Application note #15

SUBMICROSECOND DEAD TIME DETERMINATION

Keywords: dead time, 200µs, 700µs

Dead time is one of the most critical performance specifications in stopped-flow technology. Dead time is the time needed for the mixture to reach the observation point in the cuvette, from the mixing point in the system. The dead time is the ageing of the solution at the observation point. In theory the dead time is defined by the ratio of the volume of the cuvette by flow rate in the cell. Estimated dead time is always displayed in Biokine and recorded. However for very fast reactions (k>500 s-1) it is essential for the user to know the precise dead time of the system. This application note shows an easy method to determine dead time, and to check performance of the stopped flow system.

$$deadtime = \frac{DeadVolume}{Flow\ \ rate}$$

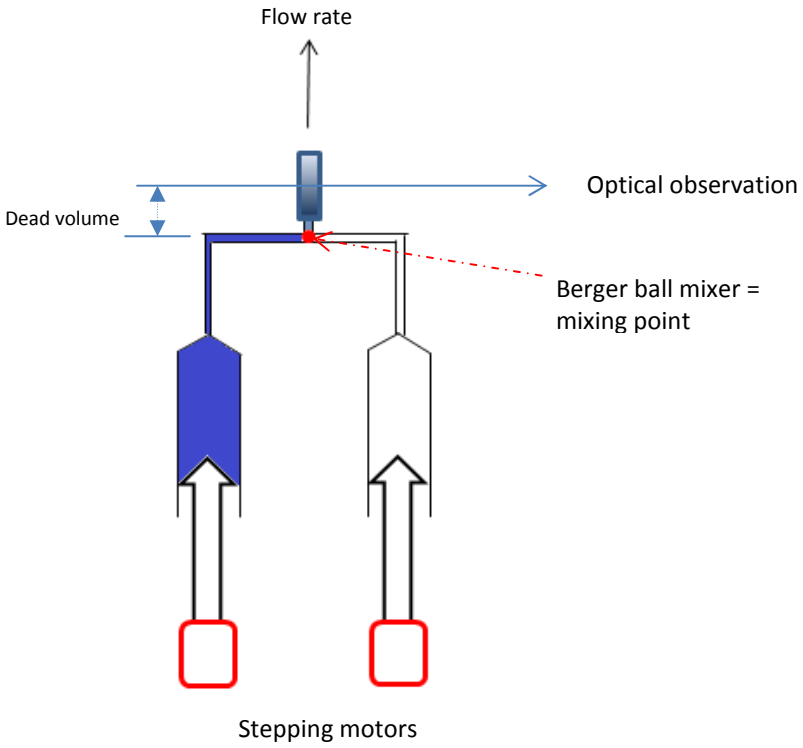

Figure 1: stopped flow scheme

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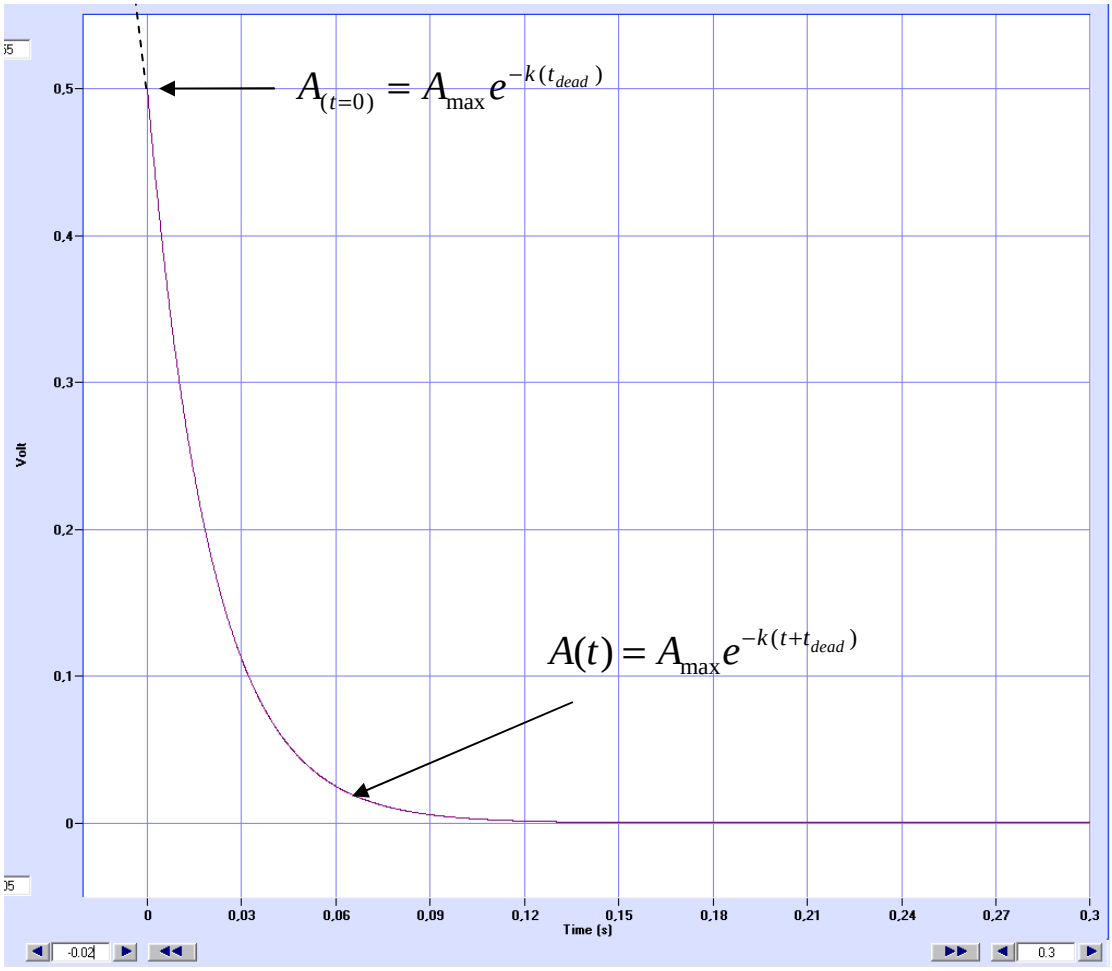
