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Dynamic NMR methodology for kinetic studies

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Abstract. NMR (Nuclear magnetic resonance) is a popular tool used to characterize the structure of molecules, which is widely used for its high precision and accurate results. Due to these advantages, the NMR technique has been developed for monitoring the changes in the components during a reaction. This is called dynamic NMR technology. The kinetic measuring of a reaction is an important application of dynamic NMR, and the unique design of the methodology allows the NMR technique to be used for the determination of kinetic data such as reaction rates. In addition, dynamic NMR has also been used to monitor the reaction process and determine the reaction mechanism. Dynamic NMR techniques have developed rapidly in recent decades and have led to new research ideas and tools in chemistry and other related fields. In this paper, some examples of kinetic properties of reactions studied by dynamic NMR methods are introduced, including a summary of their development and prospects.

Keywords: Dynamic NMR, Kinetic study, Reaction mechanism

1. Introduction

NMR (Nuclear magnetic resonance) spectroscopy is a popular technique used in analytical chemistry today. It is widely loved by chemists for its high precision, sensitivity and non-destructive nature of the sample. Nuclei capable of spinning carry the property of magnetic moments (μ), and when these nuclei are in an applied magnetic field of strength B0, they move around B_0 on top of their spin. These magnetic moments are quantised at the microscopic level, and for a nucleus with the spin quantum number I, there are 2I+1 possible states in the presence of an external magnetic field. The energies of the nuclei in these states are different, with forward-aligned nuclei having lower energies and reverse-aligned nuclei having higher energies. When a spinning nucleus in an external magnetic field is exposed to electromagnetic radiation of a specific energy, the lower energy nuclei jump to a higher energy state, a phenomenon known as nuclear magnetic resonance (NMR). Nuclei capable of spinning in a strong magnetic field will produce a resonance signal, and the NMR spectrum resulting from collecting such signals will contain information about the structure of the substance.

NMR brings accurate and informative results for the structural analysis of substances - especially small molecules - and has a wealth of applications at both organic and inorganic levels. Since the signals measured by NMR spectroscopy come from the process of energy release from nuclei in the high energy state back to the equilibrium state, i.e., the process of relaxation, during which the signals evolve according to time, the NMR process can measure time-dependent parameters. This is the hardware basis on which the NMR technique can be used for dynamic studies.

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The usual NMR pattern only gives structural information about a single state. However, due to the high sensitivity of the spin nature of the nucleus to its surroundings, NMR can sustain kinetic analyses on large time scales (10^{-3} to 10^{-7} s) [1]. The non-destructive and quantitative nature of NMR technology makes it ideally suited for studying the dynamic processes of substances, where it can be used to determine changes in the components of the reaction environment, known as dynamic NMR. As an advanced application of NMR technology, most of the dynamic NMR processes can be realised on a usual NMR spectrometer by simply designing and adjusting the corresponding NMR method.

Dynamic NMR allows the monitoring of ion exchange processes, the capture of intermediate structures, and the determination of reaction kinetics. In general, as long as nuclei with NMR activity are present and the signals are sufficiently dispersed, it is possible to monitor every compound in a dynamic process.

In the 1960s, dynamic NMR techniques were developed and used to monitor the hydration-dehydration exchange reaction of pyruvic acid [2]. Some very classical dynamic equilibrium processes can also be observed in full view by dynamic NMR, such as equilibria of keto-enol or other various reciprocal isomers.

Kinetic studies of reactions are an essential application of dynamic NMR. Kinetic studies have a non-negligible contribution to measuring reaction rate constants and activation energies, studying reaction mechanisms, and understanding and optimising reaction conditions. Although kinetic studies require precise control of reaction conditions and accurate measurement of raw material mass and time information, for dynamic NMR, this technique is quantitative, simple and fast. It has become a prevalent and practical tool in various fields. Dynamic NMR technology can study the reaction processes under the traditional equilibrium conditions and the imbalanced chemical processes occurring in similarly mixed reactions. Many helpful information can be obtained.

The aim of this paper is to introduce the application of dynamic NMR techniques to provide new tools and ideas for researchers in chemistry.

2. Application of dynamic NMR in biological environments

2.1. Application of dynamic NMR on tissue and cells

No damage to samples makes NMR possible to study biochemical processes in cells and tissues. While isotope labelling is a well-known mechanistic technique, NMR allows more accessible and more convenient isotope tracing in vivo to follow the kinetic process of reactions [3]. The simplest method is that when a nucleus transforms into its isotope, its original spectrum disappears and is replaced by a new signal. However, this method does not give access to additional information about the nucleus that is not NMR active. Hence, a better method is to look at another nucleus adjacent to the one being exchanged so that both species' full signals are available simultaneously. For example, the substitution of O for ^{18}O in phosphoric acid can lead to the displacement of ^{31}P [4].

