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Accurate Measurement of Translational Diffusion Coefficients: A Practical Method to Account for Nonlinear Gradients

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For NMR probes equipped with pulsed field gradient coils, which are not optimized for gradient linearity, the precision and accuracy of experimentally measured translational diffusion coefficients are limited by the linearity of the gradient pulses over the sample volume. This study shows that the accuracy and precision of measured diffusion coefficients by the Stejskal–Tanner spin-echo pulsed field gradient experiment can be significantly improved by mapping the gradient *z*-profile and by using the mapped calibration parameters in the data analysis. For practical applications the gradient distribution may be approximated by a truncated linear distribution defined by minimum and maximum values of the gradient. By including the truncated linear gradient distribution function in the Stejskal–Tanner equation, the systematic deviation between the fitted curve and the experimental attenuation curve decreases by an order of magnitude. The gradient distribution may be calibrated using an intense NMR signal from a sample with a known diffusion coefficient. The diffusion coefficient of an unknown sample may then be determined from a two-parameter fit, using the known gradient distribution function.

Key Words: **NMR; pulsed field gradient; gradient calibration; diffusion; self diffusion.**

INTRODUCTION

NMR structural studies of biological molecules are typically carried out at millimolar concentrations. At these high concentrations the aggregation state of molecules is generally not known. It is therefore of interest to determine the aggregation state under exactly the same conditions as those used for structure determination. One way of doing this is by measuring translational diffusion coefficients. Translational diffusion coefficients may be determined using a pulsed field gradient spin-echo experiment (*1*). Highly accurate values of diffusion coefficients may be measured by using specially designed probes with linear gradients. For NMR probes, which are optimized for shielding and short recovery times and not for gradient linearity, the precision and accuracy of experimentally measured translational diffusion coefficients are limited by the linearity of the gradient pulses over the sample volume.

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The nonlinearity of the gradients has been studied both theoretically (*2*) and experimentally (*3*). The effect of nonlinear gradients on the estimated diffusion coefficients has been analyzed (*2*) from realistic models of the gradient coil. The nonlinearity leads to a systematic deviation between the Stejskal–Tanner equation and the experimental data points. This deviation is very hard to distinguish from the situation where two components with different diffusion coefficients contribute to the signal. It has been recommended that only the central region of the detection coil, where the gradients are reasonably linear, should be used in diffusion experiments. This could be achieved by employing slice selective pulses or NMR microcells (*4*). Both of these approaches, however, reduce the signal intensity, which makes them unsuitable for conditions where the signal-to-noise ratio is a limiting factor.

Here we present another approach where the effect of nonlinear gradients is taken into account during both calibration and analysis. By this approach it is possible to use the entire sample volume and to obtain accurate values of diffusion coefficients. This results in a significant improvement in the determination of precise and accurate diffusion coefficients.

The outline of this paper is as follows. First, we use the method of Hurd *et al.* (*3*) to map the nonlinear gradients. The mapped gradient profile is then transformed into a distribution function, which may be used for accurate analysis of the attenuation curve obtained with nonlinear gradients in the diffusion measurements. Subsequently we show that for practical applications the true distribution function of gradient strengths can be approximated by a truncated linear function. The parameters describing the linear distribution function can be easily determined by measurement of a standard attenuation curve of an intense NMR signal from a sample with known diffusion coefficient. This approach provides a very simple, sensitive, and accurate method to measure diffusion despite nonlinear gradients.

THEORY

The response from a pulsed field gradient spin-echo (PFGSE) experiment, performed with linear gradients, is given by the Stejskal–Tanner equation (*1*):

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$$A = A_0\exp[-(\gamma\delta G)^2(\Delta - \delta/3)D]$$
$$= A_0\exp[-(\gamma\delta I g_{cal})^2(\Delta - \delta/3)D]. \quad [1]$$

Here A_0 is the signal area without the gradients, γ is the magnetogyric ratio of the nuclei under study, G is the gradient strength, Δ is the delay between the two gradients, δ is the duration of one of the gradient pulses, and D is the diffusion coefficient. The gradient strength, G, is proportional to the electric current, I, through the gradient coil. Typically the current is varied and the probe-specific proportionality constant g_{cal} is fitted to the data of a substance with known diffusion coefficient, typically water, in order to calibrate the gradient strength.

