Supporting Information

Chemical Analysis of Deep-Lung Fluid Derived from Exhaled Breath Particles

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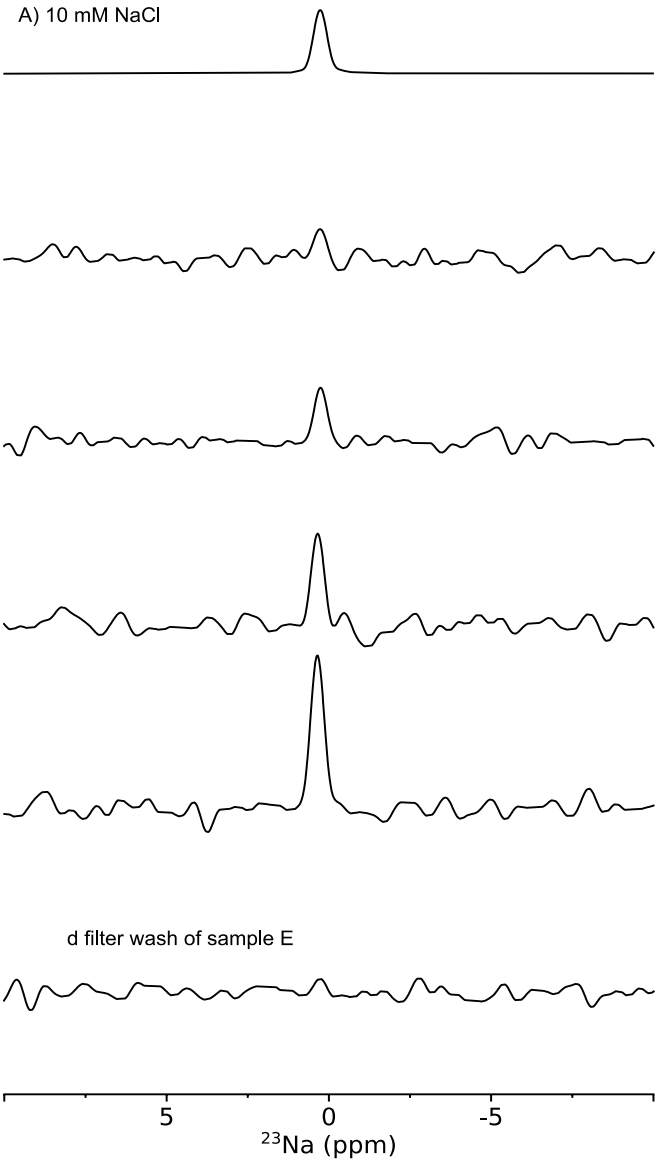

Figure S1. ^{23}Na NMR spectra used for data plotted in Figure 5, main text. The spectra were collected at 22 °C in a 7:3 ethanol:D_2O solution and correspond to dry particle volumes (DPV) derived from optical particle sizer measurements, marked for spectra B-E. Spectrum A, corresponds to a 10 mM standard with 1k (1,024) transients. For spectra B-F, 3,145,728 transients were recorded due to lower analyte concentrations. Spectrum F represents the second filter wash of the highest-concentration sample, E.

