Rapid Kinetics and Spectroscopy instruments - Technical note #53

Determination of your experimental dead time in fluorescence mode.

The *aim* of this technical note is to learn how to calculate the experimental dead time of your stopped-flow instrument. The design of stopped-flow sequence and the use of Biokine are supposed to be known.

The *dead time* of a stopped-flow experiment is the age of the solution when it reaches the observation point; in other words, it is the time for the mixed solution to go from the centre of the last mixer to the observation point. Besides total flow rate and cuvette volume, the dead time depends on many different factors. In this technical note, only influences of flow rate and cuvette volume will be discussed. Because of hydrodynamics phenomenon difficult to be taken into account for software calculations, a slight difference between the estimated dead time (given by the Biokine software) and the real dead time may be observed.

The fluorescence quenching reaction of NATA (N-acetyltryptophanamide) by NBS (N-bromosuccinimide) is presented in this note. If the concentration of NATA is sufficiently smaller than NBS then this reaction can be treated as pseudo first-order reaction.

Upon a simple dilution of NATA , the resulting signal amplitude can be assumed to represent the total amplitude of NATA that would be observed at the mixing point in the case of a real reaction kinetics. The fluorescence value at the mixing point and at the observation point are the same.
Then, a real reaction mixing is performed and the amplitude of the signal at the stop is considerably smaller than in the simple dilution experiment since the reaction mixture has already aged. From this signal amplitude and the rate constant of the reaction together with the total amplitude measured in the dilution reaction, it is possible to determine the real dead time of the experiment.

A simplified drawing of the method used for the dead time calculation is given in Fig. 1.

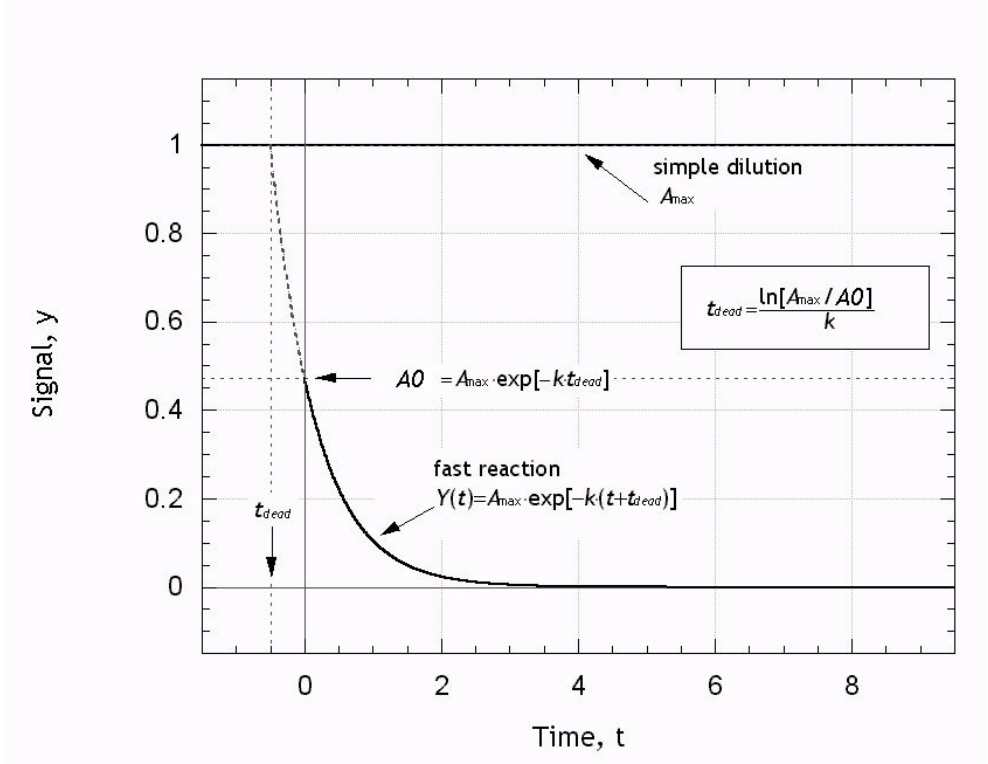

Figure 1: Principle of the dead time calculation.

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The technique to evaluate dead time in fluorescence mode presented here may be adapted to different instrumental conditions. This experiment can be performed with any optical system allowing total fluorescence detection: MOS-200, MOS250, MOS450 and J-810 and any of our SFM (SFM-20, SFM-300 and SFM-400)

Experimental conditions

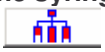
Optical system: equipped with a xenon or xenon mercury lamp (150W).

