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NUCLEAR MAGNETIC RESONANCE – ANALYTICAL APPLICATIONS AND LIMITATIONS – – A PLENARY LECTURE

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The application of nuclear magnetic resonance (NMR) in food science is fast developing due to rapid advances in NMR instrumentation and techniques. The examples of the most important applications of NMR in food analysis are briefly reviewed (high resolution NMR of liquids and solids, magnetic resonance imaging, relaxation techniques).

INTRODUCTION

Nuclear magnetic resonance (NMR) is one of the most powerful, non-destructive analytical techniques that enables studies of the molecular structure through measurement of the interaction of an oscillating radio-frequency electromagnetic field with sample immersed in a strong magnetic field. The structure of food ranges from pure liquids to solutions, gels, emulsions, foams, suspensions and solids with different content of water. From the chemical point of view food is composed of water, carbohydrates, lipids and proteins. As a consequence of physical and chemical variety, different NMR techniques are applied in food studies, including solution and solid state spectroscopy as well as NMR imaging (MRI).

The sensitivity of nucleus to investigation by an NMR spectroscopy depends on the magnitude of its magnetic moment, the natural abundance of nuclei and the strength of the applied magnetic field. Food molecules contain NMR-active nuclides, *e.g.* ^{1}H, ^{13}C, ^{31}P, ^{23}Na. Hydrogen ^{1}H has the highest receptivity at natural abundance, while carbon ^{13}C the lowest (receptivity of 1.7×10^{-4} relative to hydrogen).

Most of the NMR research in food science is performed using high-resolution or solid state instrumentation (magnetic field of 5.9-21.1 T). For industrial applications the low-cost, benchtop instruments are used, *e.g.* the MINISPEC series made by Bruker (0.4 T). MRI technique applies mainly the 31-cm bore magnets operating at 2 T.

IMPORTANT CONCEPTS OF NMR [Günther, 1996]

When a sample is placed in the static magnetic field B_0, the macroscopic magnetization vector **M** builds up as the resultant of individual magnetic moments. The application of radio-frequency pulses deflects the vector **M** from *z*-axis and induces *x,y*-magnetization. The relaxation to equilibrium is described by longitudinal relaxation (T_1, along the B_0 field, along the *z*-axis) and transverse relaxation (T_2, in the *x,y*-plane).

A chemical shift is the most important parameter of NMR and its discovery started the chemical application of nuclear magnetic resonance. The chemical shift is the difference in frequencies between a nucleus and the carrier frequency, referred to a standard and divided by carrier frequency (ppm). Computer programs developed by Advanced Chemistry Development, Inc., allow finding in the data bases or predicting the chemical shift value on the basis of a structure-fragment algorithm [ACD/HNMR..., www.acdlabs.com]. The chemical shift can be predicted theoretically by *ab initio* methods [Helgaker *et al.*, 1999]. The chemical shift is sensitive to molecular structure and dynamics, solvent, hydrogen bonding and isotopic substitution.

The NMR area (*integration*) corresponds to number of nuclei that give rise to the signal. NMR signals are splited to dublets, triplets, *etc*., due to scalar coupling (*multiplicity*). The line shape analysis enables determination of the chemical exchange rate.

The interactions between nuclei can be transmitted through bonds (*scalar coupling*) or through space (*dipolar coupling*). The *scalar coupling* is orientation independent and yields the splitting of the NMR signal. The *dipolar coupling* is orientation dependent and averages out in the solution due to rapid motions of the molecule. It is responsible for relaxation and nuclear Overhauser effect (*NOE*, an intensity change of an NMR signal induced by another, close in space, nucleus in nonequilibrium state). In solids the intermolecular dipolar couplings are responsible for line broadening, loss of chemical shifts and scalar spin-spin couplings. The special techniques (*vide infra*) allow to effort the solid NMR spectra with relatively narrow lines.

HIGH-RESOLUTION NMR

In the last two decades, a remarkable increase in sensitivity was accomplished by an increase of magnetic field (the

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NMR signal for given nucleus scales to the 7/4 power of the field strength), as well as by improvement in electronics and new probe design. Substances at the picomole level have been identified using NMR spectrometers equipped with micro-coil based probes [Lacey *et al.*, 1999]. Application of the cryoprobe leads to further significant reduction of the experiment time and the sample concentration [Flynn *et al.*, 2000].