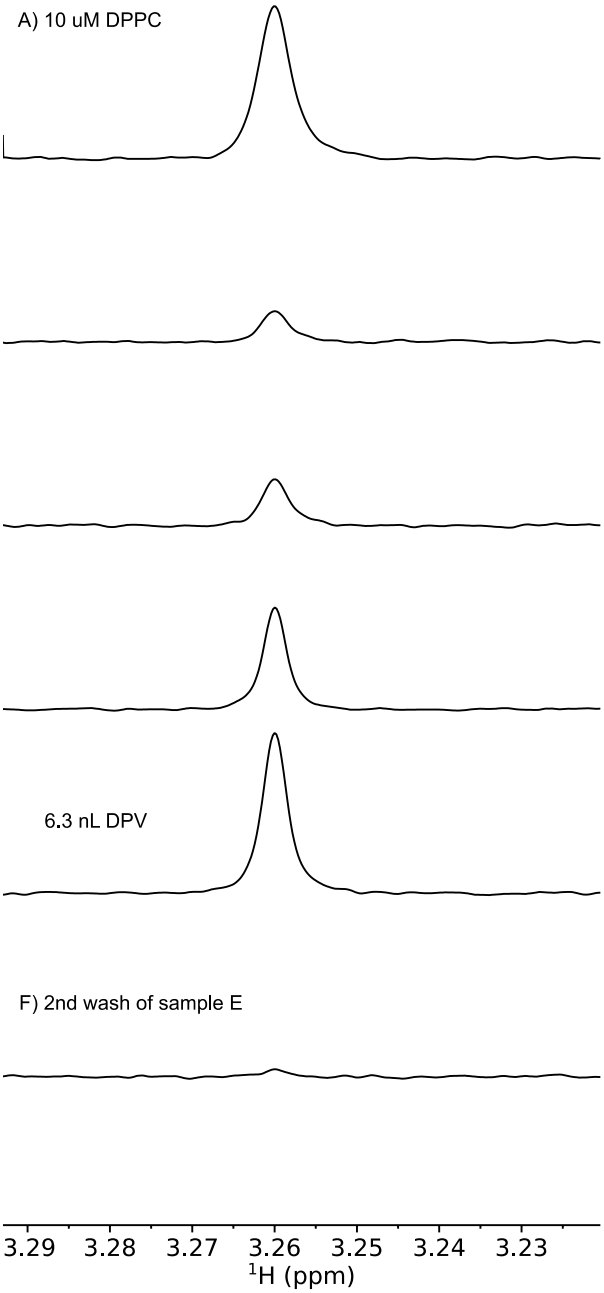

Figure S2. Choline methyl region of ^{1}H NMR spectra used for data plotted in Figure S4. (A) 7.5-μM standard sample. (B-E) Exhaled breath particles; the total dry particle volume (DPV) collected on the filters, as derived from the optical particle sizer, is marked for each of these spectra. (F) The sample from a second filter wash of the highest-concentration sample, E. All spectra were recorded at 600 MHz, 20 °C in a 7:3 ethanol:D_2O solution with a total of 128 transients and referenced to DSS. The NMR pulse sequence for selective observation of the choline methyl signal is included as Supporting Information below.

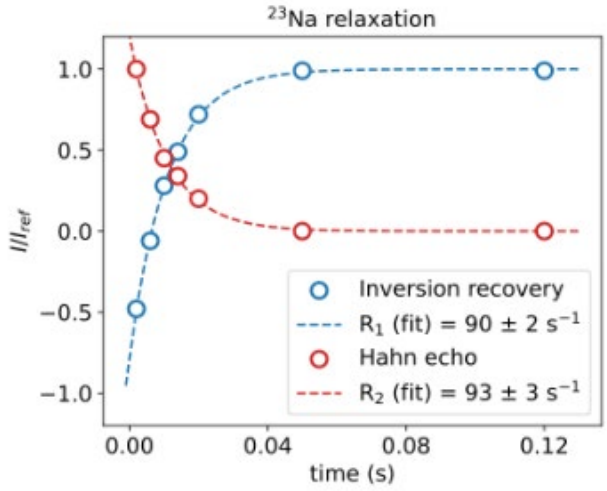

Figure S3. R_1 and R_2 measurements of the ^{23}Na signal on a sample containing 10 mM NaCl in 7:3 ethanol:D_2O, at 22 °C. A typical inversion recovery experiment was used for T_1 measurements. ^{23}Na intensities were fitted to $I(t)/I_0 = 1-A_1\exp(-R_1t)$, in which the fitted parameters, A_1 and R_1, are the preexponential factor and longitudinal relaxation rate, respectively. I(t) represents the spectral intensity for a recovery delay of duration t and I_0 corresponds to the intensity obtained in the absence of the 180° pulse. A Hahn-echo experiment was used for ^{23}Na transverse relaxation rate measurements. The data were fitted to $I(t) = A_2\exp(-R_2t)$, in which the fitted parameters, A_2 and R_2, are the preexponential factor and the transverse relaxation rate, respectively. Variable recovery delays of 2, 6, 10, 14, 20, 50 and 120 ms, and interscan delays of 1 s were used for both R_1 and R_2 measurements.

