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Supplementary Information

for

Kinetics of the Oxidation of Isoniazid with Hypochlorite Ion

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Derivation of the absorbance for spectrophotometric titration:

Suppose that a general reaction with the following stoichiometry is investigated by spectrophotometric titration:

$$\mathrm{R}_1 + \mu\mathrm{R}_2 \rightarrow \sum \nu_i \mathrm{P}_i \qquad \text{(S1)}$$

The stoichiometric coefficient of R_1 is set 1, this can always be achieved by normalizing the equation (as a consequence, the rest of the coefficients, μ and ν_i are not necessarily integers). A titration is carried out by selecting a suitable volume of the solution (V_{ini}) of reagent R_1 with concentration c_{R1} and adding the titrant solution of R_2 with concentration c_{R2} gradually. The initial amount of substance for reagent A is $V_{\mathrm{ini}}c_{\mathrm{R1}}$ for the entire titration. After the addition of titrant solution with volume V, the amount of substance for reagent R_2 is given as Vc_{R2}. There are two cases:

1. If a relatively low amount of titrant R_2 has been added, reagent A remains in excess. The final concentration of R_2 is $[R_2] = 0$, the concentration of remaining R_1 is $[R_1] = (V_{\mathrm{ini}}c_{\mathrm{R1}} - Vc_{\mathrm{R2}}/\mu)/(V_{\mathrm{ini}} + V)$. The concentrations of the products are $[P_i] = \nu_i /\mu \times Vc_{\mathrm{R2}}/(V_{\mathrm{ini}} + V)$. According to Beer's law, each substance present can contribute to the absorbance. The molar absorptivity of R_1 is $\varepsilon_{\mathrm{R1}}$, the molar absorptivity R_2 is $\varepsilon_{\mathrm{R2}}$, the molar absorptivity of product P_i is $\varepsilon_{\mathrm{P}i}$. Therefore, the absorbance reading after the addition of titrant solution with volume V is given as:

$$A = \varepsilon_{\mathrm{R1}}[\mathrm{R}_1] + \varepsilon_{\mathrm{R2}}[\mathrm{R}_2] + \sum \varepsilon_{\mathrm{P}i}[\mathrm{P}_i] = \varepsilon_{\mathrm{R2}}\frac{V_{\mathrm{ini}}c_{\mathrm{R1}} - Vc_{\mathrm{R2}}/\mu}{V_{\mathrm{ini}} + V} + \sum \frac{\varepsilon_{\mathrm{P}i}\nu_i V c_{\mathrm{R2}}}{\mu(V_{\mathrm{ini}} + V)} \qquad \text{(S2)}$$

The dilution (η) was defined in the main text as $\eta = (V + V_{\mathrm{ini}})/V_{\mathrm{ini}}$. The molar ratio of the two reactants (ξ) is simply given as $\xi = Vc_{\mathrm{B}}/(V_{\mathrm{ini}}c_{\mathrm{A}})$. With these new quantities, Eq. S2 can be successively re-arranged as follows:

$$\frac{V_{\mathrm{ini}} + V}{V_{\mathrm{ini}}}A = \varepsilon_{\mathrm{R1}}c_{\mathrm{R1}} - \frac{\varepsilon_{\mathrm{R1}}Vc_{\mathrm{R2}}}{\mu V_{\mathrm{ini}}} + \sum \frac{\varepsilon_{\mathrm{P}i}\nu_i V c_{\mathrm{R2}}}{\mu V_{\mathrm{ini}}} \qquad \text{(S3)}$$

$$\eta A = \varepsilon_{\mathrm{R1}}c_{\mathrm{R1}} - \xi\frac{\varepsilon_{\mathrm{R1}}c_{\mathrm{R1}}}{\mu} + \frac{\xi c_{\mathrm{R1}}}{\mu}\sum \varepsilon_{\mathrm{P}i}\nu_i \qquad \text{(S4)}$$

$$\eta A = \varepsilon_{\mathrm{R1}}c_{\mathrm{R1}} + \xi\left(\frac{c_{\mathrm{R1}}}{\mu}\sum \varepsilon_{\mathrm{P}i}\nu_i - \frac{\varepsilon_{\mathrm{R1}}c_{\mathrm{R1}}}{\mu}\right) \qquad \text{(S5)}$$

Therefore, if ηA is plotted as a function of ξ, a straight line is expected with intercept $\varepsilon_{\mathrm{R1}}c_{\mathrm{R1}}$ and slope $\left(\frac{c_{\mathrm{R1}}}{\mu}\sum \varepsilon_{\mathrm{P}i}\nu_i - \frac{\varepsilon_{\mathrm{R1}}c_{\mathrm{R1}}}{\mu}\right)$.

