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Research Article

APPLICATIONS OF X-RAY DIFFRACTION AND FOURIER TRANSFORM-INFRARED SPECTROSCOPY IN DRUG DEVELOPMENT, FORMULATION, AND MICROBIAL ANALYSIS

***Hussein Jabbar Ghazi Sajet, Fatimah Salah Oleiwi, Alaa Nazar Hadi and Shahba Ahmed Abbas Alwan**

Department of Applied Medical Physics, Al-Hilla University College, Iraq

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Abstract

The X-ray diffraction (XRD) technique is a very accurate instrument that may be used to determine the hydration of cementitious materials. This technique involves time-resolved quantitative analysis with an appropriate level of accuracy with the sample. The topic of microbial calcite precipitated cement hydration was being examined in this study. The first section consisted of a study of the numerous pieces of literature that were associated with XRD, a discussion of the various experimental strategies, and the selection of the appropriate method for testing the bio-concrete. Comparative analysis was performed between the conventional samples and the bio-concrete samples. The bacteria belonging to the Bacillus family, which are responsible for precipitating calcite, were selected for the testing in this study. In order to ascertain the quantity of calcite precipitation and cementitious components, Bacillus subtilis and Bacillus halodurans are utilised as analytical laboratories. An investigation of the effectiveness of cement hydration was carried out when bacteria were present. It was also described how this strategy works, as well as its advantages and disadvantages. We are able to draw the conclusion that the selection of an appropriate experimental setup that provides accurate data and a short measurement time, the appropriate evaluation of a variety of unstructured and limitations in the XRD diffraction analysis, and an effective data analysis for the periodic critical factors are all necessary for an effective analysis. There is a strong recommendation that independent methodologies be utilised in order to supplement the data obtained from the bio-concrete XRD. Through the utilisation of such an appropriate data analysis routine, progress is made, consistency of time-resolved research is maintained, and operator variability is affected, all while avoiding outcomes that are unrealistic. The future of Fourier transform infrared spectroscopy (FT-IR) in environmental microbiology is expected to feature a wide variety of revolutionary applications and developments. These include the creation of comprehensive and standardised FT-IR libraries for the purpose of precise microbial identification, the incorporation of advanced analytical techniques, the adoption of high-throughput and single-cell analysis, real-time environmental monitoring through the utilisation of portable FT-IR systems, and the incorporation of FT-IR data into ecological modelling for the purpose of gaining predictive insights into microbial responses to changes in the environment. The utilisation of these novel approaches holds the potential to considerably increase our comprehension of microorganisms and the intricate interactions that they have within a variety of ecosystems.

Keywords: X-Ray Diffraction, FTIR, Drug, Formulation, Microbial.

INTRODUCTION

Another name for the phenomenon known as self-healing is bacterial calcite precipitation in concrete. In order to address the actual issue of concrete durability, one potential option is to make use of bacteria that may self-heal cracks in concrete without the need for human intervention [1]. When stimulated, bacteria that degrade urea, such as Bacillus holodurans and Bacillus subtilis, which create the enzyme 'urease' in the rumen, have the ability to precipitate calcium carbonate (CaCO3) in concrete [2]. As a valuable atmospheric law, this occurrence of natural induction is being investigated by specialists. Concrete is a structural material that is widely utilised all over the world because it is important, versatile, diversified, and versatile. Cracking is an inevitable characteristic of concrete, and it does not matter what formulation, grit, or design is utilised; it will always occur. It has been demonstrated that cracks are responsible for the destruction of structures because they progressively allow substances such as chemicals, acid gases, water, moisture, and other potentially harmful materials like salts to pass through. As well as precipitation and humidity [3]. Because of this, all of these variables cause corrosion of the rebar and reduce the amount of time that concrete structures may remain alive. The development of fundamental self-healing agents (SH) of natural biological origin that are capable of repairing cracks and fissures in concrete is going to be necessary in order to improve various elements of concrete. On the other hand, microbial concrete has the ability to successfully cure cracks in concrete [4]. As a consequence of this, researchers have found the HS notion of these potentially hazardous fractures in abandoned structures with limited resources to be a particularly fascinating topic of study. For this reason, it is of the utmost importance to create solutions for crack restoration that are both low-cost and long-term, and that do not require any personal involvement. In order to alleviate these problems, natural SH methods can be utilised, which involve the incorporation of particular kinds of bacteria into the mixture. Bacillus subtilis has the ability to stimulate the enzymes of ureolytic bacteria like Bacillus holodurans. This process, when paired with calcium delivery systems, can result in the formation of calcium carbonate (PCC) precipitates, which are an effective means of repairing micro fractures. Concrete that has just been manufactured. Previous research has shown that bacteria-based concrete made with microsilica and metakalonie has a greater value of UPV and a higher strength than traditional concrete [5].

