A Meta-Stable Liquid Precursor Phase of Calcium Carbonate and its Interactions with Polyaspartate

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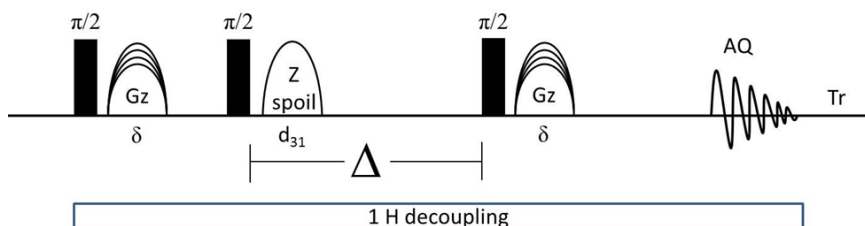
Supplement:

Fig. S.1. NMR pulse sequence used to acquire the ^{13}C diffusion data. It is a modified version of stegs1s, which is Bruker's sequence. We used 16 equidistant varying z-gradient (Gz) strengths (2% - 95%) to generate the data. The relevant variables used for our acquisition are:

Dwell time: 100 µs

Gz (100%) = 50 g/cm

Recovery time (Tr) = 25 s

Diffusion time (Δ) = 1.6 s

Gradient time (δ) = 1 ms

Z-spoil = -17.13%

Z-spoil gradient time (d31) = 1.1 ms

Acquisition time (AQ) = 4.9 s

6 mM Injections of CaCl2 (aq) into 20 mM Bicarbonate Buffer, pH 8.5
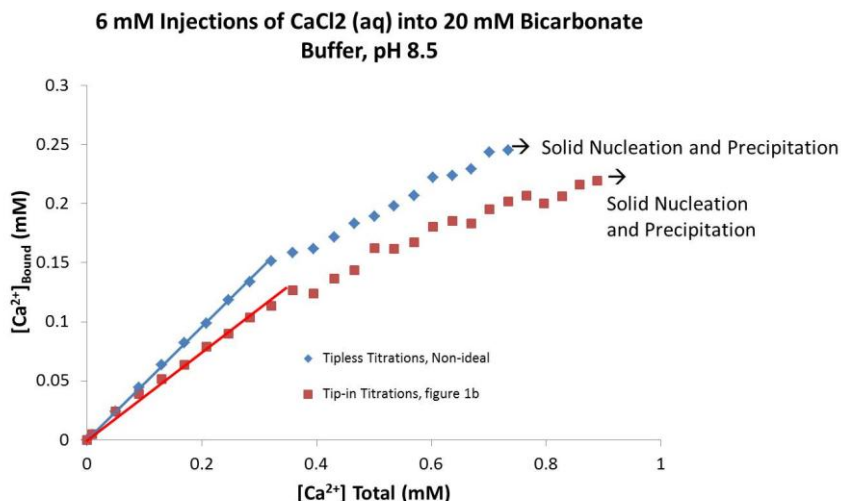

Fig. S.2. A comparison between the same titrations conducted with injection nozzle (micropipette tip) in the solution (as conducted in this paper) vs. with the nozzle just above the solution. We still see the same qualitative character to both curves. The nozzle-out method (blue, diamonds) kinetically binds more Ca^{2+} under the less ideal mixing conditions than the nozzle-in method (red, squares). Even with the presence of some kinetic binding effects, the qualitative character of the curve is maintained, including a close agreement of bound Ca^{2+} at which the phase transition occurs, and at which solid nucleation occurs. Since the nozzle is out of the solution and the qualitative character of the profile is maintained, the behavior of the profile is not due to nucleation at the nozzle tip and therefore can be discussed in a qualitative fashion.

First Emergent Phase Experiment, 6mM CaCl2 into 20 mM bicarbonate buffer, 10th injection.wmv

Second Emergent Phase Experiment, 6mM CaCl2 into 20 mM bicarbonate buffer, 10th injection.wmv

