Applied Magnetic Resonance (2023) 54:1555–1569
https://doi.org/10.1007/s00723-023-01626-8

Applied
Magnetic Resonance

ORIGINAL PAPER

# Quantitative Analysis in Continuous-Flow $^{1}$H Benchtop NMR Spectroscopy by Paramagnetic Relaxation Enhancement

**Raphael Kircher[1,2] · Sarah Mross[1,2] · Hans Hasse[1,2] · Kerstin Münnemann[1,2]**

Received: 13 August 2023 / Revised: 26 September 2023 / Accepted: 10 October 2023 /
Published online: 3 November 2023


## Abstract

Nuclear magnetic resonance (NMR) spectroscopy is an excellent tool for reaction and process monitoring. Process monitoring is often carried out online, where the analytic device is operated in flow mode. Benchtop NMR spectrometers are especially well-suited for these applications because they can be installed close to the studied process. However, quantitative analysis of a fast-flowing liquid with NMR spectroscopy is challenging because short residence times in the magnetic field of the spectrometer result in inefficient polarization buildup and thus poor signal intensity. This is particularly problematic for benchtop NMR spectrometers, where it severely limits the flow velocity in quantitative measurements. One method for increasing polarization in continuous-flow NMR spectroscopy is paramagnetic relaxation enhancement (PRE). Here, the interaction of the studied liquid with a PRE agent significantly accelerates the buildup of nuclear polarization prior to NMR detection, which enables quantitative measurements at high flow velocities. For process monitoring applications, the synthesis of robust and chemically inert immobilized PRE agents is mandatory. This was accomplished in the present work, where a new PRE agent is tested on 12 common solvents including water, acetonitrile, 1,4-dioxane, and binary mixtures with quantitative benchtop $^{1}$H NMR spectroscopy at 1 Tesla. The results show that the flow regime for quantitative measurements can be greatly extended by the use of the synthesized PRE agent.

## 1 Introduction

NMR spectroscopy offers outstanding possibilities for the investigation of technical processes, e.g. reaction and process monitoring [1–5]. A bypass approach is used often, in which a small amount of the sample is continuously pumped through a measurement loop passing through the NMR spectrometer. For an unbiased observation of the process, it is desirable that the volume of the bypass is as small as possible and that only a minimal time delay occurs between process and measurement.

Extended author information available on the last page of the article

The high flexibility and compact design of benchtop NMR spectrometers make them ideally suited for such applications [6–11], as they can be installed close to the studied process. However, the analysis of a fast-flowing liquid with benchtop NMR spectrometers is particularly challenging: the small size of the permanent magnets used in benchtop NMR spectrometers leads to an incomplete polarization buildup in flowing samples due to the short residence time of the liquid in the magnetic field. This incomplete polarization buildup prevents quantitative NMR measurements and, thus, the desired accurate analysis of the composition of mixtures.

Polarization of a sample in continuous flow can be monitored by measuring a signal $S$ at varying flow velocity and comparing it to a signal $S_0$ recorded without flow. For a laminar flow, this ratio can be described by Eq. 1 [12–15]:

$$\frac{S}{S_0} = 1 - \exp\left(-\frac{L_{\mathrm{Pol}}}{v \cdot T_1}\right) \tag{1}$$

where $v$ is the average flow velocity, $T_1$ the longitudinal relaxation time of the nuclear-spin polarization, and $L_{\mathrm{Pol}}$ the polarization length of the spectrometer. For quantitative NMR measurements in continuous-flow, residence times in the magnetic field of the spectrometer of around $5 \times T_1$ are required for complete polarization buildup, if no calibration or recalculation of polarization is applied. However, even for $^1$H nuclei, this is on the order of many seconds for most molecules, thus heavily compromising the delay time between the process and measurement. In addition to protons ($^1$H), carbon ($^{13}$C) or nitrogen ($^{15}$N) are often studied by NMR spectroscopy; these nuclei usually have significantly longer $T_1$ than protons and the effect of incomplete polarization buildup is even worse. In benchtop NMR spectrometers, which typically have rather short polarization lengths $L_{\mathrm{Pol}}$ of around 0.1 m [15], this can lead to a complete disappearance of the signal at high flow velocities.

This problem can be tackled by the addition of paramagnetic relaxation agents to the sample which drastically shorten the $T_1$ of the surrounding nuclei, resulting in the so-called paramagnetic relaxation enhancement (PRE) [16–18]. The PRE effect is mainly dependent on electron-nuclear dipole-dipole interactions, which scale with distance as $r^{-6}$ [19]. Many different PRE agents have been developed based on either stable radicals or metal complexes. They can roughly be divided into two categories: dissolved paramagnetic materials and immobilized paramagnetic materials. A major application field of PRE NMR is medical imaging, where dissolved metal complexes, based on e.g. gadolinium (III) ions are used as contrast agents [20–24]. These metal complexes are usually optimized for most efficient relaxation of water protons and yield only poor PRE efficiencies in nonpolar solvents, which reduces their applicability in process monitoring applications.

Furthermore, dissolved paramagnetic material in the sample can hamper NMR detection, because it has an influence not only on the $T_1$ of the molecules but also on their transversal relaxation time $T_2$, which can lead to an undesired broadening of the NMR lines. This is especially disturbing if the PRE agent is used in high concentrations for maximizing its effect or in continuous-flow NMR applications, where an additional broadening of NMR lines is observed due to out-flow effects of the sample during NMR acquisition [25]. It is therefore advantageous to use a packed bed

of PRE agent instead, through which the sample passes before entering the NMR coil for detection [15, 26, 27]. Immobilized free radicals (IFRs) were already used in the early 1980 s to shorten $T_1$ of $^1$H nuclear spins. In addition, immobilized and dissolved PRE agents were studied in continuous-flow high field NMR with heteronuclear detection [28, 29]. Moreover, it has been demonstrated that a larger linker, and hence a greater distance from the surface of the carrier material, can result in an improved PRE [30]. However, the radical loading of PRE agents must be high enough to ensure a $T_1$ reduction sufficient for enabling quantitative flow NMR in the short polarization length of benchtop NMR spectrometers. Thus, new synthesis strategies are required to achieve high radical loadings of PRE agents. Moreover, for process and reaction monitoring applications it is mandatory that the PRE agent is compatible with polar and nonpolar solvents. Additionally, it should be robust, chemically inert, and exhibit a good long-term stability.

