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ARTICLE *in* ANALYTICAL CHEMISTRY · MAY 2007

Impact Factor: 5.64 · DOI: 10.1021/ac0617767 · Source: PubMed

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Hyphenation of Gas Chromatography to Microcoil ^{1}H Nuclear Magnetic Resonance Spectroscopy

Marc David Grynbaum,[†] Diana Kreidler,[†] Jens Rehbein,[†] Armin Purea,[‡] Paul Schuler,[†] Walter Schaal,[‡] Harri Czesla,[†] Andrew Webb,[§] Volker Schurig,[†] and Klaus Albert*[,†]

Institute of Organic Chemistry, Chemisches Zentral-Institut, University of Tübingen, Auf der Morgenstelle 18, 72076 Tuebingen, Germany, Department of Experimental Physics 5 (Biophysics), University of Würzburg, Am Hubland, 97074 Würzburg, Germany, and Department of Bioengineering, Penn State University, 315 Hallowell Building, University Park, Pennsylvania 16802

Whereas the hyphenation of gas chromatography (GC) with mass spectrometry is of great importance, little is known about the coupling to nuclear magnetic resonance spectroscopy (NMR). The investigation of this technique is an attractive proposition because of the valuable information given by NMR on molecular structure. The experiments shown here are to our knowledge the first hyphenating capillary GC to microcoil NMR. In contrast to liquids, gases have rarely been investigated by NMR, mainly due to the experimental difficulties in handling gases and the low signal-to-noise-ratio (SNR) of the NMR signal obtained at atmospheric pressure. With advances in NMR sensitivity (higher magnetic fields and solenoidal microprobes), this limitation can be largely overcome. In this paper, we describe the use of a custom-built solenoidal NMR microprobe with an active volume of 2 μL for the NMR detection of several compounds at 400 MHz, first in a mixture, and then with full coupling to capillary GC to identify them separately. The injected amounts of each analyte in the hyphenated experiments are in the range of 15−50 μmol, resulting in reasonable SNR for sample masses of 1−2 μg.

The hyphenation of gas chromatography (GC) with mass spectrometry (MS) has evolved into one of the most important analytical techniques. However, less is known about the coupling of GC with nuclear magnetic resonance spectroscopy (NMR), although the investigation of this is an attractive proposition because of the valuable information on molecular structure obtained from NMR. In contrast to liquids,[1] gases are much less commonly investigated using NMR, due mainly to the experimental difficulties in sample handling and the low signal-to-noise ratio (SNR) obtained at atmospheric pressure.[2] High-pressure NMR, though, has been used in a wide variety of applications including on-line monitoring of chemical reactions, investigations of equilibrium systems,[3] and utilization of supercritical fluids.[4]

GC-NMR using typical carrier gases (helium, nitrogen, argon) has the advantage of the NMR spectra having no large background solvent signal. Buddrus and Herzog[5] first reported GC-NMR experiments in 1981 using a JEOL FX-100 NMR spectrometer with a large detection cell volume of 260 μL in the flow probe. For gas chromatography, they used a 2-mm-i.d. column packed with 20% squalane on Chromosorb. In further experiments,[6] they used a 4-mm-i.d. packed column (Carbowax 4000), with a heated interface discharging into an air-heated conventional rotating 5-mm NMR tube. With this setup, they were able to record NMR spectra of gaseous organic compounds of higher boiling points. Over the past 20 years, many advances in magnet and radio frequency (rf) probe technology have occurred, which lead to our reinvestigation of the utility of capillary scale GC-NMR experiments.

