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PAPER

The effects of ligand decomposition on the pseudo first-order profile of a ligand substitution reaction: a "silent killer" in the background†

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The effect of ligand decomposition on the apparent rate constant for a ligand substitution reaction where the reverse reaction is negligible is examined. When the ligand concentration remains in excess for the entirety of the reaction, the data can be fitted to a modified form of the familiar pseudo first-order rate expression. However rapid ligand decomposition may result in the ligand concentration not remaining in excess for the duration of the reaction. Data simulations show that in this latter case the data still fit remarkably well to a first-order rate equation. Plots of the apparent rate constant versus the initial ligand concentration are also linear. However the reaction will not proceed to completion despite the reverse reaction being insignificant. Furthermore intercepts in the plots of apparent rate constant versus the initial ligand concentration are obtained, in addition to misleading values of the second-order rate constants for the ligand substitution reaction. We show that kinetic coupling between the decomposition of the ligand and its complex formation reaction may easily lead to falsified conclusions. Thus, the first-order appearance of a kinetic trace does not guarantee real first-order behavior.

Introduction

Recently we carried out detailed kinetic studies on the reaction between the vitamin B_{12} derivative aquacobalamin/hydroxocobalamin (H_2OCbl^+/HOCbl) and secondary amine NONOates, R_2N-NONOates.[1] It is well established that R_2N-NONOates spontaneously decompose cleanly in a first-order process *via* a mechanism involving protonation of the NONOate itself.[2–4] Using a combination of UV-vis and NMR spectroscopy, ensuring the initial ligand concentration is in a large excess and taking into account ligand decomposition when appropriate, we showed that H_2OCbl^+/HOCbl reacts directly with R_2N-NONOates to form nitrosylcobalamin (NOCbl) and the corresponding nitrosylamine ($R_2NH/R_2NH_2^+$). The general scheme is shown in Scheme 1 for a ligand substitution reaction in which the reverse reaction is negligible compared with the forward reaction, where A is H_2OCbl^+/HOCbl and L = R_2N-NONOate. The corresponding rate expressions are as follows.

$$A + L \xrightarrow{k_1} \text{products}$$
$$L \xrightarrow{k_L} \text{decomposition products}$$

Scheme 1 General scheme for ligand substitution.

$$\frac{d[A]}{dt} = -k_1[A][L] \quad (1)$$

$$\frac{d[L]}{dt} = -k_1[A][L] - k_L[L] \quad (2)$$

Assuming that the concentration of the ligand remains in excess compared with the concentration of A during the measurement of kinetic data, the following equation applies to this system:[1]

$$\Delta Abs = Abs_{obs} - Abs_F = (Abs_0 - Abs_F)e^{\frac{k_1[L]_0}{k_L}(e^{-k_L t}-1)} \quad (3)$$

ΔAbs, Abs_{obs}, Abs_0 and Abs_F are the change in absorbance and the observed, initial and final absorbances, respectively, k_1 is the second-order rate constant for the ligand substitution reaction, k_L is the rate constant for spontaneous ligand decomposition, t is time and $[L]_0$ is the initial ligand concentration.

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† Electronic supplementary information (ESI) available: Derivation of eqn (4); plots of [A] *versus* time for $k_1[L]_0/k_L$ = 1, 0.5 and 5.0; expanded view of Fig. 5c. See DOI: 10.1039/c2nj40055c

In our experiments, eqn (3) was valid for all experimental conditions and k_L was much smaller than $k_1[L]_0$ ($k_1[L]_0$ = the pseudo first-order rate constant for the ligand substitution reaction). However it was of interest to us to determine what happens if the rate of ligand decomposition is similar to or even faster than the observed rate of the reaction between A and L under initial pseudo-first order conditions ($[L]_0$ is at least 10 times in excess). If one was, for example, unaware that ligand decomposition was occurring and had neglected to fully characterize the products of the reaction, would its presence be reflected in the goodness of fit of the experimental data to a first-order rate expression? Furthermore, what is the effect of ligand decomposition on the rate constant when the data are fitted to a first-order rate equation, not taking into account ligand decomposition? We now present numerical simulations which address these questions.

