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Spin diffusion transfer difference (SDTD) NMR: an advanced method for the characterisation of water structuration within particle networks

Valeria Gabrielli, Agne Kuraite, Marcelo Alves da Silva, Karen J. Elder, Jesús Angulo, Ridvan Nepravishta, Juan C. Munoz-García* and Yaroslav Z. Khimyak*

School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK.
Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK.
Present address: Department of Chemistry, University of Sevilla, Professor García González St, Sevilla, 41012, Spain
Present address: Sealy Center for Structural Biology and Molecular Biophysics, The University of Texas Medical Branch, Galveston, TX 77555, United States
* Corresponding authors: y.khimyak@uea.ac.uk; j.munoz-garcia@uea.ac.uk; rineprav@utmb.edu

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Introduction

Figure S1. Energy levels for a two-spin system. (a) When the two spins are equivalent, the αβ and βα states are degenerate. The dipolar coupling between the two nuclei induces an energy-conserving “flip–flop” transitions between these two states, and cross-correlation occurs. (b) When the two spins are not equivalent, the transition is not energy conserving and its probability is low. (c) When the two inequivalent spins are coupled to (many) other spins, the energy levels of the two-spin system are broadened and an overlap occurs between some of the αβ and βα levels. Cross-correlation has high probability and spin diffusion occurs (Adapted from Emsley 2009).

SDTD NMR as a tool for quantifying the spin diffusion coefficient at the interface (D_interface)

The phenomenon of spin diffusion can be described as the diffusion in space of nuclear magnetisation. It is mainly mediated by dipolar couplings and has been extensively used to retrieve a wealth of information about distances between atomic or molecular entities and for the characterisation of soft and solid materials. However, to obtain molecular spatial information it is of fundamental importance to experimentally determine the spin diffusion coefficient (D). A straightforward way to achieve this is to create a nonequilibrium spatial distribution of magnetisation along the protons of a molecular entity. Then, magnetisation is allowed to evolve freely for a specific time, and subsequently detected. During the evolution time, the spatial distribution differences of proton magnetisation will equilibrate via spin diffusion (i.e. dipolar couplings). The time to achieve the spatial equilibration of magnetisation will depend on the morphology of the molecular entity. In other words, the velocity of the equilibration step will depend on proton-proton distances and proton density.

The diffusion process of spin diffusion can be mathematically described by the Fick’s law of diffusion
\[ M(r, t) = \nabla[D(r)\nabla M(r, t)] \quad \text{Eq. S1} \]

Where \( \nabla \) is the Laplace operator, \( D \) is the diffusion gradient, \( r \) is the space vector, \( t \) is the diffusion time and \( M(r, t) \) is defined as the ratio between the z-magnetisation \( m(r, t) \) and the mass fraction of protons \( m_H(r) \)

\[ M(r, t) = \frac{m(r, t)}{m_H(r)} = \frac{m(r, t)}{(Q_HV_{\text{tot}})(r)} \quad \text{Eq. S2} \]

Where \( Q_H \) is the proton density and \( V_{\text{tot}} \) is the total volume of the molecular entity.

The solution of the diffusion equation for a point source is the Gaussian function

\[ M(r, t) = (M_0/(4\piDt)) \exp(-r^2/4Dt) \quad \text{Eq. S3} \]

While for an infinite solid the error function of the Gaussian function can be a solution for the diffusion equation

\[ M(r, t) = \frac{1}{2}M_0 \text{erfc} \left( r - r_0/\sqrt{4Dt} \right) \quad \text{Eq. S4} \]

In a two-phase system A and B where the magnetisation non-equilibrium spatial distribution is achieved by saturating selected protons on phase A and detecting it in phase B, the diffusion can still be described for each phase by the error function.

For two phases A and B.

\[ M_A(r, t) = E_A + F_A \text{erfc} \left( r - r_0/\sqrt{4D_A t} \right) \quad \text{Eq. S5} \]
\[ M_B(r, t) = E_B + F_B \text{erfc} \left( r - r_0/\sqrt{4D_B t} \right) \quad \text{Eq. S6} \]

Where \( E_A \) and \( E_B \) are the magnetisations at the interface.

