

Borate-Catalyzed Carbon Dioxide Hydration via the Carbonic Anhydrase Mechanism

SUPPORTING INFORMATION

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Rate Constant Calculations

Borate catalyzed reactions were followed by the stopped-flow pH indicator technique, in which the boric acid / borate system operates as the buffer, with thymol blue as the indicator. Under our experimental conditions the total boric acid concentration $[BA]_{total}$ is sufficiently low that $B(OH)_3$ is only in equilibrium with $B(OH)_4^-$:

$$[BA]_{total} = [B(OH)_3] + [B(OH)_4^-] \quad (S1)$$

$$K_a = \frac{[B(OH)_4^-][H^+]}{[B(OH)_3]} \quad (S2)$$

Where, K_a is the dissociation constant of boric acid.

The total reaction rate for CO_2 hydration (r , $M\ s^{-1}$) can be defined as the rate of CO_2 consumption:

$$r = -\frac{d[CO_2]}{dt} = k_{obs}[CO_2] \quad (S3)$$

Here, k_{obs} (s^{-1}) is the observed pseudo-first-order rate constant. Experimental conditions have been chosen so that $[CO_2]$ is always in large excess over $[OH^-]$, with constant $[CO_2]$ assumed over the course of all experiments. Considering contributions from the $CO_2 + OH^-$ and $CO_2 + H_2O$ reactions, as well as possible catalysis by $B(OH)_3$ and $B(OH)_4^-$, the observed rate constant can be expressed as:

$$k_{obs} = k_1 + k_2[OH^-] + k_3[B(OH)_4^-] + k_4[B(OH)_3] \quad (S4)$$

Assuming that the ionization equilibrium of the buffer and indicator are instantaneous, the initial reaction rate can be defined as follows:

$$k_{obs} = -\frac{1}{[\text{CO}_2]_0} \frac{d[\text{CO}_2]}{dt} \bigg|_{t=0} = -\frac{Q}{[\text{CO}_2]_0} \frac{dA}{dt} \bigg|_{t=0} \quad (\text{S5})$$

Where Q is the buffer factor, defined by the following relation:

$$\frac{dA}{dt} = \left(\frac{\partial A}{\partial x} \right)_t \left(\frac{d[\text{H}^+]}{dt} \right) = \frac{1}{Q} \frac{d[\text{H}^+]}{dt} = \frac{1}{Q} \frac{d[\text{CO}_2]}{dt} \quad (\text{S6})$$

The buffer factor is obtained via Eqn (S7). In this equation C_B , C_{In} are the total buffer and indicator concentrations, respectively; α_B , α_{In} are mole fractions of the base forms of the buffer and indicator, respectively; b is the optical path length; $\Delta\epsilon$ is the difference in extinction coefficients between the acid and base forms of indicator.

$$Q = \frac{\alpha_B (1 - \alpha_B) C_B}{\alpha_{In} (1 - \alpha_{In}) b C_{In} \Delta\epsilon} \quad (\text{S7})$$

Values calculated according to Eqn (S7) were found to agree well with experimental values obtained via spectrophotometric titration of the buffer-indicator system.

In general, the buffer factor is a complicated function of the states of ionization of buffer and indicator, and this quantity changes to some extent during the course of reaction¹. If a buffer and indicator have nearly identical pK_a values, variation in the buffer factor can be minimized by observing the reaction kinetics over a period in which pH does not change significantly^{1,2}. In the present work, boric acid and thymol blue have similar pK_a values, which have been published at different temperatures and ionic strengths^{3,4}. For the stopped-flow experiments reported here, kinetic data was analyzed over a short reaction period, in which the pH change was measured to be small (< 0.02 pH units), allowing us to ignore any variation in Q over the course of the reaction.

CO₂ + OH⁻ Reaction

The reaction of CO₂ with OH⁻ was followed by observing the decrease in [OH⁻], which can be derived from the absorbance change of thymol blue. Experiments were performed in the pH range 8.4 to 9.7 at 20 °C to 40 °C, in the absence of buffer.



$$K_{\text{In}} = \frac{[\text{In}^{2-}][\text{H}^+]}{[\text{HIn}^-]} \quad (\text{S9})$$

where HIn⁻ and In²⁻ are the acid and base form of thymol blue, respectively; K_{In} is the second dissociation constant of thymol blue. The absorbance of indicator can be defined as the product of the concentration and the extinction coefficient (ε) for the acid and base forms:

$$A = \epsilon_{\text{HIn}^-} [\text{HIn}^-] + \epsilon_{\text{In}^{2-}} [\text{In}^{2-}] \quad (\text{S10})$$

