
DIFFUSION-ORDERED NMR SPECTROSCOPY (DOSY) IN THE ANALYSIS OF MULTICOMPONENT MIXTURES

Hreben Mariia, Lampeka Rostyslav

DOI <https://doi.org/10.30525/978-9934-26-714-7-23>

INTRODUCTION

Modern analytical chemistry and physicochemical studies of solutions increasingly face the challenge of analysing complex multicomponent mixtures. Traditional approaches based on hyphenated separation techniques (liquid chromatography, gas chromatography, capillary electrophoresis) followed by detection suffer from several significant limitations¹. First, they require lengthy and often labor-intensive sample preparation (extraction, derivatization, preconcentration). Second, the native structure of the sample is destroyed, making it impossible to study labile systems that exist in a state of dynamic equilibrium (for example, coordination compounds with high rates of ligand exchange or processes associated with the migration of heavy metal ions in natural waters or soils)².

Nuclear Magnetic Resonance (NMR) spectroscopy is a recognized leader among non-destructive analytical methods. However, when studying real-world objects (petrochemical fractions, biological fluids, food products, or extraction mixtures), one-dimensional spectra are characterized by severe signal overlap. Multidimensional NMR spectroscopy (COSY, HSQC, HMBC) solves the signal resolution problem by correlating chemical shifts through bonds; however, it cannot distinguish signals from different molecules if their chemical structures are similar or if they are mixed in a single solution³.

An elegant and powerful solution to this problem came with Diffusion-Ordered Spectroscopy (DOSY NMR), first proposed by Johnson and

¹ Holzgrabe U. Quantitative NMR spectroscopy in pharmaceutical applications. *Progress in Nuclear Magnetic Resonance Spectroscopy*. 2010. Vol. 57. № 2. P. 229–240. DOI: <https://doi.org/10.1016/j.pnmrs.2010.05.001>.

² Johnson C. S. Jr. Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications. *Progress in Nuclear Magnetic Resonance Spectroscopy*. 1999. Vol. 34, № 3–4. P. 203–256. DOI: [https://doi.org/10.1016/S0079-6565\(99\)00003-5](https://doi.org/10.1016/S0079-6565(99)00003-5).

³ Claridge T. D. W. *High-Resolution NMR Techniques in Organic Chemistry*. 3rd ed. Elsevier, 2016. 464 p. URL: <https://www.inspectioncopy.elsevier.com/book/details/9780080999869>.

co-workers⁴. DOSY introduces a fundamentally new dimension to NMR methodology – the translational self-diffusion coefficient (D) – effectively turning the spectrometer into a “virtual chromatograph”. On a two-dimensional DOSY plot, the horizontal axis displays the classical chemical shift (spectral structural data), while the vertical axis represents the diffusion coefficient (physical data related to molecular size and shape)⁵.

The primary analytical capabilities and advantages of DOSY NMR include:

- Virtual Mixture Separation: Signals from different chemical species present in the same solution are separated onto distinct levels along the vertical axis, provided their molecular weights or hydrodynamic sizes differ by at least 10–15%.

- Non-destructiveness: Analysis is performed directly in the NMR tube without adding chemical reagents or sorbents, ensuring the preservation of thermodynamic equilibria⁶.

- Determination of Molecular Weights and Sizes: By utilizing fundamental physical relationships, experimental D values allow for the calculation of the hydrodynamic radius of molecules or nanoparticles, as well as the estimation of their molecular weight via calibration curves⁷.

- Investigation of Intermolecular Interactions: The method is highly sensitive to association, aggregation, solvation, and complexation processes.

If a small molecule binds to a large macromolecule or a heavy metal ion, its diffusion rate drops sharply, which is immediately reflected in the DOSY spectrum⁸.

Due to these unique properties, the DOSY method has become an indispensable tool in fields such as food quality control (detection of adulteration in olive oil and honey), medical diagnostics (blood plasma metabolomics), polymer chemistry, and modern coordination and environmental chemistry⁹.

⁴ Morris K. F., Johnson C. S. Jr. Diffusion-ordered two-dimensional nuclear magnetic resonance spectroscopy. *Journal of the American Chemical Society*. 1992. Vol. 114. № 8. P. 3650–3657. DOI: <https://doi.org/10.1021/ja00034a071>.

⁵ Barjat H. et al. High-resolution diffusion-ordered 2D spectroscopy (HR-DOSY). *Journal of Magnetic Resonance, Series B*. 1995. Vol. 108. № 3. P. 266–272. DOI: http://apps.isiknowledge.com/UA_GeneralSearch_input.do?product=UA&search_mode=GeneralSearchWOS:A1995RN49300008.

⁶ Johnson C. S. Jr. Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications. *Progress in Nuclear Magnetic Resonance Spectroscopy*. 1999. Vol. 34, № 3–4. P. 203–256. DOI: [https://doi.org/10.1016/S0079-6565\(99\)00003-5](https://doi.org/10.1016/S0079-6565(99)00003-5).

⁷ Barjat H. et al. High-resolution diffusion-ordered 2D spectroscopy (HR-DOSY). *Journal of Magnetic Resonance, Series B*. 1995. Vol. 108. № 3. P. 266–272. DOI: http://apps.isiknowledge.com/UA_GeneralSearch_input.do?product=UA&search_mode=GeneralSearchWOS:A1995RN49300008.

⁸ Macchioni A. et al. Determining accurate molecular sizes in solution through NMR diffusion measurements. *Chemical Society Reviews*. 2008. Vol. 37, № 3. P. 479–489. DOI: <https://doi.org/10.1039/b615067p>.

⁹ Pregosin P. S. NMR diffusion methods in inorganic and organometallic chemistry. *Spectroscopic Properties of Inorganic and Organometallic Compounds. Royal Society of Chemistry*, 2011. Vol. 42. P. 248–268. DOI: <https://doi.org/10.1039/9781849732833-00248>.

