P&L Scientific

Electrophoretic NMR Instrumentation and Methodology

Extending the capability of conventional NMR instruments

eNMR experimental setup

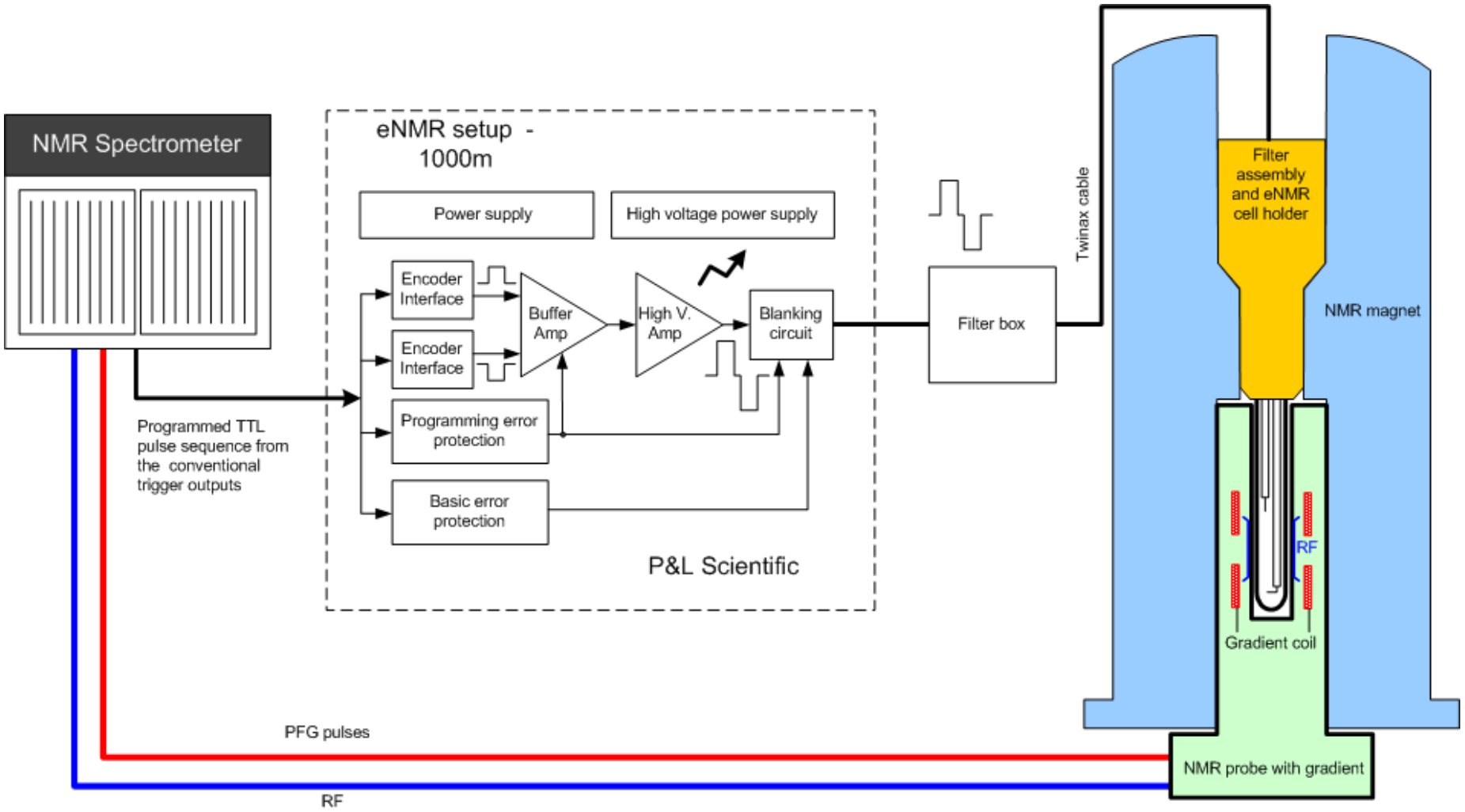
NMR spectrometer

eNMR cell

NMR magnet

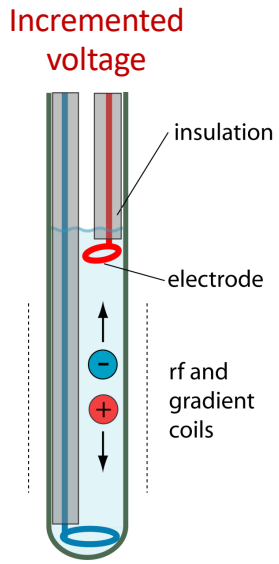
Main unit with encoder, high voltage amplifier, and the safety protection system

Experimental setup. Block diagram



The block diagram of the eNMR assembly

Methodology



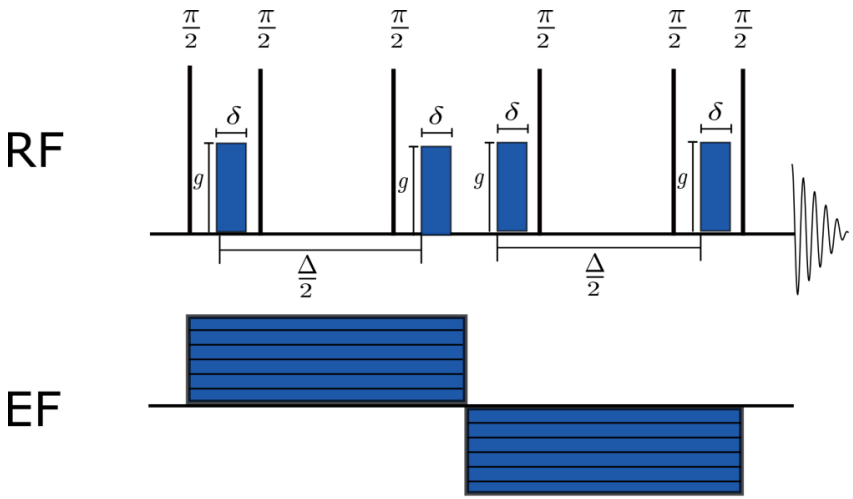
Electrophoretic NMR (eNMR)
with the voltage incremented

