

Double Kinetic Systems

AN3: Mg-Quinoline chelate formation.

Reaction of Mg^{+2} – 8-hydroxyquinoline chelate formation was used for testing of instrument performance and mixer dead time determination. This reaction is routinely used to determine the dead time of rapid mixing devices [1]. Although quantum yield of this complex is only 0.02, it has absorption maximum at 355 nm, what makes it ideal for excitation by the third harmonic of our YAG laser.

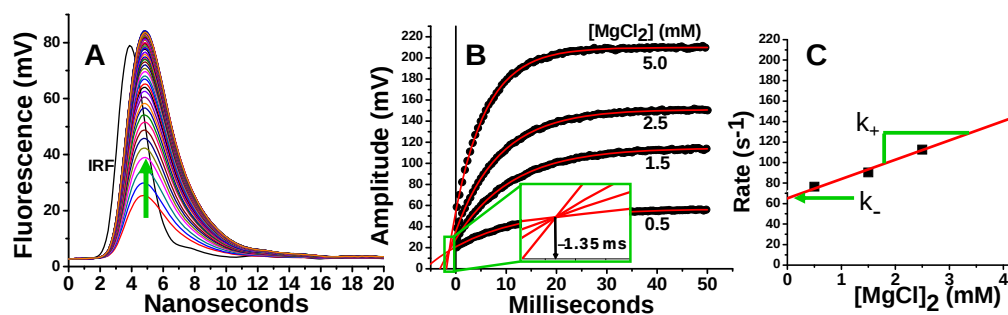
Experiments were performed at 25° C in 0.1 M TRIS buffer, pH 8.0. The solution of 0.2 mM 8-hydroxyquinoline was mixed with solution containing 1 mM, 3 mM, 5 mM, and 10 mM of MgCl_2 . Concentration of MgCl_2 was at least 10 times that of 8-hydroxyquinoline to maintain pseudo-first-order reaction conditions. The flow rate through the mixer was kept at 8 mL/s, and 100 mL of the sample from each syringe was used in one push of stopped flow. Emission was collected through a sheet polarizer oriented at the magic angle (54.7°), and a 420 nm cut-off glass filter. For each stopped-flow mix, 500 waveforms were measured and then averaged in groups of 5 waveforms, reducing time resolution to 0.5 ms. Experimental data were averaged over 20 stopped-flow mixes. A typical data set is shown in Pic. 1A.

Two strategies of data analysis were used. According first one all data for 4 concentrations of MgCl_2 were analyzed globally using single exponential model with all lifetimes linked. Recovered lifetime was 1.4 ns. Values of amplitude, obtained from this fitting are shown on Pic.1A. These values for each concentration of MgCl_2 were fitted by single exponential functions and observed rate constants were obtained. The inset of Pic. 1B shows that the four curves extrapolate to a common point at - 1.35 ms, corresponding to the mixer dead time, in good agreement with the 2 ms value calculated from the nominal dead volume and flow rate. A plot of the observed rate constant vs. the MgCl_2 concentration (Fig. 1C) shows the predicted linear dependence, permitting a direct determination of the association rate constant ($k^+ = 18.8 \text{ mM}^{-1}\text{s}^{-1}$) and the dissociation rate constant ($k^- = 44.5 \text{ s}^{-1}$), in good agreement with previously reported values [1].

Another data analysis strategy was based on FargoFit ability to include fraction kinetic in fitting model. Single exponential model was used. In order to include into account nonzero luminescence of 8-hydroxyquinoline two fractions were used – first represented 8-hydroxyquinoline and second represented product of reaction. In Kinetic Designer tab of Model Designer option “Rate conc” was used for 1->2 reaction, what represents k^+ and option “Rate” was used for 2->1 reaction what represents k^- . As initial condition value for Fraction1 was foxed as 1.0. All parameters, including amplitudes, lifetimes, rate constants, and dead time were linked. As result of this one step analysis values of rate constants and dead time were obtained. These values were almost identical to values obtained from first analysis strategy.

1. Brissette, P., D.P. Ballou, and V. Massey, Determination of the dead time of a stopped-flow fluorometer. *Anal Biochem*, 1989. **181**(2): p. 234-8.

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Pic.1. Formation of Mg^{2+}HQ complex in stopped flow ($[\text{HQ}] = 0.1 \text{ mM}$ after mix). A. Fluorescence waveforms 0.5 ms apart (average of 5) for $[\text{MgCl}_2] = 2.5 \text{ mM}$ after mix. B. Amplitudes of exponential rise, obtained from global fit of waveforms. Inset: dead-time = 1.35 ms. C. Plot of observed rate vs. $[\text{MgCl}_2]$, used to determine forward (k_+) and reverse (k_-) rate constants.