Newly developed probes have reduced the rf coil diameter significantly, leading to major improvements in NMR detection limits.[7] Two different geometrical probe designs have been employed: the saddle-type coil,[8] commonly used in conventional NMR probes, and the solenoidal-type, which is constructed by directly wrapping the rf coil around a capillary column,[9] immersing the coil into a susceptibility-matching fluid for improved field homogeneity,[10] and placing it transverse to the magnetic field. These microprobes with a solenoidal coil and an active detection volume of ~1.5 μL overcome the limited sensitivity of conventional saddle-shaped NMR probes and currently provide detection limits in the low-nanogram range. They have been used extensively in hyphenated capillary separation techniques, in which low solvent consumption additionally makes the use of fully deuterated solvents economically feasible, and solvent signal suppression in the NMR experiment is generally not required.[11−14]

* To whom correspondence should be addressed. E-mail: klaus.albert@uni-tuebingen.de. Tel.: +49-7071-2978727. Fax: +49-7071-295875.

[†] University of Tübingen.

[‡] University of Würzburg.

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10.1021/ac0617767 CCC: $37.00
Published on Web 02/24/2007

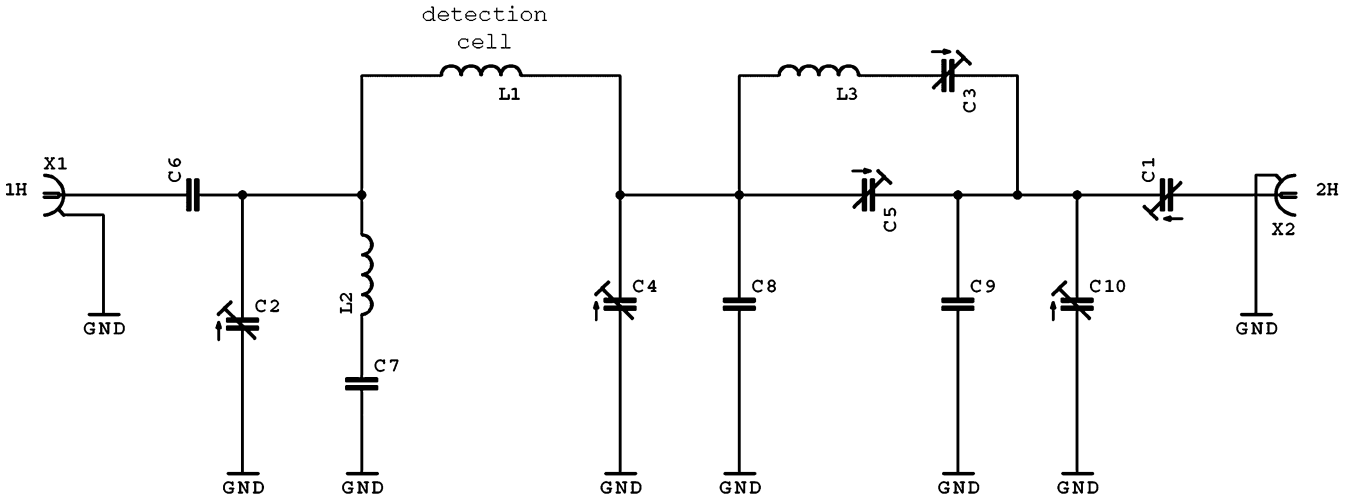

Figure 1. Radio frequency circuit of the custom-built, double-resonant solenoidal NMR microprobe.

In on-line GC-NMR experiments, as in continuous-flow HPLC-NMR,[11] the optimum flow rate is a compromise between the best chromatographic resolution and NMR sensitivity. The limited residence time of the nuclei in the flow cell reduce the spin−lattice (T_1) and the spin−spin (T_2) relaxation times and causes broadening of the line width of the signals. On the other hand, the SNR increases with higher flow rate by allowing rapid pulsing and data acquisition and avoiding saturation effects. To achieve maximum sensitivity, the sample must spend a certain time in the magnetic field before entering the flow cell to polarize the nuclear spins. The geometry and volume of the flow cell should avoid dispersion effects and maintain the quality of chromatographic separation by ensuring laminar flow. In the past few years, the development of flow cells has made significant steps forward.[11,15] In continuous-flow probes, the detection coil is directly attached to the flow cell,[1,16] so the filling factor ϕ ($\phi = V_s/V_c$, where V_s is the sample volume and V_c the NMR coil volume)[11] is optimized, resulting in an improved sensitivity. With suitable probes and shimming systems, it is possible to obtain spectra with the same resolution as with 5-mm tubes. These probes also permit a very stable lock signal. Here we report on the to our knowledge first experiments hyphenating capillary GC to solenoidal microprobe NMR.