Results and discussion

An expression was derived for ligand decomposition competing with ligand substitution, Scheme 1, when the ligand concentration is not necessarily in excess for the entirety of the data collection. The value of the resulting integral,

$$\int \frac{d[A]}{k_1[A]^2 + k_L[A]\ln[A] + k_1[A]c_1} = -t + c_2 \qquad (4)$$

cannot be determined analytically (solved by quadrature; c_1 and c_2 are constants). The derivation of eqn (4) is given in the Supporting Information.† Numerical simulations were therefore undertaken using Mathematica. In order to check that our simulation program was performing correctly, we first tested it using experimental data. Fig. 1 gives a plot of absorbance change *versus* time for the reaction between H_2OCbl^+/HOCbl (5.00×10^{-5} M) and MAHMA-NONOate (2.50×10^{-3} M) at pH 9.80. The first-order rate constant for ligand decomposition under the same conditions, k_L, was determined in an independent experiment to be 2.94×10^{-3} min^{-1}. Given that the ligand concentration remains in excess during data collection, the experimental data can be fitted to eqn (3), fixing $k_L = 2.94 \times 10^{-3}$ min^{-1} and $[L]_0$ ([MAMHA-NONOate]) = 2.50×10^{-3} M. The best fit of the data to eqn (3) is superimposed on the data, giving $k_1 = 2.49 \pm 0.09$ M^{-1} min^{-1}.

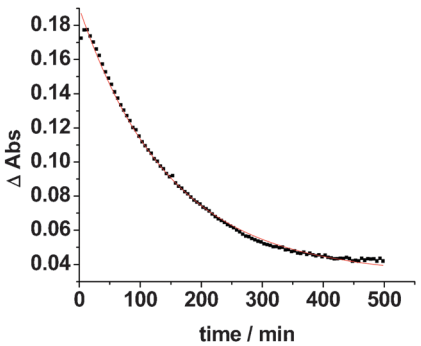

Fig. 1 Plot of change in absorbance *versus* time for the reaction between H_2OCbl^+/HOCbl (5.00×10^{-5} M) and MAHMA-NONOate (2.50×10^{-3} M) (pH 9.80, 25.0 °C, I = 1.0 M ($NaCF_3SO_3$)). The best fit of experimental data to eqn (3) superimposed on the data fixing $[L]_0 = 2.50 \times 10^{-3}$ M and $k_L = 2.94 \times 10^{-3}$ min^{-1} gives $k_1 = 2.49 \pm 0.09$ M^{-1} min^{-1}.

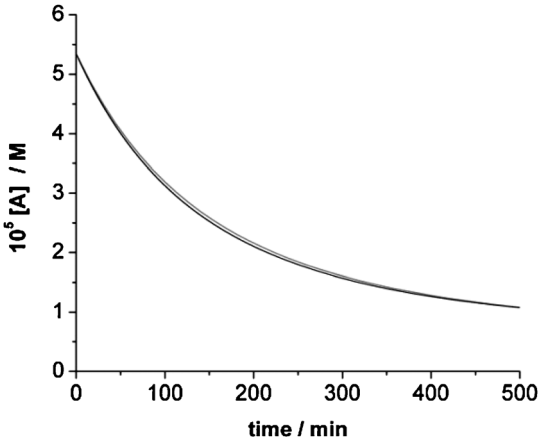

Fig. 2 Plot of concentration of A (=H_2OCbl^+/HOCbl) *versus* time for the reaction shown in Fig. 1. The best fit of the data in Fig. 1 to eqtn (3) (grey curve) is superimposed upon data simulated for $[A]_o = 5.00 \times 10^{-5}$ M, $[L]_0$ ($[MAHMA\text{-}NONOate]_0$) = 2.50×10^{-3} M, $dt = 1 \times 10^{-3}$ min, $k_L = 2.94 \times 10^{-3}$ min^{-1} and $k_1 = 2.49$ M^{-1} min^{-1} ($k_1[L]_0 = 6.22 \times 10^{-3}$ min^{-1}).

The data was subsequently simulated using Mathematica for $k_1 = 2.49$ M^{-1} min^{-1}, $k_L = 2.94 \times 10^{-3}$ min^{-1}, $[L]_0 = 2.50 \times 10^{-3}$ M, $[A]_0$ (=$[H_2OCbl^+/HOCbl]_0$) = 5.00×10^{-5} M and dt = 0.001 min, using the iterative equations (i = 0, 1, 2, 3...):

$$[A]_{(i+1)} = [A]_i - k_1 \cdot [A]_i \cdot [L]_i \cdot dt \qquad (5)$$

$$[L]_{(i+1)} = [L]_i - (k_1 \cdot [A]_i \cdot [L]_i + k_L \cdot [L]_i) \cdot dt \qquad (6)$$

The number of data points generated was 1.5×10^6 and the $[A]_{(i+1)}$ data generated plotted *versus* time. The resulting simulated data is shown in Fig. 2, superimposed upon the best fit of the data to eqn (3) taken from Fig. 1 (note that the data and fit are now expressed in terms of the concentration of A *versus* time). It can be clearly seen from this figure that there is excellent agreement between the best fit of the experimental data to eqn (3) and the simulated data using the same parameters.