Detection of displacement yields the **electrophoretic mobility**. The combination of the **self-diffusion coefficient** and the electrophoretic mobility yields the **effective charge**, a measure of ion association.[1]

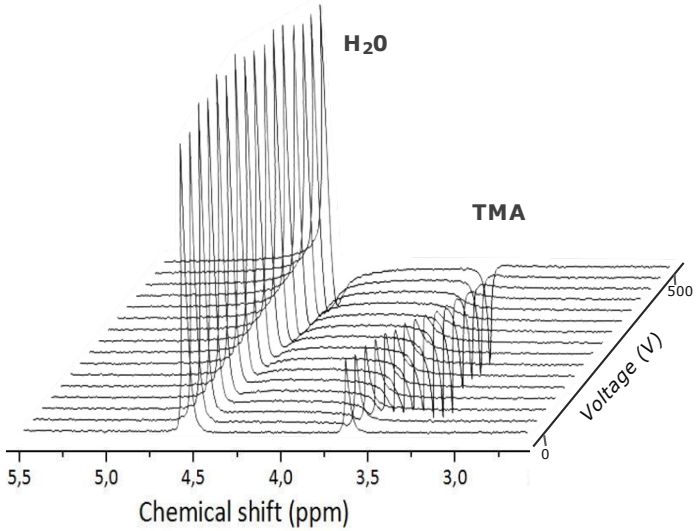
Methodology

Electrophoretic NMR (eNMR) - based on pulsed field gradients, akin to diffusion NMR

A double-stimulated-echo pulse sequence for eNMR



Resulting series of spectra

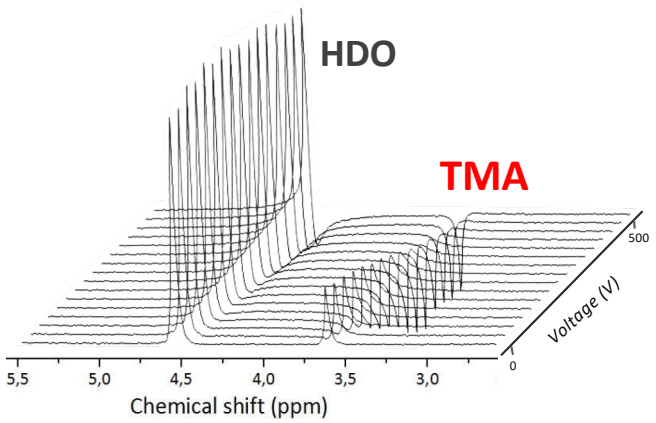


The signal is phase modulated: $\frac{S}{S_o} \propto \exp(-\gamma^2 g^2 \delta^2 D\Delta) \exp(i\phi),$ $\phi = \gamma g \delta \boldsymbol{\mu} E \Delta$

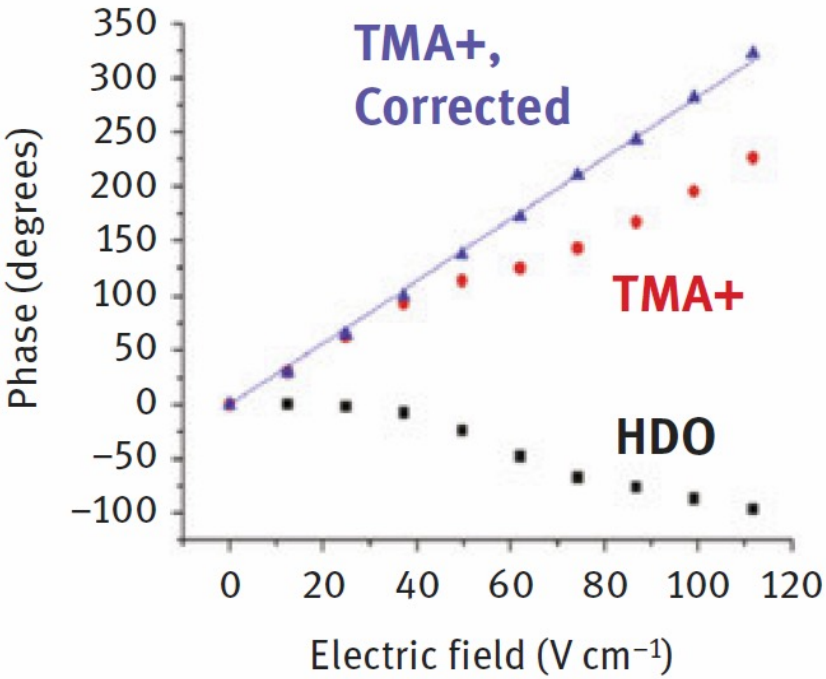
$$\mu = \frac{\phi}{\gamma \delta g \Delta_E E}$$

Experiment and Operation

Results:



Processed data [1]:



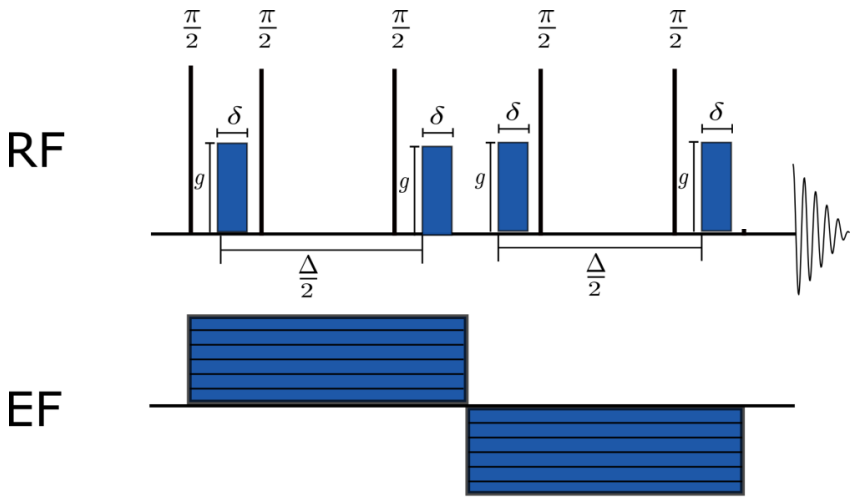
$$\mu = \frac{\phi}{\gamma \delta g \Delta_E E} \qquad z = \frac{\mu k_B T}{De}$$

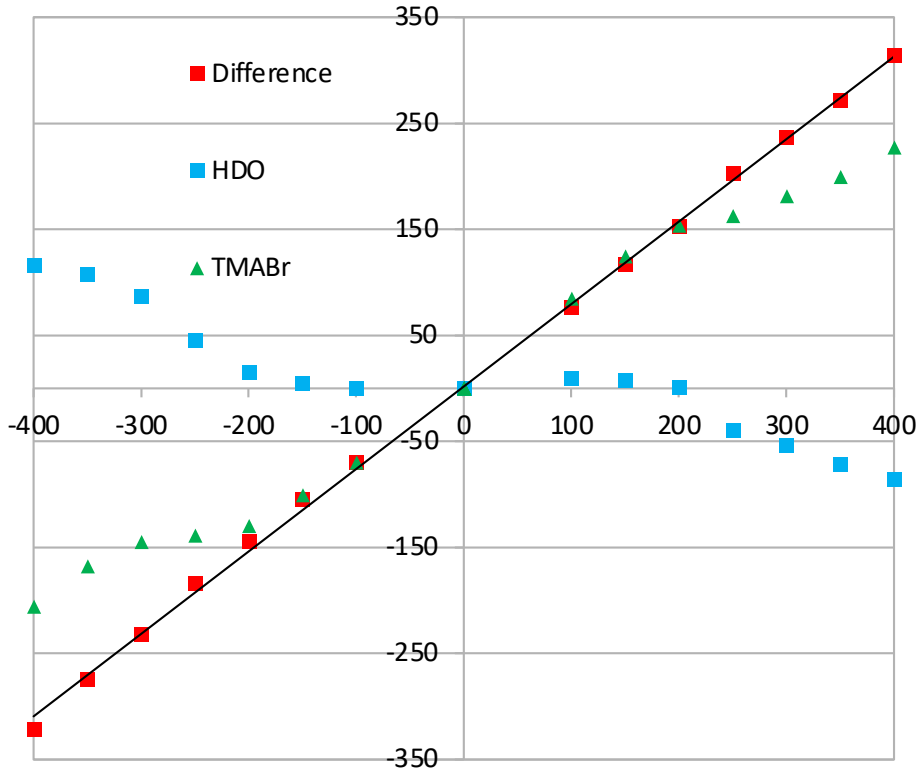
The electrophoretic mobility and the effective charge can be obtained by recording the variation of spectral phase by increasing electric field

Experimental artefacts and the way to solve it

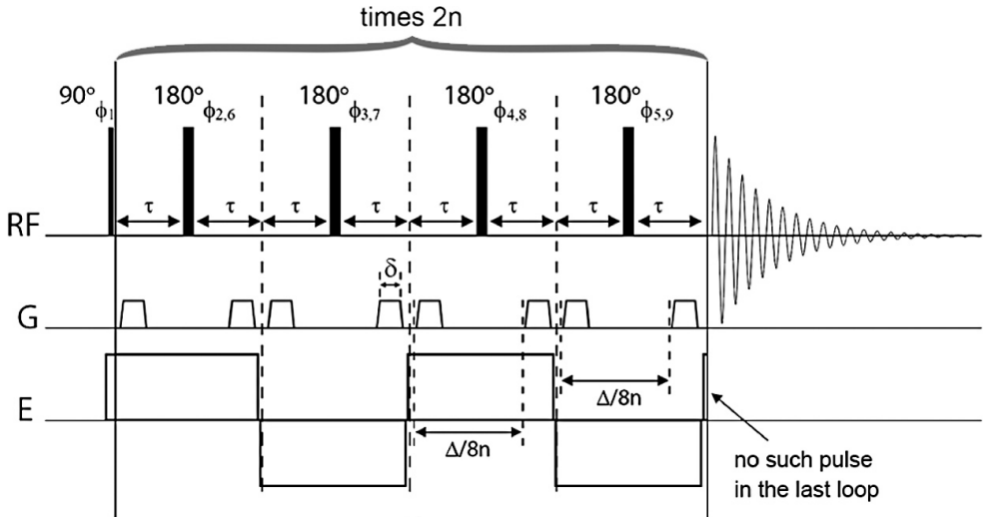
Some experimental artifacts may cause big signal attenuation and the artificial phase shift:

- **Thermal convection** in highly conductive samples
- **Electroosmosis**
- **Gas buble production due to electrolysis**