Fig. S.3. Raw Nanoparticle Tracking Analysis (NTA) video data collected of the suspected
5 metastable emergent phase at the 10th injection of 6 mM $CaCl_2$ into 20 mM bicarbonate buffer, pH
8.5 for two separate experiments. The phase is plainly visible but very sparse on the order of
hundreds of picomolar in droplet counts. The images shown in in Figure 5 of the main paper were
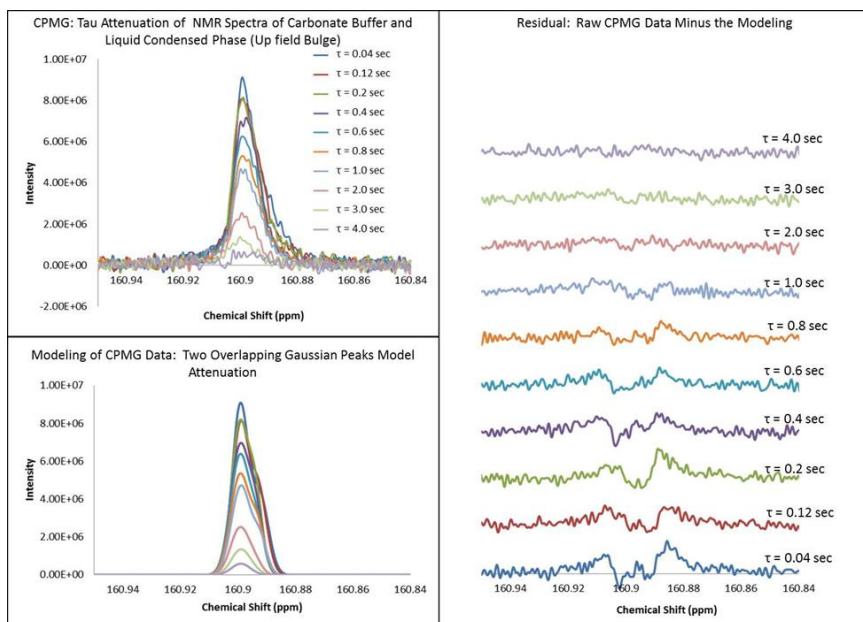taken from the second emergent phase experiment. To view, click on the description.

