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Determination of self-diffusion coefficients in mixtures with benchtop ^{13}C NMR spectroscopy via polarization transfer

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Abstract

Nuclear magnetic resonance (NMR) is an established method to determine self-diffusion coefficients in liquids with high precision. The development of benchtop NMR spectrometers makes the method accessible to a wider community. In most cases, ^{1}H NMR spectroscopy is used to determine self-diffusion coefficients due to its high sensitivity. However, especially when using benchtop NMR spectrometers for the investigation of complex mixtures, the signals in ^{1}H NMR spectra can overlap, hindering the precise determination of self-diffusion coefficients. In ^{13}C NMR spectroscopy, the signals of different compounds are generally well resolved. However, the sensitivity of ^{13}C NMR is significantly lower than that of ^{1}H NMR spectroscopy leading to very long measurement times, which makes diffusion coefficient measurements based on ^{13}C NMR practically infeasible with benchtop NMR spectrometers. To circumvent this problem, we have combined two known pulse sequences, one for polarization transfer from ^{1}H to the ^{13}C nuclei (PENDANT) and one for the measurement of diffusion coefficients (PFG). The new method (PENPFG) was used to measure the self-diffusion coefficients of three pure solvents (acetonitrile, ethanol and 1-propanol) as well as in all their binary mixtures and the ternary mixture at various compositions. For comparison, also measurements of the same systems were carried out with a standard PFG-NMR routine on a high-field NMR instrument. The results are in good agreement and show that PENPFG is a useful tool for the measurement of the absolute value of the self-diffusion coefficients in complex liquid mixtures with benchtop NMR spectrometers.

KEYWORDS

benchtop NMR, DOSY, polarization transfer, pulse sequence, self-diffusion

1 | INTRODUCTION

The self-diffusion coefficient characterizes the mobility of individual molecules and is, hence, of fundamental interest in many fields of science and engineering.[1,2] Data of self-diffusion coefficients are used, for example, to test molecular models and to characterize molecular interactions[1,3–7]; for determining hydrodynamic radii[8]; to characterize restricted diffusion in pores and cells[9–13]; and for the characterization of complex mixtures such as process streams in biotechnology, lubricants or beverages.[2,14–18] They are also used in pharmaceutical quality control for the detection of possible product frauds.[19,20]

The self-diffusion coefficient of an infinitely dilute component in a mixture is equal to the mutual diffusion coefficient.[21,22] The mutual diffusion coefficient is an extremely important property for the design of all mass transfer processes.[23,24] Methods used for predicting mutual diffusion coefficients at finite concentrations usually need data at infinite dilution as input.[24] Therefore, results from measurements of absolute values of self-diffusion coefficients of highly diluted species are directly relevant for many technical applications.

Self-diffusion coefficients can be determined experimentally with dynamic light scattering,[25] tracer measurements,[26] neutron scattering[27] and nuclear magnetic resonance (NMR) with pulsed field gradients (PFG).[28] From these methods, PFG-NMR is well established as it allows chemically resolved measurements of self-diffusion coefficients even in complex mixtures without perturbing the investigated systems.

The measurement of self-diffusion coefficients in PFG-NMR experiments can be achieved by applying two gradient pulses to spatially label the molecules. The first gradient pulse alters the precession phase and encodes the molecules in dependency of their position in the sample. The second decoding gradient pulse reverses the phase shifts in stationary molecules. However, due to the molecular motion by self-diffusion in the magnetic gradient field the phase shift cannot be fully reversed. The resulting spin echo (SE) or stimulated spin echo (STE) is attenuated depending on the magnitude of the self-diffusion coefficient of the observed molecules. The experiment is repeated several times with incrementally increasing gradient strength. The Stejskal–Tanner equation is fitted to the attenuated NMR signals from which the self-diffusion coefficient can be determined as a fitting parameter.[29] Note that flow and temperature gradients induce convection within the sample which can cause additional signal attenuation and can lead to wrong results for the self-diffusion coefficient. Special pulse sequences with flow compensation are available which tackle this issue.[30] In PFG-NMR experiments, the gradient has to be calibrated only once. Afterwards, it is a calibration free method.

For the precise determination of self-diffusion coefficients in general high-field NMR spectrometers are used, which, however, have some disadvantages: These spectrometers are expensive, require cryogenic cooling and an adapted laboratory infrastructure as well as highly trained personnel to carry out the experiments. An alternative are benchtop NMR spectrometers, which use permanent magnets and are much less expensive and more robust than high-field NMR spectrometers and do not require cryogenic cooling—but at the cost of lower resolution and sensitivity.[31–34] Currently, benchtop NMR spectrometers are available up to a field strength of about 2.5 T. Furthermore, benchtop NMR spectrometers were also developed for the purpose of teaching and education; hence, these spectrometers provide a user-friendly operation software which is suitable for non-experienced NMR users.[35] In addition, benchtop NMR spectrometers with variable temperature control up to 338.15 K have emerged on the market, which enable the measurement of self-diffusion coefficients at a wider temperature range.

The ^{1}H nucleus is mostly used for benchtop NMR measurements, especially for the determination of self-diffusion coefficients, because of its high gyromagnetic ratio resulting in high sensitivity of this nucleus. However, the investigation of multi-component mixtures is often hindered by peak overlapping problems in the ^{1}H NMR spectrum because of the low chemical shift dispersion. This is particularly serious for benchtop NMR due to the restricted field strength and thus inherently low resolution. Some pulse sequences exist, for example pure-shift or Oneshot 45, which are able to partly fix this issue and are already tested on benchtop NMR spectrometers.[36,37] There are also studies to use lanthanide shift reagents to enhance the chemical shift dispersion.[38] But the differentiation of single species and therefore the precise determination of the self-diffusion coefficient is often infeasible with benchtop NMR in mixtures.

Another way of tackling this problem is to use mathematical methods such as direct exponential curve resolution algorithm (DECRA),[39] multivariate curve resolution (MCR),[40] speedy component resolution (SCORE)[41] or a QM model-based approach.[42] However, all these methods need additional software packages and expert knowledge to apply the fitting procedure properly on the crowded ^{1}H NMR spectrum and cannot fully resolve the problems resulting from overlapping peaks. In diffusion-ordered spectroscopy (DOSY) NMR experiments, species in complex mixture are distinguished by correlating the NMR signals with corresponding self-diffusion coefficients via a data inversion process[1,43,44] (note that the acronyms PFG-NMR and DOSY experiments might be used as synonyms to describe the measurement of self-diffusion coefficients[12]). However, in ^1H NMR spectra where peak overlap is severe, the discrimination of different molecules by DOSY is hampered by large uncertainties in the determined self-diffusion coefficients.

Hence, the usage of nuclei with greater chemical shift dispersion, such as the ^{13}C nucleus, is highly desirable.[45–48] However, ^{13}C NMR experiments suffer from a low signal-to-noise ratio (SNR) due to the low natural abundance and gyromagnetic ratio of ^{13}C. Furthermore, the low gyromagnetic ratio requires the application of stronger magnetic field gradients than for the diffusion encoding via ^1H nuclei. In addition, the spin-lattice relaxation time T_1 of ^{13}C nuclei is much longer than that of ^1H nuclei, leading to long measurement times.