The aim of this work was to develop a new PRE agent that matches the above-mentioned design criteria. Its PRE efficiency should be high enough to ensure complete $^1$H polarization buildup in benchtop NMR spectrometers even at high flow velocities. Therefore, a packed bed of a solid PRE agent with high radical loading was synthesized and applied in continuous-flow quantitative benchtop $^1$H NMR spectroscopy within this work. Aminopropyl-grafted controlled porous glass (CPG) was used as solid support material because of its high chemical inertness and good flow characteristics. Polyethyleneimine was first coupled to the CPG to multiply the binding sites for the coupling of nitroxide radicals [31] to achieve a sufficiently high radical loading. Thereafter, glycidyloxy-tetramethylpiperidinyloxyl (GT) radicals were coupled to the binding sites of the polymer. To examine the PRE effect of the synthesized material, several liquids commonly used as solvents in industrial processes, were investigated with respect to their polarization buildup in contact with the synthesized PRE agent. Furthermore, two binary mixtures (acetonitrile+water and acetonitrile+1,4-dioxane) were studied in quantitative continuous-flow $^1$H NMR experiments with varying flow velocities to demonstrate a sufficient PRE efficiency of the new material.

## 2 Materials and Methods

Chemicals used in the presented synthesis and in continuous-flow benchtop NMR are listed in Table 1 and were used without further purification. Water was obtained using an Elix Essential 5 purification system from Merck.

The synthesis was previously published by our group in detail [31]. Aminopropyl-CPGs (350 mg) were added to ethanol (5 × 2 mL) and centrifuged (5 min at 2500 rpm). 1,4-Butanediol diglcyidyl ether (283.15 mg) was dissolved in methanol (3.5 mL) and dry aminopropyl-CPGs were added. The reaction mixture was rotated in a tube rotator for 24 h at room temperature. Water (5 × 15 mL) and later ethanol (2 × 15 mL) were added and centrifuged (5 min at 2500 rpm). Polyethyleneimine (molecular mass of 25 000 g mol$^{-1}$, 8.75 g, 20 mM) was dissolved in methanol and BDGE-grafted CPGs were added. The reaction mixture was rotated in a tube rotator for 24 h at room temperature. Water (5 × 15 mL)

**Table 1** Chemicals used in the presented synthesis and in continuous-flow NMR experiments are listed with respective suppliers and purities

| Chemical | Supplier | Purity / g $g^{-1}$ |
|---|---|---|
| Aminopropyl-CPG | Biosearch Technologies | |
| 4-Glycidyloxy-2,2,6,6-tetramethylpiperidinyloxyl | TCI | > 0.950 |
| 1,4-Butanediol diglycidyl ether | Sigma Aldrich | > 0.950 |
| Polyethyleneimine | Sigma Aldrich | > 0.990 |
| Methanol | Sigma Aldrich | ≥ 0.999 |
| Ethanol | Sigma Aldrich | ≥ 0.999 |
| Acetonitrile | Roth | ≥ 0.999 |
| Acetone | Sigma Aldrich | ≥ 0.998 |
| Ethylene glycol | Roth | ≥ 0.995 |
| Benzonitrile | Sigma Aldrich | ≥ 0.998 |
| 1-Propanol | Sigma Aldrich | ≥ 0.995 |
| 2-Propanol | Sigma Aldrich | ≥ 0.999 |
| Tetrahydrofuran | Sigma Aldrich | ≥ 0.999 |
| Pyridine | Fisher Scientific | ≥ 0.999 |
| 1,4-Dioxane | Sigma Aldrich | ≥ 0.998 |

and later ethanol (2 × 15 mL) were added and centrifuged (5 min at 2500 rpm). GT (399.56 mg, 0.5 mM) was dissolved in methanol and added to previously prepared PEI-grafted CPGs. The reaction mixture was rotated in a tube rotator for 24 h at room temperature. Water (5 × 15 mL) was added and CPGs were separated by filtration. The product was stored in water (2.0 mL) at 277 K.

Radical loading of the synthesized PRE agent was studied with a MicroESR-X-Band spectrometer from Bruker. Analysis was performed using micro capillaries from Blaubrand intraMark with an inner diameter of 1 mm and a sample volume of around 4 µL. Samples were sealed with Leica Microsystems Critoseal capillary tube sealant. The concentration of immobilized radicals was determined by comparison of EPR integrals with aqueous solutions of dissolved GT. Acquisition parameters were set to: microwave power 15 mW, modulation coil amplitude 1.0 G, and receiver gain 12 dB; and an average of 16 scans was used [31].

A benchtop NMR spectrometer (Spinsolve Carbon, manufactured by Magritek) operating at a proton resonance frequency of 43 MHz was used for all NMR experiments. Inversion-recovery NMR measurements were performed in microprobe NMR tubes purchased from Norell with an outer diameter of 2.5 mm, which were used to minimize the sample volume. For samples in static contact with the synthesized PRE agent, the supernatant was removed several times after brief centrifugation, so that finally no liquid without static contact with the PRE agent was in the active volume of the NMR spectrometer. Parameters were set with the standard $T_1$ measurement protocol supplied by Magritek. The relaxation delay between several excitations was chosen to be sufficiently high for complete equilibration of the nuclear spins. An average of 8 scans was used. The relative

error of calculated $T_1$ values from successive NMR measurements is less than 1% in this work.

