Determining carbon-carbon connectivities in natural abundance organic powders using dipolar couplings

M. Dekhil, G. Mollica, T. Texier Bonniot, F. Ziarelli, P. Thureau and S. Viel

Electronic Supplementary Information

1 Predicted DQ intensities

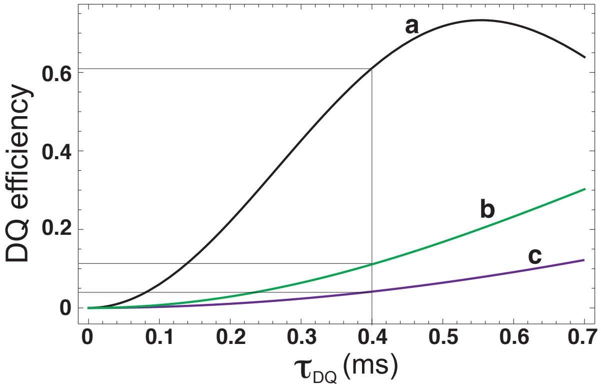

Figure 1: Predicted DQ efficiency of 2D dipolar-based ^{13}C-^{13}C correlation experiments as a function of the DQ excitation time τ_{DQ}. The curve *a* was plotted for a dipolar coupling corresponding to a ^{13}C-^{13}C distance of 154 pm, *i.e.* typical longest single bond distances found between two sp^3 carbons. Curves *b* and *c* were plotted for dipolar couplings corresponding to non covalently bonded carbons, *i.e.* ^{13}C-^{13}C distances of 220 pm and 260 pm, which correspond to typical distances found between two sp^2 and sp^3 carbons, respectively.

2D dipolar-based ^{13}C-^{13}C correlation spectra can be recorded using a short DQ excitation time τ_{DQ} leading exclusively to correlations peaks between carbons covalently bonded. In fact, as shown in Figure 1 for the $R20_2^9$ symmetry and $\tau_{DQ} = 0.4$ ms, the predicted intensity of correlation peaks arising from ^{13}C-^{13}C pairs separated by more than one covalent bond are at least 5.5 times weaker. Therefore for NA samples these correlations would be lost in the noise of the spectrum and hence undetected in the 2D ^{13}C-^{13}C DQ dipolar correlation spectrum.

The predicted DQ efficiency was plotted using the following equation:

$$s(\tau_{\mathrm{DQ}}) = \left[\frac{1}{2} - \frac{1}{x\sqrt{8}}\left(F_c\left(x\sqrt{2}\right)\cos 2\theta + F_s\left(x\sqrt{2}\right)\sin 2\theta\right)\right] \tag{1}$$

where

$$x = \sqrt{\frac{2\theta}{\pi}} \tag{2}$$

$$\theta = \frac{3}{2}\kappa b_{ij}\tau_{\mathrm{DQ}} \tag{3}$$

b_{ij} represents the dipolar coupling constant and κ corresponds to the recoupling scaling factor. F_c and F_s are the Fresnel integrals. For the $R20_2^9$ symmetry, the recoupling scaling factor $\kappa = 0.174$.[1]

