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FFC APPLICATION NOTE: FINGERPRINTING

Fast Field Cycling as fingerprinting technique

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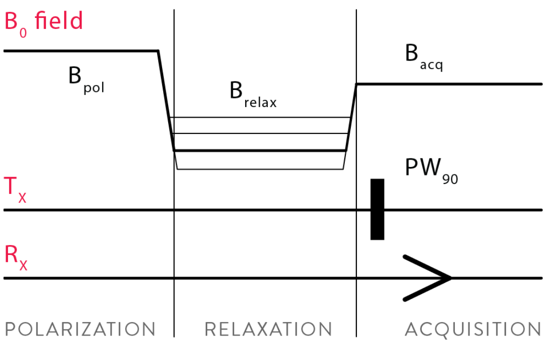
Introduction

Fast field cycling (FFC) NMR relaxometry is a low-field magnetic resonance technique which measures the dependence of the spin-lattice relaxation rate $\mathbf{R_1}$ (= $\mathbf{1/T_1}$) on the magnetic field over a wide range of field strengths with just one instrument. The important information extracted from the relaxation dispersion curves (NMRD profiles) concerns molecular motions (molecular dynamics) described by means of spectral density $\mathbf{J(\omega)\propto R_1}$.

FFC technique

The Stelar relaxometer works by fast electronic switching of the magnetic field from an initial polarizing magnetic field ($\mathbf{B_{POL}}$), where the equilibrium of nuclear magnetization is attained in about $4\mathbf{T_1}$, to a field of interest (relaxation field; $\mathbf{B_{RELAX}}$) at which the nuclear spins relax to the new equilibrium state with a characteristic relaxation time constant $\mathbf{T_1}$. After a delay time, τ, the $\mathbf{B_{RELAX}}$ is switched to the field of acquisition ($\mathbf{B_{ACQ}}$) and the NMR signal is detected after a $\pi/2$ RF pulse (**Fig.1**).
The magnetic field dependence of $1/\mathbf{T_1}$ is shown in the graphical form as a Nuclear Magnetic Resonance Dispersion (NMRD) profile (**Fig.2**).
Each point of the NMRD profile (i.e. a certain $\mathbf{B_{RELAX}}$) is obtained detecting the NMR signal using a number of different delay times τ [1].

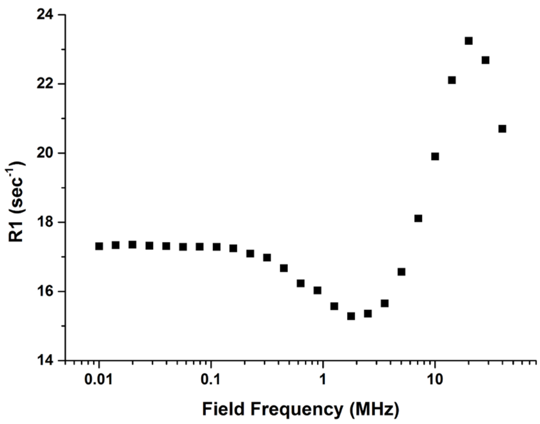


Application of FFC NMR as fingerprinting technique

Fast field cycling NMR relaxometry is a versatile technique that provides an analytical method for discriminating between different products that otherwise might be mistaken. The fingerprinting method relies on the fact that each material or substance (at a specified temperature) presents a peculiar NMRD profile that fully characterizes it. However, changes in $\mathbf{1/T_1}$ might not be evident at single magnetic field strengths but when studied over a wide range of values, especially at lower fields, such changes become quite easy to spot. The NMRD profiles can also be used for quality control purposes. FFC technique is applicable to several fields including ***material science***, ***food science***, ***pharmaceutical*** and may be a suitable study tool that has not yet been tested for many other applications waiting to be discovered.

⇠ **FIG. 1 (LEFT):**
Fast Field Cycling NMR method.

⇣ **FIG. 2 (BELOW):**
Example of NMRD profile.
A Gadolinium-based contrast agent measured from 0.01MHz to 40MHz (from in-house data).



