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# Application of a Microcoil Probe Head in NMR Analysis of Chemicals Related to the Chemical Weapons Convention

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A 1.7-mm microcoil probe head was tested in the analysis of organophosphorus compounds related to the Chemical Weapons Convention. The microcoil probe head demonstrated a high mass sensitivity in the detection of traces of organophosphorus compounds in samples. Methylphosphonic acid, the common secondary degradation product of sarin, soman, and VX, was detected at level 50 ng (0.52 nmol) from a 30- $\mu$ L water sample using proton-observed experiments. Direct phosphorus observation of methylphosphonic acid with  $^{31}\text{P}\{^1\text{H}\}$  NMR experiment was feasible at the 400-ng (4.17 nmol) level. Application of the microcoil probe head in the spiked sample analysis was studied with a test water sample containing 2–10  $\mu\text{g/mL}$  of three organophosphorus compounds. High-quality  $^1\text{H}$  NMR,  $^{31}\text{P}\{^1\text{H}\}$  NMR, 2D  $^1\text{H}$ – $^{31}\text{P}$  fast-HMQC, and 2D TOCSY spectra were obtained in 3 h from the concentrated 1.7-mm NMR sample prepared from 1 mL of the water solution. Furthermore, a 2D  $^1\text{H}$ – $^{13}\text{C}$  fast-HMQC spectrum with sufficient quality was possible to measure in 5 h. The microcoil probe head demonstrated a considerable sensitivity improvement and reduction of measurement times for the NMR spectroscopy in identification of chemicals related to the Chemical Weapons Convention.

The Chemical Weapons Convention (CWC),<sup>1</sup> signed in 1993, is the latest and most determined attempt to bring to an end the development, production, stockpiling, and use of chemical weapons. The Technical Secretariat of the Organisation for Prohibition of Chemical Weapons (OPCW)<sup>2</sup> is the governing body that implements the CWC internationally. Designated laboratories, nominated by the Director General of the OPCW, uphold the analytical capability to verify scheduled chemicals such as chemical warfare agents, their precursors, and degradation products in various matrixes (e.g., aqueous or organic liquid, soil, sand,

concrete, paint, or rubber). Verification of a scheduled chemical in a sample requires confirmation with at least two analytical, preferably spectrometric, methods. Therefore, the verification analysis has employed major analytical techniques like mass spectrometry combined with gas and liquid chromatography (GC/MS, LC/MS), infrared spectroscopy (IR), and nuclear magnetic resonance spectroscopy (NMR) for this task.<sup>3–5</sup>

Due to the modest sensitivity compared to mass spectrometric techniques, NMR spectroscopy has played a minor role in the CWC-related analysis. However, recent research in NMR spectroscopy has emphasized improvement in NMR sensitivity. Some of these technologies have also been adapted by the spectrometer manufacturers and have become commercially available. The most widespread of these technologies aimed for enhanced NMR sensitivity is concerned with the cryogenic probe heads.<sup>6</sup> In this technology, the receiver coil and the signal route to the preamplifier are cooled with cold helium gas. This leads to a higher  $Q$  factor for the coil, and the thermal noise in the rf circuitry is minimized, resulting in an enhancement to the signal-to-noise ratio. The cryogenic probe head systems are nowadays provided by all major NMR manufacturers. The second technology that deals with improved NMR sensitivity concerns miniaturization of the rf coil design. The sample volume in these microcoil probe heads ranges from a few microliters to a few nanoliters.<sup>7</sup> The confined coil volume leads to a high filling factor,<sup>8</sup> giving a high mass sensitivity in the NMR signal observation. NMR manufacturers have also taken the microcoil probe heads in their selection.<sup>9–11</sup> There have also been some promising demonstrations of how hyperpolarization can facilitate acquisition of a single-scan spectrum with a high

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- (1) Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction; signed in January 1993; printed and distributed by the Provisional Technical Secretariat of the Preparatory Commission for the Organisation for the Prohibition of Chemical Weapons ([http://www.opcw.org/docs/cwc\\_eng.pdf](http://www.opcw.org/docs/cwc_eng.pdf)). The Depositary of this Convention is the Secretary-General of the United Nations, from whom a certified true copy can be obtained.
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- (10) gH(X) Nano Probes of Varian Inc. Palo Alto, CA.
- (11) MicroProbes of Bruker BioSpin, Fällanden, Switzerland.

signal-to-noise ratio.<sup>12</sup> There is already one system on the market that employs dynamic nuclear polarization,<sup>13</sup> but the applicability of this technology in the CWC-related analysis is yet to be demonstrated.

Owing to their provided high sensitivity and the now-matured technology, the cryogenic probe heads are an essential part of the NMR instrumentation in biomolecular research.<sup>6</sup> The applicability of the technology in environmental analysis has also been demonstrated,<sup>14</sup> and therefore, these probe heads have also been taken in use by some of the designated laboratories. One paper concerning the application of the cryogenic probe head in the CWC-related analysis has been very recently published.<sup>15</sup> However, the high costs of the cryogenic probe head system may prevent acquisition of this technology by smaller laboratories in the field. The microcoil probe heads, on the other hand, are much more affordable compared to the cryogenic probe heads. An additional benefit in going to smaller volumes is the reduction of the “solvent noise” for electrically conductive solvents such as salt-containing solutions,<sup>16</sup> which would be beneficial particularly in the decontamination solution analysis.<sup>17</sup> The cryogenic probe heads can suffer the loss of the sensitivity advantage over the conventional probe heads in analysis of samples with high ion strength and electrical conductivity,<sup>18,19</sup> which may be a problem in the decontamination solution analysis.

The applicability of microcoil probe heads in analysis of CWC-related chemicals was tested using a 1.7-mm triple-channel (<sup>1</sup>H, <sup>31</sup>P, <sup>13</sup>C) inverse probe head purchased from Bruker BioSpin. The inverse configuration of the probe head offers an optimal sensitivity in proton observation. As the large part of the CWC-related chemicals has phosphorus, the probe head was optimized, by the manufacturer, also for phosphorus observation. There is also a smaller, 1-mm-diameter microcoil probe head available<sup>20</sup> that would offer even higher mass sensitivity, but from the point of sample preparation, the 1.7-mm probe head with a 30-μL sample volume was considered more practical. The detection limits of the micro coil probe head in both proton and phosphorus observation in analysis of CWC-related chemicals were tested with methylphosphonic acid (MPA), a secondary degradation product of chemical warfare agents with phosphorus (Table 1). In addition, the applicability of the microcoil probe head in the proficiency test-related analysis was tested with a water sample from the 22nd OPCW Proficiency Test containing three characteristic degradation products of chemical warfare agents; ethylphosphonic acid (EPA), ethyl methylphosphonic acid (EMPA), and MPA (Table 1).