At \( t=0 \)

\[ E_A + F_A = M_{A,0} \quad \text{Eq. S7} \]
\[ E_B + F_B = M_{B,0} = 0 \quad \text{Eq. S8} \]

Using the interface condition \( E_A = E_B \) and the flux equilibrium at the interface \( j_A(r_0, t) = j_B(r_0, t) \) it can be shown that

\[ M_B(r, t) = (M_{A,0} \sqrt{D_A Q_{HA}}/\sqrt{D_A Q_{HA}} + \sqrt{D_B Q_{HB}}) \text{erfc} \left( r - r_0/\sqrt{4D_B t} \right) \quad \text{Eq. S9} \]

If the \( D_A >> D_B \). This can simplify the equation to

\[ M_B(r, t) = M_{A,0} \text{erfc} \left( r - r_0/\sqrt{4D_B t} \right) \quad \text{Eq. S10} \]
Notably, to detect the magnetisation in phase B we can use the $^1$H NMR STD technique. The $^1$H STD NMR pulse program has been used successfully in the biomolecular field in the last 20 years. The main advantage of STD NMR is being a ligand-observed NMR technique, hence using small molecules as reporters of the macromolecular environment in an easy, inexpensive and robust way. In this paper, we explore the use of $^1$H STD NMR to access the interfacial spin diffusion phenomenon rationalised using Fick’s 2nd law of diffusion. Importantly, to relate the $^1$H STD NMR experiment to Eq. S10 the following considerations apply:

1. The small molecule is constantly in fast exchange between free and bound species in the phase B. Indeed, the overall exchange constant ($k_{ex}=k_{on}+k_{off}$) between the free and bound states is expected to be high due to a $k_{on}$ that can be considered at the diffusion limit and a $k_{off}$ that is expected to be relatively high so that $k_{ex} \gg D_{\text{Interface}}$

2. The half-life time of the instantaneous small molecule/macromolecule interaction is expected to be short compared to the interfacial diffusion time. In order to achieve the interface condition, several cycles of association dissociation for the small molecule will take place before the magnetisation can be efficiently transported through phase A (macromolecule) and subsequently to phase B (small molecule) in a continuous fashion, and detected in phase B.

3. In phase B, the spin diffusion of the small molecule in the free state is the same as the molecular diffusion and approaches the diffusion limit so there is virtually no difference between spin diffusion coefficient $D_{sp}$ (no matter transport is involved) and molecular diffusion coefficient $D$ (matter transport is involved). However, the relaxation of small molecules via spin diffusion is highly inefficient. Indeed, the small molecules that received the magnetisation through interacting with the macromolecule will maintain that magnetisation for a long time before relaxing. This will create again a new non-equilibrium spatial distribution of magnetisation through the protons of phase B that can be described by the error function and can be related to Eq. S10. As the interfacial spin diffusion is the slowest process it will be rate limiting for the entire process. Indeed, it is safe to substitute $D_B = D_{\text{Interface}}$ in Eq. S10.

4. Finally, $D_{\text{Interface}}$ can be obtained experimentally by varying the saturation time in the $^1$H NMR STD pulse sequence, selecting specific protons of phase A and detecting the
diffusion of magnetisation in the phase B through the proportionality \( M_B (r, t) \propto \frac{I}{I_0} \).

**Materials and methods**

**Sample preparation**

**Gels prepared in D\(_2\)O**

Dispersions of TEMPO-oxidised cellulose nanofibrils (OCNF), corn starch (CS) and enzymatically produced cellulose (EpC) at different concentrations were prepared in D\(_2\)O. OCNF of a degree of oxidation of \( \sim 25\% \), produced from purified softwood fibre and processed via high pressure homogenization, was kindly provided by Croda. These were further purified by dialysis against ultra-pure water (DI water, 18.2 MO cm) and stirred at room temperature for 30 min. Then the dispersion was acidified to pH 3 using HCl solution and dialysed against ultra-pure water (cellulose dialysis tubing MWCO 12400) for 3 days with the DI water replaced twice daily. The dialysed OCNF suspension was processed with mechanical shear (ULTRA TURRAX, IKA T25 digital, 30 minutes at 6500 rpm) and the pH was adjusted to 7 using NaOH solution. This suspension was further dialysed to remove any remaining salts and dispersed using a sonication probe (Ultrasonic Processor, FB-505, Fisher), via a series of 1 s on 1 s off pulses for a net time of 60 min at 30\% amplitude in an ice bath, and subsequently freeze-dried.

