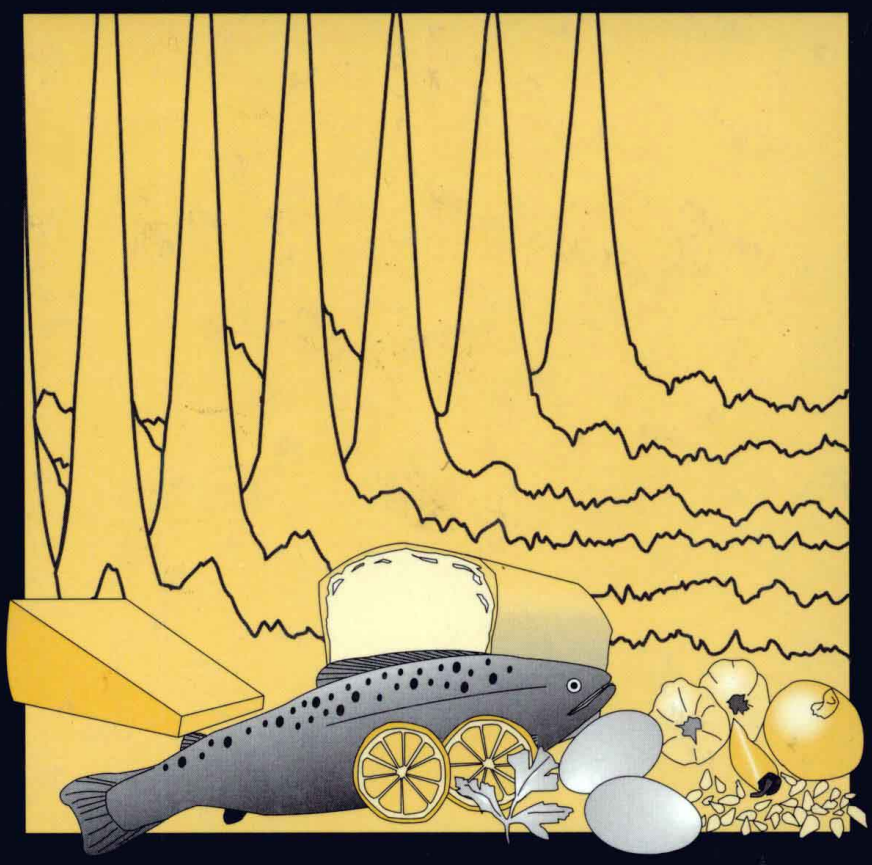
ADVANCES IN MAGNETIC RESONANCE IN FOOD SCIENCE



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Advances in Magnetic Resonance in Food Science

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Advances in Magnetic Resonance
in Food Science

Preface

The fourth International Conference on Applications of Magnetic Resonance in Food Science was held in Norwich, UK, between 7th and 9th September 1998. The meeting attracted 120 scientists from 20 countries and was thus a truly international occasion. The success of the Conference is reflected in the high quality of the oral and poster presentations which it attracted. This volume contains the material given in the oral presentations; the science covered in the fifty posters is additional.

The Conference comprised major and minor oral contributions divided among five symposia which, taken together, ably demonstrate the protean nature of magnetic resonance techniques in dealing with problems arising in many areas of food science. The order of the chapters in this volume shows a parallelism to that in which the lectures were given at the Conference.

Symposium A covered Magnetic Resonance in Food: The Developing Scene; the first four chapters relate to this Symposium. Symposium B dealt with Water, Ions and Small Molecules in Food; Chapters 5 to 10 relate to this material. The following eight chapters belong to the largest of the Symposia, Symposium C, which was devoted to Functional Constituents of Food. Chapters 19 to 21 are from Symposium D, which dealt with Signal Treatment and Analysis in Magnetic Resonance. The topics presented in Symposium E, relating to Applications of Magnetic Resonance to Food Processing and Engineering, are covered in the final five chapters of this volume.

The Editors wish to express their gratitude to the authors for the prompt submission of their camera-ready copy manuscripts and to the production staff at the Royal Society of Chemistry for their kind co-operation in the genesis of this volume.

Contents

Magnetic Resonance in Food: The Developing Scene

From Solid–Liquid Ratios to Real Time Tomography – The Development of NMR in Food Applications

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1 INTRODUCTION

Most food materials are of complex chemical composition, heterogeneous structure, and reactive. i.e. just like the biological materials from which most of them arise. Food technology is the developing skill which allows all these variables to be understood, controlled and manipulated to produce nutritious, attractive, entertaining and, most of all, safe food.