1. Practical Applications of DOSY in the Analysis of Complex Systems

1.1. Oils: Olive Oil as a Case Study (*Lipid Matrices*)

Extra Virgin Olive Oil (EVOO) is a high-value commodity on the global market whose quality is strictly regulated. The primary chemical components of the oil are triglycerides (TAGs >95%), composed predominantly of oleic, linoleic, and palmitic acid residues. A common form of adulteration involves diluting EVOO with cheaper refined vegetable oils (such as sunflower, soybean, corn, or rapeseed oils).

Because TAGs from different vegetable oils feature identical glycerol backbones and similar fatty acid chains, their signals in NMR spectra overlap heavily in the regions of glycerol protons (4.1–4.3 ppm) and aliphatic chains (0.8–2.4 ppm). However, using DOSY makes it possible to differentiate these components based on subtle differences in their self-diffusion coefficients, which stem from varying degrees of unsaturation (number of double bonds) and the specific micro-viscosity of the lipid environment¹⁰.

Furthermore, DOSY NMR enables direct visualization of minor olive oil components (squalene, tocopherols, sterols), which have significantly lower molecular weights (300–400 g/mol). Thanks to their substantially higher diffusion molecular weights (300–400 g/mol). Thanks to their substantially higher diffusion coefficients, the peaks of these minor components form a distinct, high-diffusion layer on the 2D plot, allowing for their quantitative analysis without prior saponification of the oil.

1.2. Natural Honey (*Carbohydrate Matrices*)

Natural honey is an extremely concentrated aqueous carbohydrate solution (up to 80–85% dry matter), where the main components are the monosaccharides D-fructose and D-glucose existing in tautomeric equilibrium. The primary challenge in the NMR analysis of honey is the presence of hundreds of severely overlapping multiplet signals from CH-ring protons within a narrow chemical shift window ranging from 3.2 to 4.5 ppm¹¹.

To resolve this issue, modern modifications of the technique – such as Pure Shift DOSY (e.g., based on the PSYCHE pulse sequence) – are employed¹². This approach completely eliminates homonuclear spin-spin coupling, converting complex multiplets into sharp, individual singlets.

¹⁰ Dais P., Hatzakis E. Quality assessment and authentication of virgin olive oil by NMR spectroscopy: A critical review. *Analytica Chimica Acta*. 2013. Vol. 765. P. 1–27. DOI: <https://doi.org/10.1016/j.aca.2012.12.003>.

¹¹ Dinca O. R. et al. Geographical and botanical origin discrimination of Romanian honey using complex stable isotope data and chemometrics. *Food Analytical Methods*. 2015. Vol. 8. № 2. P. 401–412. DOI: <https://doi.org/10.1007/s12161-014-9903-x>.

¹² Foroozandeh M. et al. Ultrahigh-Resolution Diffusion-Ordered Spectroscopy. *Angewandte Chemie International Edition*. 2016. Vol. 55. № 50. P. 15579–15582. DOI: <https://doi.org/10.1002/anie.201609676>.

On a Pure Shift DOSY 2D plot, carbohydrate signals are clearly stratified according to their degree of polymerization: monosaccharides (fructose, glucose) possess the highest D values, disaccharides (sucrose, maltose) occupy an intermediate position, and trisaccharides (melezitose, raffinose) shift toward lower diffusion coefficients.

This allows for the straightforward detection of added artificial invert or starch syrups containing high-molecular-weight oligosaccharides (dextrins), which exhibit abnormally low diffusion rates¹³.

1.3. Coordination Chemistry and Metal Complexes

In modern organometallic and coordination chemistry, DOSY NMR serves as an indispensable tool for resolving solution speciation, identifying dynamic aggregation behaviour, and evaluating metal–ligand complexation parameters without disturbing delicate chemical equilibria.

Solution Speciation in Transition Metal Complexes

The determination of exact solution structures for transition metal complexes is often hampered by rapid ligand exchange, dynamic equilibria between multiple species (e.g., monomer–dimer or neutral–ionic equilibria), and severe NMR signal overlap. DOSY NMR directly addresses these challenges by correlating chemical shifts with hydrodynamic volume.

For instance, in nickel(II) coordination chemistry, DOSY was pivotal in establishing speciation trends for 2-substituted vs. 2,9-disubstituted phenanthroline-Ni(II) complexes¹⁴. By measuring self-diffusion coefficients (D), researchers can unambiguously discriminate between mononuclear complexes, halogen-bridged dimeric species, and free uncoordinated ligands in solution. The spatial steric bulk at the 2,9-positions directly dictates whether a complex remains strictly monomeric or undergoes concentration-dependent self-association, a distinction easily tracked via step-changes in measured D values.

Similarly, DOSY NMR proves crucial in soft-matter photocatalytic systems. In studies evaluating cobalt-catalyzed photo transformations within aqueous micellar environments¹⁵, DOSY tracks the partition and compartmentation of cobalt catalysts and organic substrates inside surfactant micelles (e.g., SDS or Triton X-100). Because a free catalyst diffuses rapidly compared to a massive surfactant micelle, measuring the reduction in D upon adding micellar media

¹³ Dinca O. R. et al. Geographical and botanical origin discrimination of Romanian honey using complex stable isotope data and chemometrics. *Food Analytical Methods*. 2015. Vol. 8. № 2. P. 401–412. DOI: <https://doi.org/10.1007/s12161-014-9903-x>.

¹⁴ Mateos-Calbet A. et al. 2-Substituted vs 2,9-Disubstituted Phenanthroline-Ni(II)-halides: Speciation Control and Structural Elucidation in Solution. *Inorganic Chemistry*. 2025. Vol. 64. № 34. P. 17093–17097. DOI: <https://doi.org/10.1021/acs.inorgchem.5c02704>.

