passing infrared radiation through a sample and measuring the amount of light absorbed at specific frequencies. Fourier transform (FT) infrared (FTIR) spectrometers have revolutionized the field, offering significant advantages in speed and efficiency compared to dispersive instruments. The core of an FTIR spectrometer is the interferometer, which utilizes a beamsplitter to divide the source radiation into two beams. These beams travel down separate paths, reflect off mirrors, and recombine at the beamsplitter. The path length difference between the two beams determines whether they interfere constructively or destructively, resulting in a signal recorded by the detector. This signal, known as an interferogram, is then mathematically transformed (using the Fourier transform) into an IR spectrum. To obtain the sample's specific absorption profile, two interferograms are collected: one with the sample in the beam path and another without. The ratio of these transformed interferograms yields the sample's IR transmission spectrum. In most cases, the sample is positioned between the interferometer and the detector for optimal analysis.

Advancements

Attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) is a powerful analytical technique employed in pharmaceutical science due to its speed, minimal sample preparation requirements, and non-destructive nature. ATR-FTIR utilizes the principle of total internal reflection, where an infrared beam reflects entirely off an internal surface when the incident angle surpasses a critical value. This allows for the characterization of a material's surface functional groups without complex sample manipulation. In the realm of pharmaceuticals, ATR-FTIR finds applications in structural Analysis (Characterizing the structure and properties of biological systems like the Stratum Corneum), non-invasive Drug Release Studies (Monitoring the release of drugs from semi-solid formulations without disrupting the sample integrity), Drug Diffusion Measurement (Quantifying drug diffusion from pharmaceutical systems such as polymers and films), drug Penetration Assessment (Investigating the penetration of drugs into artificial and biological membranes, aiding in the design of targeted delivery systems) and drug-macromolecule interaction studies (Elucidating interactions between drugs and various macromolecules, including synthetic, semi-synthetic, and native biomolecules). This analytical power, coupled with its ease of use, makes ATR-FTIR a valuable tool for pharmaceutical scientists across various research and development stages (Dole et al., 2011).

Fourier Transform-Infrared (FT-IR) spectroscopy offers a powerful window into the microbial world. Micro-FTIR spectroscopy allows researchers to analyze the molecular composition of specific microorganisms or their components within a sample with high spatial resolution. This technique is particularly valuable for studying microscopic features or isolated microbial structures. Conversely, Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectroscopy excels at analyzing bulk samples, including microorganisms in their natural state (e.g., powdered or granulated forms) with minimal preparation. Together, these FT-IR techniques provide invaluable insights into the intricate biochemical processes underlying microbial interactions that shape ecosystems and biogeochemical cycles (Kassem et al., 2023).

Fourier-Transform Infrared Spectroscopy (FTIR) offers a powerful analytical tool for archaeologists due to its versatility. Requiring minimal sample amounts (milligrams or micrograms),

it can analyze a wide range of materials, including organic and inorganic, crystalline and amorphous. This allows archaeologists to identify and compare the composition of diverse archaeological finds, from fired clays and bone fragments to fibers and dyes. This broad applicability makes FTIR a valuable asset for any archaeological laboratory (Margaris, 2020).

2.4 UV-Visible Spectroscopy

Due to its inherent advantages, ultraviolet-visible (UV-Vis) spectroscopy has emerged as a powerful tool for pollutant detection in aqueous environments. These advantages include high precision, fast analysis times, minimal sample disruption, environmental friendliness, affordability, and the potential for portable instrumentation. Underpinning the quantitative analysis capabilities of UV-Vis in water quality monitoring is the Lambert-Beer Law. This law dictates the interaction between light and a light-absorbing medium. A monochromatic beam of light strikes the sample, and as it travels through the medium, a portion of the light energy is absorbed. This absorption reduces the intensity of the transmitted light, with the extent of this reduction being directly proportional to the thickness of the absorbing medium (Kot et al., 2021).