2 Experimental recoupling efficiencies

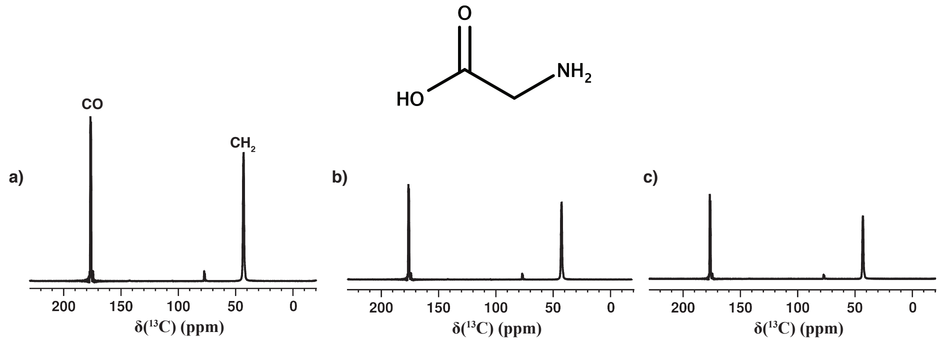

Figure 2: DQ filtered ^{13}C spectra of [U-^{13}C]-α-glycine obtained for different recoupling schemes: $R20_2^9$ (a), SPC5 (b) and POST-C7 (c). All spectra were recorded with a MAS frequency of 10 kHz and $\tau_{DQ} = 0.4$ ms.

Figure 2 shows the experimental DQ recoupling efficiency of the $R20_2^9$ DQ recoupling pulse sequence obtained for a DQ recoupling time (τ_{DQ}) of 0.4 ms. The experimental DQ recoupling efficiencies of two standard ^{13}C-^{13}C DQ recoupling experiments, *i.e.* POST-C7 and SPC5, are also shown.

Table 1 summarises the theoretical and experimental DQ efficiencies obtained with $\tau_{DQ} = 0.4$ ms for these three DQ recoupling pulse sequences. The experimental DQ efficiencies were obtained by scaling the integrated DQ-filtered spectra with respect to the integrated CP spectrum of [U-^{13}C]-α-glycine. The theoretical DQ efficiencies were calculated using Equation 1 and with $b_{ij} = 2200 \times 2\pi$ rad.s^{-1}, $\tau_{DQ} = 0.4$ ms; $\kappa = 0.174$,[1] $\kappa = 0.155$[2] and $\kappa = 0.137$[3] were used for $R20_2^9$, POST-C7 and SPC5, respectively.

	DQ efficiency (%)	
	Theoretical	Experimental
$R20_2^9$	0.64	0.49
POST-C7	0.57	0.25
SPC5	0.48	0.28

Table 1: Theoretical and experimental DQ efficiencies for $R20_2^9$, POST-C7 and SPC5 DQ recoupling pulse sequences. The experimental DQ efficiencies were measured on [U-^{13}C]-α-glycine at a MAS frequency of 10 kHz.

Overall, for $\tau_{DQ} = 0.4$ ms the experimental DQ recoupling efficiency is 1.7 higher for $R20_2^9$ with respect to POST-C7 or SPC5. As a result, ^{13}C-^{13}C dipolar DQ experiments recorded using the $R20_2^9$ scheme require experimental durations 2.9 times shorter with respect to standard recoupling experiments and, therefore, 2D ^{13}C-^{13}C DQ dipolar correlation spectra of NA samples can now be recorded in a practicable amount of time.

It should be noted that because all isotropic chemical shift terms are symmetry-forbidden in the case of the $R20_2^9$ symmetry, this recoupling experiment is less sensitive to chemical shift offsets with respect to POST-C7 or SPC5 recoupling experiments.[1,4] The main weakness of RN_n^ν pulse sequences is the extreme sensitivity to the precise value of the radio-frequency phase shift ϕ.[4,5] Nevertheless, thanks to the latest advances in NMR hardware, effective phase shifts are now possible, and thus valuable RN_n^ν recoupling pulse sequences, such as $R20_2^9$, can be conceived.

3 Experimental details

NMR experiments were performed on a Bruker 9.4 T magnet (400 and 100 MHz for the ^{1}H and ^{13}C Larmor frequency, respectively) operated by an AVANCE III HD NMR spectrometer and equipped with a Bruker 4-mm double-resonance ^{1}H/^{13}C CP-MAS probe. Approximately 80 mg of sample can be loaded into a 4 mm rotor providing the best compromise between spinning-frequency and sensitivity for NA samples at 9.4 T.