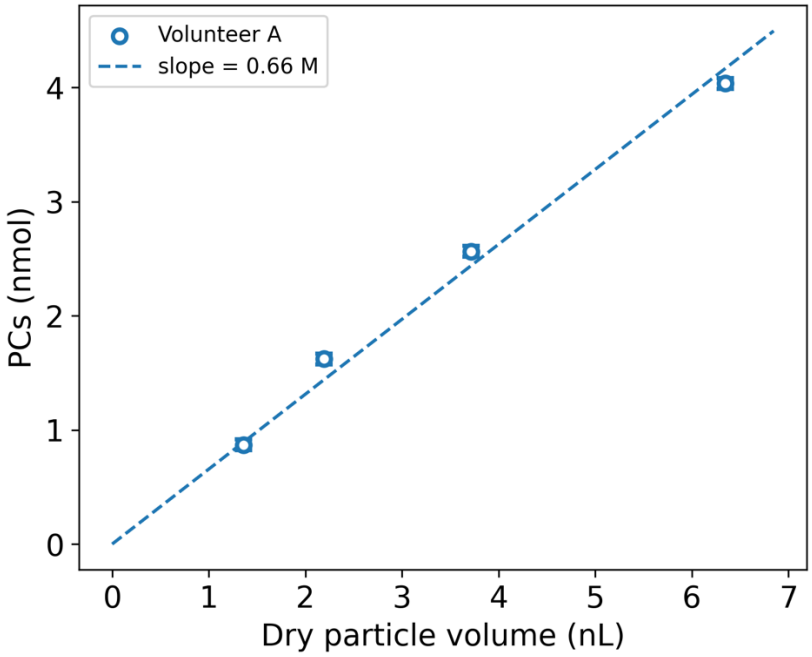

Figure S4. Plot of corresponding measurements of PCs for the same samples that were used for sodium quantification in breath particles (see Figure 5, main text).

