PFG NMR and internal magnetic field gradients in plant-based materials

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Abstract

In this contribution, it is demonstrated that inner magnetic field gradients can seriously affect the results of stimulated echo PFG NMR experiments on plant-based materials even if there is no notable content of paramagnetic substances. Such effects could be observed both in experiments on water in pharmaceutical grade cellulose powder materials and on eggplant fruit tissue. In both cases, it was observed that the effects of internal magnetic field gradients led to different relative values of the diffusion coefficient compared to values obtained with a gradient-compensating pulse sequence. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Water transport phenomena in plant-based materials are of great importance in many areas of science and technology ranging from plant physiology over food science to wood and textile industries. Both intact plant tissue materials and isolated structural materials from plant tissue such as cellulose exhibit a rich internal structure on the nm and μm scale. These structures strongly affect water transport behaviour in the respective systems. PFG NMR is a very powerful method for studying water transport properties in materials with structures on length scales on the range of 100 nm to about 100 μm [1,2]. Especially three properties of the PFG diffusometry method are important for such materials:

- It allows to study diffusion processes on a time scale between about 1 ms and about 1 s with a well-defined observation time. Time-dependent studies of the diffusion coefficient allow the identification of structural barriers on the length scales corresponding to the respective diffusive displacements.
- As a non-invasive method, PFG NMR allows also to monitor changes of the transport properties within one sample undergoing changes in its internal structure [3].
- As a bulk method, PFG NMR measures average properties over a volume of several 100 μl to some ml. Such a volume is big enough to be representative even for materials with irregular structures on the μm scale.

Several applications of PFG NMR studies to water transport in plant-based materials have been reported during the last few years [4–8]. In Refs. 4 and 5, the dynamics of water in the interaction with native cellulose fibres was studied. Comparative PFG NMR studies on the water dynamics in modified cellulose materials such as microcrystalline cellulose (MCC) or amorphous cellulose are not known to us although such materials recently received much attention in studies by other methods such as SANS [9]. Due to tremendous differences in the macroscopic properties of moistened powder preparations of MCC and native cellulose, which are observed in the use of these materials in pharmaceutics processing [10,11], we recently embarked on a study of the water transport behaviour in such materials. The evaluation of this study is not yet completed and will be

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reported in a later paper. Here, we only will report observations concerning the surprisingly strong effects of inner magnetic field gradients on the performance of stimulated echo PFG NMR and the merits of a gradient-compensating 5-pulse sequence according to Cotts et al. 1989 [12] in overcoming these problems.

Artifacts or susceptibility-induced signal losses in NMR experiments on plant-based materials have been reported quite often for imaging [13–16]. For diffusometry, some experiments aiming at internal gradients due to the susceptibility difference between water and cellulose were conducted [4] in a fully water-saturated cellulose pulp. There, only a minor internal gradient was observed.

2. Materials and methods

Native cellulose pharmaceutical grade powder Elcema P100 was obtained from Degussa AG, Frankfurt/Main. Microcrystalline cellulose Vivapur type 102 was obtained from Rettenmeier, Ellwangen. The eggplant (black melanzana type) was obtained in a local supermarket.

The preparation of water-cellulose mixtures was performed manually either by stirring with a PVC stick in a small rectangular plastics container or in a standard ceramic mortar. No significant differences in the homogeneity of samples resulting from the various mixing methods were observed. The eggplant sample was cut out of the fruit with a knife and had the shape of a rectangular prism. Bruising of the samples was performed by manual pressing between the fingers.

PFG NMR on the samples was performed on a home-built 400 MHz PFG spectrometer [17] using both a standard stimulated echo PFG sequence and the 5 pulse PFG sequence [12] (Fig. 1). The diffusive signal attenuation in this sequence is given as

$$\Psi(G) = \frac{S(G)}{S(0)} = \exp(-\gamma^2 \delta^2 G^2 D(4\tau_2 + 6\tau_e - \frac{2}{3} \delta))$$

$$= \exp(-F(G) D)$$

With $\tau_e$ denoting the time interval between the 90°- and 180°-pulses and $\delta$ the duration of the gradient pulses and with the gradient pulses centered within the time intervals between the 90° and 180° pulses.