¹⁵ Serebrennikova O. O. et al. Aqueous micellar environment impacts the Co-catalyzed phototransformation: A case study. *ACS Catalysis*. 2025. Vol. 15. № 3. P. 1820–1831. DOI: <https://doi.org/10.1021/acscatal.4c05678>.

quantifies the binding affinity of the metal complex to the micellar core vs. the bulk aqueous phase.

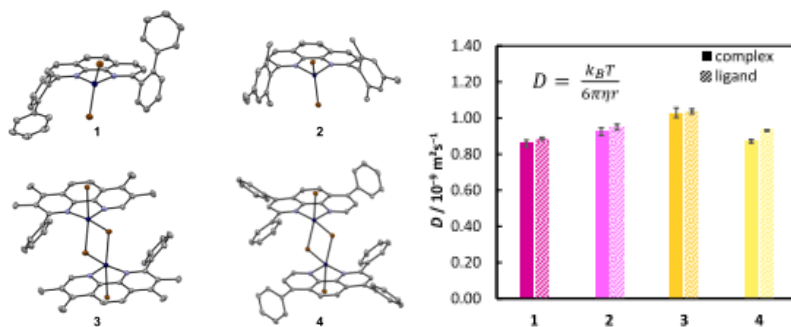


Fig. 1. Solid state and solution behavior of complexes 1–4 (red-Br, blue-Ni). (A) sc-XRD structures of complexes 1–4 (thermal ellipsoids at 50% probability) (E) Diffusion coefficients of complexes 1–4 and their free ligands in CDCl_3 solution, as determined by DOSY NMR¹⁴

Discrimination of Mononuclear and Binuclear Uranyl (UO_2^{2+}) Complexes

The study of uranyl (UO_2^{2+}) coordination chemistry in solution is critically important for designing selective extractants, nuclear waste sorbents, and functional photoluminescent materials. The linear dioxo uranyl cation coordinates ligands in its equatorial plane, frequently forming higher-order oligomers or hydroxo-/oxo-bridged complexes depending on the ligand geometry, stoichiometry, and pH.

In our recent work on novel hydroxyl-functionalized 1,2,4-triazole-based oligomers as uranyl chelators¹⁶, 2D DOSY NMR played a decisive role in deciphering complex coordination behaviour. The nitrogen-rich triazole cores equipped with hydroxyl pendant groups present multiple binding sites capable of engaging uranyl ions in both 1:1 and 2:2 (or 2:1) binding modes.

Standard 1D NMR spectra of these reaction mixtures display broadened, highly complex multiplet patterns due to overlapping proton signals from distinct coordination species in solution. By applying DOSY NMR:

– *Unambiguous Speciation*: Mononuclear UO_2L^1 , UO_2L^2 , $\text{UO}_2(\text{L}^3)_2$ (or hydroxyl-bridged) complexes were clearly separated along the diffusion axis into distinct horizontal tracks.

¹⁶ Khomenko D. M. et al. Novel hydroxyl-functionalized 1,2,4-triazole-based oligomers: synthetic approach, photoluminescence and coordination behaviour as uranyl chelators. *CrystEngComm*. 2026. Vol. 28. № 22. P. 3486–3498. DOI: <https://doi.org/10.1039/D6CE00233A>.

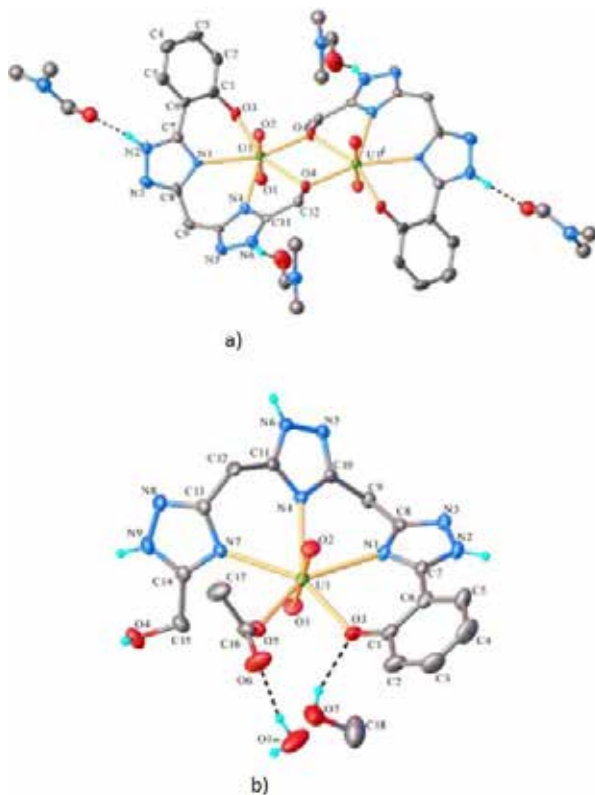


Fig. 2. Examples of complexes with different ratio between ligand and coordination atom a)1:1; b)1:2¹⁷

– *Hydrodynamic Volume Correlation:* Owing to the significant increase in molecular weight and effective hydrodynamic radius upon dimerization/binuclear complex formation, the binuclear uranyl species exhibited systematically lower self-diffusion coefficients $D(\text{binuclear})$ compared to their mononuclear counterparts $D(\text{mononuclear})$.

– *Stoichiometric Determination:* Comparing the ratio of D values $D(\text{complex}) / D(\text{free ligand})$ against modified Stokes-Einstein estimations allowed for the explicit determination of the complex oligomeric state in

¹⁷ Khomenko D. M. et al. Novel hydroxyl-functionalized 1,2,4-triazole-based oligomers: synthetic approach, photoluminescence and coordination behaviour as uranyl chelators. *CrystEngComm*. 2026. Vol. 28. № 22. P. 3486–3498. DOI: <https://doi.org/10.1039/D6CE00233A>.

solution without relying on single-crystal X-ray diffraction, which may not always represent the predominant liquid-phase species.

