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A new method for suppressing the central transition in $I = 3/2$ NMR spectra with a demonstration for ^{23}Na in bovine articular cartilage

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Abstract

The splitting and the lineshape of the satellite transitions of ^{23}Na are measures of the residual quadrupolar interaction and its distribution, which are related to the degrees of order and binding of sodium in biological tissues. However, these transitions are often masked by the stronger signals of the central transition and the isotropic sodium ions. A way to suppress the central signals, while preserving the lineshape and the intensity of the satellites, is suggested and tested on a liquid crystal and on bovine articular cartilage.

Keywords: ^{23}Na NMR; Zero order filtered NMR; Selective pulses; Residual quadrupolar interaction; Articular cartilage

1. Introduction

The measurement of the satellite transitions of sodium nuclei in biological systems is desirable owing to the facts that they can provide information about the degree of order and the amount of bound sodium. We have previously shown that the splitting between the satellites in articular cartilage is a sensitive measure of the depletion of the proteoglycans—an effect occurring in osteoarthritis [1,2]. However, owing to their large linewidth and the presence of free sodium ions, these transitions are masked by the large central transition in the single pulse experiment. The signal arising from isotropic sodium, as well as the central transition of the sodium ions in an anisotropic environment, can be suppressed by double and zero order filtering [3–5] techniques. In these techniques satellite transitions appear in opposite phases, and as a result of the distribution of the residual quadrupolar splittings, their lineshapes depend on the parameters of the pulse sequences. All these methods suffer from inferior signal-to-noise ratio (SNR) relative to the single pulse experiment. Clearly, a method whose sole effect would be to suppress the central transition peak without affecting the lineshape of the satellite transitions is desirable, and is the aim of the current work.

2. Theory

In the present study we introduce a pulse sequence that leads to the suppression of the central peak in the quadrupolar-split ^{23}Na spectrum and of the single peak of the isotropically rotating sodium ions. It is based on three basic ideas: (a) A linear combination of spectra where the satellites have the same phase, while the central peaks have opposite phases that can bring about the suppression of the central peak. Such spectra can be a result of the spin dynamics $T_{1,-1} \rightarrow T_{1,-1}$, where the central and the satellite peaks have the same phase, and $T_{3,-1} \rightarrow T_{1,-1}$, where the phase of the central peak is opposite to that of the satellites. (b) The linear combination can be made independent of the residual quadrupole

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interaction by creating $T_{3,0}$ and $T_{1,0}$ tensors by a single selective pulse on the central peak. (c) Since the ^{23}Na spectrum of free sodium in an isotropic environment is a singlet coinciding with the central peak of the quadrupolar-split sodium, a pulse can be selective for the latter and non-selective for the former, forming only $T_{1,\pm1}$ tensors that are filtered out by zero order filtering. Thus, the pulse sequence used in the current study (Fig. 1) consists of two pulses: a selective pulse followed by a non-selective one. The sequence starts with selective excitation of the central transition with phase cycling that retains only zero order. As explained in the Appendix A, assuming on-resonance irradiation one has to consider only the evolution of the zero order first rank tensor $(T_{1,0})$ into a third rank tensor of the same coherence $(T_{3,0})$. The $T_{2,0}$ tensor, which may cause distortion of satellite lineshapes is not formed. Subsequent application of a hard pulse converts the $T_{1,0}$ and $T_{3,0}$ tensors into a single quantum coherences $(T_{1,-1}, T_{3,-1})$ whose proportion depends on the flip angles of both pulses. These single quantum tensors evolve during the acquisition time into the detectable tensor $T_{1,-1}$. With the appropriate proportions between them a suppression of the central transition is obtained without affecting the lineshape of the satellite transitions, with only a small reduction (10%) in their intensity in comparison with a single pulse experiments. We designate, for abbreviation, the pulse sequence in Fig. 1 "CPS," since it brings about the central peak suppression.