Benchtop $^1$H NMR spectroscopy with PRE agent was performed with a container manufactured of Polyether ether ketone (PEEK) that was positioned directly in front of the NMR-active volume of the benchtop NMR spectrometer to store the PRE agent [15]. Inner diameter of this PEEK container and of the PEEK capillary running through the benchtop NMR spectrometer was 1 mm. 300 mg of PRE agent was filled in the PEEK container, which results in a filling height of around 50 mm. Samples were fed to the set-up using a HPLC pump purchased from Flusys that was additionally equipped with a mass flow meter (Mini CORI-FLOW) purchased from Bronkhorst. NMR experiments were performed with a single-pulse free induction decay (FID) sequence using short acquisition times of around 400 ms. The recycle delay between individual scans was set to 3 s, which ensured a full exchange of the liquid in the active volume of the RF coil. The standard deviation in successive continuous-flow NMR data is overall less than 2% in this work. NMR data without flow was recorded with a recycle delay of 30 s.

## 3 Results and Discussion

### 3.1 Continuous-flow Benchtop $^1$H NMR Spectroscopy without PRE Agent

In benchtop $^1$H NMR experiments, the problem of incomplete polarization buildup occurs already at moderate flow velocities. This is illustrated in Fig. 1 by results calculated with Eq. 1 for a polarization length of 0.1 m typical for benchtop NMR

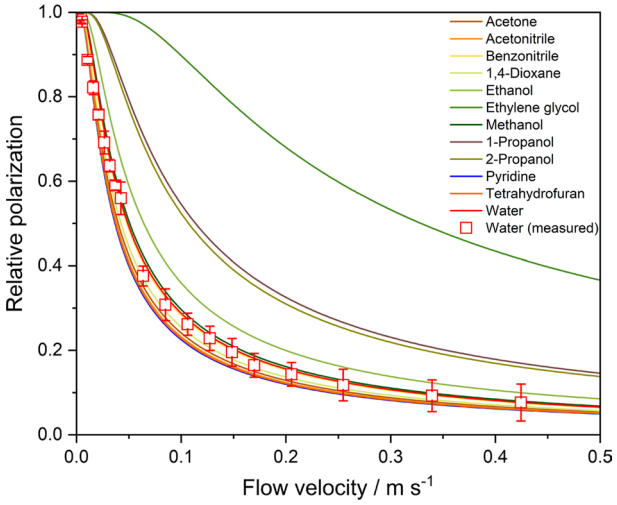


**Fig. 1** Relative polarization of protons of 12 common solvents versus flow velocity. Lines represent calculations using Eq. 1. Squares are experimental results for water measured in a 1 Tesla benchtop NMR spectrometer and were obtained as the arithmetic mean of three individual acquisitions. Error bars indicate the standard deviation

spectrometers, for 12 common solvents based on the $T_1$ values given in Table 2. For molecules with several distinguishable protons, the highest $T_1$ was used. In addition, experimental results are presented for water using a PEEK capillary with 1 mm inner diameter, which was simply inserted into the bore of the flow probe. Experimental data is shown as squares and was obtained as the arithmetic mean value of three individual NMR acquisitions. As can be seen in Fig. 1, they agree very well with the predictions based on Eq. 1.

Figure 1 shows that already moderate flow velocities well below 0.1 m $s^{-1}$ generally result in drastic polarization losses in continuous-flow benchtop $^1$H NMR applications without PRE agents. The relative polarization decreases exponentially with increasing flow velocity, corresponding to around 80–90% signal loss at a flow velocity of 0.4 m $s^{-1}$ for most of the studied solvents. The decrease in relative polarization is most pronounced for solvents with long $T_1$. However, bringing the solvents in contact with a PRE agent drastically shortens their $T_1$ (denoted as $T_1^{RM}$ in Table 2) and greatly accelerates the polarization buildup.

### 3.2 Continuous-flow Benchtop $^1$H NMR Spectroscopy with PRE Agent

#### 3.2.1 Synthesis of New PRE Agent

Controlled porous glasses (CPGs) were chosen as solid support material for the PRE agent because they are robust, chemically inert, exhibit a good flow characteristic, and are commercially available in high quality. In this work, we have used an aminopropyl-functionalized CPG with a pore size of 30 nm and particle size of 70 to 140 µm, which possesses a high surface area and, thus, a high number of binding sites. However, direct coupling of GT on the CPG resulted in an immobilized radical concentration of only 1.5 mM, which is far too low for a high PRE efficiency with CPG-based materials [31]. Therefore, the number of available binding sites had to be significantly increased, which was achieved by first coupling a cross-linked polymer

**Table 2** Overview of measured longitudinal relaxation times $T_1$ of common solvents at 1 Tesla and 28.5°C. For molecules with several distinguishable protons, the highest $T_1$ is listed. $T_1^{RM}$ denotes measured values of the investigated solvents in static contact with the synthesized PRE agent

| Solvent | $T_1$/s | $T_1^{RM}$/s | PRE $(T_1\text{-}T_1^{RM})/T_1$ |
|---|---|---|---|
| Water | 3.10 | 0.05 | 0.98 |
| Acetonitrile | 3.99 | 0.05 | 0.99 |
| Ethanol | 2.36 | 0.06 | 0.97 |
| Ethylene glycol | 0.46 | 0.12 | 0.74 |
| Benzonitrile | 3.16 | 0.06 | 0.98 |
| Acetone | 3.82 | 0.13 | 0.97 |
| Methanol | 2.99 | 0.15 | 0.95 |
| 1-Propanol | 1.34 | 0.16 | 0.88 |
| 2-Propanol | 1.42 | 0.17 | 0.88 |
| Tetrahydrofuran | 4.07 | 0.19 | 0.95 |
| Pyridine | 4.13 | 0.09 | 0.98 |
| 1,4-Dioxane | 3.58 | 0.21 | 0.94 |

exhibiting many binding sites (polyethyleneimine, PEI) to the CPG before attaching the stable nitroxide radical GT. We have also synthesized solid packed beds of this type for hyperpolarized NMR by Overhauser dynamic nuclear polarization (ODNP), where immobilized free electrons are also essential. For ODNP-enhanced NMR, the aim was to achieve radical loadings in the range of 20 to 30 mM [31]. Here, our aim was to further increase the concentration of radicals to optimize their PRE efficiency. The synthesis of the new PRE agent is shown in a simplified scheme in Fig. 2. The CPG has multiple aminopropyl groups on the surface and in the pores, but, for simplicity, Fig. 2 shows only one of these binding sites of the CPG.