If the ligand exchange rate is slow on the NMR time scale, two isolated diffusion layers are simultaneously observed on the DOSY spectrum, allowing for the precise determination of the complex stoichiometry¹⁸. In the case of fast chemical exchange, a single time-averaged ligand signal is observed; its position along the diffusion axis allows for the calculation of thermodynamic stability constants and the determination of the degree of uranyl polymerization in aqueous phases¹⁹.

1.4. Pharmaceutical Analysis and Quality Control

In modern pharmacy, DOSY NMR serves as a rapid method for detecting counterfeit and falsified products without destroying the commercial formulation of the drug. For example, analysing tablet formulations of erectile dysfunction medications or antibiotics makes it possible – in a single 15–20 minutes experiment – to identify the Active Pharmaceutical Ingredient (API), quantitatively evaluate excipients (fillers such as lactose, starch, magnesium stearate), and detect the presence of dangerous impurities or illegally added active substances. Because the API and excipients differ significantly in molecular weight, their signals on a 2D DOSY plot are clearly separated along the vertical axis, even when total overlap occurs in the methyl or aromatic regions of a standard 1D spectrum.

2. Environmental Science and Contaminant Dynamics

In environmental research, DOSY NMR has emerged as an indispensable non-destructive analytical method for dissecting complex environmental matrices – such as Natural Organic Matter (NOM), soil extracts, and aquatic sediments – and monitoring pollutants from molecular interactions to global carbon cycles.

– **Probing Natural Organic Matter (NOM) Supramolecular Structures:** NOM consists of polydisperse, heterogeneous mixtures of humic and fulvic acids. Standard 1D ¹H or ¹³C spectra yield broad, featureless “humps” due to extreme spectral crowding. DOSY NMR separates these supramolecular aggregates along the diffusion dimension according to their hydrodynamic radii without altering non-covalent hydrophobic or electrostatic networks^{20, 21}.

¹⁸ Khomenko D. M. et al. Novel hydroxyl-functionalized 1,2,4-triazole-based oligomers: synthetic approach, photoluminescence and coordination behaviour as uranyl chelators. *CrystEngComm*. 2026. Vol. 28. № 22. P. 3486–3498. DOI: <https://doi.org/10.1039/D6CE00233A>.

¹⁹ Urbank C., Vondung L. Accurate Molecular Size Determination by Diffusion Ordered NMR Spectroscopy Based on an Improved Diffusion Model. *Chemistry – A European Journal*. 2026. Art. e71471. DOI: <https://doi.org/10.1002/chem.71471>.

²⁰ Zheng G., Price W. S. Direct hydrodynamic radius measurement on dissolved organic matter in natural waters using diffusion NMR. *Environmental Science & Technology*. 2012. Vol. 46. № 3. P. 1675–1680. DOI: <https://doi.org/10.1021/es202809e>.

²¹ Simpson A. J., McNally D. J., Simpson M. J. NMR spectroscopy in environmental research: From molecular interactions to global processes. *Progress in Nuclear Magnetic Resonance Spectroscopy*. 2011. Vol. 58. № 3–4. P. 97–175. DOI: <https://doi.org/10.1016/j.pnmrs.2010.09.001>.

– **Contaminant–Matrix Interactions and Bioavailability:** DOSY is uniquely equipped to characterize non-covalent binding between organic pollutants (e.g., pesticides, pharmaceuticals, polycyclic aromatic hydrocarbons [PAHs], per and polyfluoroalkyl substances via ^{19}F -DOSY) and humic matrices²². When a small pollutant molecule binds to a high-molecular-weight NOM assembly, its observed diffusion coefficient drops sharply toward that of the NOM supramolecular host. Measuring this change provides quantitative thermodynamic data on binding constants, speciation, bioavailability, and mobility in soils and natural waters²³.

– **Identification of Organic Phosphorus Species in Soil Extracts:** Soil phosphorus composition directly governs ecosystem fertility and aquatic eutrophication. In alkaline soil extracts (NaOH-EDTA), ^{31}P NMR signal assignment is frequently hindered by severe peak overlap in the monoester region and chemical shift drift caused by variable pH and paramagnetic ions (Fe^{3+} , Mn^{2+}). Applying ^{31}P -DOSY NMR separates organic phosphorus compounds (such as *myo*- and *scyllo*-inositol hexakisphosphates, phosphonates, and nucleic acid fragments) based on their mobility, providing accurate peak identification in dense soil spectra²⁴.

3. Multidimensional 3D-DOSY Spectroscopy

While 2D DOSY is a powerful technique, it encounters significant limitations when dealing with ultra-complex mixtures where signals overlap severely in the chemical shift (F_1) domain species possess similar diffusion coefficients (D). When two overlapping peaks decay at slightly different rates, monoexponential decay fitting fails, producing severe mathematical artifacts or averaged diffusion values along the vertical axis.

To overcome this bottleneck, **3D-DOSY Spectroscopy** was developed by combining standard 2D correlation pulse sequences (e.g., COSY, TOCSY, HSQC, HMQC, NOESY, or 2DJ) with pulsed field gradient diffusion encoding.

Concatenated 3D-DOSY Experiments (e.g., *DOSY-COSY*, *DOSY-HSQC*)

– **Resolves Overlapping Signals:** The main advantage is the ability to accurately measure diffusion coefficients even when proton signals overlap heavily in standard 1D spectra.

²² Gauthier J. R., Mabury S. A. Experimentally Determined Aqueous Diffusion Coefficients of PFAS Using ^{19}F NMR Diffusion-Ordered Spectroscopy. *ACS EST Water*. 2024. Vol. 4. № 10. P. 4615–4624. DOI: <https://doi.org/10.1021/acsestwater.4c00631>.

²³ Mazzei P., Piccolo A. Quantitative Evaluation of Noncovalent Interactions between Glyphosate and Dissolved Humic Substances by NMR Spectroscopy. *Environmental Science & Technology*. 2012. Vol. 46. № 11. P. 5939–5946. DOI: <https://doi.org/10.1021/es300265a>.