2. If reagent R_2 has been added by excess, then R_1 is not present any more $[R_1] = 0$. The concentration of remaining R_2 is $[R_2] = (Vc_{\mathrm{R2}} - \mu V_{\mathrm{ini}}c_{\mathrm{R1}})/(V_{\mathrm{ini}} + V)$, whereas the concentrations of the products are $[P_i] = \nu_i V_{\mathrm{ini}} c_{\mathrm{R1}}/(V_{\mathrm{ini}} + V)$. The absorbance signal then equals to:

$$A = \varepsilon_{\mathrm{A}}[\mathrm{R}_1] + \varepsilon_{\mathrm{B}}[\mathrm{R}_2] + \sum \varepsilon_{\mathrm{P}i}[\mathrm{P}_i] = \varepsilon_{\mathrm{R2}} \frac{Vc_{\mathrm{R2}} - \mu V_{\mathrm{ini}} c_{\mathrm{R1}}}{V_{\mathrm{ini}} + V} + \sum \frac{\varepsilon_{\mathrm{P}i} v_i V_{\mathrm{ini}} c_{\mathrm{R1}}}{(V_{\mathrm{ini}} + V)} \tag{S6}$$

As in the previous case, this equation can be successively re-arranged as follows:

$$\frac{V_{\mathrm{ini}} + V}{V_{\mathrm{ini}}} A = \frac{\varepsilon_{\mathrm{R2}} V c_{\mathrm{R2}}}{V_{\mathrm{ini}}} - \mu \varepsilon_{\mathrm{R2}} c_{\mathrm{R1}} + c_{\mathrm{R1}} \sum \varepsilon_{\mathrm{P}i} v_i \tag{S7}$$

$$\eta A = \varepsilon_{\mathrm{R2}} c_{\mathrm{R1}} \xi + \left(c_{\mathrm{R1}} \sum \varepsilon_{\mathrm{P}i} v_i - \mu \varepsilon_{\mathrm{R2}} c_{\mathrm{R1}} \right) \tag{S8}$$

Again, if ηAbs is plotted as a function of ξ, a straight line is expected with intercept $\left(c_{\mathrm{R1}} \sum \varepsilon_{\mathrm{P}i} v_i - \mu \varepsilon_{\mathrm{R1}} c_{\mathrm{R2}} \right)$ and slope $\varepsilon_{\mathrm{R2}} c_{\mathrm{R1}}$.

Therefore, it has been established that the points in the plot will lie on either of the two straight lines depending on whether they have been measured at an excess of R_1 or R_2. The common point (intersection) of the straight lines is found at the value of ξ_{c} where the ηA values are equal.

$$\varepsilon_{\mathrm{R1}} c_{\mathrm{R1}} + \xi_{\mathrm{c}} \left(\frac{c_{\mathrm{R1}}}{\mu} \sum \varepsilon_{\mathrm{P}i} v_i - \frac{\varepsilon_{\mathrm{R1}} c_{\mathrm{R1}}}{\mu} \right) = \varepsilon_{\mathrm{R2}} c_{\mathrm{R1}} \xi_{\mathrm{c}} + \left(c_{\mathrm{R1}} \sum \varepsilon_{\mathrm{P}i} v_i - \mu \varepsilon_{\mathrm{R2}} c_{\mathrm{R1}} \right) \tag{S9}$$

This equation can be simplified by division with c_{R1} and then re-arranged to give:

$$\xi_{\mathrm{c}} \left(\frac{1}{\mu} \sum \varepsilon_{\mathrm{P}i} v_i - \frac{\varepsilon_{\mathrm{R1}}}{\mu} - \varepsilon_{\mathrm{R2}} \right) = \sum \varepsilon_{\mathrm{P}i} v_i - \mu \varepsilon_{\mathrm{R2}} - \varepsilon_{\mathrm{R1}} \tag{S10}$$

Then a simple division gives the ξ_{c} value where the intersection of the two straight lines occurs:

$$\xi_{\mathrm{c}} = \mu \tag{S11}$$

Therefore, the intersection of the two straight lines gives the stoichiometric coefficient of reagent R_2. A plot based on this method is given in Eq. 2 of the main article.

