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Karlsson, Magnus; Ardenkjær-Larsen, Jan Henrik; Lerche, Mathilde Hauge

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Hyperpolarized ^{133}Cs is a sensitive probe for real-time monitoring of biophysical environments†

M. Karlsson, * J. H. Ardenkjær-Larsen and M. H. Lerche *

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^{133}Cs NMR is a valuable tool for non-invasive analysis of biological systems, where chemical shift and relaxation properties report on changes in the physical environment. Hyperpolarization can increase the liquid-state ^{133}Cs NMR signal by several orders of magnitude and allow real-time monitoring of physical changes in cell based systems.

^{133}Cs NMR has proven to be a valuable tool in studies of complex molecular systems since the physical properties of the Cs^+ ion is highly sensitive to its surroundings.[1] ^{133}Cs NMR has successfully been applied to study Na^+–K^+ pump activity using Cs^+ as an analog for K^+. By measuring the influx in intact and compromised membranes[2] the rate of accumulation of intracellular Cs^+ can be modeled. Also, localization of potential contrast agents can be readily studied by induction of changes in the ^{133}Cs chemical shift and line width.[3,4]

Cesium has only one stabile isotope, ^{133}Cs, with spin 7/2 and a gyromagnetic ratio of approx. 1/8 the value of ^{1}H, making ^{133}Cs a moderately sensitive nucleus in NMR. Cesium is the heaviest of the stabile alkali metals with a large ion radius leading to a highly polarizable electron cloud that makes its chemical shift sensitive to the surroundings. It is a quadrupolar nucleus, but the quadrupolar moment is low, resulting in narrow NMR line widths and slow relaxation rates.[5] This allows resolution of the magnetic resonance signal of Cs in different cellular compartments on the basis of chemical shift and/or magnetic relaxation properties.[1]

The scope of NMR has been extended by hyperpolarization methods, which improve sensitivity and allow real-time probing of biological systems.[6] The sensitivity improvement is achieved by a temporary re-distribution of nuclear magnetization of nuclei in molecular probes. When studied in the liquid-state, this *ex situ* magnetization often has lifetimes of tens of seconds or, sometimes, several minutes. Most hyperpolarization studies in the liquid-state have been performed using ^{13}C NMR; however, other spin 1/2 nuclei, *e.g.* ^{1}H, ^{15}N, ^{89}Y, ^{29}Si and ^{107,109}Ag, and the spin 1 nucleus ^{6}Li have been reported as possible hyperpolarized liquid-state probes.[7] Here, we investigate the feasibility of hyperpolarizing ^{133}Cs using dissolution dynamic nuclear polarization (dDNP)[8] to increase the sensitivity and allow real-time applications using ^{133}Cs NMR.

Cesium salts are generally highly soluble, much more so than other alkali metal salts of, *e.g.*, sodium or potassium. Cesium acetate was chosen for this study for the possibility of directly comparing the performance to the ^{13}C labelled counter ion (acetate). Using the principles of sample conditions developed for ^{13}C-DNP[9] cesium acetate samples were prepared in 4.7 M with ethylene glycol as a glassing agent and 15 mM trityl. It is, however, straightforward to make cesium DNP samples also from the more common laboratory chemicals cesium chloride and cesium hydroxide. Cesium chloride can be prepared to (at least) 3.8 M solution in 1 : 1 water/ethylene glycol and cesium hydroxide can be prepared to approximately 8 M in ethylene glycol. Both preparations will form a glass upon rapid freezing.

The strong NMR signal of ^{133}Cs permits polarization build-up to be directly monitored as it is created in the solid state. A ^{133}Cs polarization of more than 50% could be achieved with a polarization build-up time constant of approx. 20 minutes (Fig. 1A, ESI†). A rather large increase in solid-state polarization, approx. 2.5 times, is achieved with the addition of gadolinium (Gd^{3+}) in the form of ProHance, a commercial MR contrast agent (solid-state polarization is 22% without Gd^{3+}).