Due to its limited range within the electromagnetic spectrum, visible light differs from other forms of radiation in the energy frequency of its photons. However, the ultraviolet-visible (UV-Vis) region, encompassing wavelengths from approximately 180-800 nm, is frequently utilized for the analysis of organic molecules. Absorption within this region primarily involves electronic level transitions, although vibrational transitions can also contribute. Transitions requiring higher energy input, such as $n \rightarrow \sigma^*$ and $\sigma \rightarrow \sigma^*$, are typically associated with UV absorption, while $\pi \rightarrow \pi^*$ transitions correspond to UV-Vis absorption. The portion of the molecule responsible for these electronic transitions is termed the chromophore.

Advancements

In instances where a molecule exhibits transparency in the UV-Vis region, various derivatization techniques can be employed to enhance detection sensitivity and, in some cases, selectivity. Several analytical strategies are employed to enhance detection sensitivity, including Schiff base formation, complexation reactions, and enzymatic/catalytic transformations. Additionally, techniques like azodye derivatization, charge transfer complexation, ion-pair analysis, and solid-phase derivatization can further improve signal strength. Furthermore, preconcentration/enrichment steps utilizing solvent or sorbent extraction or membrane separation can be implemented to magnify the target analyte for more effective detection. Recent advancements in UV-Vis spectrophotometry focus on improving sensitivity, precision, and accuracy while simultaneously minimizing analysis costs and time.

Advancements in UV-Vis Detection Technology

Ultraviolet-visible (UV-Vis) detection has undergone significant advancements in recent years. The development of novel detectors has focused on enhancing detection sensitivity, minimizing limitations, and improving data acquisition speed.

Traditional Detectors

Photomultiplier tubes (PMTs): Highly sensitive PMTs offer exceptional low-light detection and high wavelength resolution due to their compatibility with narrow slit widths. These detectors have been widely employed for UV and visible region analyses.

Modern Detector Advancements

Photodiode Array (PDA) Detectors: PDAs excel as multichannel detectors due to their ability to monitor the entire UV-Vis spectrum simultaneously. They can rapidly capture complete spectra, making them ideal for applications like HPLC and flow analysis where transient signals are present. Additionally, PDAs offer advantages like high photon flux quantification, high quantum

efficiency, and a superior height-to-width aspect ratio. However, limitations include significant dark current and high read noise.

Charged-Coupled Devices (CCDs): CCDs were developed to address the limitations of PDAs. These silicon chip-based detectors convert light into an electrical signal and boast advantages like low dark count rate, low read noise, and high UV-Vis quantum efficiency, making them exceptional spectroscopic detectors.

Emerging Technologies

Light-Emitting Diodes (LEDs): LEDs, featuring p-n junctions that emit narrow light bands under forward bias, offer several advantages. Their low power consumption, reduced size, high sensitivity, and broad wavelength response with a high signal-to-noise ratio make them attractive options. Notably, their direct pulse-duration-modulated output eliminates the need for expensive A/D converters. These benefits extend beyond simple absorbance measurements, making them suitable for flow-through optical sensors in chromatography and flow analysis applications.

NanoDrop Technology: This technology utilizes a proprietary sample-retention system, enabling analysis with minimal sample volumes (approximately 1 μL). These microvolume spectrophotometers employ a unique design where the sample is precisely positioned between two optical surfaces. Surface tension forces create a liquid column with a controllable path length, eliminating the need for sample dilution across a broad concentration range.

These advancements in UV-Vis detection technology have significantly expanded analytical capabilities, offering researchers greater sensitivity, faster data acquisition, and improved performance in various scientific disciplines (Passos & Saraiva, 2019).