To prepare the OCNF dispersions for NMR investigation, OCNF powder and water were weighted to provide the desired weight concentrations of OCNF, and then probe sonicated for 30 min at 20\% amplitude using pulses of 1 s on and 2 s off, using an ultrasonic processor vibracell VCX 130 sonicator. On the other hand, CS samples were first gelatinized in a boiling water bath for 30 minutes. The CS samples were sonicated for 2 min at 40\% amplitude using 1 s on 2 s off pulses.

For the H\(_2\)O titration experiments, OCNF 1 wt\% dispersions were prepared using MilliQ® water and D\(_2\)O of 99.9 atom \% D to achieve the desired H\(_2\)O/D\(_2\)O ratio (5:95, 10:90, 20:80 and 30:70). For the variable gelator concentration experiments (OCNF and EpC at 0.5, 1 and 2 wt\%), the samples were prepared by dilution from the 2 wt\% dispersions to avoid error propagation.
OCNF 1 wt% gels prepared in mixtures of D\textsubscript{2}O and alcohol-OD

First, stock dispersions of OCNF 2 wt% were prepared by redispersing OCNF powder in D\textsubscript{2}O by probe sonication for 1 min at 30% amplitude using 1 s on 1 s off pulses, using an ULTRA TURRAX, IKA T25 digital sonicator. Subsequently, all the gels were prepared by dilution of the OCNF 2wt% dispersions using the corresponding alcohol-OD and D\textsubscript{2}O weight concentrations. D\textsubscript{2}O (151882) and 2-propanol-OD (615080) were purchased from Sigma-Aldrich. Ethanol-OD and methanol-OD were purchased from Cambridge Isotopes Lab, Inc.

Nuclear magnetic resonance (NMR) spectroscopy

Solution state NMR experiments were performed using a Bruker Avance I spectrometer equipped with a 5 mm triple resonance probe operating at frequency of 499.69 MHz (\textsuperscript{1}H). Saturation transfer difference (STD) NMR experiments of CS and OCNF dispersions were acquired at 298 K using a train of 50 ms Gaussian shaped pulses for selective saturation of the gelator particles, using an on-resonance frequency of 0 and -1 ppm for CS and OCNF dispersions, respectively, and an off-resonance frequency of 50 ppm. For the CS 15 wt% dispersion, saturation times ranging from 50 ms to 5 s were employed. For the experiments carried out on OCNF dispersions in water (i.e. H\textsubscript{2}O titrations and variable OCNF concentration), STD NMR experiments were performed using saturation times ranging from 100 ms to 8 s. A constant time length per scan (saturation time + recycle delay) of 8 s was used. Depending on saturation time, STD NMR experiments were performed with 128 scans or less (with a minimum of 16 scans), in inverse relation to the saturation time, and 8 dummy scans.

Variable concentration STD NMR experiments for EpC were carried out using a Bruker Avance II 800 MHz spectrometer equipped with a 5 mm inverse triple-resonance probe. The experiments were acquired at 298 K at saturation times ranging from 100 ms to 8 s, using a constant time length per scan (saturation time + recycle delay) of 8 s. The on- and off-resonance frequencies were set to -1 and 50 ppm, respectively. Depending on saturation time, STD NMR experiments were performed with 512 scans or less, in inverse relation to the saturation time, and 8 dummy scans.