Combing Eqns (S9) and (S10) yields:

$$[\text{OH}^-] = \frac{(\epsilon - \epsilon_{\text{HIn}^-}) K_{\text{w}}}{(\epsilon_{\text{In}^{2-}} - \epsilon) K_{\text{In}}} \quad (\text{S11})$$

Figure S5 shows a plot of the observed rate constant (*k*_{obs}) versus [OH⁻], demonstrating a good linear fit. Rate constants *k*₂, obtained from the slope of *k*_{obs} versus [OH⁻] are provided in Table S1 from 20 °C to 40 °C. Fitting these rate constants to the Arrhenius equation (Figure S6) provides an activation energy of 57.8 kJ mol⁻¹ and the rate constant expression *k*₂ [M⁻¹ s⁻¹] = 9.88×10¹³exp(-6956/*T* [K]), which are in agreement with values previously reported in the literature.

CO₂ + H₂O Reaction

The rate constant for reaction of CO₂ with H₂O was measured in imidazole buffer solutions with 4-nitrophenol as indicator, at around pH 7.0. At this pH reaction of CO₂ with the hydroxide ion is minor, but cannot be ignored. In order to account for the effect of the buffer on CO₂ hydration the overall reaction rate was measured at different buffer concentrations, with the true rate constant then determined by extrapolating to zero buffer concentration. Experiments were also run at the same conditions in the presence of boric acid to see if B(OH)₃ can catalyze CO₂ hydration (at around pH 7 B(OH)₃ is the dominant boron compound).

Temperature-dependent rate constants, k_1 (s⁻¹), are listed in Table S2, obtained by extrapolating rate constants from Figure S1 to zero buffer concentration, then subtracting the contribution due to the hydroxyl mechanism, $k_2[\text{OH}^-]$, using rate constants determined above. Our measured values for k_1 are in general agreement with available literature data [8, 10]. Figure S1 also indicates that B(OH)₃ is not a significant catalyst for CO₂ hydration, by comparison of the experimental results with and without boric acid. An inverse log plot of rate constants for the CO₂ + H₂O reaction are plotted in Figure S7, where we observe a good linear relationship corresponding to $k_1 [\text{s}^{-1}] = 4.07 \times 10^6 \exp(-5584/T [\text{K}])$, with activation energy of 46.4 kJ mol⁻¹.

References

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Table S1: Rate constants for the CO₂ + OH⁻ reaction

T (°C)	20	25	30	35	40
k_2 (M ⁻¹ s ⁻¹)	5200±150	6830±200	10340±300	15900±370	22700±720

Table S2: Rate constants for the CO₂ + H₂O reaction

T (°C)	25	30	35	40
k_1 (s ⁻¹)	0.0298±0.0007	0.0409±0.0008	0.0553±0.0011	0.0729±0.0015