Applications

UV-vis spectroscopy offers a valuable toolbox for chemical engineers due to its versatility and affordability. This non-destructive technique analyzes a broad range of organic compounds and select inorganic species by measuring light absorbance or transmittance across a wavelength spectrum. Applications include quantitative analysis, reaction kinetics investigation, and elucidating reaction mechanisms at the molecular level. Furthermore, hyphenated techniques like HPLC-UV-vis and UHPLC-UV-vis enable both identification and quantification of compounds in liquid streams. Coupling UV-vis with mass spectrometry provides comprehensive identification of all present species. Additionally, UV-vis diffuse reflectance spectroscopy extends the analysis to solid and powdered materials (Rocha et al., 2018).

2.5 Raman Spectroscopy

The convergence of biomedical and nanobiotechnological research has opened exciting avenues for exploration. Studies on antioxidant carotenoids, disease-specific molecular markers, and microalgal production for biofuels all require a deep understanding of biological processes at the cellular level. This is where Raman spectroscopy (RS) emerges as a powerful tool. RS offers a unique window into cellular events by exploiting the Raman scattering phenomenon. Unlike Rayleigh scattering, where the light frequency remains unchanged, Raman scattering involves a shift in the scattered photon's frequency, revealing information about the sample's molecular composition. Notably, Stokes shifts, where the scattered photon loses energy (longer wavelengths), are more prevalent.

Several variations of RS exist, each leveraging Raman scattering differently. The choice of technique depends on factors like sample complexity and target molecule concentration. Conventional RS forms the foundation for a diverse "family tree" of advanced techniques, including Surface-enhanced Raman Spectroscopy (SERS), Coherent Anti-Stokes Raman Spectroscopy (CARS), and Spatially Offset Raman Spectroscopy (SORS). While conventional RS utilizes a continuous wave

(CW) laser, techniques like CARS and SRS employ pulsed lasers for nonlinear optical interactions. Notably, CARS microscopy offers high-resolution, label-free imaging of endogenous chemical structures, proving valuable in various biomedical fields.

Advancements

A key limitation of RS is its inherently weak signal. Fortunately, techniques like Resonance-enhanced Raman Scattering (RRS) address this challenge. By matching the laser excitation wavelength to a molecule's absorption peak, RRS significantly enhances the Raman signal of specific bands, leading to more detailed analysis. (Allakhverdiev et al., 2022).

Surface-enhanced Raman spectroscopy (SERS) offers a powerful analytical tool for scientists due to its exceptional sensitivity. This technique amplifies the weak Raman scattering signal of molecules adsorbed on specific nanostructured materials. The amplification arises from a combination of plasmon-mediated enhancement of the local electric field and potential chemical effects between the analyte and the substrate. This dramatic increase in sensitivity allows SERS to act as a fingerprint technique for low-concentration analytes (Han et al., 2021; Stiles et al., 2008).

Application

The continued development and application of these Raman spectroscopy techniques are poised to significantly advance our understanding of biological processes at the cellular level, propelling further breakthroughs in medicine and biotechnology. Consequently, SERS finds applications in a broad range of fields including surface and interface chemistry, catalysis research, nanotechnology developments, biological and biomedical studies, food science analysis, and environmental monitoring

2.6 X-ray Spectroscopy

X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) emerges as a cornerstone technique for exploring surface properties, particularly in the crucial first 10 nanometers. This capability extends across various scientific and engineering disciplines. Understanding a material's surface chemistry, structure, and interfacial behavior is paramount when evaluating its corrosion resistance under diverse environmental conditions. Early surface reactions primarily occur within this nanoscale region, highlighting the necessity for tools adept at probing such depths. XPS stands out as a preferred method, delivering detailed chemical compositions of material surfaces within this 10 nm window. Additionally, XPS offers elemental identification and detection sensitivity as low as 0.1-1 atomic percent (except for H and He), solidifying its position as a unique tool for surface characterization, specifically in corrosion studies. Notably, XPS facilitates the investigation of surface reactions under vacuum environments, with the ability to resolve down to a single monolayer.