Figure 2 shows that the coupling reactions in the presented immobilization procedure of GT take place exclusively via the reaction of epoxy and amino groups in all three synthesis steps. This is a selective reaction that can be carried out under mild conditions. Here, reactions were solely performed at 293 K in methanol for a period of 24 h, which resulted in a stable radical concentration of 120 mM in the synthesized paramagnetic packed bed. The paramagnetic-packed bed showed no degradation in the entire series of continuous-flow experiments. The long-term stability of CPG-based packed beds with immobilized nitroxide radicals and PEI-linker has previously been investigated by our group for ODNP-enhanced benchtop NMR spectroscopy [31]. During the storage of the PRE agent in acetone, water, diethyl ether, acetonitrile, and 3-pentanone, we observed no leakage of the radicals into the solvents over a period of 50 days. Furthermore, we have shown that the developed PRE agent can tolerate acidic conditions, which is highly beneficial for process monitoring applications.

To analyze the PRE efficiency of the synthesized material, $T_1$ of 12 common solvents was measured in static contact with the synthesized PRE agent ($T_1^{RM}$) and without the PRE agent. Table 2 lists $T_1$ of investigated solvents, which were measured directly in the benchtop NMR spectrometer. The experimental results were obtained as the arithmetic mean of the results of three individual measurements. $T_1$ of the studied solvents was reduced by at least 74% in contact with the PRE agent, and for most solvents significantly more, e.g. the $T_1$ for water and acetonitrile was

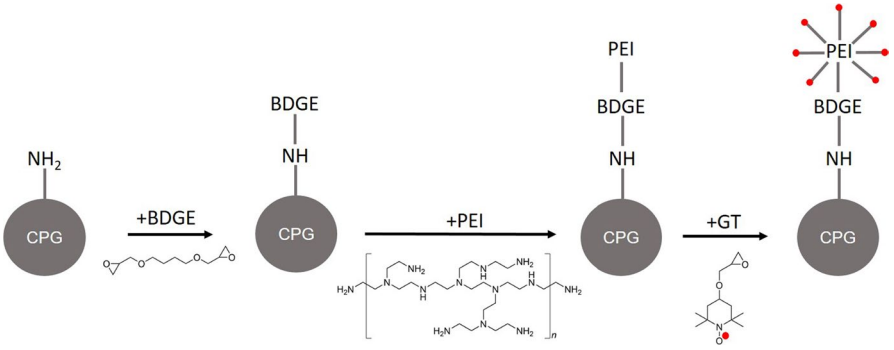


**Fig. 2** Scheme of the three-step synthesis of the new PRE agent: (1) coupling of BDGE on aminopropyl-functionalized CPGs; (2) coupling of PEI on BDGE-grafted CPGs; (3) immobilization of GT on PEI-grafted CPGs. All reactions are solely performed in methanol at ambient temperature

reduced by 98% to 50 ms in static contact with the PRE agent. For most of the solvents, the PRE efficiency is around 90%, which is remarkable because the solvents exhibit different polarities. Only for molecules with a rather short native $T_1$ is the PRE efficiency reduced, because here other relaxation pathways are involved, which reduce the possible paramagnetic relaxation. All solvents show $T_1^{RM}$ values in contact with the radical matrix of less than 200 ms, which significantly shortens the time required for complete polarization buildup.

### 3.2.2 Experimental Set-up

For demonstrating the applicability of the new PRE agent in continuous-flow benchtop NMR spectroscopy, quantitative $^1$H NMR measurements were performed at different flow velocities. The corresponding set-up with incorporated PRE agent is shown in Fig. 3. The liquid to be analyzed first passes through a packed bed of the PRE agent where the accelerated polarization buildup takes place before entering the NMR-active volume.

Data were always acquired with a 1 Tesla benchtop NMR spectrometer from Magritek (Spinsolve Carbon, proton frequency of 43 MHz), into which a 1 mm inner-diameter PEEK capillary containing the flowing liquid sample was placed. The PRE agent was installed in a PEEK container with 1 mm inner diameter positioned directly prior the NMR coil in the flow path. NMR experiments were performed with acquisition times of 400 ms to minimize out-flow effects that occur

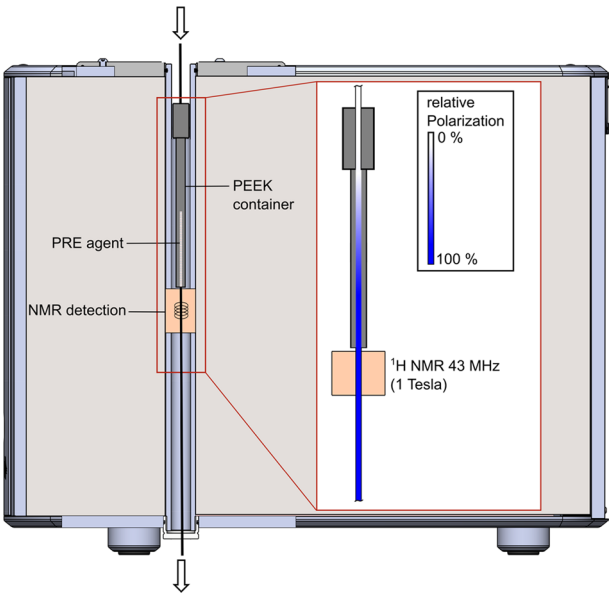


**Fig. 3** Set-up for continuous-flow benchtop NMR spectroscopy with PRE agent. The direction of the liquid flow is indicated by arrows. Insert: visualization of accelerated polarization buildup in the flow path with PRE agent

when the sample is already leaving the active volume of the NMR spectrometer during acquisition.

### 3.2.3 Polarization in Continuous-Flow Benchtop $^{1}$H NMR Spectroscopy

To demonstrate the effect of the PRE agent on polarization buildup in continuous-flow benchtop $^{1}$H NMR experiments, we compared experiments with and without PRE agent. Measurements were performed on two binary liquid systems (system 1: acetonitrile+water and system 2: acetonitrile+1,4-dioxane) of different compositions with the experimental set-up shown in Fig. 3.