²⁴ Mazzei P., Piccolo A. Quantitative Evaluation of Noncovalent Interactions between Glyphosate and Dissolved Humic Substances by NMR Spectroscopy. *Environmental Science & Technology*. 2012. Vol. 46. № 11. P. 5939–5946. DOI: <https://doi.org/10.1021/es300265a>.

– **Higher Structural Precision:** Dispersing signals across two frequency dimensions (^1H - ^1H or ^1H - ^{13}C) enables independent decay fitting for each cross-peak, ensuring precise component identification.

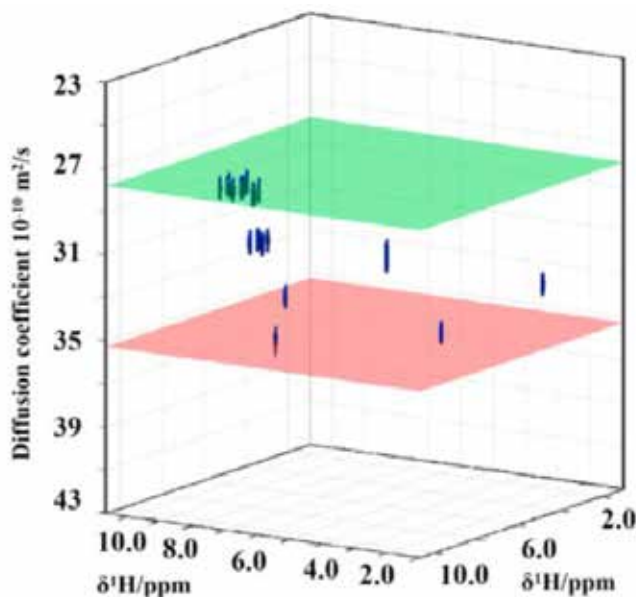


Fig. 3. Typical spectrum of 3D-DOSY-COSY of organic compounds in coal chemical wastewater²⁵

Internally Encoded IDOSY Sequences (e.g., 2DJ-IDOSY, HSQC-IDOSY)

This strategy overcomes the signal loss and long acquisition times of conventional 3D experiments:

- **Doubles Sensitivity (S/N):** Embedding diffusion gradients into pre-existing delays (like TOCSY/NOESY mixing times) eliminates the 50% signal loss typical of standard pulse sequences.
- **Drastically Faster Acquisition:** Reduces experimental runtimes from several hours down to just a few minutes.
- **Greater Efficiency:** Minimizes overall sequence delays and simplifies phase cycling requirements.

²⁵ Ma H. et al. Utilizing 3D DOSY NMR in the characterization of organic compounds in coal chemical wastewater. *Magnetic Resonance Letters*. 2022. Vol. 2. № 2. Art. 100021. DOI: <https://doi.org/10.1016/j.mrl.2021.100021>.

4. Experimental Part

4.1. Materials and Methods

DOSY NMR experiments on honey and oil samples were performed on an 800 MHz Bruker Neo dual-purpose solid/liquid-state spectrometer at the University of Edinburgh, School of Chemistry, while the study of actinide complexes (in this case, uranyl) was carried out on a Bruker Avance NEO 400 MHz spectrometer in Kyiv.

Olive oil samples were analysed as solutions in CDCl_3 . Honey samples were prepared by dissolving 50 mg of honey in 550 μl D_2O ²⁶. Uranyl complexes were studied in DMSO-d_6 under strict thermal control of the system at $T = 298\text{K}$ ¹². To measure diffusion in oil and honey samples, the ledbpgp2s pulse sequence (Bruker stimulated echo with bipolar gradient pulses and longitudinal eddy current delay, LED) was used. The number of gradient steps was at least 16, with the gradient amplitude varying linearly from 5% to 95%. Optimal values for the gradient duration and diffusion time were adjusted individually for each system. For uranyl complexes, the stebpgp1s pulse sequence was employed, keeping all other acquisition parameters identical.

4.2. Results and Discussion

Mathematical processing and fitting of signal intensity decay curves were performed using Bruker TopSpin / Dynamics Center software.

A. Results of Olive Oil Analysis

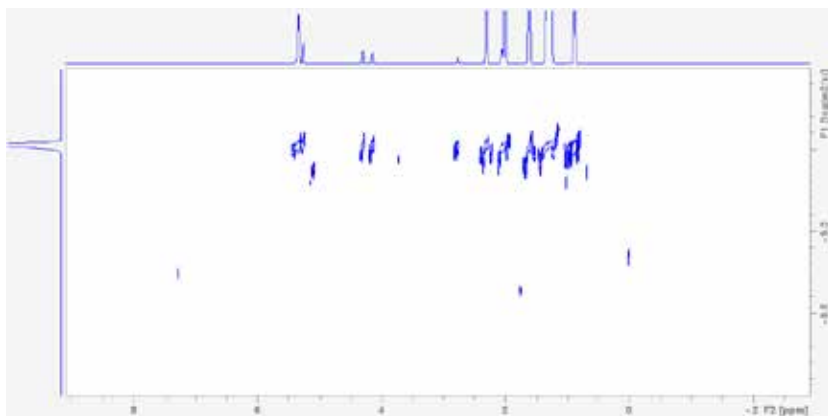


Fig. 4. Full 2D DOSY NMR spectrum of olive oil

²⁶ Gauthier J. R. et al. Exploring the Composition and Authenticity of Honey and Syrup Samples Using Quantitative NMR Spectroscopy and Principal Component Analysis in an Upper-Year Undergraduate Analytical Environmental Course. *Journal of Chemical Education*. 2023. Vol. 100. № 1. P. 161–169. DOI: <https://doi.org/10.1021/acs.jchemed.2c00501>.