Corresponding Author:** ***Hussein Jabbar Ghazi Sajet,
Department of Applied Medical Physics, Al-Hilla University College, Iraq.

Importance of X-ray Crystallographic Studies in Drug Discovery

X-ray diffraction has shown to be an effective method for obtaining details about the molecular structure and composition of crystalline polymers found in the cell walls of

algae and fungi. Algae cellulose molecular architecture was revealed by X-ray diffraction and electron microscopy in conjunction with one another [6]. Glucans and chitin are present in the cell walls of many different genera of yeast, according to X-ray diffraction research [7]. Chitosan is the main component of the cell walls of the dimorphic fungus Mucor rouxii, according to Bartnicki-Garcia and Nickerson. Chitin is present in yeast cell walls, as shown by X-ray diffraction, however it is estimated to constitute a negligible fraction of the total glucosamine in the cell wall [8]. From the zoospore stage all the way to the adult hyphae of the fungus Allomyces, Aronson and Preston's X-ray diffraction research revealed the evolution of cell wall structural components. Hurst showed that yeasts extracted with different solvents and Escherichia coli have orientated lipid in their cell walls and membranes based on measurement of electron diffraction lines. The identification of G-quadruplexes was a watershed moment in X-ray crystallography. The architectures of these higher-order deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules are unlike those of any other known DNA type [9]. Discovering medications that target DNA and RNA quadruplexes from specific genes requires both biological insight and high-resolution X-ray crystallographic structures. In 2008, another significant find was the crystal structure of the complex between two well-studied medicines, ketoconazole and erythromycin, and the human cytochrome P450 (CYP)3A4. Half of all drugs on the market are affected by CYP3A4's metabolic effects. The crystal structure of the CYP3A4-ligand interaction revealed important details about the binding stoichiometry (2:1 ketoconazole:CYP3A4 and multiple binding modes for erythromycin), the two different open conformations that occur when both ketoconazole and erythromycin are present, and the relevant binding kinetics. The discovery of the high-resolution three-dimensional X-ray structures of symbolic members of all enzymes in the type II fatty acid pathway early in 2015 provided a great resource to direct antibacterial drug research. The catalytic activity, ligand recognition, and selectivity/specificity with respect to chain length were all illuminated by these structures. Similar X-ray crystallographic structural investigations of CO2-liganded metalloenzyme carbonic anhydrase have therapeutic uses in the development of anti-cancer, anti-fungal, anti-bacterial, anti-glaucoma, and anti-convulsant medicines [10].

Application of Powder X-ray Diffraction (PXRD) to the Study of Drugs, Their Formulation, and Polymorphism