Table S3: Rate constants for the CO₂ + B(OH)₄⁻ reaction

T (°C)	25	30	35	40
k_3 (M ⁻¹ s ⁻¹)	35.3±2.0	51.6±2.3	81.6±2.7	128.9±4.4

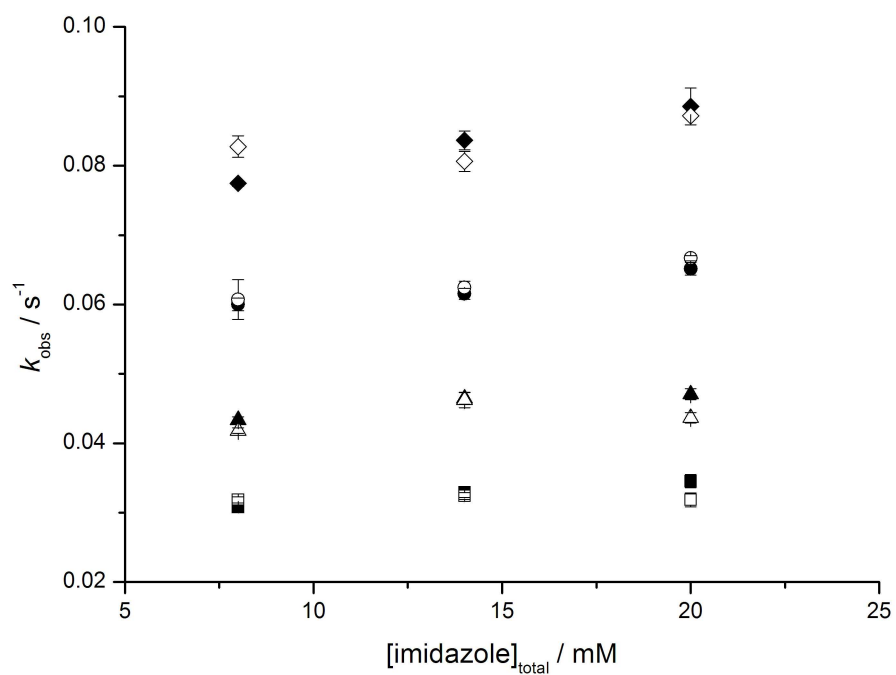


Figure S1: CO₂ hydration rate versus buffer (imidazole) concentration at pH 6.8. Solid symbols: without boric acid; filled symbols: 4.0 mM boric acid added (■/□: 25 °C; ▲/△: 30 °C; ●/○: 35 °C; ◆/◇: 40 °C).

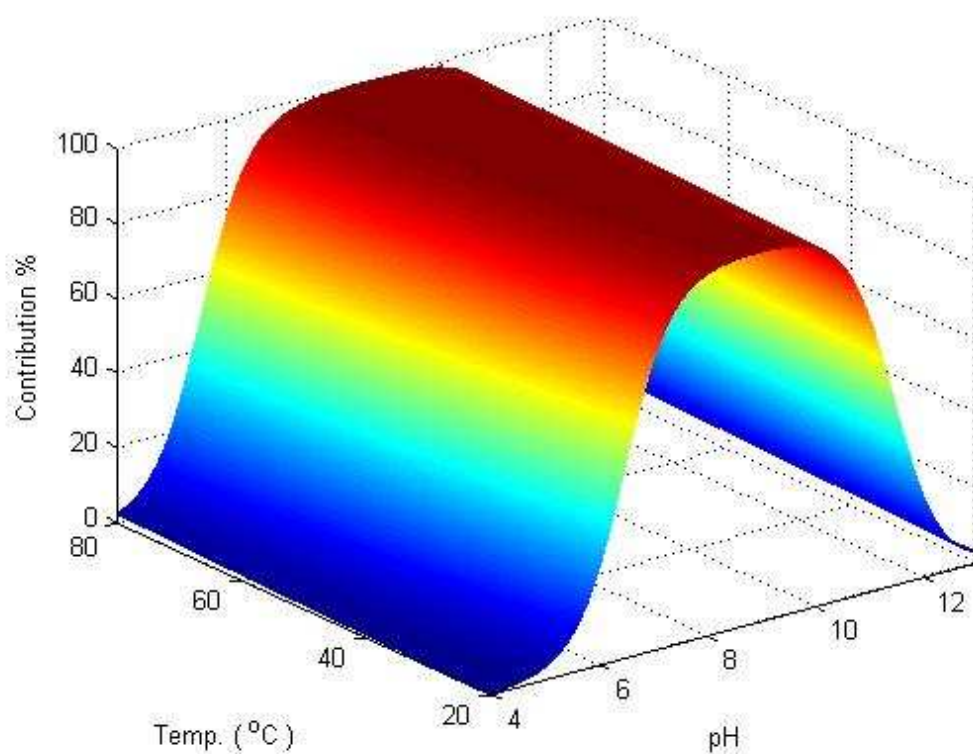
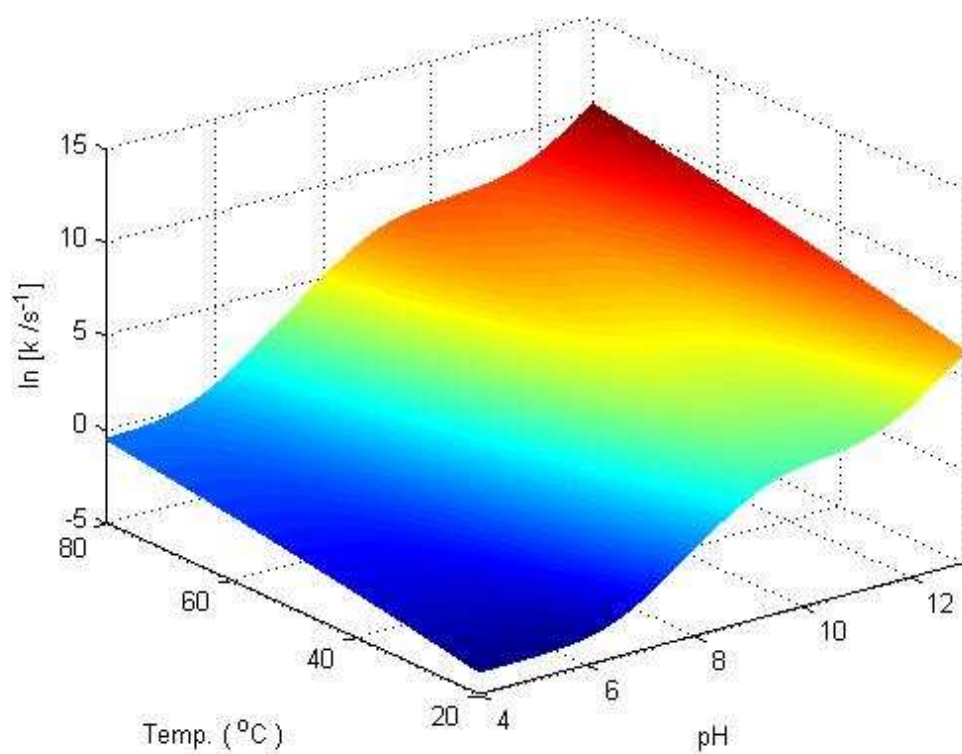


