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Probing DNP Enhancement for Biological Samples



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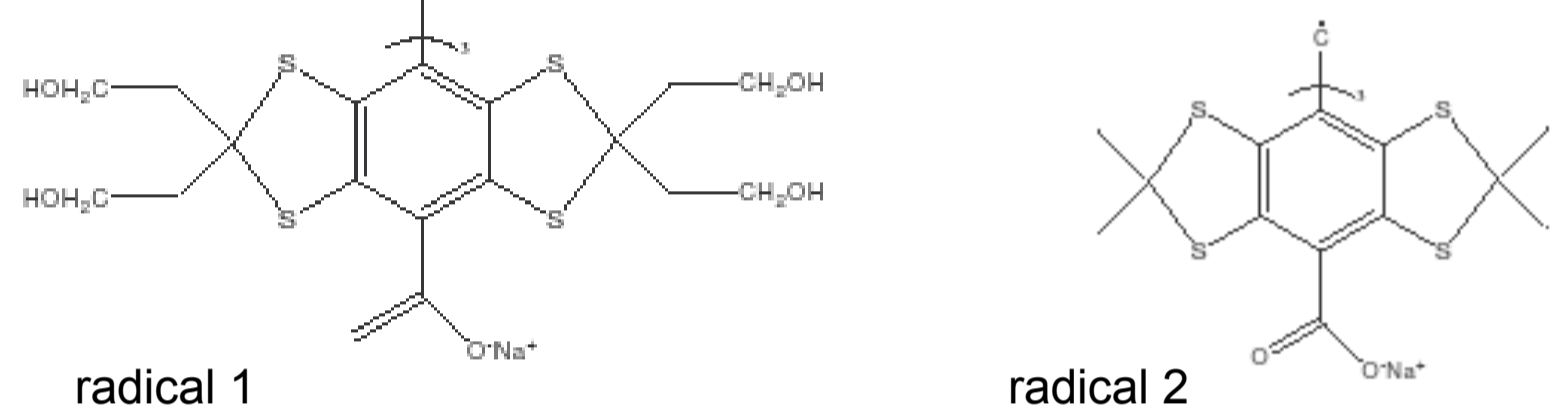
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Introduction

