Practical Aspects of Nuclear Magnetic Resonance Application

Subjects: Spectroscopy

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Nuclear magnetic resonance (NMR) is a primary method of measurement according to metrology standards. It has been proven to have good reproducibility. NMR spectra are evaluated numerically, including chemical shifts, coupling constants, and signal integrals, which lead to quantitative results.

Keywords: nuclear magnetic resonance (NMR) spectroscopy ; food ; cosmetics ; pharmaceuticals ; structure determination

1. Introduction

In 1952, Felix Bloch and Edward Mills Purcells were awarded the Nobel Prize for their discovery of nuclear magnetic resonance signals in 1946 [1][2][3]. During the same year, Varian Associates from Palo Alto, CA, USA released the first commercial nuclear magnetic resonance spectrometer. Additionally, Bruker, founded by Günther Laukien in Karlsruhe, Germany, built the first commercial nuclear magnetic resonance pulse spectrometers. In the subsequent years, nuclear magnetic resonance (NMR) spectroscopy has rapidly advanced in various application fields. Where NMR spectroscopy was primarily utilized for determining the structure and purity of synthesis products exclusively in the chemical industry in the 1970s, it has since grown in popularity within the medical industry for exploring and imaging human organs.

Through technological advancements, particularly in metrological implements and computer technology, NMR has evolved significantly in terms of stability, reproducibility, and sensitivity. This has formed the foundation for high-resolution imaging, automated processes, and the standardization of analytical procedures. The spectrum of NMR applications encompasses, nowadays, the identification and structure elucidation of organic and biochemical molecules, the quantitative detection of single or multiple analytes, and non-targeted screening when paired with chemometric methods. Notably, there is a rising trend in the development of methods focusing on food adulteration, including origin determination, production methods, and the verification of declared contents. This indicates that NMR spectroscopy is one of the most flexible current analytical techniques [4].

NMR is a primary method of measurement according to metrology standards [5]. It has been proven to have good reproducibility. NMR spectra are evaluated numerically, including chemical shifts, coupling constants, and signal integrals, which lead to quantitative results. For the targeted evaluation of NMR spectra, i.e., the quantification of known components in a sample, the intensities of different signals recorded under properly adjusted and identical experimental conditions are directly proportional to the number of nuclei giving rise to their respective resonance signals. There are three possible approaches to quantitative NMR: adding an internal standard (ISTD) of known concentration to the analysis sample [6]; comparing the analysis sample to another sample with an external standard (PULCON method—Pulse-Length-Based Concentration Determination [7]); or sending a calibrated, synthetic signal to be recorded with the analyte sample spectrum that mimics the signal of an internal standard. (ERETIC method—Electronic Reference To access In vivo Concentrations [8][9]). In view of these facts, it is peculiar that the term NMR *spectroscopy* is still commonly used despite being inconsistent with linguistics as well as with IUPAC definitions: in Ancient Greek, *σκοπέω* (skopéō) means “to see” and *μέτρέω* (métréō) means “to measure”. The IUPAC Gold Book defines spectroscopy and spectrometry as follows: spectroscopy is the study of physical systems by the electromagnetic radiation with which they interact or that they produce and spectrometry is the measurement of such radiations as a means of obtaining information about the systems and their components [10]. Maybe chemists should “promote” NMR from spectroscopy to spectrometry, minding the quality of NMR data and avoiding contradictory terms. Despite these considerations, the orthodox term spectroscopy is used in this publication.

NMR is utilized in food surveillance and quality control and several articles have highlighted the potential of this technique before [11][12][13][14]. Additionally, ^{2}H NMR spectroscopy—site-specific natural isotope fractionation (SNIF) as another NMR

technique—has been proven to be an effective tool in wine analytics, regarding geographical origin, since the 1990s [15]. Methods for ^{1}H NMR in food surveillance have been published only in the last two decades. Monitoring also raises questions about the determination of origin and authenticity. In addition to established methods, such as isotope mass spectrometry, NMR spectroscopy is also a promising approach to authenticate and assess the quality of food and cosmetics. In 2003, Le Gall and Colquhoun [4] summarized the fundamental suitability of NMR for origin determination. Owing to the numerous analysis options in this field, development is progressing rapidly. Thus, samples can be subjected to identifications, structure elucidations, and quantifications, as well as authenticity and origin determinations based on their spectra. A further advantage is that, under defined conditions, it is possible to retrospectively evaluate past samples analyzed since all spectral information is stored and can be accessed. Numerous possibilities have been reported in the literature, many of which demonstrate the use of chemometric methods.