Figure 4 shows the results of the recorded signal integral of acetonitrile for four different compositions of system 1 (acetonitrile+water) at flow velocities up to 0.04 m s$^{-1}$. Figure 5 shows the results for water in system 1. The presented results were obtained from three individual acquisitions, where the recorded signal integral was normalized by dividing it by the number of protons in the studied functional group. Error bars (smaller than symbol size) indicating standard deviation are not shown for clarity purposes.

The signal integrals of acetonitrile and water decrease significantly with increasing flow velocity without PRE agent. At a flow velocity of 0.04 m s$^{-1}$ signal integrals of acetonitrile are reduced by around 50% for all studied mixtures without PRE agent compared to the signal without flow. The signal integrals of water were reduced by around 40% due to the shorter $T_1$ of water. In addition, as the concentration of water in the mixture decreases, the polarization problem of the water protons becomes less severe. Low concentrations of water in this binary system result in shorter $T_1$ of water [32], which slightly improves the buildup of its polarization.

This trend can also be observed in the measurements with PRE agent. For water, the signal integral is almost stable over the examined flow velocity range at a comparable value to the measurement without flow. The reduction of the signal integrals

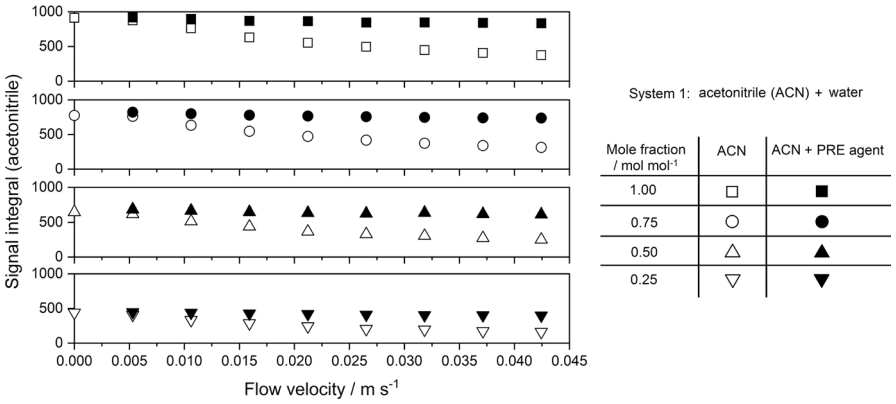


**Fig. 4** Comparison of $^{1}$H NMR signal integrals of acetonitrile (ACN) for mixtures in system 1 (acetonitrile+water) recorded with the benchtop NMR spectrometer (open symbols) and with PRE agent (closed symbols). Symbols are experimental results and were obtained as the arithmetic mean value of three individual acquisitions

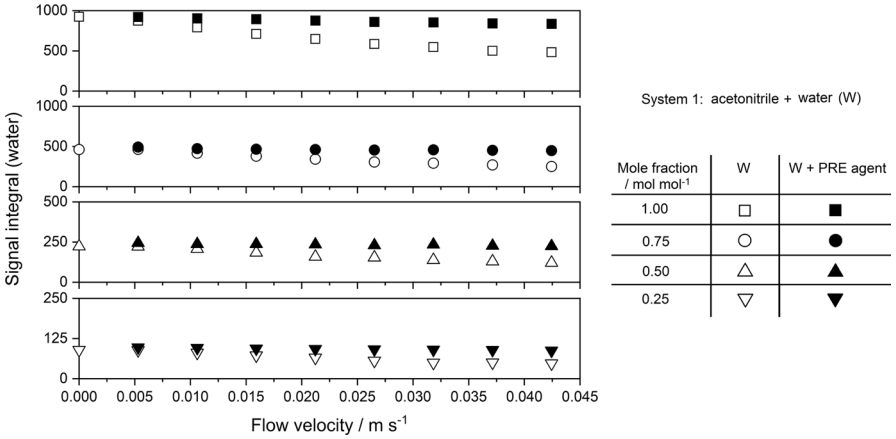


**Fig. 5** Comparison of $^{1}$H NMR signal integrals of water (W) for mixtures in system 1 (acetonitrile+water) recorded with the benchtop NMR spectrometer (open symbols) and with PRE agent (closed symbols). Symbols are experimental results and were obtained as the arithmetic mean value of three individual acquisitions. The scale of the signal integral for compositions with mole fractions of water $\leq 0.50$ mol mol$^{-1}$ is increased

for both components at the highest studied flow rate is less than 10%. However, a small decrease in signal for both components with increasing flow velocity can still be observed, although the polarization buildup must be complete given the short $T_1^{RM}$ of water and acetonitrile in contact with the PRE agent. This small drop in signal integrals can be attributed to out-flow effects. Nevertheless, signals for both components can be maintained at values comparable to data measured without flow over the entire velocity range.

Figures 6 and 7 show the results of the same experiments for system 2 (acetonitrile+1,4-dioxane).

The results recorded with the benchtop NMR spectrometer without PRE agent show again an ineffective polarization buildup for both components. The signal of acetonitrile is reduced by around 60% at a flow velocity of 0.04 m s$^{-1}$ for all studied concentrations and for 1,4-dioxane by around 50% compared to the respective signal without flow. By using the PRE agent, the signal integral was comparable to the signal without flow and, therefore, exhibited a high SNR in the $^{1}$H NMR spectrum for both components at all studied flow velocities. The small signal decrease that is still observable in the measurements with PRE agent is again caused by out-flow effects during NMR acquisition and is not due to incomplete polarization buildup in this range of flow velocities.