**Table 1. Chemical Warfare Agent Degradation Products Spiked in the 22nd OPCW Proficiency Test Water Sample<sup>a</sup>**

Chemical	Abbreviation	Origin	Structure
Methylphosphonic acid [993-13-5] Schedule 2.B.4	MPA	Secondary hydrolysis product of CWAs containing Me-P moiety	
Ethylphosphonic acid [6779-09-5] Schedule 2.B.4	EPA	Secondary hydrolysis product of CWAs containing Et-P moiety	
Ethyl methylphosphonic acid [1832-53-7] Schedule 2.B.4	EMPA	Characteristic hydrolysis product of VX	

<sup>a</sup> The CAS numbers are shown in brackets. Schedule codes are from the CWC.

**Table 2. MPA Amounts and Concentrations in the Detection Limit Samples**

MPA amount (ng)	sample dimension/ volume (mm/mL)	MPA amount (nmol)	MPA concentration (μg/mL)	MPA concentration (μM)
50	1.7/0.03	0.52	1.7	17.4
400	1.7/0.03	4.17	13.3	138.9
900	1.7/0.03	9.37	30.0	312.4
8000	1.7/0.03	83.31	266.7	2777.1
8000	5.0/0.60	83.31	13.3	138.9

## EXPERIMENTAL SECTION

**Sample Preparation.** MPA (purity 98%) was purchased from Fluka. Deuterium oxide (>99 d%) was purchased from Spectra Stable Isotopes. Formic acid (Suprapur) was purchased from Merck. Water purified with Elgastat UHQ system (Elga) was used in the detection limit sample preparation.

For the detection limit studies, a set of stock solutions were prepared containing different amounts of MPA in water. The pH of the stock solutions was adjusted with 0.1% formic acid. NMR sample solutions were prepared by taking 0.5 mL of stock solution and adding 0.1 mL of D<sub>2</sub>O. NMR samples were prepared either in 1.7-mm NMR tubes (Bruker BioSpin) or 5-mm NMR tubes (HIP-7, Wilmad). The recommended sample volumes for these tubes are 30 and 600 μL, respectively. The required volume of the NMR sample solution was transferred to the NMR tubes. A Hamilton 1700 series 100-μL removable-needle syringe with a special needle (11 cm, blunt tip, 22s gauge) was used to transfer the solution in a 1.7-mm NMR tube. The sample was centrifuged with a hand-operated two-bucket centrifuge in order to settle the whole sample volume into the bottom of the tube. The sample constituents are presented in Table 2.

The 22nd OPCW Proficiency Test samples (two organic liquid samples and one water sample) were prepared by the Military Institute of Chemistry and Radiometry, Poland.<sup>21</sup> The water sample contained three relevant chemicals, MPA, EMPA, and EPA (Table 1) at levels of 2, 2, and 10 μg/mL, respectively. The sample contained also 450 μg/mL of different poly(ethylene glycols) (PEG) as an interfering impurity. One milliliter of the sample was

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transferred into a vial (Waters Alliance Total Recovery) and concentrated in accordance with the recommended operating procedures<sup>3</sup> by evaporation using nitrogen gas flow to the final volume of 20  $\mu\text{L}$ . Ten microliters of  $\text{D}_2\text{O}$  was added, and the solution was transferred into a 1.7-mm NMR tube.

**NMR Experiments.** The experiments were carried out at 11.75-T using a Bruker DRX 500 NMR spectrometer equipped with a 1.7-mm inverse  $z$ -gradient triple-resonance ( $^1\text{H}$ ,  $^{31}\text{P}$ ,  $^{13}\text{C}$ ) probe head (abbreviated to TXI) at 290 K. Typical  $90^\circ$  pulse lengths for  $^1\text{H}$ ,  $^{31}\text{P}$ , and  $^{13}\text{C}$  were 4.5, 13.0, and 8.8  $\mu\text{s}$ , respectively. Additionally, a 5-mm  $z$ -gradient inverse broadband probe head (abbreviated to BBI), and 5-mm  $z$ -gradient quadruple-nucleus ( $^{31}\text{P}$ ,  $^{19}\text{F}$ ,  $^{13}\text{C}$ ,  $^1\text{H}$ ) probe head (abbreviated to QNP) were used for comparison. For the BBI probe head, the typical  $90^\circ$  pulse lengths for  $^1\text{H}$  and  $^{31}\text{P}$  were 6.5 and 13.5  $\mu\text{s}$ , and for the QNP probe head, 10.6 and 8.5  $\mu\text{s}$ , respectively.

The  $^1\text{H}$  NMR spectra were measured using  $90^\circ$  excitation pulse, and the repetition time (acquisition time + relaxation delay) was set for 7.2 s corresponding to five times the  $T_1$  of  $\text{H}_\text{a}$  protons of MPA (1.44 s) measured with the inversion–recovery experiment. The transmitter frequency for proton was 4.7 ppm, and the spectrum width was set for 12 ppm. Presaturation ( $\gamma B_2 = 50$  Hz) was applied, if not stated otherwise, during the relaxation delay (6 s) on water resonance. Eight dummy scans were used prior to acquisition.

The 1D  $^1\text{H}$ – $^{31}\text{P}$  HSQC experiment<sup>17,22</sup> was used for selective detection of MPA proton resonance. The experiment was optimized according to the observed  $J_{\text{HP}}$  coupling in MPA by setting the polarization transfer delay  $\Delta$  to  $1/(2 \times 16.8$  Hz), and the repetition time was set for  $1.269T_1$ .<sup>23</sup> The transmitter frequencies for proton and phosphorus were 4.7 and 30 ppm, respectively. The spectrum width was set for 12 ppm. 1D  $^1\text{H}$ – $^{31}\text{P}$  HSQC spectra were acquired with 768 scans (8 dummy scans), giving the total measurement time of 26 min.

$^{31}\text{P}\{^1\text{H}\}$  NMR spectra were acquired using  $45^\circ$  excitation pulse angle. The repetition time was set for 1.3 s according to the Ernst angle equation<sup>24</sup> based on the  $T_1$  of phosphorus in MPA (3.75 s) measured with the inversion–recovery experiment. The transmitter frequency for phosphorus was 30 ppm, and the spectrum width was set for 60 ppm. Sixteen dummy scans were used prior to acquisition.