To understand and control anything requires that firstly measurements must be made, and it is easy to write down the information that is required to build a predictive model of the behaviour of any food product or process, (and processes not only involve the fabrication and assembly of products, but also their degradation during storage, use and consumption).

We need to know:

What are the molecules present, and how many of each?
Where are they within the product?
Are they stationary or moving, and if so at what speed?
What are they reacting with?

- and though it is reasonable to construct models to simplify the study of each of these questions, it would be ideal if we could have a single instrument, to measure all of them, in real time and on the immediate subject of interest.

Fortunately, the food technologist is not alone in asking these questions. It is also what all medical researchers would like to know, and they have the added problem that their subjects of interest are not cheap or easily and frequently manufactured and dissected. So a non-invasive route to all of these measurements is also advantageous to all of us.

Fortunately, Physical Science gave us the route to the solution of these problems over 50 years ago by the discover of nuclear magnetism, and the demonstration of nuclear magnetic resonance (NMR). The solution to all our measurement problems are encapsulated in two basic equations. Viz.

$$M = \left(N\gamma^2 h^2 I(I+1)B\right) / 3kT \qquad (1)$$

and

$$\frac{1}{T_2} = C\left(3\tau + \frac{5\tau}{1+\omega^2\tau^2} + \frac{2\tau}{1+4\omega^2\tau^2}\right) \qquad (2)$$

The first tells us that if we can measure the net magnetisation of nuclei, they will be labelled by their specific magnetic moment (γ), and we can count how many (N). Also, by controlling the external field (B), we can expose internal field shifts giving molecular information, or even gross position in space; and if all these were constant we could even measure temperature (T).

The second tells us that the decay of magnetisation gives direct information on the molecular correlation times (τ) and therefore movement of the nucleus and the molecule in which it is contained.

All our measurement problems are over. It's just a question of doing it! The real rate of progress in solving our measurement problems has been in the hands of electronic engineers, physicist and latterly computer scientists, who have identified how to extract the parameters and latterly how to reassemble complex data sets to provide the molecular and structural information we need. We still don't have machines that can answer all our questions but before we complain we should now review what we have done with what they have already provided.

I will approach this on an approximately chronological basis, but like most scientific developments, progress is rarely linear but transfers developments from one field to another on an entrepreneurial basis.

2 THE CONTINUOUS WAVE ERA

The early instruments used relatively low magnetic fields corresponding to ≤ 60 MH_z nuclear frequencies for protons and used continuous wave radio frequencies for excitation. There were two parallel developments, firstly in search of information on molecular structure - "high resolution" which operated well on solution spectra of mobile molecules. Liquid foods (and drinks) were open to study, and many of us, as undergraduates, first learnt the principles of chemical shifts and spin coupling from the spectra of alcohol and sugar solutions. The first protein spectra was published in 1957[1]. Certainly, by the 1960s Unilever was examining high resolution spectra of tea, only to find that molecular complexity rather than intrinsic resolution limited the interpretation of the spectra.

Even 30 years later, with advanced in high field and pulsed Fourier Transform instruments, it is still not easy (see Figure 1).

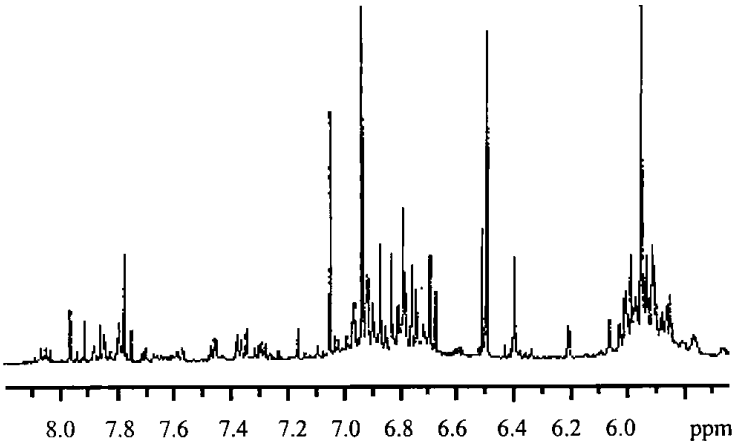

Figure 1 *Proton NMR Spectrum of a Black Tea Extract: Expansion showing polyphenol Signals*

Broad band instruments were capable of identifying relaxation time differences of solids and liquids by analysis of lineshapes, with immediate impact on food formulation in the areas of fats and oils, and water. Most edible fats exist as mixtures of liquid and crystalline forms at body temperature so that the proportions of each can be measured directly from NMR lineshape (Figure 2).