The D\textsubscript{2}O/alcohol-OD OCNF gels were characterised by high-resolution magic angle spinning (HR-MAS) using a solid-state Bruker Avance III spectrometer operating at a \textsuperscript{1}H frequency of 400.22 MHz with a triple resonance HR-MAS probe (\textsuperscript{1}H, \textsuperscript{31}P, \textsuperscript{13}C). All samples were spun at 6
kHz. HR-MAS NMR was required for these samples due to large $^1$H peak broadening precluding enough resolution in the absence of magic angle spinning. The large spectral broadening of the D$_2$O/alcohol-OD OCNF gels is due to their high viscosity leading to very strong dipolar couplings, particularly for D$_2$O/ethanol-OD and D$_2$O/2-propanol-OD at high alcohol concentrations.

Saturation transfer difference (STD) NMR experiments were carried out by $^1$H selective irradiation (on-resonance) of OCNF peaks (2.3-2.5 ppm). A train of 50 ms Gaussian-shaped pulses were employed for saturation, with a field strength of 50 Hz. STD NMR experiments using 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 s saturation times were carried out, using a total relaxation time of 8.1 s. The off-resonance frequency was set to 56 ppm.

The STD spectra ($I_{STD}$) were obtained by subtracting the on- ($I_{sat}$) to the off-resonance ($I_0$) spectra. To determine the STD response or STD factor ($\eta_{STD}$), the peak intensities in the difference spectrum ($I_{STD}$) were integrated relative to the peak intensities in the off-resonance spectrum ($I_0$). The SDTD build-up curves were obtained by normalising all the STD factors against the highest value (usually corresponding to the longest saturation time).

**Simulation of the SDTD build-up curves**

To obtain a good fit of the SDTD build-up curve, it is essential to achieve a good sampling of both the lag phase and the plateau of the curve. To do so, using saturation times ranging from tens of milliseconds to 6-8 seconds is advised. The SDTD build-up curves were represented as a function of the square root of the saturation time and simulated in Matlab (Script 1) using Eq. 2. Here, the dependent variable is the normalized intensity of the NMR observable and the independent variable is the square root of the saturation time (in ms), $r$ is the minimum distance of the grid (in nm), $D$ is the spin diffusion rate (in nm$^2$/ms) at the particle-solvent interface, $erfc$ is the complementary error function, $C$ is the proportionally constant of the fit, and $b$ is a parameter to centre the function around $x$. Notably, the growth rate of the SDTD curve presents a proportional and inversely proportional relationship to the spin diffusion rate $D$ and the minimum distance $r$, respectively, both related to the degree of solvent structuration within the gel network. Hence, faster spin diffusion rates $D$ and shorter distances $r$ reflect increased solvent structuration.
Script 1. Matlab script used in this work to plot the SDTD data vs the square root of saturation time ($\sqrt{t_{\text{sat}}}$) and carry of the fit to Eq. 2.

function [fitresult, gof] = createFit(SQRT_tsat, SDTD)
%CREATEFIT(SQRT_TSAT,SDTD)
% Create a fit.
%
% Data for 'SDTD_fit' fit:
% X Input : SQRT_tsat
% Y Output: SDTD
% Output:
% fitresult : a fit object representing the fit.
% gof : structure with goodness-of fit info.
%
% See also FIT, CFIT, SFIT.

% export data, introduce path to your xlsx file in fname.
% SQRT_tsat and SDTD data must be in the first and second column, respectively, of fname
fname = 'PATH/filename.xlsx';
data = readmatrix(fname);
SQRT_tsat = data(:,1);
SDTD = data(:,2);
scatter(SQRT_tsat,SDTD);

% Fit: 'untitled fit 1'.
[xData, yData] = prepareCurveData( SQRT_tsat, SDTD );

% Set up fittype and options.
ft = fittype( 'C*erfc(r/(sqrt(4*D*x)))-b)', 'independent', 'x', 'dependent', 'y' );
opts = fitoptions( 'Method', 'NonlinearLeastSquares' );
opts.Display = 'Off';
opts.Lower = [-Inf 0 0.2];
opts.StartPoint = [1 0.00012 1 0.2];
opts.Upper = [Inf 0.1 1 0.2];