Experimental artefacts and the way to solve it

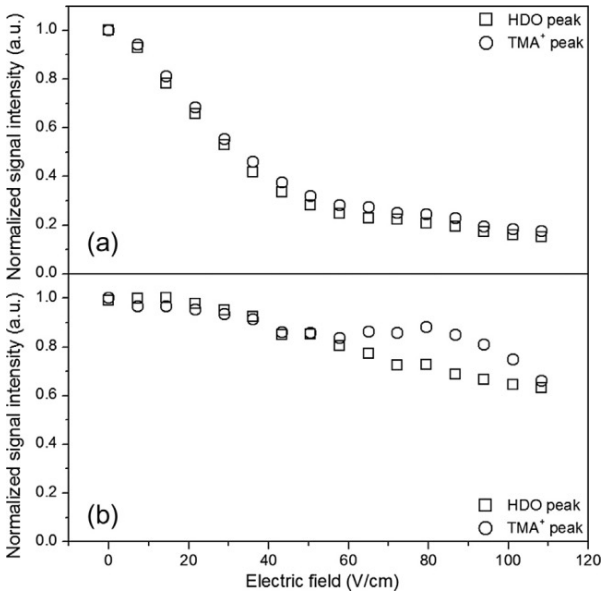


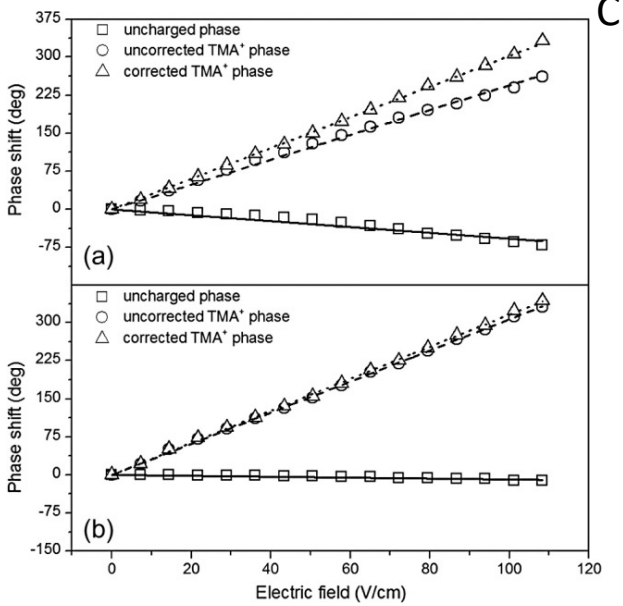
A **fast switching** of the **electric field may** counteracts some of these issues [5]

DSTE

CPMGER

Reduced intensity loss





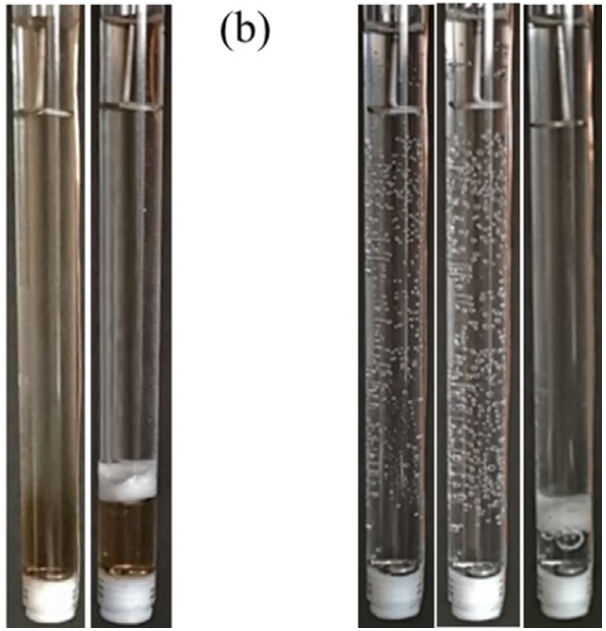
CPMGER
n=1

n=7

Reduced phase shift for uncharged reference when increasing n

Experimental artefacts and solutions

(a) (b)

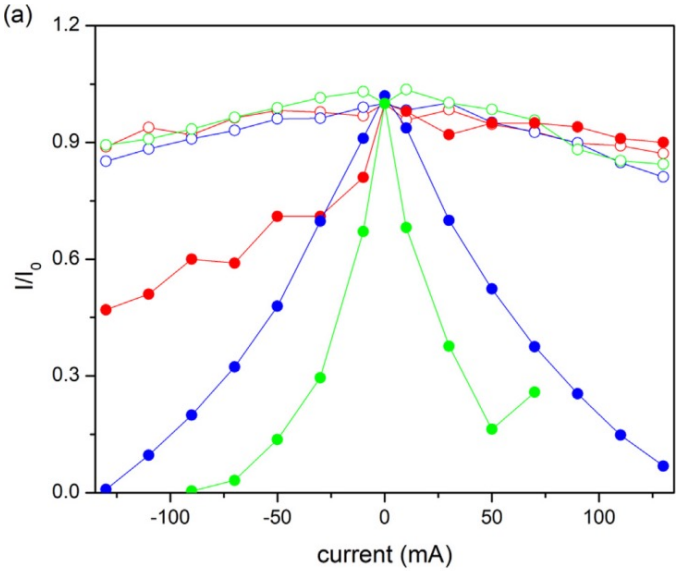


100 mM TMABr in D_2O

PNIPAM with 200 mM NaSCN in D_2O solution.

a) The porous plug retains the electrode reaction products out of the sensitive region. TMABr
b) Gas bubbles are confined by the plug at the bottom. PNIPAM sample

Attenuation of the signal with and without the plug

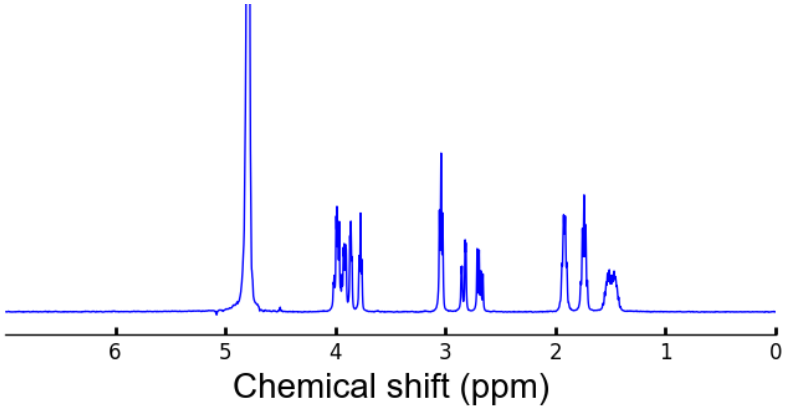


The symmetric construction of the eNMR cell and the filter plug helps [2]. The current stabilization mode in eNMR unit is required

