NMR

Exploring the Potential of Nuclear Magnetic Resonance to Enhance Pharmaceutical Research

Innovation with Integrity

Understanding the structural properties, residual impurities and degraded products of pharmaceutical formulations in solid form is a key part of the drug characterization process. Studying polymorphs in non-crystalline solids presents a challenge. Researchers at the Indian Institute of Chemical Technology (IICT), one of the constituent laboratories of the Council of Scientific and Industrial Research (CSIR), Ministry of Science & Technology, Government of India, are applying innovative nuclear magnetic resonance (NMR) techniques to solid-state pharmaceutical analysis backed by powder X-ray diffraction studies, with very promising results. Under the leadership of Dr. Jagadeesh Bharatam, the team at the IICT is working to develop solid and solution-state NMR methods for applications focusing primarily on the evaluation, characterization and structure-based design of molecules.

NMR at the CSIR-IICT

NMR is the technology of choice for the CSIR-IICT. According to Dr. Bharatam: *"The power of NMR lies in its ability to sample a wide range of molecular dynamics and reaction intermediates, and its capacity to study analytes in both solid- and solution-state conditions."*

The ultra-high resolution methods based on homo-decoupling strategies developed by Dr. Bharatam's group have been applied to drug sample analysis from across the pharmaceutical industry, ensuring that reliable results are obtained with sufficient levels of data integrity. Such aims may explain why the IICT is the only U.S. Food and Drug Administration (FDA)-inspected NMR facility of all India's government laboratories. Therefore, it is now one of the most highly sought after NMR facilities for the world's pharmaceutical companies.

Typical tasks from industry clients include the analysis of inter-batch variation and identification of drug polymorphisms, which refers to drugs with the same chemical structure but different crystalline structures, as well study of biosimilars in solution-state. For example, the IICT team has applied this technique to establish the two-dimensional structure of a tuberculosis antibiotic and characterization of the critical insulin-IS1 interaction in the presence of a prion-derived tetrapeptide stabilizer.

In pharmaceutical industries, polymorphs are relevant not only for the selection of the best solid material to carry through the various stages of drug development, including the choice of dosage and of excipients suitable for drug development and marketing, but also in terms of intellectual property protection and/or extension. There are many examples of polymorphisms in drugs, with more than 50 percent of active pharmaceutical ingredients (APIs) estimated to have more than one polymorphic form. This includes common medicines such as paracetamol, omeprazole, and chloramphenicol palmitate. When it comes to drugs that are released from a polymer matrix, the density of the crosslinked polymers must be established using NMR (for example, ^{13}C). The IICT regularly conducts crucial solid-state NMR experiments of this kind.

Bruker and the IICT have also collaborated to drive overall progress in solid-state NMR, particularly for the first experimental demonstration of Hadamard NMR spectroscopy in the solid-state.

NMR from the ground up

Dr. Bharatam's NMR journey began during his PhD years. He recalls: *"My supervisor and I built our own wide-line NMR spectrometer by purchasing a B25 electromagnet from Bruker and fabricating all the necessary electronics around that. Once it was complete, we used the spectrometer to study molecular dynamics and phase-transitions in a range of molecular solids."*

"It will come as no surprise that things have changed considerably in the past 40 years. When I was getting started, the demand for high-resolution outputs was non-existent and the main activities were focused on molecular dynamics, such as relaxometry and dispersion studies. Today, all instruments are black boxes, which means they are easier to use and give access to intriguing molecular details for two- and three-dimensional solids."

Looking at solution-state

When using solution-state ^{1}H NMR (proton NMR), the obtained spectra appear in a range from zero to 12 or 14 ppm. Despite this narrow range, complex splitting patterns often appear due to the tendency for protons to exist in chemical bonds that can interconnect with one another, resulting in overlapping multiplet resonance signals. This spectral complexity needs to be resolved for unambiguous assignment of the resonance signals.

The pulse sequences developed at the IICT are to circumvent the spectral complexity due to the overlapping multiplets of the NMR signals. The outcome of employing the developed homodecoupling pulse sequences enhances spectral resolution and facilitates the unambiguous determination of molecular structures.

Homodecoupling works by suppressing atomic spin and eliminating interactions between coupled nuclei, giving rise to the singlet peaks that are more typical of ^{13}C spectra (which run up to 200 ppm). This approach simplifies peak assignment and streamlines the characterization of complex molecules.

Dr. Bharatam
Dr. Bharatam is Chief Scientist and Professor-AcSIR at the Center for Magnetic Resonance of the CSIR-IICT in Hyderabad, India.

Dr. Jagadeesh Bharatam started his career as a PhD student, working with NMR in the School of Physics at the University of Hyderabad in 1988. He graduated in 1994 and continued his work in Mik Pintar's group at the University of Waterloo in Ontario, Canada, collaborating with chemical engineers to study porous materials and molecular dynamics in restricted geometries. He then moved to Russel Bowers group at the University of Florida, USA, to work on hyperpolarized NMR techniques, including ^{129}Xe NMR and thermally polarized systems. In 1998, he accepted an independent faculty position at the CSIR-IICT Hyderabad, India, where his focus has remained on developing NMR methods and applications.