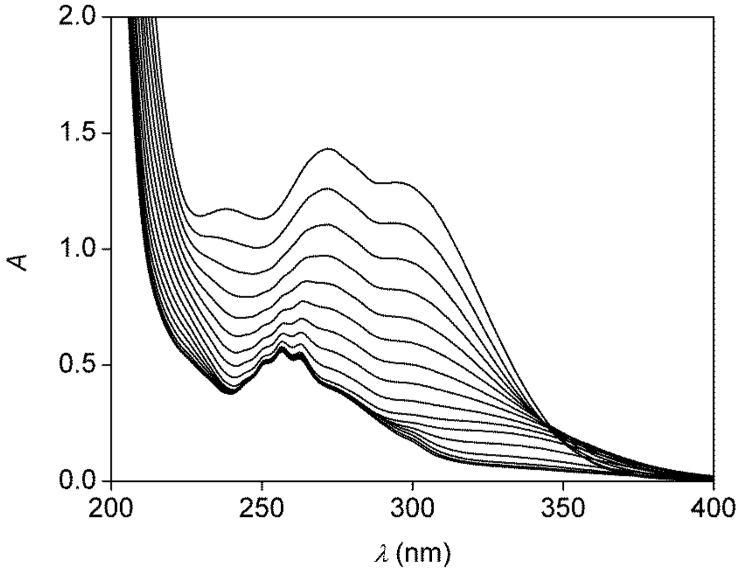

Fig. S1 Stoichiometry determination in the oxidation of isoniazid with hypochlorite ion by spectrophotometric titration. Initial sample: [INH] = 0.30 mM. Titrant concentration [OCl^-] = 1.0 mM. [OH^-] = 10 mM, T = 25 °C, *l* = 1.00 cm. Titrant increment volume: 100 μl. The letter *A* represents absorbance.

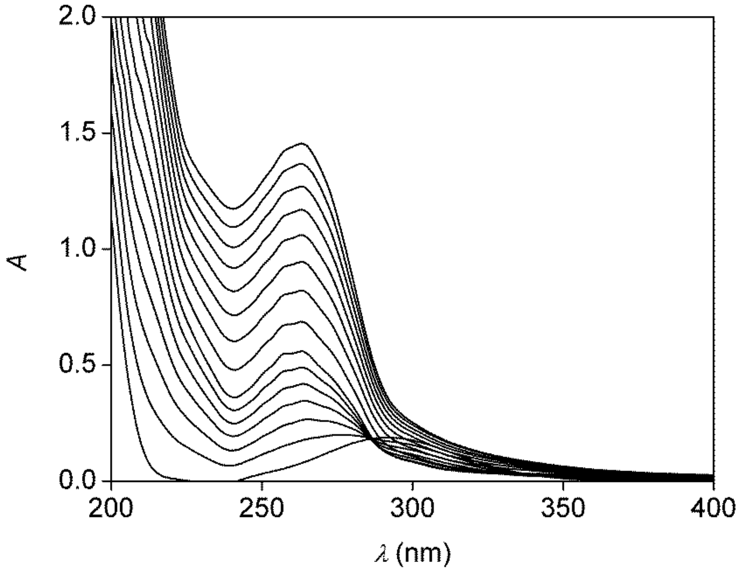

Fig. S2 Stoichiometry determination in the oxidation of isoniazid with hypochlorite ion by spectrophotometric titration. Initial sample: [OCl^-] = 0.64 mM. Titrant concentration: [INH] = 1.00 mM. [OH^-] = 10 mM, T = 25 °C, *l* = 1.00 cm. Titrant increment volume: 100 μl. The letter *A* represents absorbance.