A full description of the echo attenuation in a stimulated echo sequence under the action of external gradient pulses and a temporally constant internal, spatially inhomogeneous gradient has not yet been found, although some limiting cases have recently been studied [18]. For a spatially constant background gradient $G_o$, the spin echo attenuation in the stimulated echo is given as [19]

$$A_{STE, diff}(G) = \exp\left(-\gamma^2 D \left\{ G^2 \delta^2 \left( \Delta - \frac{\delta}{3} \right) \right. \right.$$  

$$+ G_o^2 \tau_1^2 \left( \tau_2 - \frac{1}{3} \tau_1 \right) - GG_o \delta \left( \tau_1^2 \right.$$  

$$\left. + \tau_2^2 + \delta(t_1 + t_2) + \frac{2}{3} \delta^2 - 2\tau_1 \tau_2 \right) \right)$$

with $\Delta$ denoting the time interval between the two gradient pulses with $t_1$ denoting the time between the excitation pulse and the first gradient pulse and $t_2$ the time between the last gradient pulse and the echo (all other parameters as denoted in Fig. 1A).
In the experiments reported here, we worked for the 5-pulse sequence with $t_e$ of 350 μs and a gradient duration $\delta$ of 200 μs. Gradient strengths were chosen so as to obtain a maximum $F(G)$ value of $1.4 \cdot 10^{-9}$ s/m$^2$ with ten equidistant steps in the squared gradient values. For each gradient value, 16 echoes were averaged using a full phase cycle for the spin echoes. For the stimulated echo, a $\tau_1$ time of 600 μs and a gradient duration $\delta$ of 300 μs were used. The gradient values were chosen so as to achieve again a maximal diffusive echo attenuation factor of $1.4 \cdot 10^{-9}$ s/m$^2$ when neglecting the influence of the internal gradient. Again 10 gradient steps were used. For each gradient value, 4 echoes were averaged using a CYCLOPS phase cycle. Relaxation delays used in both sequences were 2 s.

A test of the importance of internal gradients in the samples when working at low fields was performed in a 22 MHz MRS 6 relaxometer (obtained from Jozef Stefan Institute, Ljubljana, Slovenia). As this spectrometer offered no pulsed gradient facilities, only a comparison between $T_1$ values obtained by saturation-recovery and stimulated echo experiments was run in this case.

NMR imaging was conducted on a Siemens Magnetom clinical MRI scanner located at the University of Ulm by means of a standard multislice spin echo technique. The slice thickness in the images was 1 mm, and the in-plane resolution was $(0.488 \text{ mm})^2$.

### 3. Results

In Fig. 2, PFG NMR results for water diffusion in cellulose powder preparations with a water content used in pharmaceutics processing are plotted both for the stimulated echo sequence and for the gradient-compensating 5-pulse sequence [12]. For both methods, an observation-time dependence of the water-self-diffusion is observed. However, the strength of the effect is quite different: in the 5-pulse-sequence only a minor change of the water diffusivity by about 20% is observed. In the stimulated echo, however, a decrease by a factor of 2 is observed for the same range of observation times. Furthermore, the signal intensity available in stimulated echo experiments with long diffusion times was found to be much smaller than the attenuation expected on the basis of the $T_1$ values of much more than 1 s. Comparing to other studies of water-diffusion in cellulose, we find that both the absolute values and the small observed time-dependence are in quite good accordance with the findings of earlier studies in the cellulose pulp system where the internal gradients were analyzed and found to be minor [4]. However, a much stronger observation-time dependence of the diffusion coefficient was reported for water and cyclohexane in dried and rewetted cellulose beads [20]. The strength and shape of the observation-time dependence of the diffusion coefficients in these studies are quite similar to our observations on the basis of the simple stimulated echo. As the same pulse sequence was also used in these experiments, this raises the question whether the data there might have been distorted by internal gradient effects despite the relatively good correspondence to an analysis based on pore-size effects presented in the cited articles.

A first tentative explanation of these phenomena on the basis of paramagnetic surface effects due to metal ions or processing-induced free radicals in the cellulose-powder could be readily dismissed both on the basis of ESR results in which no paramagnetic species at all were detected and
on the basis of the chemical analysis of the material as required for pharmacotechnical applications.