To overcome these problems, polarization transfer methods have been developed, which transfer the high and rapidly restored polarization of ^1H nuclei to ^{13}C nuclei. These methods considerably improve the sensitivity of ^{13}C NMR spectroscopy and greatly reduce the experimental time because fewer scans are required. Moreover, the experiments can be repeated at a high rate as the repetition time for the polarization transfer is dictated by the T_1 of the ^1H instead of that of the ^{13}C nuclei which further reduces the experimental time. However, in the diffusion encoding step of a PFG-NMR experiment, the ^{13}C nuclei still relax with their inherent relaxation time. This is advantageous because the longer relaxation times T_1 and T_2 of the ^{13}C nucleus can be used to achieve longer diffusion times which is beneficial for the measurement of macromolecules. Several pulse sequences have been described in the literature in which polarization transfer techniques were coupled to ^{13}C DOSY sequences: INEPT-DOSY, DEPT-DOSY and DEPTSE as well as the 3D sequence HSQC-iDOSY.[49–56] The mentioned pulse sequences are frequently implemented on high-field NMR spectrometers.

However, to the best of our knowledge, ^{13}C PFG-NMR with signal enhancement by polarization transfer methods has not yet been studied on benchtop NMR spectrometers. In addition, the often used polarization transfer pulse sequences INEPT and DEPT sacrifice the signals of quaternary carbons. In contrast, the less popular polarization transfer pulse sequence PENDANT (abbreviation for ‘polarization enhancement that is nurtured during attached nucleus testing’) of Homer & Perry combines the features of sufficient signal enhancement and the selective excitation of functional carbon groups (CH_3, CH_2 and CH) with the additional ability to detect quaternary carbons.[57,58]

In this work, the polarization transfer sequence PENDANT is combined with the PFG-SE sequence in order to obtain absolute values of self-diffusion coefficients via ^{13}C NMR spectroscopy on a 1 T benchtop NMR spectrometer.[29] The SE sequence was chosen here rather than the more common STE sequence due to its simplicity and because there is no significant problem with homonuclear coupling for ^{13}C nuclei (unless labelled samples are measured). Furthermore, the ^{13}C spin–spin relaxation time T_2 is nearly the same as the ^{13}C spin–lattice relaxation time T_1 for small molecules allowing the use of the SE sequence.[49,59] The new combined pulse sequence is called PENPFG. The PENPFG experiment was implemented on a 1 T benchtop NMR spectrometer and was applied to determine the self-diffusion coefficients of acetonitrile (MeCN), ethanol (EtOH) and 1-propanol (PrOH) in the pure substances as well as in their three binary mixtures and in their ternary mixture at different compositions. All experiments were carried out at ambient pressure and at $T = 301.65$ K, which is the operating temperature of the benchtop NMR spectrometer that was used. For comparison, the measurements were repeated with a high-field NMR spectrometer using standard PFG-NMR routines, which we had previously used for studying many other systems and which were validated by comparison with literature data.[3,23,24] For completeness, the PENPFG sequence was also implemented on the high-field spectrometer, and the corresponding results are reported here and included in the comparison. The aim of this work is to demonstrate that the absolute value of the self-diffusion coefficient can be precisely and reliably determined by PENPFG on a benchtop NMR spectrometer.

2 | EXPERIMENTAL SECTION

2.1 | Hardware and experimental procedure

The PENPFG experiment is a combination of the polarization transfer sequence PENDANT (signal enhancement) and the PFG-SE sequence (diffusion encoding) as illustrated in Figure 1. The PENDANT sequence, including the evolution delays and pulse phases, was implemented as published by Homer & Perry. The evolution delays are given by Equations (1) and (2).

$$d_1 = \frac{1}{4\,^1J_{C,H}}, \tag{1}$$

$$d_2 = \frac{5}{8\,^1J_{C,H}}. \tag{2}$$

Here, $^1J_{C,H}$ is the coupling constant between the carbon and the scalar coupled proton. The coupling constant was set to $^1J_{C,H} = 140$ Hz for all PENPFG experiments. The value was determined by experiments and is a

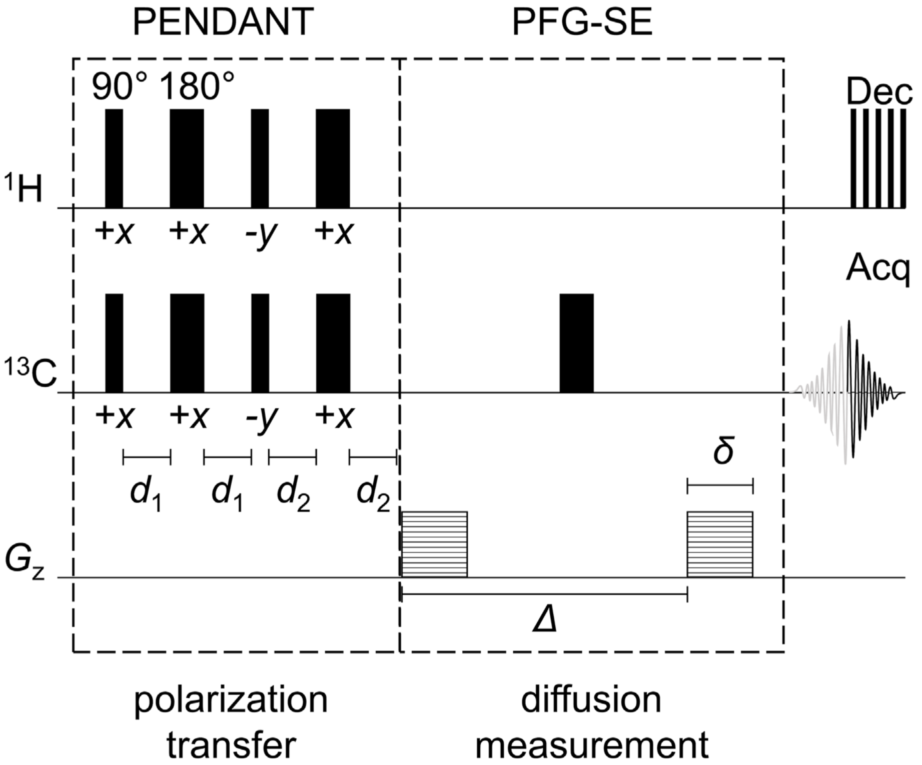

FIGURE 1 Illustration of the PENPFG pulse sequence for the measurement of self-diffusion coefficients with ^{13}C NMR spectroscopy, consisting of the polarization transfer pulse sequence PENDANT for signal enhancement and the PFG-SE sequence for diffusion encoding. The delays d_1 and d_2 depend on the ^{13}C–^{1}H $^1J_{C,H}$-coupling constants. Δ denotes the diffusion time and δ the gradient pulse duration.

compromise for $^1J_{C,H}$ so that CH_3, CH_2 and CH groups are all enhanced. The enhancement does not reach the possible maximum for each group, but this is not necessary because only the relative signal attenuation is considered during the PFG-NMR experiments. In the PENPFG experiment, the first 90° pulse of the original PFG-SE sequence is replaced by the PENDANT sequence. The refocusing 180° pulse of the regular PFG-SE sequence is following which separates the two gradient pulses for the phase encoding. Decoupling (WALTZ-16) is applied during the acquisition.