In the field of structural biology, the scanning calorimetric X-ray diffraction (SCXRD) technique has proven to be an indispensable instrument for the identification of novel biomimetic active pharmaceutical ingredients (APIs) and target molecules. It has been useful in determining the different atoms that are present in a crystal as well as the particular locations of those atoms, as well as the electron densities, bond lengths, and angles. For certain medications, acquiring a single crystal might be a difficult endeavour, and the majority of these medications are found in the form of microcrystalline material. In situations like these, powder X-ray diffraction (PXRD) comes to the rescue and offers an alternative method for determining the structure, purity [11], efficacy, and effectiveness of a wide variety of materials in a variety of fields, including pharmaceutical science. In contrast to SCXRD, which offers information on the structure of a single crystal, PXRD is able to provide information about the structure of a microcrystalline substance that is present in bulk. The pattern that is acquired from the PXRD analysis contains the average diffraction for a large number of crystallites that are randomly orientated within the sample. A plot of the diffracted intensities versus the angle of the detector, denoted as 2-theta (2-θ), is shown as a representation of this phenomenon. Confirming if a substance of interest is crystalline or amorphous is another function that PXRD can do effectively. Crystalline material is characterised by a periodic arrangement of atoms in three-dimensional space, often referred to as 3-D space. The formation of the well-defined high intensity Bragg peaks is caused by the fact that X-rays are only scattered in certain directions as they pass through the framework that has been constructed. Amorphous materials are composed of atoms that lack periodicity and are generally arranged at short-range order distances consisting of two to five angstroms and medium-range order distances ranging from five to twenty angstroms [12]. The X-rays are scattered in a variety of directions, resulting in a substantial bump that is distributed throughout a broad range of two degrees of angle (θθ). For many active pharmaceutical ingredients (APIs), the amorphous form has substantial advantages over the crystalline form. These advantages include increased oral bioavailability, superior dissolution rate, and greater solubility. Because of this advantage, various amorphous active pharmaceutical ingredients (APIs) that possess the pharmacokinetic features that are required have been developed and approved. On the other hand, because certain amorphous systems have a larger free energy, they have a tendency to be thermodynamically unstable, which results in the crystallisation of the active pharmaceutical ingredient (API), particularly when they are subjected to high humidity [13]. As a result, PXRD has been used to determine the beginning of crystallisation in amorphous active pharmaceutical ingredients (APIs) and has the capability to establish a detection limit for the crystalline content in medication formulations. This limit is normally on the range of a few percent (0.2–5%) crystallinity by mass, and it can be much lower when synchrotron X-ray sources are utilised [14, 15].

Fourier Transform-Infrared (FTIR) spectroscopy

FTIR spectroscopy has shown to be an invaluable instrument in a wide range of biological research endeavours across a variety of scientific fields. In the discipline of microbiology, it has been of great use in the identification of microorganisms in a timely manner, which has contributed to the timely diagnosis of infections and the adoption of appropriate treatment approaches. A greater understanding of the physiology and behaviour of microorganisms has been achieved as a result of this technology, which has also made it possible to conduct in-depth investigations of the structures, metabolic activities, and responses of microorganisms to any changes in their environment. In addition, Fourier transform infrared spectroscopy has been an indispensable contributor to the monitoring and evaluation of the dynamics of microbial communities in a variety of situations [16, 17]. The management of water quality, the evaluation of ecosystem health, and the identification of microbiological pollution have all benefited from this. For the purpose of metal-pollutant bioremediation, Fourier transform infrared spectroscopy (FTIR) has made it possible to conduct an exhaustive investigation of the ways in which microorganisms interact with metal pollutants. This has been helpful in gaining a better knowledge of the mechanisms that are involved in the transformation, detoxification, and sequestration of metals.

Additionally, in the field of organic pollutant bioremediation, Fourier transform infrared spectroscopy (FTIR) has shown to be an invaluable instrument for analysing the interactions that take place between microorganisms and organic pollutants. The metabolic transformations and degradation pathways that are involved in the bioremediation process have been uncovered as a result of this [18].

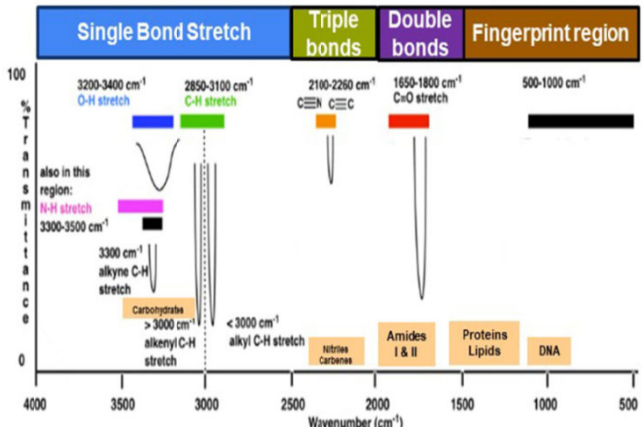

Figure 1. Typical infrared values for several types of bonding. In the mid-infrared spectrum, the region spanning from 500 to 1,500 cm−1 is referred to as the fingerprint region. This region is responsible for providing molecular fingerprints that are peculiar to particular molecules