A further experimental fact that could not be explained on the basis of paramagnetic effects is the strong dependence of the internal magnetic field gradient effects on the water loading of the cellulose samples: While even stronger gradient effects were observed for cellulose with smaller water addition, no significant gradient effects were found in pastes with water loadings over 3 ml/g for native cellulose and over 2 ml/g for MCC. This finding can be easily understood when the observed internal magnetic field gradients are attributed to tiny air bubbles in the paste. The magnetic susceptibilities of water and air are $-9 \cdot 10^{-6}$ and $+0.4 \cdot 10^{-6}$, respectively. This corresponds to a field difference of 9.4 ppm between both materials. The magnetic field gradient in the water surrounding an air bubble is localized on a length scale on the order of the diameter of the bubble. At field strength of 9.4 T and with air compartments of an approximate diameter of 50 $\mu$m, this leads to values of about 1.8 T/m for the inner magnetic field gradients. For smaller bubble dimensions, the gradient strength will increase proportional to the inverse of the length scale. However, these magnetic field gradients are not constant over the diffusive displacement of a water molecule traveling over an observation time of several 100 ms. Therefore, no straightforward theory is available to describe the common action of the internal magnetic field gradient and the external gradient on the echo amplitude. However, we can at least qualitatively discriminate some cases for the diffusion in the internal magnetic field gradients when applying a stimulated echo PFG sequence:

- For short observation times, the diffusive shift is small compared to the length scale of the inner magnetic field gradient, the internal gradient can be considered to be constant over the observation time. In this case, the diffusive attenuation of the echo is due to a superposition of the spatially variable constant inner gradient and the external, uniform gradient pulse. The echo attenuation in this case obeys the relationship as in the case of the joint action of a constant and a pulsed external magnetic field gradient.

- For intermediate observation times, the magnetic field gradient will change on the length scale of the diffusive shift but it will be constant during the short intervals where a transverse magnetization is present in the system. Furthermore, some correlation between the gradient values experienced in the first and the second interval with transverse magnetization will exist. This case was recently carefully studied [18, 21]. In Ref. 18, a quantitative analysis on the basis of a Gaussian distribution of effective internal gradients was performed and an expression for deviation of the measured diffusion coefficients in a stimulated echo PFG experiment from the “true” diffusion coefficients was derived.

- For long observation times with diffusive shifts much greater than the gradient length scale, there will be no more correlation between the gradients experienced during the first and the second transverse magnetization period. We can expect that most of the spins will have sampled the full range of possible gradient amplitudes over the sample. Under these conditions, the assumption of a Gaussian distribution of the inner magnetic field gradients (as in Ref. 18) over the sample is no longer valid and thus we find an increasingly smaller decrease in the measured diffusion. A theory for this general case is not available yet.

The decrease of the stimulated-echo diffusion coefficients is initially faster for the MCC paste while at longer echo times, the diffusion coefficients in the native cellulose powder show a stronger decrease. This behaviour can be attributed to different length scales of the gradients which are consistent with the appearance of the two powders in electron microscopy (Fig. 3). The MCC grains exhibit a rough surface and a rich internal pore structure on length scales of some $\mu$m. The Elecema powder, by contrast comes along in flakes with a much broader size distribution that

Fig. 3. Electron microscopic appearance of the cellulose materials native powder cellulose (A) and MCC (B). Note the rough outer structure and the many access points to the grain in the case of MCC and the smooth surface of the native cellulose grain.
exhibit a relatively smooth surface over length scales of up to several 10 μm and capillary-shaped internal pores. We therefore can expect the size of air bubbles in the Elcema material to be typically bigger than in the MCC as the smooth outer sides of Elcema allow the formation of bubbles extending over the whole outside of a grain while the bubbles in MCC typically will be confined to the space between two crystallites. Furthermore, the water loading in the Elcema paste is bigger which also should lead to a bigger bubble-bubble-distance.

Based on the observations in cellulose powders, it is worthwhile to ask whether there are similar effects also in natural plant tissues and thus should be considered as a possible source of artifacts in NMR diffusometry studies on plants, agricultural materials and foodstuffs in general. As a test tissue, we chose eggplant fruit tissue as it has an obvious content of air pores and it is easy to cut macroscopically homogenous samples out of the fruit tissue which exhibits no obvious macroscopic structure over large parts of the fruit. Further indications for the presence of strong internal magnetic field gradients in the eggplant come from MRI experiments. As can be seen from the images in Fig. 4, the eggplants exhibit much shorter relaxation times than most other fruits and vegetables.