PENPFG and ^{1}H PFG-NMR (for the evaluation of PENPFG) experiments were carried out on a benchtop NMR spectrometer from Magritek (Spinsolve Carbon) with a magnetic field strength of $B_0 = 1\,\mathrm{T}$ corresponding to a ^{1}H Larmor frequency of $\nu_0 = 42.5\,\mathrm{MHz}$. The benchtop NMR spectrometer is equipped with a gradient coil with a maximum gradient strength of $G = 15.7\,\mathrm{G\,cm^{-1}}$ and operates at a temperature of $T = 301.65\,\mathrm{K}$. The gradient is aligned perpendicular to the NMR tube in order to reduce influences from convection. Standard sample tubes with an outer diameter of $d = 5.0\,\mathrm{mm}$ from Magritek were used for the measurements. The PENPFG experiment was implemented with the software Spinsolve Expert (Magritek) for the operation on the benchtop NMR spectrometer and is referred to as ^{13}C BT PENPFG in the following. The code of the pulse sequence is given in the Supporting Information. For the reference ^{1}H PFG-NMR experiments the PFGSTE sequence (referred to as ^{1}H BT PFGSTE here) was used as provided by the standard Magritek operating software Spinsolve. The self-diffusion coefficients of the species were determined from the acquired NMR signals with a modified Stejskal–Tanner equation according to Equation (3):

$$I_i = I_{0,i} \exp\left(-D_i \gamma^2 G^2 \left(\delta_{\mathrm{eff}}^2 \left(\Delta - \frac{\delta_{\mathrm{eff}}}{3}\right)\right)\right). \qquad (3)$$

Here, I is the signal intensity, I_0 is the signal intensity without gradient, γ is the gyromagnetic ratio of the investigated nucleus, G is the gradient strength and Δ is the diffusion time. As the applied magnetic field gradient has a trapezoidal shape, δ_{eff} (which is defined as the sum of the gradient pulse duration δ and the gradient ramp time τ_{grad} as shown in Equation (4)) needs to be used in the Stejskal–Tanner equation to assume a rectangular pulse. The linear ramp prevents distortion of the acquired NMR signal. A complete Stejskal–Tanner equation, which also includes the correction of the trapezoidal shape of the gradient pulse, is given in the Supporting Information. However, the correction can be neglected as τ_{grad} is small.

$$\delta_{\mathrm{eff}} = \delta + \tau_{\mathrm{grad}}. \tag{4}$$

For ^{1}H BT PFGSTE and for ^{13}C BT PENPFG, the diffusion time was set to $\Delta = 50$ ms. The gradient strength was incrementally increased in 16 steps ranging from $G = 1.8$ to $15.7\,\mathrm{G\,cm^{-1}}$ for both cases (in equal steps of G^2). The gradient ramp time τ_{grad} was set to $\tau_{\mathrm{grad}} = 0.1$ ms for all experiments. The gradient pulse duration δ of ^{13}C BT PENPFG was longer than in the ^{1}H BT PFGSTE because of the lower gyromagnetic ratio of the ^{13}C nucleus and the benchtop NMR spectrometer's weak maximum gradient strength. In order to obtain a NMR signal attenuation of approximately 90 to 95% at the strongest gradient strength the duration of the gradient pulse δ was set to values between $\delta = 2$ to 7 ms for ^{1}H BT PFGSTE and to values between $\delta = 9$ to 23 ms for ^{13}C BT PENPFG, respectively. The diffusion time Δ includes the gradient pulse duration δ in order to keep the absolute diffusion time constant for each experiment. However, the effective Δ (defined as the delay between the end of the dephasing and the beginning of the rephasing gradient) is different. Note that the short gradient pulse (SGP) approximation needs to be considered if porous systems are investigated.[60–62] Spectra of ^{1}H BT PFGSTE were acquired with four scans and an acquisition time (time for acquiring the FID) of 3.2 s. For ^{13}C BT PENPFG, 128 scans and an acquisition time of 3.2 s were applied. In all experiments, a relaxation delay of 25 s was used to ensure sufficient relaxation of the sample with at least five times T_1 regarding the relaxation time of the ^{1}H nuclei. This set of parameters results in a total measurement time of about 14 h for one ^{13}C BT PENPFG experiment, which could be reduced by the modification of the repetition delay and the number of gradient steps. In comparison, the measurement of self-diffusion coefficients on ^{13}C nuclei without polarization transfer is not feasible because it requires at least a doubling of the number of scans, which is incompatible with the lock stability of the benchtop NMR spectrometer during this long measurement time.

For the validation of the results obtained on the benchtop NMR spectrometer, ^{1}H and ^{13}C PFG-NMR as well as PENPFG experiments were also performed on a high-field NMR spectrometer with a superconducting magnet and a magnetic field strength of $B_0 = 9.4$ T corresponding to a ^{1}H Larmor frequency of $\nu_0 = 400.25$ MHz (Bruker Biospin magnet Ascend 400, console Avance III HD 400, probe BBFO). Special sample tubes optimized for diffusion measurements with an outer diameter of $d = 2.5$ mm (Deutero) were used to prevent radiation damping and to reduce sample convection. The temperature of the high-field NMR spectrometer was set to $T = 301.65$ K to match the operation temperature of the benchtop NMR spectrometer. ^{1}H and ^{13}C PFG-NMR experiments were executed with STE pulse sequences with bipolar pulsed gradients (called ^{1}H HF PFGSTE and ^{13}C HF PFGSTE in the following, respectively). These sequences were already implemented (as stebpgp1s) in the spectrometer's operating software TopSpin. The PENPFG sequence was manually implemented on the high-field NMR spectrometer with TopSpin (referred to as ^{13}C HF PENPFG). All sequences on the high-field NMR spectrometer apply the SMSQ10.100 gradient pulse. Self-diffusion coefficients of the species were determined with Equation (3) for ^{13}C HF PENPFG experiments while a modified Stejskal–Tanner equation was used for the evaluation of ^{1}H HF PFGSTE and ^{13}C HF PFGSTE. This modified Stejskal–Tanner equation is displayed in Equation (5):

$$I_i = I_{0,i} \exp\left(-D_i \gamma^2 G^2 \left(\Delta - \frac{\delta}{3} - \frac{\tau}{2}\right)\right). \tag{5}$$

In Equation (5), τ is the correction parameter for the usage of bipolar gradients and was chosen as $\tau = 0.2$ ms. Similar to the benchtop NMR spectrometer experiments the diffusion time was set to $\Delta = 50$ ms in all experiments. The gradient strength G of the high-field NMR spectrometer was incremented in 16 steps from $G = 2.3$ to $43.1\,\mathrm{G\,cm^{-1}}$ (again in equal steps of G^2). To obtain a NMR signal attenuation of approximately 90 to 95% at the strongest gradient strength the duration of the gradient pulse δ was adjusted between $\delta = 0.6$ to 1.0 ms for ^{1}H HF PFGSTE and to values between $\delta = 2.1$ to 7.0 ms for ^{13}C HF PFGSTE and ^{13}C HF PENPFG, respectively. Each high-field diffusion experiment consisted of 16 scans with an acquisition time of 5 s for ^{1}H HF PFGSTE and 4 s for ^{13}C HF PFGSTE as well as for ^{13}C HF PENPFG. The repetition delays for ^{1}H HF PFGSTE, ^{13}C HF PFGSTE and ^{13}C HF PENPFG are given in the Supporting Information and are set to ensure a complete relaxation of the sample ($5 \times T_1$). The molecule with the longest T_1, which was determined in the pure component, is considered to be decisive for the repetition delays in the mixture.