In standard gradient probes the gradient strength is not constant over the active sample volume. This can lead to systematic errors in the measured diffusion coefficients because the attenuation curve is no longer described by the Stejskal–Tanner equation. To account for the nonlinearity of the gradient we show here how it reflects in the attenuation curve. The expectation value of the distance a molecule has moved during the diffusion delay is

$$\sqrt{\langle z^2\rangle} = \sqrt{2Dt}. \quad [2]$$

For water diffusion at 25°C, this corresponds to an expectation value of circa 20 μm during a typical experiment with a diffusion time of about 100 ms. For the following, we consider the gradient to be constant over this distance. This prerequisite leads to a great simplification, since the experimental decay curve can be considered a superposition of several ideal Stejskal–Tanner curves with different gradient strengths represented by different values of g_{cal}. Over the active volume of the sample there is a distribution of gradient strengths represented by a distribution function $\rho(g_{cal})$. The response from a PFGSE experiment, performed with a distribution of gradient strengths, is given by an integral over g_{cal}. In practice we use the limiting values g_{cal}^{min} and g_{cal}^{max} as the integration range since $\rho(g_{cal})$ is assumed to be zero outside this range.

$$A = A_0 \cdot \frac{\int \rho(g_{cal}) \cdot \exp[-(\gamma\delta I g_{cal})^2(\Delta - \delta/3)D]dg_{cal}}{\int \rho(g_{cal})dg_{cal}} \quad [3]$$

Here I is the current through the gradient coil during the gradient pulses. When the logarithm of the echo intensity is plotted as a function of the square of the gradient strength a multiexponential decay is observed as a deviation from the theoretical straight line. This is a consequence of the distribution of gradient strengths. The width of the distribution is reasonably narrow for standard PFG probes. The gradient is about 25% weaker at the ends compared to the central region of the detection coil (*2*, *5*) in probes designed for biomolecular NMR.

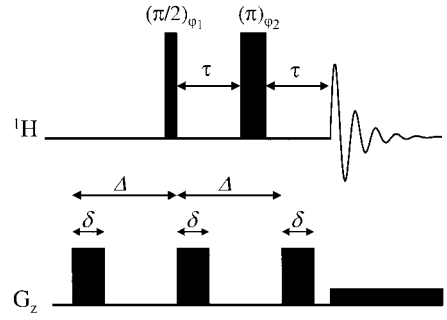

FIG. 1. Stejskal–Tanner gradient spin-echo pulse sequence for diffusion experiments. For gradient stability a gradient prepulse with the same polarity and a delay Δ equal to the separation of diffusion encoding gradients were used. Proton pulses were phase cycled using an 8-step phase cycle ($\varphi 1 = (x, -x)$, $\varphi 2 = (2x, 2y, 2(-x), 2(-y))$, acq $= (x, -x, x, -x, -x, x, x, -x)$). The 90° pulse width was 7 μs, the gradient pulse length δ was 2 ms, and Δ was 50 ms. The pregradient has two effects. First, it purges all transverse magnetization from the previous FID, and second, it makes the two diffusion encoding gradient pulses more equal. A weak gradient applied during acquisition was used to map the gradient z-profile.

By performing a gradient spin-echo experiment with a weak gradient during the acquisition on a sample with an intense signal, we show that it is possible to obtain spatial resolution along the z-axis in a 1D-imaging manner. From this experiment it is possible to obtain a good estimate of the distribution of gradient strengths, represented by the distribution function $\rho(g_{cal})$, as described below.

EXPERIMENTAL

Pulsed field gradient NMR translational diffusion measurements were carried out using the Stejskal–Tanner spin-echo experiment with a gradient prepulse (*6*, *7*). The prepulse creates a steady state for the PFG amplifier and makes the second and the third pulses, used in gradient experiment, identical. This is a more general way of achieving equal gradient pulses than adjusting the length of one of the gradient pulses. An additional weak gradient during the acquisition enabled the spatial resolution along the z-axis. The pulse sequence for the Stejskal–Tanner spin-echo with gradient during acquisition is shown in Fig. 1. All experiments were done using a Varian unity 600 spectrometer. The inverse detection probe equipped with a z-axis gradient coil was used. Typically 60 linearly spaced values of gradient strength were used in the range from 0 to 0.3 T/m. The duration of PFG pulses was 2 ms and a 50-ms refocusing delay was used. All spectra were baseline corrected.