Figure S2: CO₂ hydration rate constant and mechanism contribution as a function of pH (4 – 13) and temperature (20 °C to 80 °C) with 0.65 M total added boric acid [top: total catalyzed hydration rate; bottom: contribution of B(OH)₄⁻ ion catalysis].

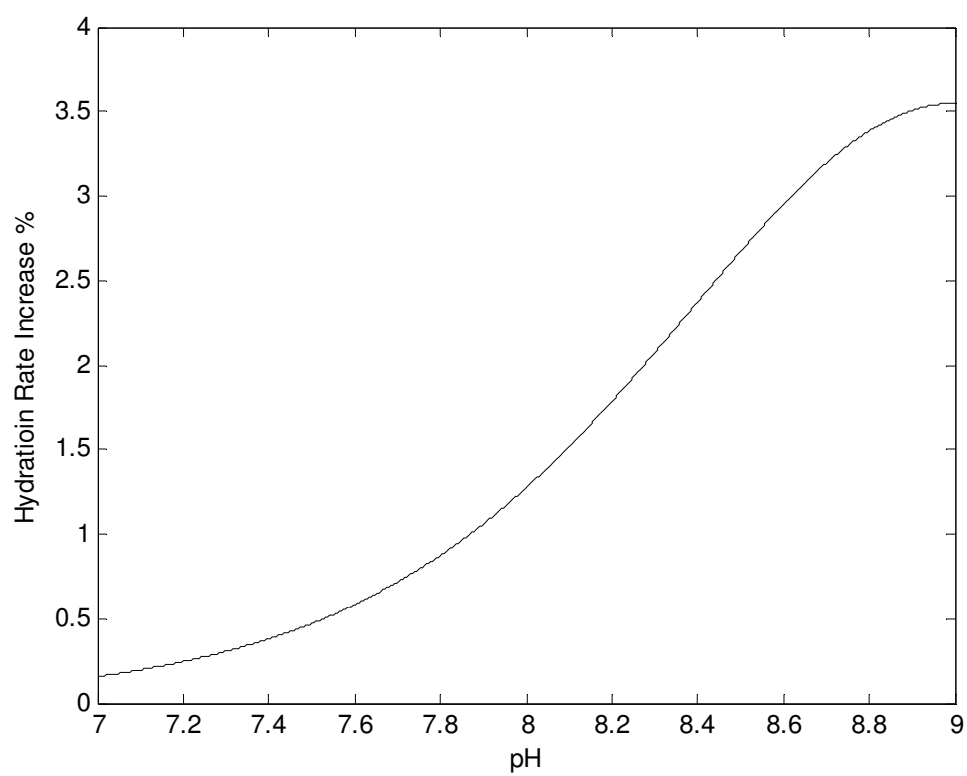


Figure S3: Increase in the CO₂ hydration rate by borate in seawater (0.4 mM boron, 15 °C).