From previous work, it is known that the expanded relative uncertainty for the measurement of self-diffusion coefficients with the high-field NMR spectrometer used in this work can be estimated to be 2%.[3] All experiments were repeated three times to calculate the root mean squared error (RMSE) of the self-diffusion coefficients which are depicted as error bars in this work. The baseline and the phase of the acquired spectra of all experiments from the benchtop NMR and the high-field NMR spectrometer were manually corrected with MestRenova (Mestrelab Research). The analysis of the signal attenuation was also executed in MestRenova by direct peak integration. The determination of the self-diffusion coefficients with the Stejskal–Tanner equations (Equation (3) and (5), respectively) was performed with nonlinear least-square fits using MATLAB's (MathWorks Inc.) *lsqnonlin* solver.

2.2 | Chemicals and studied mixtures

Table 1 gives an overview of the chemicals that were used in this study, including their formulas, the suppliers and the specified purities. In this study, the self-diffusion coefficients of the molecules were determined in pure components as well as in binary and ternary mixtures. The molecular structures of the components are not complex; however, they are suitable to demonstrate the methodology because the ^{1}H NMR spectrum's complexity is considerably increasing from pure components to ternary mixtures. Figure 2 gives an overview of the mixtures investigated in this work. The samples were prepared gravimetrically using a laboratory balance (AG204, Mettler Toledo Inc.) with an accuracy of ± 0.0001 g. The total mass of each sample was approximately $m = 2$ g, from which the required amount of sample for the NMR experiments was taken.

3 | RESULTS AND DISCUSSION

Figure 3 presents ^{1}H NMR spectra of an equimolar mixture of MeCN + EtOH + PrOH acquired with a benchtop NMR spectrometer and a high-field NMR spectrometer to illustrate the problem of peak overlap in the benchtop NMR spectrometer due to reduced spectral resolution. The peak nomenclature and the assignment of the protons of the single species to the corresponding NMR signals is included. Note that there is a peak splitting observed for the OH groups of EtOH and PrOH (H4/H8) because the sample is free of water.

The signals acquired at high-field are well resolved, sharp and well separated enabling quantification by direct integration. In contrast, the signals of the different species strongly overlap in the benchtop ^{1}H NMR spectrum. Especially, the CH_3 group signals of EtOH and PrOH as well as the CH_2 group signal of PrOH in the region of 0.5 to 2 ppm cannot be distinguished from each other. The same holds for the CH_2 group signals of both alcohol molecules in the area around 3.5 ppm. Standard analysis methods, such as a direct integration of peak areas, fail in this situation. Only the signal of MeCN can be clearly identified due to the singlet signal of its CH_3 group. This illustrates that the distinction of molecules and the integration of the signals is often challenging in benchtop ^{1}H NMR spectra, even for only moderately complex systems.

Figure 4 displays ^{13}C NMR spectra of the same mixture, for which the ^{1}H NMR spectra are shown in Figure 3, without and with polarization transfer acquired with the benchtop NMR spectrometer. Due to the large chemical shift dispersion of ^{13}C NMR, the signals of all components can be easily distinguished. However, measurements relying only on the thermal polarization of ^{13}C spins (top panel in Figure 4) at the low magnetic field strength of the benchtop NMR spectrometer result in low SNR and long measurement times because of the huge number of scans required for sufficient signal accumulation. This problem is amplified by the long T_1 relaxation time of ^{13}C resulting in a total measurement time for the ^{13}C NMR spectrum of 3.2 h. Figure 4 also shows a ^{13}C NMR spectrum acquired using polarization transfer with PENDANT. This increases the SNR by a factor of about 3.5. The theoretical maximum enhancement of the SNR of 4 is not reached because an average $^1J_{C,H}$-coupling constant was chosen. Note that the signal of the CN group of MeCN at around 120 ppm is not clearly visible in the ^{13}C NMR spectrum regardless the application of

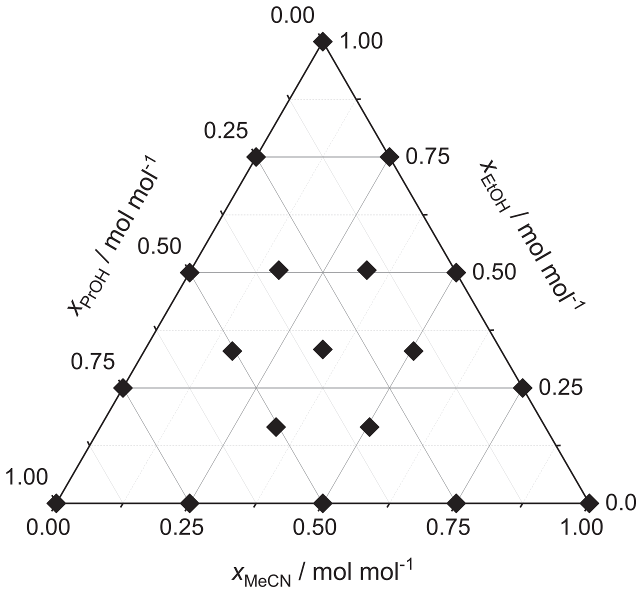

FIGURE 2 Overview of the studied mixtures consisting of acetonitrile (MeCN), ethanol (EtOH) and 1-propanol (PrOH).

Chemical	Formula	Supplier	Purity
Acetonitrile (MeCN)	C_2H_3N	Carl Roth	≥ 99.9%
Ethanol (EtOH)	C_2H_6O	Merck	≥ 99.9%
1-Propanol (PrOH)	C_3H_8O	Honeywell Specialty Chemicals	≥ 99.5%

TABLE 1 Chemicals used in this work including the suppliers and the purities as specified by the suppliers.