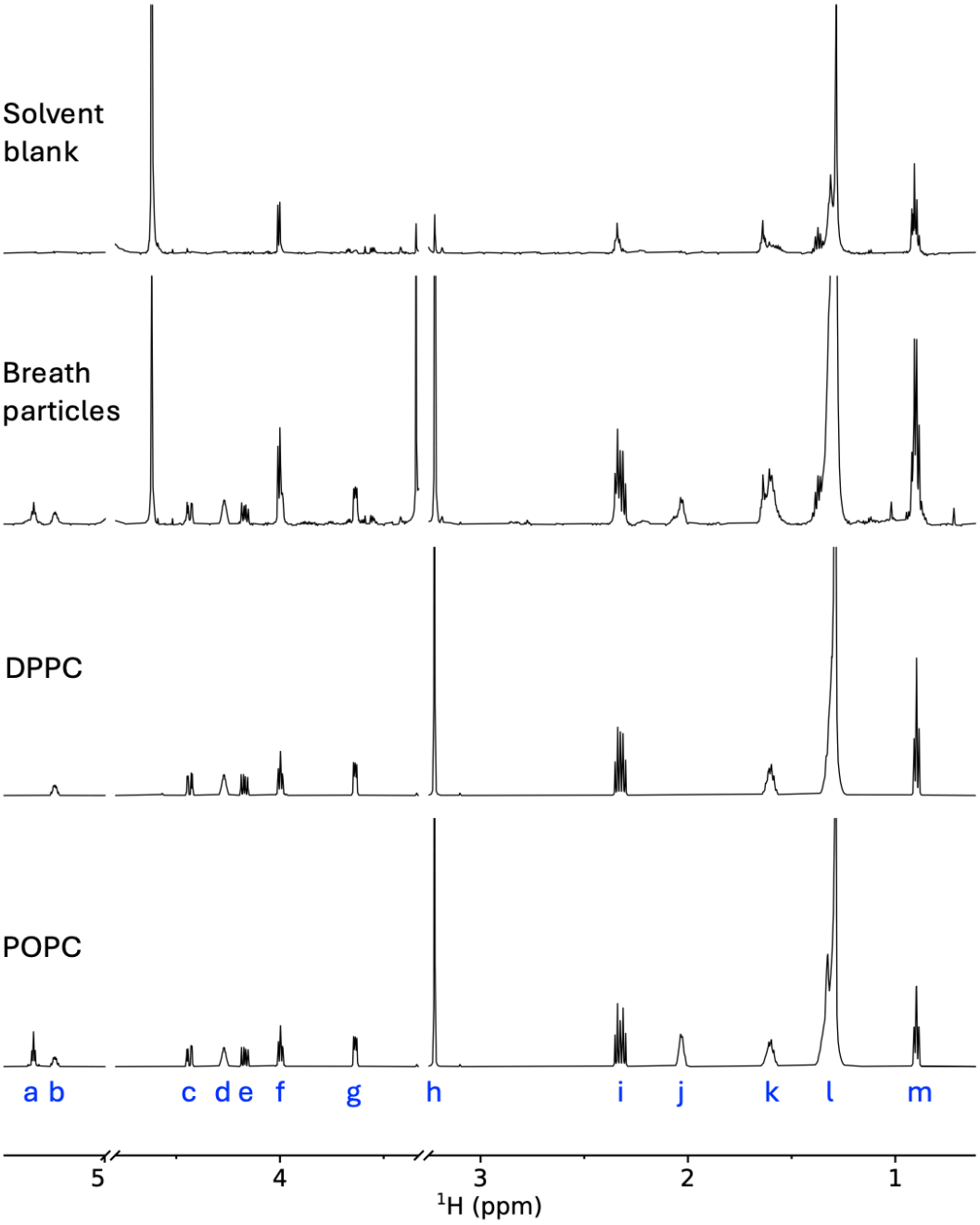

Figure S5. ^{1}H NMR spectra of POPC, DPPC, exhaled breath particles, and solvent blank (methanol-d_4, ≥99.96 %D, Sigma-Aldrich, 535435), all recorded at 20 °C. DPPC and POPC samples were prepared at a concentration of 13 mg/mL and recorded in 5-mm NMR tubes (DEUTEROTUBE, Boroeco-5-7) with each spectrum resulting from 64 transients. For exhaled breath and solvent blank samples, 10,240 transients were collected both in capillaries (ID: 1.15 mm, CORNING, 9530-2). Transients were recorded on a Bruker Avance NEO 600 MHz instrument equipped with a cryogenic probe at the rate of 30 per minute. The DPPC and POPC spectra are scaled down by a factor of 10 for visual clarity.