The pulsed field gradients were calibrated using the Varian standard doped water sample (1% H_2O in D_2O + 1 mg/ml $GdCl_3$) and a literature value of 1.90 10^{-9} m^2/s for the HDO diffusion coefficient in D_2O at 25°C (*8*).

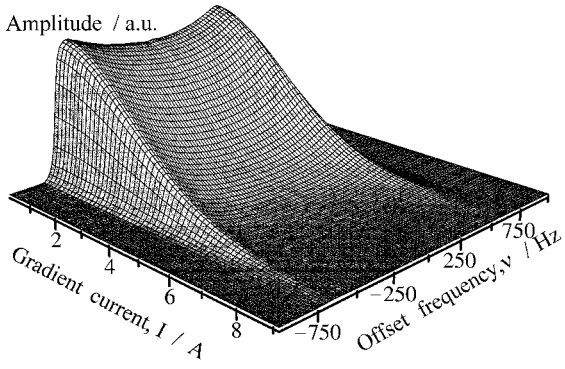

FIG. 2. Results from the Stejskal–Tanner experiment using a gradient during the accquisition from a Varian standard doped water sample (1% H_2O in D_2O + 1 mg/ml $GdCl_3$) at 600 MHz. The slope of the profile is steepest in the middle of the sample where the gradient is the strongest. The current through the gradient coil was arrayed in 60 steps between 0 and circa 10 A.

The approximate profile of the gradient strength along the *z*-axis was studied by performing a GSE experiment with a gradient during the acquisition period performed on a sample described above which has an intense signal with known diffusion coefficient. Gradient profile spectra were recorded using the Stejskal–Tanner gradient spin-echo pulse sequence and a 1 mT m^{-1} gradient during the acquisition period, typically 20 ms. Closed form integrals of the Stejskal–Tanner equation with different gradient distribution functions were evaluated analytically using Mathematica v.3.0 and the attenuation curves were analyzed using the nonlinear regression package of the same system.

RESULTS

The Stejskal–Tanner gradient spin-echo experiment with a weak gradient during the acquisition (Fig. 1) was used to measure attenuation profiles at different values of the current in the gradient coils. The results are presented in Fig. 2 as a three-dimensional data set. The signal intensity is shown as a variable of the gradient current I and of the offset frequency ν. The gradient varies in the sample along the vertical *z*-axis. The offset frequency is related to the *z*-coordinate in such a way that ν is 0 at the maximum value of the gradient strength at the center of the gradient coil. The width of the active volume corresponds to a range of ν of about 1.2 kHz. In the central region of the active volume (close to $\nu = 0$), the signal is attenuated faster with increasing current I through the gradient coil than it does closer to the ends of the active volume, corresponding to offset frequencies around ±600 Hz. This is due to stronger gradients in the central region.

The spectra were subdivided into 400 slices each 4 Hz wide and integrated, resulting in 400 lists of integrated intensities as a function of the current through the gradient coil. Each list corresponds to a certain position along the *z*-axis in the detection coil. The parameters g_{cal} and A_0 of Eq. [1] were fitted to each list with the diffusion coefficient, D, fixed to the literature value 1.90 10^{-10} m^2 s^{-1} (*7*). The estimated g_{cal} values are shown in Fig. 3A and intensities A_0 in Fig. 3B. The right edge of the profile corresponds to the top of the detection coil. The gradient *z*-axis profile is quite flat and shows about 25% drop at both edges (±8 mm). The metric scales shown in Figs. 3A and 3B were derived from the data in Fig. 3A as

$$z(\nu_k) = \sum_{i=1}^{k} \frac{\Delta\nu}{\gamma I_{acq} g_{cal}(\nu_i)} + C. \qquad [4]$$

Here $\Delta\nu$ is the width of a slice in the integration, in our case 4 Hz, and I_{acq} is the current through the gradient coil during acquisition. The arbitrary constant C was adjusted so that zero mm corresponds approximately to the frequency where the gradient strength is highest.