The technique allows for analysis of compositional variations within thin films (less than 10 nm) by manipulating the take-off angle of ejected electrons or by employing different X-ray excitation energies. This approach avoids film destruction during analysis. XPS further enables the study of surface film passivity, its breakdown in aqueous environments, adhesion properties, mass transport phenomena, and selective oxidation. This technique has proven instrumental in elucidating the critical role of chromium and molybdenum in stainless steels, particularly regarding their passivation mechanisms.

However, XPS analysis encounters limitations when dealing with films exceeding 10 nm in thickness. These limitations can manifest as artifacts, including preferential etching of specific elements, alterations in oxidation states, ion implantation, and atomic mixing during the ion beam sputtering process used for depth profiling. Additionally, insulating specimens can present challenges due to surface charging, leading to peak shifts, broadening, and asymmetry, potentially compromising the accuracy and complexity of the analysis.

The power of XPS and its application

Understanding the interplay between a material's surface chemistry, structure, and interface is critical for predicting its corrosion behavior in various environments. Since initial surface reactions often occur within the top few nanometers, analytical tools with nanoscale sensitivity are essential. X-ray photoelectron spectroscopy (XPS) emerges as a preferred technique due to its exceptional surface sensitivity (within 10 nm). It offers detailed chemical composition information, elemental identification, and detection limits as low as 0.1-1 atomic percent (except for H and He). This unique combination makes XPS an invaluable tool for surface characterization, particularly in corrosion evaluation.

XPS is a powerful technique for investigating surface reactions at the atomic level. Its high surface sensitivity allows for the analysis of changes occurring within the topmost atomic layer (monolayer) under vacuum conditions. By varying the take-off angle of ejected electrons or utilizing different X-ray excitation energies, XPS can analyze the composition variation within a thin film (less than 10 nm) without compromising its integrity. This capability allows for investigations into film passivity, breakdown in aqueous environments, adhesion mechanisms, mass transport phenomena, and selective oxidation processes. XPS has played a crucial role in elucidating the role of chromium and molybdenum in stainless steels and the underlying mechanisms of passivity.

However, XPS has limitations when dealing with films exceeding 10 nm in thickness. Techniques like ion beam sputtering, used to determine film depth, can introduce artifacts such as preferential etching of elements, modification of oxidation states, ion implantation, and atomic mixing. Additionally, analyzing insulating specimens can be challenging due to surface charging, leading to peak shifts, broadening, and asymmetry, potentially hindering accurate analysis.

The following equation describes the fundamental principle of XPS, where EB represents binding energy, EK signifies kinetic energy, h is Planck's constant, ν is the X-ray photon frequency, and φ is the spectrometer's work function (both constant for a specific instrument).

$$EB=h\upsilon-EK-\varphi$$

This equation assumes electron energy levels as "frozen orbitals," implying minimal change between the initial (ground) and final (excited) states. In reality, the measured binding energies deviate slightly from theoretical values by a few electron volts (eV) due to "relaxation energy" (ER). This energy difference arises from the rearrangement of electrons and their energy levels during the photoemission process (Krishna & Philip, 2022).

X-ray absorption spectroscopy (XAS) advancements

X-ray Absorption Spectroscopy (XAS) offers a powerful toolkit for probing the electronic structure and local atomic arrangements (short-range order) in materials. This versatility makes it highly valuable across diverse scientific disciplines, from physics and chemistry to materials science, biology, and medicine. Notably, unlike X-ray Diffraction (XRD) or X-ray Photoelectron Spectroscopy (XPS), XAS measurements traditionally rely on synchrotron radiation sources due to the specific requirements for high-intensity, tunable X-rays. However, recent advancements in laboratory-based XAS spectrometers are transforming accessibility. These instruments enable researchers to analyze samples relevant to contemporary research within practical timeframes. This innovation addresses a major limitation of synchrotron radiation research – the restricted access for many research groups.

The development of laboratory XAS instruments can be categorized into three generations. The second generation, emerging in the 1980s, paved the way for broader application across research fields. These instruments utilized curved crystals in conjunction with high-power X-ray tubes, achieving the required spectral resolution and photon flux for transmission mode measurements.

