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Report on the Doctoral thesis of Mrs Şeyma Alçiçek

It has been a pleasure to read Şeyma Alçiçek's doctoral thesis on chemical and biochemical applications of Zero- and Ultralow-field nuclear magnetic resonance (ZULF-NMR). ZULF-NMR is a recently developed methodology which allows chemically informative NMR spectra to be obtained without the use of large and expensive high-field NMR magnets. Instead of the conventional inductive detection of NMR signals, ZULF-NMR uses atomic magnetometers to detect nuclear magnetism directly, in extremely low magnetic fields. The detection of such extremely weak magnetic signals is a technological feat by itself. In her thesis, Ms Alçiçek analyses the ZULF-NMR signals in terms of the spin-spin coupling networks of the chemical compounds which compose the sample. She has paid special attention to the ZULF-NMR of phosphorus compounds, which was previously an unexplored area. On the way she came across several interesting spectroscopic effects, such as the effects of chemical exchange on ZULF-NMR spectra. She has also demonstrated the use of parahydrogen-based hyperpolarization techniques in conjunction with ZULF-NMR. She assesses the feasibility of ZULF-NMR as a relatively cheap and compact form of chemical and biochemical analysis.

After an introductory chapter, Chapter 2 presents the theoretical background of NMR in general and ZULF-NMR in particular. Basic NMR concepts such as spin, precession, relaxation, and nuclear induction are presented clearly and simply. Although these sections are very clear and well-written, there are a few misunderstandings that I will be happy to discuss with Ms Alçiçek during her thesis defence. The chapter continues with the theoretical background of ZULF, giving example for several different coupling topologies. This section has clearly received careful thought and is very nicely written. The chapter concludes with a section on parahydrogen-induced polarization, including a discussion of how the Pauli principle leads to the existence of the hydrogen spin isomers, and the presentation of several experimental results in order to demonstrate the theoretical concepts. This chapter is an impressive and wide-ranging piece of work. Ms Alçiçek may have got a little carried away while writing it since it contains much material which goes well beyond the "theoretical background" rubric of the chapter. In some respects, this single chapter almost qualifies as a PhD thesis by itself.

Chapter 3 gets down to business with the ZULF-NMR of organophosphorus compounds - a completely unexplored area before Ms Alçiçek's work. The contents of this chapter are based on a publication in *J. Phys. Chem. Letters* with Alçiçek as first author. The chapter describes the experiments in detail, including informative equipment descriptions and useful photos. The displayed experimental results and accompanying simulations are very fine. My main criticism is that the method for simulating the ZULF-NMR spectra is not as clearly described as the experimental descriptions. The chapter concludes with some statements about the potential of ZULF-NMR for metabolic studies in living systems, perhaps even tumour differentiation. Such speculations are possible but I would have expected more analysis of the limitations of the technique in practice, for example its relatively poor signal-to-noise ratio, and a quick comparison with other available methods. This is a topic that deserves a realistic appraisal and I expect to explore this topic with Ms Alçiçek during her thesis defence.

Chapter 4 discusses the ZULF-NMR of various biomolecules, with particular attention to chemical exchange effects. The contents of this chapter are also based on a publication in *J. Phys. Chem. Letters* with Alçiçek as first author. This is a very interesting piece of work, showing how chemical processes in the material of interest may modify the appearance of the ZULF-NMR spectra. As far as I know, this phenomenon was not discussed before. The work contains numerous interesting and very well-presented experimental results, and accompanying simulations.

Chapter 5 discusses the combination of ZULF-NMR with the variety of parahydrogen-based hyperpolarization known as SABRE. The chapter starts with another detailed and informative presentation of the instrumentation (including parahydrogen generation, the hydrogenation setup, and the ZULF-NMR equipment) followed by a series of fine experimental results on pyridine and its derivatives. It is truly impressive that SABRE enhances the nuclear magnetization sufficiently strongly to allow the detection of 15N in natural abundance, in ultralow magnetic field. This is a real achievement. Numerical simulations are also shown. These are in good agreement with the experimental results but the lack of detail on the simulation methodology is somewhat disappointing. The chapter closes with more speculations about the possible application of SABRE-enhanced ZULF for analytical applications, but again, these statements are not accompanied by a realistic appraisal of signal-to-noise ratio and consideration of alternative methods.

Chapter 6 proceeds to discuss the use of various parahydrogen-based hyperpolarization methods in which exchangeable protons are used to relay polarization between different molecules. Again, the description of the apparatus and the experimental procedures is exemplary. A series of carefully presented experimental results is shown for the low-field NMR case. The section on the enhancement of ZULF-NMR by PHIP-X is groundbreaking in the sense that, as far as I know, this experiment had not been done before. The experiment and the results are described in exemplary detail.

Chapter 7 covers ZULF NMR relaxometry, i.e. the study of nuclear spin relaxation processes in the ZULF regime. The effect of paramagnetic agents such as molecular oxygen and copper ions on nuclear spin relaxation times are studied in ultralow magnetic field. The description of the experiment and the results is again very fine, but it would have been better to plot the relaxation rate constants (inverses of the relaxation time constants) against concentration, rather than the relaxation time constants themselves. Paramagnetic relaxation normally leads to a linear dependence of relaxation rate constant on concentration of the paramagnetic agent, with a slope known as the relaxivity. It would have been interesting to see if that linear relationship also holds in the ZULF regime. A remarkable section follows, in which human blood is studied by ZULF-NMR.

Chapter 8 summarises the thesis and gives an outlook of the prospects for ZULF-NMR in the analytical and biological sciences. This section is full of enthusiasm and optimism. However it does lack any sort of comparison with available alternatives, which is a weakness, in my opinion.

In summary, I find this thesis quite remarkable, It covers a very broad range of science, taking in physics, engineering, chemistry, and biology. The thesis contains several novel scientific contributions and describes several strong and original advances to the emerging field of ZULF-NMR. In fact the thesis fulfils the requirements for an ordinary PhD several times over, and holds a very high academic level throughout.

In my opinion this thesis richly deserves the award of a "distinction".

Yours sincerely

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