Since all spectral information is recorded in a core-specific manner, it is possible to quantify ingredients, make comparisons, discriminate, or classify, for example, when evaluating the authenticity of foodstuffs or determining the origin and variety of specific products. Furthermore, non-target analysis allows a fast and highly selective sample screening with significant information gain. Key features of the NMR technique are its often low sample preparation requirement and acceptable measurement time, enabling a high sample throughput. The NMR spectra of, e.g., food products, usually contain numerous signals and, therefore, are highly informative. This may appear initially disadvantageous for classical spectral evaluation. However, chemometric techniques, such as multivariate data analysis, can aid in the visualization and assessment of the data.

Chemometrics involves the use of mathematical and statistical methods in chemistry. This discipline of formal logic can be used to plan experimental designs or evaluate experimental measurement data [16]. For analyses of multivariate data, different methods could be used, e.g., principal components analysis, cluster analysis, and multiple linear regression. The fundamental concept of many chemometric techniques rests on the application of latent variables, e.g., principal components. These variables are depicted in the space of the original data to simplify intricate and voluminous data sets and uncover any obscure dependencies [17]. In addition, using this technique, analytical measurement data can be easily graphically portrayed and interpreted. The initial analysis of NMR spectra with chemometrics was presented in 1971 by Kowalski et al. [18]. Over the years, chemometrics became increasingly popular, partly due to more powerful computers. Subsequently, publications featured methods such as linear discriminant analysis [19] and the classification of objects into specific groups, which is referred to as the Soft Independent Modelling of Class Analogy (SIMCA) [20]. These methods can be found in numerous publications and textbooks [21][22][23][24][25][26][27][28][29][30][31][32].

2. Sample Preparation

The examination of food, cosmetics, and pharmaceuticals using NMR typically requires little sample preparation. This research focuses on liquid-state NMR. A liquid sample for NMR analysis should be a one-phase, non-turbid solution (otherwise signal broadening can result). Filtration or centrifugation can yield clear measurement samples. The weighed portion of the extracted or dissolved original sample material should be precisely known. For quantification with an external standard, the total volume of the sample (original material plus solvent) should be precisely known. If extraction is used to prepare the sample, a solvent ensuring the optimal and reproducible solubility of the target analytes should be selected. Systematic underdetermination, e.g., due to incomplete but well-reproducible extraction, can be compensated with correction factors ascertained during method validation.

High-throughput analyses for general quality monitoring are preferably conducted using sample extracts or solutions. Due to the price of the miniature rotors (the sample vessels) used in MAS-NMR (magic angle spinning, solid-state NMR), this is rather uneconomic for high throughput samples; thus, MAS-NMR is used only for specific analyses of solid or viscous samples (e.g., oncological biopsies, gel studies) if the sample should not or cannot be dissolved.

For instance, fats and oils are usually weighed, blended with deuterated chloroform (including tetramethylsilane as an internal standard), and measured directly. For the quantitative or chemometric analysis of samples containing labile/sensitive substances, the usage of mitigated chloroform (thoroughly stripped of phosgene and hydrogen chloride traces) is advised here to prevent the deterioration of target substances and/or skewed spectra due to pH-induced irregular chemical shifts [33].

Aqueous matrices, such as soft drinks, fruit juices, or milk, are mixed with D_2O (including trimethylsilyl propionic acid as an internal standard)—if necessary after pH adjustment—before direct measurement [34][35]. Ethanol-containing drinks, such as beer, spirits, or wine, can be quantified by adding a buffer and, if required, making a pH adjustment [36][37][38]. For solid food, cosmetics, or mixtures of pharmaceuticals, extractions (aqueous or with organic solvents, depending on the

analytical purpose) are needed prior to the preparation of the measurement solution. One significant advantage of NMR analyses over other methods is that they often do not necessitate the complicated isolation and purification of individual substances during sample preparation. High-field NMR instruments, which are widely used, offer high resolution (half-width of a signal compared to spectral width), enabling the assessment of complex mixtures by univariate and multivariate analysis, even with minimal sample preparation.