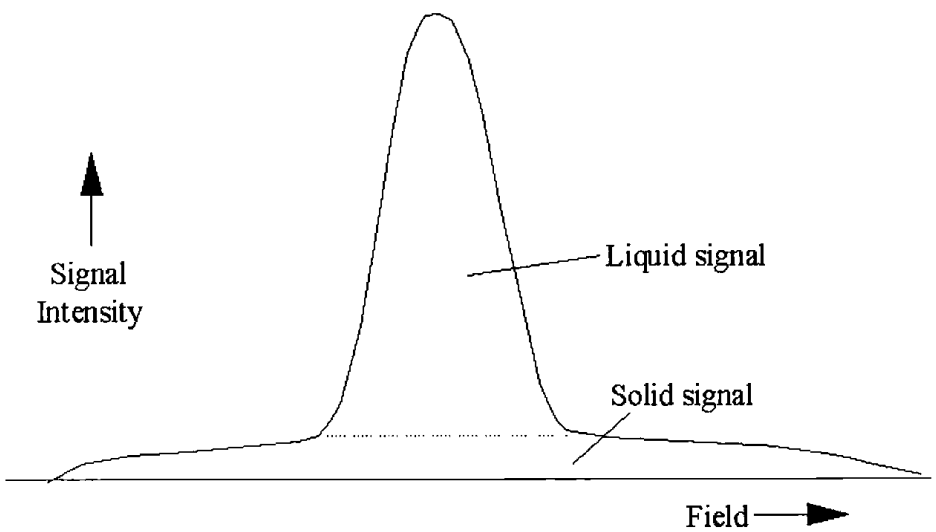

Figure 2 *Continuous Wave NMR Signal from a Sample Containing a Mixture of Solid and Liquid Phases.*

Table 1 *A Comparison of the analytical D_2O contents of crisps with the concentration of D_2O detected by NMR (~1969)*

Total D_2O content	D_2O concentration detected i.e. bound water concentration	Solid-like water concentration
19.8	16.1	3.7
10.1	0.9	9.2
6.5	0.6	5.9
5.8	0.5	5.3

A simple method, allowing the quantification of amounts of solid and liquid fats as a function of blending source and temperature has been of enormous commercial value. It probably pays my salary. Observations of water in dried foods showed not dissimilar spectra. Some of the water appeared to be solid at temperatures well above its freezing point. Table 1 shows early results of deuteron studies in potato crisps. The 'liquid' water appears somewhere between 5 and 10% w:w, which is the same point where crispness is lost. It appeared that NMR could now measure textural properties and the search for 'bound water' was afoot.

But continuous wave experiments were slow, signal to noise was awful and we needed to speed things up.

3 PULSED NMR STUDIES

It is amazing to think that as late as 1968, Ernst was still doubtful that pulsed nuclear excitation followed by Fourier Transform of the resulting decay signal would become a regular way of conducting NMR. His doubts related to the difficulty in collecting sufficient detailed data to produce high resolution spectra. But for 'broad line' practitioners there was no such problem. The collection of decay rates provided direct access to relaxation times (T_1 and T_2) and was much faster and could be averaged. The CW instruments were superseded by pulse machines with simple averagers, and as many pulse sequences as one could afford or build oneself. Solid-liquid ratios became cheap and almost on-line, and the study of water in foods and model systems began in earnest.

3.1 "Water binding"

As early as 1954[2], it was reported that the line width of water was increased by the presence of biological material (deoxyribonucleic acid). Broad line studies also showed that food gels and biological tissues had anomalously broad water peaks, and the simplistic explanation that all the water was more solid-like, bound or less structurally mobile was briefly advanced. This did not last long after the realisation that rapid exchange according to the Zimmermann-Britten model was the probable reason. i.e.

$$\frac{1}{T_{2obs}} = X_B \frac{1}{T_{2B}} + (1 - X_B)\frac{1}{T_{2F}} \qquad (3)$$

where X_B relates to the proportion of water "bound " to substrates and T_{2obs}, T_{2B} and T_{2F} relate to observed, bound and free water relaxation time.