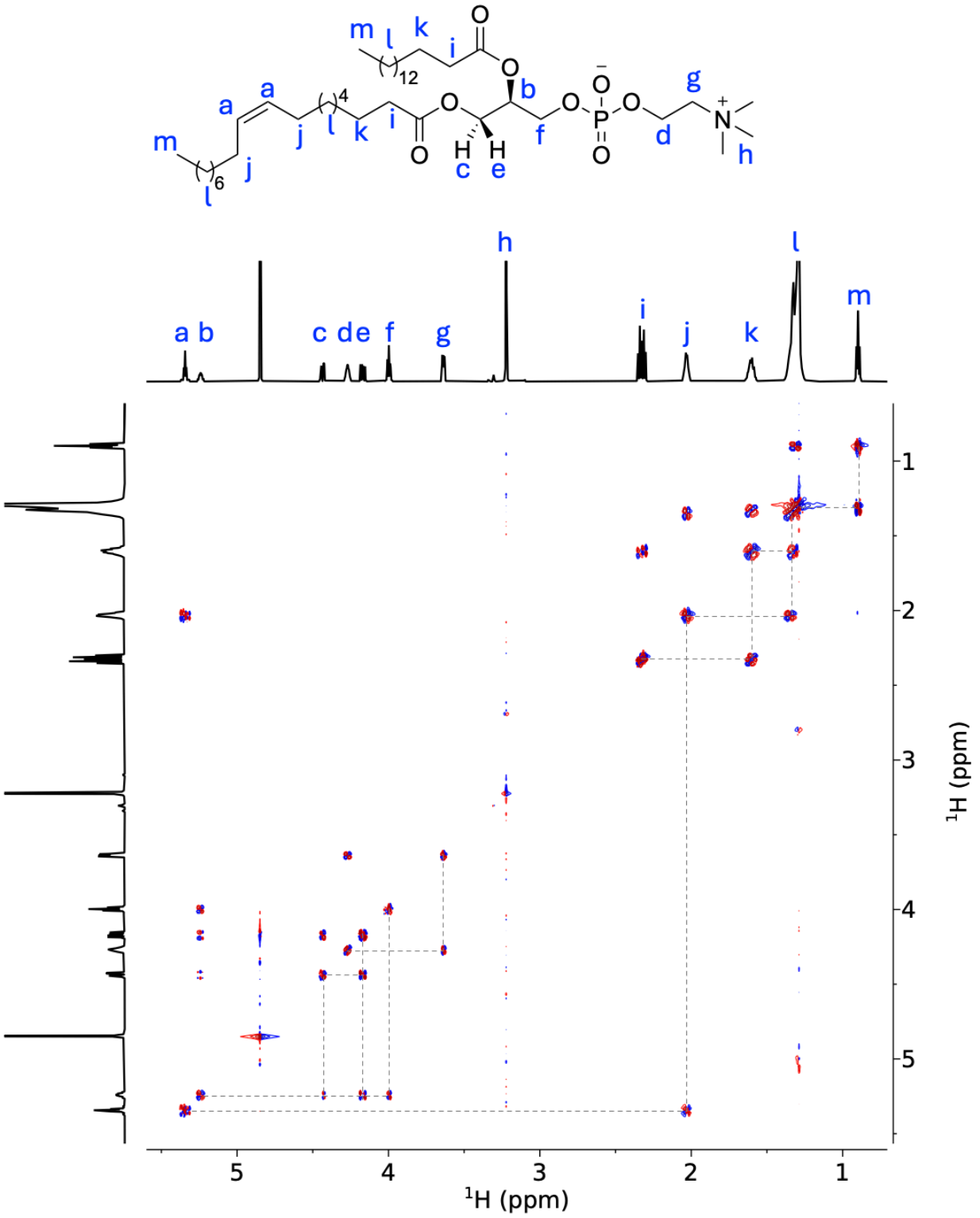

Figure S6. ^{1}H-^{1}H DQF-COSY spectrum of POPC (13 mg/mL; 0.5 mL in a 5 mm tube) in methanol-d_4 at 25 °C. 1024 increments with 8 scans per increment were collected on a Bruker 600-MHz Avance NEO instrument equipped with a cryogenic probe.