EXPERIMENTAL SECTION

Chemicals. Diethyl ether, tetrahydrofuran, acetone, and dichloromethane (LiChrosolv gradient grade) were purchased from Merck (Darmstadt, Germany).

Gas Chromatography. The chromatographic separations were performed on a Fractovap Series 2350 (Carlo Erba Strumentazione, Milan, Italy) gas chromatograph employing a SE-54 column (50 m, 250-μm i.d., 0.25-μm film thickness). Helium 5.0 (Air Liquide Deutschland GmbH, Duesseldorf, Germany) was used as carrier gas at a pressure of 1 bar, resulting in a flow rate of 0.72 mL/min. The injector (splitless mode) temperature was set to 250 °C and the column oven to 60 °C.

NMR. All NMR experiments were recorded on a Bruker ARX 400 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). The outlet of the gas chromatograph was connected to a 2-μL active volume, custom-built, double-resonant solenoidal microprobe using a 3-m fused-silica transfer capillary (50-μm i.d. to prevent extensive diffusion). Universal glass connectors (Klaus Ziemer GmbH, Langerwehe, Germany) were employed for all capillary connections. The NMR spectrometer was controlled by an O2 workstation (Silicon Graphics) and XWIN-NMR software (Bruker Biospin GmbH, Rheinstetten, Germany). Data analysis was carried out with XWIN-NMR 3.5. The ^{1}H flip angle (16 dB attenuation/50 W amplifier, 6.5 μs) was optimized to give maximum NMR signal. Spin−lattice (T_1) relaxation times were determined using an inversion recovery sequence.

RESULTS AND DISCUSSION

All NMR experiments were carried out using a custom-built, double-resonant solenoidal microprobe with proton and lock channels. The rf circuit is shown in Figure 1 and has been characterized extensively for other types of microprobes.[1,17] The probe coil (L1) was an 8-turn solenoid wound with 200-μm-diameter copper wire, a length of 4 mm, an inner diameter of 1.5 mm, and an active volume of 2 μL. The proton tank circuit (C2, C4, C6, C8) and the lock tank circuit (C1, C4, C8, C9, C10) were isolated using rf traps (L2, C7; L3, C3, C5): an isolation of 20 dB was achieved. The flow cell was made of Duran, 0.8-mm i.d., and placed inside the probe coil. The coil was immersed in susceptibility-matching fluid (FC-43) for magnetic susceptibility matching.[10]

Probe performance was initially tested using standard liquid samples. A spectral line width test used a solution of 10% chloroform in acetone-d_6 (commonly termed the "Hump test"), with the full width at half-maximum of the chloroform signal width measured at 0.8 Hz, and the signal width at the height of the ^{13}C satellites a value of 12 Hz. The sensitivity of the probe was