% Fit model to data.
[fitresult, gof] = fit( xData, yData, ft, opts );

% Plot fit with data.
figure( 'Name', 'SDTD_fit' );
h = plot( fitresult, xData, yData );
legend( h, 'SDTD vs. SQRT_tsat', 'SDTD_fit', 'Location', 'NorthEast', 'Interpreter', 'none' );
% Label axes
xlabel( 'SQRT_tsat', 'Interpreter', 'none' );
ylabel( 'SDTD', 'Interpreter', 'none' );
grid on

Script 2. Python script to plot the SDTD data vs the square root of saturation time (SQRT_TSAT) and carry of the fit to Eq. 2.

#!/usr/bin/env python3
#
# -*- coding: utf-8 -*-

@author: Dr Juan C. Muñoz García

import pandas as pd
import matplotlib.pyplot as plt
import scipy as sc
from scipy.optimize import curve_fit
from scipy.stats.distributions import t
from scipy import special
import numpy as np

# Loading data
fname = '/Users/jcmunoz/Documents/UEA/test_SDTD_fit_Matlab.xlsx'
data = pd.read_excel(fname, delimiter='\t').astype(float)
SQRT_TSAT = data.iloc[:,1]
SDTD = data.iloc[:,2]
x = SQRT_TSAT
y = SDTD

# Plot experimental data points
plt.plot(x,y,'bo',markersize='5')
plt.xlabel('Saturation time$^{1/2}$ [ns$^{1/2}$]', fontsize = '15', fontstyle='normal')
plt.ylabel('SDTD', fontsize = '15', fontstyle='normal')

# Defining function for curve fit
def model(x, C, D):
    return C*special.erfc(r/(2*sc.sqrt(D*x))-b)

# Set initial C and D values.
init_guess = [1, 0.00025] # follows order of parameters defined for model. C first, D second
# Set r in nm (usually 0.2-0.3 nm) and b (typically between 0 and 3)
r = 0.200
b = 1

# Perform curve fit
ans, cov = curve_fit(model, x, y, p0 = init_guess, absolute_sigma=False)

# Set confidence level = 100*(1-alpha)
alpha = 0.05 # 95% confidence level = 100*(1-alpha)
n = len(y) # number of data points
p = len(ans) # number of parameters
dof = max(0, n - p) # number of degrees of freedom

# student-t value for the dof and confidence level
tval = t.ppf(1.0-alpha/2., dof)

# Plot fit

t = np.linspace(1,90)
plt.plot(t, model(t, ans[0], ans[1]), label="model")

# Print C and D results with 95% confidence level. p0 = C. p1 = D in nm2/ms
for i, p, var in zip(range(n), ans, np.diag(cov)):
    sigma = var**0.5
    print('p{0}: {1} +/- {2}'.format(i, p, sigma*tval))

# Print goodness of fit parameters
modelPredictions = model(x, *ans)
absError = modelPredictions - y
SE = np.square(absError) # squared errors
MSE = np.mean(SE) # mean squared errors
RMSE = np.sqrt(MSE) # Root Mean Squared Error, RMSE
R_squared = 1.0 - (np.var(absError) / np.var(y))
print('RMSE:', RMSE)
print('R-squared:', R_squared)
print()
plt.savefig('SDTD.svg')
Results

![Figure S2](image1.png)

**Figure S2.** Off-Resonance (red) and STD (blue) HDO peak for (a) OCNF 1wt% and (b) CS 15wt% at 5 s saturation time and 298 K.

![Figure S3](image2.png)

**Figure S3.** SDTD NMR build-up curves of the HDO (circles, squares, rhomboids and triangles) and alcohol (stars and crosses) peaks in OCNF 1 wt% gels prepared in D$_2$O/MeOD (a) D$_2$O/EtOD (b) and D$_2$O/2PrOD (c) cosolvent mixtures of 10 wt% (black symbols), 30 wt% (red symbols), 50 wt% (green symbols) and 60 wt% (blue symbols) alcohol content. Note the faster growth of the SDTD build-up curves for HDO compared to the alcohols in all the gels.