2D  $^1\text{H}$ – $^{31}\text{P}$  fast-HMQC spectrum from the proficiency test sample was measured with the modified experiment.<sup>25</sup> Number of scans was 16 per increment; the number of dummy scans was 64. Spectral widths in the proton and phosphorus dimensions were 10 and 60 ppm, respectively. The transmitter frequencies for proton and phosphorus were 4.7 and 30 ppm. The 2D spectrum was acquired with  $8\text{k} \times 100$  points. Total measurement time was 29 min. Further experimental details are described elsewhere.<sup>25</sup>

2D  $^1\text{H}$ – $^{13}\text{C}$  fast-HMQC spectrum from the proficiency test sample was measured with the original phase-sensitive pulse sequence.<sup>26</sup> Number of scans was 128 per increment; the number of dummy scans was 256. Spectral widths in the proton and carbon

dimensions were 10 and 80 ppm, respectively. The transmitter frequencies for proton and carbon were 4.7 and 40 ppm. The repetition time was set for 1.0 s. Presaturation ( $\gamma B_2 = 50$  Hz) was applied during the relaxation delay (590 ms) on water resonance. Polarization transfer delay  $\Delta$  was set for  $1/(2 \times 145$  Hz). Ernst angle pulse was  $128.77^\circ$ . Gradient pulse strengths were 28.0, 28.0, and 14.1 G/cm. Quadrature detection in the  $F_1$  dimension was accomplished with a gradient-based echo–antiecho selection, resulting in a phase-sensitive spectrum. The 2D spectrum was acquired with  $4\text{k} \times 128$  points. Total measurement time was 4 h, 43 min. Further experimental details can be found elsewhere.<sup>26</sup>

2D TOCSY<sup>27,28</sup> spectrum from the proficiency test sample was measured with 4 scans per increment; the number of dummy scans was 64. The repetition time was 2.6 s, and the mixing time was 60 ms. Spectral widths were 8 ppm in both dimensions. The transmitter frequency for proton was 4.7 ppm. The 2D spectrum was acquired with  $4\text{k} \times 400$  points. Quadrature detection in the  $F_1$  dimension was accomplished with a time-proportional phase increment, resulting in a phase-sensitive spectrum. Total measurement time was 1 h, 14 min.

Processing of the spectra was conducted using spectrometer operating software (TopSpin 1.3, Bruker BioSpin). Line broadenings (LB) of 0.1, 1.0, and 3.0 Hz were used in apodization of  $^1\text{H}$  NMR, 1D  $^1\text{H}$ – $^{31}\text{P}$  HSQC, and  $^{31}\text{P}\{^1\text{H}\}$  NMR spectra, respectively. The phase-sensitive 2D spectra were processed with squared cosine apodization on both dimensions (i.e., using QSINE window function with SSB = 2). The 2D  $^1\text{H}$ – $^{31}\text{P}$  fast-HMQC spectrum was weighted with QSINE function using SSB parameters 3.47 and 5.52 for  $F_2$  and  $F_1$  dimensions prior to magnitude calculation on  $F_1$  dimension. The number of real points after Fourier transform for the  $^1\text{H}$  NMR, 1D  $^1\text{H}$ – $^{31}\text{P}$  HSQC,  $^{31}\text{P}\{^1\text{H}\}$  NMR, 2D  $^1\text{H}$ – $^{31}\text{P}$  fast-HMQC, 2D  $^1\text{H}$ – $^{13}\text{C}$  Fast-HMQC, and 2D TOCSY spectra were 32k, 32k, 128k,  $8\text{k} \times 512$ ,  $8\text{k} \times 512$ , and  $4\text{k} \times 1\text{k}$ , respectively. The signal-to-noise calculations were performed with the specific calculation tool in TopSpin (sinocal). The signal region was set for the resonance of interest, and the noise windows of 1 and 5 ppm were used for proton-observed and phosphorus-observed spectra, respectively.

## RESULTS AND DISCUSSION

The spiking level in the proficiency test samples is typically 1–10  $\mu\text{g}/\text{mL}$ , which is rather low from the point of NMR analysis. A great amount of research has been made in preceding decades in the field of sample preparation to offer solutions for the sample enrichment. The methodology ranges from simple nitrogen gas flow evaporation to the application of solid-phase extraction and related sorption methods.<sup>3,5,29–31</sup> Still, there have been numerous times in the course of the proficiency tests that preparation of an enriched 5-mm NMR sample has not been feasible. The reason has been that the volume of the original (typically organic) sample has been rather low, e.g., 5 mL.<sup>21</sup> As this sample must be divided among all analytical techniques available (e.g., LC/MS, GC/MS,

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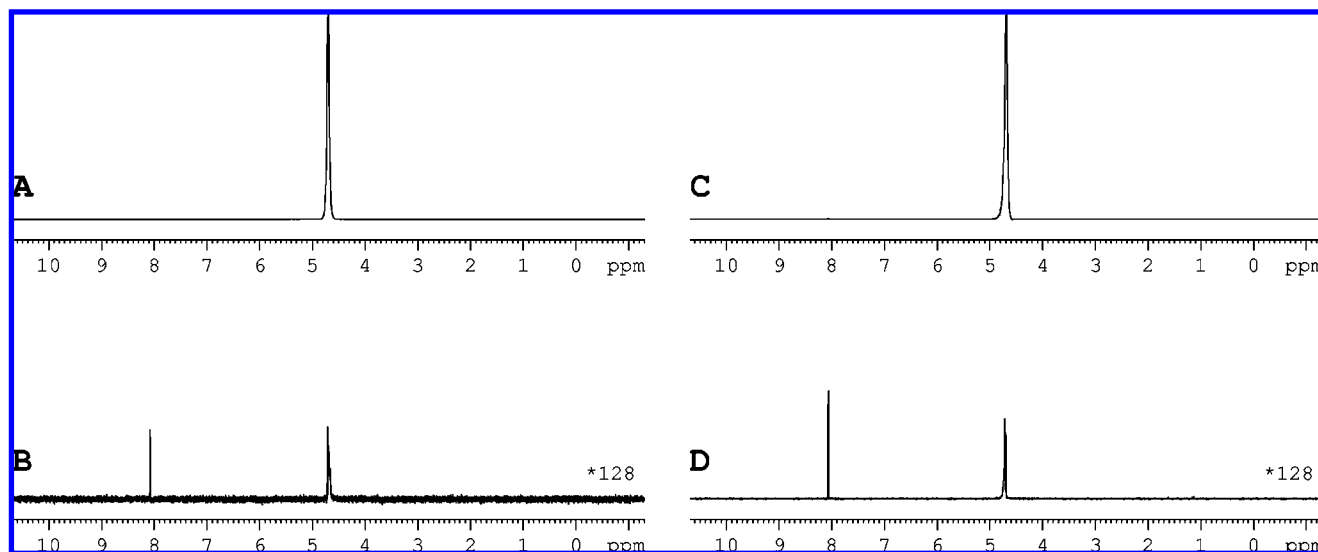
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**Figure 1.**  $^1\text{H}$  NMR spectra from the detection limit test samples containing  $13.3\ \mu\text{g/mL}$  MPA in  $\text{H}_2\text{O}$  with 0.1% formic acid (see Table 2). The spectra were measured with the 1.7-mm TXI probe head without (A) and with (B) presaturation applied on the water resonance, and with the 5-mm BBI probe head without (C) and with (D) presaturation. The number of scans was four, and rf field strength was 50 Hz for presaturation. The signal-to-noise ratio of the water signal was 11279:1 in (A), 30:1 in (B), 46556:1 in (C), and 154:1 in (D). Receiver gain was the same in all the experiments. The formic acid resonance is shown at 8 ppm. The amount of MPA was roughly 20 times higher in the 5-mm sample than in the 1.7-mm sample; a weak resonance from MPA is shown at 1.2 ppm in the presaturated  $^1\text{H}$  NMR spectrum from the 5-mm sample (D).