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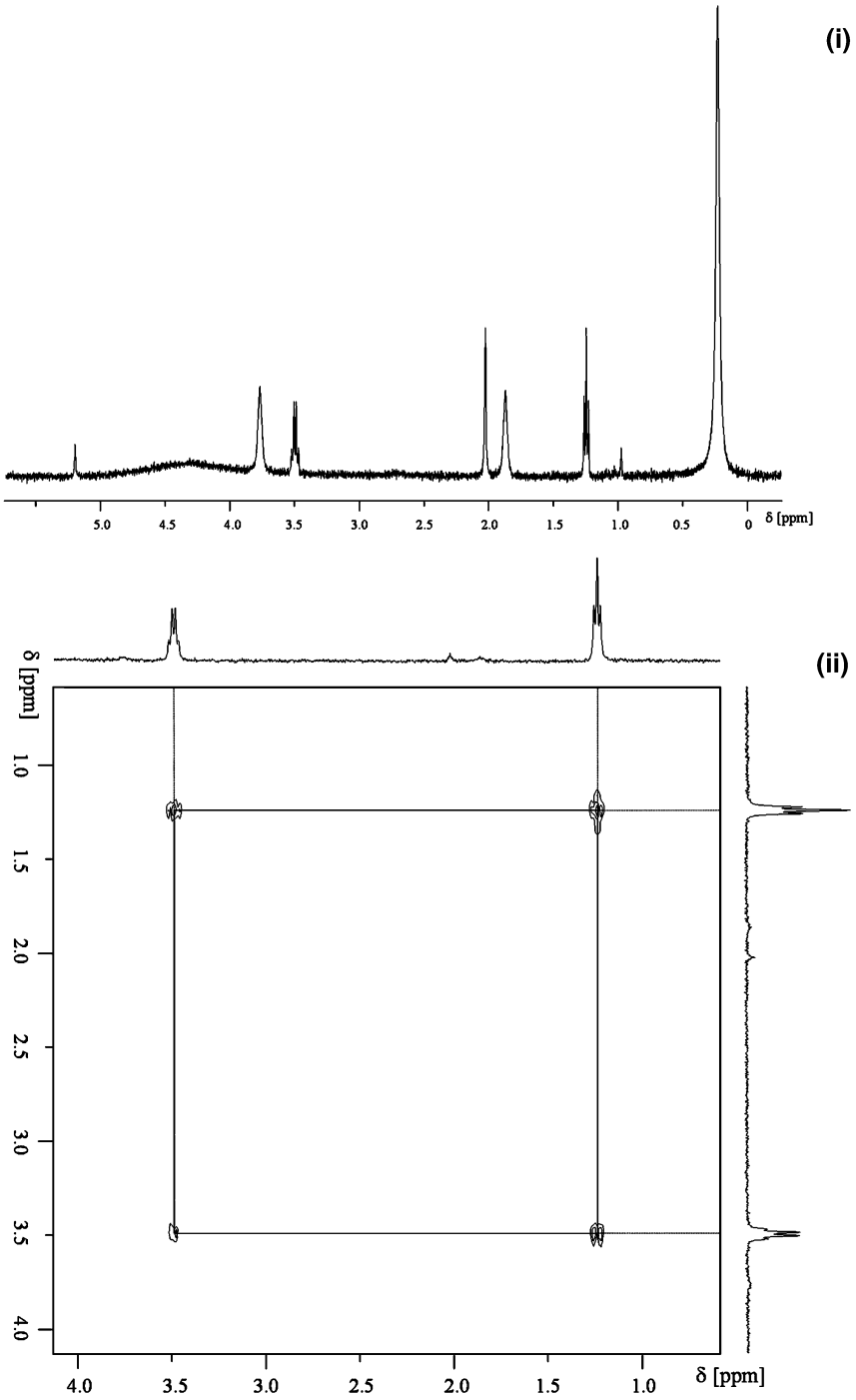

Figure 2. (i)^{1}H NMR spectrum of a gaseous mixture of methane (a), diethyl ether (b), tetrahydrofuran (c), acetone (d), and dichloromethane. Data acquisition parameters: 64 transients with a spectral width of 5618 Hz and 16k time domain points. The relaxation delay was set to 5 s. The measurement time was 6 min 59 s. (e). (ii)^{1}H,^{1}H COSY spectrum of diethyl ether. Data acquisition parameters: 4 transients with 1k complex data points and a spectral width of 4132 Hz. The measurement time was 39 min 16 s.

measured using a 10 mM sucrose solution in D_2O and ACN-d_3 (90:10 *v:v*). A single scan gave an SNR for the anomeric proton of 13.8: 1, corresponding to a limit of detection (LOD) of 1.86 μg. The spectral resolution is comparable to the results achieved with commercially available solenoidal microprobes, although the LOD is slightly poorer.

