UNIVERSITY
OF WARSAW
Faculty of Chemistry

WCh
Faculty of Chemistry
University of Warsaw

Warsaw, 16th September 2022

Evaluation of Mrs. Seyma Alcicek's Ph.D. thesis

In this letter, I give my assessment of the Ph.D. thesis by Seyma Alcicek, entitled "Zero- and Ultralow-Field NMR for Chemical and Biochemical Analysis" that was supervised by Professor Szymon Pustelny and submitted on the Faculty of Physics, Astronomy and Applied Computer Science at the Jagiellonian University in Kraków in June 2022.

The Ph.D. thesis by Seyma Alcicek is devoted to liquid-state nuclear magnetic resonance studies conducted in the regime of magnetic fields much below Earth's magnetic field, in which spectra are mainly determined by the indirect spin-spin interaction. In her hundred fifty pages thesis, the PhD candidate presented a broad scope of NMR studies including analysis of biomolecules spectra, efficient methods of *para*-hydrogen induced hyperpolarization, and relaxation caused by paramagnetic substances. My overall impression of the results presented by the Ph.D. candidate in her thesis is very positive. The strengths of the thesis are a clear background introduction, understanding of the theory necessary for successful simulation of the spectra, detailed description of the carried out experiments, correct analysis of collected data, and high editorial quality of the text. Moreover, the results described in chapters 3 and 4 were published in the Journal of Physical Chemistry Letters last year. Nonetheless, some relatively minor points in the thesis require clarification, and I encourage the candidate to refer to my comments given below. I summarized my comments according to the sequence of chapters in the doctoral thesis.

In the second chapter, the Ph.D. candidate described in a textbook style the theoretical basis of NMR, starting from the case of the high field, with which most spectroscopists are familiar, and then discussed the nearly-zero magnetic field case. Although the high-field NMR is not an objective of the thesis, a brief description of interactions with the magnetic field given in the thesis deserves clarification. In particular, section 2.1.6.1 introduces an interaction between the nuclear magnetic dipole moment and the magnetic field. I would call it nuclear magnetic shielding (denoted as σ) rather than chemical shift (δ). Shielding is specific to a given nucleus placed in a molecule. On the contrary, chemical shift depends on the reference molecule, *e.g.*, chemical shifts of fluorine in NF_3 and HF measured relative to $CFCl_3$ have opposite signs. However, it does not follow that 19F is shielded in one case and in the other is deshielded. Instead, the electrons in these molecules reduce the strength of the field near the nucleus in all three compounds listed above.

Eq. (2.15) in the isotropic motion case implies that the induced field has the same direction as the external field. This disagrees with the statement, "This circulating current generates a magnetic field opposed to the external field (Lenz law), called an induced field."

At the beginning of the next section (2.1.6.2), there is a statement that "**un**like a chemical shift, J-coupling is magnetic-field independent." Unless in special circumstances, both quantities are generally considered field-independent (see, for example, ^{59}Co NMR studies described in *Phys. Chem. Chem. Phys.*, **22**, 8485-8490, 2020).

The third chapter describes zero-field NMR of organophosphorus compounds, *e.g.*, trimethyl phosphate and phosphite. In section 3.2.2, the magnetic shield's attenuation is estimated at ~60 dB. This impressive number, in my opinion, requires additional comment about the efficiency of suppressing the unwanted magnetic field generated by other external sources. As far as I understand, the shim coils actively shielded the sample. However, I did not find any further details in the text. Alternatively, shims could be static, and the effect of the unwanted external magnetic field caused baseline drift and was removed by fitting as described in section 3.2.3. Another technical detail is the question of the impact of the relatively small bandwidth of the sensor on the intensities of the NMR signals. Were any deviations from the peak intensities found by simulations in Mathematica observed? *E.g.*, are the peaks at high frequency small in Fig. 3.6 because of the nature of the compound, or is this a consequence of the sensor bandwidth?