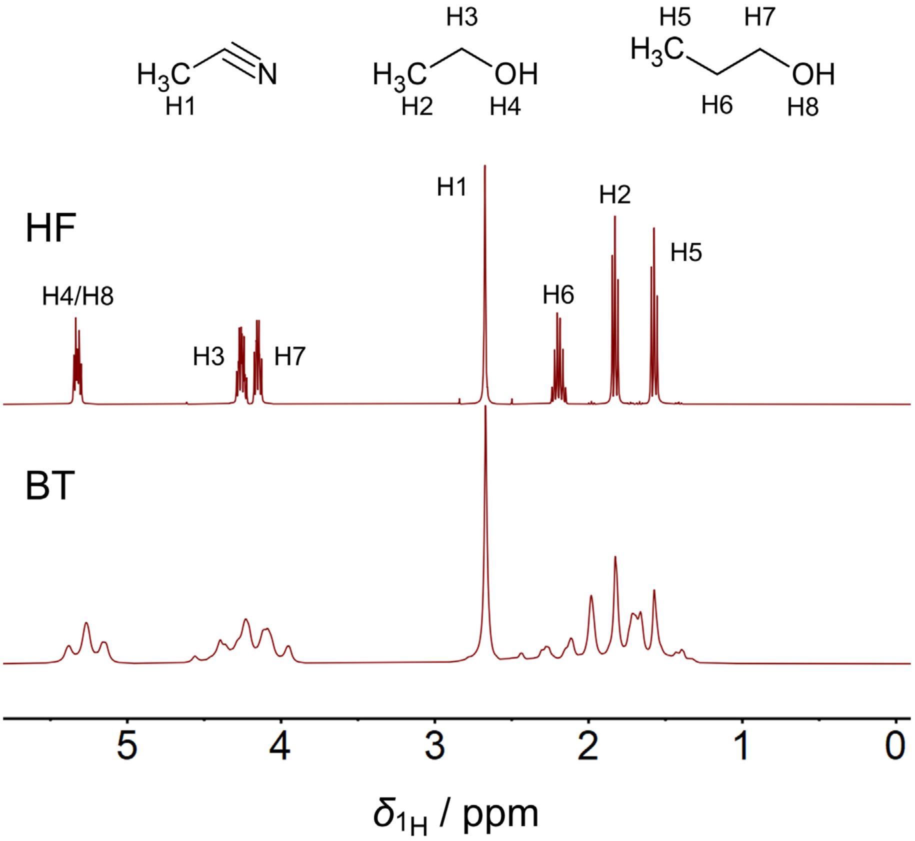

FIGURE 3 Comparison of ^{1}H NMR spectra of an equimolar mixture of MeCN + EtOH + PrOH acquired in a high-field (HF, top) and a benchtop NMR spectrometer (BT, bottom). Also the peak assignment is indicated.

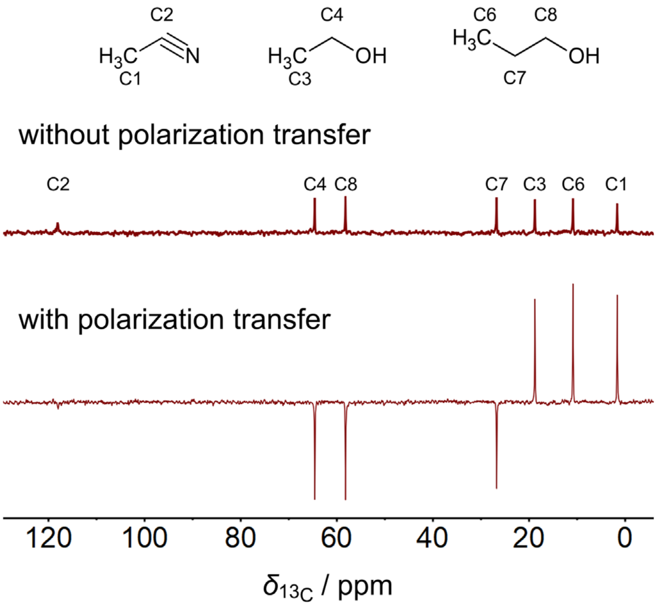

FIGURE 4 Comparison of ^{13}C NMR spectra of an equimolar mixture of MeCN + EtOH + PrOH acquired without (top) and with the polarization transfer sequence PENDANT (bottom) in a benchtop NMR spectrometer. Also the peak assignment is indicated.

polarization transfer. This observation is attributed to the fact that the T_2 relaxation time for the CN group is short, leading to a broadening of this peak. The polarization transfer is not able to enhance the CN group signal due to the lack of scalar-coupled protons attached directly at the respective carbon atom.

The total measurement time for the ^{13}C PENDANT NMR spectrum is considerably shortened (total measurement time 0.9 h) due to two reasons: The signal enhancement reduces the necessary number of scans, and also, the repetition time is reduced due to the shorter T_1 time of protons. In addition, PENDANT allows a discrimination between the CH_3, CH_2 and CH groups of the organic molecules that result in either positive or negative peaks in the spectrum (cf. Figure 4). This is highly beneficial for the identification of components in complex multicomponent mixtures.

3.1 | Pure components

Figure 5 displays self-diffusion coefficients of the pure components MeCN, EtOH and PrOH measured with different experiments with the high-field and with the

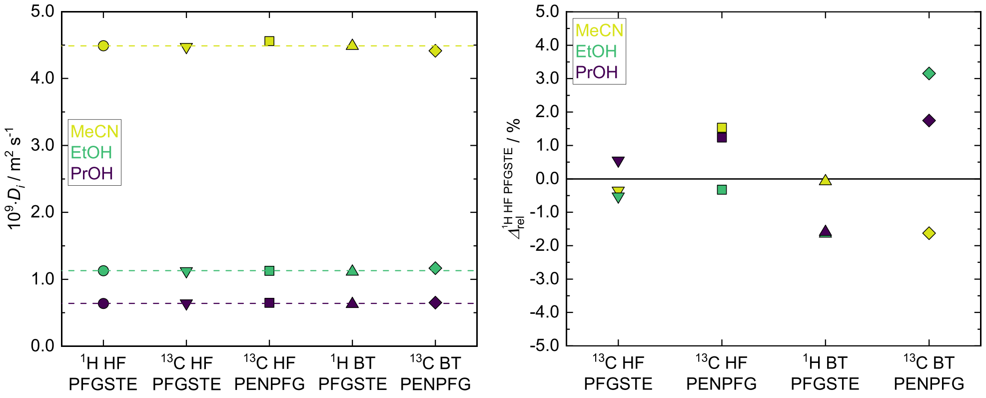

FIGURE 5 Left: Comparison of the experimental results of different NMR techniques for the self-diffusion coefficients of the pure components MeCN, EtOH and PrOH at $T = 301.65$ K. The symbols indicate results obtained with the different methods and instruments (HF high-field, BT benchtop; PFGSTE standard experiment, PENPFG experiment with polarization transfer). The dashed horizontal lines represent the reference measurement with ^{1}H HF PFGSTE. All error bars are within the symbol size (further details are given in the Supporting Information). Right: Relative deviations of the results obtained by different NMR techniques compared with the reference method ^{1}H HF PFGSTE.

benchtop NMR spectrometer. The numerical results are given in the Supporting Information. Furthermore, the error bars calculated by the RMSE and the errors caused by the fit of Stejskal–Tanner equation are shown in the Supporting Information. In each experiment, only the signals of the CH_3 groups were used for the analysis of the self-diffusion coefficients. The self-diffusion coefficients obtained with ^{1}H HF PFGSTE are considered as the ground truth in this work and are used to evaluate the results of the other experiments. They are additionally depicted as horizontal lines in Figure 5. The relative deviations of all experiments is provided on the right side of Figure 5.

As expected, the self-diffusion coefficient of MeCN is the highest due to its molar mass of $M_{\mathrm{MeCN}} = 41.05\,\mathrm{g\,mol^{-1}}$, which is the lowest of the three substances studied. PrOH has the lowest self-diffusion coefficient due to its high molar mass of $M_{\mathrm{PrOH}} = 60.09\,\mathrm{g\,mol^{-1}}$ and because it forms H-bonded clusters.

The results for the self-diffusion coefficients of all studied substances obtained with the different methods are in excellent agreement. The relative deviation of the results from the reference value from ^{1}H HF PFGSTE is below 3%. The results from ^{13}C HF PENPFG agree well with those from ^{1}H HF PFGSTE and ^{13}C HF PFGSTE experiments. Therefore, it can be concluded that the combination of the polarization transfer sequence PENDANT with the selected diffusion encoding sequence PFG-SE is suitable for the accurate measurement of self-diffusion coefficients.