A comparison between ^{13}C and ^{133}Cs solid-state polarization build-up was performed using 1-^{13}C enriched cesium acetate (Fig. 1A and C). Under conditions used in this study (3.35 T and approx. 1.4 K) the DNP mechanism is expected to be thermal mixing. Thus, a ratio of 1.56 between ^{133}Cs and ^{13}C polarization is expected under fully polarized conditions and in the high temperature approximation (see the ESI† for details on calculations). Samples without addition of Gd^{3+}, however, showed

Technical University of Denmark, Department of Electrical Engineering, Center for Hyperpolarization in Magnetic Resonance, Building 349, DK-2800 Kgs Lyngby, Denmark. E-mail: mhauler@elektro.dtu.dk, mkarls@elektro.dtu.dk; Tel: +45 53624555, +45 53646555

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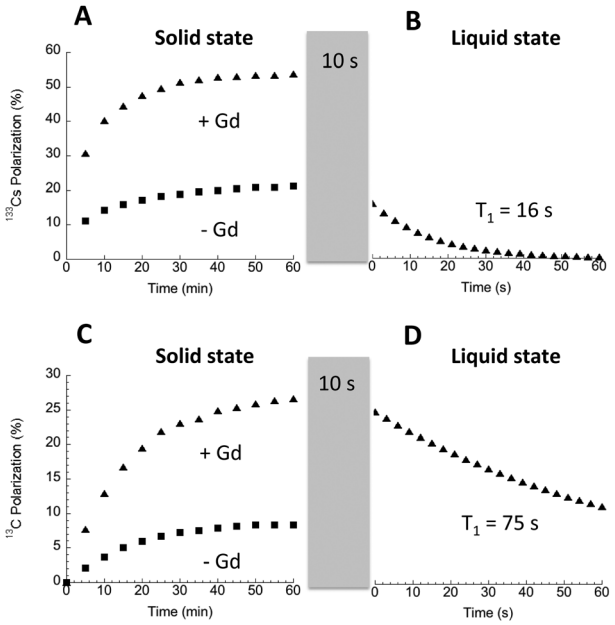

Fig. 1 Solid-state polarization build-up of the ^{133}Cs^{13}CAc signal at 3.35 T and 1.4 K followed by dissolution and 10 s transfer to liquid-state polarization decay monitored at 9.4 T and 318 K. (A) Solid-state ^{133}Cs polarization build-up as a function of time. Filled triangles (with Gd^{3+}) and filled squares (without Gd^{3+}). (B) Liquid-state ^{133}Cs polarization as it decays over time with a T_1 of 16 s. (C) Solid-state [1-^{13}C]Ac polarization build-up as a function of time. Filled triangles (with Gd^{3+}) and filled squares (without Gd^{3+}). (D) Liquid-state [1-^{13}C]Ac polarization as it decays over time with a T_1 of 75 s.

an approx. 2.5 times higher ^{133}Cs polarization compared to ^{13}C, whereas when Gd^{3+} was added the ^{133}Cs polarization was only approx. 1.9 times higher. We have currently no explanation for this discrepancy; however, the fact that Gd^{3+} and higher radical concentration (data not shown) brings the ratio closer to an equal spin temperature may suggest that the ESR line width of the trityl radical is too narrow for ^{13}C for efficient DNP in this sample. For quadrupolar nuclei, often only the central transition is observed[10,11] in the weak pulse limit. If the pulse is not properly calibrated this would lead to unexpected signal intensity for ^{133}Cs (see further discussion on this point in the ESI†). Here we compare the nuclear polarizations, calculated from the ratio between the hyperpolarized and the thermal signal, and therefore this effect should not influence the ratio between the ^{133}Cs and ^{13}C polarizations.

The polarization buildup rate of ^{133}Cs followed a bi-exponential path and was fast compared to the polarization rate of ^{13}C, reaching 30% polarization already after 5 min. The polarization of both ^{133}Cs and ^{13}C was strongly increased by adding Gd^{3+} to the sample.

After dissolution in an appropriate solvent ^{133}Cs decayed with a relaxation time constant of 16 s (Fig. 1B, Table 1), resulting in 16% polarization of ^{133}Cs calculated relative to its thermal polarization at 9.4 T, 318 K, 10 s after dissolution. A back-calculation based on the measured T_1 at 9.4 T and a transfer time of 10 s revealed that almost half of the solid state signal could not be accounted for (Fig. 1A and B). This significant loss of ^{133}Cs polarization was in contrast to the [1-^{13}C]acetate signal, which was quantitatively accounted for (Fig. 1C and D).