The quantitative ^{1}H NMR measurement requires a good signal-to-noise ratio (at least 35:1), pulse width of 90° and the pulse repetition equal to five values of the longest longitudinal relaxation T_1 [Leyden & Cox, 1977]. If one component is present at much lower concentration than another, the dynamic range test for signal amplitudes is advised to avoid the distortion of small signals. ^{13}C quantitative NMR measurements are more demanding because of NOE and widely different relaxation times. To avoid the NOE enhancement the inverse ^{1}H-gated decoupling must be applied and repetition time must be at least 10 times longer than acquisition time [Wehrli & Wirthlin, 1985].

The analysis of overcrowded spectra is possible by means of two-dimensional (and three- and four-dimensional) NMR techniques. They allow spreading the spectral information out in plane and correlating pairs of resonances. The correlation applying scalar coupling *via* one or more bonds can be homonuclear (COSY, TOCSY) or heteronuclear (HMQC, HMBC). The correlations applying interactions of nuclei close in space are also widely used (NOESY, ROESY). Two-dimensional spectra are particularly useful in studies of biomolecules (proteins, enzymes, nucleic acids, carbohydrates and membranes) [Wüthrich, 1986; Evans, 1995].

SOLID STATE NMR

Solid-state NMR develops very fast and applies a variety of techniques [Laws *et al.*, 2002]. One of the most important and widely used is cross-polarization technique coupled with magic angle spinning (CP/MAS) that can rapidly yield one-dimensional spectra of compounds containing spin ½ nuclei of low abundance (*e.g.* ^{13}C, ^{15}N, ^{31}P, ^{29}Si). Signal areas obtained by CP/MAS strongly depend on parameter (contact time) used for acquisition and cannot be applied for quantitative analysis. Solid state ^{13}C NMR have been used to study intact seeds [Bardet & Foray, 2003], the structure-function relationships of starches and other polysaccharides [Tavares *et al.*, 2003].

MRI OF FOOD

MRI can be applied in many fields of food processing and storage. Food can be non-invasively and in real time tested in transparent to radiofrequency waves containers and if it is free of ferromagnetic components. Magnetic resonance imaging (MRI) applies external linear magnetic field gradients for the association of each spatial region with characteristic magnetic resonance frequency of nucleus. In food only water and lipids produce adequate signals for imaging. The signal intensity can alter due to different concentration of water molecules, relaxation time (T_1 and T_2-weighted images), diffusion coefficient, or flow velocity [Olendorf & Olendorf, 1991; Hennel & Kryst-Widźgowska, 1995]. Water relaxation times depend on quality factors such as protein and polysaccharide aggregation, gelatinization, pH and solid to liquid ratio [Hills, 1995; Kerr *et al.*, 1998]. Imaging is very useful in monitoring of the formation of ice during food freezing. Loss of the proton mobility as a result of phase transition is detected by the decrease of signal strength [Hills, 2000].

NMR RELAXATION STUDIES OF FOOD

NMR relaxation time measurements provide a possibility to view the food microstructure. Many experiments are possible using a low-resolution NMR spectrometer system (MINISPEC, Bruker). Hahn-spin-echo-sequence is used for the simultaneous determination of oil and moisture content with the upper limit of water of about 15% [Guthausen *et al.*, 2002]. The determination of fat or oil content in fresh food with high water content is possible when pulsed-field-gradient spin-echo-method is used. This experiment can distinguish different components in a sample on the basis of their diffusion coefficients, even if T_2 relaxation times are equal. Field gradient NMR is used to suppress unwanted signals in studies of water droplet size distribution in oil (margarine, butter) [Fourel *et al.*, 1995] as well as in measurements of oil droplet size distribution on oil-in-water emulsion (mayonnaise, dressings) [Goudappel *et al.*, 2001]. T_2 measurements and difusion experiments are very useful for characterizing gels (*e.g.* yogurts) [Hinrichs *et al.*, 2003].

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