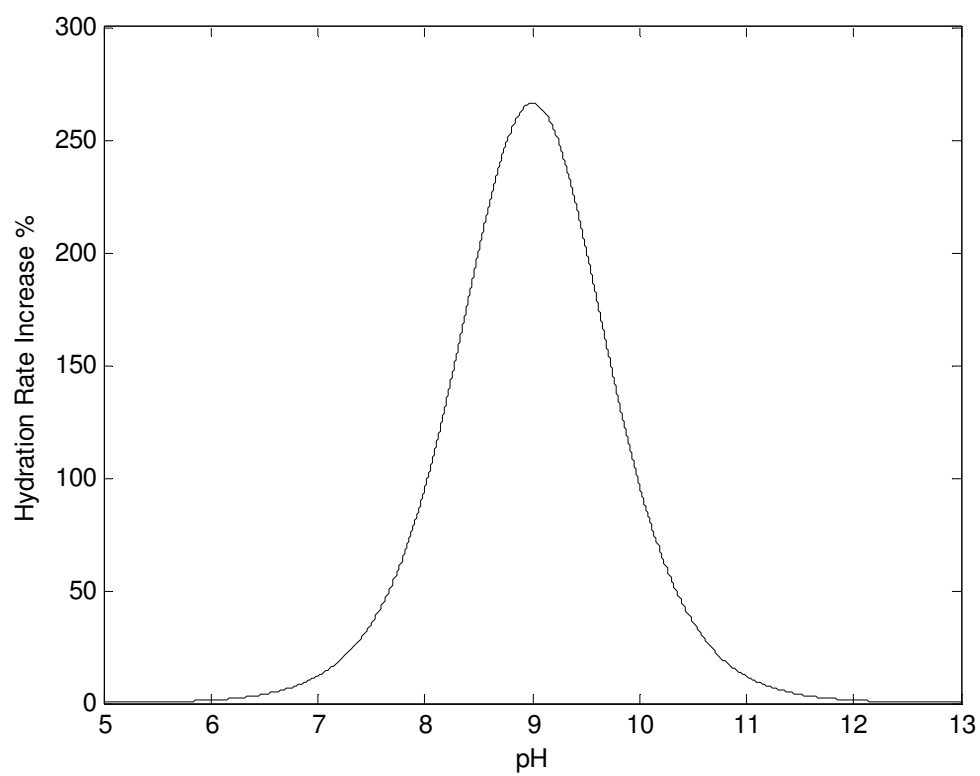


Figure S4: Increase in the CO₂ hydration rate by borate in Mono Lake (30 mM boron, 15 °C).

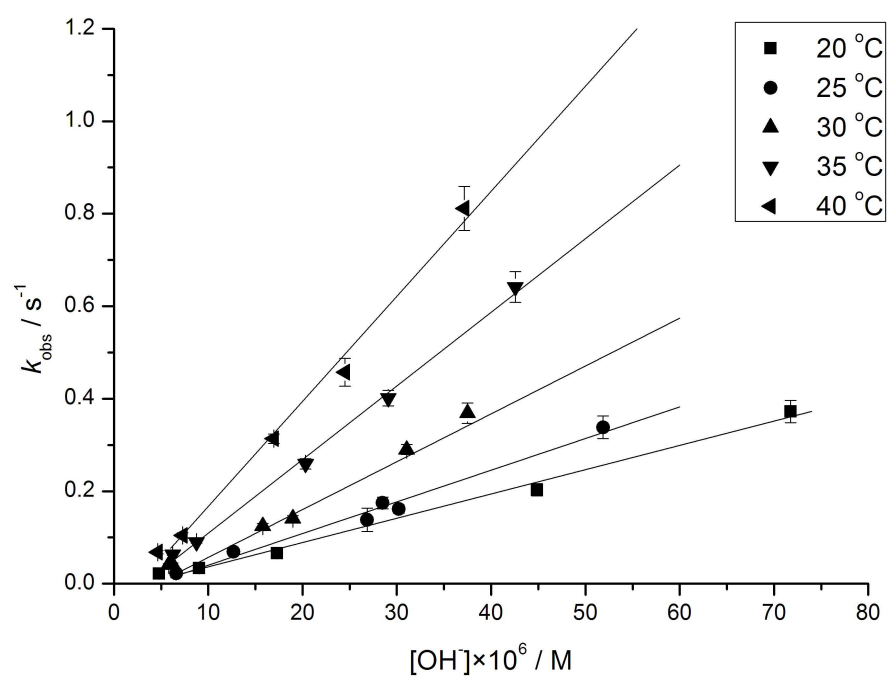


Figure S5: Uncatalyzed CO_2 hydration (k_{obs}) rate versus $[\text{OH}^-]$ at 20 °C to 40 °C.