We then set about simulating data for $k_1[L]_0/k_L$ in the range 1000–0.05, where the ligand is in ten times excess compared with the complex (the lowest value typically used by experimentalists to obtain pseudo first-order behavior); that is, from conditions where ligand decomposition is negligible compared to the reaction between A and L through to conditions where ligand decomposition is considerably faster than the reaction between A and L. Selected simulated data are shown in Fig. 3, with $[A]_0 = 5.00 \times 10^{-5}$ M, $[L]_0 = 5.00 \times 10^{-4}$ M, $k_1 = 12.4$ M^{-1} min^{-1}, $k_1[L]_0/k_L$ = 5.0–0.5 ($k_L = 6.22 \times 10^{-6}$–0.124 min^{-1}) and dt = 0.001 min. It is clear from Fig. 3 that for decreasing $k_1[L]_0/k_L$ values (increasing relative rate of ligand decomposition), the final state of the reaction is reached more rapidly. In addition, the reaction does not go to completion ($[A]_F \neq 0$) when ligand decomposition is important. For example, when $k_1[L]_0/k_L = 1.0$, [A] is $\sim 2 \times 10^{-5}$ M after 1500 min, (*i.e.*, the reaction proceeds ~60% towards completion). From these simulation results it is clear that if ligand decomposition is important, even though the reverse rate

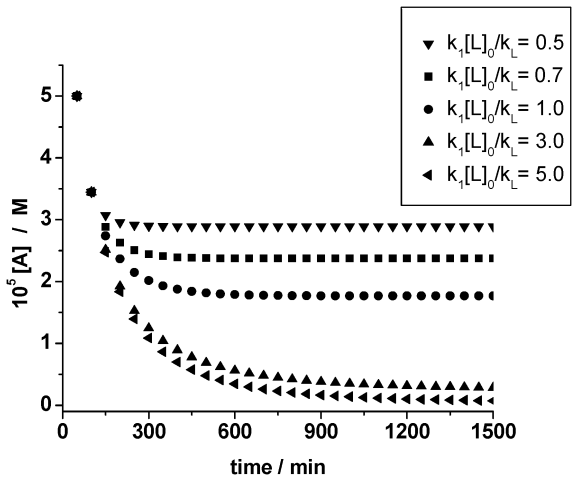

Fig. 3 Simulated data showing the concentration of A *versus* time for the reaction given in Scheme 1 at various $k_1[L]_0/k_L$ ratios. The following parameters were fixed in the simulations: $[L]_0 = 5.00 \times 10^{-4}$ M, $[A]_0 = 5.00 \times 10^{-5}$ M, $dt = 1 \times 10^{-3}$ min, and $k_1 = 12.4$ M^{-1} min^{-1} ($k_1[L]_0 = 6.22 \times 10^{-3}$ min^{-1}). Only selected data points are shown for clarity.

constant is negligible (the formation constant is large), the reaction will not proceed to completion even with 10 times excess ligand.

In the absence of ligand decomposition and with excess ligand concentrations ($\geq 10[A]_0$), plots of [A] *versus* time fit well to a first-order rate equation. It was therefore of interest to determine what effect ligand decomposition has on the fit of the data to a first-order rate equation. Fig. 4 gives fits of the data shown in Fig. 3 to a first-order rate equation (data fitted for $\sim$5 half lives). Individual plots for $k_1[L]_0/k_L = 0.5$, 1.0 and 5.0 are given in Fig. S1–S3 in the Supporting Information.† The corresponding rate constants, k_{1st}, calculated by fitting the