IR, NMR), the aliquot spared for the NMR analysis has simply been too small to gain any significant sample enrichment for the 5-mm NMR sample. Therefore, the proficiency test sample aliquot is mainly used directly as the NMR sample. The low concentration of the relevant chemicals in the NMR sample results in exceptionally long measurement times, and intense sample background resonances further hinder the interpretation of  $^1\text{H}$  NMR through signal overlapping and cause poor dynamic range. The measurement of a  $^1\text{H}$  NMR spectrum with signal-to-noise ratio above the limit of detection (3:1)<sup>32</sup> with a 5-mm conventional probe head can take close to 20 min.  $^{31}\text{P}\{^1\text{H}\}$  NMR spectrum will not typically suffer from background signals, but the low level of analytes will result in that measurement of the spectrum with signal-to-noise ratio above the limit of detection can take close to 6 h.<sup>25</sup> These typical measurement times needed in the proficiency test analysis with a conventional 5-mm probe head were used as the starting point in the detection limit determination for the microcoil probe head.

The detection limit tests were conducted with water samples containing trace amounts of MPA (Table 2). These samples contained an intense water resonance, which would cause a poor dynamic range in the  $^1\text{H}$  NMR analysis. Theoretically, the lower absolute amount of the solvent in the 1.7-mm NMR sample should make the signal suppression easier. The solvent suppression efficiency between the 1.7-mm TXI probe head and the 5-mm probe head optimized for proton observation (5-mm BBI) was tested with the simple presaturation method. Two samples, the 1.7- and 5-mm NMR samples, were prepared, both containing  $13.3\ \mu\text{g/mL}$  MPA (Table 2). A comparison of the water signal intensities in the  $^1\text{H}$  NMR spectra with and without presaturation measured with the 1.7-mm TXI probe head demonstrated ~375-fold intensity reduction in four scan experiments (Figure 1A,B).

The result with the 5-mm BBI probe head (Figure 1C,D) showed a similar intensity reduction factor, but due to higher amount of the solvent in the active area of the coil, the presaturated water signal intensity was ~5 times more intense. As the residual solvent signal is less intense, it should be easier to optimize the receiver gain for the observation of the analytes in trace amounts. However, it has also been reported that microcoil probe heads are more prone to radiation damping effects than conventional probe heads,<sup>33</sup> which can affect to the signal suppression efficiency with suppression methods that employ selective pulses<sup>34</sup> or excitation sculpting.<sup>35</sup>

The proton sensitivity test with the 1.7-mm TXI revealed that 50 ng of MPA was detectable with the  $^1\text{H}$  NMR experiment in under 20 min (Figure 2A), which demonstrates a high mass sensitivity. While the high solvent signal can be sufficiently suppressed with presaturation or other suppression schemes, there can be also situations where the sample background completely overlaps the resonances of interest. In these cases, the 1D  $^1\text{H}$ – $^{31}\text{P}$  HSQC experiment<sup>17,22</sup> offers an excellent method in selective screening of organophosphorus compounds, and the background (solvent and other impurities) can be effectively eliminated. The coherence selection with gradient pulses will also reject half of the signal, which must be taken into account. Still, MPA was observable at level 50 ng with intensity equal to the limit of detection under 0.5 h with the 1D  $^1\text{H}$ – $^{31}\text{P}$  HSQC experiment optimized specifically for MPA observation (Figure 2B).

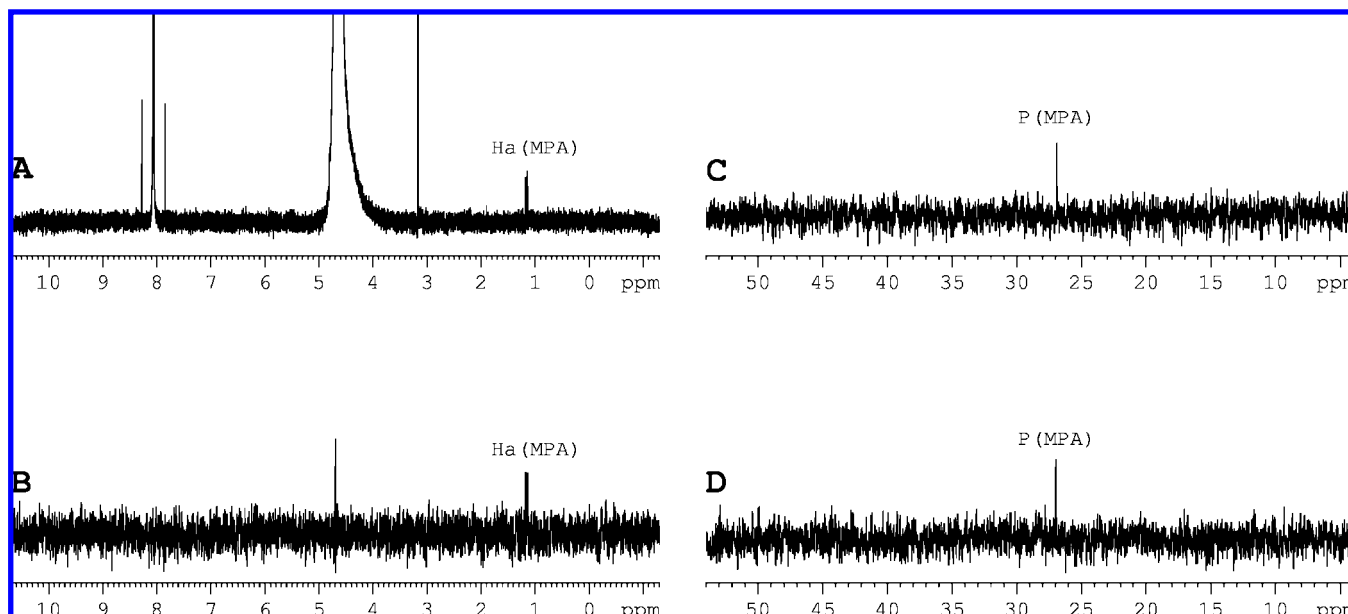
The 1.7-mm TXI probe head was also manufactured for phosphorus observation. The limit of detection in phosphorus observation was tested with the  $^{31}\text{P}\{^1\text{H}\}$  NMR experiment. The