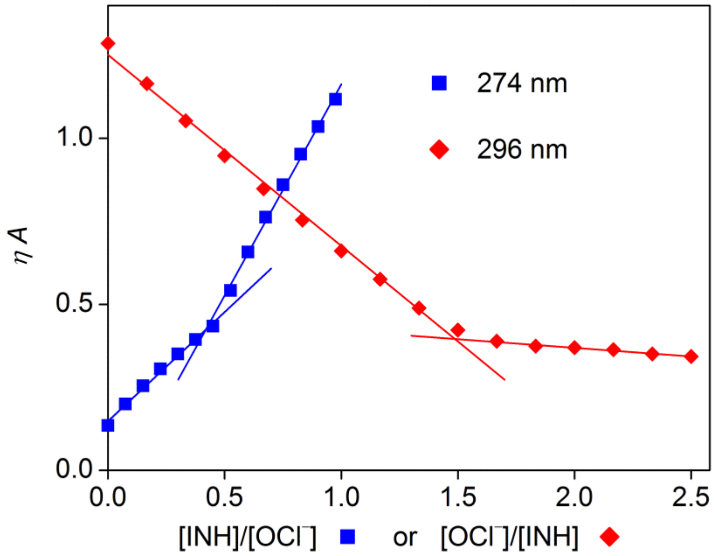

Fig. S3 Stoichiometry determination in the oxidation of isoniazid with hypochlorite ion by spectrophotometric titration. $[\mathrm{OH}^-]_\mathrm{T}$ = 10.0 mM, *T* = 25 °C, *l* = 1.00 cm. For red points: Initial sample: $[\mathrm{OCl}^-]$ = 0.64 mM. Titrant concentration: [INH] = 1.00 mM. $[\mathrm{OH}^-]$ = 10 mM, T = 25 °C, *l* = 1.00 cm. Titrant increment volume: 100 µl. For blue points: Initial sample: [INH] = 0.30 mM. Titrant concentration $[\mathrm{OCl}^-]$ = 1.0 mM. $[\mathrm{OH}^-]$ = 10 mM, T = 25 °C, *l* = 1.00 cm. Titrant increment volume: 100 µl.

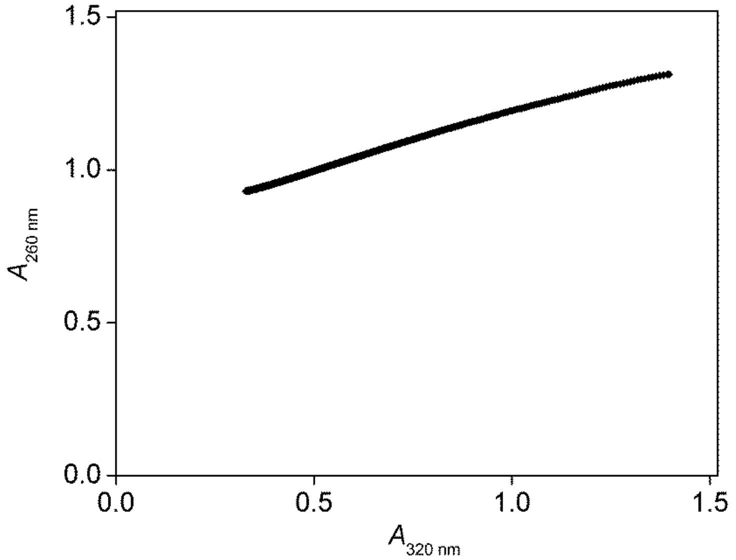

Fig. S4 Absorbance correlation plot between data measured at 260 and 320 nm from the spectral stopped-flow experiments shown in Fig. 1 during the oxidation of isoniazid with hypochlorite ion. [INH] = 0.50 mM, $[\mathrm{OCl}^-]$ = 1.0 mM, $[\mathrm{OH}^-]_\mathrm{T}$ = 10.0 mM, *T* = 25.0 °C, *l* = 1.00 cm; *t* = 0.01, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0 s. The letter *A* represents absorbance.