When the gradient profile is transformed into en effective distribution function, $\rho(g_{cal})$, it is important to account also for the spatial dependence of the receptivity of the detection coil. As can be seen from Fig. 3B, the regions just outside of the detection coil (±8 mm in this case) also contribute to the signal. This was achieved by dividing the g_{cal} values of Fig. 3A

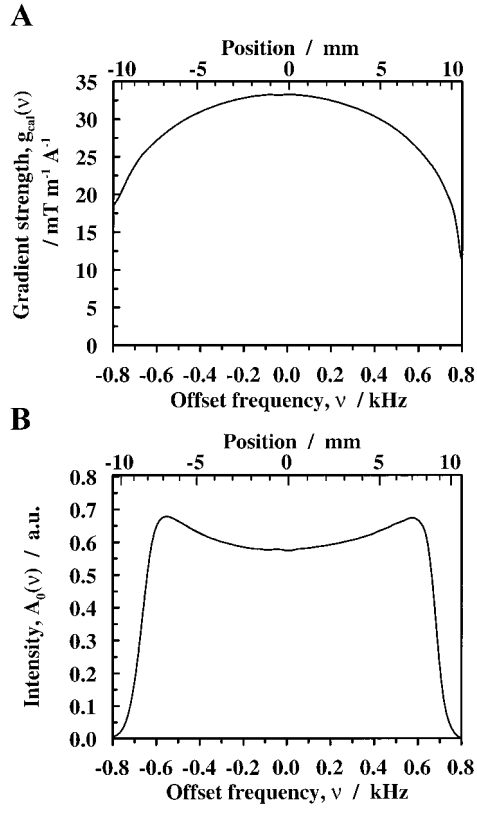

FIG. 3. Gradient *z*-profile obtained from analyzing the water profile attenuation. The 1.6-kHz broad profile was divided into 400 slices and the decay of integral of each slice was fitted using the Stejskal–Tanner equation with constant gradient strength. (A) The gradient strength *z*-profile. (B) The signal intensity *z*-profile.

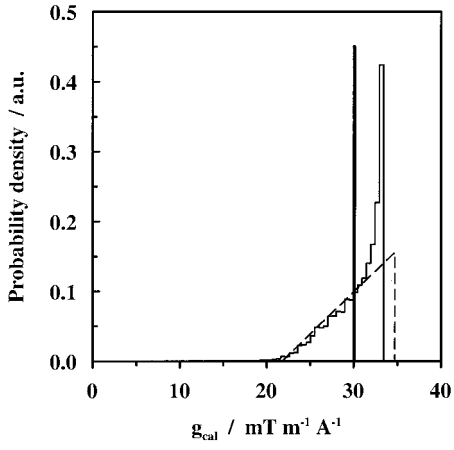

FIG. 4. Gradient distribution functions. The histogram describes the distribution function $\rho(g_{cal})$ as determined from the gradient profile (see text). The thick solid peak indicates the value of the g_{cal} parameter as determined by fitting A_0 and g_{cal} of Eq. [1] to the experimental attenuation curve. The dashed triangle indicates the distribution function obtained when assuming a truncated linear distribution (Eq. [5]) and fitting the parameters A_0, g_{cal}^{min}, and g_{cal}^{max} of Eq. [5] inserted into Eq. [3] to the experimental attenuation curve.

into 68 bins of equal width, with a total span between zero and the highest observed value. For each observation of a g_{cal} value the corresponding intensity, A_0, was added to the relevant bin. The results are shown as the histogram in Fig. 4.

Comparing the Distribution and Experimental Data

Figure 5 shows the experimental results when no gradient was applied during the acquisition together with the fitted curves of Eqs. [1] and [3]. For a homogeneous gradient a linear attenuation curve should be observed when the logarithm of the intensity is plotted against the square of the gradient. The difference between experimental points and the fitted curve of Eq. [1] (constant g_{cal}) is typical for nonlinear gradients (*2*).

As will be discussed below, the $\rho(g_{cal})$ function was chosen as a truncated linear function in Eq. [3]. The corresponding residuals are shown in Fig. 5B. For our Varian PFG probes the sum of squared residuals is at least a factor of 20 smaller when Eq. [3] is used instead of Eq. [1]. This reduces the error in the estimate of the diffusion coefficient and the intensity without gradients by more than a factor of 4, when the signal-to-noise ratio is the limiting factor. It should be noted that systematic errors are suppressed more effectively than random errors by the suggested method, so the accuracy is believed to improve even more than the precision.