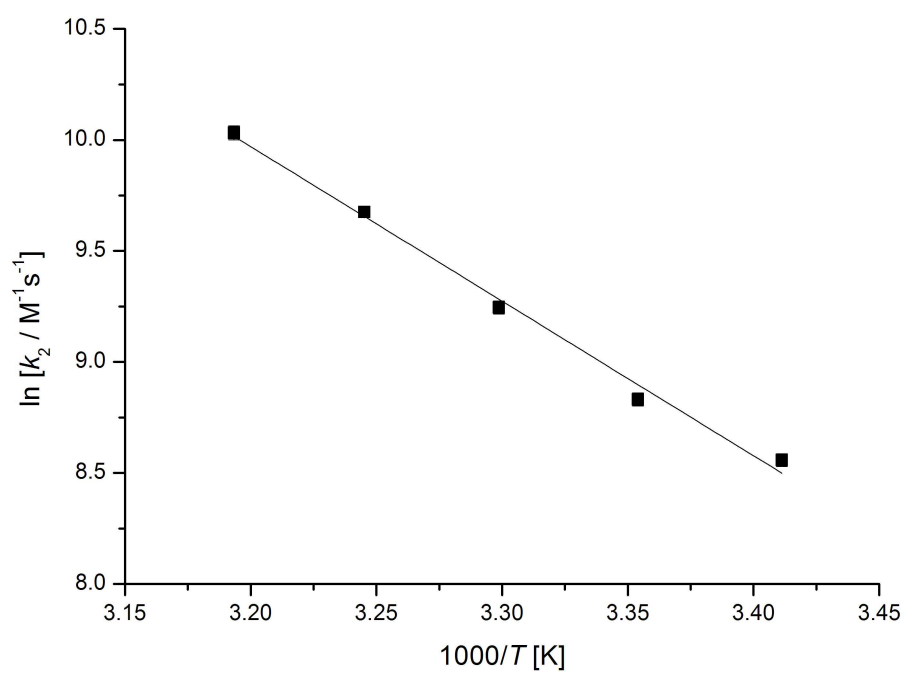


Figure S6: Arrhenius plot of k_2 versus $1000/T$.

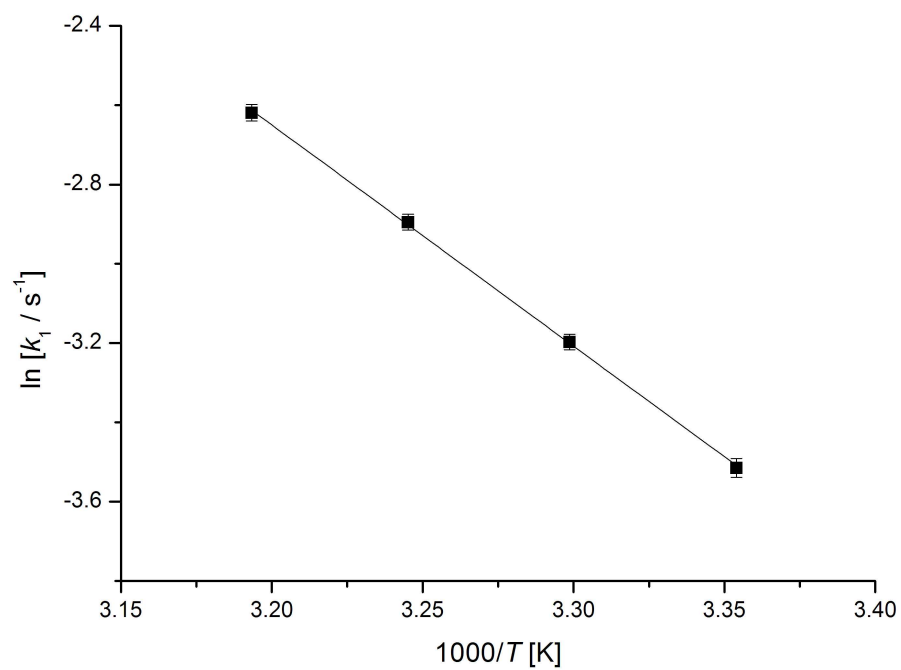


Figure S7: Arrhenius plot of $\ln k_1$ versus $1000/T$.