This development significantly impacted research areas like catalysis.

The newest, third generation of laboratory XAS instruments encompasses two distinct design concepts. One innovation involves the use of spherical crystals in the Johann geometry for monochromatization. The second approach employs the von Hamos geometry for simultaneous spectral measurement, utilizing either a graphite crystal with exceptional integral reflectivity or segmented crystals.

3. Emerging Techniques

3.1 Terahertz Spectroscopy

Terahertz (THz) radiation, occupying the electromagnetic spectrum between microwaves and infrared, presents unique opportunities for various scientific and technological applications. Generating THz radiation necessitates specialized techniques. Traditional methods include Fourier-transform infrared (FTIR) spectrometers, offering versatility with both broadband and narrow-band options. For pulsed THz generation, electron accelerators, air/laser-induced plasmas, and photo-Dember emitters find application. Early contributions came from gas lasers, enabling continuous-wave (CW) operation and high power output. However, limitations in size and cost hampered widespread adoption. Research efforts have since yielded a diverse range of sources, including Gunn diodes, photonic devices utilizing light-matter interactions, superlattice structures, and optical rectification techniques. Broadly, these sources can be classified as accelerator-based or laser-based. While accelerator-based sources provide highly focused THz radiation, laser-based alternatives offer advantages in compactness and efficiency. Current advancements are directed towards developing high-power THz sources through improved electron accelerators and cost-effective approaches employing multimode laser diodes. This ongoing development is crucial for unlocking the full potential of THz technology across various disciplines (Feng & Otani, 2021).

Applications

Terahertz time-domain spectroscopy (THz-TDS) has emerged as a powerful tool for investigating biological molecules. Studies have shown distinct terahertz absorption fingerprints for DNA, proteins like bovine serum albumin, and structural proteins like collagen. This technique offers advantages over existing methods, allowing researchers to probe not only nucleic acids and proteins but also carbohydrates. Notably, THz spectroscopy, when combined with protein folding studies, can unveil reactions occurring at incredibly fast timescales (femtoseconds) during crucial protein conformational changes. While attempts have been made to analyze the secondary structure of peptides (short protein chains) using THz radiation, challenges in interpreting spectra from dried or frozen samples limit its definitive application in this area. Moving forward, THz-TDS holds significant promise for analyzing protein tertiary structure (3D folding) if the collective vibrational modes of the protein fall below 10 THz. This would enable differentiation between various protein bonds based on their unique terahertz frequencies, leading to a deeper understanding of protein structure and function (Falconer & Markelz, 2012).

Unlike X-rays, THz waves can safely penetrate many non-conducting materials like clothing and paper due to their non-ionizing nature and low power levels. This unique property offers significant advantages for security screening applications. THz technology excels in material differentiation. Clothing appears transparent to THz waves, revealing hidden objects beneath. Additionally, THz imaging distinguishes between plastics and ceramics, overcoming the limitations of X-ray's limited contrast. Furthermore, femtosecond THz pulses enable high-resolution 3D imaging, capable of resolving fine details like individual pages within a sealed envelope.

THz security screening boasts another key advantage: spectroscopic analysis. Terahertz waves can detect the unique chemical "fingerprints" of concealed objects, even when they are sealed or hidden beneath clothing. This spectroscopic capability offers unparalleled detection capabilities

for security personnel.

In conclusion, terahertz technology presents a revolutionary approach to security screening. Its ability to safely penetrate materials, differentiate materials, and perform 3D imaging with spectroscopic analysis makes it a powerful tool for identifying concealed objects and enhancing security measures (Wietzke et al., 2010).