Next, gases were introduced into the probe. This was achieved by filling 300 μL of various volatile (bp < 65 °C) solvents into a GC-sealed vial and piercing the transfer capillary to the NMR through the septum. Methane was injected into the vial with a 50-mL syringe, thus forcing the gaseous phase into the transfer capillary and the probe.

After the pressure had decreased and the flow had stopped, NMR spectra were recorded as shown in Figure 2i.

The frequency scale in Figure 2i is calibrated to methane at 0.232 ppm[18] and shows dichloromethane (5.20 ppm), tetrahydrofuran (1.87 and 3.77 ppm), acetone (2.03 ppm), diethyl ether (1.25

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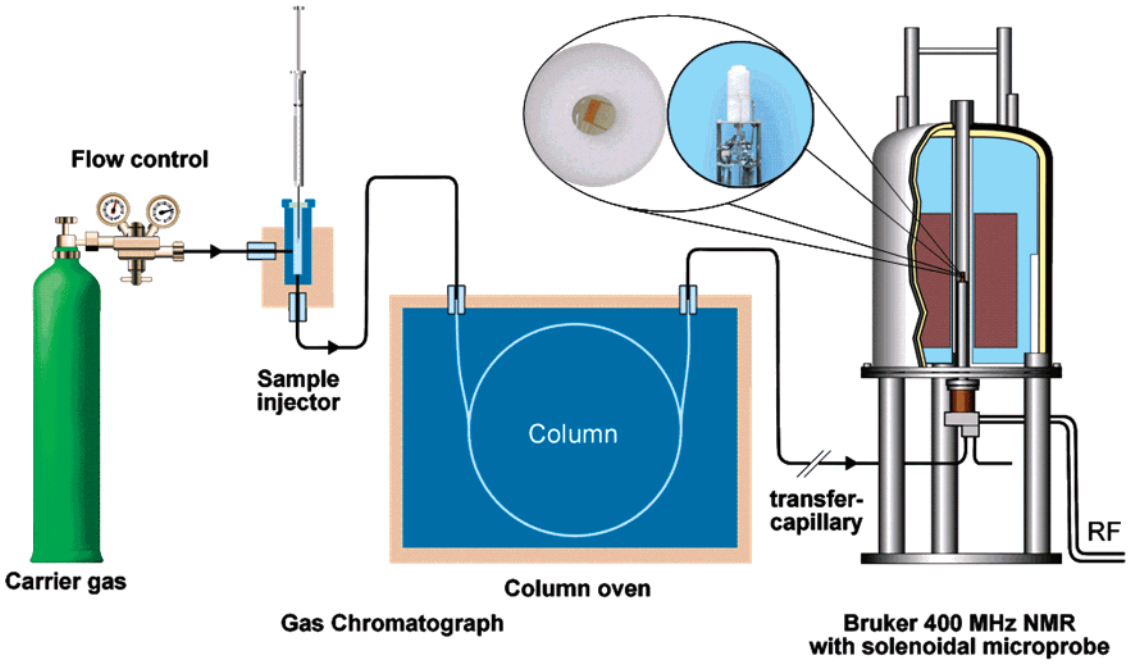

Figure 3. Instrumental setup for the GC-NMR coupling.