- *Mode* : fluorescence
- *Excitation wavelength*: 280 nm
- *Detection*: using a 320 nm cut-off filter, or select excitation wavelength at 320 nm

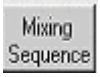
SFM (fill the 2 last syringes) **:**

- Syringe 1 : NATA 20 µM
- Syringe 2 : NBS 1.4 mM or water
- Cuvette: any FC cuvette
- Mixer : HDS or Ball Mixer
- Total flow rate : 9.5 ml/s.

Filling the syringes

Click on  and fill the syringe.

Mixing sequence configuration

Click on 

to edit your driving sequence then click on SFM options in the same window to configure the cuvette , delay line and valve lead.

System configuration

In the main menu choose Install, Device

- Choose optical system
- Go back in the main menu choose Install, Stopped Flow Config
- Select SFM and its corresponding serial port
- Select the size of the syringes
- Select the advanced mode
- Accept the parameters by clicking on the **OK** button

Optical system control:

- In the main menu choose **Device, Transient Recorder**.
  - Set the illumination wavelength to 280 nm.
  - Insert the cut-off filter in the PM tube or select the emission wavelength.

Acquisition setup

- Click on the 

Button to load the acquisition setup window.
  - Choose the fluorescence mode using the up and down button.
  - Do not select the Reference Diode check box.
  - Check the trigger box.
  - Choose a single time base.
  - Choose a 20 µs sampling period.
  - Choose a 0.15 s end time.
- Push some solution from the syringe 1 (with NATA) to fill the cuvette with the NATA solution.
- click on the HV state panel then on Auto to get 5V answer from the photomultiplier. In order to get a good sensitivity it is advised to get at least 4 or 5V. If the response given by the photomultiplier is too low, close the setup window and increase the High voltage then return to the setup window and repeat the operation. In case of a MOS-200 or a J-715, adjust the HV externally.

Dilution reaction

One syringe must be filled with water (e.g. S2), another one with NATA (e.g. S1, Fig. 2).

Driving sequence :

- Click on the Mixing Sequence button and edit the driving sequence as shown in Fig. 2.
- Click on the Ready button to activate the shot control.
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Acquisition:

- Click on the **start** button of the scope (with trigger), '**waiting for trigger**' is then displayed.
- Click on the **start** button to start the shots.
- The dilution is now displayed in blue in figure 4, all graphical tools can be used.