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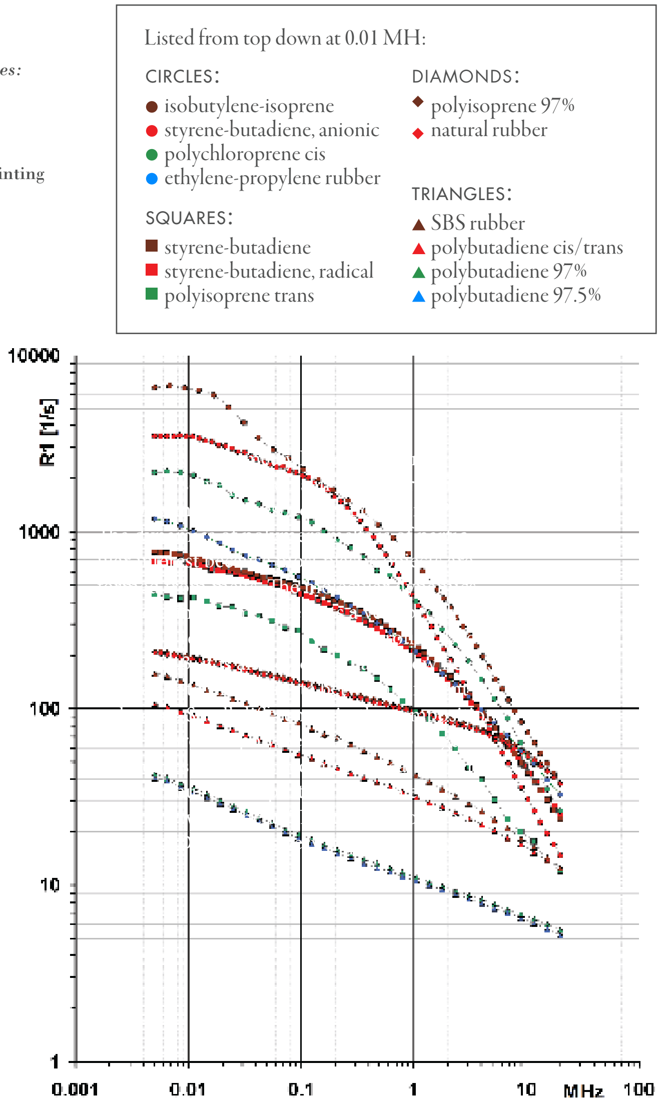
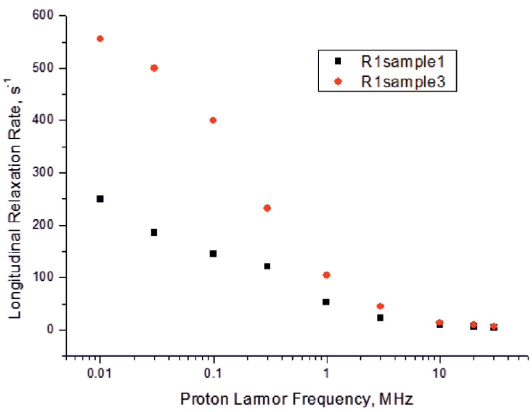
Material science / polymers

FFC NMR relaxometry has often been employed to solve problems in characterization of complex materials, especially polymers [2-6]. The dynamics of polymers melts is of particular interest because most plastic objects are formed from melts. The molecular dynamics of a certain polymer presents a very peculiar behavior, reflected in its NMRD profile.
Looking at **Fig. 3**, it is also evident that, at low fields, it is easier to discriminate between different polymers. Small differences in the composition or structure of a polymer may lead to large differences in the desired mechanical properties and thus it is important to be able to differentiate between them. The information from the NMRD profiles is very important because, due to the close correspondence between FFC results and rheological studies, changes in mechanical properties of a polymer are reflected in changes in its NMRD profile [6].
Another important point is that small deviations in manufacturing procedure may lead to a polymer product not meeting the required performance parameters. **Fig. 4** shows as FFC can distinguish between two samples of the same polymer, made at two different manufacturing sites and presenting different mechanical properties. The NMRD profiles of the two samples reveal large difference at lower magnetic fields strengths that are not evident at higher fields (at which most fixed field relaxometers work). Many polymers produced in factories are synthetically crosslinked because this procedure makes them more elastic and polymers gain the desired mechanical properties. The crosslinking procedure can be monitored by the polymer NMRD profiles as different crosslink densities would lead to differences in the corresponding NMRD profiles [7, 8].
These applications for polymer characterization are exploitable by the polymer industry and could be developed to become standard analytical tools. The information from the NMRD profiles can be exploited to control polymer melt processing in industry and to distinguish 'good' products from 'bad' ones.