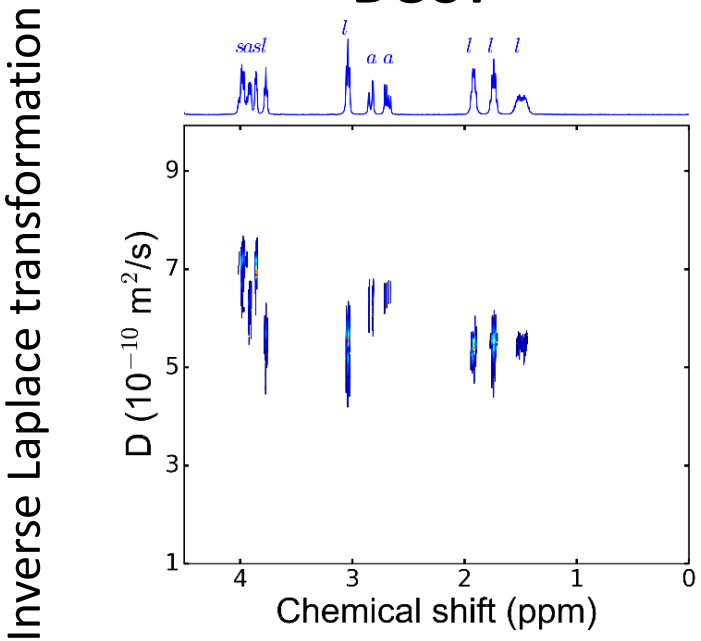
Application. 2D electrophoretic mobility ordered spectroscopy (2D-MOSY) [3]

A mixture of L-Lysine (l), L-Serine (s) and L-Aspartic acid (a)

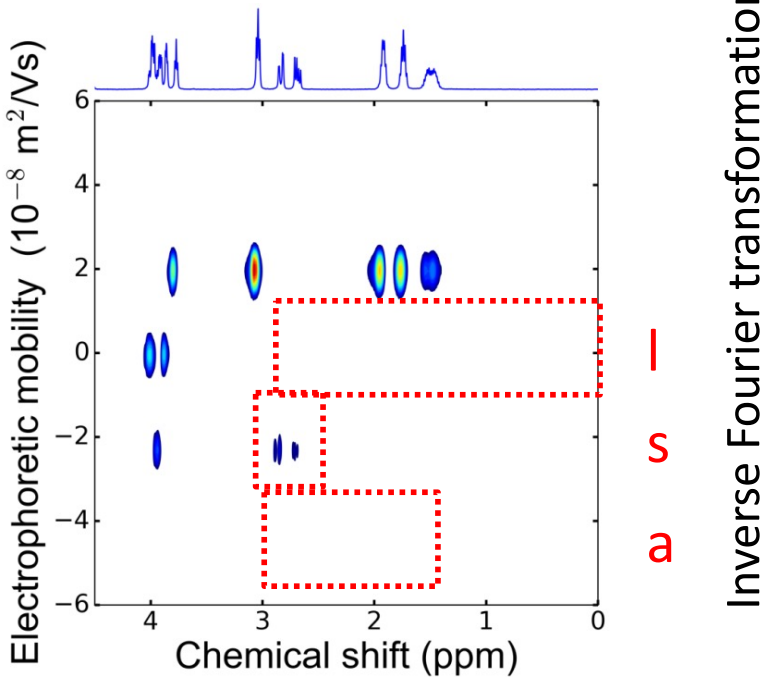
Diffusion Ordered SpectroscopY (DOSY)