3. NMR Spectroscopy

The NMR spectra of fat-containing matrices in solvents such as $CDCl_3$ can be recorded directly. However, in the ^1H NMR spectroscopy of matrices containing water or ethanol, the H_2O protons or the protons of the methyl or methylene group of ethanol dominate the spectrum to an extent that makes electronic signal amplification for the purpose of detecting minor components impossible. However, these signals may be effectively attenuated through appropriate presaturation, allowing the detection of trace-level constituents via electronic amplification [36][39][40]. This approach can be employed to uncover adulteration within a given spectrum (e.g., the use of methanol to adulterate spirits). Furthermore, collecting spectra in a consistent manner is critical for automated assessments and chemometric analysis. Among other things, it is important to ensure accurate phasing, an accurate baseline, a consistent smoothing factor, a constant signal half-width, and the chemical shift of pH-sensitive components. This required reproducibility of spectral data is a fundamental prerequisite for mathematical operations in chemometrics. The great amount of information and data density found in high-resolution NMR spectra often presents an issue, even for current computer systems; therefore, data reduction is frequently performed. A common approach is the binning or bucketing method [36]. This involves dividing the spectra into small segments, usually of constant width, and determining the total signal area of each segment. The resulting matrix of integrals can be processed via chemometric procedures. For ^1H NMR spectra, segments of 0.01 to 0.05 ppm are typically useful for static bucketing. It is advisable to exclude regions of solvent signals.

Protium (^1H) is the ideal candidate for NMR due to its near 100% natural abundance, its high gyromagnetic ratio of 42.6 MHz/T, and its diverse presence in organic molecules. Being a spin ½ nucleus, protium yields very sharp resonance signals compared to nuclei with a higher spin. Other than ^1H, other spin ½ nuclides, such as ^{13}C, ^{15}N, ^{19}F, and ^{31}P, are potential candidates for qNMR but certain factors are limiting their usefulness: ^{13}C has a natural abundance of only 1.1% and a gyromagnetic ratio of 10.7 MHz/T, the resulting severely lower detection efficiency compared to ^1H necessitates higher concentrations or a high number of accumulated scans. On the other hand, ^{13}C NMR spectra show signals for every carbon, which is useful for analyte identification. If broadband ^{13}C $\{^1H\}$ decoupling is used, the ^{13}C signal intensities are not quantitative due to the potential nuclear Overhauser effect. Additionally, ^{15}N has a natural abundance of only 0.36% and a gyromagnetic ratio of only −4.32 MHz/T, again, leading to a severely low detection efficiency compared to ^1H. The abundance (~100%) of ^{19}F and its gyromagnetic ratio (40.1 MHz/T) allow for the fast acquisition of highly sensitive spectra and fluorine's chemical shift range is very wide, compared to the shift range of ^1H; thus, even with mostly only a few fluorine atoms in a target molecule, the specific shift is a strong aid for identification. With an isotopic abundance of ~100% and a rather high gyromagnetic ratio (17.2 MHz/T), ^{31}P is also a useful nuclide for routine NMR, especially focusing on bioorganic molecules and certain complexes. For more detailed aspects of hetero-nuclei NMR, see [41][42][43].

A key aim of method development should be to ensure properly set and equal experimental conditions (acquisition parameters), yielding optimal spectra and good comparability between spectra (e.g., for quantification with an external standard). There are important parameters to optimize for quantitative accuracy. A sufficient delay time for full relaxation (of the evaluated nuclei) between subsequent excitation pulses is ensured, avoiding specific correction factors. A spectral width encompassing approx. 3 ppm of clean baseline to both sides of the region with signals is ensured, easing better phase and baseline correction. An excitation frequency is set centrally in the region with signals, improving overall equal excitation. Optimized excitation pulses (preferably 90° pulses) of known duration and power (especially for aqueous samples with varying electrolyte concentrations) are important for comparability between sample spectra and external standards (PULCON method). Overly strong signals from protonated solvents or water can be attenuated by signal suppression; then, (weaker) signals relevant for evaluation can be acquired better.

NMR spectra intended for quantification or chemometric evaluation generally benefit from the following processing steps: a zero filling doubling the number of real data points, a light exponential line broadening (<0.5 · FWHM, the full width at half maximum of a clean resonance) to improve the signal-to-noise ratio, and 0^{th}-order phase correction resulting in pure absorption mode signals; spectra needing a 1^{st}-order phase correction should be re-acquired with better parameters because 1^{st}-order phasing often leads to hard-to-adjust baseline distortions. The baseline correction should ensure a flat baseline (for empty regions), with the median of the noise at zero.

In many cases, simple quantification is possible by evaluating a 1D spectrum; the spectra of complex mixtures can be evaluated by precisely identifying the relevant signals with a JRES (J-resolved 2D NMR spectra) first and, then, using the 1D spectrum for quantification. If signals are superimposed, a guided curve fit (deconvolution) based on prior knowledge about the target signal will lead to improved quantitative accuracy.

For more details, see the cited literature, e.g., [40][44][45][46].

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