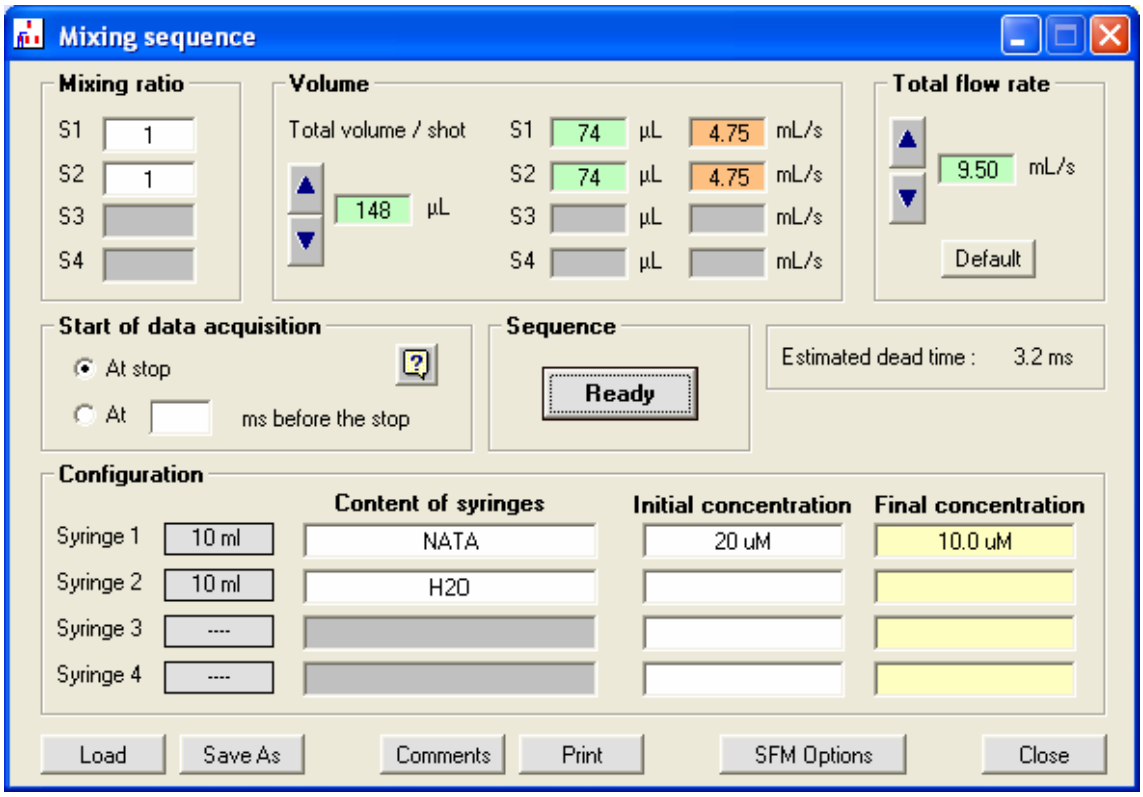

Figure 2: Driving sequence of the dilution reaction as written for a SFM-20 stopped-flow mixer model.

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Fluorescence quenching of NATA

One syringe must be filled with NBS (e.g. S2), another one with NATA (e.g. S1, Fig. 3).

Driving sequence:

- Click on the Mixing Sequence button and edit the driving sequence shown in Fig. 3.
- Click on the Ready button to activate the shot control.

Acquisition:

- Click on the **start** button of the scope (with trigger), '**waiting for trigger**' is then displayed.
- Click on the **start** button to start the shots.
- The kinetics is now displayed (red in Fig. 4), all graphical tools can be used.

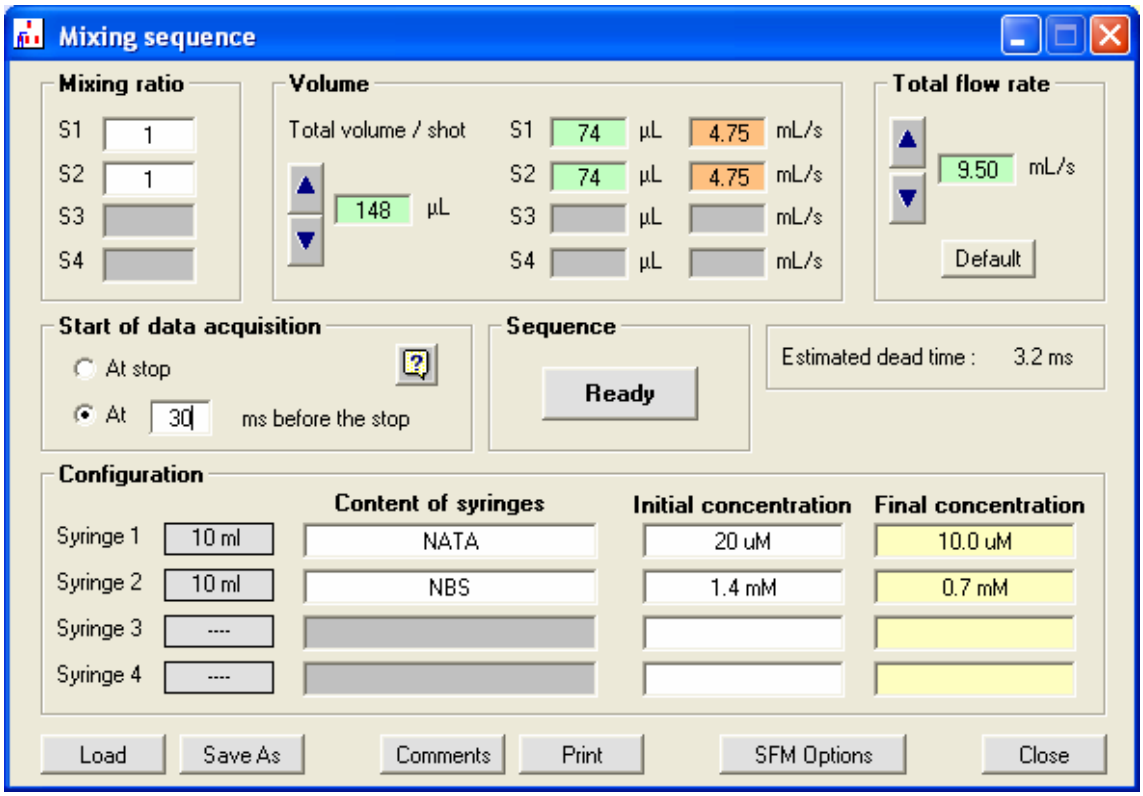

Figure 3: Driving sequence of the fluorescence quenching of NATA by NBS as written for a SFM-20 stopped-flow mixer model.