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**Figure 2.**  $^1\text{H}$  NMR spectrum (A) and 1D  $^1\text{H}$ – $^{31}\text{P}$  HSQC spectrum (B) from the detection limit test sample containing 50 ng of MPA in  $\text{H}_2\text{O}$  with 0.1% formic acid,  $^{31}\text{P}\{^1\text{H}\}$  NMR spectra from the detection limit test samples containing 400 ng of MPA in  $\text{H}_2\text{O}$  with 0.1% formic acid (C), and 900 ng of MPA in  $\text{H}_2\text{O}$  with 0.1% formic acid (D). The spectra were measured with the 1.7-mm TXI probe head. The measurement times were 17 min (number of scans 128) for the  $^1\text{H}$  NMR spectrum, 25 min for the 1D  $^1\text{H}$ – $^{31}\text{P}$  HSQC spectrum, and 5 h, 56 min (number of scans 16k) and 30 min (number of scans 1328) for the  $^{31}\text{P}\{^1\text{H}\}$  NMR spectra, respectively. The signal-to-noise ratios are equal or above the limit of detection (3:1) in all the spectra (6:1, 3:1, 4:1, and 4:1, respectively).

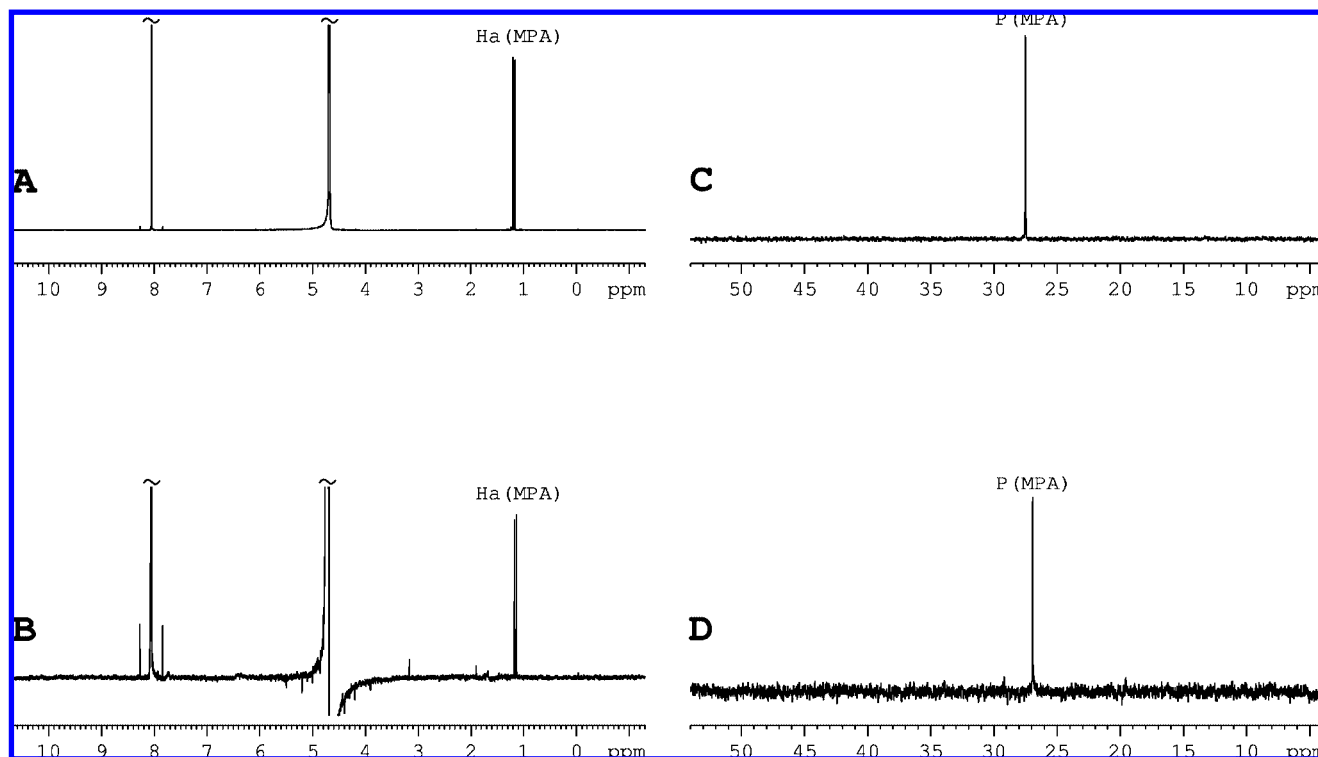
results showed that MPA was detectable at a level of 400 ng when a long,  $\sim 6$  h of measurement time was used (Figure 2C). While these long acquisitions are expected in the proficiency test-related NMR analysis, a shorter measurement time is of course more desirable. If a good quality spectrum could be measured within 0.5 h, it would make a considerable reduction to the total NMR analysis time. Therefore, the phosphorus limit of detection was also tested with shorter measurement times. A signal-to-noise ratio above the limit of detection was attainable with the 0.5-h measurement time restriction, when the amount of the MPA was 900 ng (Figure 2D). As a proton observed experiment like  $^1\text{H}$  NMR, 1D  $^1\text{H}$ – $^{31}\text{P}$  HSQC, or 2D  $^1\text{H}$ – $^{31}\text{P}$  fast-HMQC can also be measured from this amount of analyte within dozens of minutes instead of hours, a reasonably fast throughput time in the NMR analysis should be feasible if the level of relevant chemicals can be concentrated above  $1\text{ }\mu\text{g}$  in the sample.