↑ **FIG. 3 (UP):**
NMRD profiles of different polymers from 0.005 MHz to 20 MHz.
From in-house data.



↑ **FIG. 4 (UP):**
NMRD profiles of two identical polymers produced by two different manufacturing sites which displayed different mechanical properties. From in-house data.

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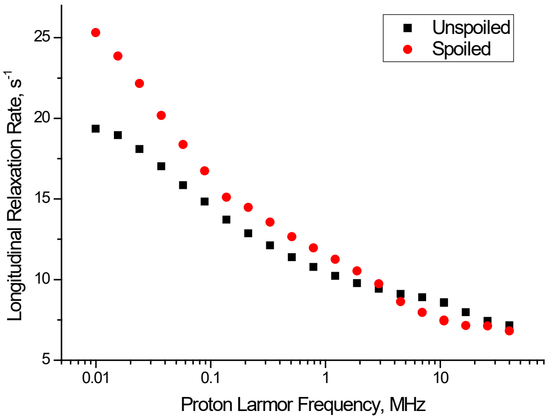
Food science

Food is a complex matrix for which NMRD has shown diagnostic utility. The NMRD profile is sensitive to dehydration, oxidation, spoilage, and the addition of additives including adulterants that may lead to fraudulent products [9-15].

Milk based products

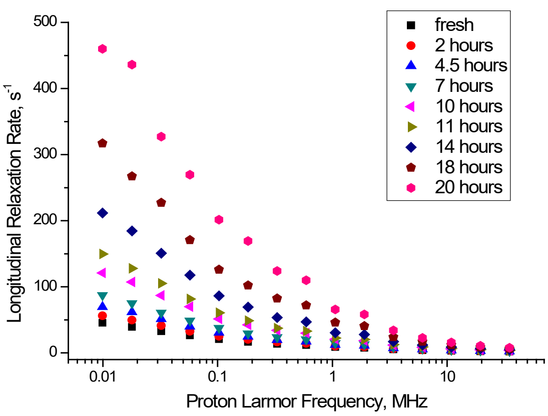
Milk sours when bacterial fermentation transforms the sugars to lactic acid. Acid may denature proteins present and drive protein aggregation both of which affect the NMRD profile. Fermentation and spoilage can be monitored by relaxometry, as reported from an in-house study on a refrigerated milk-based product (**Fig.5**). In these cases, low magnetic field measurements are a critical advantage and the profile shape is diagnostic.



> **Fig. 5:** *NMRD profiles of an unbranded milk-based refrigerated drink product before and after artificial spoilage (acidification). From in-house data.*

Meat

Meat has a short shelf-life and thus needs to be stored rigorously at cold temperatures. NMRD can show how quickly meat, such as the pork loins shown in Fig.6, can dehydrate over a period of 20 hours. After 12 days the pork loins have lost a lot of water (as shown by the NMRD in **Fig.** 7). A more detailed analysis of **Fig.** 7 reveals quadrupolar dips or peaks in both NMRD: this phenomenon is due to magnetization transfer from water protons to ^{14}N nuclei at a short range, leading to an increase in water proton relaxation [16]. This occurs when one of the nuclear quadrupolar energy levels matches the ^{1}H Larmor frequency thus producing the quadrupolar dips/peaks observed in the NMRD profile.