Furthermore, an estimate of X_B could be envisaged by connecting with the observation that NMR saw a fraction of water with reduced mobility, but not frozen, below the freezing point. Solutes clearly affect the mobility of some water, if not all of it[3]. Real foods showed the same behaviour and furthermore the processes of rigor mortis and cooking were reflected in the water proton relaxation behaviour[4]. As data collection improved, so did the number of apparent water relaxation times, from 2 to 3 and up to 5, where the number of fittable parameters exceeded the reasonable number of domains where water could be thought to reside.

An alternative approach, transforming the decay curve to a relaxation time spectrum was proposed[5], where the origin of the complex decay can be related to the spatial heterogeneity of the sample over scales of 10's of microns (Figure 3).

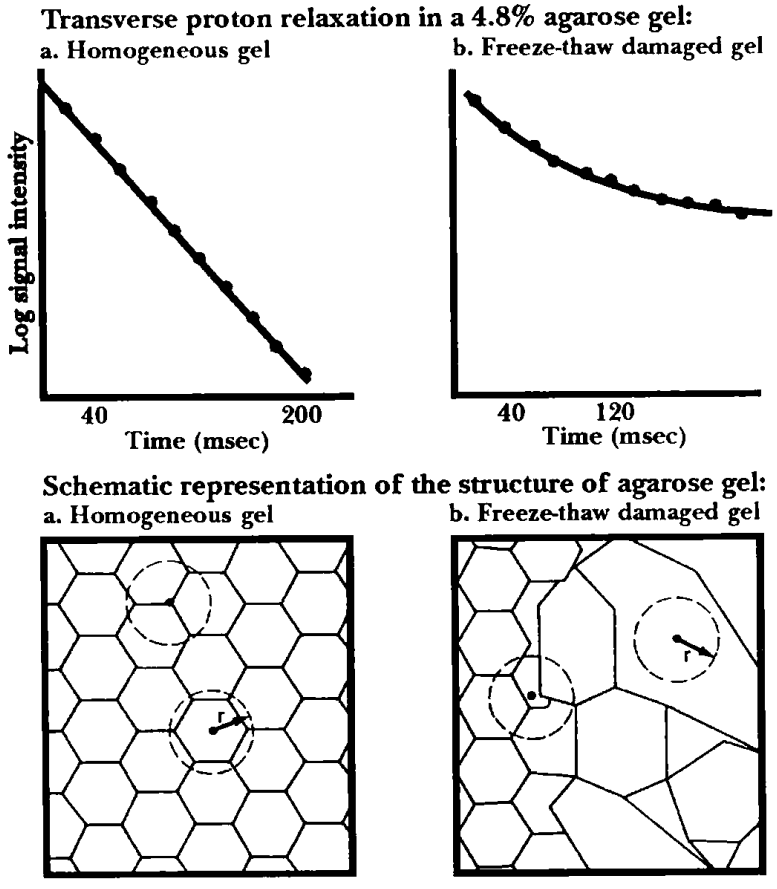

Figure 3 *Spatial Heterogeneity and Diffusion Lengths*

The NMR machine can now be used as structure measurement tool, capable of measuring the effect of processes relevant to food processing.

The approach has been investigated and developed extensively by Brian Hills et al[6] and will be referred to in a later lecture. The relaxation time spectrum has also been correlated with the sensory impression of juiciness in the mouth. Not surprisingly, the water least influenced by the architecture of the food is released the most quickly.

3.2 Water droplets

Many foods e.g. margarine, low fat spreads, dressing, are oil continuous emulsions, containing disperse droplets of aqueous solution. The stability, both microbiological and physical, and even the mouthfeel are dependent on the droplet size distribution. The insertion of controlled field gradients pulses within a normal echo train causes the refocussed signal to be dominated by the diffusion of water rather than its intrinsic relaxation time (Figure 4). If diffusions is restricted (by the boundaries of a droplet) then anomalous diffusion is observed from which the droplet sizes can be estimated[7].