It is founded on the notion that molecules absorb particular frequencies of infrared light, which correspond to the vibrational frequencies of their chemical bonds. This principle is the foundation of Fourier transform infrared spectroscopy. Whenever a sample is subjected to infrared light, the molecules that are contained within the sample absorb this light at specific frequencies, which in turn causes the bonds that are contained within the molecules to start vibrating. The vibrational frequencies of various types of bonds, such as C-H, O-H, and N-H bonds, are distinct from one another, which results in the formation of distinctive patterns observed in the absorption of infrared light. Through the process of measuring the intensity [19] of the light that is absorbed at different wavelengths, an FT-IR spectrometer is able to generate a distinctive spectrum representing the material. The functional groups and molecular structures that are present in the sample can be identified with the help of this spectrum, which, in essence, provides a "fingerprint" that can be utilised for both qualitative and quantitative examination [20].

In addition to having applications in a wide range of scientific fields, such as chemistry, physics, materials science, and biology, Fourier transform infrared spectroscopy (FT-IR) can be utilised to investigate a wide variety of specimens, such as solids, liquids, and gases. The range of infrared radiation covers electromagnetic radiation with frequencies ranging from 14,300 to 20 cm−1. The most significant vibrational frequencies for the majority of molecules [21, 22] are found within the mid-infrared spectrum, which involves frequencies ranging from 4,000 to 400 cm−1. Four distinct regions may be found within this particular range. These regions are as follows: (1) the single bond region, which spans from 2,500 to 4,000 cm−1, (2) the triple bond region, which spans from 2,000 to 2,500 cm−1, (3) the double bond region, which spans from 1,500 to 2,000 cm−1, and (4) the fingerprint region, which spans from 600 to 1,500 cm−1 (Figure 1).

Table 1. A representation of some of the most common peaks that may be found in FT-IR spectra, together with the functional groups and reference wavenumbers that correlate to them

Assignment	Frequency (cm⁻¹)
C-H stretching peaks	
Alkane C-H stretching	2,850–2,960
Alkene C-H stretching	3,010–3,100
Alkyne C-H stretching	3,300
O-H and N-H stretching peaks	
Alcohols and phenols O-H stretching	3,200–3,600
Carboxylic acids O-H stretching	2,500–3,300
Amines N-H stretching	3,300–3,500
C=O stretching peaks	
Ketones and aldehydes C=O stretching	1,680–1,750
Carboxylic acids C=O stretching	1,700–1,750
Amides C=O stretching	1,630–1,690
C-N stretching peaks	
Aromatic C-N stretching	1,300–1,350
Aliphatic C-N stretching	1,000–1,300
C=C stretching peaks	
Alkene C=C stretching	1,620–1,680
Aromatic C=C stretching	1,450–1,600
N-H bending peaks	
Amines N-H bending	1,560–1,640
Amides N-H bending	1,550–1,670
C-H bending peaks	
Alkanes C-H bending	1,370–1,470
Alkenes C-H bending	960–1,200
O-H bending peaks	
Alcohols and phenols O-H bending	1,350–1,450
Fingerprint region peaks	700–900

Microbial Identification

It has been demonstrated that FT-IR spectroscopy can be utilised for the purpose of identifying microbes by utilising their individual spectral fingerprints. The FT-IR spectra of microbial cells, such as those of bacteria, yeast, and fungi, have unique peaks that represent the presence of particular biomolecules (lipids, proteins, and carbohydrates) and the functional groups that they contain [23, 24]. The identification of the microorganisms can be accomplished by comparing these spectral patterns to reference spectra that are stored in databases. Microorganisms are made up of a wide variety of biomolecules, including proteins, lipids, nucleic acids, and carbohydrates, all of which display distinct absorption bands in the infrared spectrum. For the purpose of identifying bacteria, the FT-IR spectra of bacterial samples can be compared to spectral databases or reference spectra. This allows researchers to identify bacterial species or strains based on the distinctive spectral characteristics of the bacteria. The ability of Fourier transform infrared spectroscopy (FT-IR) to detect bacteria is dependent on the fact that the chemical composition and structure of bacterial macromolecules differ between various species and strains, which results in diverse infrared absorption