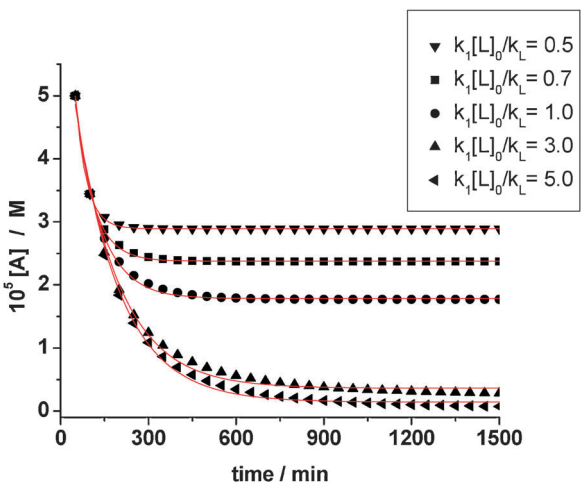

Fig. 4 Best fits of the simulated data at a range of $k_1[L]_0/k_L$ values assuming that the reaction fits a first-order equation (that is, not taking into account ligand decomposition). The simulated data has been fitted for the first 5 half lives of the reaction. The following parameters were fixed in the simulations: $[L]_0 = 5.00 \times 10^{-4}$ M, $[A]_0 = 5.00 \times 10^{-5}$ M, $dt = 1 \times 10^{-3}$ min and $k_1 = 12.4$ M^{-1} min^{-1}. Selected data points are shown for clarity. There was no weighting of the data in the fits.

Table 1 Rate constants, k_{1st}, obtained by fitting the data to a simple first-order rate equation (that is, not taking into account ligand decomposition) and the corresponding R^2 values for data simulated for a range of $k_1[L]_0/k_L$ ratios. The simulated data have been fitted for the first 5 half lives of the reaction. The following parameters were fixed in the simulations: $[L]_0 = 5.00 \times 10^{-4}$ M, $[A]_0 = 5.00 \times 10^{-5}$ M, $dt = 1 \times 10^{-3}$ min and $k_1 = 12.4$ M^{-1} min^{-1}

10^3 k_L (min^{-1})	$k_1[L]_0/k_L$	10^3 k_{1st} (min^{-1})	R^2	Percentage of Reaction Completed
124.4	0.05	126.4	1.0000	4.8
62.20	0.1	64.20	0.99998	9.4
20.73	0.3	22.73	0.99986	25.3
12.44	0.5	14.46	0.99962	38.2
8.89	0.7	10.94	0.99928	48.6
6.220	1.0	8.363	0.99865	60.9
2.073	3.0	5.216	0.99644	92.6
1.244	5.0	5.187	0.99747	97.8
0.0622	100	5.849	0.99988	100.0
0.00622	1000	5.894	0.99993	100.0

data to a first-order rate expression, and the R^2 values for these fits are summarized in Table 1, in addition to data for other values of $k_1[L]_0/k_L$. Importantly, the data fit extremely well to a first-order rate equation ($R^2 \geq 0.996$) *regardless of whether ligand decomposition is significant or not*. As expected, when the rate constant for ligand decomposition is much smaller than $k_1[L]_0$ ($k_1[L]_0/k_L \geq 100$), the fit of the data to a first-order rate expression is very good ($R^2 \geq 0.99988$) and the reaction proceeds to completion. While one might initially expect the value of k_{1st} to be equal to $k_1[L]_0$ ($= 6.22 \times 10^{-3}$ min^{-1}) at high values of $k_1[L]_0/k_L$ (that is, where ligand decomposition is negligible), the limiting value of $k_{1st} = 5.89 \times 10^{-3}$ min^{-1} is expected, given that the ligand concentration is only 10 times in excess, and therefore the free ligand concentration decreases from 5.00×10^{-4} M ($k_1[L]_0 = 6.22 \times 10^{-3}$ min^{-1}) to 4.50×10^{-4} M ($k_1[L]_0 = 5.56 \times 10^{-3}$ min^{-1}) during the reaction, 5.89×10^{-3} min^{-1} being the average of these two values. (Note that a simulation with $[L]_0$ 100 times in excess with other parameters remaining the same gave $k_1[L]_0 = 6.19 \times 10^{-3}$ min^{-1}.) The fit to a first-order rate equation also improves at low $k_1[L]_0/k_L$; however the value of k_{1st} is significantly larger than that of the reaction (*e.g.* $k_{1st} = 0.1264$ min^{-1} *versus* $k_1[L]_0 = 5.89 \times 10^{-3}$ min^{-1} at $k_1[L]_0/k_L = 0.05$). Hence under these latter conditions the reaction rate appears over 20 times faster than the ligand substitution reaction itself due to ligand decomposition.