The mass sensitivity of the 1.7-mm TXI probe head was compared against two 5-mm probe heads: one optimized for proton observation (5-mm BBI) and one for phosphorus observation (5-mm QNP). Two samples, the 1.7- and 5-mm NMR samples, were prepared, both containing  $8\text{ }\mu\text{g}$  of MPA (Table 2). The sample volumes were the recommended volumes for these corresponding sample tubes to guarantee easy shimming for attaining a spectrum with good line shapes. The spectra were measured from the samples with identical acquisition parameters. Using these experimental arrangements, the results indicate a 7.7-fold higher signal-to-noise ratio in the  $^1\text{H}$  NMR spectra for the 1.7-mm TXI probe head compared to the 5-mm BBI (Figure 3A,B). With  $^{31}\text{P}\{^1\text{H}\}$  NMR spectra, the signal-to-noise gain was 3.6-fold higher with the 1.7-mm TXI compared to the 5-mm QNP (Figure 3C,D). These values are, particularly in proton observation, higher than the theoretical values would be according to the “1/coil-size” dependency of the signal-to-noise ratio.<sup>8</sup> The reason could be the

difference of the coil design (other than the diameter), and that the absolute amount of MPA in the active area of the coil is smaller in the 5-mm probe heads compared to the 1.7-mm probe head. However, from the point of practical sample preparation, these figures represent a good example of the advantage in mass sensitivity.

It was also considered if the used probe head has an affect on the observed  $T_2$  or  $T_2^*$  values and, therefore, to the attainable signal-to-noise ratio. It could be reasoned that the more confined sample volume of the microcoil probe head can help to obtain optimal shims more easily, thus giving narrower line shapes in the spectrum. The line widths of the MPA proton resonance were analyzed from the detection limit test samples containing  $13.3\text{ }\mu\text{g/mL}$  MPA (Table 2). The samples were analyzed using all three probe heads, and the measured  $^1\text{H}$  NMR spectra were processed without apodization. The observed line widths were 0.78, 0.72, and 0.76 Hz with the 1.7-mm TXI, 5-mm BBI, and 5-mm QNP, respectively. Based on these results, there were not any significant differences in the  $T_2^*$  values. As the microcoil probe heads are more prone to the radiation damping effects,<sup>33</sup> the analytes with high concentration can have broadened line shapes. This was tested by observing the line width of MPA proton resonance in the  $^1\text{H}$  NMR spectrum from the detection limit test sample containing  $266.7\text{ }\mu\text{g/mL}$  MPA with the 1.7-mm TXI probe head. The line width was somewhat broadened (0.91 Hz), but still sufficiently narrow. In conclusion, the small sample volume should not affect the appearance of the analyte resonances in the studied concentration range.

As the above results indicate, CWC-related chemicals can be observed even at dozens of nanograms levels in the sample. But it should be emphasized that the microcoil probe heads are mass sensitive, not concentration sensitive. As can be seen



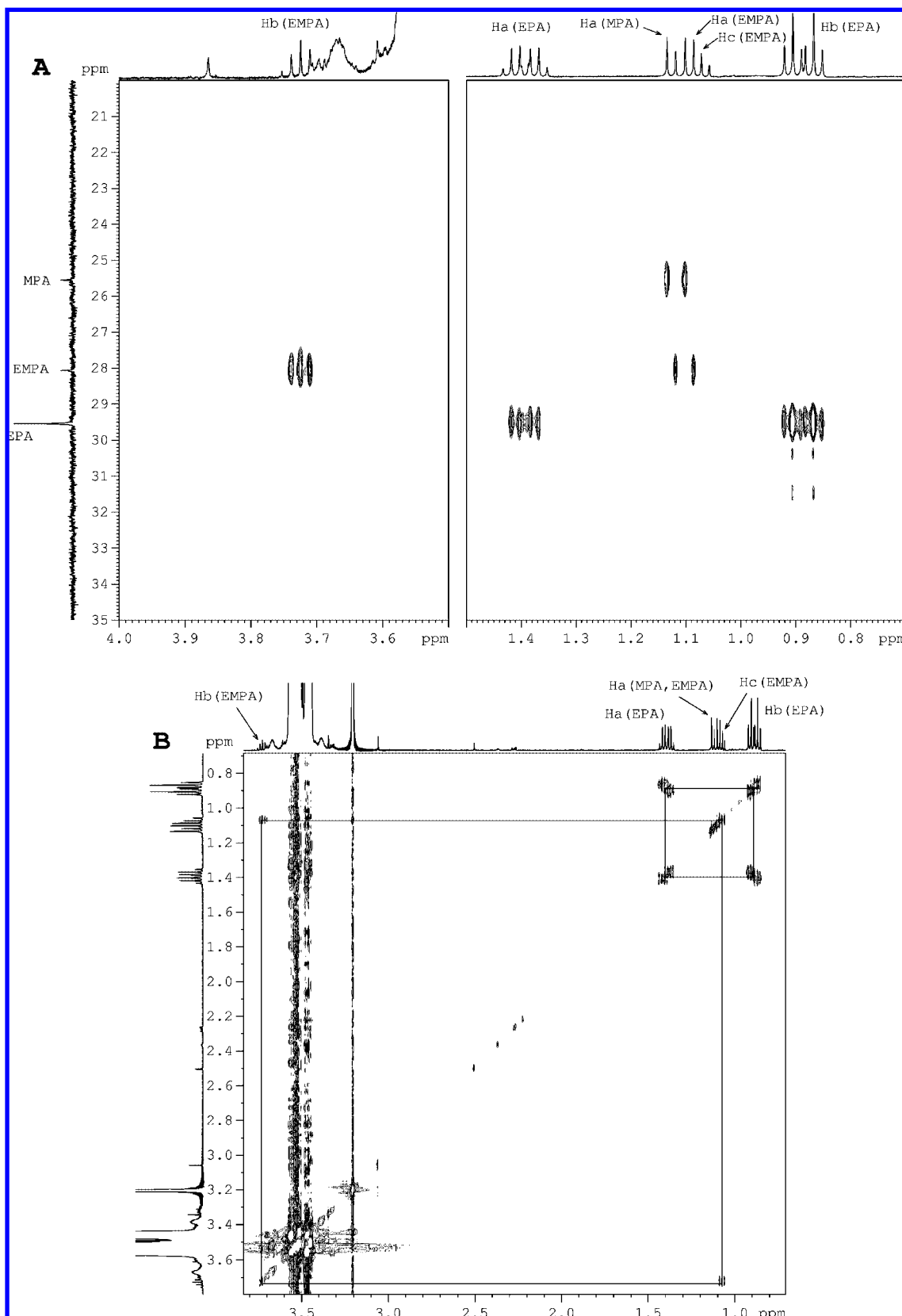
**Figure 3.**  $^1\text{H}$  NMR and  $^{31}\text{P}\{^1\text{H}\}$  NMR spectra from detection limit samples containing  $8\ \mu\text{g}$  of MPA in  $\text{H}_2\text{O}$  with 0.1% formic acid.  $^1\text{H}$  NMR spectrum from the 1.7-mm NMR sample measured with the 1.7-mm TXI probe head (A), and  $^1\text{H}$  NMR spectrum from the 5-mm NMR sample measured with the 5-mm BBI probe head (B),  $^{31}\text{P}\{^1\text{H}\}$  NMR spectrum from the 1.7 mm NMR sample measured with the 1.7 mm TXI probe head (C), and  $^{31}\text{P}\{^1\text{H}\}$  NMR spectrum from the 5-mm NMR sample measured with the 5-mm QNP probe head (D). The measurement times were 17 min (number of scans 128) for the  $^1\text{H}$  NMR spectra and 5 h, 56 min (number of scans 16k) for the  $^{31}\text{P}\{^1\text{H}\}$  NMR spectra. The signal-to-noise ratios in the spectra are 868:1, 112:1, 113:1, and 31:1, respectively.