## 4 Quantification in Continuous-Flow Benchtop $^{1}$H NMR Spectroscopy

Figures 8 and 9 show the results of the quantitative analysis of continuous-flow measurements with and without the PRE agent. Open symbols represent the results without the PRE agent, whereas closed symbols indicate the results with the PRE

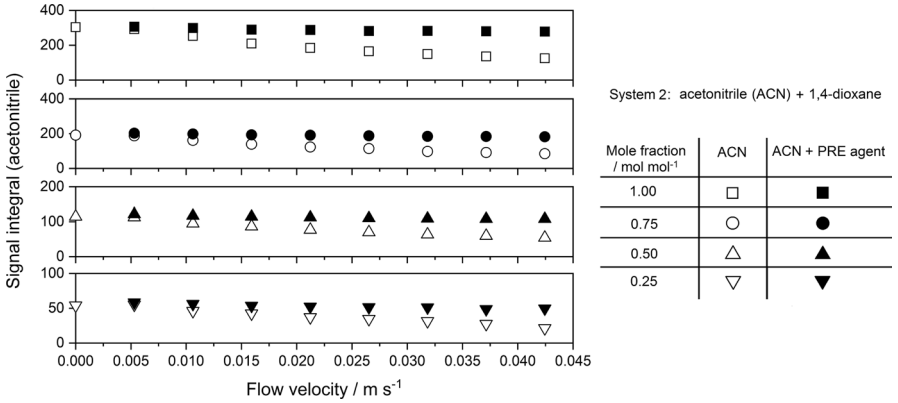


**Fig. 6** Comparison of $^{1}$H NMR signal integrals of acetonitrile (ACN) for mixtures in system 2 (acetonitrile+1,4-dioxane) recorded with the benchtop NMR spectrometer (open symbols) and with PRE agent (closed symbols). Symbols are experimental results and were obtained as the arithmetic mean value of three individual acquisitions. The scale of the signal integral for compositions with mole fractions of acetonitrile ≤ 0.50 mol mol$^{-1}$ is increased

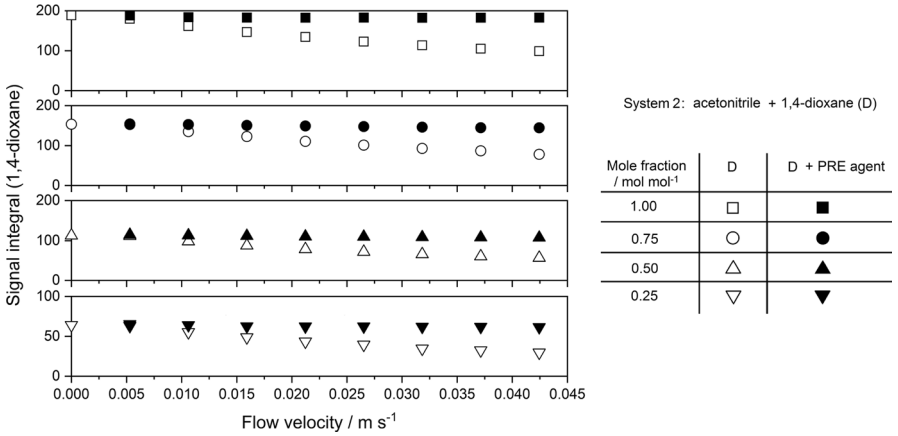


**Fig. 7** Comparison of $^{1}$H NMR signal integrals of 1,4-dioxane (D) for mixtures in system 2 (acetonitrile+1,4-dioxane) recorded with the benchtop NMR spectrometer (open symbols) and with PRE agent (closed symbols). Symbols are experimental results and were obtained as the arithmetic mean value of three individual acquisitions. The scale of the signal integral for the composition with a mole fraction of 1,4-dioxane of 0.25 mol mol$^{-1}$ is increased

agent. The dashed lines in the figures represent the ground truth, which corresponds to the mole fractions of the original sample weight. To calculate the mole fraction, the signal integrals shown in Figs. 4, 5, 6 and 7 were normalized by dividing each signal integral by the sum of the signal integrals of both components present in the mixture. Calculated mole fractions are shown for three compositions of the studied systems, c.f. Figure 8 for system 1 (acetonitrile+water) and Fig. 9 for system 2 (aceontrile+1,4-dioxane).

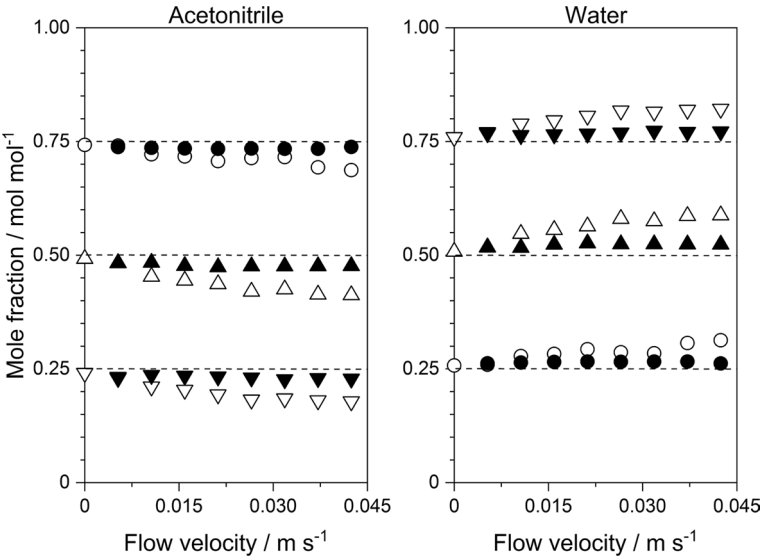


**Fig. 8** Quantification of $^{1}$H NMR data for system 1 (acetonitrile+water). Left panel: acetonitrile mole fractions, right panel: water mole fractions. Dashed lines represent the mole fraction of the original sample weight of the prepared mixtures: (○) 0.75 mol mol$^{-1}$ acetonitrile/0.25 mol mol$^{-1}$ water, (△) 0.50 mol mol$^{-1}$ acetonitrile/0.50 mol mol$^{-1}$ water, (▽) 0.25 mol mol$^{-1}$ acetonitrile/0.75 mol mol$^{-1}$ water. Open symbols show data for the benchtop NMR set-up without PRE agent. Closed symbols show data for the presented set-up with PRE agent

In quantitative $^{1}$H NMR measurements performed with PRE agent, a higher quantification accuracy of the ground truth of the prepared mixture was achieved compared to measurements without PRE agent for both systems in the entire velocity range. In Fig. 8, measurements without PRE agent showed a relative error of around 11% in the quantification of the mole fractions of acetonitrile and water for all compositions and flow velocities in system 1, whereas the relative error is severely reduced in measurement with PRE agent to about 4.5%. Figure 9 shows that there is only a small difference in quantification with and without PRE agent in system 2 in the studied range of flow velocities: i.e. without PRE agent the relative error was around 1.7%, whereas the relative error is reduced in measurements with PRE agent to about 1.5%. In system 2, the $T_1$ of both components is similar, which leads to almost the same decrease in the recorded NMR signal for both substances (c.f. Figs. 6 and 7). However, for systems with a slightly larger difference in $T_1$ this is not the case, resulting in inaccurate quantification as can be seen from the evaluation of system 1.