Table 1 Polarization and T_1 of ^{133}Cs and [1-^{13}C]acetate (^{13}CAc). Solid state polarization is measured at 3.35 T and 1.4 K. Liquid state polarization is measured 10 s after dissolution at 9.4 T and 318 K

Nuclei	T_1 (s)	Solid state polarization (%)	Liquid state polarization (%)
^{133}Cs^{13}CAc with Gd, in D_2O	16 ± 1	54 ± 2	16 ± 1
^{133}Cs^{13}CAc without Gd, in D_2O	16 ± 1	22 ± 2	5 ± 1
^{133}Cs^{13}CAc with Gd, in D_2O	75	28	25
^{133}CsAc with Gd, in H_2O	14 ± 1	54 ± 2	14 ± 1

We cannot currently explain this large, however reproducible, loss, but better control over the magnetic field and temperature of the environment during sample transfer may make up for this loss. Another possible explanation could be that the hyperpolarised ^{133}Cs signal reflects enhancement of only the central transition, whereas the liquid state thermal signal (used as a reference to calculated polarisation) covers all transitions.

Since $^{133}Cs^+$ relaxation is dominated by the quadrupolar mechanism, employing a deuterated solvent only marginally affects the relaxation rate (Table 1). The radical and Gd-complex additives were in the dissolution diluted to the extent where T_1 of ^{133}Cs does not depend on these sample additives.

The sensitivity and response time of hyperpolarized ^{133}Cs to its chemical and physical surroundings were studied. Temperature, pH and ionic strength were varied with time, and the corresponding chemical shift change of hyperpolarized ^{133}Cs was monitored (Fig. 2A). The ^{133}Cs chemical shift changed linearly with 0.1 ppm $^{\circ}C^{-1}$ within the investigated range. The temperature change was easily monitored with ^{133}Cs NMR on a sub-second timescale. ^{133}Cs chemical shift has been reported to increase with pH at very basic pH.[12] No chemical shift changes were observed in the physiological relevant pH 3–8 neither with hyperpolarized ^{133}Cs nor with thermal ^{133}Cs NMR. The chemical shift of ^{133}Cs was, however, highly sensitive to ion strength (Fig. 2B). Doubling the ion strength resulted in a ^{133}Cs chemical shift change of more than 1 ppm. The chemical shift was referenced to 5 M CsCl in a capillary with water.

To investigate whether hyperpolarized ^{133}Cs could be a quantitative reporter of cellular impairment an electroporation yeast cell model was set up. Hyperpolarized ^{133}CsCl was added in increasing concentrations to intact and electroporated yeast cells (Fig. 3A). In the electroporated yeast cells two distinct ^{133}Cs populations could be measured due to a chemical shift difference (7.7 ppm) between the different environments of the Cs^+ ions. The signals were measured in real-time over the course of the 30 s experiment with 0.5 s resolution (Fig. 3B). This chemical shift difference most likely reflects intra- and extracellular ^{133}Cs since only the extracellular ^{133}Cs signal was detected in non-electroporated yeast cells (Fig. 3B, inset). Increase in extracellular ^{133}Cs led to a linear increase in the intracellular ^{133}Cs, suggesting free diffusion between the intra- and extracellular compartments (Fig. 3C). With a constant amount of biological material the intra- and extracellular signals were directly proportional (1 ± 0.05%) over the measured range of extracellular concentrations. At the highest extracellular