DOSY



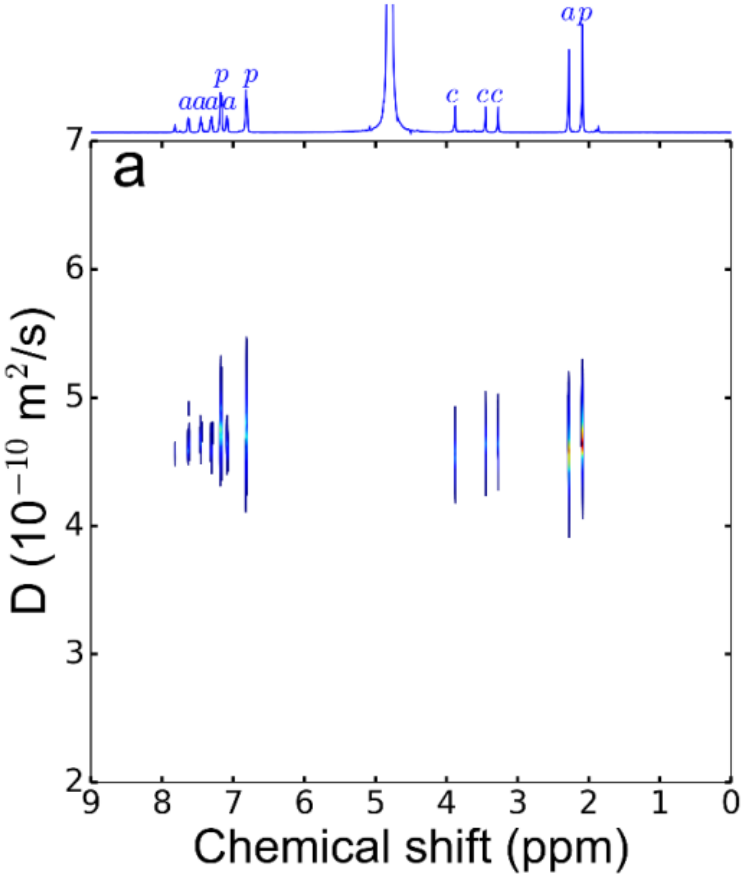
MOSY



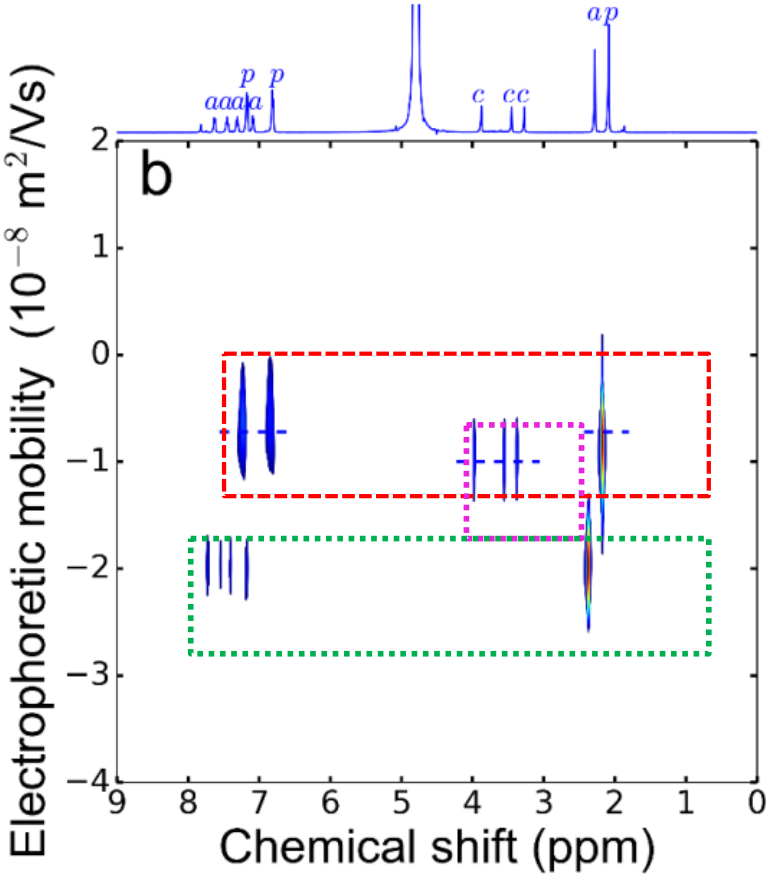
eNMR 2D-MOSY for analytical applications [3]