There is also an excellent agreement of the values obtained with ^{1}H BT PFGSTE and ^{13}C BT PENPFG with the reference values from ^{1}H HF PFGSTE proving the benchtop NMR spectrometer's reliability to deliver precise self-diffusion coefficients. It should be noted that the acquisition with ^{1}H BT PFGSTE is only possible in simple systems, in which peaks do not overlap. This approach fails already for the mixture of the three solvents as shown in Figure 3. Therefore, ^{13}C BT PENPFG expands the horizon of systems for which diffusion coefficients can be measured with benchtop NMR spectrometers. All in all, Figure 5 shows that the new method PENPFG delivers reliable results on a benchtop NMR spectrometer.

3.2 | Binary mixtures

In the studies of binary mixtures, we only compare ^{13}C BT PENPFG experiments with the reference experiments carried out with ^{1}H HF PFGSTE. Figure 6 shows the measured self-diffusion coefficients of MeCN, EtOH and PrOH in the three binary mixtures. The numerical results are given in the Supporting Information. Again, only the CH_3 groups of the specific molecules were considered for the determination of the self-diffusion coefficients. The diagrams in Figure 6 display the self-diffusion coefficients of each component in dependency of the mixture's composition. Because of the small differences between the self-diffusion coefficient of EtOH and PrOH, the ordinate of the respective diagram is magnified by a factor of 10 for better visualization. In all mixtures, the component with the higher self-diffusion coefficient as pure component has also the higher value in the binary mixtures. By increasing the mole fraction of the fast diffusing component, the self-diffusion coefficients of both components gradually increase.

In the binary mixtures MeCN + EtOH and MeCN + PrOH, the self-diffusion coefficient of MeCN substantially

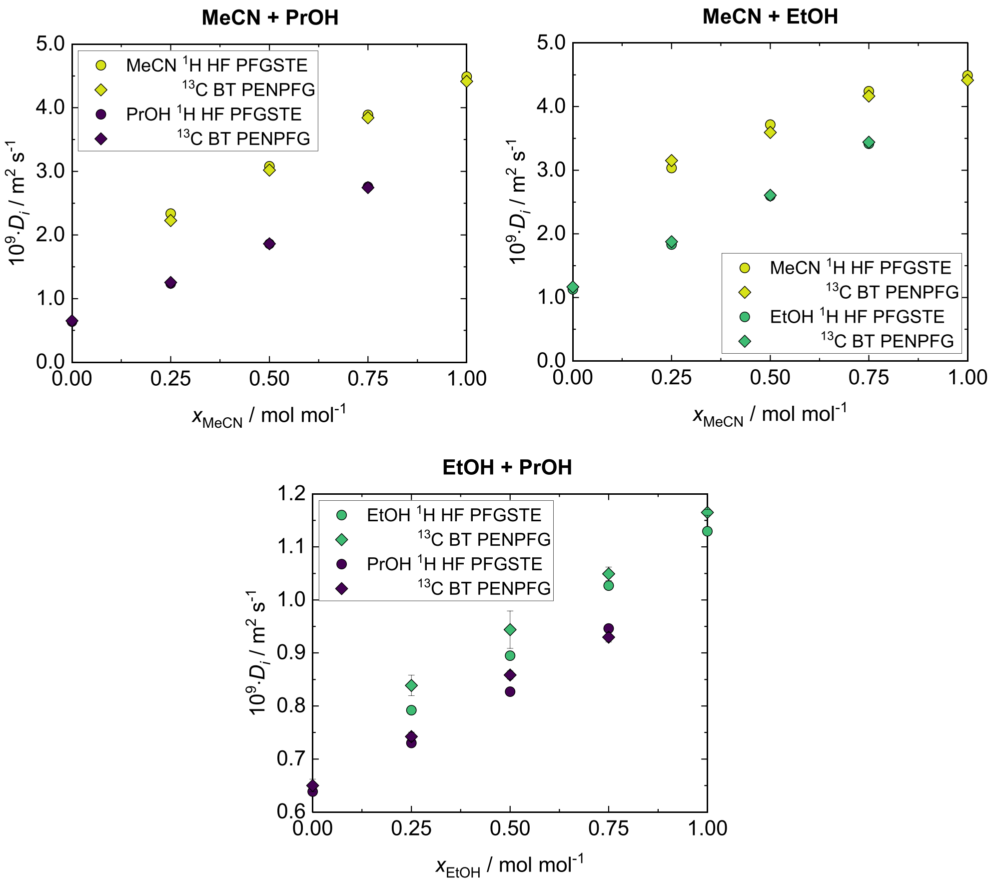

FIGURE 6 Comparison of results of measurements of self-diffusion coefficients of MeCN, EtOH and PrOH in the binary mixtures MeCN + PrOH, MeCN + EtOH and EtOH + PrOH at $T = 301.65$ K. The diamonds stand for results obtained with ^{13}C BT PENPFG, the circles for the results from ^{1}H HF PFGSTE. For the binary mixture EtOH + PrOH, the vertical scale is magnified by a factor of 10. Error bars are only shown if they are larger than the symbol size.

exceeds those of EtOH and PrOH. These two mixtures show a similar behaviour: The relative small molecule MeCN forms no hydrogen bonds in the bulk phase resulting in a fast molecular motion. However, in the binary mixture EtOH + PrOH the components EtOH and PrOH have similar molar masses and associate via hydrogen-bonding. This results in very similar self-diffusion coefficients.

The results from ^{13}C BT PENPFG are in very good agreement with the results from ^{1}H HF PFGSTE. The mean relative deviation of the results from ^{13}C BT PENPFG to the reference values from ^{1}H HF PFGSTE is typically below 3%. A maximum relative deviation of 6% to the reference values is identified for EtOH in the binary system EtOH + PrOH. However, the ability to distinguish between the two alcohols EtOH and PrOH, which have similar molar masses as well as molecular dynamics and therefore similar self-diffusion coefficients, shows that the benchtop NMR spectrometer in combination with the PENPFG sequence is a powerful tool.

3.3 | Ternary mixtures

Figure 7 depicts the self-diffusion coefficients of the seven investigated ternary mixtures of MeCN + EtOH + PrOH obtained with ^{13}C BT PENPFG and ^{1}H HF PFGSTE. The numerical results are summarized in the Supporting Information. Self-diffusion coefficients obtained from the CH_3 groups of MeCN, EtOH and PrOH were used for the comparison of ^{13}C BT PENPFG with the reference measurements ^{1}H HF PFGSTE. In the parity plot in Figure 7 the self-diffusion coefficients of each species obtained with ^{13}C BT PENPFG are plotted against the results from ^{1}H HF PFGSTE. An overview of all self-diffusion coefficients of the pure components MeCN, EtOH and PrOH as well as the obtained values in the binary and ternary mixtures determined with ^{13}C BT PENPFG is given in the Supporting Information.