in Table 2, the 1.7-mm detection limit samples have quite high MPA concentrations. The difference in the signal-to-noise ratio measured from samples with identical concentration can be seen, for example, by comparing the  $^{31}\text{P}\{^1\text{H}\}$  NMR spectra in Figure 2C and Figure 3D. The spectrum measured with the 5-mm QNP probe head has over 7 times higher signal-to-noise ratio than in the spectrum measured with the 1.7-mm TXI probe head. The crucial point in the application of the microcoil probe head is preparation of a small-volume sample in which the relevant chemicals have been concentrated. Direct analysis of concentration-limited samples, like proficiency test samples containing relevant chemicals at a level of  $1\text{--}10\ \mu\text{g}/\text{mL}$ , with a microcoil probe head is not practical. However, as a sample having few micrograms of analytes is more than sufficient for the microcoil probe head analysis, it means that 1 mL of a liquid-state proficiency test sample, a typical aliquot volume allocated for the NMR analysis, contains enough the chemicals of interest. In most cases, a simple nitrogen gas flow concentration is sufficient for liquid samples for reduction of the sample volume while maintaining a high recovery of the relevant chemicals.

Application of the microcoil probe head in the proficiency test-related analysis was tested with 1 mL of the 22nd OPCW Proficiency Test water sample. The sample preparation involved a nitrogen gas flow concentration of the sample below the required 1.7-mm NMR sample volume. After addition of  $\text{D}_2\text{O}$  for lock reference, the sample was analyzed with the  $^1\text{H}$  NMR and  $^{31}\text{P}\{^1\text{H}\}$  NMR experiments. All three CWC-related chemi-

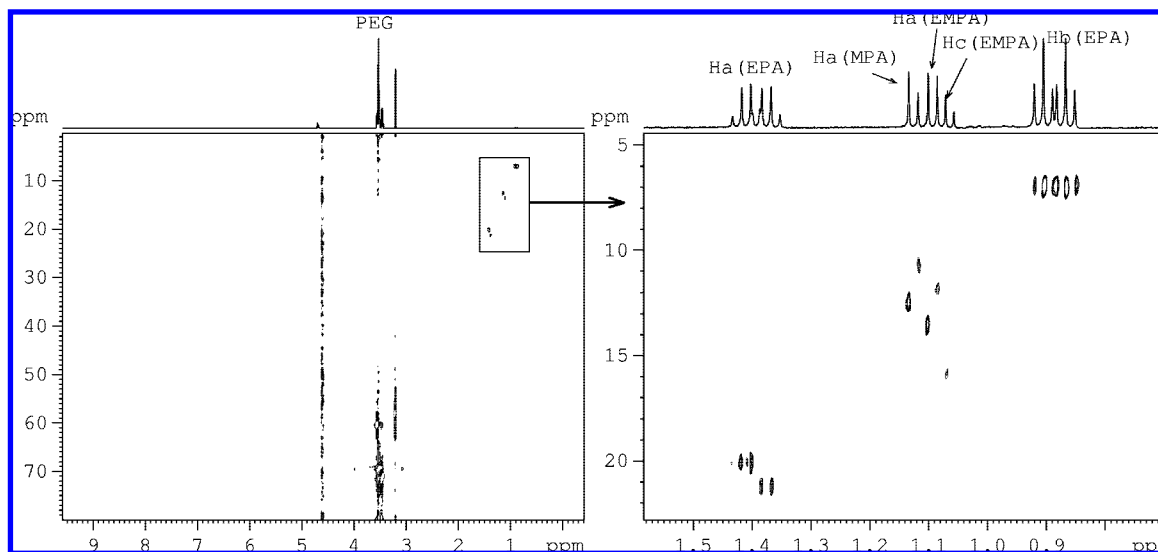
cals were retained in the sample with good recovery, and the spectra with sufficient signal-to-noise ratio were possible to measure within 0.5 h (Figure 4). This demonstrates remarkable saving in the measurement time compared to, for example, direct analysis of a proficiency test sample with  $^{31}\text{P}\{^1\text{H}\}$  NMR using 5-mm conventional probe heads.<sup>25</sup> In order to confirm the assignment of Hb(EMPA), which was partially overlapped by the PEG resonances, the 2D  $^1\text{H}\text{--}^{31}\text{P}$  fast-HMQC spectrum<sup>25</sup> was measured. After 0.5-h measurement, all the resonances from the relevant chemicals bearing  $J_{\text{PH}}$  coupling were detected with good signal-to-noise ratio (Figure 4A). The assignment of Hc(EMPA) needed still confirmation; therefore, as the resonances of the relevant chemicals were not completely overlapped, it was tested as to how a standard 2D TOCSY experiment can aid the assignment. The 2D TOCSY spectrum presented a clear correlation between the Hb(EMPA) and Hc(EMPA), confirming the structure (Figure 4B). Moreover, correlations between Ha(EPA) and Hb(EPA) were visible.