Analysis of components of a drug

DOSY



MOSY

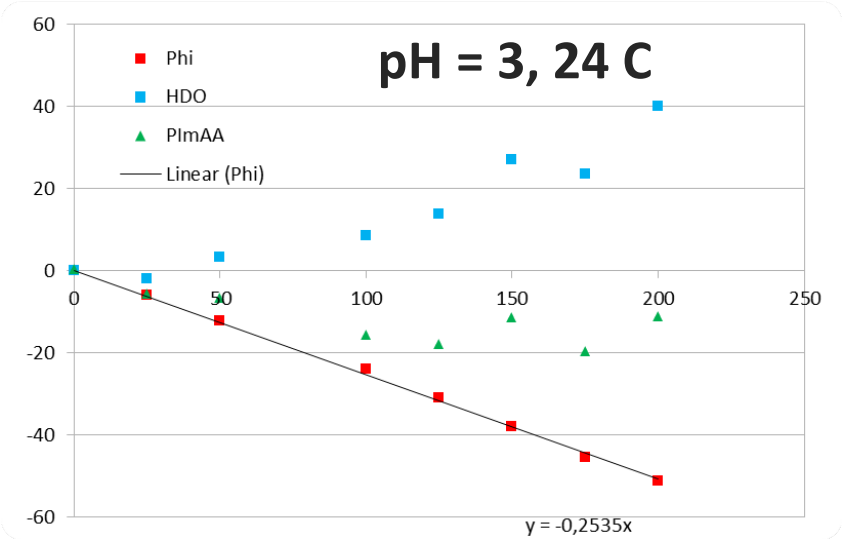


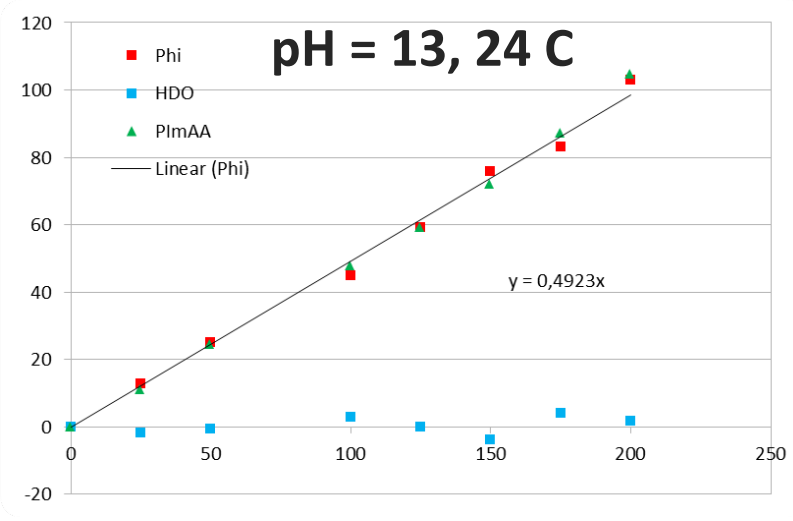
Not separated

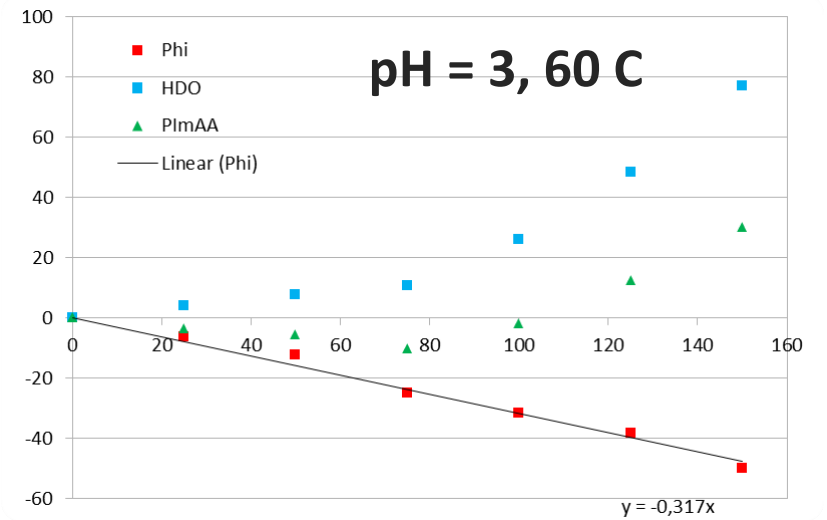
Separated

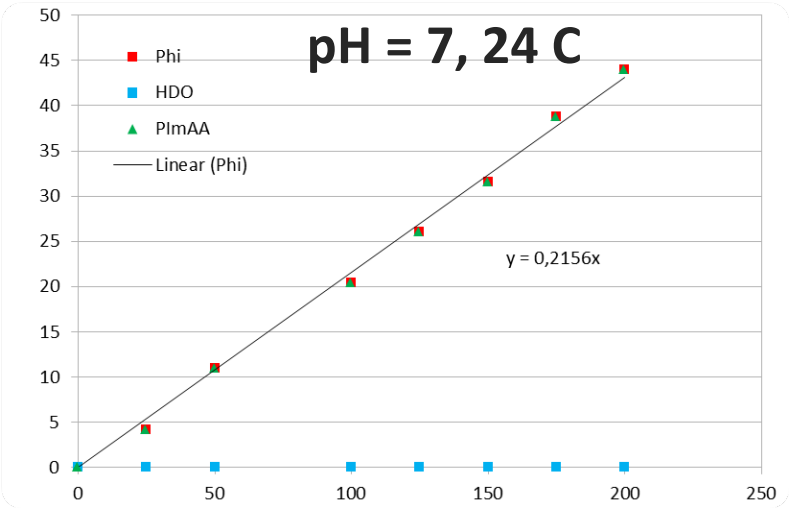
Application examples

Investigation of zwitterionic behaviour in different pH



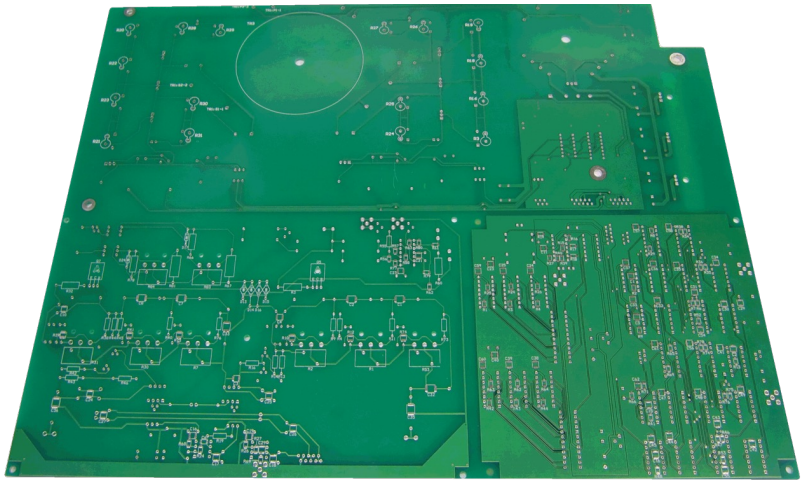






Specifications of eNMR unit

Output voltage:	0 to ±1000 V
Digital To Analogue converter size: 2×12 bit	
Output current	
at ±1000 V:	0 to ±50 mA
at ±500 V:	0 to ±200 mA
Output power:	
Peak power	300 W
Mean power	30 W
Minimum/maximum pulse length:	500 µs / 30 s
Duty cycle:	30 %
Output pulse shapes:	Rectangular
Slew rate:	Greater then 25 V / µs
Settling time (to 2%):	Less than 200 µs for 2 kV step
Stability	
Drift with time	Less than 100 ppm/hr, noncumulative
Drift with temperature	Less than 300 ppm/°C
RF Filters	10 MHz low pass
Power consumption:	80 W for 220 V AC
Dimensions of the main unit:	430×340×90 (fits in standard 19'' rack)
Weight:	7 kg



Key features

- Well-established scientific background and proven technology
- Operates as add-on for any conventional NMR spectrometer and probe; requires no additional hardware or software
- Advanced sample cell and RF filter system
- Straightforward embedding of high voltage pulses in conventional NMR pulse programs
- Probe and user protection system

Selected applications of electrophoretic NMR

Physical chemistry – ion pairing and association in simple and complex (polyelectrolytes) ionic mixtures.

Batteries and fuel cells - chemically selective measurement of ionic migration.

Biochemistry – biomolecular charge and association.