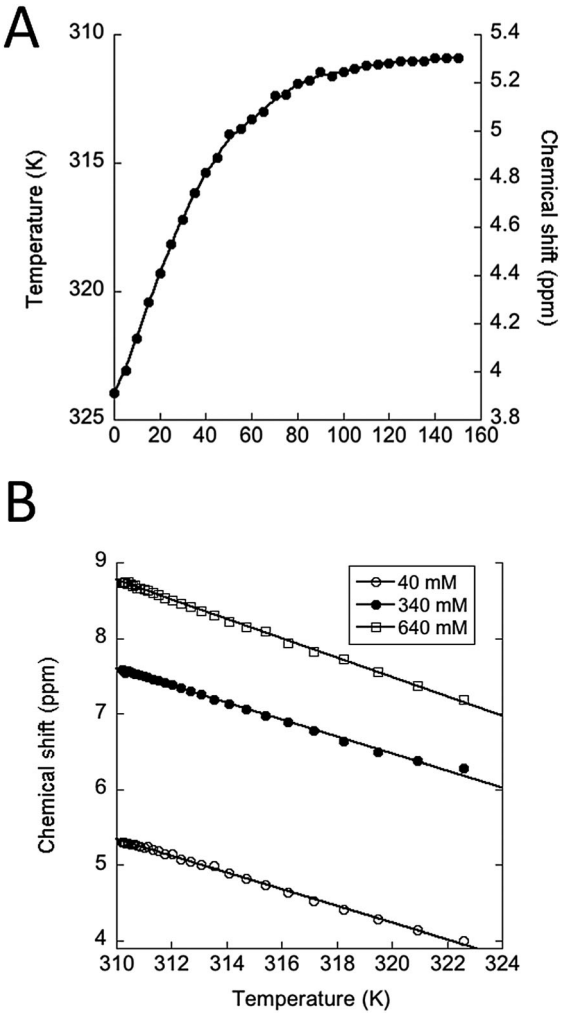

Fig. 2 ^{133}Cs chemical shift perturbations induced by changes in the physical environment. (A) Temperature changes monitored over time with ^{133}Cs chemical shift. (B) Changes in osmolality measured over a physiologically relevant temperature range monitored with ^{133}Cs chemical shift.

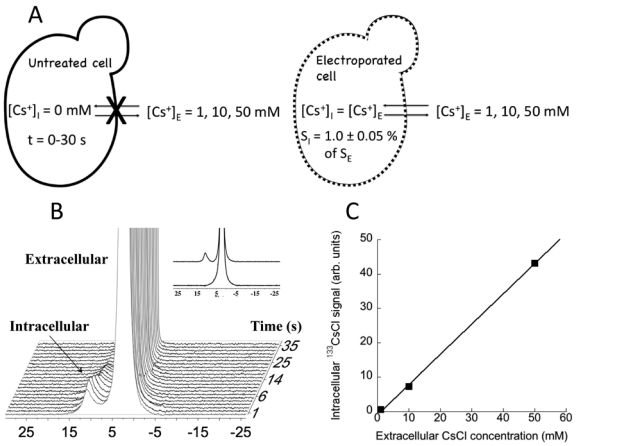

Fig. 3 ^{133}Cs as a marker of membrane impaired cells. (A) Yeast cellular model. (B) Uptake of ^{133}Cs in electroporated yeast cells. The chemical shift difference between the extracellular and intracellular ^{133}Cs signal is approx. 7.7 ppm. The inset shows a comparison between a spectrum of electroporated cells (top) and a spectrum of non-electroporated cells (bottom). (C) Intracellular ^{133}Cs signal in membrane impaired yeast cells is directly proportional to the concentration of extracellular ^{133}CsCl.

concentration (50 mM) it was possible with extensive signal averaging to measure the intracellular ^{133}Cs signal in a thermal NMR spectrum (data not shown).

In conclusion, hyperpolarized ^{133}Cs NMR has been developed into a highly polarized liquid state molecular probe and studied as a sensitive, fast and specific marker for probing cellular membrane impairment. Its 54 orbited electrons magnetically shield the NMR active $^{133}Cs^+$ ion, which makes it highly sensitive to both chemical and magnetic environments, leading to direct and real-time detection of its intracellular accumulation by a distinct change in chemical shift. In seconds, hyperpolarized ^{133}Cs NMR provides a background free measure of access to the intracellular environment. In contrast to thermal ^{133}Cs NMR where active transport of the Cs^+ takes place over the longer scanning times, only membrane-disrupted cells provide signals from hyperpolarized $^{133}Cs^+$. Such a molecular marker may come into play in the development and optimisation of treatments in several types of modern health threats where membrane impairment plays a crucial role. In particular, it may be a diagnostic tool in the fight against antibiotic resistant bacteria where an important group of antibiotics (antimicrobial peptides, AMPs) display their action by disruption of the bacterial membrane *via* pore-formation or unspecific membrane permeabilization.[13] Similarly, this tool may find use in oncological research where reversible tissue electroporation serves as a method to bypass the cellular barrier and introduce drugs into targeted tissue.[14] Supervision of the efficiency and further optimisation of these types of treatments are important since cytotoxic drugs and radiation are deleterious also to healthy tissue and physicochemical properties of drugs can prevent their penetration through the plasma cell membrane even when delivered in the targeted area.[15]

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