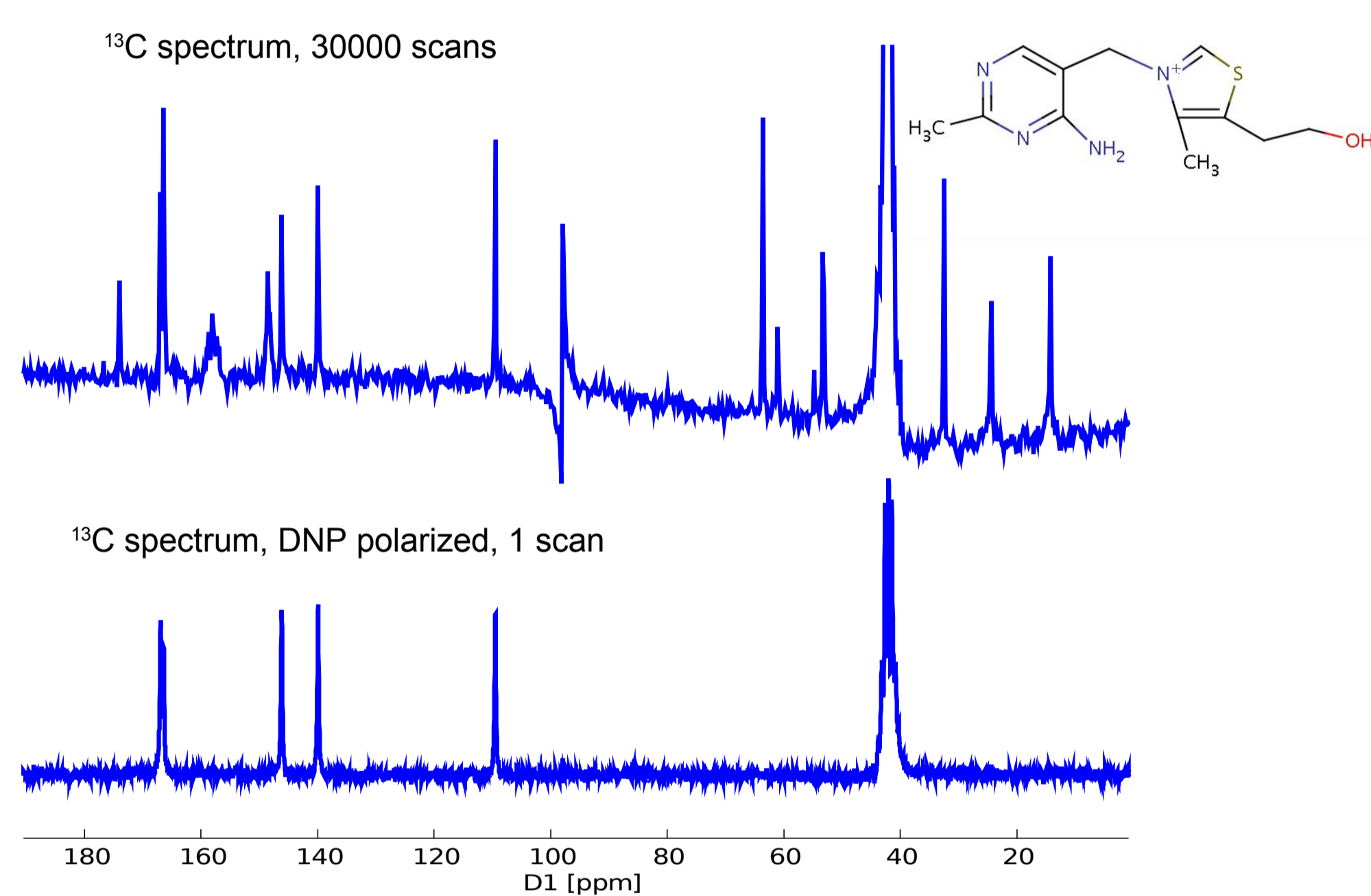
Dynamic Nuclear Polarisation (DNP) is used to transfer the high spin polarization of unpaired electrons to coupled nuclear spins. This is achieved by doping samples with stable free radicals and microwave irradiation of the EPR lines of the radical. In the past signal enhancements of ~200 were achieved in the solid state [1,2]. In supercritical ethylene enhancements in the range of 40-60 were observed [3]. With a more recent implementation of a DNP polarizer where samples are polarized at low temperature and subsequently dissolved in hot solvent and transferred to a NMR spectrometer enhancements of ~10,000 were reported [4].

In this work we have used the recently developed HyperSense® DNP system from Oxford Instruments based on the design described in [4] to evaluate the possible gain in sensitivity for a variety of biologically relevant samples, including metabolites, vitamins and different fruit juices. Signal enhancements for up to 13,000 were observed. We have examined the effect of different radicals, water vs. MeOH as solvents and the composition of the samples to optimise enhancement factors.