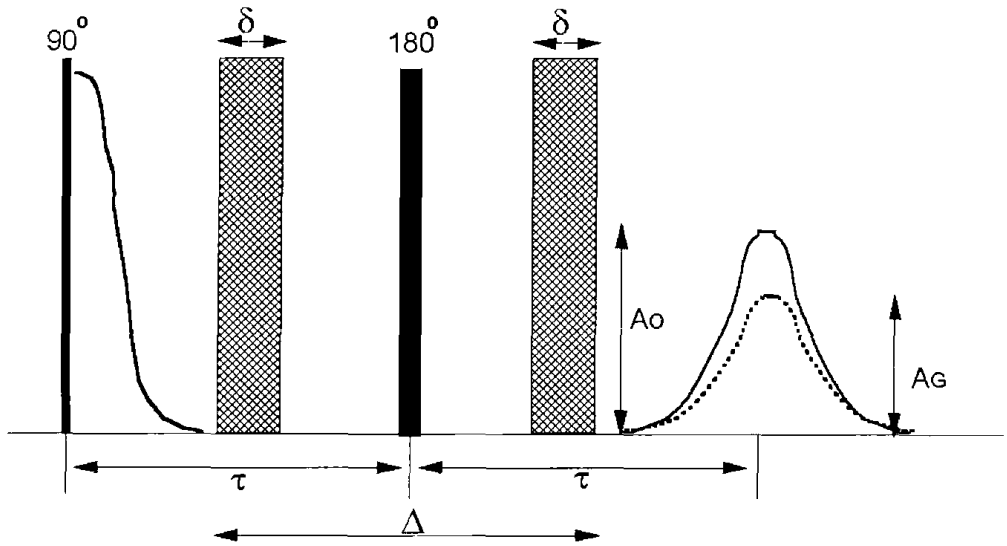

Figure 4 *Pulse sequence for determination of self diffusion*

3.3 Diffusion in Foods

The interest in diffusion rates is not limited to emulsions. Essentially, the self diffusion coefficient of any small molecule in a matrix of any other is vital to the reaction rates of deteriorative processes, flavour retention, and migration between components. All

of these can be measured by the pulsed field gradient method. Figure (5) shows a recent and surprising result, that the water within a "glassy" polysaccharide still exhibits considerable diffusive motion, and is quite independent of the glassing of the polymer itself[8].

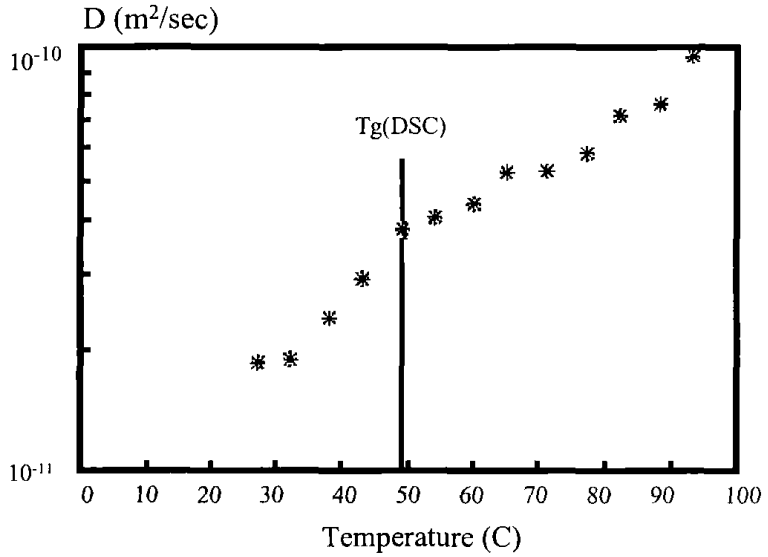

Figure 5 *Self Diffusion Coefficient of the Water in 81% Pullulan*

4 FOURIER TRANSFORMED HIGH RESOLUTION SPECTRA

Ernst's early worries were unfounded. As computer power developed, and NMR spectroscopists found clever ways of enhancing signal to noise, FT-NMR became the dominant experiment and allowed amazing results to be delivered. As early as 1970, Stahl and McNaught showed that with a little hydrolysis to reduce viscosity, proton NMR could be the most effective method of analysing starches and chemically modified food ingredients[9].

Access to natural abundance C^{13} spectra made even further possibilities available, such as this detailed assignments of hyaluronate polymer resonance. Chemical shift and line width changes can be quantitatively measured as association or conformational changes take place.

Wüthrich reported the first NMR structural determination of a protein in solution in 1985[10]. All of these advances now allow structural studies of macromolecule unfolding to be measured under conditions relevant to commercial processing.

Developments in the medical field showed that ^{31}P spectra could be obtained in whole cells and organs[11]. This was seized upon by the food industry to monitor pre to post rigor changes in most muscle tissue, and the location and turnover of phosphates added to enhance water retention in processing and freezing.

All these high resolution phenomena rely on sufficient molecular mobility to allow resonances to be observed. But many food systems contain real solids, crystalline