Nuclear magnetic resonance methods – the key to determining the structure of organic substances

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Abstract. This article is intended to help an organic chemists and industrial engineers to choose a suitable method for the analysis of substances with complex structure and its control in industry. In the paper the current level of the NMR development and analytical abilities of the different NMR techniques is considered. It is noticed, that as a rule the results of the one-dimensional NMR are quite difficult to interpret in the case of a complex molecular structure. The main principles and different varieties of two-dimensional NMR techniques are described.

1. Introduction

At the present time various physicochemical methods of analysis are organic systems are widely developed [1-4]. The structural study of the organic compounds is a very special and complex task, and Nuclear Magnetic resonance spectroscopy is known to be an unique method that makes a substantial contribution to the solution of this problem.

NMR is a method based on the resonance absorption of electromagnetic waves by a substance in a constant magnetic field caused by nuclear magnetism. NMR was discovered in 1946 independently by two groups of researchers of American physicists headed by F. Bloch and E. Purcell. The NMR phenomenon is based on the fact that the nuclei of atoms of many elements (like electrons) have their own magnetic moment (spin). [5] From the description of the “traditional” NMR method and the experience of its practical use, certain limitations of the classical NMR experiment become obvious. The increase in the size of the molecule, the complication of its structure lead to a critical complication of the type of the observed NMR spectrum. As an example the ¹³C and ³¹P NMR spectra of bis-(2-chloroethyl)-9,10-dihydro-(11-bromine,11-nitroethano)-anthracene-12-ylphosphonate in CDCl₃ solution is included (Figure 1) [6].

Two-dimensional NMR is able to solve this problem. [7]

2. Overview of modern NMR techniques.

The history of two-dimensional NMR dates back to 1971, when George Jinner proposed and set up an experiment in which the spectral peak is determined not by one but by two frequencies. As a result of the first action, the excitation of one type of nuclei occurs, then, the second effect promotes the
transfer of magnetization to neighboring nuclei, depending on whether the nuclei of one element or two different elements are exposed to the first and second effects, distinguish between homonuclear and heteronuclear two-dimensional NMR. In this case, the spins of the nuclei interact with each other through a chemical bond through a spin-spin interaction or through a dipole-dipole mechanism. The peak position is determined by the chemical shift or resonance frequencies of the interacting spins.[7]

Figure 1. $^{13}$C and $^{31}$P NMR spectra of bis-(2-chloroethyl)-9,10-dihydro-(11-bromo-11-nitroethano)-anthracene-12-ylphosphonate in CDCl$_3$ solution.

The two-dimensional NMR experiment includes (Figure 2):
- the preparation period during which the spins come to a state of thermodynamic equilibrium; its duration is fixed; it ends with an radiofrequency impulse ($p_1$) that excites the first spin A;
- the period of evolution or the period of indirect measurement $t_1$, preceded by one or more exciting radiofrequency pulses ($p_2$), during which the total magnetization evolves;
- mixing period, during which several pulses are produced, which ensure the transfer of magnetization from one spin to another;
- free induction decay (FID), during which (duration of time $t_2$) registration takes place.
During the two-dimensional NMR experiment, several consecutive measurements of the same sample are made, while the time $t_1$ increases with each subsequent experiment according to the linear law. The initial value $t_1=0$, and with each subsequent experiment it increases by $\Delta t$. A feature of the period of evolution is that the excited state of the spin is not immediately detected by the receiving coils, so it is also called the period of "indirect measurement". During the mixing period, the magnetization of nuclei A is transferred to the nuclei B.

Two-dimensional NMR is represented by several methods, the main of which are:

- **COSY (CorellationSpectroscopY)** correlation $^1$H – $^1$H spectroscopy, which allows estimating magnetic interactions between protons through electrons of chemical bonds separating them.

- **HSQC (Heteronuclear Single Quantum Correlation)** – correlation $^1$H – $^{13}$C spectroscopy, which allows estimating the magnetic interactions of the proton and the carbon atom, with which it is directly connected, that is, for the C–H Bonds;

- **HMQC (Heteronuclear Multiple Quantum Coherence)** – correlation $^1$H – $^{13}$C (or $^{15}$N) spectroscopy, which allows estimating the direct spin-spin interactions of the proton and the carbon atom, with which it is directly connected, that is, for the C–H Bonds. It is protons that are defined nuclei in this method;

- **COLOC (COrrelation of LOng range Coupling)** — correlation $^1$H – $^{13}$C (or $^{15}$N) spectroscopy in which interacting nuclei are separated by two or three bonds (rarely, by a large number of bonds). The detectable nucleus is the $^{13}$C (or $^{15}$N);

- **HMBC (Heteronuclear Multiple Bond Correlation)** —correlation $^1$H – $^{13}$C (or $^{15}$N) spectroscopy in which interacting nuclei are separated by two or three bonds (rarely, by a large number of bonds). In this technique, the detected nucleus is protons;

- **NOESY (Nuclear Overhauser Effect SpectroscopY)** – spectroscopy of the nuclear Overhauser effect between $^1$H nuclei located close in space (up to 3.5 E), but not necessarily connected by a chain of covalent bonds;

- **ROESY (Rotating Overhauser Effect SpectroscopY)** – variation of the nuclear Overhauser effect spectroscopy;

Nowadays, this area of analysis is intensively studied and new methods of two-dimensional NMR appear. [7]

Two-dimensional NMR NOESY methods can successfully solve quite complex problems, such as the study of the three-dimensional structure of proteins and proteins, complementing x-ray diffraction analysis [8,9,10]. The methods of two-dimensional spectroscopy are also successfully used in classical organic chemistry to determine and (or) confirm the structure of synthesized substances.