The DQ dipolar recoupling was accomplished using a pulse sequence with the symmetry $R20_2^9$ and based on the element $\mathcal{R}^0 = 90_0 270_0$.[1,4,5] The experiments were performed at a MAS frequency of 10 kHz, leading to a ^{13}C nutation frequency of 100 kHz during the recoupling sequence. The DQ recoupling time was set to 0.4 ms. Proton decoupling was not applied during the ^{13}C recoupling[6,7] while swept-frequency TPPM[8] decoupling with a ^{1}H nutation frequency of 90 kHz was used during the t_1 and t_2 delays. Pure absorption 2D spectra were obtained by using the States-TPPI procedure. It should be noted that the $R20_2^9$ symmetry lead to the so-called "γ-encoded" recoupling, which permits to freely vary the t_1 evolution time, independently of the sample spinning-frequency.[4,5]

For the POST-C7 and SPC5 experiments, a ^{1}H Lee-Goldburg decoupling,[4] with a nutation frequency of 90 kHz, was applied during the DQ recoupling time.

[U-^{13}C]-α-glycine: [U-^{13}C]-α-glycine sample was purchased from Cambridge Isotopes and used with no further purification. Lee-Golburg decoupling with a ^{1}H nutation frequency of 90 kHz was applied during the ^{13}C POST-C7 and SPC5 recoupling. All the recoupling experiments ($R20_2^9$, POST-C7 and SPC5) were performed using identical experimental parameters, except for the recoupling ^{13}C nutation frequency, which is imposed by the symmetry of the recoupling pulse sequence.

L-tyrosine ethyl ester: L-tyrosine ethyl ester was purchased from Sigma Aldrich and used as supplied. The 2D ^{13}C-^{13}C correlation spectrum was recorded using a total of 27 t_1 increments with 1600 scans each were collected with a spectral width of 25 kHz and 28 kHz in the DQ and SQ dimensions, respectively. Ramped cross-polarisation of duration 2 ms was used to generate ^{13}C magnetisation and the recycle delay was set to 3 s. The total experimental time of the experiment was 72 hours (3 days).

Cellulose: Cellulose type 20 sample was purchased from Sigma Aldrich and used as supplied. A total of 20 t_1 increments with 2048 scans each were collected with a spectral width of 10 kHz in the DQ and SQ dimensions. Ramped cross-polarisation of duration 1.0 ms was used to generate ^{13}C magnetisation and the recycle delay was set to 3.2 s. The total experimental time of the experiment was 72 hours (∼3 days).

Poly(methyl methacrylate) (PMMA): PMMA was purchased from Sigma Aldrich and used as supplied. The DQ/SQ ^{13}C correlation spectrum was recorded using the $R20_2^9$ pulse sequence and a recoupling time of 400 μs. A total of 38 t_1 increments with 1024 scans each were collected with a spectral width of 50.5 kHz and 25.25 kHz in the DQ and SQ dimensions, respectively. Ramped cross-polarisation of duration 3.0 ms was used to generate ^{13}C magnetisation and the recycle delay was set to 2 s. The total experimental time of the experiment was 43 hours (∼1.8 days).