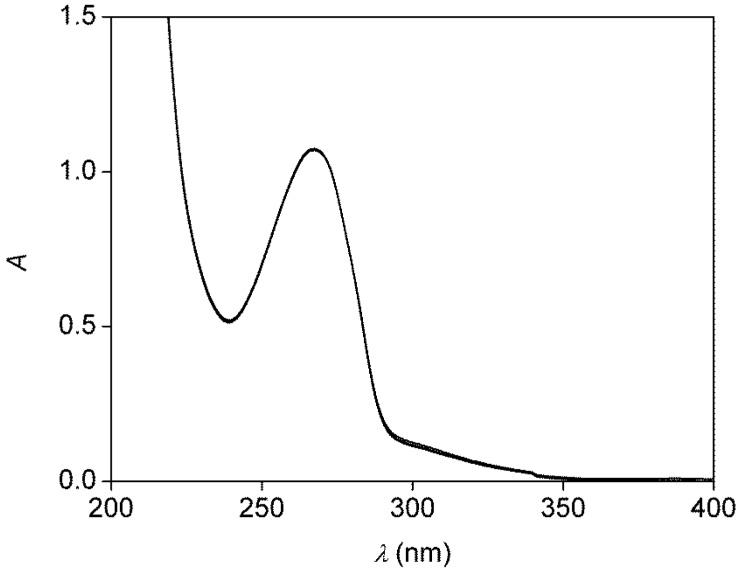

Fig. S5 Spectral observations in the oxidation of isonicotinic acid with hypochlorite ion. [INA] = 0.50 mM, [OCl^-] = 0.50 mM, [OH^-]$_T$ = 10.0 mM, T = 25 °C, l = 1.00 cm, total experiment time: 50 minutes. The letter A represents absorbance.

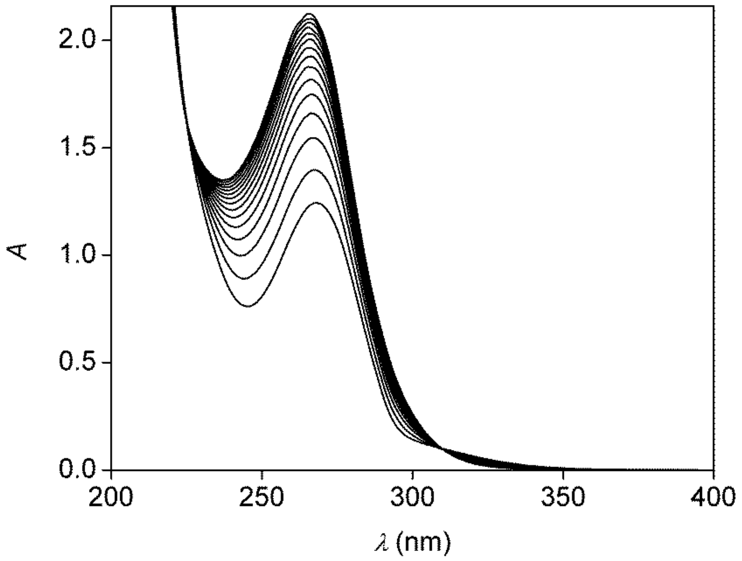

Fig. S6 Spectral observations in the oxidation of isonicotinic amide with hypochlorite ion. [INM] = 0.50 mM, [OCl^-] = 0.50 mM, [OH^-]$_T$ = 10.0 mM, T = 25 °C, l = 1.00 cm, total experiment time: 50 minutes. Consecutive spectra are recorded in every 3.5 min. The letter A represents absorbance.