## 5 Conclusion

In this work, a new-immobilized PRE agent for improved paramagnetic relaxation in continuous-flow benchtop NMR spectroscopy was synthesized and tested. The PRE agent was synthesized using controlled porous glass particles with a pore size of 30 nm. By grafting the CPG surface with butanediol diglycidyl ether

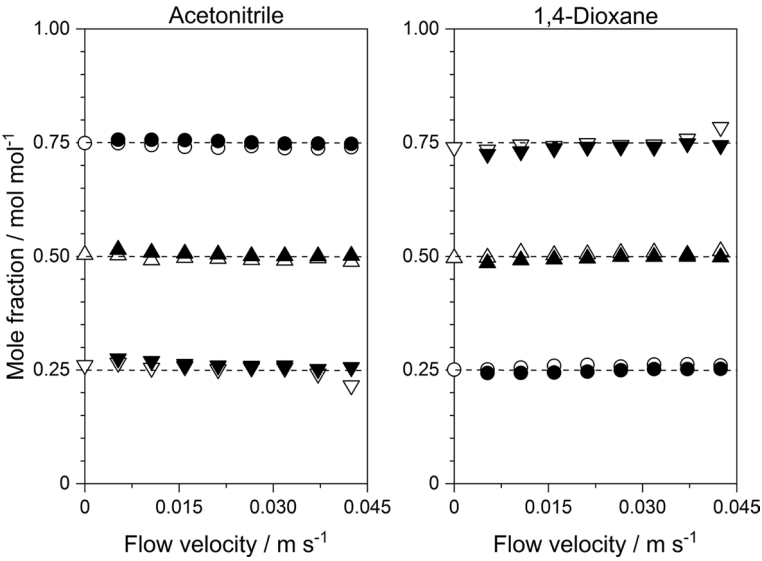


**Fig. 9** Quantification of $^{1}$H NMR data for system 2 (acetonitrile+1,4-dioxane). Left panel: acetonitrile mole fractions, right panel: 1,4-dioxane mole fractions. Dashed lines represent the mole fraction of the original sample weight of the prepared mixtures: (○) 0.75 mol mol$^{-1}$ acetonitrile/0.25 mol mol$^{-1}$ 1,4-dioxane, (△) 0.50 mol mol$^{-1}$ acetonitrile/0.50 mol mol$^{-1}$ 1,4-dioxane, (▽) 0.25 mol mol$^{-1}$ acetonitrile/0.75 mol mol$^{-1}$ 1,4-dioxane. Open symbols show data for the benchtop NMR set-up without PRE agent. Closed symbols show data for the presented set-up with PRE agent

and polyethyleneimine, the binding sites available for immobilizing the nitroxide radical TEMPO were multiplied. The resulting PRE agent with a radical loading of 120 mM shows a high PRE efficiency and no degradation in the entire series of experiments. The new PRE agent was tested in continuous-flow benchtop $^{1}$H NMR experiments at flow velocities for which significant signal loss of 50–70% occurs when no PRE agent is used. The results show that accelerated polarization buildup occurs over the whole studied velocity range with application of the PRE agent. Using the PRE agent clearly improved the results of quantification in cases where components in the mixture had different $T_1$. Thus, the new PRE agent is useful for applications in reaction and process monitoring with benchtop NMR spectrometers. Future work will focus on the application of the new PRE agent for the improved detection of heteronuclei (such as $^{13}$C) in process engineering applications.

**Author Contributions** Conceptualization, R.K., H.H. and K.M.; methodology, R.K. and K.M.; software, S.M. and R.K.; validation, S.M. and R.K.; investigation, R.K.; writing—original draft preparation, R.K.; writing—review and editing, R.K., H.H. and K.M.; visualization, R.K. and S.M.; supervision, H.H. and K.M.; project administration, H.H. and K.M.; funding acquisition, K.M. and H.H. All authors have read and agreed to the published version of the manuscript.

**Funding** Open Access funding enabled and organized by Projekt DEAL. The authors thank the German Research Foundation (DFG) for financial support within the Collaborative Research Center SFB 1527 "High Performance Compact Magnetic Resonance – HyPERiON".

**Data Availability** Data is available in the manuscript and can be obtained from the authors upon request.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