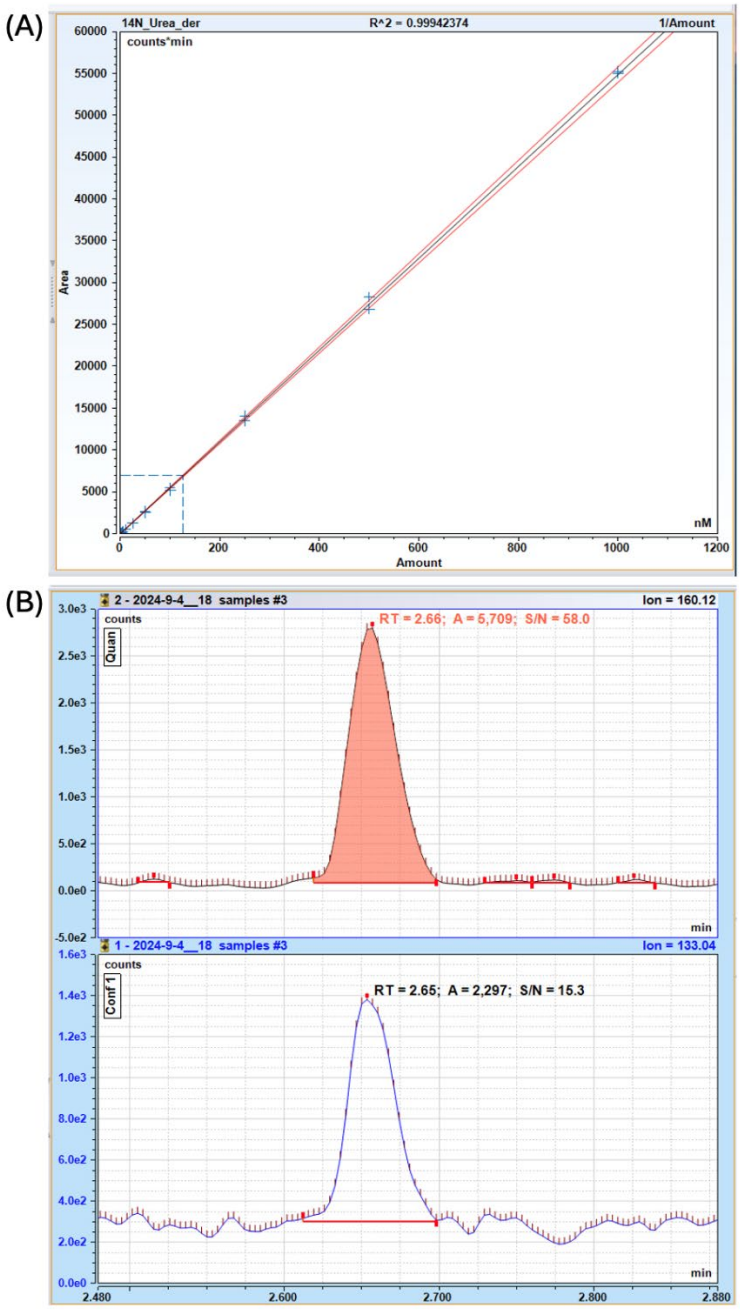

Figure S7. (A) Calibration plot for the ^{14}N urea derivative ranging from 1 nM to 1 µM, 5 µL injections, linear curve fit with 1/x weighing, and plotting ± 99.5% confidence intervals (B) LC-MS chromatogram of a 5 µL injection of a 1 nM ^{14}N urea derivative standard solution, quantitative ion and confirming MRMs, integrated using Chromeleon's Genesis.

Bruker pulse program used for PC quantification using ^{1}H NMR

```
#include <Grad.incl>

1 ze
2 30m
  20u pl1:f1 BLKGRAD
  d1
  50u UNBLKGRAD
  p1 ph1
  4u
  p16:gp1
  d16 pl0:f1
  (p12:sp1 ph2:r):f1
  4u
  p16:gp1
  d16 pl0:f1
  4u
  go=2 ph31
  30m mc #0 to 2 F0(zd)
  4u BLKGRAD
exit

ph1=0
ph2=0 1
ph31=0 2

;pl1    : f1 channel - power level for pulse (default)
;p1     : f1 channel - 90 degree high power pulse
;pl0    : 1000 dB
;p16    : 500 us
;gpz1   : 35%
;gpnam1 : SMSQ10.100
;p12    : 20 ms
;spnam1 : Gaus1.1000
;spoffs1: center at 3.26 ppm in 7:3 EtOH:D2O at 20C, referenced to DSS
;d16    : 200 us
;ds     ; 4
;ns     ; 128
;aq     ; 1 s
;d1     : relaxation delay; 1 s
;TopSpin version 4.1.4
```

Parameters for ^{23}Na quantification

To optimize signal-to-noise ratio, T_1 of sodium was first measured in 7:3 ethanol:D_2O mixture, yielding a value of 11.1 ms (Figure S1). The repetition time (T_R) was then set to 1.5 x T_1 = 16.65 ms with 16.55 ms allocated for acquisition and 0.1 ms for interscan delay. Transients were collected with an optimal flip angle of 77°, calculated for this T_R value using the Ernst angle equation below.

$$A_{ernst} = cos^{-1}(e^{-T_R/T_1})$$

Since this repetition time is unusually short, the actual repetition rate was initially limited by a few tens of milliseconds of default delays in standard Bruker pulse sequences. To achieve the intended repetition rate, the standard pulse-acquire program was modified to remove these delays. The modified pulse program is shown below, implemented with version 2.1 of the TopSpin software operating an Avance III 600 MHz console. To prevent automatic rescaling during data acquisition due to integer word length overflow, the spectra were recorded as 3 separate spectra of 1,048,576 scans each, and then combined.

Bruker pulse program used for ^{23}Na quantification

```
1 ze
2 d1
  5u
  p1 ph1
  go=2 ph31
  30m wr #0
  1m zd
exit

ph1=0 2 2 0 1 3 3 1
ph31=0 2 2 0 1 3 3 1

;p1 : f1 channel – excitation high power 77 deg pulse
;d1 : relaxation delay; 0.1 ms
;aq : 16.5 ms
;TopSpin version 2.1
```

Bin cutoff for TSI-3330 optical particle sizing measurements (instrument default)

Bin number	Cut Point (μm)
1	0.300
2	0.374
3	0.465
4	0.579
5	0.721
6	0.897
7	1.117
8	1.391
9	1.732
10	2.156
11	2.685
12	3.343
13	4.162
14	5.182
15	6.451
16	8.031
17	10.00