The group also uses Bruker instruments to conduct residual dipolar coupling (RDC) NMR experiments in weakly aligned organic-solvent/polymer-gel media. RDCs as orientation restraints provide long-range orientation information for magnetic dipole-to-dipole interaction vectors in a reference frame, for samples dissolved in the weakly aligned in the organic-solvent/polymer solvent medium. Unlike NOE-based short range structural information, the RDC-based approach provides long range structural correlation and allows researchers to elucidate molecular structures much more precisely, including insights into relative stereochemical qualities. These advantages highlight RDC NMR as a powerful tool for pharmaceutical applications and the IICT has capitalized on it accordingly.

Dr. Bharatam explains: *"It is a kind of translational research, even though we are developing these methods in the basic research realm. These advances are not confined to our lab, or any single lab. In fact, these developments are happening in parallel across the globe. Once these technologies prove they are versatile and easily applicable, they quickly become routine. This is fantastic, because leading techniques should not be confined to any given group; they should be accessible to all."*

Density functional theory

The IICT team is also involved in synthesizing peptides that mimic natural peptides. In nature, proteins adopt 3D-folded structures, which dictate protein functionality, and are comprised of various secondary structure elements. These secondary structures are organized in a way that exposes important functional groups in a determined fashion. Some drugs and drug analogs are designed with inspiration from these peptides in mind, but the issue of enzymatic degradation represents a real threat. Dr. Bharatam explains: *"If the molecule is recognized by an enzyme, then its function may be changed or prevented. We can overcome this issue by structure-based designing of biomolecular analogs comprised of certain unnatural amino acids. But the question is, where does this process begin?"*

The usual approach is to start with theoretical calculations covering molecular angles and resistances that position secondary element structures and thereby the functional sidechains as needed. This process requires computation and quantum chemical calculations. Once the design is complete, this is sent to an organic chemistry expert at the IICT to produce the compound and send it back to the NMR team. Upon receipt, the product is analyzed via NMR to establish whether the initial design has been achieved or not. Fine-tuning can then be carried out as needed.

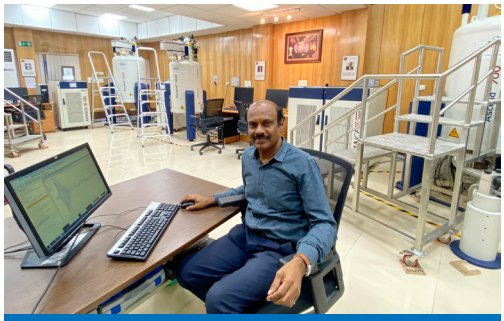
The Bruker partnership

Dr. Bharatam added: *"I'd like to give my thanks to the Bruker team, who gives fantastic support to our research programs when it is needed. I would describe myself as an instrumentalist, and we work very much on a peer-to-peer basis. Our partnership goes back many years, and we feel far more like partners than customers; we have co-authored papers, for example. Our country boasts a long history in NMR-based research, and we look forward to celebrating a fruitful future."*

Looking ahead

Looking forward, Dr. Bharatam adds that his focus is on the development of portable NMR instruments for on-site applications, as well as high-resolution solid-state NMR studies of energy materials. The team has already made sizeable steps towards the goal of portable instrumentation, but the question now is how to make these devices appropriate for point-of-care applications, which are yet to be defined. The magnet needs to be well-stabilized in these cases because it is extremely sensitive to fluctuations in temperature.

Dr. Bharatam concludes: *"Spectrum simplification represents another important goal. In accomplishing such impressive feats, few things are certain. But one thing we can predict with confidence is that innovative methods will lead the way – and powerful partnerships supported by world-class instrumentation will play a major role in developing these methods."*



Introducing the CSIR-IICT

Established in 1944, CSIR-IICT's vision is to contribute to the acquisition of knowledge that supports applications in chemistry and chemical technology through the Analytical & Structural Chemistry department, which is comprised of four centers for NMR, separation science, mass spectrometry, X-ray. The CSIR-IICT acts as the backbone of the department by playing a pivotal role in daily activities and research programs designed to support industry, government departments, and entrepreneurs.

Hyderabad is a world-class pharmaceutical hub with a large number of drug manufacturers producing and distributing pharmaceutical products to meet global demand. Many of the manufacturers remain loyal to the CSIR-IICT because of its expertise in both solid- and solution-state analyses.

The CSIR-IICT is studying the application of NMR in pharmaceutical chemistry, including upscaling and the development of new molecules and natural products. In addition to its own research programs, the NMR center of the CSIR-IICT plays a major role in two important areas: firstly supporting in-house projects, which are typically research and development, and secondly tending to the analytical needs of external clients. The NMR team consists of four scientists and a technical support team of 10 further staff equipped with a total of nine Bruker NMR spectrometers ranging from 300 to 700 MHz, as well as a dedicated solid-state facility.

For more information on the CSIR-IICT, please visit:
https://www.iict.res.in/

For more information on Bruker's NMR solutions systems, please visit:
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