The rotating frame spin Hamiltonian relevant to the evolution during the selective excitation is given in Eq. (1):

$$H = \omega_1 I_x + \frac{\omega_Q}{6}(3I_z^2 - I^2), \tag{1}$$

where $\omega_1 = \gamma B_1$ is the nutation frequency and ω_Q is the quadrupolar interaction. Following the choice of basis operators suggested by Hancu et al. [6] we numerically solved the Liouville equation for the density matrix (see Appendix A). The dependence of the intensities of the NMR transitions on the flip angles of the selective and the hard pulses show that complete suppression of the central transition is possible with a hard pulse flip angle equal to or smaller than 41.8°. The variation of the peak intensities with changing the selective pulse flip angle χ^{sel} (given by $\omega_1 t_p$, where t_p is the selective pulse length) for a hard pulse flip angle of 41.8° is shown in Fig. 2. In Fig. 3 the dependence of the intensity of the satellite peaks on the ratio of the nutation frequency, ω_1, and ω_Q is shown. Three important facts are evident: (a) for odd integral multiples of 90° the central transition vanishes while the satellites ones are at their maximum; (b) the maximum intensity of the satellites transitions approaches a value

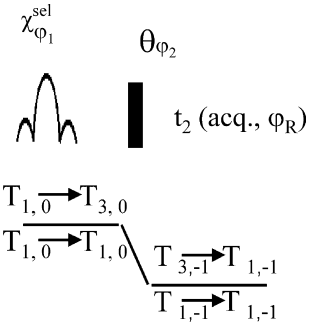

Fig. 1. The CPS pulse sequence designed to suppress the ^{23}Na central peak by selective excitation and zero order filtering. χ^{sel} is a selective pulse while θ is a non-selective one. The pulses phases were cycled according to: $\varphi_1 = m^*90°$, $\varphi_2 = k^*90°$, $\varphi_R = k^*90°$ with m and k assume the values {0,1,2,3} independently.

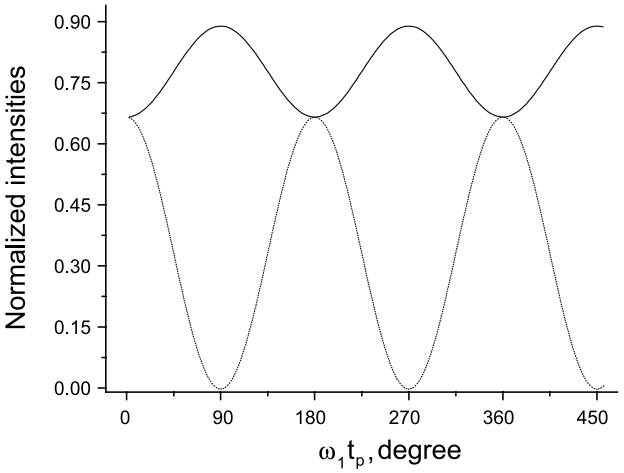

Fig. 2. Theoretical simulation of the dependence of the satellites (upper trace) and the central transition (lower trace) intensities on the soft pulse tilt angle, using 41.8° for the hard pulse and ignoring the effects of relaxation. The normalization factors were the corresponding transitions intensities observed after a single 90° pulse.

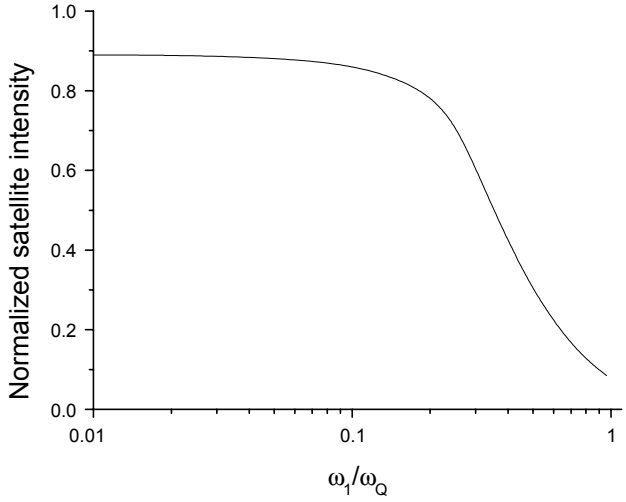

Fig. 3. The satellite peak intensity as a function of the ratio between the radio frequency intensity and the residual quadrupolar interaction. The intensity was normalized to its value in a single non-selective 90° pulse experiment.