**Ethical Approval** Not applicable.

**Conflict of interest** The authors declare no competing interests.



## References

1. M. Goldbach et al., Preparation of grignard reagents from magnesium metal under continuous flow conditions and on-line monitoring by NMR spectroscopy. Tetrahedron Lett. **57**, 122–125 (2016)
2. A. Friebel, E. von Harbou, K. Münnemann, H. Hasse, Reaction monitoring by benchtop NMR spectroscopy using a novel stationary flow reactor setup. Ind. Eng. Chem. Res. **58**, 18125–18133 (2019)
3. P. Giraudeau, F.-X. Felpin, Flow reactors integrated with in-line monitoring using benchtop NMR spectroscopy. React. Chem. & Eng. **3**, 399–413 (2018)
4. D.A. Foley et al., NMR flow tube for online NMR reaction monitoring. Anal. Chem. **86**, 12008–12013 (2014)
5. F. Dalitz, L. Kreckel, M. Maiwald, G. Guthausen, Quantitative medium-resolution NMR spectroscopy under non-equilibrium conditions, studied on the example of an esterification reaction. Appl. Magn. Reson. **45**, 411–425 (2014)
6. H.-Y. Yu, S. Myoung, S. Ahn, Recent applications of benchtop nuclear magnetic resonance spectroscopy. Magnetochemistry **7**, 121 (2021)
7. T. Castaing-Cordier, D. Bouillaud, J. Farjon, P. Giraudeau. In: *Chapter four - recent advances in benchtop nmr spectroscopy and its applications* (ed. Webb, G. A.) , Vol. 103 of Annual Reports on NMR Spectroscopy 191–258 (Academic Press, 2021)
8. L.L. Wald, P.C. McDaniel, T. Witzel, J.P. Stockmann, C.Z. Cooley, Low-cost and portable MRI. J. Magn. Reson. Imaging **52**, 686–696 (2019)
9. T.A. Beek, Low-field benchtop NMR spectroscopy: status and prospects in natural product analysis. Phytochem. Anal. **32**, 24–37 (2020)
10. C.Z. Cooley et al., Two-dimensional imaging in a lightweight portable MRI scanner without gradient coils. Magn. Reson. Med. **73**, 872–883 (2014)
11. T. Guallart-Naval et al., Portable magnetic resonance imaging of patients indoors, outdoors and at home. Scientific Reports **12**, (2022)
12. F. Dalitz, M. Cudaj, M. Maiwald, G. Guthausen, Process and reaction monitoring by low-field NMR spectroscopy. Prog. Nucl. Magn. Reson. Spectrosc. **60**, 52–70 (2012)
13. G. Krüger, A. Birke, R. Weiss, Nuclear magnetic resonance (NMR) two-phase mass flow measurements. Flow Meas. Instrum. **7**, 25–37 (1996)
14. N. Zientek, K. Meyer, S. Kern, M. Maiwald, Quantitative online NMR spectroscopy in a nutshell. Chemie Ingenieur Technik **88**, 698–709 (2016)
15. R. Kircher, H. Hasse, K. Münnemann, High flow-rate benchtop NMR spectroscopy enabled by continuous overhauser DNP. Anal. Chem. **93**, 8897–8905 (2021)
16. I. Solomon, Relaxation processes in a system of two spins. Phys. Rev. **99**, 559–565 (1955)
17. N. Bloembergen, L.O. Morgan, Proton relaxation times in paramagnetic solutions. effects of electron spin relaxation. J. Chem. Phys. **34**, 842–850 (1961)

18. A. Abragam, The Principles of Nuclear Magnetism (Clarendon Press, Oxford, 1961)
19. T.M. Carr, W.M. Ritchey, Toward an optimal paramagnetic relaxation agent for $^{31}$P NMR spectroscopy. Spectrosc. Lett. **13**, 603–633 (1980)
20. R.B. Lauffer, Paramagnetic metal complexes as water proton relaxation agents for NMR imaging: theory and design. Chemical Reviews **87**, 901–927 (1987)
21. N. Schork et al., NMR relaxivities of paramagnetic, ultra-high spin heterometallic clusters within polyoxometalate matrix as a function of solvent and metal ion. ChemPhysChem **23**, (2022)
22. A.C. Venu et al., NMR relaxivities of paramagnetic lanthanide-containing polyoxometalates. Molecules **26**, 7481 (2021)
23. P. Caravan, J.J. Ellison, T.J. McMurry, R.B. Lauffer, Gadolinium(III) chelates as MRI contrast agents: structure, dynamics, and applications. Chem. Rev. **99**, 2293–2352 (1999)
24. P. Caravan, Strategies for increasing the sensitivity of gadolinium based MRI contrast agents. Chem. Soc. Rev. **35**, 512 (2006)
25. A.M.R. Hall et al., Practical aspects of real-time reaction monitoring using multi-nuclear high resolution FlowNMR spectroscopy. Catal. Sci. Technol. **6**, 8406–8417 (2016)
26. D. Bruck, R. Dudley, C. Fyfe, J.V. Delden, Sample magnetization using immobilized free radicals for use in flow NMR systems. J. Magn. Reson. **1969**(42), 51–59 (1981)
27. H.H. Fischer et al., Quantification studies in continuous-flow $^{13}$C nuclear magnetic resonance spectroscopy by use of immobilized paramagnetic relaxation agents. J. Phys. Chem. **107**, 4879–4886 (2003)
28. Y. Zhang, D.A. Laude, Immobilized free-radical substrates for magnetization of carbon-13 nuclei in flow NMR measurements. J. Magn. Reson. **1969**(87), 46–55 (1990)
29. A. Bara-Estaún et al., Paramagnetic relaxation agents for enhancing temporal resolution and sensitivity in multinuclear FlowNMR spectroscopy. Chem. Eur. J. **29**, (2023)
30. H.C. Dorn, T.E. Glass, R. Gitti, K.H. Tsai, Transfer of $^{1}$H and $^{13}$C dynamic nuclear polarization from immobilized nitroxide radicals to flowing liquids. Appl. Magn. Reson. **2**, 9–27 (1991)
31. R. Kircher, S. Mross, H. Hasse, K. Münnemann, Functionalized controlled porous glasses for producing radical-free hyperpolarized liquids by overhauser DNP. Molecules **27**, 6402 (2022)
32. A. Friebel, T. Specht, E. von Harbou, K. Münnemann, H. Hasse, Prediction of flow effects in quantitative NMR measurements. J. Magn. Reson. **312**, 106683 (2020)



## Authors and Affiliations

**Raphael Kircher[1,2] · Sarah Mross[1,2] · Hans Hasse[1,2] · Kerstin Münnemann[1,2]**

✉ Kerstin Münnemann
kerstin.muennemann@rptu.de

Raphael Kircher
rkircher@uni-mainz.de

Sarah Mross
sarah.mross@rptu.de

Hans Hasse
hans.hasse@rptu.de

[1] Laboratory of Engineering Thermodynamics (LTD), RPTU Kaiserslautern, Erwin-Schrödinger-Straße 44, Kaiserslautern 67663, Germany

[2] Laboratory of Advanced Spin Engineering - Magnetic Resonance (LASE-MR), RPTU Kaiserslautern, Gottlieb-Daimler-Straße 76, Kaiserslautern 67663, Germany