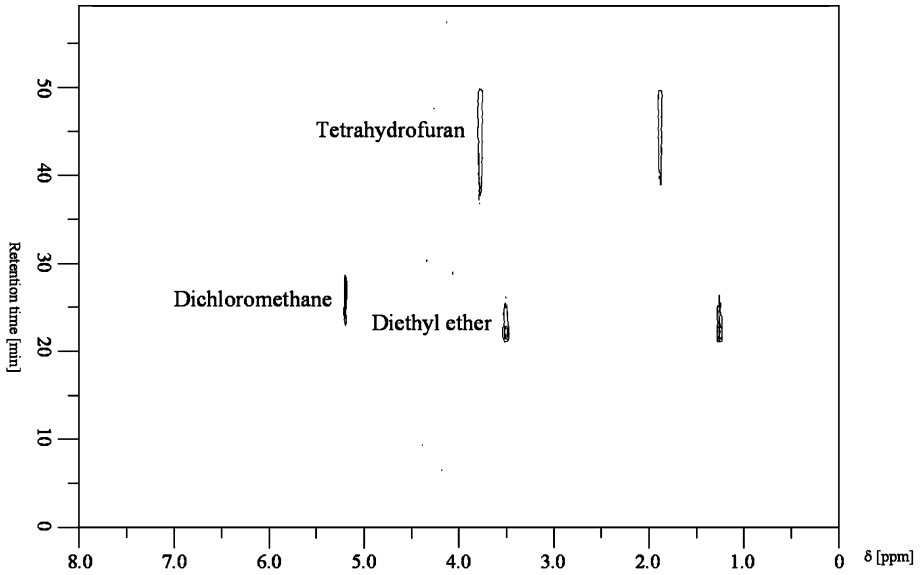

Figure 4. Contour plot of the GC-NMR separation of diethyl ether, dichloromethane and tetrahydrofuran. Data acquisition parameters: 32 transients with 4k time domain points and a spectral width of 5618 Hz were accumulated with a relaxation delay of 500 ms. During the separation, 128 rows with an acquisition time of 28 s per row were recorded.

and 3.50 ppm), and methane (0.23 ppm). The triplet and quartet of diethyl ether are resolved, which shows that small coupling constants can be measured. The signal at 0.98 ppm derives from an impurity from the natural gas, and the broad signal at ~4.4 ppm derives either from impurities in the susceptibility-matching fluid (FC-43) or from atmospheric moisture.

Figure 2ii shows a COSY spectrum of diethyl ether recorded in the gas phase. It was obtained by accumulating just four signal averages, showing the feasibility of recording 2D spectra in the gas phase using microprobes; 2D spectra often provide crucial information for a full structure elucidation.

Under static conditions, the T_1 value for methane was measured to be 51 ms and for diethyl ether 0.7 s for the CH_3 protons and 0.8 s for the CH_2 protons. These values are much lower in the gaseous phase than in the liquid phase due to a very effective spin-rotation relaxation mechanism. The advantage of the short T_1 values is that, under flowing conditions, the gaseous samples become polarized much more quickly.

Finally, the gas chromatograph was coupled to the NMR spectrometer. This was achieved by connecting the GC column to a transfer capillary, which was connected to the inlet capillary to the NMR on the other end. Figure 3 shows the instrumental setup.

A splitless injection of 10 μL of diethyl ether, dichloromethane, and tetrahydrofuran (3.3 μL each) leads to the pseudo 2D plot depicted in Figure 4. This contour plot shows the ^{1}H chemical shift axis (F_2 dimension) vs the retention time.