The comparison of the results of ^{13}C BT PENPFG with the corresponding results from ^{1}H HF PFGSTE shows very good agreement. The maximum relative deviation does not exceed 5%, and the mean relative deviation

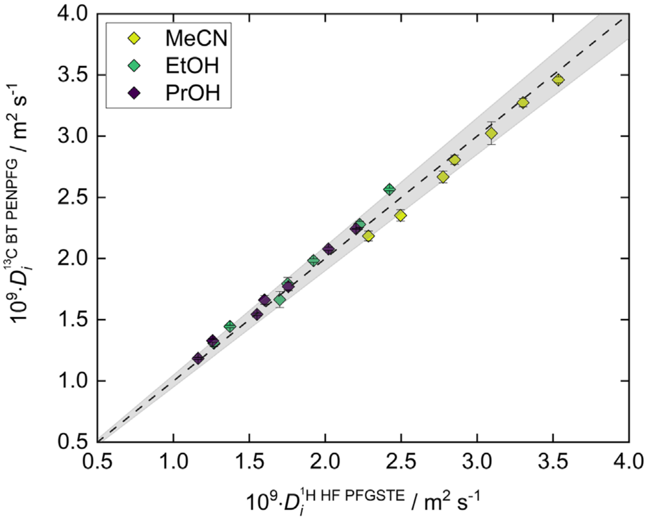

FIGURE 7 Self-diffusion coefficients of MeCN, EtOH and PrOH in ternary mixtures of MeCN + EtOH + PrOH at $T = 301.65$ K measured with ^{13}C BT PENPFG. The parity plot compares the results from ^{13}C BT PENPFG to the reference values from ^{1}H HF PFGSTE. The grey shaded area indicates relative deviations below 5%.

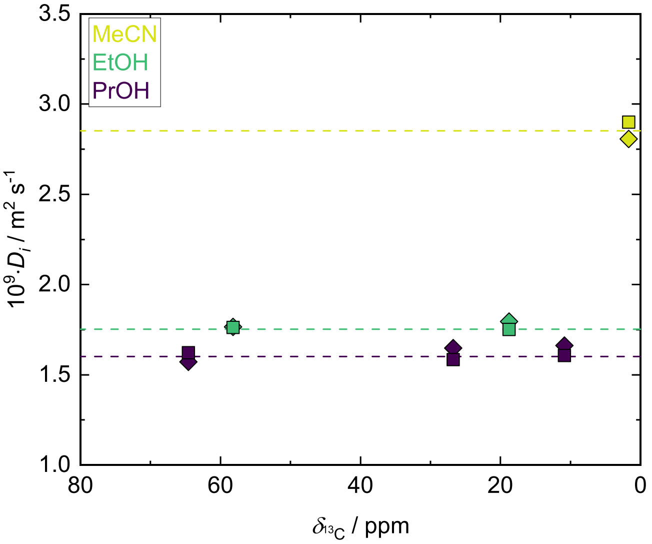

FIGURE 8 DOSY representation of an equimolar mixture of MeCN + EtOH + PrOH at $T = 301.65$ K. The squares and diamonds indicate results from ^{13}C HF PENPFG and ^{13}C BT PENPFG. The dashed horizontal lines represent the reference measurement with ^{1}H HF PFGSTE, obtained by the analysis of the CH_3 group of the specific molecules. All error bars are within the symbol size.

is again only about 3%. The comparison shows that the benchtop NMR spectrometer in combination with the PENPFG experiment is able to measure precisely the self-diffusion coefficients of a complex mixture.

Figure 8 shows the self-diffusion coefficients obtained from the analysis of all individual peaks of the ^{13}C NMR spectrum of the ternary system MeCN + EtOH + PrOH by ^{13}C BT PENPFG and ^{13}C HF PENPFG. The numerical results are given in the Supporting Information. For the reference experiment ^{1}H HF PFGSTE, only the CH_3 groups of the specific molecules were considered. They are depicted as horizontal lines in Figure 8. The resulting 2D NMR spectrum allows a differentiation of the molecules in the mixture due to their specific self-diffusion coefficients (DOSY spectrum).

Despite the fact that MeCN has two carbon atoms, only one value is shown in Figure 8 because the ^{13}C SNR of the CN group is insufficient for quantitative evaluation. All values are in good agreement with the corresponding reference values from the high-field NMR spectrometer indicated by the horizontal lines. In addition, the results obtained by ^{13}C BT PENPFG are in very good agreement with the results of ^{13}C HF PENPFG. It can therefore be concluded that differentiation between MeCN, EtOH and PrOH in a DOSY experiment is possible by using a benchtop NMR spectrometer in combination with the ^{13}C BT PENPFG experiment. This differentiation is even successful for EtOH and PrOH which have very similar self-diffusion coefficients.

4 | CONCLUSIONS

In this work, a new pulse sequence, PENPFG, was developed which combines the proton-carbon polarization transfer sequence PENDANT with the diffusion encoding sequence PFG-SE. The sequence enables the determination of self-diffusion coefficients in mixtures on benchtop NMR spectrometer by ^{13}C nuclei as it combines the following advantages: high chemical shift dispersion, signal enhancement and fast measurement times. The new sequence was tested by measuring the self-diffusion coefficients of MeCN, EtOH and PrOH as pure components as well as in binary and ternary mixtures on a 1 T benchtop NMR spectrometer. For reference, the same systems were investigated by high-field ^{1}H and ^{13}C PFG-NMR and, where possible, by ^{1}H PFG-NMR experiments on the benchtop instrument. Good agreement was found in all cases. All experiments were performed at 301.65 K, the operating temperature of the benchtop NMR system used. Recently, however, benchtop NMR spectrometers have become available that operate at temperatures up to 338.15 K, which greatly increases the applicability of the new method. Moreover, new benchtop NMR spectrometers with higher magnetic field strengths are expected to become available in the near future from which the new method will greatly benefit. They will allow to further reduce the measurement time and to study compounds at high dilution. The new methodology is especially attractive for small laboratories that do not have the suitable infrastructure for expensive high-field NMR spectrometers. In addition, expert knowledge is not required for operating benchtop NMR spectrometers and applying the new method for measuring self-diffusion coefficients.