Parametric Models of the Distribution Function

From fitting distributions of correlation times to fluorescence lifetime or polarization anisotropy decay data, it is well known that various distribution functions with a small number of parameters may reproduce the experimental data equally well. This fact can be used constructively by realizing that almost any shape function with a small number of adjustable parameters can be used to describe the distribution function also in the present case. Although not physically correct, if it is appropriately parameterized, it can still be used in the data analysis without introduction of new sources of errors. We have considered a number of different shapes, e.g., two component, gaussian, truncated gaussian, and trapezoidal distribution functions, which all result in analytical solutions to Eq. [3]. As expected, all considered distribution functions give much better agreement with experimental data (data not shown) than the model with a constant gradient.

For practical applications a simple truncated linear gradient profile was found to be adequate to describe the distribution of the gradient strength. Parameters of that distribution function were obtained by fitting the ordinary PFGSE experiment using a normalized linear distribution function:

$$\rho(g_{cal}) = \begin{cases} 0, & g_{cal} < g_{cal}^{min},\ g_{cal} > g_{cal}^{max} \\ \dfrac{2(g_{cal} - g_{cal}^{min})}{(g_{cal}^{max} - g_{cal}^{min})^2}, & g_{cal}^{min} < g_{cal} < g_{cal}^{max} \end{cases} . \quad [5]$$

This distribution function corresponds to an analytical closed form equation for the PFGSE attenuation curve, obtained by substituting Eq. [5] into Eq.[3] and integrating:

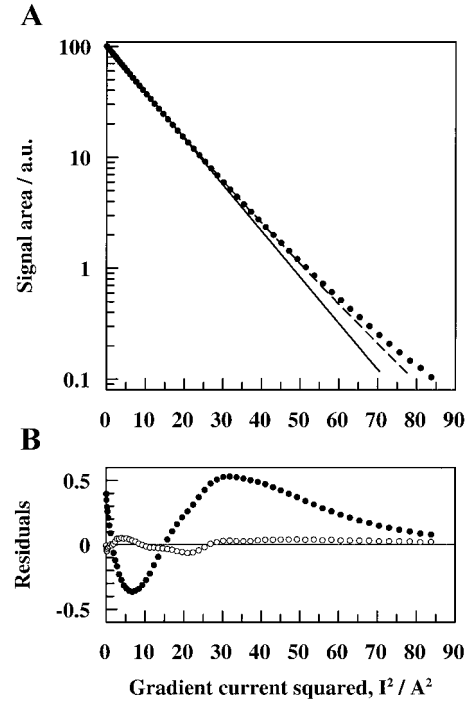

FIG. 5. Stejskal–Tanner experiment using a Varian standard doped water sample (1% H_2O in D_2O + 1 mg/ml $GdCl_3$) at 600 MHz proton frequency along with the best fitting Stejskal–Tanner curve for constant gradient (—) and linear truncated distribution of gradient strengths (- - -). Residuals relative to the Stejskal–Tanner function for constant gradient (●) and linear truncated distribution of gradient strengths (○) are shown in B.

$$A = \frac{2A_0}{(g_{\text{cal}}^{\max} - g_{\text{cal}}^{\min})^2}\left(\frac{\exp(-q \cdot g_{\text{cal}}^{\min 2})}{2q} - \frac{\exp(-q \cdot g_{\text{cal}}^{\max 2})}{2q} - \frac{\sqrt{\pi}\, g_{\text{cal}}^{\min}}{2\sqrt{q}}(\text{erf}(\sqrt{q} \cdot g_{\text{cal}}^{\max}) - \text{erf}(\sqrt{q} \cdot g_{\text{cal}}^{\min}))\right),$$

where

$$q = \gamma^2 I^2 \delta^2 D\left\{\Delta - \frac{\delta}{3}\right\}. \quad [6]$$

The fitting involves three parameters A_0, $g_{\text{cal}}^{\min}$, and $g_{\text{cal}}^{\max}$. The values of the parameters of linear distribution function obtained by fitting the attenuation curve using the distribution function in Eq. [5] are $g_{\text{cal}}^{\min} = 21.8\ \text{mT m}^{-1}\ \text{A}^{-1}$ and $g_{\text{cal}}^{\max} = 34.7\ \text{mT m}^{-1}\ \text{A}^{-1}$, corresponding to a distribution of gradient strengths between 0.22 and 0.35 T/m at the maximum gradient current (10 A).