The fourth and fifth chapters are devoted to the effect of the chemical exchange on zero-field NMR spectra. In particular, the influence of pH of the solution and proton-deuteron exchange in urea, analysis of spectra of amino acids, and signal amplification by reversible exchange involving parahydrogen. For me, in section 4.3.2, there is a missing comment about comparison between zero-field results and the high-field studies of urea proton exchange rate. Fig. 4.2 clearly shows the disappearance of nitrogen-15 labeled urea peak at low and high pH, but it is unclear if the coalescence point of the line is roughly in agreement with the literature, *e.g.*, 100−103 references in the thesis. This information could allow judgment about the possible effect of the field strength effect on the exchange rate. Please also notice that, in fact, there are at least two exchange rates as you are dealing with at least three chemical entities, *i.e.*, that shown in Eq. 4.1 and 4.2 on page 72, resulting in several nonequivalent protons coupling to nitrogen, so this is a very complex system.

At the EUROMAR conference in Utrecht this year, I had the pleasure to meet the Ph.D. candidate presenting the poster entitled "Parahydrogen-based Hyperpolarization of Biomolecules via Chemical Exchange," which covered the results presented in her Ph.D. thesis in chapter six. Our discussion during the conference strengthened my conviction about the Ph.D. candidate's high fluency in the NMR field. Furthermore, the high degree of hyperpolarization of biomolecules by chemical exchange of labile protons obtained by the Ph.D. candidate shows promising progress in wider usage of hyperpolarization in the NMR community and may find application in many magnetic resonance studies going far beyond the ultralow-field conditions making the work very valuable.

The seventh chapter is devoted to NMR studies of relaxation due to paramagnetic species such as oxygen and copper salts in the ultra-low magnetic field. Section 7.3.1 discusses the effect of successive freeze-pump-thaw cycles on trimethyl phosphate and formic acid. It would be helpful to give even a raw estimation of the oxygen content in that section. As the article describing the results is being prepared for publication, I would suggest an improvement in the control of the oxygen content. The Ph.D. candidate used a vacuum line in her studies. Why not add an additional small flask with air, reduce the pressure, and saturate the sample using the expanded gas? This method requires a minor modification of the experimental setup and gives fairly precise (within a few percent) information about the oxygen content in the sample.

In the bottom half of the page 122, the reader is redirected to section 7.2.2 regarding the biexponential decay of the signal. Actually, section 7.2.2 does not contain any information about it. Could the Ph.D. candidate give more details? For example, did she find this kind of decay in her studies, and how she treated it? This should be visible, for instance, from the line shape and the T_1 relaxation curve.

In section 7.3.2, it would also be helpful to compare the experimental T_1 relaxation time of water at zero-field conditions with theory and explicitly state the theoretically expected ^{1}H longitudinal relaxation time of water at the low-field conditions. On page 124, there is a T_1 relaxation time of water shown. Could the Ph.D. candidate comment on why the relaxation time for pure water increased with the field?

In summary, the Ph.D. thesis written by Seyma Alcicek's covers a broad range of topics of great importance to the NMR community and contains many interesting experimental results. My comments about the thesis covered the points that were unclear for me and did not affect my very high assessment of the Ph.D. thesis. Therefore, the thesis satisfies all criteria required for awarding a Ph.D. degree to the candidate (according with the act "Prawo o szkolnictwie wyższym i nauce"; Dz. U. z 2020 r., poz. 85 z późn. zm.). Moreover, I am applying for a distinction of Seyma Alcicek's Ph.D. thesis because she collected and analyzed low-field NMR spectra of numerous biologically relevant molecules and then experimentally showed how to make their observation feasible and useful by application of hyperpolarization techniques.

Digitally signed by
Piotr Garbacz;
Uniwersytet
Warszawski
Date: 2022-09-21
16:04:13

Dr hab. Piotr Garbacz
Nuclear Magnetic Resonance Laboratory
Faculty of Chemistry
ul. Ludwika Pasteura 1, 02-093 Warsaw
University of Warsaw
tel. 22 55 26 346
e-mail: pgarbacz@uw.edu.pl