Sugar nucleotides are biological phosphates that are extremely important for cellular metabolic processes, and it is of great interest to study their fate in living organisms [5]. Sugar nucleotides have NMR-active phosphorus elements within them to facilitate the tracing of kinetic processes, and information on their synthetic methods and structural modifications with their analogues can provide new therapeutic ideas in pharmacology. The heterocarbon reactions of sugars and the keto-enol equilibrium processes involved are well suited to be monitored using dynamic NMR, allowing multiple reaction conditions to be easily varied to obtain more valid information.

Dynamic NMR techniques can also be used to monitor the state of molecules inside and outside the cell and processes such as transmembrane transport. This method was first used by Conlon & Outhred to measure the rate of water exchange between the inside and outside of the cell membrane on erythrocytes, as the chemical shifts vary slightly due to the different environments inside and outside the cell [6]. In addition, dynamic NMR can also be used to measure enzyme activity, monitor the fate of essential molecules such as ATP(Adenosine triphosphate), and determine the energy barrier of proton exchange processes.

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2.2. Dynamic NMR used in drug development process

Due to the complexity of biological systems, the drug development process is very costly in terms of time and money, and the application of dynamic NMR technology has dramatically increased the efficiency of development and reduced costs. NMR is excellent at all stages of drug development, and Dynamic NMR makes it possible to monitor intracellular biochemical kinetic processes with the advantage of not damaging the analyte. NMR-active isotopes of H, C, P, N and other elements commonly found in biomolecules exist, and the ability of NMR technology to monitor the processes of several elements simultaneously makes tracking in vivo reactions more convenient and informative.

In drug discovery, dynamic NMR is excellent for monitoring the behaviour of ligands, confirming whether the ligand possesses appropriate selectivity and specificity and whether it can accurately bind to the protein target. Dynamic NMR is the best means of examining protein-ligand interactions. It can provide kinetic data such as dissociation constants and reaction rates, find ligand-binding sites, resolve the 3D structure of ligand complexes, and monitor their movement in the human body. It can also be used to find ligand binding sites, resolve the three-dimensional structure of ligand complexes, and monitor their movement in the human body [1].

The HTS (High throughput screening) step of drug discovery can also be combined with dynamic NMR technology. HTS-NMR does not require high specificity for the proteins to be screened and can be tailored to the functional needs of the protein. A significant advantage of HTS-NMR for fragment-based screens is its ability to detect weak binding ligands [7].

In metabolomics, dynamic NMR techniques can characterise drug toxicity and selectivity [8]. However, although NMR techniques have good resolution, they are only sometimes the best choice within metabolomics as their sensitivity is not very high and can only resolve the most abundant metabolites.

3. Application of dynamic NMR in the inorganic field

Dynamic NMR is one of the preferred methods to study the dynamic rearrangement of atoms in reactions related to transition metal complexes. dynamic NMR spectroscopy allows quantitative calculation of the relevant activation parameters, identification of the exchange processes and helps to optimise the final rearrangement model. Eight-coordinate rhenium(V) polyhydride systems have many simultaneous kinetic processes in which phosphorus and hydrogen atoms are involved in rearrangement and exchange inside and outside the ligand [9]. Dynamic NMR makes it easy to study this phenomenon with great clarity, to characterise the dynamics quantitatively, and to obtain information about the changes in the ligand.

Although most of the content of this paper discusses dynamic processes related to chemical reactions, some dynamic physical changes can also be studied using dynamic NMR. Maillet et al. published the results of their dynamic NMR analyses of liquid transport processes in the porous media material silica (glass) in 2022 [10]. A porous medium is a pervasive substance with many pores that can be filled with liquids or gases, such as sponges, wood, and bones. Separating fluids from porous media or enabling fluids to penetrate porous media is the focus of such substances being investigated, such as petroleum extraction from natural environments, fluid transfer processes in the human body, etc. The unique time dependence of NMR gives more quantitative results than neutron imaging and X-ray techniques. Due to the nature of porous media, non-destructive studies of their internal structure are very important, and Dynamic NMR allows for the study of the exchange rate and diffusion parameters of liquids, in this case, silica and water, in such porous materials as well as the evaluation of the material's properties.