which is 90% of that obtained in an experiment that uses a single non-selective 90° pulse (c) For $\omega_1/\omega_Q < 1/6$ the frequency of the oscillation depends only on the r.f. nutation frequency and not on the residual quadrupole interaction. It should be noted that, although Fig. 3 was calculated for $\chi^{sel} = \omega_1 t_p = 90°$, the intensities of the satellite transitions are not sensitive to ω_Q as long as $\omega_1/\omega_Q < 1/6$, and are dependent only on $\chi^{sel} = \omega_1 t_p$ (Fig. 2). Thus, one may expect that it should be possible to suppress the central transitions without distorting the lineshape of the satellite transitions as a result of a distribution of a residual quadrupolar interaction. For hard pulse flip angles smaller than 41.8° the soft pulse tilt angle that suppresses the central transition is smaller than 90° and the intensities of the satellite transitions.

Preliminary calculations of the effects of the longitudinal relaxation indicate that, when the selective pulse length is comparable to the longitudinal relaxation time, the pulse lengths of both the selective and the non-selective pulses required for the central peak suppression become smaller than their optimal values (90° and 41.8°, respectively), leading to reduced intensities of the satellite transitions. These restrictions set a lower limit of $(3T_1)^{-1}$ to the value of ω_1. This, together with the requirement of $\omega_1 < \omega_Q/6$, implies that reliable lineshapes for the satellite peaks can be obtained for $\omega_Q > 2/T_1$. For connective tissues such as cartilage this condition translates into $\omega_Q/2\pi > 130$ Hz. It is interesting to note that, in addition to the suppression of the central transition of the ^{23}Na which experiences anisotropic motion, the pulse sequence (Fig. 1) also suppresses the single peak of ^{23}Na which experiences isotropic fast motion. In the latter case the first soft pulse χ^{sel} acts as a non-selective 90° pulse, forming single quantum coherences $T_{1,\pm1}$ which are eliminated by phase cycling.

Some caution should be paid to the effect of the inhomogeneities of B_0 and r.f. magnetic fields. Our preliminary theoretical results show that the former type of inhomogeneity (and offset from resonance) may lead to two types of difficulties: (a) formation of a second rank zero order tensor that may distort the satellites' lineshapes, (b) reduction of the intensity of the tensor $T_{3,0}$, that either results in poor suppression of the central peak (i.e., a very small reduction of its intensity relative to the satellite peaks) or, by changing the length of the non-selective pulse, will give an improved suppression, but with a reduced intensity of all the peaks. From the discussion above (Figs. 2 and 3 and related text) it is clear that the effect of the r.f. inhomogeneity would be to reduce the intensities of the satellite peaks while maintaining their shape. Another undesirable effect of the r.f. inhomogeneity is a reduction of the central peak suppression.

The CPS pulse sequence can also be applied to ^{23}Na in an environment where the motion is isotropic but slow (compared to $1/\omega_0$) so that the transverse magnetization decays bi-exponentially, giving a super-Lorentzian lineshape. However, a discussion of this topic is outside the scope of the present publication.

3. Experimental

The liquid crystal used in our experiments was 24.6% w/w aqueous solution of sodium linoleate [7]. Prior to measurements the sample was left to align in the magnetic field for at least a couple of hours. For the articular cartilage experiment a cartilage-bone of plug 8 mm in diameter was excised from bovine femoral condoyl and was immersed in fluoroinated oil (Fluorinert, FC-77, 3M) for the NMR measurements. The experiments were carried out on a Bruker Avance 360WB system supplied with a 500 W transmitter and a solenoid that could deliver 4.5 μs 90° pulses at the sodium frequency.