A part of the CWC-related chemicals (e.g., mustards and their precursors and degradation products) have no phosphorus, but it would give more confidence if spectral data from an additional nucleus could be applied in the identification of those chemicals. The  $^{13}\text{C}\{^1\text{H}\}$  NMR and 2D  $^1\text{H}\text{--}^{13}\text{C}$  correlation experiments<sup>36</sup> are crucial part in the NMR structural elucidation of organic chemicals, but so far the low concentration of the relevant chemicals in the proficiency test-related analysis has hindered the use of these experiments. Recently, Cullinan and co-workers<sup>15</sup> have demonstrated that cryogenic probe heads



**Figure 4.** 2D  $^1\text{H}$ – $^{31}\text{P}$  fast-HMQC spectrum (A) and 2D TOCSY spectrum (B) from the 22nd OPCW Proficiency Test water sample containing a mixture of chemicals, MPA, EPA, and EMPA, measured with the 1.7-mm TXI probe head. The  $^1\text{H}$  NMR and  $^{31}\text{P}\{^1\text{H}\}$  NMR spectra are shown as projections. The measurement times were 9 min (number of scans 64) for the  $^1\text{H}$  NMR spectrum, 21 min (number of scans 1024) for the  $^{31}\text{P}\{^1\text{H}\}$  NMR spectrum, 29 min for the 2D  $^1\text{H}$ – $^{31}\text{P}$  fast-HMQC spectrum, and 1 h, 14 min for the 2D TOCSY spectrum. The signal-to-noise ratios in the  $^{31}\text{P}\{^1\text{H}\}$  NMR spectrum for EPA, EMPA, and MPA are 22:1, 4:1, and 4:1, respectively. The 2D  $^1\text{H}$ – $^{31}\text{P}$  fast-HMQC spectrum shows clear cross peaks for the relevant organophosphorus compounds; the cross peak for Hc(EMPA) is missing due to absence of  $J_{\text{HP}}$  coupling. The 2D TOCSY spectrum presents a clear correlation between Hb(EMPA) and Hc(EMPA), and between Ha(EPA) and Hb(EPA).





**Figure 5.** 2D  $^1\text{H}$ – $^{13}\text{C}$  fast-HMQC spectrum from the 22nd OPCW Proficiency Test water sample containing a mixture of chemicals, MPA, EPA, and EMPA, measured with the 1.7-mm TXI probe head. The  $^1\text{H}$ – $^{13}\text{C}$  correlation of Hb(EMPA) could not be assigned due to the high intensity of the close-by PEG cross peaks. The tilt caused by  $J_{\text{PH}}$  and  $J_{\text{PC}}$  is most prominent in the Ha cross peaks of the chemicals (see the expanded part of the spectrum). The measurement time for the spectrum was 4 h, 43 min.

offer sufficient sensitivity to acquire  $^{13}\text{C}$  shift data with indirect detection and by that give more details about the chemicals to support the identification. This data could also be compared against the  $^{13}\text{C}\{^1\text{H}\}$ NMR library spectra of the OPCW central analytical database, OCAD.<sup>2</sup> Therefore, it was tested if it is feasible to measure  $^1\text{H}$ – $^{13}\text{C}$  correlation data from the proficiency test sample with the 1.7-mm TXI probe head. The original fast-HMQC experiment<sup>26</sup> was optimized for detection of a phase-sensitive 2D  $^1\text{H}$ – $^{13}\text{C}$  correlation spectrum. Presaturation was applied on water resonance during the relaxation delay to suppress the intense water signal. The results indicated that almost all correlations from the relevant chemicals were shown in the spectrum in less than 5 h of measurement (Figure 5). However, the Hb(EMPA) could not be assigned due to the intense PEG cross peaks that were close to the presumed location ( $\delta_{\text{C}} \sim 62$  ppm, data from the OCAD) of the Hb(EMPA) cross peak. While this experiment demonstrated that the sensitivity of the 1.7-mm TXI was sufficient to acquire the  $^1\text{H}$ – $^{13}\text{C}$  correlation data within reasonable measurement time, it clearly pointed out, that if the sample contains intense background signals from organic chemicals, the applicability of the 2D  $^1\text{H}$ – $^{13}\text{C}$  correlation spectrum can be limited in identification. The level of interfering organic chemicals in the sample must be reduced with sample preparation procedures before measurement of the 2D  $^1\text{H}$ – $^{13}\text{C}$  correlation spectrum. In this way, the identification of the relevant cross peaks is more straightforward, and the dynamic range can be matched better for the level of the relevant chemicals.

In addition to the considerable mass sensitivity improvement and measurement time saving, the microcoil probe head can offer additional benefits in the proficiency test-related analysis. The small volume of the sample offers the possibility of making the first analysis from any liquid-state proficiency test samples without consuming the original solution too much. While this first sample may not be used for identification of the relevant chemicals, it can be used to get the first view to the sample constituent and give crucial information about the sample

matrix that could hamper other analytical techniques. For example, a high level of hydrocarbons in the sample can cause problems in GC/MS analysis. As the presence of hydrocarbons can be rapidly detected with  $^1\text{H}$  NMR, this information would assist the sample preparation to use proper sample purification methods for the sample prior to the GC/MS analysis. The small volume also offers flexibility in preparation of the concentrated sample by giving possibilities to incorporate the sample enrichment into the general sample preparation strategy. For example, soil samples are typically extracted with water and dichloromethane in order to extract the relevant chemicals. The water extract needs concentration and solvent exchange prior to GC/MS analysis.<sup>3,5</sup> After the extract has been concentrated to a small volume, an aliquot can be taken from this sample for the NMR analysis while the rest of the solution could need further sample preparation like derivatization<sup>37</sup> before it is analyzable by the other technique. In this way, no extra time is needed for NMR sample preparation, while the mass spectrometric techniques owing to their high sensitivity can easily cope with the divide of the concentrated sample.

## CONCLUSIONS

The microcoil probe head technology is a feasible and cost-effective alternative to the cryogenic probe head technology to improve mass sensitivity of NMR spectroscopy in the analysis of trace amounts of chemicals related to the CWC. With aid of the well-established sample preparation procedures, it is possible to make an enriched sample for NMR analysis, and the mass sensitivity of a microcoil probe head makes possible the measurement of standard NMR experiments employed in the proficiency test-related analysis with considerably shorter measurement times. Additionally, the improved mass sensitivity facilitates applications of many NMR experiments, like  $^1\text{H}$ – $^{13}\text{C}$

(36) Croasmun, W. R.; Carlson, R. M. K., Ed. *Two-Dimensional NMR Spectroscopy. Applications for Chemists and Biochemists*; VCH Publishers: New York, 1994.

correlation experiments, that have not been feasible before without the use of the cryogenic probe heads.<sup>15</sup> The broader arsenal of NMR experiments at our disposal gives more flexibility and confidence to the NMR analysis of the proficiency test samples and other CWC-related samples.

The presented approach involving the use of a microcoil probe head is not limited to the CWC-related chemicals, but should also be useful in, for example, analysis of organic phosphorus<sup>38</sup> and pesticides<sup>39</sup> in environmental samples.

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