Trityl radicals used for the polarisation

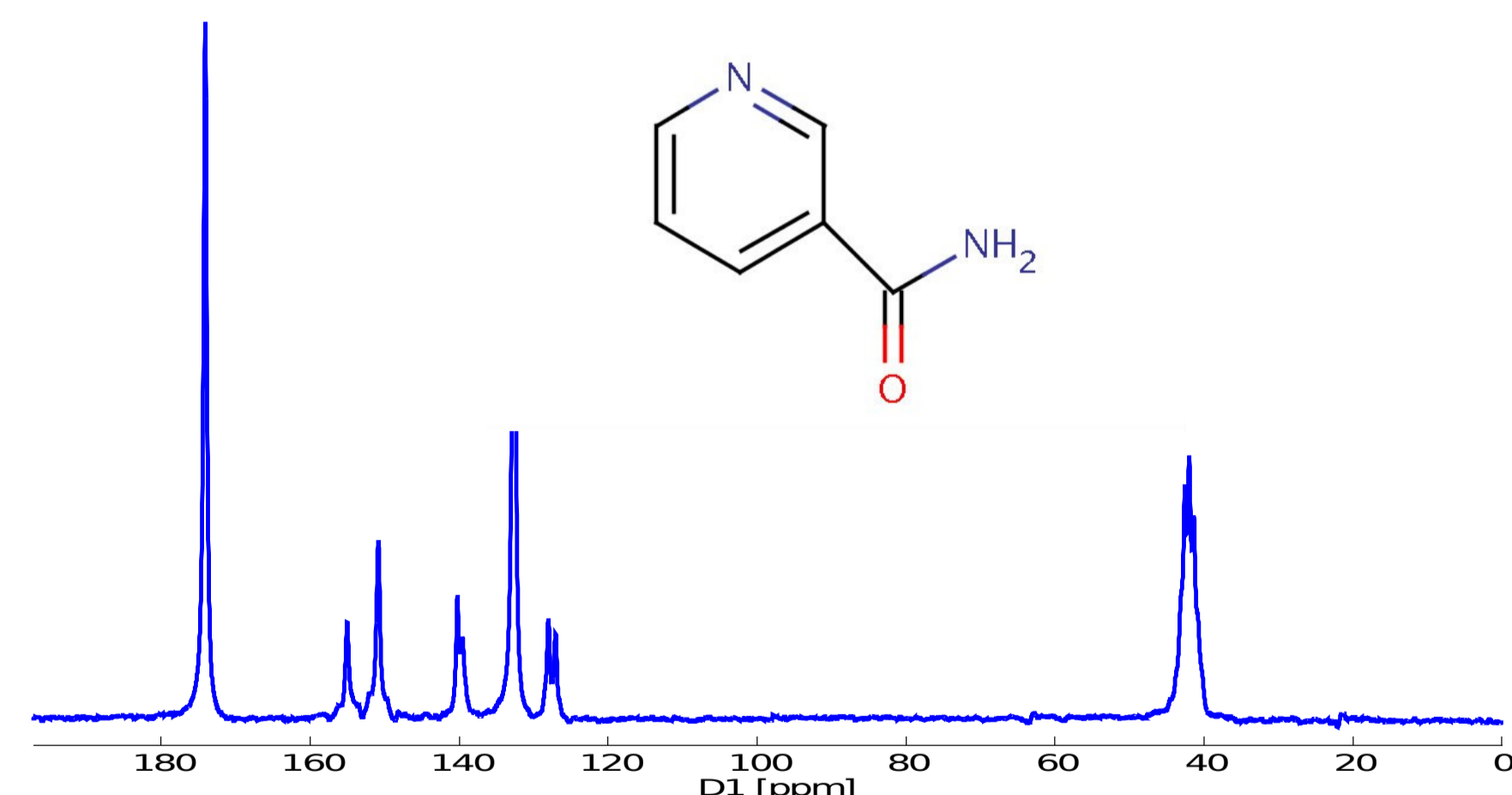


Signal enhancements for vitamin B1



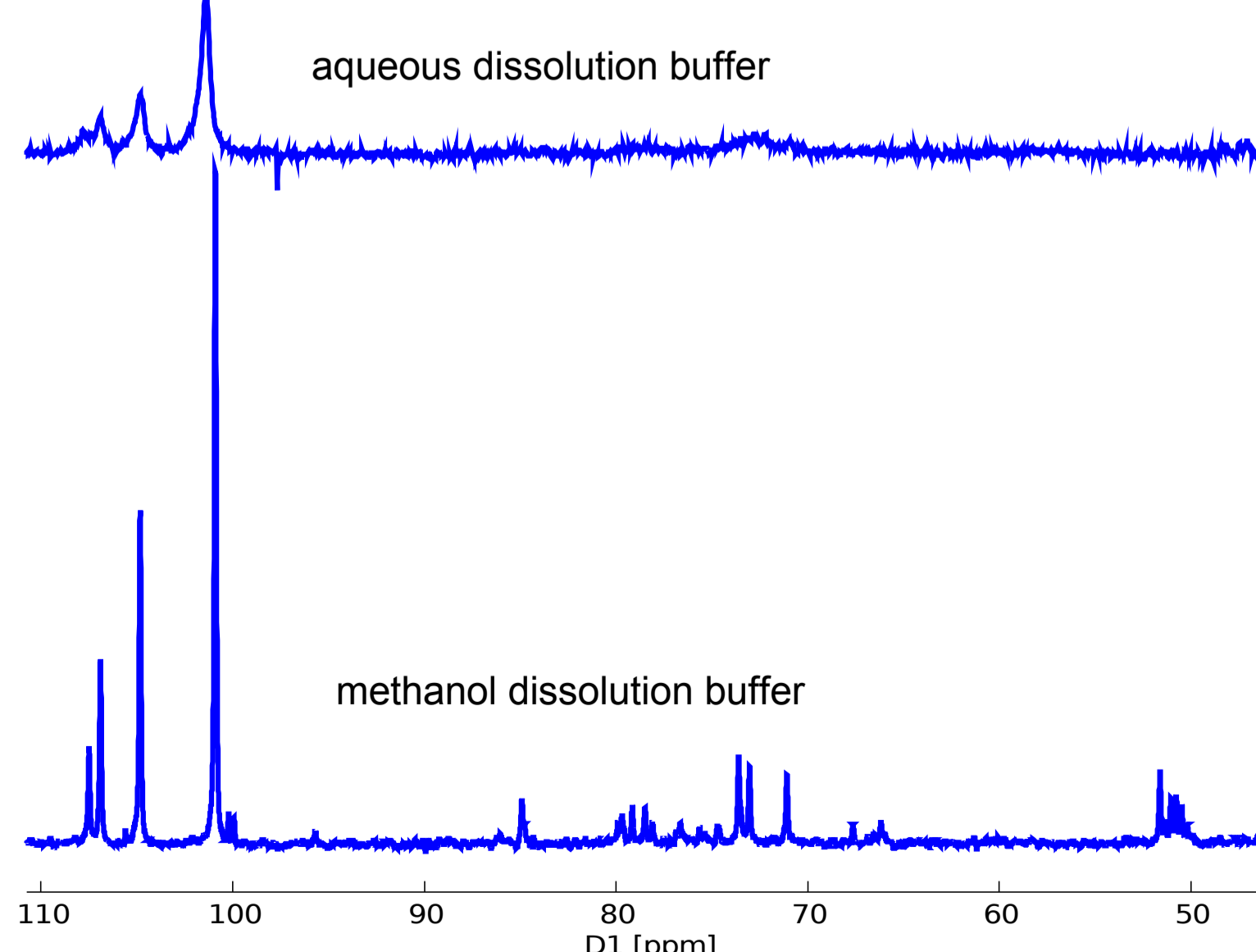
Comparison of a ¹³C spectra of vitamin B1 (thiamin) recorded on a 500 MHz system (11.75T) top with 30000 scans, bottom with one scan after DNP polarisation. The sample was polarised at 1.5K over 90 min. in a glass state using radical 1. Spectra were recorded with the same sample under identical conditions. An enhancement factor of approximately 1200 was achieved.

Signal enhancements for vitamin B3



¹³C spectra of vitamin B3 (niacinamide) recorded on a 500 MHz system (11.75T) after polarisation at 1.5 K over 90 min using radical 1. An enhancement factor >10000 was achieved.