4. Introduction to dynamic 2D-NMR technology

In general, most of the objects being studied are first-order reactions or pseudo-primary reactions that have been designed to be so. If one wants to study more complex reactions, one needs to use 2D-NMR techniques, take into account a more comprehensive range of conditions affecting the course of the reaction, and perform more rigorous calculations and analyses. Part of the reason for not choosing to simplify the experimental conditions may be that the reactions under study are more focused on practical

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applications than in the laboratory, such as large-scale industrial production or rigorous modelling of processes in organisms. 2012, Susanne et al. studied the hydrolysis of acetic anhydride under realistic secondary conditions suitable for an industrial production environment, and their experiments required a more comprehensive range of conditions to be taken into account, such as concentration of substrate and reaction temperature, and developed a more complex kinetic model that was simulated using mathematical methods (see Figure 1) [11]. In 2022, Jaroszewicz et al. proposed a very detailed 2DNMR kinetic model to monitor the enzymatically-catalysed hydrolysis of n-α- benzoylarginine ethyl ester (BAEE) [12].

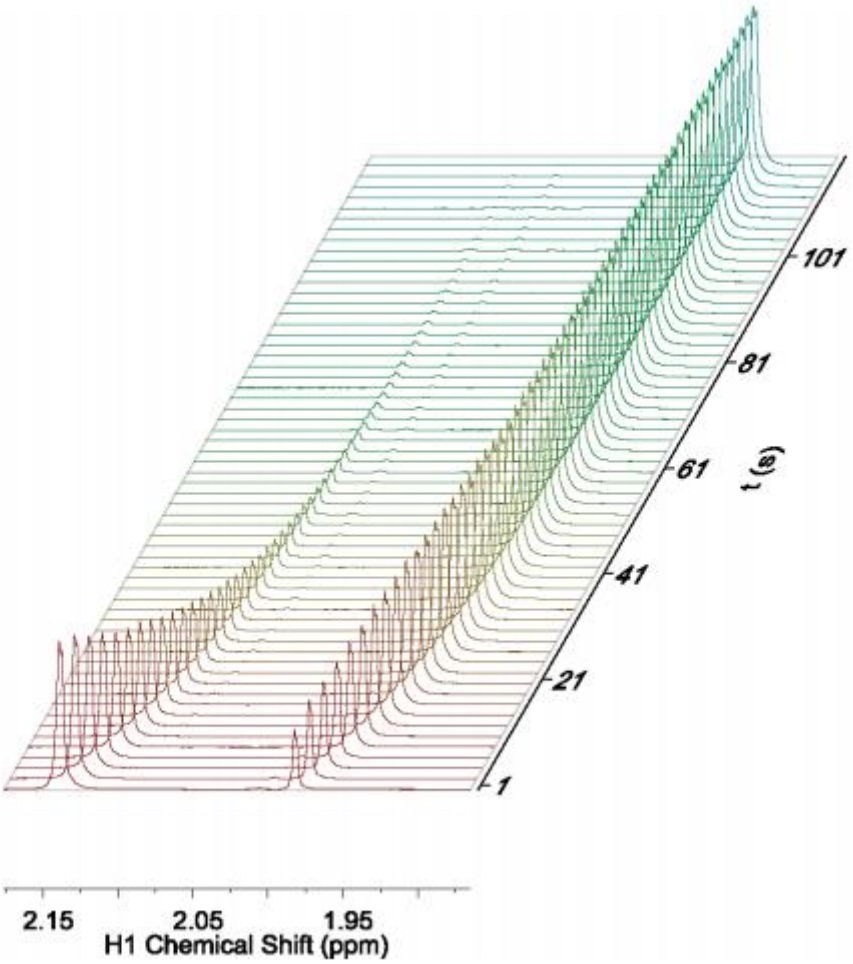

Figure 1. The 1H dynamic NMR spectra of the acetic anhydride hydrolysis reaction at 0.47M and 25°C [11].

5. Conclusion

This paper focuses on the more cutting-edge applications of dynamic NMR in recent years and provides a preliminary summary of its applications. The acquisition of NMR spectra maintains the integrity of the sample, and the NMR signals are acquired on a time-based basis, which together contributes to the advantages of dynamic NMR over techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS), which are often shared with NMR. Since the idea of dynamic NMR was proposed 70 years ago, this technique has been developed rapidly and widely used, especially in the determination of kinetic data of the reaction; dynamic NMR has a hard-to-replace position. In addition to the above two points, Dynamic NMR has many other advantages. It has a wide range of applications in organic and inorganic fields and can even analyse physical change processes. Although the sensitivity

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of the dynamic NMR technique is not top-notch, its resolution is excellent, and very high-quality structural information can be obtained within the range of signals that can be monitored. It is simple and easy to apply, and the results are accurate. Unfortunately, the content of the inorganic part and the application in living organisms of dynamic NMR discussed is still superficial and unable to show the full picture. The limits of practical application of dynamic NMR technology are far beyond this, and it is expected that with the progress of research and the development of more sophisticated analytical tools, dynamic NMR can be used in more complex environments or to obtain more refined experimental data.

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