4. Results and discussion

We tested our method on two systems that are distinguished significantly by their distribution of the quadrupolar interaction. One is a liquid crystal, where the molecules are well-aligned parallel to the magnetic field and thus the distribution of the quadrupolar interactions is narrow, giving a well-resolved spectrum (Fig. 4), and the other is bovine articular cartilage, where the sodium ions are oriented with the collagen fibers and thus the quadrupolar interaction distribution is broad, giving a poorly resolved spectrum (Fig. 5). The upper trace in Fig. 4 represents the ^{23}Na spectrum of the liquid crystal obtained by applying a single non-selective pulse. The lower trace in the figure was obtained by

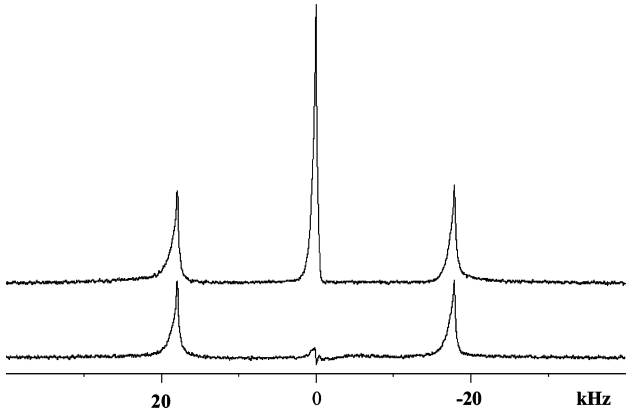

Fig. 4. ^{23}Na spectra of sodium linoleate liquid crystal using a single non-selective pulse of 4.7 μs (upper trace) and the CPS pulse sequence (lower trace). For the CPS pulse sequence the hard pulse tilt angle was 40° and the selective pulse parameters were $\omega_1/2\pi = 1250$ Hz, pulse length 200 μs and a sinc pulse shape. For the two spectra the repetition and the acquisition times were 200 and 80 ms, respectively. The number of accumulations was 16.

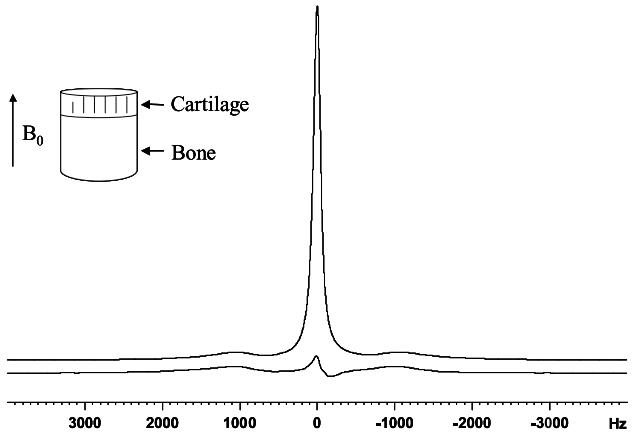

Fig. 5. ^{23}Na spectra of sodium in articular cartilage aligned with the collagen fibers parallel to the magnetic field. The upper trace was obtained using a single 90° non-selective pulse of 17 μs. The lower trace is a spectrum recorded using the CPS pulse sequence with a hard pulse tilt angle of 34° and a selective pulse of $\omega_1/2\pi = 32$ Hz and length of 7 ms. A rectangular pulse was used. For the two spectra the repetition and the acquisition times were 250 and 100 ms, respectively. The number of accumulations was 16,384.

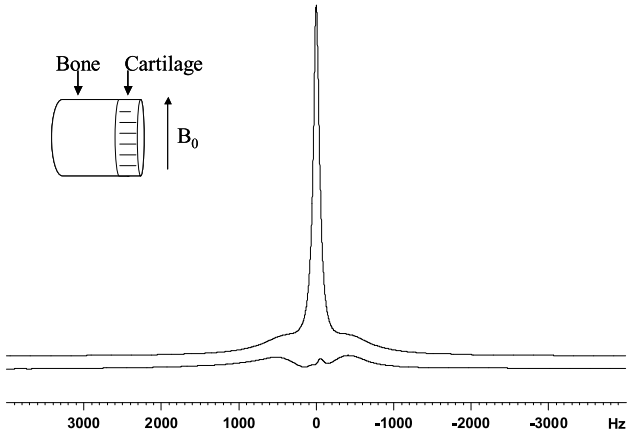

Fig. 6. ^{23}Na spectra of sodium in articular cartilage aligned with the collagen fibers perpendicular to the magnetic field. The upper trace was obtained using a single 90° non-selective pulse of 16 μs. The lower trace is a spectrum recorded using the CPS pulse sequence with a hard pulse tilt angle of 38° and a selective pulse of $\omega_1/2\pi = 33$ Hz and pulse length 7 ms. A rectangular pulse was used. For the two spectra the repetition and the acquisition times were 250 and 100 ms, respectively. The number of accumulations was 16,384.