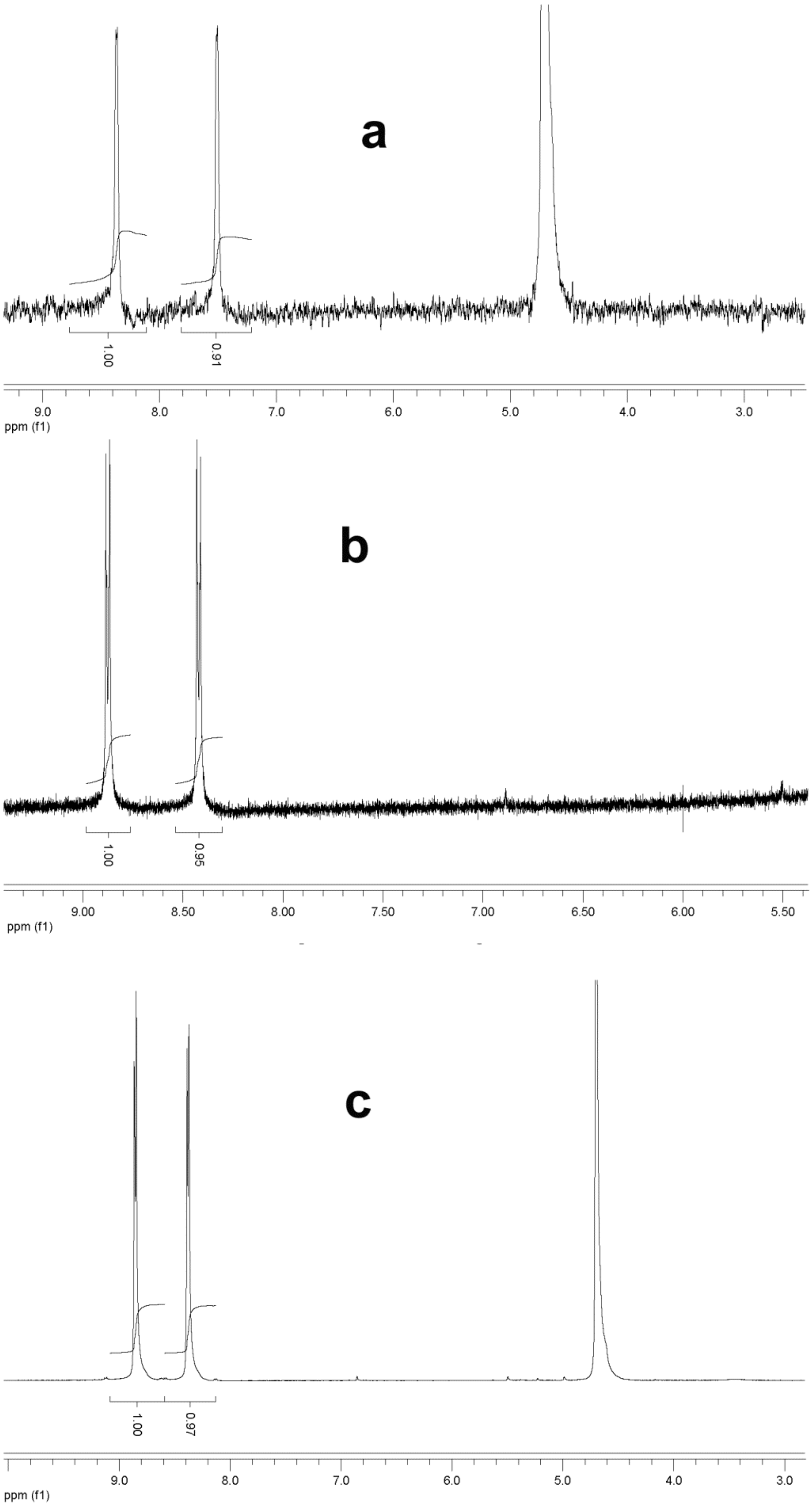

Fig. S7 NMR spectra: isoniazid in basic medium (**a**), isoniazid with excess NaOCl after the completion of the reaction (**b**), spectrum b + isonicotinic acid (**c**)