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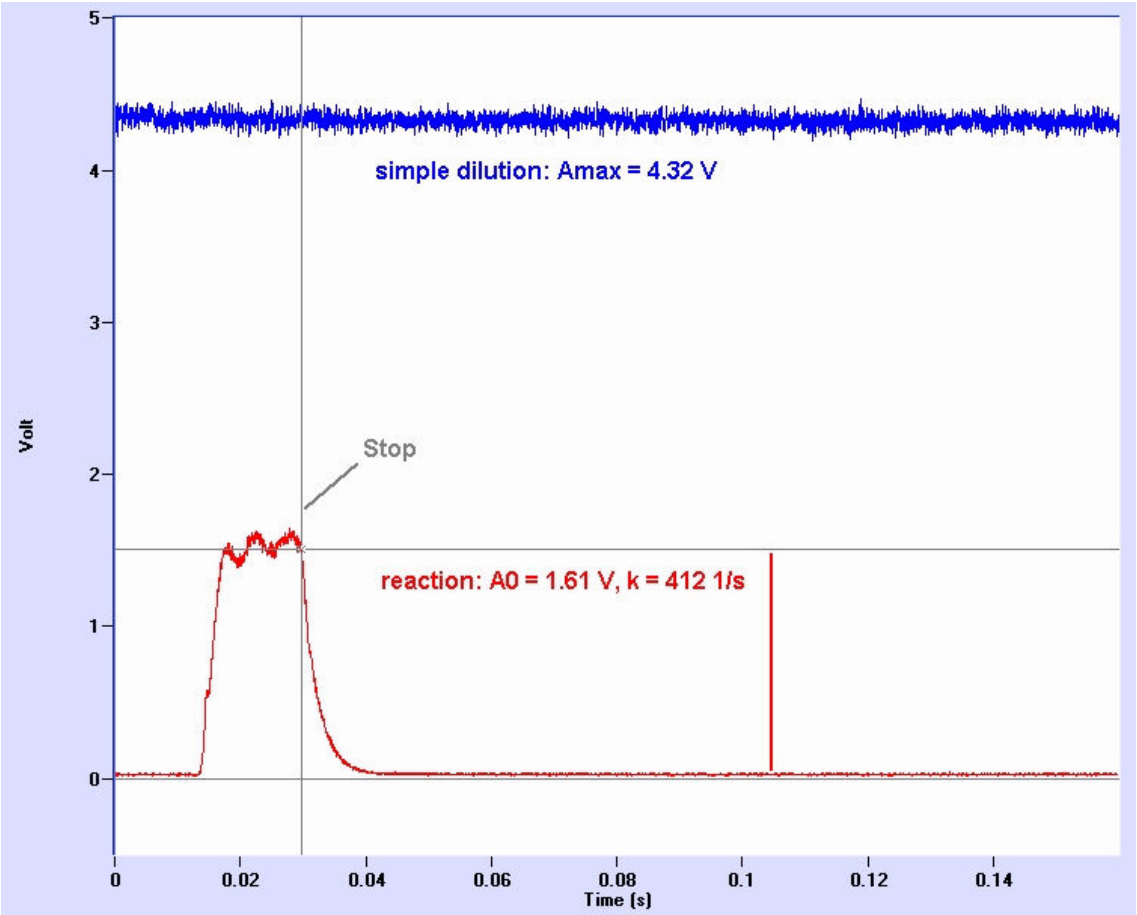

Figure 4: Traces of dilution (blue) and fluorescence quenching (red) of NATA.

Evaluation of the experimental dead time

The amplitude and the rate constant of the kinetics are obtained by means of simplex fit option of Biokine.

From the dilution we get Amax: 4.32 V
From the kinetics we get A_0 = 1.61 V and k = 412 s^{-1}.

As indicated in Fig. 1, the experimental dead time is calculated by the following formula:

$$t_{dead} = 1/k \cdot \ln(A_{max}/A_o)$$

Therefore, **the experimental dead time in this experiment is 2.4 ms.** In the experiment described in this note, a FC-08 and HDS mixer were installed in the SFM-20. The estimated dead time given by Biokine software was 3.2 ms, thus, very close to the experimental one.

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