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Fig. S.4. An example of our modeling technique. Raw data of an attenuating signal was collected (upper left). Gaussian distributions were used to model the bulging asymmetry observed in the NMR spectra due to the fact that there is exchange between the carbonate and bicarbonate species in solution (bottom left). The overlapping Gaussian model was then subtracted from the raw data to yield a residual (right). The residual suggests that some of the signal at the "foot" of the raw data peaks were not modeled. This is consistent with the raw data peaks having some Lorenzian character that a Gaussian would clip at the edges. The edge data is not as important as the overlap and exchange between the two phases present and therefore was not modeled. Once overlapping Gaussian distributions are modeled representing each of the phases, they can be used to solve for the T_2 relaxation of each phase. A similar technique was used to deconvolute the Pulse-Field Gradient Stimulated Echo (PFG-STE) data and determine its attenuation for a diffusion measurement of the two phases.
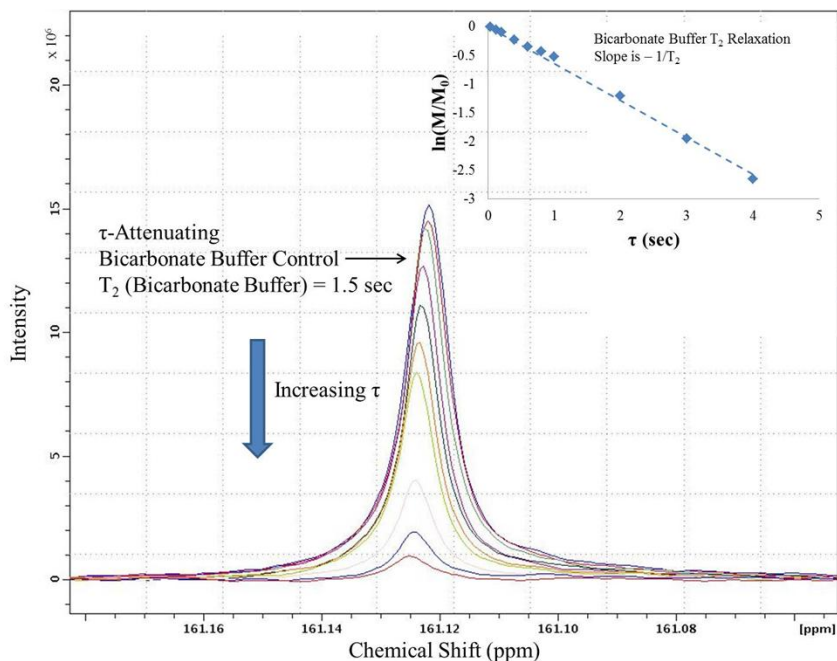
Fig. S.5. The τ-induced attenuation of the ^{13}C peak during the Carr- Purcell Meiboom-Gill (CPMG) T_2 relaxation experiment of the bicarbonate peak of the 20 mM, pH 8.5, bicarbonate control solution titrated with injections of nanopure water. Using the attenuation of the intensity of this one-phase system and the known values of the increasing τ used in the looped pulse sequence, the T_2 relaxation of the ^{13}C (carbonates/bicarbonates) in this phase can be determined by using equation 1 (shown in the main paper).
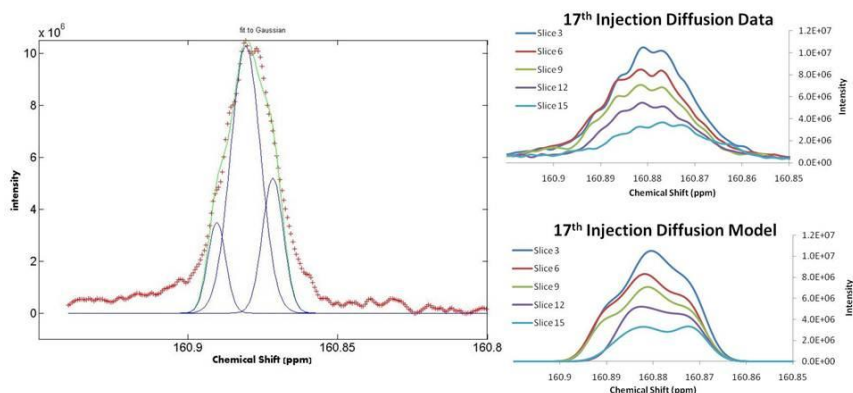
Fig. S.6. The real data and the modeling of the ^{13}C diffusion during the 17th injection without polyaspartic acid additive. Only slices 3, 6, 9, 12, and 15 are shown for clarity. Left) The raw data of the 3rd slice of the widened ^{13}C NMR peak after the first-order phase transition modeled with