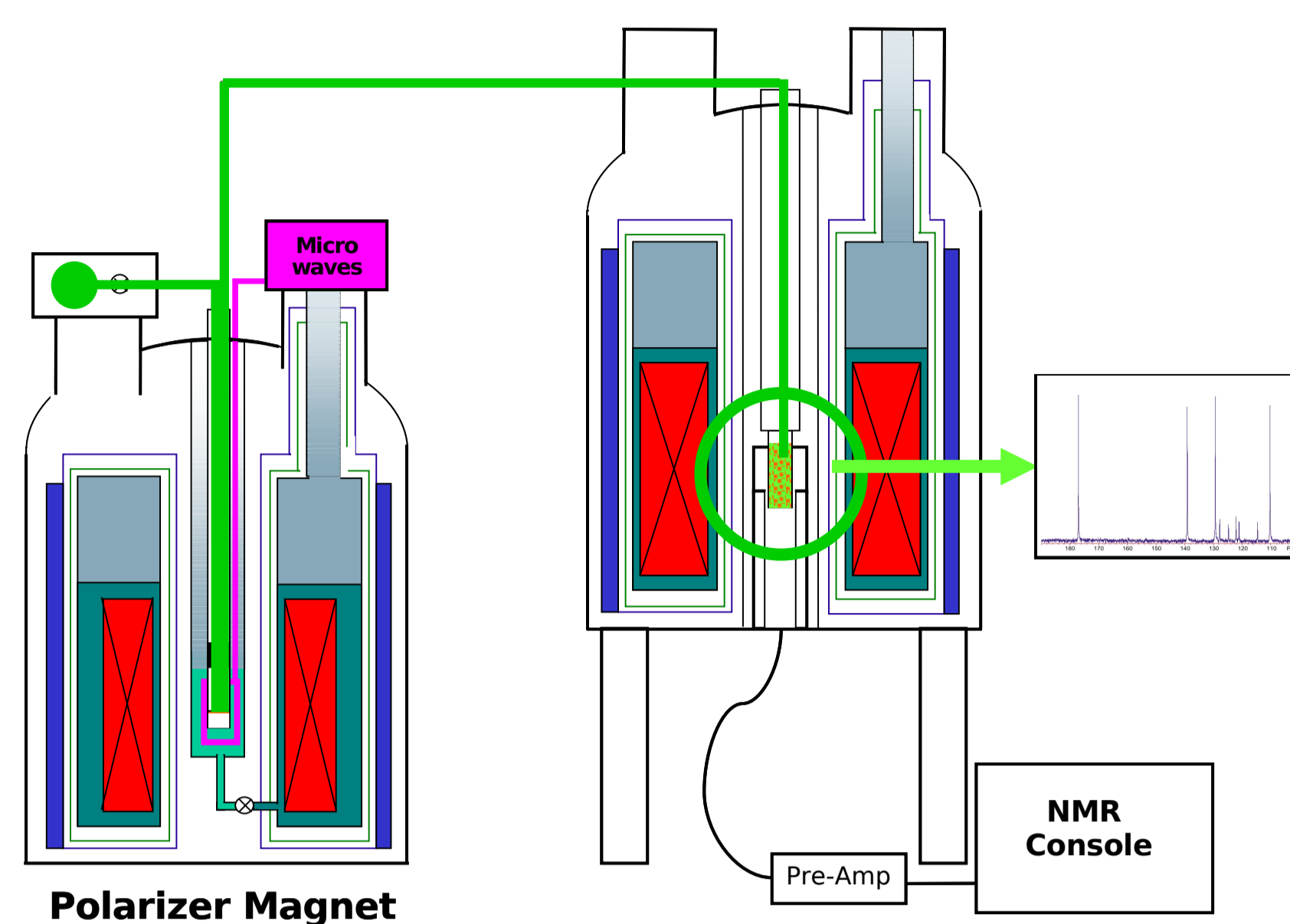
One-scan ¹³C spectra of fruit juices



To test the potential of DNP for the analysis of biological samples a series of juices was analysed. The figure shows one-scan ¹³C spectra of an apple juice which was dried and redissolved in a mixture of DMSO and water.