Analytical chemistry – electrophoretic analysis of complex ionic mixtures

Pharmaceutical chemistry – release and association of charged drugs

Metallorganic chemistry – the structure of supramolecular complexes from the observed charge

Conclusions

eNMR together with diffusion NMR provide a powerful tool to study the ionic solutions and complexation/binding of ions with macromolecules in solution. It is especially good at detecting weak interactions

Two dimensional eNMR (MOSY) is superior than DOSY in conventional chemical analysis and has great potential to be used in drug discovery and metabolomics

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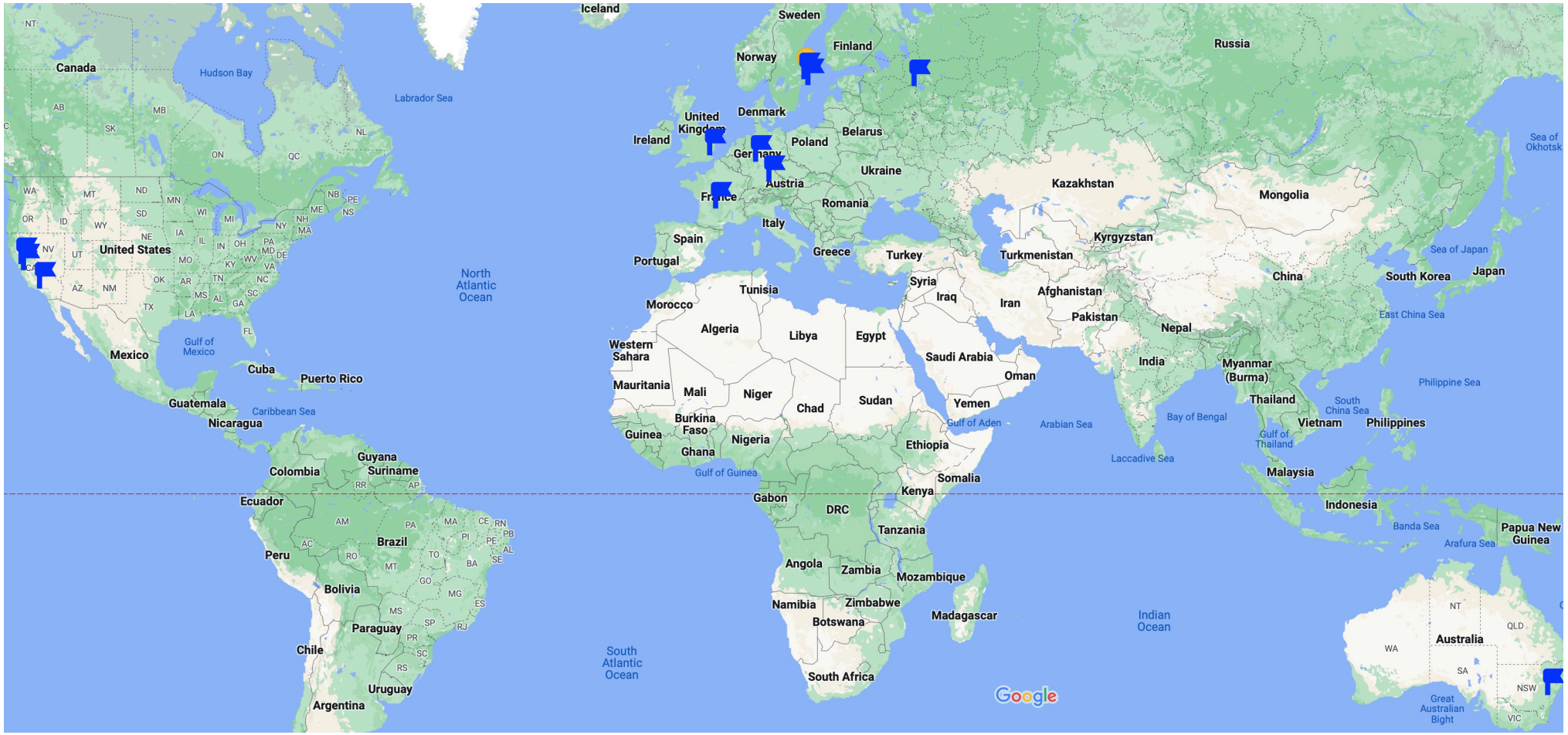
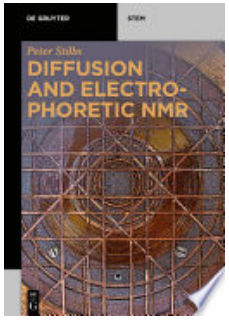
References



Selected literature



1. Peter Stilbs. Diffusion and Electrophoretic NMR, Series: De Gruyter STEM 2019, ISBN : 3-11-055165-9 ISBN : 3-11-055153-5

2. Yuan Fang, Pavel V Yushmanov, Istvan Furo, Improved accuracy and precision in electrophoretic NMR experiments. Current control and sample cell design; July 2020, Journal of Magnetic Resonance
3. Y. Fang, P. V. Yushmanov, and I. Furó, Assessing 2D electrophoretic mobility spectroscopy (2D MOSY) for analytical applications, *Magn. Reson. Chem.* **55** DOI: 10.1002/mrc.4558 (2017).
4. M. Giesecke, F. Hallberg, Y. Fang, P. Stilbs, and I. Furó, Binding of monovalent and multivalent metal cations to polyethylene oxide in methanol probed by electrophoretic and diffusion NMR, *J. Phys. Chem. B* **120** 10358−10366 (2016).
5. M. Bielejewski, M. Giesecke and I. Furó, On electrophoretic NMR. Exploring high conductivity samples, *J. Magn. Reson.* **243** 17-24 (2014).
6. L. Patel, O. Mansour, M. Crossman, P. Griffiths, Electrophoretic NMR characterization of charged side chain cationic polyelectrolytes and their interaction with the anionic surfactant, sodium dodecyl sulfate, *Langmuir* 2019, 35, 28, 9233-9238