New experimental method IR-DOSY reveals molecular structure and size

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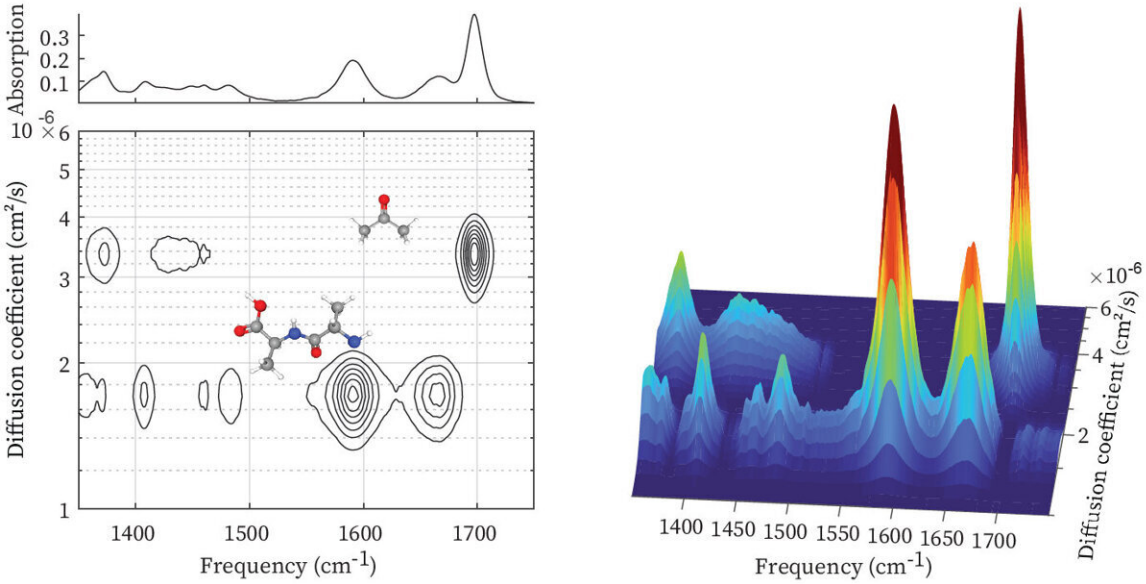


IR-DOSY spectra of a mixture of acetone and dialanine, showing which IR peak belongs to which compound. Credit: HIMS

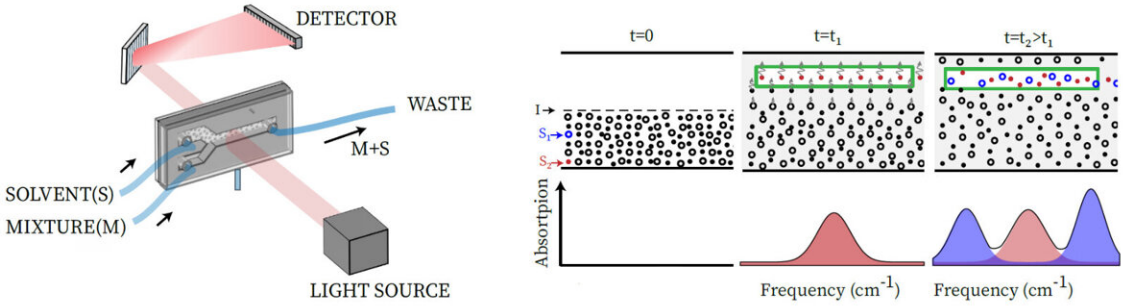
Researchers at the University of Amsterdam have developed a novel approach to infrared spectroscopy that enables simultaneous characterization of molecular structure and size. Called Infrared Diffusion-Ordered Spectroscopy (IR-DOSY), the method nicely separates molecules with different sizes into distinct sets of IR peaks.

Reporting on IR-DOSY in a paper that has just been accepted by

Angewandte Chemie, the researchers foresee analytical applications in fields as diverse as proteins, polymers, pharmaceuticals and biomedicine. They are currently developing a first version of a practical chemical probe implementing the IR-DOSY concept.

Infrared (IR) spectroscopy is an important workhorse in the analysis of chemical compounds. It helps to identify molecules based on their functional groups and spatial conformation. In general, IR spectroscopy is not sensitive to the size of the molecules. Inspired by an already existing approach in NMR spectroscopy, the Amsterdam researchers now applied the principle of diffusion ordered spectroscopy to IR.