Typically experimentalists carry out kinetic experiments with the ligand in excess (5–100 times or higher) and plot the data in the form of k_{1st} *versus* initial ligand concentration plots. We were therefore also interested in comparing the slopes, intercepts and goodness of fit (R^2) of these plots under three conditions—when the rate of ligand decomposition is much smaller than the rate of the reaction between A and L ($k_1[L]_0 \geq k_L$), the rate of ligand decomposition is larger than the rate of the reaction between A and L ($k_1[L]_0 \leq k_L$), and when the $k_1[L]_0 : k_L$ ratio changes from less than 1 to greater than 1 as the ligand concentration increases. Simulated data was therefore obtained for each of these conditions and the resulting plots of k_{1st} (assuming the data fit a first-order rate equation) *versus* initial ligand concentration are given in Fig. 5. Importantly, once again the simulated data at each

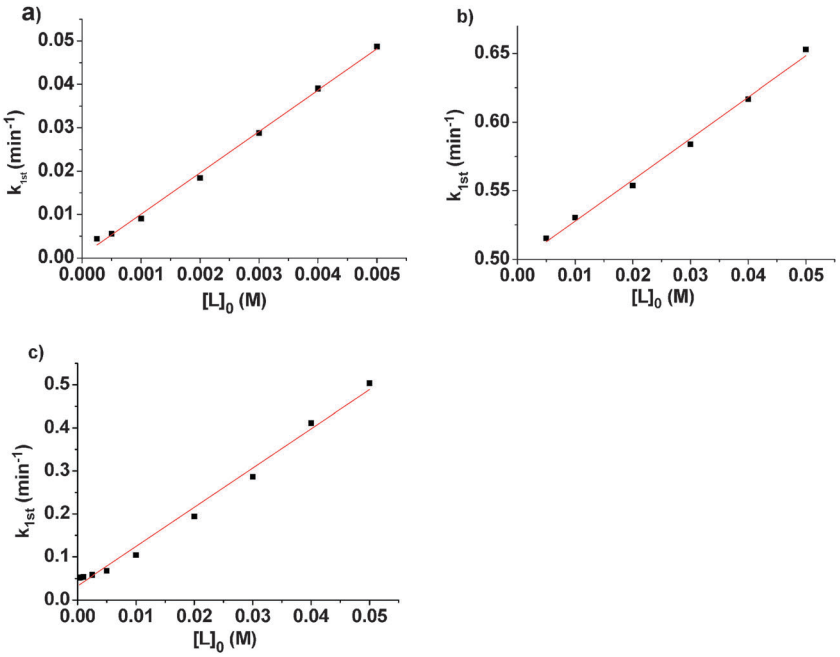

Fig. 5 Simulated plots of the first-order rate constant, k_{1st}, *versus* the initial ligand concentration for (a) $k_L = 3 \times 10^{-3}$ min^{-1}, $[L]_0 = 2.5 \times 10^{-4}$–$5 \times 10^{-3}$ M, (b) $k_L = 0.5$ min^{-1}, $[L]_0 = 5 \times 10^{-3}$–5×10^{-2} M, and (c) $k_L = 5 \times 10^{-2}$ min^{-1}, $[L]_0 = 2.5 \times 10^{-4}$–$5 \times 10^{-2}$ M. The parameters $k_1 = 10$ M^{-1} min^{-1} and $[A]_0 = 5 \times 10^{-5}$ M were used in all three simulations. Fitting the data to a straight line gives slopes 9.53 ± 0.23 (a), 3.02 ± 0.11 (b) and 9.12 ± 0.32 M^{-1} min^{-1} and intercepts equal to 0 within the error of the data (a), 0.4974 ± 0.0033 (b) and 0.0330 ± 0.0075 (c) min^{-1}. R^2 values are 0.997 (a), 0.994 (b) and 0.989 (c). There was no weighting of the data in the fits.