patterns. [25] These patterns make it possible to differentiate and identify individuals. Principal Component Analysis (PCA), Hierarchical Clustering Analysis (HCA), Linear Discriminant Analysis (LDA), Stepwise Discriminant Analysis (SDA), and Ward's algorithm are some of the advanced statistical analysis methods that researchers use in order to improve the accuracy and efficiency of bacterial detection and classification using Fourier Transform Infrared Spectroscopy (FT-IR). Establishing strong and trustworthy models for the identification of bacteria can be accomplished through the creation of spectrum libraries or databases that cover a wide variety of bacterial species. Because the absorption patterns of functional groups in biological molecules are observed in this particular region as sharp fundamental vibrations, rather than broad overtones or harmonics, which are found in the near-IR, the majority of bacterial [26] samples have been observed to fall within the mid-IR region (4,000 to 600 cm−1) in the process of bacterial identification. This is primarily due to the fact that the mid-IR region encompasses the majority of the frequency range. When it comes to the use of Fourier transform infrared spectroscopy (FT-IR) for the identification of microorganisms, one of the most significant issues is the complexity of the samples that are being used. This complexity can result in overlapping spectral signals and difficulty in achieving correct identification (Wenning and Scherer, 2013).

Furthermore, the absence of standardised techniques and vast databases can be a barrier to the identification of microorganisms in a consistent and accurate manner, particularly for microorganisms that have been investigated less or that have been identified more recently [27]. It is possible that the resolution of infrared spectroscopy is not sufficient to differentiate between closely related microbial species or strains in certain circumstances. This would restrict the effectiveness of the technique in terms of exact microbiological identification. Within each species, there can be great variability due to genetic, environmental, and phenotypic factors. Microbial species exhibit a significant amount of variation, and this diversity can be found within each species. In order to capture this variability in an FT-IR library, it would be necessary to include a large variety of strains and circumstances, which would entail substantial efforts to collect data and conduct sampling. In order to ensure the reproducibility and comparability of spectral data, it is essential to ensure that sample preparation, data collecting, and analytical processes are standardised across all of the different laboratories. Inconsistencies in spectral profiles can be caused by variations in experimental methodologies, which makes it difficult to construct an FT-IR library that is dependable and consistent. In addition, the analysis and interpretation of complicated spectrum data from a wide variety of microbial species calls for the utilisation of sophisticated computational tools and a high level of knowledge.

Conclusion

The processes of drug discovery, design, and formulation all benefit significantly from the application of X-ray structural characteriszation techniques. At each and every stage, it is absolutely necessary to conduct an accurate study of the crystal structures of biological target and biological target-substrate complexes. Nevertheless, these models are not without their shortcomings. There is a possibility that crystal structures will be overanalyzed, which may result in the formation of biassed hypotheses and an indefinite number of subsequent experiments. However, prior to making conclusions that are definitive in the field of drug development, it is necessary to conduct an in-depth analysis of the structures of both small molecules and macromolecules, as well as their physiologically significant interactions, using methodologies that are complementary to one another. As a spur for innovation and the investigation of fresh solutions, the difficulties that are experienced when utilising FT-IR in the field of microbiology serve as a catalyst. In order to set the path for an exciting future in microbiological research, it is necessary to address these issues through the formation of interdisciplinary collaborations and the advancement of technical improvements. The scope of microbial analysis will expand as FT-IR technology continues to advance and its integration with other techniques becomes more seamless. This will allow us to uncover previously hidden insights into the communities of microbes, their functions, and the roles they play in shaping our understanding of the dynamics and sustainability of ecosystems. Studies of drug delivery systems and big biological components like proteins can benefit from the application of these techniques. Additionally, these approaches can be utilised to investigate the crystallisation processes of pharmaceuticals that are derived from solutions.

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