applying the CPS pulse sequence with a soft pulse $\chi^{sel} = \omega_1 t_p = 90°(\omega_1 = 1250\,\text{Hz}, t_p = 200\,\mu\text{s})$ and a hard pulse of 40°. A good suppression of the central transition is observed with the intensities of the satellite transitions being 84% of their single pulse experiment values. These results are in good agreement with the theoretical predictions for a relaxation-free situation and were compared with the one obtained by the previously described method [3,8] of magic angle double quantum filtered (DQF) (i.e., DQF with the last two pulses set to 54.7°). This method gave a spectrum (not shown) with reasonable suppression of the central band, and with the satellite transitions in antiphase to each other having intensities that are 44% of their single pulse value. This value is about half of the value of the current method.

The good resolution observed in the liquid crystal sample is not very common in biological systems where sodium NMR spectroscopy is often applied. Thus, in order to examine whether the satellites lineshapes are reliable even in the presence of a wide distribution of quadrupolar splittings, we performed our measurements on a sample of bovine articular cartilage. Single-pulse and CPS spectra of an excised cartilage-bone plug at two orientations relative to the magnetic field are shown in Figs. 5–7. When the sample is aligned with its collagen fibers parallel to the magnetic field, a splitting of 1900 Hz is observed for the single-pulse experiment (Fig. 5 upper trace). However, when aligned perpendicular to the magnetic field the satellite peaks appear as unresolved shoulders superimposed on the central transition peak (Fig. 6 upper trace). It is worth noting that, although the lineshape is broadened as a result of a distribution of

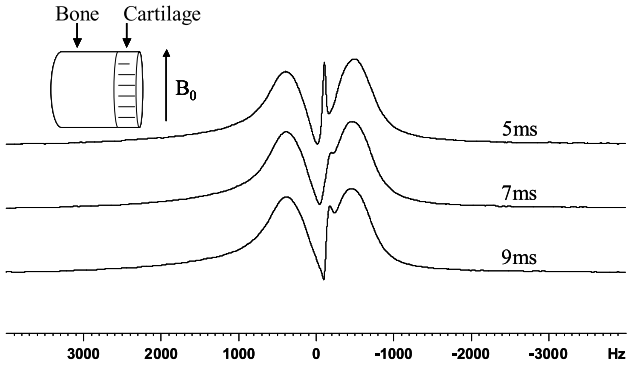

Fig. 7. ^{23}Na spectra of sodium in articular cartilage aligned with the collagen fibers perpendicular to the magnetic field. All three spectra were obtained with selective pulses of $\chi^{sel} = \omega_1 t_p = 90°$. Thus, for the pulse lengths of 5, 7, and 9 ms the values of $\omega_1/2\pi$ were 50, 35.7, and 27.8 Hz, respectively. The corresponding non-selective pulses were rectangular with tilt angles of 33°, 29°, and 25°, respectively. The number of accumulations for each spectrum was 12,288. The repetition and acquisition times were 250 and 100 ms, respectively.

quadrupolar interactions, the dependence of the spectrum on the sample orientation with respect to the magnetic field indicates that a significant level of macroscopic order exists in the tissue. The application of the CPS sequence suppresses the central peak and gives a clear splitting of 1900 and 920 Hz for the parallel and perpendicular orientations, respectively (Figs. 5 and 6 lower traces). The ratio between the splittings for the two orientations is close to the ratio of 2.0 expected for ordered collagen fibers. Since in the perpendicular orientation the resolution is poor and thus one cannot get the satellites spectrum lineshape from the single pulse