**Table S1.** Calculated values for the C and D parameters obtained from the fit to Eq. 2 of the SDTD build-up curves of the HDO peak for the OCNF 1 wt% and CS 15 wt% dispersions. An r value of 0.2 nm and a b value of 1 were kept constant during the fit. The errors associated to each C and D value are shown in parenthesis and correspond to the 99% confidence level. The $R^2$ values of each fit to Eq. 2 are shown.

|       | OCNF 1 wt%       | CS 15 wt%       |
|-------|------------------|-----------------|
| C     | 1.14 (± 0.13)    | 1.12 (± 0.14)   |
| D (nm$^2$/ms) | 9.80E-05 (± 1.13E-05) | 1.28E-04 (± 1.70E-05) |
| $R^2$ | 0.9951           | 0.9952          |
**Table S2.** Calculated values for the $C$ and $D$ parameters obtained from the fit to Eq. 2 of the SDTD build-up curves of the HDO peak for OCNF 1 wt% dispersions prepared with different concentrations of H$_2$O. An $r$ value of 0.2 nm and a $b$ value of 1 were kept constant during the fit. The errors associated to each $C$ and $D$ value are shown in parenthesis and correspond to the 99% confidence level. The $R^2$ values of each fit to Eq. 2 are shown.

| HDO   | OCNF 1 wt%       |
|-------|------------------|
|       | <1% | 5% | 10% | 20% | 30% |
| C     | 1.14 (± 0.13) | 1.15 (± 0.11) | 1.13 (± 0.15) | 1.13 (± 0.18) | 1.11 (± 0.12) |
| $D$ (nm$^2$/ms) | 9.80E-05 (± 1.13E-05) | 9.36E-05 (± 9.35E-06) | 9.64E-05 (± 1.31E-05) | 9.66E-05 (± 1.59E-05) | 9.93E-05 (± 1.12E-05) |
| $R^2$ | 0.9951 | 0.9963 | 0.9930 | 0.9897 | 0.9952 |

**Table S3.** Calculated values for the $C$ and $D$ parameters obtained from the fit to Eq. 2 of the SDTD build-up curves of the HDO peak for EpC dispersions at different concentrations. An $r$ value of 0.2 nm was kept constant during the fit. A $b$ value of 1 was used to fit the EpC SDTD curves. The errors associated to each $C$ and $D$ value are shown in parenthesis and correspond to the 99% confidence level. The $R^2$ values of each fit to Eq. 2 are shown.

| Gelator conc. | EpC |
|---------------|-----|
|               | 0.5 wt% | 1 wt% | 2 wt% |
| $C$           | 1.24 (± 0.06) | 1.19 (± 0.04) | 1.24 (± 0.04) |
| $D$ (nm$^2$/ms) | 8.85E-05 (± 3.06E-06) | 9.25E-05 (± 2.97E-06) | 8.88E-05 (± 5.40E-06) |
| $R^2$         | 0.9942 | 0.9953 | 0.9949 |

**Table S4.** Calculated values for the $C$ and $D$ parameters obtained from the fit to Eq. 2 of the SDTD build-up curves of the HDO peak for OCNF dispersions at different concentrations. An $r$ value of 0.2 nm was kept constant during the fit. A $b$ value 2 was used to fit the OCNF SDTD curves. The errors associated to each $C$ and $D$ value are shown in parenthesis and correspond to the 99% confidence level. The $R^2$ values of each fit to Eq. 2 are shown.

| Gelator conc. | OCNF |
|---------------|-----|
|               | 0.5 wt% | 1 wt% | 2 wt% |
| $C$           | 0.76 (± 0.06) | 0.69 (± 0.04) | 0.65 (± 0.04) |
| $D$ (nm$^2$/ms) | 3.93E-05 (± 3.06E-06) | 4.86E-05 (± 2.97E-06) | 5.91E-05 (± 5.40E-06) |
| $R^2$         | 0.9964 | 0.9975 | 0.9940 |
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