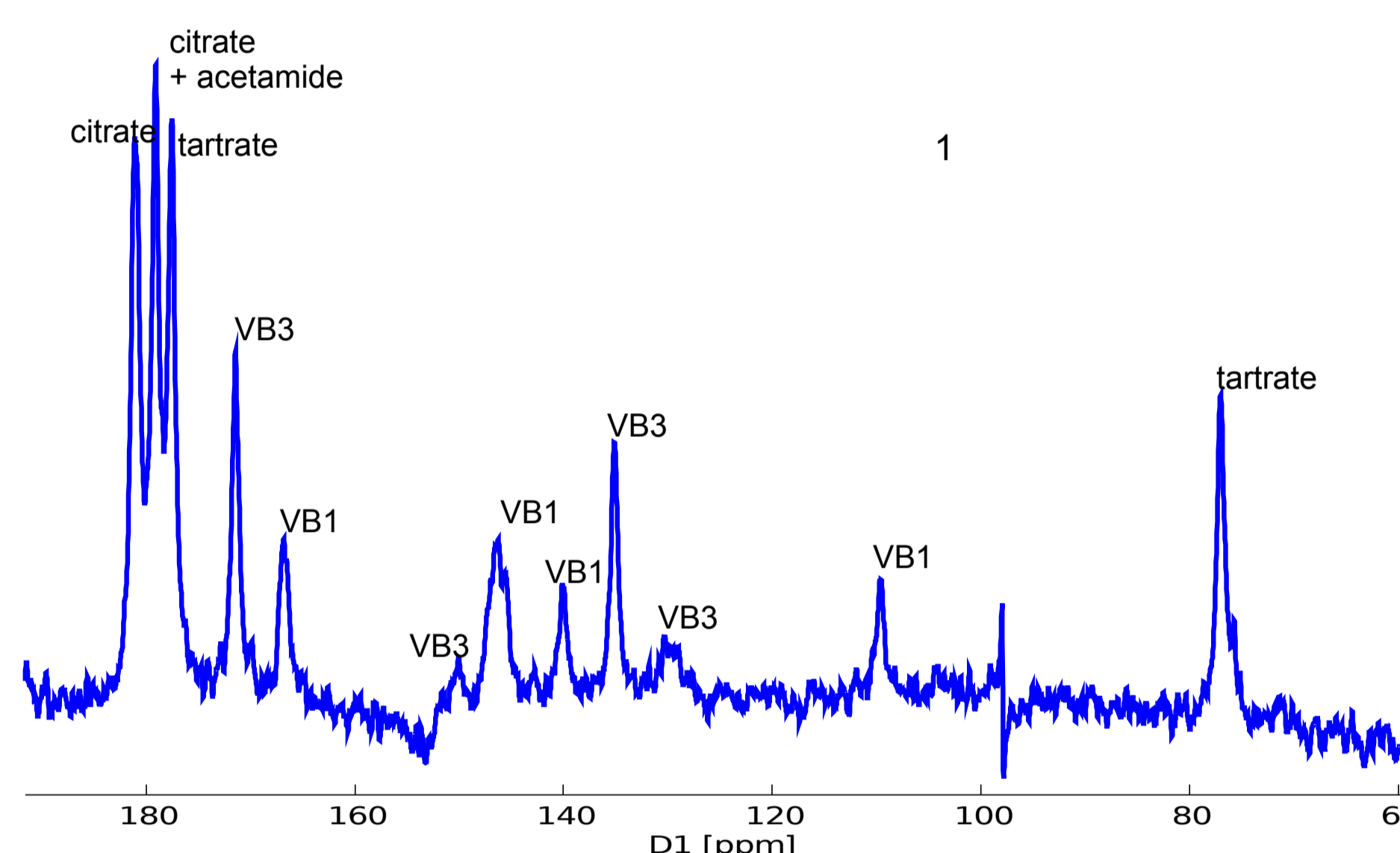
Top: The sample was dissolved in water after 90min of polarisation using radical 1. **Bottom:** The sample was dissolved with methanol after an overnight polarisation with radical 1. Dissolution in methanol has significant advantages for the transfer of the sample into the NMR tube owing to the better flow characteristics of methanol compared to water leading to significantly lower line widths in the spectrum.

The HyperSense® DNP Polariser



Left: Schematic representation of the HyperSense® DNP polariser: The sample is polarised at low temperature (~1.5K) in a superconducting magnet (3.35T) by MW irradiation at ~94 GHz. The polarised sample is dissolved in the polarisation magnet and transferred to a magnet at higher field (11.75T) where a ¹³C or ¹⁵N signal is detected. Right: HyperSense® DNP polariser.

One-scan ¹³C spectrum of a compound mixture



A mixture of 5 compounds (citrate, acetamide, tartrate, vitamins B1 and B3) was used to test the potential of DNP for metabolomics samples. An enhancement of approx 800 was achieved after 90 min of polarisation time at 1.5K using radical 1. The overall signal enhancement was lower than compared to enhancement obtained for the individual compound spectra. Further improvement may include an optimised radical concentration and measures to obtain a reduced line width in spectra, e.g. the use of methanol as solvent or a flow system.