5 three Gaussian peaks. A single large peak (center model) with similar width as the buffer and 7th injection system is not sufficient to model the morphology of the spectral peak. Additional peaks (upfield, downfield) were modeled to account for the morphology. Upper right) The real attenuation data for the system during a diffusion NMR experiment. Bottom right) the sum of the Gaussian modeling of the attenuation using the 3 Gaussian peaks. Due to the asymmetric
10 attenuation, and the inability to model the system with a singular Gaussian distribution, there is justification for use of the additional Gaussian peaks and additional information about the properties of the upfield widening (bicarbonate direction) can be obtained. In the case of the submitted publication, we found that the upfield widening diffused slower than the other two Gaussian models, suggesting the presence of a distinct phase.
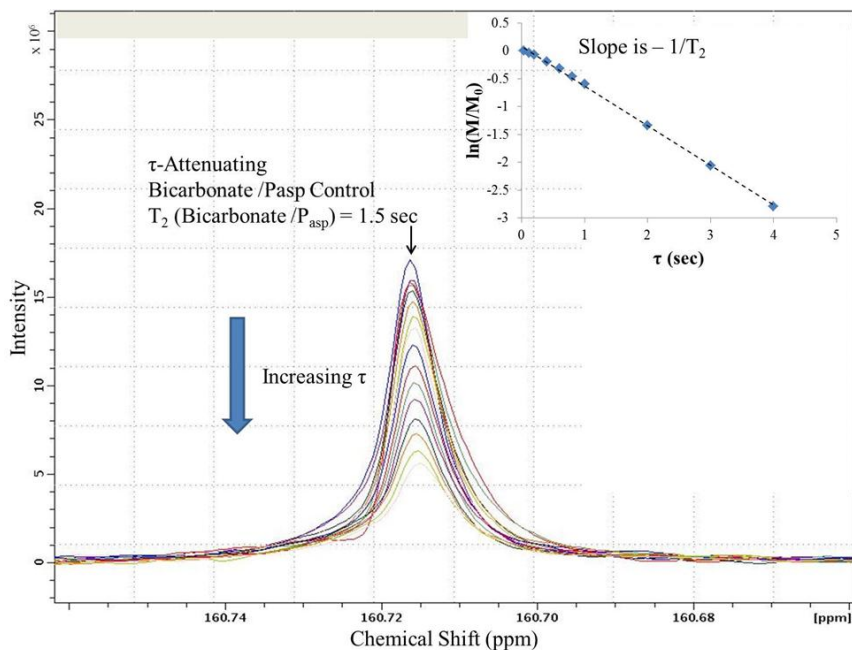
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Fig. S.7. The τ-induced attenuation of the ^{13}C peak during the Carr- Purcell Meiboom-Gill (CPMG) T_2 relaxation experiment of the bicarbonate peak of the 20 μg/ml , M.W. 14,000 g/mol Pasp control solution titrated with 30.15 mM bicarbonate buffer, pH 8.5. Using the attenuation of the intensity
5   of this one-phase system and the known values of τ used in the looped pulse sequence, the T_2 relaxation can of the ^{13}C (carbonates/bicarbonates) in this phase can be determined by using equation 1 (shown in the main paper).
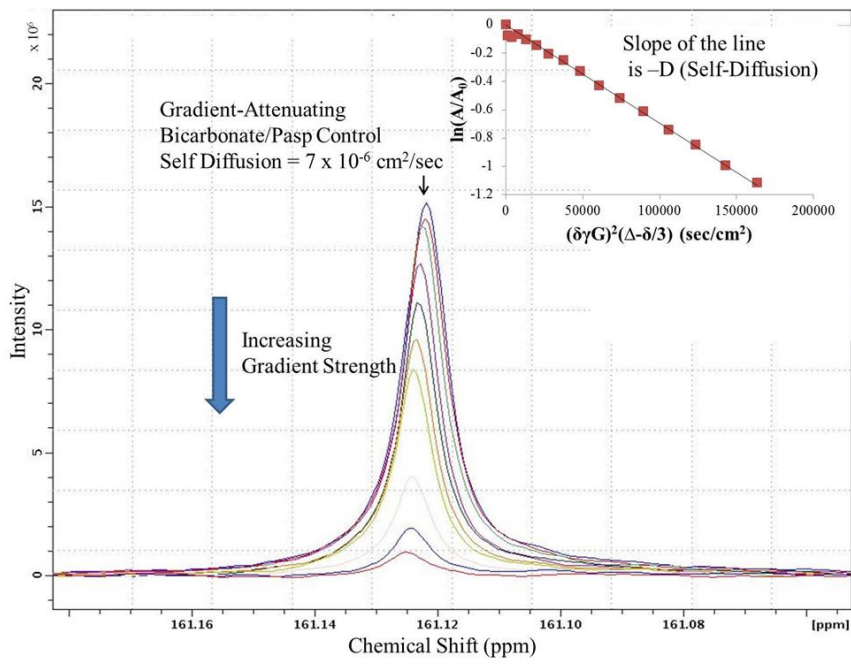
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Fig. S.8. The gradient-induced attenuation during the Pulse-Field Gradient Stimulated-Echo (PFG-STE) T_2 relaxation experiment of the bicarbonate peak of the 20 µg/ml polyaspartic acid sodium salt (M.W. 14,000 g/mol) control solution titrated with 300 mM bicarbonate buffer, pH 8.5,