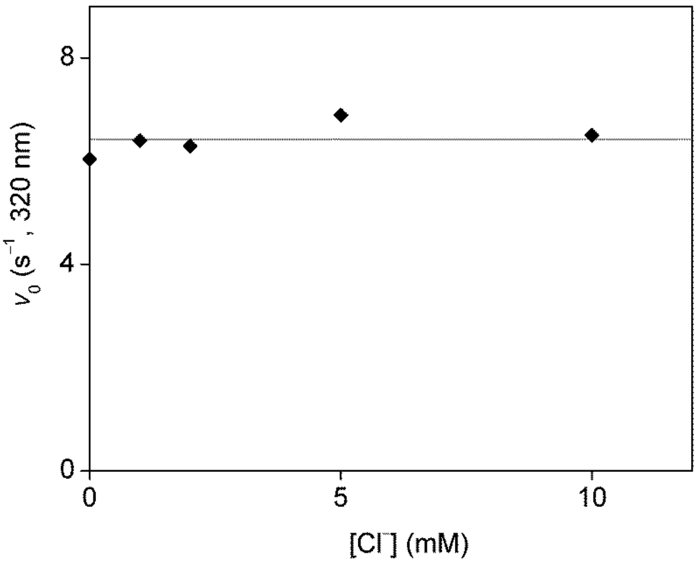

Fig. S8 Initial rate as a function of chloride concentration in th reaction of isoniazid with hypochlorite ion. [INH] = 0.50 mM, [OCl$^-$] = 1.00 mM, [OH$^-$]$_T$ = 10.0 mM, T = 25.0 °C, l = 1.00 cm.

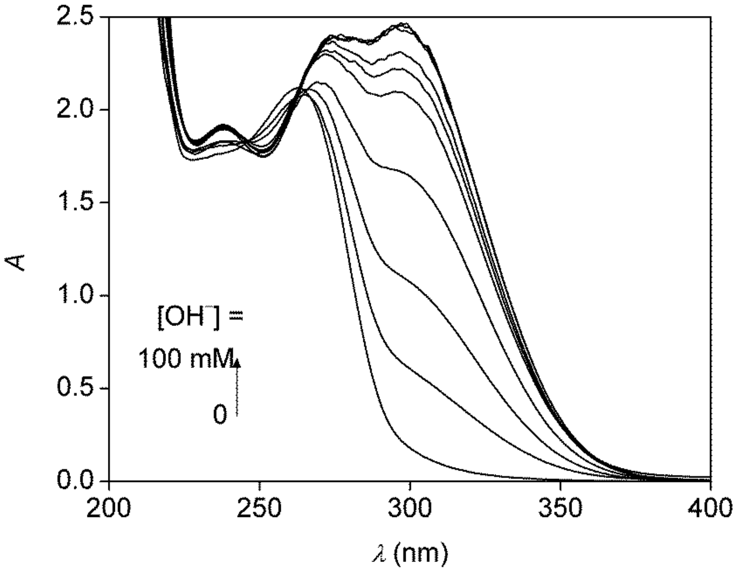

Fig. S9 UV-vis spectrum of isoniazid at different hydroxide ion concentrations. [INH] = 0.50 mM, T = 25 °C, l = 1.00 cm. The letter A represents absorbance.

Table S1 Results from the initial rate calculations

[INH]	$[\mathrm{OCl}^-]_0$	$[\mathrm{OH}^-]_0$	v_0/A_0	k
mM	mM	mM	s^{-1}	$\mathrm{M}^{-1}\mathrm{s}^{-1}$
0.5	1	0.5	19.53363	25284.76
0.5	1	1.5	13.8846	26148.39
0.5	1	4.5	8.63694	31523.07
0.5	1	9.5	3.77325	24880.87
0.5	1	24.5	1.80293	27813.21
0.5	1	49.5	1.17333	35373.27
0.5	1	74.5	0.77231	34652.66
0.5	1	99.5	0.63123	37614.96
0.5	1	109.5	0.64663	42340.29
0.5	1	124.5	0.53473	39736.34
0.5	0.1	49.5	0.102485	30896.95
0.5	0.17	49.5	0.199074	35303.69
0.5	0.19	49.5	0.223895	35525.93
0.5	0.22	49.5	0.264271	36214.46
0.5	0.5	49.5	0.623053	37567.31
0.5	0.75	49.5	1.033162	41530.02
0.5	0.9	49.5	1.210784	40558.24
0.5	1	49.5	1.299567	39179.03
0.5	1.2	49.5	1.604495	40309.94
0.5	0.1	9.5	0.284811	18780.48
0.5	0.25	9.5	0.687023	18120.96
0.5	0.5	9.5	1.623896	21415.99
0.5	0.75	9.5	2.74202	24107.9
0.5	0.85	9.5	3.059353	23733.44
0.5	1	9.5	3.826806	25234.01
0.5	1.15	9.5	4.122397	23637.52
0.5	1.25	9.5	4.746321	25037.85
0.5	1.3	9.5	4.611849	23392.77
0.5	1.5	9.5	5.435251	23893.42
0.01	1	9.99	1.474364	10147.38
0.04	1	9.96	1.455027	9988.591
0.1	1	9.9	2.015355	13763.97
0.25	1	9.75	1.8715	12616.2
0.4	1	9.6	1.804491	12005.1
0.5	1	9.5	1.779371	11733.2
0.05	1	9.95	4.162892	28553.25
0.1	1	9.9	4.211765	28764.47
0.25	1	9.75	3.938888	26552.93
0.4	1	9.6	3.792327	25229.97
0.5	1	9.5	3.585647	23643.81
			average	34061.69
			standard deviation	7206.428