At the Figure 3 we listed typical examples of heteronuclear experiments 4 -trichloromethyl-1-phenyl-1,2,3-triazole by the use of two methods.: $^1$H – $^{13}$C HMQC and. $^1$H – $^{13}$C HMBC [11].

Traditionally, NMR spectra of solids have rather wide peaks, which greatly complicates their interpretation. [5] Homonuclear 2D NMR study of nanoparticles can successfully overcome this
The authors of the article [12] investigated diamonds in an amount not exceeding $10^{21}$ g with quantum sensors in the form of vacancies occupied by nitrogen atoms by $^1$H and $^{19}$F NMR spectroscopy methods. Pulsed NMR sequences were used to measure homonuclear decoupling and spin diffusion.

**Figure 3.** $^1$H – $^{13}$C HMQC (a), and HMBC (b) heteronuclear spectra of 4-trichloromethyl-1-phenyl-1,2,3-triazole.
The best measured NMR line width of the liquid sample was ~1 ppm, mostly limited by molecular diffusion. In order to reduce the influence of diffusion, it was performed by solid-state high-resolution NMR with the use of homolateral interchange, which allowed 20 times narrowing the line width of the NMR [13]. It is noted however, that there is a resolution limit of a spectrum, determined by the ratio of signal to noise causes by errors in the Fourier transformation. The discrepancy in the spectral resolution of the present measurement of nanoscale NMR and the measured volume width of the NMR line can be overcome, for example, by immobilization of sample molecules in nanocapsules in the liquid state of NMR, such as liposomes, micelles or polymer shells [12]. This makes it possible to directly apply NMR in Microbiology. [12]

Since the 1980s, industrial control laboratories have used desktop NMR spectrometers with sub-ppm spectral resolution. They are much less demanding working conditions and the installation site.

3. Results and discussion
However, their significant drawback is the low sensitivity and significant overlap of peaks in the NMR spectra of $^1$H due to the fact that the magnetic fields of these devices are relatively weak (1-2 T) [15]. The article [16] considers the difficulties of online control of production and laboratory processes using NMR spectroscopy. A brief theoretical consideration of the NMR method and the resulting main limitations for real-time analysis in production is presented. On the one hand, the existing NMR devices, dedicated to the needs of the industry, have low sensitivity, on the other – devices designed for research laboratories due to its technical parameters and high cost is not suitable for use in industry. At the same time, the significant progress of the last decade in the development of cheaper, small and light permanent magnets based on rare earth materials that use Halbach and one-sided configurations will be mentioned [16]. Currently, powerful cryogen-free superconducting magnets are available on the market, which opens up the prospect of developing cheaper and more compact devices. Based on this, it should be expected that in the near future NMR spectroscopy will be widely used for on-line monitoring of industrial raw materials and products, as well as the real-time chemical reaction progress. Flow-through NMR analyzers are already used in the oil industry for the analysis of crude oil and borehole fluid directly at the site [16].

Another key to solve the problem of the improvement of the quality of desktop NMR instruments for industrial use is the use of hyperpolarized NMR $^{13}$C($^1$H)[14, 16]. Using the method of reversible exchange (SABRE) hyperpolarization based on hydrogen vapor increases the sensitivity of the 1D and 2D $^{13}$C($^1$H) NMR spectra of these devices. This calls for the recording of informative spectra for substances with low concentrations. It is reported [16, 17] that for 26 mmol of pyridine in deuterated methanol (CD$_3$OD), the increase in the $^1$H NMR signal to 17,000 times was achieved within a few seconds, which corresponds to the polarization levels of $^1$H P = 5.9%. The comparable levels of amplification can be achieved in both deuterated and proton solvents, but currently the enhanced (SABRE) signals of the analyte predominate due to the relatively weak thermally polarized solvent response. The use of SABRE also allows obtaining$^{13}$C NMR spectra in the natural presence of isotopes in a single scan, as in the case of hyperpolarized $^{13}$C NMR spectra of 4-methylpyridine with a concentration of tens of millimoles. Now the corresponding signal amplification factors are increased up to 45 500 times (P = 4.0%) and are achieved in just 15 seconds [17].

Recently, the American company Thermo Fisher Scientific has launched a serial production of a new generation of compact NMR spectrometers [18]. It allows obtaining information about the structure of molecules, using less than 40 microliters of the studied solution. In addition, the new device costs $ 20,000, which is about a tenth of the cost of traditional NMR spectrometers. "We designed a device that really changes the "landscape" of NMR spectroscopy. Now scientists will be able to place the NMR system directly on his laboratory table," - said the head of the work on the creation of PicoSpin-45 John Price of a new generation of compact NMR spectrometers [19]. It allows obtaining information about the structure of molecules, using a very small volume of the studied solution – less than 40 microliters.
Adiabatic pulses [19, 20], ultrafast 2D NMR [21, 22], fast droplet size distribution (DSD) measurements [21, 22, 23] and reaction monitoring in medium-resolution NMR spectroscopy [24, 25] are among the potential methods that can expand NMR applications in the industrial sector.

Compact NMR equipment and the technique development, led to the creation of not only desktop but mobile NMR devices [26] allowing rapid analysis of water and milk for the presence of bacteria.

4. Conclusion.
Most organic chemists who began their work back in the 1980s remember bulky NMR spectrometers, which occupied an entire room. These instruments were difficult to maintain and not always reliable. Now the method has undergone radical improvements, based both on the revolutionary achievement of George Jinner, and latest advances in materials science, electronics and computer engineering. Today the NMR spectrometers have become more compact, and the era of desktop and portable instruments has come. These modern instruments allow solving difficult tasks associated with determining the complex structure of the big molecules, industrial, environmental and food analysis of different solids, solutions and disperse systems. Current progress in NMR technique makes it possible to solve a wider range of analytical tasks, previously inaccessible or very time-consuming.

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