3.2 Plasmonic Sensing

In recent decades, plasmonic sensing has emerged as a powerful and versatile technique with applications across diverse fields. It plays a crucial role in chemical and biological sensing, industrial process monitoring, environmental engineering, and even pushes the boundaries of imaging with super-resolution capabilities. Notably, its non-invasive nature has made plasmonic sensing a cornerstone of nanobiotechnology and the development of biomedical nanodevices. This technology's impact is evident in the substantial growth of the global smart healthcare market, reaching a value of 293 billion USD.

The performance of plasmonic technologies hinges on two key factors: the meticulously crafted shape of the employed nanostructures and the intricate characteristics of the interface between these nanomaterials and their surrounding environment. Deviations from perfectly spherical nanoparticles, such as sharper corners or non-symmetrical shapes, can significantly amplify the localized electromagnetic fields near the surface. These localized field modifications exhibit exquisite sensitivity, even to minute changes in the surrounding dielectric environment. Consequently, plasmonic nanostructures act as highly sensitive optical probes. They can detect and reveal the presence of trace amounts of active molecules at the interface between the metal and the surrounding dielectric medium.

Surface plasmon resonance (SPR) has become a cornerstone technology for diverse photonic devices, encompassing demultiplexers, splitters, filters, and crucially, sensors. SPR sensors offer compelling advantages over traditional methods, including unrivalled sensitivity, rapid response times, and a compact footprint. Their operation hinges on the principle that the resonance wavelength of surface plasmons – collective electron oscillations at a metal-dielectric interface – shifts upon interaction with the surrounding environment. This shift is exquisitely sensitive to changes in the effective refractive index (RI) of the sensor structure, triggered by the introduction of different materials. The sensitivity of the sensor can then be meticulously calculated based on this measured RI change.

Optimizing the effectiveness of SPR sensors necessitates careful consideration of several key parameters: sensitivity, resolution, and signal-to-noise ratio (SNR). Sensitivity remains paramount in the design process. Recent advancements have witnessed the exploration of various plasmonic RI sensor configurations, including those incorporating slit waveguides coupled with nanodisk resonators, ring resonators, hexagonal cavities, slit cavities, and strategically placed single defects. Furthermore, high dielectric constant materials, such as silver (Ag) and gold (Au), are favored choices due to their ability to support both localized and propagating surface plasmon modes (Divya et al., 2022).

Advancements

Localized surface plasmon resonance (LSPR) describes the collective oscillation of electrons in metallic nanoparticles upon interaction with an electromagnetic field. These LSPR modes exhibit a unique sensitivity to the incident light field due to their highly responsive nature, allowing for direct excitation from free space without the need for critical momentum matching, unlike surface plasmon resonance (SPR) techniques.

Two crucial aspects governing LSPR sensitivity for sensing applications considered are:

Analyte Adsorption: The intricate shapes and surface properties of nanostructures significantly influence how target molecules (analytes) bind to them. This interaction depends heavily on both the surface area and energy of the nanostructure, ultimately impacting the intensity, polarization, and local electromagnetic fields associated with the LSPR peak.

Nanoparticle Properties: The size and geometry of the metallic nanoparticles within the dispersion dictate the wavelength and symmetry of the LSPR peak. These characteristics are highly sensitive to the surrounding environment, making them ideal for sensing applications. Changes in analyte concentration directly modify the surrounding refractive index, consequently impacting the LSPR band (peak position, intensity, or width). By monitoring these changes, plasmonic sensors can effectively detect the presence and concentration of target analytes (Duan et al., 2021).

Application

Localized surface plasmon resonance (LSPR) offers a powerful toolbox for biosensing due to its ability to enhance light-matter interactions. This translates to highly sensitive techniques like SERS, SEIRA, and surface-enhanced fluorescence. Furthermore, LSPRs can be used as miniaturized sensors due to their excitation by simple light sources and shallow penetration depth, making them ideal for real-time multiplex detection in microfluidic devices. Beyond biosensing, plasmonically functionalized thin films are emerging as robust monitoring solutions for harsh industrial environments due to their durability, sensitivity, and all-optical readout capabilities (Mejía-Salazar & Oliveira Jr, 2018; Tittl et al., 2014).