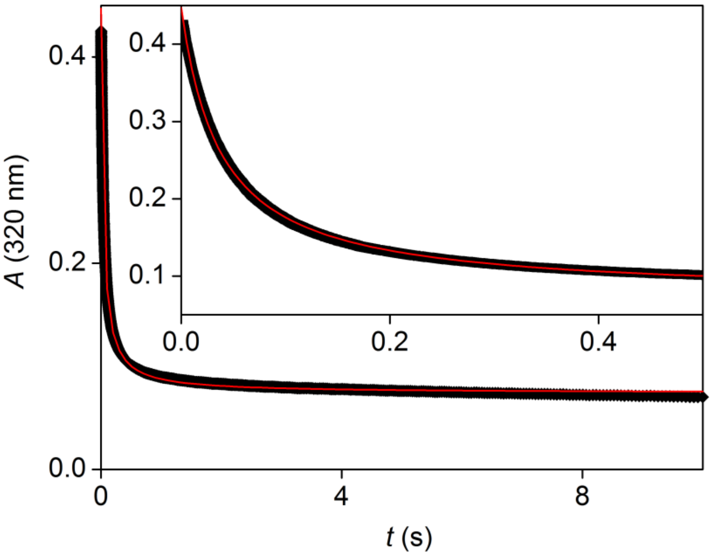

Fig. S10 Example of fitting experimental data to Eq. 17. [INH] = 0.50 mM, [OCl$^-$] = 1.00 mM, $[OH^-]_T$ = 10.0 mM, T = 25.0 °C, l = 1.00 cm. The letter A represents absorbance.

Table S2 Parameters determined from the numerical fitting using Eq. 19

$[INH]_0$	$[OCl^-]_0$	$[OH^-]_0$	P1	stdev	P2	stdev	P3	stdev	P4	stdev
mM	mM	mM	dimensionless		s^{-1}	s^{-1}	dimensionless		dimensionless	
0.50	1.00	50	0.077	0.002	0.426	0.009	0.945	0.001	0.2558	0.0002
0.50	1.25	50	0.04727	0.002	0.35	0.01	0.97	0.001	0.265	0.0003
0.50	1.15	50	0.06775	0.002	0.42	0.01	0.953	0.001	0.255	0.0003
0.50	0.85	50	0.05285	0.0007	0.228	0.003	0.9574	0.0005	0.257	0.0001
0.50	1.50	50	0.44	0.008	3.2	0.04	0.684	0.007	0.2975	0.0003
0.50	1.30	50	0.337	0.005	2.16	0.02	0.746	0.004	0.28	0.0002
0.50	0.75	50	0.0408	0.0001	0.1502	0.0005	0.9682	0.0001	0.31837	0.00004
0.85	1.00	50	0.3122	0.0009	1.554	0.003	0.771	0.001	1.104	0.001
0.75	1.00	50	0.1532	0.0003	0.633	0.001	0.896	0.0002	0.6643	0.0001
0.60	1.00	50	0.0576	0.0006	0.233	0.002	0.9618	0.0004	0.361	0.0001
0.50	1.00	50	0.071	0.001	0.34	0.005	0.9493	0.0009	0.2847	0.0002
0.40	1.00	50	0.079	0.002	0.52	0.02	0.934	0.002	0.209	0.0002
0.25	1.00	50	0.044	0.002	0.55	0.02	0.945	0.002	0.1453	0.0002
0.10	1.00	50	0.0083	0.0006	0.27	0.02	0.977	0.002	0.1391	0.0001