As a recommendation for practical applications we suggest that one should calibrate the gradient by fitting the parameters A_0, $g_{\text{cal}}^{\min}$, and $g_{\text{cal}}^{\max}$ of Eq. [6] to an attenuation curve of an intense NMR signal from a sample with known diffusion coefficient. When later analyzing an attenuation curve of interest one should use the calibrated values of $g_{\text{cal}}^{\min}$ and $g_{\text{cal}}^{\max}$ and fit only A_0 and the diffusion coefficient, D, of Eq. [6] by any nonlinear fitting routine.

DISCUSSION

The suggested protocol (in the following called distribution function model) compares favorably to previously published methods to deal with nonlinear gradients in diffusion measurements. Tillet *et al.* (*4*) suggested that only the central region of the gradient coil should be used to suppress the effect of nonlinear gradients. In their application they used only 22% of the active volume, reducing the signal intensity by a factor of 4. The distribution function method makes use of the whole active volume and is therefore expected to give four times higher signal-to-noise ratio, resulting in approximately four times better precision in the diffusion coefficient. Another advantage is that the distribution function method is very easy to implement, i.e., one does not have to calibrate any shaped pulses. Compared to the situation when the problem of nonlinearity of the gradient is ignored, the distribution function method yields approximately a 20-fold reduction of the sum of squared residuals for intense signals (estimated from the data shown in Fig. 5). This reduction reflects that a systematic error has been essentially removed.

Another approach for dealing with the gradient nonlinearity problem in NMR studies of translational diffusion has been to only use the initial part of the attenuation curve. This approach gives unnecessary limitations to the measurement precision. In DOSY experiments (*9*) it even becomes impossible to use, since the signals may have very different degrees of attenuation. In such a situation the use of Eq. [1] would lead to significant systematic errors in the determined diffusion coefficient, which are avoided by the use of Eq. [6]. In applications where one tries to estimate the molecular weight distribution from diffusion measurements, the distribution of gradient strengths is confounded with the molecular weight distribution. For such applications a consideration of the gradient distribution is necessary.

Others before us have estimated the gradient nonlinearity by similar methods (*3*). Here we show for the first time how a distribution function, either as a histogram or as an analytical function approximating the histogram, can account for the nonlinear gradients in the data analysis. In principle it is possible to obtain the distribution function, $\rho(g_{\text{cal}})$, by an inverse Laplace transformation of $A(I)$, but we believe that the present approach is more robust. A different alternative to map nonlinear gradients would be the approach taken by Jerschow and Bodenhausen (*5*) for estimating the spatial distribution of RF fields. Their equation for the true z-coordinate as a function of the apparent z-coordinate is closely related to $g_{\text{cal}}(\nu)$.

Other NMR applications where gradients are used, e.g., coherence selection or purging of transverse magnetization, are not critically dependent upon perfectly linear gradients. We have shown that the gradient nonlinearity can easily be taken into account in diffusion experiments. These observations should have an impact on gradient probe design. The gradient does not have to be perfectly linear but should rather be optimized for parameters like gradient strength, recovery time, and improved gradient shielding. We have shown here that moderate nonlinearity of gradients can easily be handled and accounted for in precise and accurate diffusion experiments.

CONCLUSIONS

The profile and distribution of gradient strengths can be measured in a simple NMR experiment. The effect of the nonlinear gradient profile is easily handled during data analysis. This is achieved by approximating the gradient strength distribution function by a truncated linear function, with parameters that are estimated in a separate experiment. The method enables measurement with full signal intensity of precise and accurate diffusion coefficients also using standard gradient probes.

ACKNOWLEDGMENTS

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Note added in proof. At 800 MHz, a diluted H_2O sample (0.04% H_2O in D_2O, 1 mg/ml $GdCl_3$) should be used for calibration to avoid radiation damping.

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