3. Conclusion

Spectroscopic techniques play a critical role in Earth Science research, providing a comprehensive toolbox for material characterization. Mass spectrometry (MS) excels in high-throughput analysis and detection of low-abundance molecules. Nuclear Magnetic Resonance (NMR) offers unparalleled insights into biomolecular structure and dynamics. Attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) and Fourier Transform-Infrared (FT-IR) spectroscopy offer powerful solutions for pharmaceutical science and microbial analysis respectively. Ultraviolet-visible (UV-Vis) spectroscopy with its recent advancements finds continued relevance. Resonance-enhanced Raman Scattering (RRS) and Surface-enhanced Raman spectroscopy (SERS) provide exceptional sensitivity for detailed analysis. X-ray photoelectron spectroscopy (XPS) emerges as a cornerstone for surface characterization, particularly crucial for understanding corrosion behavior. X-ray Absorption Spectroscopy (XAS) offers a powerful tool for probing material properties across diverse disciplines. Terahertz (THz) time-domain spectroscopy (THz-TDS) presents unique opportunities for biological analysis. Finally, plasmonic sensing has emerged as a powerful and versatile technique with widespread applications. In conclusion, the ever-evolving suite of spectroscopic techniques continues to revolutionize our understanding of Earth Science materials.

References

Allakhverdiev, E. S., Khabatova, V. V., Kossalbayev, B. D., Zadneprovskaya, E. V., Rodnenkov, O. V., Martynyuk, T. V., Maksimov, G. V., Alwasel, S., Tomo, T., & Allakhverdiev, S. I. (2022). Raman spectroscopy and its modifications applied to biological and medical research. Cells, 11(3), 386.

Divya, J., Selvendran, S., Raja, A. S., & Sivasubramanian, A. (2022). Surface plasmon based plasmonic sensors: A review on their past, present and future. Biosensors and Bioelectronics: X, 11, 100175.

Dole, M. N., Patel, P. A., Sawant, S. D., & Shedpure, P. S. (2011). Advance applications of Fourier transform infrared spectroscopy. Int. J. Pharm. Sci. Rev. Res, 7(2), 159-166.

Duan, Q., Liu, Y., Chang, S., Chen, H., & Chen, J.-h. (2021). Surface plasmonic sensors:

Sensing mechanism and recent applications. Sensors, 21(16), 5262.

Eisenreich, W., & Bacher, A. (2007). Advances of high-resolution NMR techniques in the structural and metabolic analysis of plant biochemistry. Phytochemistry, 68(22), 2799-2815. https://doi.org/https://doi.org/10.1016/j.phytochem.2007.09.028

Falconer, R. J., & Markelz, A. G. (2012). Terahertz spectroscopic analysis of peptides and proteins. Journal of Infrared, Millimeter, and Terahertz Waves, 33, 973-988.

Feng, C.-H., & Otani, C. (2021). Terahertz spectroscopy technology as an innovative technique for food: Current state-of-the-Art research advances. Critical Reviews in Food Science and Nutrition, 61(15), 2523-2543.

Haag, A. M. (2016). Mass Analyzers and Mass Spectrometers. In H. Mirzaei & M. Carrasco (Eds.), Modern Proteomics – Sample Preparation, Analysis and Practical Applications (pp. 157-169). Springer International Publishing. https://doi.org/10.1007/978-3-319-41448-5_7

Han, X. X., Rodriguez, R. S., Haynes, C. L., Ozaki, Y., & Zhao, B. (2021). Surface-enhanced Raman spectroscopy. Nature Reviews Methods Primers, 1(1), 87.