References

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Bruker Pulse Program

```
;R20nu9n2_2D

;2D SQ-DQ correlation experiment based on R2092 symmetry
;Suitable for 10 kHz MAS frequency

;Avance III version
;parameters:
;d1 : recycle delay
;d0 : min. delay in t1 evolution
;d4 : z-filter delay, typically 20 us (used for ZGOPTNS=-Dzfilter)
;pl1 : f1 power level for X CP pulse
;pl11 : for recoupling sequence (Nutation Freq=13*cnst31 in Hz)
;p1 : excitation pulse at plw11
;sp0 : proton power level during contact
;pl2 : =0, not used
;pl12 : for 1H excitation and decoupling
;p3   : 1H excitation pulse at plw12
;p15  : CP contact time
;pcpd2 : decoupling pulse f2 at plw12, pcpd = 2*P3-0.2us used by TPPM and SPINAL
;spnam0 : for CP on 1H e.g. ramp.64
;cpdprg2: decoupling f2, e.g. tppm15, SPINAL64. swTPPM
;cnst31: MAS frequency=10kHz
;l10 : number of R20 cycles for DQ excitation & reconversion
;l0 :  R20 increments for DQ excitation&reconversion=l0*(TD1-1)
;ns    : 16*n
;in0 : inf1
;mc2 : STATES-TPPI
;nd0 : 1
;WDW : F1 QSINE 3,  F2 QSINE 2 or EM
;use "xau xfshear rotate" to shift spectrum suitably along f1

;$COMMENT=SQ-DQ experiment
;$CLASS=Solids
;$DIM=2D
;$TYPE=cross polarisation
;$SUBTYPE=homonuclear correlation
;$OWNER=Bruker

define pulse pul90
"pul90=((0.25s/cnst31)/10)"
define pulse pul270
"pul270=((0.75s/cnst31)/10)"
define loopcounter count       ;for STATES-TPPI procedure
  "count=td1/2"                ;and STATES cos/sin procedure

"d31=(1s/cnst31)"
define delay excite
"excite=(2*d31*l10)/10"
define delay reconv
"reconv=(2*d31*l10)/10"
define delay recoup
"recoup=(4*d31*l10)/10"
```

```
;set t1 increment
"d0=1u"
"in0=inf1"

;cnst11 : to adjust t=0 for acquisition, if digmod = baseopt
"acqt0=1u*cnst11"


#include <rot_prot.incl>
            ;protect for too slow rotation

 ze
1 10m
"cnst27=-180*(d0+0.1u)/d31"       ;calculate t1 dependent phase shift for
reconversion
  1m ip18+cnst27
  1m ip17+cnst27
  1m ip16+cnst27
  1m ip15+cnst27
2 d1 do:f2                     ;F2 decoupler off

#include <p15_prot.incl>
            ;make sure p15 does not exceed 10 msec
            ;let supervisor change this pulseprogram if
            ;more is needed
#include <aq_prot.incl>
            ;allows max. 50 msec acquisition time, supervisor
            ;may change  to max. 1s at less than 5 % duty cycle
            ;and reduced decoupling field


 (p3 pl12 ph1):f2
  (p15 pl1 ph2):f1 (p15:sp0 ph10):f2
  1u pl11:f1
  (p1 ph4):f1

  ;1u pl11:f1 ; switch to R20 RF condition for cpdprg1

3 pul90:f1 ph11        ;R20 excitation
  pul270:f1 ph12
  pul90:f1 ph13
  pul270:f1 ph14
lo to 3 times l10                 ;l0 DQ excitation block
4 0.1u cpds2:f2
  d0                    ;F2 decoupling during DQ evolution: tppm15, or SPINAL64
  0.1u do:f2

5 pul90:f1 ph18                  ;R20 reconversion
  pul270:f1 ph17
  pul90:f1 ph16
  pul270:f1 ph15
  lo to 5 times l10           ;l0 DQ reconversion block

  (p1 ph5):f1 (1u cpds2):f2  ;
```

```
  gosc ph31            ;start ADC with ph31 signal routing
  1m do:f2
  1m ip15
  1m ip16
  1m ip17
  1m ip18
  lo to 2 times ns
  100m wr #0 if #0 zd
  1m  ip11
  1m  ip12
  1m  ip13
  1m  ip14
  lo to 1 times 2
  id0
  lo to 1 times count
HaltAcqu, 1m
exit

ph1= 1
ph2= 0
ph4= 3
ph5 = 1 2 3 0 2 3 0 1 3 0 1 2 0 1 2 3
ph10=0

ph11 = (float,45.0)  81
ph12 = (float,45.0)  261
ph13 = (float,45.0)  279
ph14 = (float,45.0)  99
ph15 = (float,90.0)  189
ph16 = (float,90.0)  9
ph17 = (float,90.0)  351
ph18 = (float,90.0)  171

ph31= 2 1 0 3 3 2 1 0 0 3 2 1 1 0 3 2
```