In the presented 2D DOSY spectrum of olive oil, a clear separation between the main triglyceride lipid matrix and minor components (squalene) is observed. Upon artificial addition of an adulterant, the formation of additional tracks is detected; however, signal discrimination remains challenging. Following spectral processing with Dynamics Center, enhanced signal resolution is achieved.

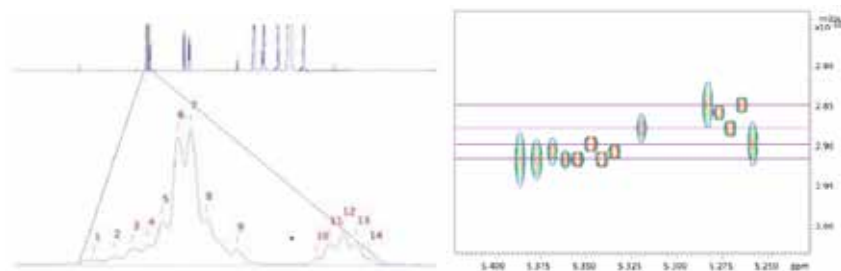


Fig. 5. Fragment of the report generated by Dynamics Center

In the Fig. 5 we see the zoom spectra of the same olive oil, as in Fig. 4. It can be seen that even severely overlapping signals can be resolved using DOSY NMR spectroscopy. We can see at least 4 compounds with different diffusion coefficients fig. 5 (right pic.).

B. Results of Honey Analysis

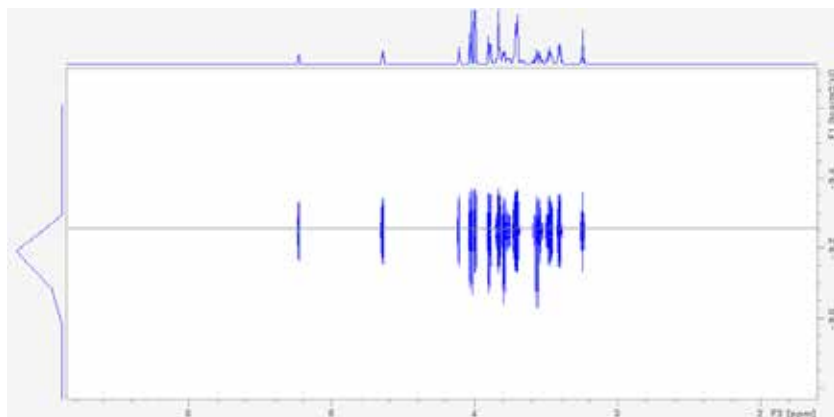


Fig. 6. DOSY NMR spectrum of floral honey

The spectrum demonstrates that resolving the primary components – such as glucose, fructose, and sucrose – is not feasible because these sugars (glucose and fructose) share the same molecular weight.

Consequently, the next stage of investigating these matrices will involve employing PSYCHE-iDOSY to enhance resolution.

C. Uranyl Coordination Compounds

To investigate uranyl complex systems, a series of DOSY NMR experiments was conducted with various ligands. The chemical formulas of these compounds are listed in fig. 7.

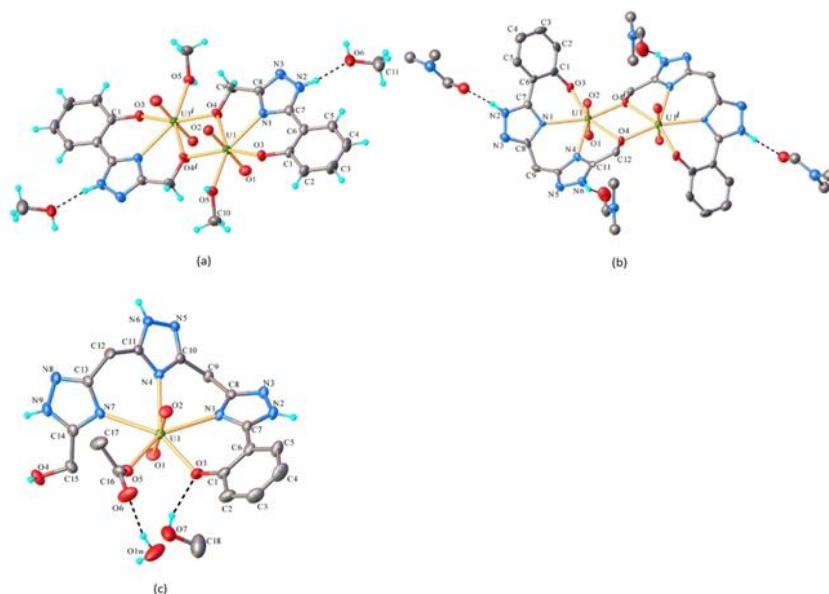


Fig. 7. Formulas of three complexes in solid state: a) $(\text{UO}_2)_2(\text{L}^1)_2$; b) $(\text{UO}_2)_2(\text{L}^2)_2 \cdot 4\text{DMF}$; c) UO_2L^3 ²⁷

The resulting experimental spectra is presenting in Fig. 8, and the values for the self-diffusion coefficients of both coordinated species are summarized in Table 1.

²⁷ Khomenko D. M. et al. Novel hydroxyl-functionalized 1,2,4-triazole-based oligomers: synthetic approach, photoluminescence and coordination behaviour as uranyl chelators. *CrystEngComm*. 2026. Vol. 28. № 22. P. 3486–3498. DOI: <https://doi.org/10.1039/D6CE00233A>.

