Chapter 1

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1 Introduction

Since the development of the high-resolution NMR spectrometer in the 1950s, NMR spectra have been a major tool for the study of both newly synthesized and natural products isolated from plants, bacteria etc. In the 1980s a second revolution occurred. The introduction of reliable superconducting magnets combined with newly developed, highly sophisticated pulse techniques and the associated Fourier transformation provided the chemist with a method suitable for determining the 3-dimensional structure of very large molecules, e.g. biomacromolecules such as oligopeptides, in solution. An interesting development of NMR spectroscopy is demonstrated in Figure 1-1. It shows a nearly linear increase of the used magnetic field strength of superconducting magnets with time, this relation seems to be valid at least until the end of this millennium.

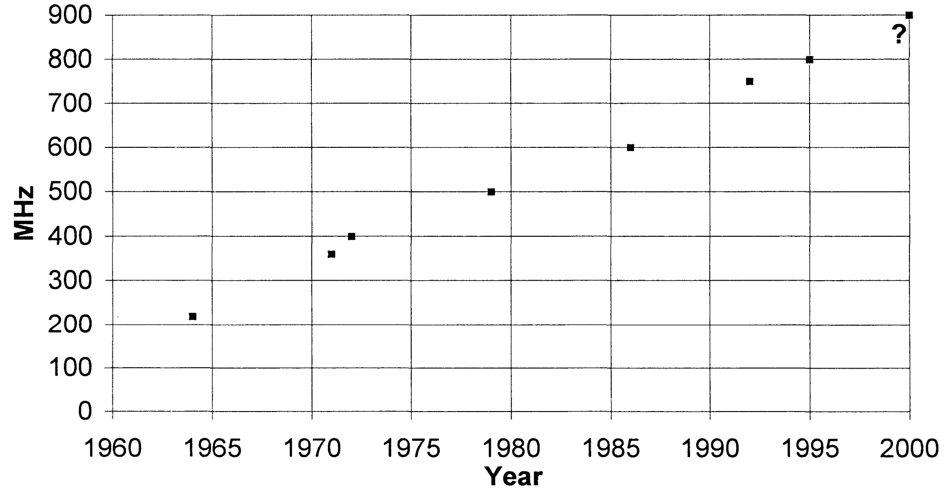

Figure 1-1: Evolution of the magnetic-field strength of superconducting magnets.

Since drugs in clinical use are mostly synthetic or natural products, NMR spectroscopy has been mainly used for the elucidation and confirmation of structures. For the last decade, NMR methods have been introduced to quantitative analysis in order to determine the impurity profile of a drug, to characterize the composition of drug products, and to investigate metabolites of drugs in body fluids. For pharmaceutical technologists, solid-state measurements can provide information about polymorphism of drug powders, conformation of drugs in tablets etc. Micro-imaging can be used to study the dissolution of tablets, and whole-body imaging is a powerful tool in clinical diagnostics. Taken together, this review will cover applications of NMR spectroscopy in drug analysis, in particular methods of international pharmacopeias, pharmaceutics and pharmacokinetics. The authors have repeated many of the methods described here in their own laboratories.

1.1 The Instrument

Organic compounds are composed basically of the elements hydrogen, carbon, phosphorus, nitrogen and oxygen. Additionally, there are the halogens fluorine, chlorine, bromine and iodine and sometimes metal atoms. Each of these elements has an isotopic nucleus which can be detected by the NMR experiment. The low natural abundance of ^{15}N and ^{17}O in nature prevents NMR being routinely applied to these elements without the use of labeled substances, but ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectroscopy are daily routine work. Many instruments are equipped with a so-called QNP (quattro nuclei probe) for sequential NMR analysis of ^{1}H, ^{13}C, ^{31}P and ^{19}F, without the hardware having to be changed. Modern NMR spectrometers are available up to field strengths of 18.8 Tesla or a proton resonance frequency of 800 MHz. Routine analysis is made at proton frequencies between 300 and 500 MHz.

Depending on the kind of experiments, the high-field instruments allow analysis of concentrations down to some µg/ml, but the “normal“ case is a concentration of 1–100 mg/ml. The data are recorded using 32-bit ADCs (analog-digital converters). This results in a high spectral dynamic range.

1.2 Principles

The NMR experiment makes the direct observation of atoms possible. The integral of an NMR signal is strictly linearly proportional to the amount of atoms in the probe volume. The signals are a measure of molar ratios of molecules, independently of the molecular weight. There are no response factors such as those in UV-detection caused by varying extinctions dependent on molecular structures; non-linear calibration curves such as those found with light-scattering detectors are unknown to NMR spectroscopy.