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REFERENCES

[1] C. S. Johnson, *Progr. Nuclear Magn. Res. Spectrosc.* **1999**, *34*(3–4), 203.
[2] G. Pagès, V. Gilard, R. Martino, M. Malet-Martino, *Analyst* **2017**, *142*(20), 3771.
[3] D. Bellaire, H. Kiepfer, K. Münnemann, H. Hasse, *J. Chem. Eng. Data* **2020**, *65*(2), 793.
[4] J. T. Bullerjahn, S. von Blow, G. Hummer, *J. Chem. Phys.* **2020**, *153*(2), 24116.
[5] G. A. Fernández, J. Vrabec, H. Hasse, *Int. J. Thermophys.* **2005**, *26*(5), 1389.
[6] A. Muhammad, G. D. Carmine, L. Forster, C. D'Agostino, *ChemPhysChem* **2020**, *21*(11), 1101.
[7] I. N. Tsimpanogiannis, O. A. Moultos, L. F. M. Franco, M. B. de M. Spera, M. Erdős, I. G. Economou, *Mol. Simul.* **2018**, *45*(4-5), 425.
[8] E. R. McCarney, C. J. Breaux, P. M. Rendle, *Magn. Res. Chem.* **2020**, *58*(7), 641.
[9] I. A. Avilova, V. I. Volkov, *Membranes* **2023**, *13*(6), 567.
[10] V. Busignies, P. Porion, B. Leclerc, P. Evesque, P. Tchoreloff, *European J. Pharmaceut. Biopharmaceut.* **2008**, *69*(3), 1160.
[11] L. Forster, M. Lutecki, H. Fordsmand, L. Yu, C. D'Agostino, *Mol. Syst. Des. Eng.* **2020**, *5*(7), 1193.
[12] J. Krger, M. Avramovska, D. Freude, J. Haase, S. Hwang, R. Valiullin, *Adsorption* **2021**, *27*(3), 453.
[13] E. O. Stejskal, *J. Chem. Phys.* **1965**, *43*(10), 3597.
[14] E. Förster, C. C. Fraenza, J. Kstner, E. Anoardo, H. Nirschl, G. Guthausen, *Measurement* **2019**, *137*, 673.
[15] A. M. Gil, I. Duarte, E. Cabrita, B. J. Goodfellow, M. Spraul, R. Kerssebaum, *Anal. Chim. Acta* **2004**, *506*(2), 215.
[16] Y. S. Hong, C. H. Lee, *J. Agricult. Food Chem.* **2005**, *54*(1), 219.
[17] G. S. Kapur, S. Berger, *Tribotest* **2000**, *6*(4), 323.
[18] J. P. M. van Duynhoven, G. J. W. Goudappel, G. van Dalen, P. C. van Bruggen, J. C. G. Blonk, A. P. A. M. Eijkelenboom, *Magn. Res. Chem.* **2002**, *40*(13), S51.
[19] G. Assemat, B. Gouilleux, D. Bouillaud, J. Farjon, V. Gilard, P. Giraudeau, M. Malet-Martino, *J. Pharmaceut. Biomed. Anal.* **2018**, *160*, 268.
[20] V. Gilard, S. Trefi, S. Balayssac, M.-A. Delsuc, T. Gostan, M. Malet-Martino, R. Martino, Y. Prigent, F. Taulelle, *in NMR Spectroscopy in Pharmaceutical Analysis*, Elsevier, Amsterdam, NL **2008**, 269–289.
[21] E. L. Cussler, *Diffusion: Mass Transfer in Fluid Systems*, Cambridge Series in Chemical Engineering, Cambridge University Press, Cambridge, UK **2009**. https://books.google.de/books?id=w_RXbN61PrEC
[22] H. Weingrtner, *Ber. Bunsenges. Phys. Chem.* **1990**, *94*(3), 358.
[23] D. Bellaire, O. Großmann, K. Múnnemann, H. Hasse, *The J. Chem. Thermodyn.* **2022**, *166*, 106691.
[24] D. Bellaire, K. Münnemann, H. Hasse, *Chem. Eng. Sci.* **2022**, *255*, 117655.
[25] J. Stetefeld, S. A. McKenna, T. R. Patel, *Biophys. Rev.* **2016**, *8*(4), 409.
[26] J. H. Wang, *J. Am. Chem. Soc.* **1951**, *73*(2), 510.
[27] J. R. D. Copley, S. W. Lovesey, *Rep. Progr. Phys.* **1975**, *38*(4), 461.
[28] H. Weingrtner, *Annu. Rep. Prog. Chem., Sect. C: Phys. Chem.* **1994**, *91*, 37.
[29] E. O. Stejskal, J. E. Tanner, *J. Chem. Phys.* **1965**, *42*(1), 288.
[30] A. Jerschow, N. Müller, *J. Magn. Res.* **1997**, *125*(2), 372.
[31] T. A. Beek, *Phytochem. Anal.* **2020**, *32*(1), 24.
[32] F. Dalitz, M. Cudaj, M. Maiwald, G. Guthausen, *Progr. Nuclear Magn. Res. Spectrosc.* **2012**, *60*, 52.
[33] B. Gouilleux, J. Farjon, P. Giraudeau, *J. Magn. Res.* **2020**, *319*, 106810.
[34] K. Singh, B. Blümich, *TrAC Trends Anal. Chem.* **2016**, *83*, 12.
[35] S. D. Riegel, G. M. Leskowitz, *TrAC Trends Anal. Chem.* **2016**, *83*, 27.
[36] E. R. McCarney, R. Dykstra, P. Galvosas, *Magn. Reson. Imaging* **2019**, *56*, 103.
[37] M. Nilsson, G. A. Morris, *Chem. Commun.* **2007**, *9*, 933.
[38] A. K. Rogerson, J. A. Aguilar, M. Nilsson, G. A. Morris, *Chem. Commun.* **2011**, *47*(25), 7063.
[39] W. Windig, B. Antalek, *Chemom. Intell. Lab. Syst.* **1997**, *37*(2), 241.
[40] L. C. M. V. Gorkom, T. M. Hancewicz, *J. Magn. Res.* **1998**, *130*(1), 125.
[41] M. Nilsson, *J. Magn. Reson.* **2009**, *200*(2), 296.
[42] E. Steimers, Y. Matviychuk, D. J. Holland, H. Hasse, E. von Harbou, *Magn. Res. Chem.* **2022**, *60*(12), 1113.
[43] M. Khajeh, A. Botana, M. A. Bernstein, M. Nilsson, G. A. Morris, *Anal. Chem.* **2010**, *82*(5), 2102.
[44] K. F. Morris, C. S. Johnson, *J. Am. Chem. Soc.* **1992**, *114*(8), 3139.
[45] P. Stilbs, *Anal. Chem.* **1981**, *53*(13), 2135.
[46] K. Vermillion, N. P. J. Price, *J. Magn. Res.* **2009**, *198*(2), 209.
[47] M. Yemloul, V. Castola, S. Leclerc, D. Canet, *Magn. Res. Chem.* **2009**, *47*(8), 635.
[48] M. Zhou, V. Frydman, L. Frydman, *Macromolecules* **1997**, *30*(18), 5416.
[49] A. Botana, P. W. A. Howe, V. Car, G. A. Morris, M. Nilsson, *J. Magn. Res.* **2011**, *211*(1), 25.
[50] J. Hou, Y. He, P. Sabatino, L. Yuan, D. Redwine, *Magn. Res. Chem.* **2016**, *54*(7), 584.
[51] D. Li, R. Hopson, W. Li, J. Liu, P. G. Williard, *Organic Lett.* **2008**, *10*(5), 909.
[52] D. Li, I. Keresztes, R. Hopson, P. G. Williard, *Accounts Chem. Res.* **2008**, *42*(2), 270.

[53] A. S. McLachlan, J. J. Richards, A. R. Bilia, G. A. Morris, *Magn. Res. Chem.* **2009**, *47*(12), 1081.

[54] N. E. Schlörer, E. J. Cabrita, S. Berger, *Angew. Chem. Int. Ed.* **2002**, *41*(1), 107.

[55] B. Vitorge, D. Jeanneat, *Anal. Chem.* **2006**, *78*(15), 5601.

[56] D. Wu, A. Chen, S. JJ. Charles, *J. Magn. Reso., Ser. A* **1996**, *123*(2), 215.

[57] J. Homer, M. C. Perry, *J. Chem. Soc. Chem. Commun.* **1994**, *4*, 373.

[58] J. Homer, M. C. Perry, *J. Chem. Soc. Perkin Trans. 2* **1995**, *3*, 533.

[59] A. M. Torres, G. Zheng, W. S. Price, *Magn. Res. Chem.* **2009**, *48*(2), 129.

[60] P. Linse, O. Soderman, *J. Magn. Res., Ser. A* **1995**, *116*(1), 77.

[61] C. Malmborg, D. Topgaard, O. Sderman, *J. Magn. Res.* **2004**, *169*(1), 85.

[62] W. S. Price, P. Stilbs, O. Sderman, *J. Magn. Res.* **2003**, *160*(2), 139.

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