DNP Enhancement factors for different samples

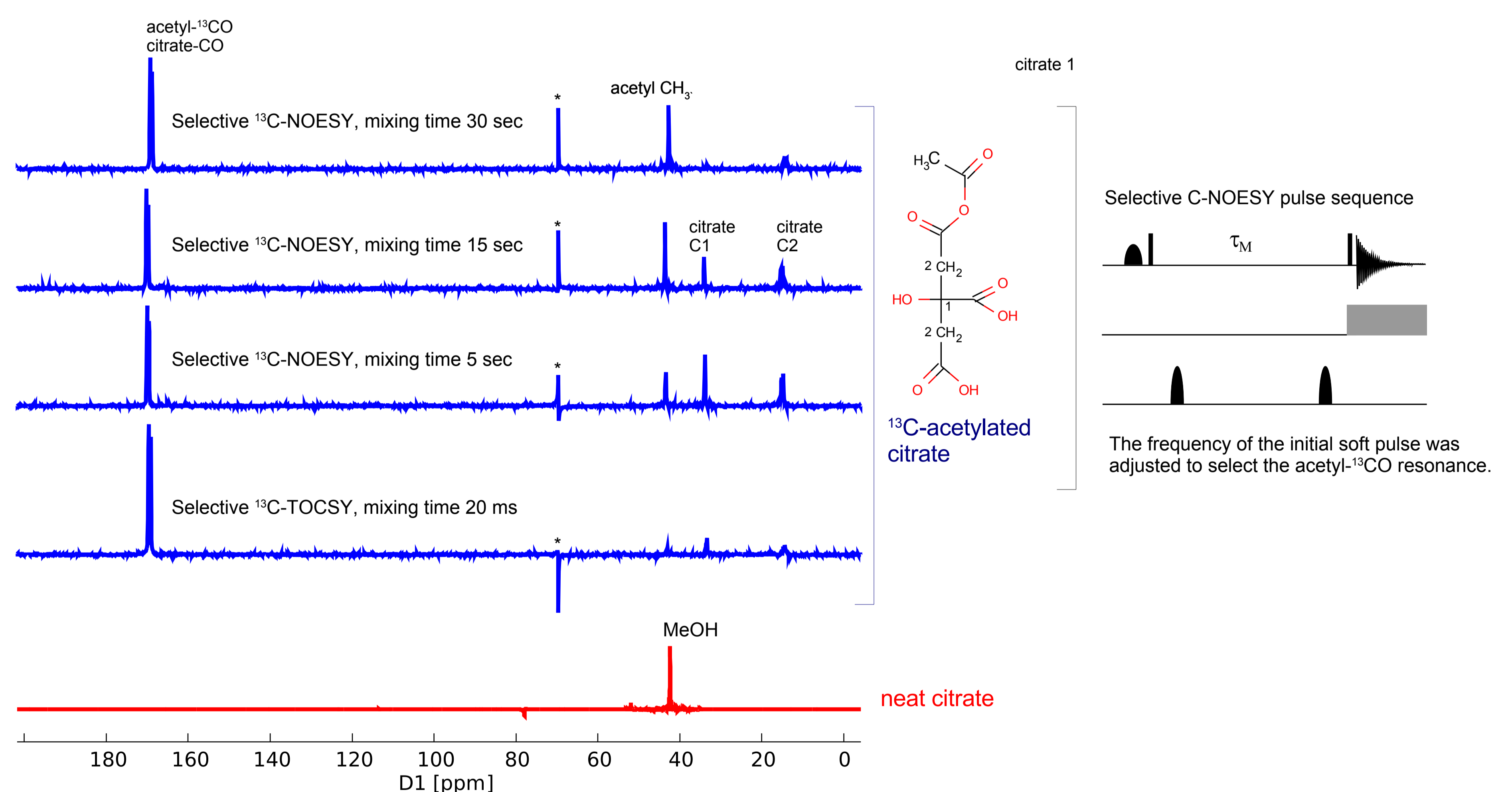
Compound	Enhancement factor	Radical
Vitamin B1	1200	1
Vitamin B3	13000	1
Acetamide	12000	1
Formic acid	6600	1
Citric acid	260	1
Phthalic acid	4800	1
Tartaric acid	840	1
Arginine	2700	1
Histidine	300	1
Glucose	0	1
Citrate	0	1
Glucose	1000	2
Juices	700 – 2000	1

Enhancement factors were estimated by comparing spectra of identical samples recorded with DNP enhancement vs. large numbers of scans. Signal integrals were considered to account for variations in signal line width.

Radical 1 was superior for most samples due its significantly higher solubility in water. Radical 2 caused sample precipitation for most of the test samples.

Glucose is an interesting exception where no signal was observed for radical 1 whereas radical 2 lead to a modest signal enhancement.

Transfer of Polarisation after DNP enhancement using acetylated compounds



Different schemes of polarisation transfer from DNP enhanced signals were evaluated. For this purpose citrate was acetylated using a ¹³CO-labelled acetyl group. While neat citrate shows no signal after DNP enhancement owing to the fast relaxation of its ¹³C-atoms, all citrate signals can be observed after C-TOCSY or C-NOESY polarisation transfer from acetyl-¹³CO. The C-NOESY polarisation transfer is significantly more effective than the C-TOCSY transfer and shows a significant enhancement of citrate atoms 1 and 2 even after 15 sec of mixing time.

References

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