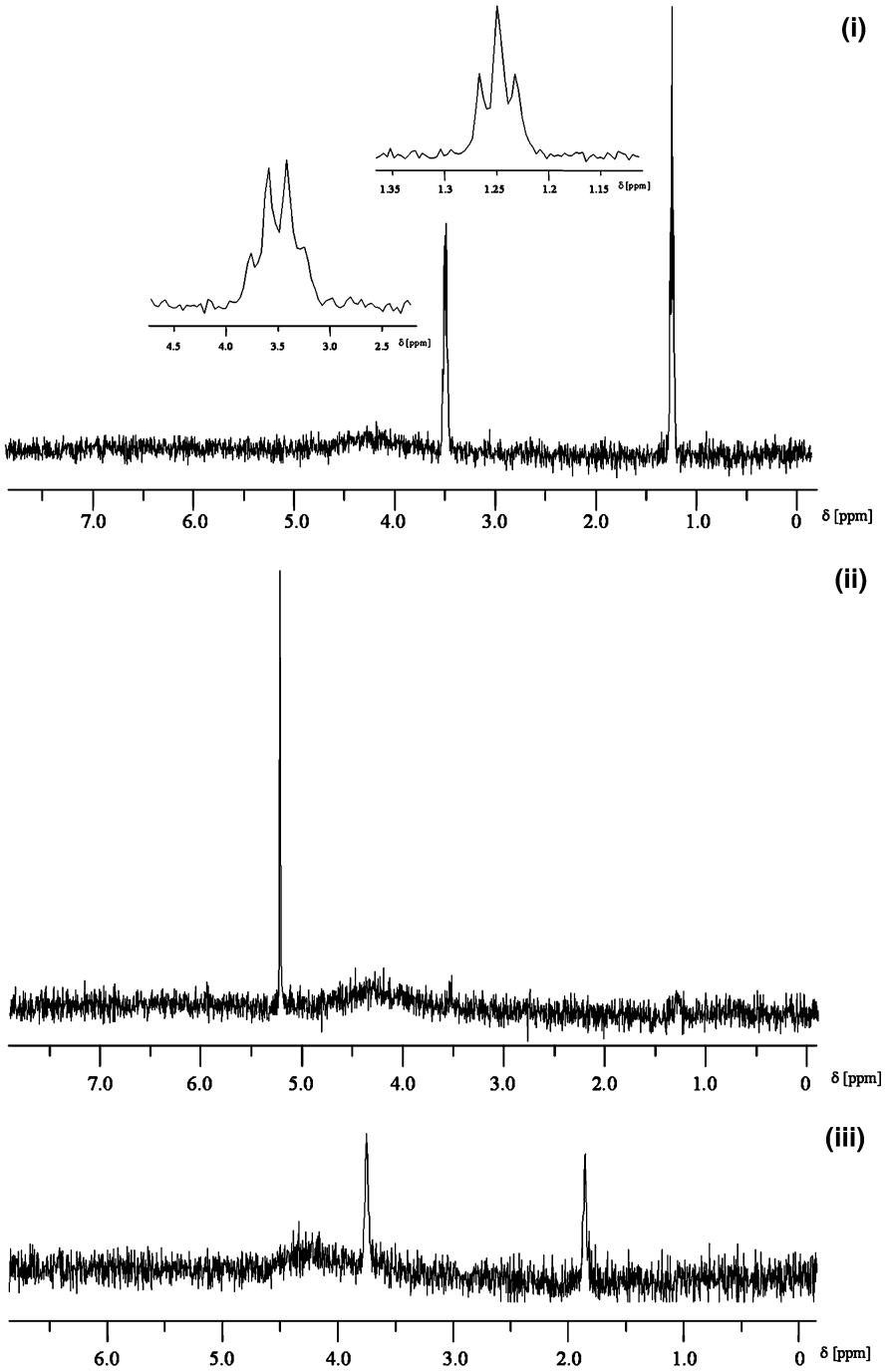

Figure 5. ^{1}H NMR spectra extracted from Figure 4 of (i) diethyl ether, (ii) dichloromethane, and (iii) tetrahydrofuran.

The flow rate in the GC-column is 0.72 mL/min. The residence time τ in the detection cell can be calculated according to

$$W_{\text{flow}} = W_{\text{stationary}} + 1/\tau; \quad W = \text{signal half-width}$$

The signal half-widths were derived from the diethyl ether spectra recorded in stopped-flow and continuous-flow mode. This leads to a residence time in the detection cell of 0.625 s.

The flow rate is a compromise between high chromatographic resolution and sufficient SNR for NMR detection. It does not represent the van-Deemter optimum, but higher flow rates would lead to too short residence times in the detection cell and very broad NMR linewidths.