> **Fig. 6:** *NMRD profiles of pork loin open to air at ambient temperature over a twenty hour time period.*

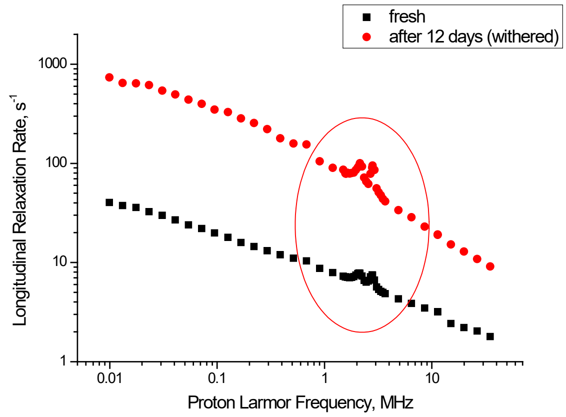


Fig. 7: *NMRD profiles of an unbranded milk-based refrigerated drink product before and after artificial spoilage (acidification). From in-house data.*

Balsamic vinegar (italian)

NMRD profiles have been acquired for characterizing the age of balsamic vinegar (TBVM, **Fig.** 8, [14]). TBVM is a protected designation of origin product and its cost on the market is rather high in accordance with its ageing process.

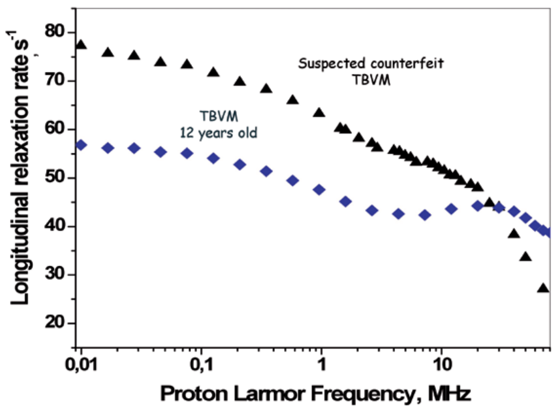


Fig. 8: *NMRD profiles for 12yrs aged balsamic vinegar (blue) and counterfeit product (black) [14].*

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Parmesan cheese

FFC NMR has applied to characterize high quality italian Parmesan cheese produced following strict criteria (**Fig. 9**, from in-house data).

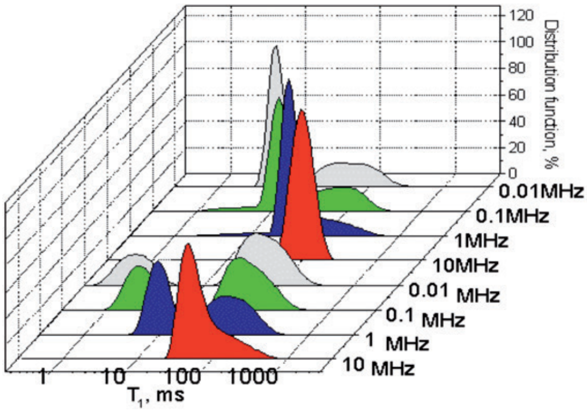

FIG. 9:
T_1 distributions of Parmesan cheese taken from parts of the crust and of the core obtained using an inverse Laplace algorithm.
From in-house data.

Pharmaceutical

FFC relaxometry has recently been used to assess the authenticity of drugs. In [17] it is reported the case of Viagra and it is shown that using FFC it is easy to discriminate between original and counterfeit product using only the information from the NMRD profiles. It has been assessed that, in the case of the counterfeit product, the relaxation process is bi-exponential in the whole frequency range, while for the original product the relaxation is always mono-exponential.
Exploiting only this simple and qualitative observation it has been possible to distinguish between the original and coun-terfeit drug. In [17] it reported that other scientific methods, such as vibrational spectroscopy and Atomic Force Microscopy (AFM), were used to differentiate between original and counterfeit Viagra but results did not show sufficient differences to be used for the identification. FFC relaxometry instead, emerges as an advantageous method of identitication of counterfeit drugs.

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