ligand concentration fitted well to a first-order rate equation for all conditions. Furthermore the resulting plots of k_{1st} *versus* $[L]_0$ are linear, regardless of whether ligand decomposition is significant. Fig. 5a represents the case where $k_1[L]_0 \geq k_L$, except at the lowest concentration point ($k_1[L]_0 = 2.5 \times 10^{-3}$–$5 \times 10^{-2}$ min^{-1}, $k_L = 3 \times 10^{-3}$ min^{-1}). Under these conditions the slope (9.53 ± 0.23 M^{-1} min^{-1}), which would be equal to k_1 in the absence of ligand decomposition, is still smaller than k_1 ($= 10$ M^{-1} min^{-1}). Clearly ligand concentrations must be significantly larger than the complex concentration for the slope to approach k_1. The intercept was, within the error of simulated data, $= 0$ min^{-1}, as would be expected if ligand decomposition was negligible. In Fig. 5b ligand decomposition is rapid compared with the A + L reaction ($k_1[L]_0 \leq k_L$; $k_1[L]_0 = 5 \times 10^{-2}$–0.5 min^{-1}; $k_L = 0.5$ min^{-1}). This time the plot of k_{1st} *versus* $[L]_0$ has a slope (3.02 ± 0.11 M^{-1} min^{-1}) considerably less than k_1—that is, the apparent rate of the reaction is slower when ligand decomposition is rapid. One can rationalize this observation on the basis that under these conditions, the concentration of the ligand drops significantly during the reaction despite the ligand concentration initially being in excess compared with the complex concentration, resulting in a slower apparent reaction rate. The intercept is now large (0.4974 ± 0.0033 min^{-1}) and approaches the value of k_L (0.5 min^{-1}). Finally, Fig. 5c gives a plot of k_{1st} *versus* $[L]_0$ for the scenario in which the $k_1[L]_0 : k_L$ ratio increases from <1 to ~ 1 to > 1 as $[L]_0$ is increased ($k_1[L]_0 = 2.3 \times 10^{-3}$–0.5 min^{-1}; $k_L = 5 \times 10^{-2}$ min^{-1}). In this case the data at higher $k_1[L]_0 : k_L$ ratios dominates the fit, and the slope is slightly less than k_1 (9.12 ± 0.32 M^{-1} min^{-1}), as expected. Once again even higher ligand concentrations are required if the slope is to approach k_1. The intercept is also significant (0.0330 ± 0.0075 min^{-1}). Looking closely at this plot (an expanded version of Fig. 5c is given in the Supporting Information†), one observes that the data curves upwards—that is, the slope of the plot increases as $[L]_0$ increases. This is expected based on the slopes of the simulated data shown in Fig. 5a and b, which increases as the $k_1[L]_0 : k_L$ ratio increases. To summarize, for k_{1st} *versus* $[L]_0$ plots the apparent value of the first-order rate constant, k_{1st}, decreases as ligand decomposition becomes increasingly important. Furthermore, when substantial ligand decomposition occurs, the intercept of these plots will be significant even if the reaction of interest, A + L → A − L, has a large formation constant. Hence while kineticists typically attribute non-zero intercepts in plots of apparent first-order rate constant *versus* initial ligand concentration to the reaction of interest being reversible and/or a competing reaction occurring, another explanation is that significant ligand decomposition occurs during the time course of the reaction. Finally, a related reaction scheme of the type A + B → C where A also additionally spontaneously decomposes has been reported for the coupling of diazonium salts with 2-naphthol-6,8-disulfonic acid.[5] In this case data was collected under pseudo-first order conditions with B in excess, simplifying treatment of the data.

Conclusions

The effect of ligand decomposition on the apparent rate constant for a ligand substitution reaction (A + L → A − L) has been evaluated. If the ligand concentration does not change significantly and remains in excess for the entirety of the reaction, eqn (3) can be used to fit the data. However, if the rate of ligand decomposition is comparable in magnitude to

the rate of the reaction of interest ($k_1[L]_0 \sim k_L$), eqn (3) is no longer valid. Numerical simulations showed that even in the latter case the data will still fit extremely well to a first-order rate equation, despite ligand decomposition being significant. The reaction will not proceed to completion, however, and the apparent first-order rate constant obtained upon fitting the data to a first-order reaction will be larger than the pseudo first-order rate constant for the ligand substitution reaction itself as a consequence of ligand decomposition. Experimentists unaware that significant ligand decomposition occurs in addition to the ligand substitution reaction will not be alerted by the fit of the data to a first-order rate expression, hence our referral to the competitive ligand decomposition as a "silent killer". The slope obtained from plots of apparent first-order rate constant *versus* initial concentration of the ligand (that is, the apparent second-order rate constant) will be less than k_1 if ligand decomposition is significant, and a non-zero intercept will be observed even when the reaction between A and L has a large formation constant (the reverse reaction is negligible). This work highlights the importance of fully characterizing the reaction products and determining the extent to which a reaction proceeds towards completion in addition to determining rate constants, and provides an alternative explanation for the observation of intercepts in plots of apparent first-order rate constants *versus* ligand concentration.

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