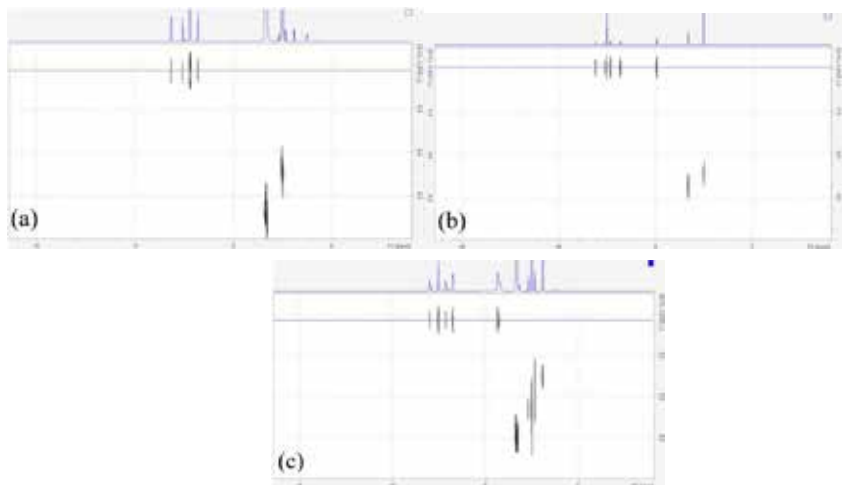


**Fig. 8. ^1H DOSY NMR spectra of complexes in DMSO-d_6 :
 (a) **1** ($(\text{UO}_2)_2(\text{L}^1)_2$), $C = 7.2 \text{ mM}$; (b) **[2-4DMF]** ($(\text{UO}_2)_2(\text{L}^2)_2$),
 $C = 6.1 \text{ mM}$ and (c) **3** (UO_2L^3), $C = 2.1 \text{ mM}$ ²⁸**

Table 1

Self-diffusion coefficients of **H2L1–3 and their complexes **1–3** calculated using the DOSY signals of the molecular framework**

Compound	Concentration, mM	$D \text{ r}$
H_2L^1	33.02	3.22
H_2L^2	23.2	2,56
H_2L^3	21.4	1.97
1	7.2	2.03
[2•4DMF]	6.1	1.99
3	2.1	2.20
DMSO-d_6		6.63*
H_2O		7.78*

**independent on compound concentration.*

An analysis of Table 1 demonstrates that upon ligand coordination to the uranyl ion, the diffusion coefficient decreases. ^1H DOSY NMR studies of complexes **1–3** (Fig. 8, Table 1) revealed that the diffusion coefficients of

²⁸ Khomenko D. M. et al. Novel hydroxyl-functionalized 1,2,4-triazole-based oligomers: synthetic approach, photoluminescence and coordination behaviour as uranyl chelators. *CrystEngComm*. 2026. Vol. 28. № 22. P. 3486–3498. DOI: <https://doi.org/10.1039/D6CE00233A>.

compounds 1 and [2•4DMF] are, as expected, virtually identical (2.03×10^{-10} and $1.99 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$, respectively) and differ from that of complex 3 ($2.20 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$). This fact is in good agreement with the dimeric structure of compounds 1 and 2 versus the more compact mononuclear structure of complex 3.

CONCLUSIONS

1. Two-dimensional Diffusion-Ordered NMR Spectroscopy (DOSY) is a highly efficient and versatile physical-chemical tool for analyzing complex liquid mixtures of various origins.

2. In the study of lipid and carbohydrate matrices (olive oil, honey), the capability of the DOSY method (particularly its Pure Shift modifications) to perform rapid quality and authenticity control without sample destruction or the use of harmful chemical reagents was demonstrated, fully aligning with the principles of green analytical chemistry.

3. In the field of actinide coordination chemistry, taking uranyl complexes as an example, measuring translational self-diffusion was proven to reliably identify the formation of complex species, determine their stoichiometry, and reveal their dynamic characteristics directly in the solution phase, bypassing the complicated stage of single-crystal isolation.

4. Future prospects for this direction lie in the implementation of Ultrafast DOSY pulse sequences, which enable the acquisition of diffusion maps in a single scan. This opens up unique opportunities for real-time monitoring of fast chemical reactions and liquid-liquid extraction kinetics.

SUMMARY

In modern chemical analysis and materials science, non-destructive molecular profiling and dynamic characterization of complex multicomponent liquid systems become increasingly relevant. This paper focuses on the comprehensive analysis of the current state and practical applications of two-dimensional Diffusion-Ordered NMR Spectroscopy (DOSY). The study outlines the theoretical foundations of the method, based on encoding the spatial position of nuclei using Pulsed Field Gradients (PGF). The concept of "virtual chromatography" is demonstrated across three distinct system classes: plant lipid matrices (olive oil), concentrated carbohydrate systems (natural honey), and coordination compounds. By measuring individual translational self-diffusion coefficients (D), this approach enables the simultaneous verification of food authenticity, detection of adulterants, and elucidation of labile complex stoichiometry, stability constants, and heavy metal oligomerization states in solution without prior physical separation.

ACKNOWLEDGEMENTS

We sincerely acknowledge the School of Chemistry at the University of Edinburgh for their financial support of our research. We express our thanks to Prof. Dušan Ugrin for his advice and assistance in recording NMR spectra.