Jespersen, N. (2006). Chapter 5 General principles of spectroscopy and spectroscopic analysis. In S. Ahuja & N. Jespersen (Eds.), Comprehensive Analytical Chemistry (Vol. 47, pp. 111-155). Elsevier. https://doi.org/https://doi.org/10.1016/S0166-526X(06)47005-7

Kassem, A., Abbas, L., Coutinho, O., Opara, S., Najaf, H., Kasperek, D., Pokhrel, K., Li, X., & Tiquia-Arashiro, S. (2023). Applications of Fourier Transform-Infrared spectroscopy in microbial cell biology and environmental microbiology: advances, challenges, and future perspectives. Frontiers in Microbiology, 14, 1304081.

Kot, P., Muradov, M., Gkantou, M., Kamaris, G. S., Hashim, K., & Yeboah, D. (2021). Recent advancements in non-destructive testing techniques for structural health monitoring. Applied Sciences, 11(6), 2750.

Krishna, D. N. G., & Philip, J. (2022). Review on surface-characterization applications of X-ray photoelectron spectroscopy (XPS): Recent developments and challenges. Applied Surface Science Advances, 12, 100332.

Li, C., Chu, S., Tan, S., Yin, X., Jiang, Y., Dai, X., Gong, X., Fang, X., & Tian, D. (2021). Towards Higher Sensitivity of Mass Spectrometry: A Perspective From the Mass Analyzers [Review]. Frontiers in Chemistry, 9. https://doi.org/10.3389/fchem.2021.813359

Li, S., Lafon, O., Wang, W., Wang, Q., Wang, X., Li, Y., Xu, J., & Deng, F. (2020). Recent Advances of Solid-State NMR Spectroscopy for Microporous Materials. Advanced Materials, 32(44), 2002879. https://doi.org/https://doi.org/10.1002/adma.202002879

Margaris, A. V. (2020). Fourier transform infrared spectroscopy (FTIR): Applications in archaeology. In Encyclopedia of global archaeology (pp. 4350-4353). Springer.

Markwick, P. R. L., Malliavin, T., & Nilges, M. (2008). Structural Biology by NMR: Structure, Dynamics, and Interactions. PLOS Computational Biology, 4(9), e1000168. https://doi.org/10.1371/journal.pcbi.1000168

Meer, F. v. d. (2018). Near-infrared laboratory spectroscopy of mineral chemistry: A review. International Journal of Applied Earth Observation and Geoinformation, 65, 71-78. https://doi.org/https://doi.org/10.1016/j.jag.2017.10.004

Mejía-Salazar, J., & Oliveira Jr, O. N. (2018). Plasmonic biosensing: Focus review. Chemical Reviews, 118(20), 10617-10625.

Passos, M. L., & Saraiva, M. L. M. (2019). Detection in UV-visible spectrophotometry: Detectors, detection systems, and detection strategies. Measurement, 135, 896-904.

Rocha, F. S., Gomes, A. J., Lunardi, C. N., Kaliaguine, S., & Patience, G. S. (2018). Experimental methods in chemical engineering: Ultraviolet visible spectroscopy—UV-Vis. The Canadian Journal of Chemical Engineering, 96(12), 2512-2517.

Stiles, P. L., Dieringer, J. A., Shah, N. C., & Van Duyne, R. P. (2008). Surface-enhanced Raman spectroscopy. Annu. Rev. Anal. Chem., 1, 601-626.

Tittl, A., Giessen, H., & Liu, N. (2014). Plasmonic gas and chemical sensing. Nanophotonics, 3(3), 157-180.

Wietzke, S., Jansen, C., Krumbholz, N., Peters, O., Vieweg, N., Jördens, C., Scheller, M., Romeike, D., Jung, T., & Reuter, M. (2010). Terahertz spectroscopy: A powerful tool for the characterization of plastic materials. 2010 10th IEEE International Conference on Solid Dielectrics,

Wong, Y. A., Martins, V., Lucier, B. E., & Huang, Y. (2019). Solid-State NMR Spectroscopy: A Powerful Technique to Directly Study Small Gas Molecules Adsorbed in Metal–Organic Frameworks. Chemistry–A European Journal, 25(8), 1848-1853.

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