5 yielding a final concentration of 18 µg/ml pasp and 30.15 mM bicarbonate. Using the attenuation of the intensity of this one-phase system and the known increases in gradient strength for each experimental slice, the self-diffusion of the ^{13}C (and therefore the carbonates/bicarbonates) in this phase can be determined by using equation 2 (shown in the main paper).
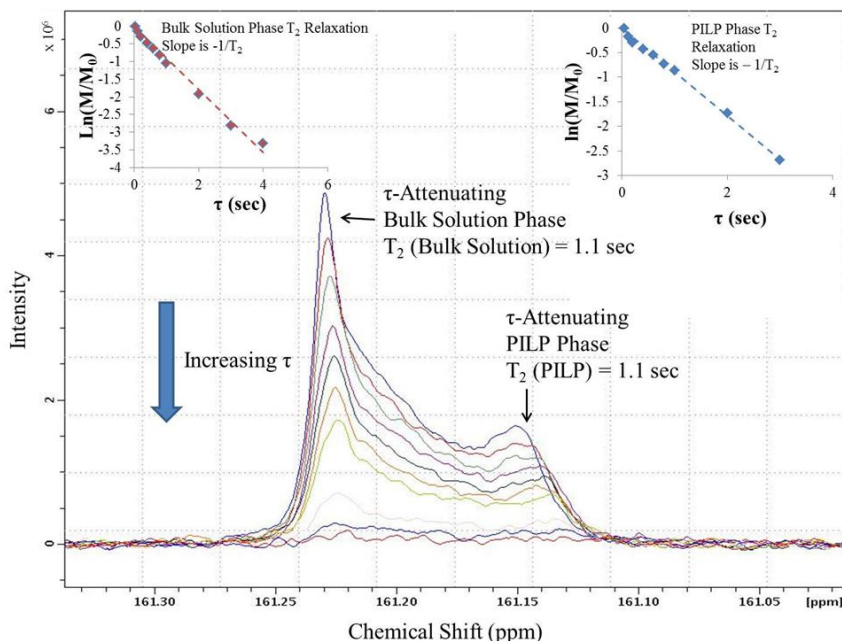
Fig. S.9. The τ-induced attenuation of the ^{13}C peak during the Carr- Purcell Meiboom-Gill (CPMG) T_2 relaxation experiment of the bicarbonate peak of the 10 mM CaCl$_2$, 20 µg/ml polyaspartic acid sodium salt (M.W. 14000 g/mol) solution titrated with 17 injections of 300 mM bicarbonate buffer,
5   pH 8.5. The final concentration of the resulting solution is 9 mM CaCl$_2$, 18 µg/ml pasp, 30.15 mM bicarbonate buffer, pH 8.5. An additional phase emerges (PILP phase). Using the observed attenuation of the intensities of both phases due to known increases in τ used in the looped pulse sequence, the T_2 relaxation of the ^{13}C (carbonates/bicarbonates) in both distinct phases can be determined by using equation 1 (shown in the main paper).
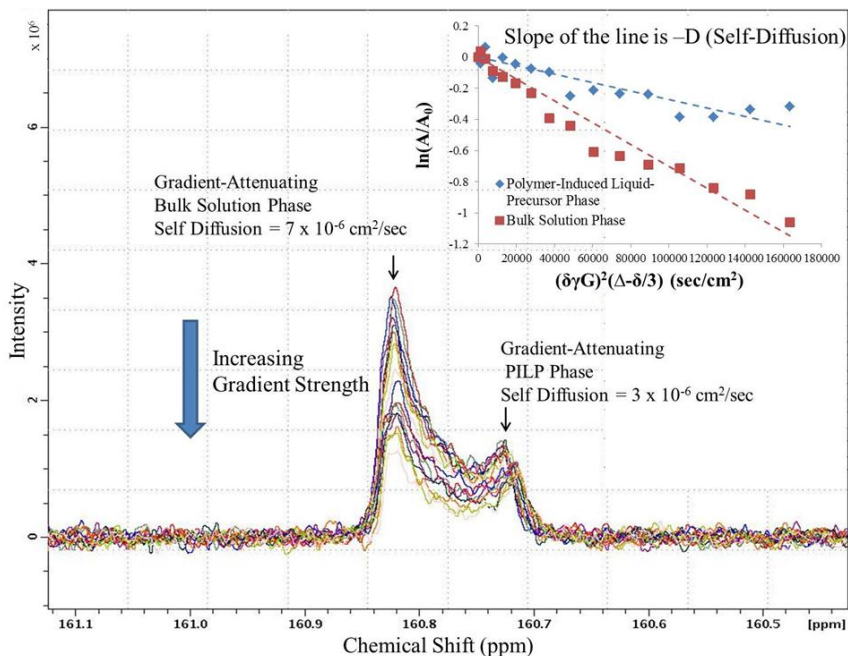
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Fig. S.10. The gradient-induced attenuation during the Pulse-Field Gradient Stimulated-Echo (PFG-STE) diffusion measurement experiment of the bicarbonate peak of the 20 µg/ml polyaspartic acid sodium salt (14,000 g/mol M.W.) solution titrated with 300 mM bicarbonate buffer, pH 8.5, yielding a final concentration of 18 µg/ml pasp and 30.15 mM bicarbonate buffer. Using the attenuation of the intensity of this two-phase system and the known increases in gradient strength for each attenuated experimental slice, the self-diffusion of the ^{13}C (and therefore the carbonates/bicarbonates) in these phases can be determined by using equation 2 (shown in the main paper). The carbonates/bicarbonates in the suspected PILP phase are diffusing at less than half the rate of the carbonates/bicarbonates in the bulk solution.