Averaging 32 transients (over 28 s) leads to partial loss of chromatographic resolution. However, although several rows in the retention time dimension show overlapping signals of diethyl ether and dichloromethane, this technique allows a clear distinction between the different analytes. The total amounts of analytes are 32 μmol of diethyl ether, 52 μmol of dichloromethane, and 40 μmol of tetrahydrofuran. We also experimented with smaller injection volumes, and the SNR was still sufficient to obtain all spectra at half the injection volume (data not shown). The contour

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plot also shows that the analytes do not condense in the flow cell (in which case the NMR spectra would remain at constant amplitude over time).

To obtain a more detailed interpretation, ^{1}H NMR spectra can be extracted from the maximums of the contour plot corresponding to the maximum signal intensity of each peak. Figure 5 shows the extracted ^{1}H NMR spectra of diethyl ether, dichloromethane, and tetrahydrofuran. Each spectrum corresponds to 32 signal averages, which took 28 s each to acquire. Even in the gaseous continuous-flow mode, the multiplets of diethyl ether are resolved and the coupling constants can be derived from the extracted ^{1}H NMR spectrum.

The preliminary results obtained are very promising for future experiments. Critical to such experiments will be an increase in the SNR of NMR detection, because longer detection times lead to a loss of the chromatographic resolution. There are several methods by which the SNR may be increased, such as increasing the volume of the flow cell, improvement of the filling factor ($V_{coil}/V_{flow\ cell}$), and operating at higher magnetic fields.

With respect to the last point, it is important to recognize that these experiments have been performed at a relatively low field strength, 400 MHz. With 800-MHz systems in routine use, one would obtain a ~4-fold increase in SNR, corresponding to 16 times less signal averaging, meaning that spectra could be acquired in ~1 s. This would allow very fine chromatographic resolution to be obtained.

Novel techniques such as positioning a capillary column filled with chiral chemical shift reagent close to the active volume of the detection cell in the NMR probe (similar to Gd(III) chelate filled columns employed for ^{13}C continuous-flow quantification studies carried out in our group)[19] will be investigated. This would enable, to our knowledge, the first chiral GC- and LC-NMR experiments for the analysis of enantiomeric compounds, e.g., pheromones.

A major technical challenge in this technique is heating of the transfer capillary and the probe head. With boiling points above 65 °C, problems arise in the form of partial condensation in the capillary connections. In considering the potential assembly of a heating unit to minimize this problem, one is constrained by the requirement that such an apparatus must operate in a strong magnetic field. Possible solutions include the transfer capillary being heated by a bifilar coil constructed from zero-susceptibility wire. In addition, we are investigating alternative susceptibility matching media, since FC-43 evaporates at temperatures between 165 and 185 °C and shows hazardous decomposition above 200 °C. The NMR registration of GC fractions of higher boiling points will be described at a later date.

CONCLUSION

The presented results demonstrate the potential of the hyphenation of capillary GC to solenoidal-type microprobe ^{1}H NMR detection as a technique for the analysis of volatile compounds. Although sensitivity, in contrast to GC/MS, is still a major challenge when coupling GC to the relatively insensitive NMR detection, continuous-flow ^{1}H NMR measurements of a small amount of sample were successfully carried out, allowing immediate structure identification of the eluted analytes, as demonstrated in this paper. Even 2D NMR experiments, which are often indispensable for unambiguous structure elucidation of unknown compounds, can be performed. Continuous-flow GC-NMR mode needs 1−2 μg of sample for successful NMR detection at 400 MHz, but instrumental improvements as well as higher field strengths are anticipated to bring this number down substantially.

ACKNOWLEDGMENT

The authors thank Dr. Norbert Welsch from Welsch & Partner (Tübingen, Germany) for assistance with the preparation of the figures. This work was supported by the Deutsche Forschungsgemeinschaft (Graduiertenkolleg " Chemie in Interphasen" Grant 441), Bonn-Bad-Godesberg, Germany.

Received for review September 20, 2006. Accepted January 22, 2007.

AC0617767