experiment, it is necessary to have a more general test that will validate the lineshapes obtained by the CPS method. This is achieved by examining the effect of the selective pulse length and intensity on the obtained lineshape. We have chosen to perform this second type of tests under more stringent conditions than the former one (Figs. 5 and 6) by selecting a specimen of articular cartilage that had a splitting of only 1700 Hz for the parallel orientation (spectrum not shown). The CPS spectra of this sample, oriented perpendicularly to the magnetic field, were recorded using selective pulse lengths over the range of 5–9 ms ($\chi^{\mathrm{sel}} = \omega_1 t_{\mathrm{p}} = 90°$) and are shown in Fig. 7. The same lineshape is observed for the three spectra with a splitting of 830 Hz, consistent with the 1700 Hz value obtained for the parallel orientation. This result indicates that the same distribution of quadrupolar interactions determines both the single non-selective 90° pulse and the CPS spectra. For the sample parallel orientation the intensities of the satellite transitions are about 76% of their values in the single pulse experiment (Fig. 4). The deviations of the optimal pulse tilt angles from their theoretical relaxation-free values, as well as the reduction in the intensities of the satellite transitions, can be attributed to the effect of the longitudinal relaxation during the soft pulse. For the experiment on the articular cartilage the ratio between the slow component of the longitudinal relaxation time $T_{1\mathrm{s}}$ (measured by inversion recovery) and the duration of the soft pulse, t_{p}, was: $T_{1\mathrm{s}}/t_{\mathrm{p}} \sim 1.4$. This value is much smaller than its liquid crystal counterpart ($T_{1\mathrm{s}}/t_{\mathrm{p}} \sim 28$) and thus the deviation from the relaxation-free situation is larger. For articular cartilage aligned with the magnetic field the suppression of the central transitions using three-pulse magic angle DQF method [3,8] yielded satellite peak intensities smaller than 50% of the values obtained in a single pulse experiment. This value is smaller than 76% of the satellite transitions intensities that are retained in the present method. Furthermore, the spectra lineshapes in the DQF experiments relied heavily on the preparation time [3,10] contrary to the current method (Fig. 7).

5. Conclusions

The pulse sequence presented here enables the suppression of the ^{23}Na central peak while maintaining the single-pulse lineshapes and intensities of the satellite transitions without distortions owing to either r.f. or residual quadrupolar interaction distributions.

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Appendix A

The theoretical calculations are based on the solution of the following equation of motion of the density matrix [9]:

$$\frac{\mathrm{d}}{\mathrm{d}t}\rho = -\mathrm{i}[H,\rho] = -\mathrm{i}H^{x}\rho, \tag{A.1}$$

where H is the Hamiltonian given in Eq. (1). The formal solution of Eq. (A.1) is given by:

$$\rho(t) = \mathrm{e}^{-\mathrm{i}tH^{x}}\rho(0) = \mathrm{e}^{Bt}\rho(0) = U\rho(0) \tag{A.2}$$

with $\rho(0)$ being the value of the density matrix at $t = 0$. Since the initial condition in our experiment is that only $T_{1,0} \neq 0$, and the excitation is on-resonance, the basis that describes the spin dynamics is [6,9]: $T_{1,0}, T_{1,1}(s), T_{2,1}(a), T_{2,2}(a), T_{3,0}, T_{3,1}(s), T_{3,2}(s), T_{3,3}(s)$. Please note that the tensor $T_{2,0}$ is not included, indicating that it may not evolve from $T_{1,0}$. The explicit expression for the matrix B was given by Hancu et al. [6]. The matrix U was calculated numerically. The satellite and central transitions intensities that are normalized by their corresponding values in a single non-selective 90° pulse were obtained by the expressions shown in Eq. (A.3):

$$\begin{aligned}\text{Central transition} &= \sqrt{2}d^{1}_{-1,0}(\theta)U_{1,1}(t_{\mathrm{p}}) \\ &\quad - \sqrt{3}d^{3}_{-1,0}(\theta)U_{1,5}(t_{\mathrm{p}}), \\ \text{Satellite transition} &= \sqrt{2}d^{1}_{-1,0}(\theta)U_{1,1}(t_{\mathrm{p}}) \\ &\quad + \sqrt{\frac{4}{3}}d^{3}_{-1,0}(\theta)U_{1,5}(t_{\mathrm{p}}),\end{aligned} \tag{A.3}$$

$d^{l}_{m,m}(\theta)$ are the appropriate Wigner matrices elements and θ is the non-selective pulse (Fig. 1) tilt angle.

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