Diffusion coefficients measured on freshly excised eggplant tissue at different observation time are plotted in Fig. 5 both for the stimulated echo and for the 5-pulse sequence. Again—like in the case of cellulose—we find that the internal magnetic field gradient leads to systematically too low diffusion coefficients in the case of the stimulated echo experiments. More important, however, is the change of the measured diffusion coefficients in the tissue after the bruising: while the stimulated echo experiment suggests higher self-diffusion coefficients, the 5-pulse sequence reveals a decrease of the self-diffusion coefficient. This finding can be explained by the fact that in the bruising process the number of air bubbles and thus the strength of the internal magnetic field gradients are drastically reduced. The lower self-diffusion coefficients revealed by the 5-pulse-sequence are due to loss of water in the bruising process which comes along with smaller distances between the diffusion barriers for the remaining water in the tissue.

As 400 MHz is presently still a relatively high field for diffusometry experiments, we were also interested in the question of how important the observed phenomena are in the case of lower magnetic fields where the gradients should be proportionally smaller. Therefore we conducted a comparison of the magnetization decay in a stimulated-echo experiment where the evolution time $T_2$ was varied with the $T_1$ values obtained in a standard saturation-recovery experiment. For a simple fluid sample without internal magnetic field gradients, the signal in both these experiments should decay exponentially with $T_1$. However, this simple behaviour was not observed in our experiments at 22 MHz on a powder cellulose sample with a water loading of 1 ml/g. The results are given in Fig. 6. Obviously, the magnetization decay in the stimulated-echo experiment is much faster than...
in the saturation-recovery experiment. The non-exponential form of the decay curve of the stimulated echo can be attributed to the short length scale of the magnetic field gradients in the sample: For longer $\tau_2$-values, the spins have mapped the whole gradient profile and thus the gradient-induced signal attenuation is correspondingly lower. The short length scale of the susceptibility variations in partially water-saturated cellulose powders leads to especially strong internal gradients, which already play a major role at 22 MHz. A similar experiment with the eggplant tissue didn’t show a significant gradient effect in the stimulated echo decay at 22 MHz as the cells are much bigger than the cellulose grains.

4. Discussion

Both distortion effects and signal attenuation in MRI on plant-based materials have been reported by several groups [2,14,15] and also the bruising-induced gain in signal intensity was described in this context [13]. Even diffusion-weighted NMR imaging with alternating gradient MRI sequences was already described for fruit tissue samples [22]. Despite such reports, the strong effect on the value of the diffusion coefficient in the stimulated echo PFG NMR experiment is quite surprising as in our stimulated echo experiments there are very short time intervals with transverse magnetization in the sample and the externally applied magnetic field gradients are on the order of 1 T/m or even higher.

While the importance of controlling the effects of internal magnetic field gradients in mineralic porous materials (where they are typically due to the content of para- and ferromagnetic phases) is well known, problems with internal magnetic field gradients in plant-based materials have not yet been considered as a major error source in PFG NMR. However, the strong discrepancy between diffusion coefficients obtained using the stimulated echo and the 5-pulse method clearly demonstrates that this can be a major source of errors and even mask the effect of changes in the diffusion behaviour in the sample that are accompanied by simultaneous changes in the internal magnetic field gradients.

The 5-pulse sequence is more difficult to implement on many PFG spectrometers as it needs the capability of fast switching of bipolar gradients, which typically need also some real-time-balancing for achieving sufficiently stable echo positions. Furthermore, one also has to ensure that the RF pulse angles are correctly adjusted (which is only of minor importance in a stimulated echo experiment) as otherwise parasitic echoes (formed e.g. on the pathway sketched in Fig. 7) might again lead to systematically too low diffusion coefficients and to distortions in the echo attenuation curves. Despite these additional complications it seems to be advisable to use this sequence for reliable diffusometry experiments on plant materials wherever possible. A test for correct pulse angle adjustment can be made by comparing the echo attenuation curves for both the
stimulated echo and the 5-pulse sequence at a short diffusion time. If a bipolar gradient facility is not available, a rough test on the reliability of the diffusion measurements can be made by comparing the decay of the stimulated echo as a function of $\tau_2$ in the absence of gradient pulses with the longitudinal relaxation behaviour of the same sample as observed by means of a standard relaxometry experiment (such as inversion-recovery or saturation-recovery). If the stimulated-echo decay is faster than the longitudinal relaxation observed in the standard experiment, stimulated echo diffusometry experiments at long observation times in the same sample can be expected to lead to diffusion coefficients that are systematically too low (at least as long as dipolar correlation effects DCE can be excluded as a reason for the faster decay of the stimulated echoes; this should be the case as this effect has up to now only been observed in complex fluids such as polymer melts [23] and liquid crystals [24] but not in simple fluids such as water and oil).

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