Here, the molecules present in a sample are separated based on their diffusion behavior prior to spectral analysis. IR-DOSY relies on the fact that the diffusion of a molecule is determined completely by its size—a concept that was first established by Albert Einstein in his 1905 classic paper on the Brownian motion of microscopic particles.



Schematic representation of the IR-DOSY setup (left) and its operation (right). A sample solution (M) and pure solvent (S) are pumped into a channel (4 mm wide). The flow rates of the sample solution and solvent are the same, so at the midpoint of the channel an interface I establishes. When measuring the IR absorption in the solvent-filled half (the green bordered region), a time-

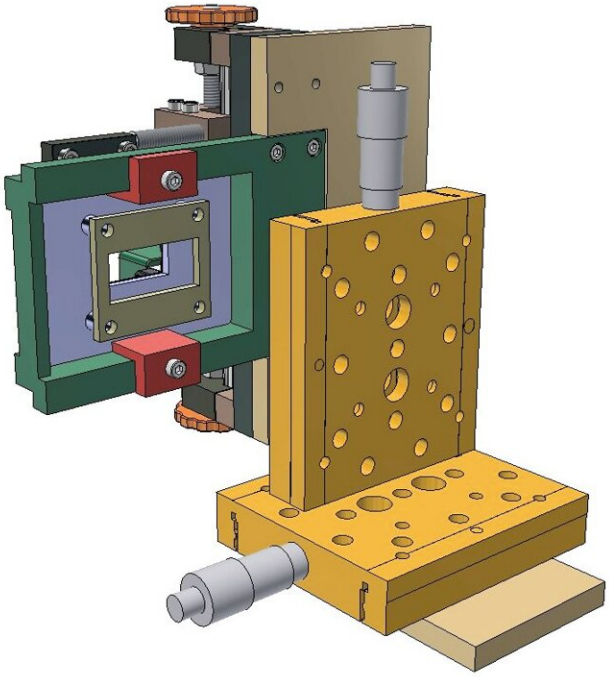
dependent spectrum is observed in which the absorption peaks of the smaller S2 molecules (red) appear before those of the larger S1 molecules (blue). Credit: HIMS

The IR-DOSY spectrometer creates a spatially inhomogeneous distribution of solute molecules using a simple yet effective flow method that transports both the mixture and pure solvent into a sample chamber. After the stopping the flow, the solute molecules start to diffuse into the pure solvent region, at a rate that depends on their diffusion coefficient.

The infrared absorption is measured at a position in the chamber where there was initially only solvent. As time progresses, the diffusing solute molecules start appearing in the IR beam. In this way, for all type of molecules the individual IR spectra are recorded at different moments in time, depending on their sizes. IR-DOSY thus produces a two-dimensional spectrum with the IR frequency along one axis and the diffusion constant (or equivalently, the size) along the other axis.

Proteins, polymers and nanoparticles

In their *Angewandte Chemie* paper, the researchers argue that although the separating power of IR-DOSY is less than that of typical chromatographic methods, it has the advantage that no prior knowledge is required of the chemical structure of the compounds present in the sample. The separating power might even be increased by adding an electrophoresis device to actively separate the species in the sample solution.



Design of the IR-DOSY probe. Credit: UvA Technology Centre

Among the applications presented in the paper is the analysis of protein aggregates and fibrils. Here, IR-DOSY makes it possible to simultaneously investigate monomers, oligomers, and fibrils, which typically coexist in a sample. Polymers and plastic nanoparticles constitute another interesting field of research since samples usually contain many different molecules of many sizes.

The size-selectivity and structure-sensitivity could also render IR-DOSY useful in the pharmaceutical and biomedical domains. For instance it has potential to detect trace amounts of small molecules present in

pharmaceutical products.

In the biomedical context, it could for instance be used to detect and structurally characterize low-molecular weight species in human blood serum. In all cases, the IR-DOSY analysis provides valuable information about the size or size distribution of the molecules or molecular aggregates in a sample.

More information: Giulia Giubertoni et al, Infrared Diffusion-Ordered Spectroscopy Reveals Molecular Size and Structure, *Angewandte Chemie International Edition* (2022). DOI: 10.1002/anie.202213424

Provided by University of Amsterdam