1.2.1 Spectra

The frequency at which an NMR signal appears depends mainly on the magnetic field strength. For example, protons have a resonance frequency of 300 MHz at 7.05 Tesla. The chemical environment of an active nucleus leads to a small shift in the resonance frequency, the "chemical shift". Functional groups find their expression in the chemical shift. The result is an intensity/frequency diagram, the NMR spectrum. This collection of more or less separated NMR signals is analogous to intensity/time diagrams in chromatography, in which one component is represented by one signal. In ^{1}H NMR spectroscopy, each H atom leads to at least one signal. Since most molecules of analytical interest contain more than one H atom, spectra are more complex than chromatograms. What is crucial to the information taken from NMR spectra is the spectral dispersion, which is a linear function of the magnetic field strength. The homonuclear spin coupling of protons leads to a low dispersion of ^{1}H NMR spectroscopy. In ^{1}H NMR spectra of complex mixtures, it is often not possible to detect single components, but the sum of functional groups in the mixture can be determined. In ^{13}C NMR spectra, the dispersion is much higher. The low natural abundance of the NMR-active ^{13}C isotope has a detrimental effect on the sensitivity, but signals are singlets after heteronuclear decoupling. The high spectral dispersion makes parts of the ^{13}C NMR spectra directly comparable to chromatograms. An example is the carbonyl region in a ^{13}C NMR spectrum of a lipid mixture, where each fatty acid is represented by a specific signal.

^{31}P NMR spectroscopy is the method of choice for phospholipids or any other phosphorus-containing compound. Most phospholipids contain only one phosphorus atom, so the ^{31}P NMR spectrum of lecithin reads like an HPLC chromatogram. There are some advantages in comparison with HPLC: specific detection of the phosphorus nucleus, high dispersion and high dynamics. The role of ^{31}P NMR spectroscopy will be discussed later in detail.

1.2.2 Response

The area of an NMR signal is directly proportional to the molar amount of the detected isotope. The ratio between two different signals of one molecule should be 1:1, the number of represented atoms being taken into account. In practice, there are differences caused by different relaxation times. This is the time an excited atom needs to fall to the ground state. In case of heteronuclear decoupling, the nuclear Overhauser effect can cause response factors as well. These response problems are influenced by the measuring parameters, they disappear or minimize with the correct (problem-oriented) choice. Within a family of atoms in similar chemical surroundings, e.g. all end-positioned methyl groups in the ^{13}C NMR spectra of fatty acid-containing material, these effects may be neglected. The same applies to corresponding carbonyl groups, but it is incorrect to compare areas of carbonyl and methyl signals without an appropriate experimental design or experimentally determined response factors. The response factors change from ±10% in ^{1}H NMR spectra up to ±50% in ^{13}C NMR spectra, and this is in fact advantageous in comparison to HPLC/UV detection.

1.2.3 Reproducibility

During an NMR experiment, there is no contamination of sample and probe head. The electronic stability of NMR spectrometers is very good. The spectra of a stable sample stored in a sealed tube are reproducible over many years with a variation of less than 1%. These facts allow minima expenditure on validation measurements when using NMR methods.

1.2.4 Calibration

Like all classical quantitative analysis methods, NMR spectroscopy needs calibration, calibration standards and a validation procedure. The standard techniques are used for calibration: external calibration, the standard addition method and the internal standard method. A fourth is a special NMR calibration method, the tube-in-tube technique. A small glass tube (capillary) containing a defined amount of standard is put into the normal, larger NMR tube filled with the sample for analysis. In most cases, there are slight differences in the chemical shift of corresponding signals of the same molecule in the inner and outer tube. The spectrum shows two signals at different frequencies, and evaluation of the signal ratio allows quantification.

1.3 Experimental

Methods marked with “SSL“ were developed by Spectral Service. To illustrate the published NMR methods, some spectra shown in the Figures were recorded by Spectral Service and replace those of the original studies. The 300-MHz NMR spectra were measured at Spectral Service GmbH, Cologne (Germany) on an NMR Spectrometer AC-P 300, at 7.05 Tesla (BRUKER, Karlsruhe, Germany) equipped with automated sample changer and QNP head for nuclei ^{1}H, ^{13}C, ^{19}F and ^{31}P. The data processing was performed using BRUKER WIN NMR 5.0 software under Microsoft Windows 95.

1.4 Acknowledgment

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