Bibliography

1. Holzgrabe U. Quantitative NMR spectroscopy in pharmaceutical applications. *Progress in Nuclear Magnetic Resonance Spectroscopy*. 2010. Vol. 57. № 2. P. 229–240. DOI: <https://doi.org/10.1016/j.pnmrs.2010.05.001>.
2. Johnson C. S. Jr. Diffusion ordered nuclear magnetic resonance spectroscopy: principles and applications. *Progress in Nuclear Magnetic Resonance Spectroscopy*. 1999. Vol. 34, № 3–4. P. 203–256. DOI: [https://doi.org/10.1016/S0079-6565\(99\)00003-5](https://doi.org/10.1016/S0079-6565(99)00003-5).
3. Claridge T. D. W. High-Resolution NMR Techniques in Organic Chemistry. 3rd ed. Elsevier, 2016. 464 p. URL: <https://www.inspectioncopy.elsevier.com/book/details/9780080999869>.
4. Morris K. F., Johnson C. S. Jr. Diffusion-ordered two-dimensional nuclear magnetic resonance spectroscopy. *Journal of the American Chemical Society*. 1992. Vol. 114. № 8. P. 3650–3657. DOI: <https://doi.org/10.1021/ja00034a071>.
5. Barjat H. et al. High-resolution diffusion-ordered 2D spectroscopy (HR-DOSY). *Journal of Magnetic Resonance, Series B*. 1995. Vol. 108. № 3. P. 266–272. DOI: http://apps.isiknowledge.com/UA_GeneralSearch_input.do?product=UA&search_mode=GeneralSearchWOS:A1995RN49300008.
6. Macchioni A. et al. Determining accurate molecular sizes in solution through NMR diffusion measurements. *Chemical Society Reviews*. 2008. Vol. 37, № 3. P. 479–489. DOI: <https://doi.org/10.1039/b615067p>.
7. Pregosin P. S. NMR diffusion methods in inorganic and organometallic chemistry. Spectroscopic Properties of Inorganic and Organometallic Compounds. *Royal Society of Chemistry*, 2011. Vol. 42. P. 248–268. DOI: <https://doi.org/10.1039/9781849732833-00248>.
8. Dais P., Hatzakis E. Quality assessment and authentication of virgin olive oil by NMR spectroscopy: A critical review. *Analytica Chimica Acta*. 2013. Vol. 765. P. 1–27. DOI: <https://doi.org/10.1016/j.aca.2012.12.003>.
9. Dinca O. R. et al. Geographical and botanical origin discrimination of Romanian honey using complex stable isotope data and chemometrics. *Food Analytical Methods*. 2015. Vol. 8. № 2. P. 401–412. DOI: <https://doi.org/10.1007/s12161-014-9903-x>.

10. Foroozandeh M. et al. Ultrahigh-Resolution Diffusion-Ordered Spectroscopy. *Angewandte Chemie International Edition*. 2016. Vol. 55. № 50. P. 15579–15582. DOI: <https://doi.org/10.1002/anie.201609676>.
11. Mateos-Calbet A. et al. 2-Substituted vs 2,9-Disubstituted Phenanthroline-Ni(II)-halides: Speciation Control and Structural Elucidation in Solution. *Inorganic Chemistry*. 2025. Vol. 64. № 34. P. 17093–17097. DOI: <https://doi.org/10.1021/acs.inorgchem.5c02704>.
12. Serebrennikova O. O. et al. Aqueous micellar environment impacts the Co-catalyzed phototransformation: A case study / O. O. Serebrennikova et al. *ACS Catalysis*. 2025. Vol. 15. № 3. P. 1820–1831. DOI: <https://doi.org/10.1021/acscatal.4c05678>.
13. Khomenko D. M. et al. Novel hydroxyl-functionalized 1,2,4-triazole-based oligomers: synthetic approach, photoluminescence and coordination behaviour as uranyl chelators. *CrystEngComm*. 2026. Vol. 28. № 22. P. 3486–3498. DOI: <https://doi.org/10.1039/D6CE00233A>.
14. Urbank C., Vondung L. Accurate Molecular Size Determination by Diffusion Ordered NMR Spectroscopy Based on an Improved Diffusion Model. *Chemistry – A European Journal*. 2026. Art. e71471. DOI: <https://doi.org/10.1002/chem.71471>.
15. Zheng G., Price W. S. Direct hydrodynamic radius measurement on dissolved organic matter in natural waters using diffusion NMR. *Environmental Science & Technology*. 2012. Vol. 46. № 3. P. 1675–1680. DOI: <https://doi.org/10.1021/es202809e>.
16. Simpson A. J., McNally D. J., Simpson M. J. NMR spectroscopy in environmental research: From molecular interactions to global processes / A. J. Simpson, D. J. McNally, M. J. Simpson. *Progress in Nuclear Magnetic Resonance Spectroscopy*. 2011. Vol. 58. № 3–4. P. 97–175. DOI: <https://doi.org/10.1016/j.pnmrs.2010.09.001>.
17. Gauthier J. R., Mabury S. A. Experimentally Determined Aqueous Diffusion Coefficients of PFAS Using ¹⁹F NMR Diffusion-Ordered Spectroscopy. *ACS EST Water*. 2024. Vol. 4. № 10. P. 4615–4624. DOI: <https://doi.org/10.1021/acsestwater.4c00631>.
18. Mazzei P., Piccolo A. Quantitative Evaluation of Noncovalent Interactions between Glyphosate and Dissolved Humic Substances by NMR Spectroscopy. *Environmental Science & Technology*. 2012. Vol. 46. № 11. P. 5939–5946. DOI: <https://doi.org/10.1021/es300265a>.
19. Ma H. et al. Utilizing 3D DOSY NMR in the characterization of organic compounds in coal chemical wastewater. *Magnetic Resonance Letters*. 2022. Vol. 2. № 2. Art. 100021. DOI: <https://doi.org/10.1016/j.mrl.2021.100021>.

20. Gauthier J. R. et al. Exploring the Composition and Authenticity of Honey and Syrup Samples Using Quantitative NMR Spectroscopy and Principal Component Analysis in an Upper-Year Undergraduate Analytical Environmental Course. *Journal of Chemical Education*. 2023. Vol. 100. № 1. P. 161–169. DOI: <https://doi.org/10.1021/acs.jchemed.2c00501>.

Information about the authors:

Hreben Mariia,

Postgraduate Student

Taras Shevchenko National University of Kyiv

60, Volodymyrska Street, Kyiv, Ukraine

Lampeka Rostyslav,

Doctor of Chemical Sciences, Professor,

Head of the Department of Inorganic Chemistry

Taras Shevchenko National University of Kyiv

60, Volodymyrska Street, Kyiv, Ukraine