The reaction used to determine the dead time of the stopped flow system is the reduction of DCIP with ascorbic acid. The concentration of ascorbic acid is in large excess to monitor a pseudo first order kinetic. The fit of the data is with a single exponential equation:

$$A(t) = A_{\max} e^{-k(t+t_{dead})}$$



At t=0s, the reaction is observed at the dead time (t_{dead}), and the absorbance will depend on the constant rate k.

$A_{t_{dead}} = A_{\max} e^{-kt_{dead}}$: using the Neperien logarithm this gives:

$$Ln(A_{t_{dead}}) = Ln(A_{\max}) - kt_{dead}$$

Doing kinetics with different concentrations of ascorbic acid allows us to determine $Ln(A_{t\ dead})$ and the constant rate (k) for each trace.

The graph $Ln(A_{t\ dead})$= f(k) allows us to determine the slope which corresponds to the experimental dead time.

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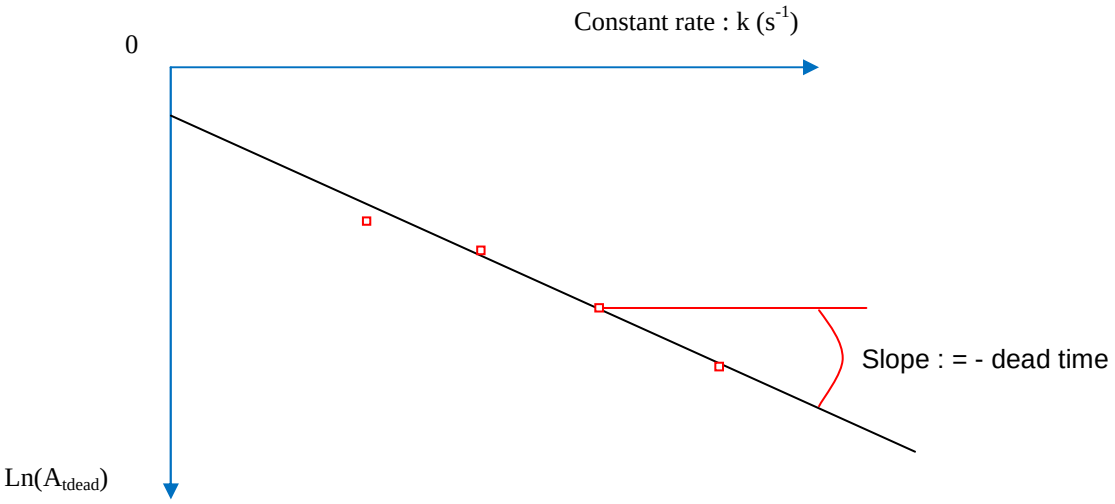
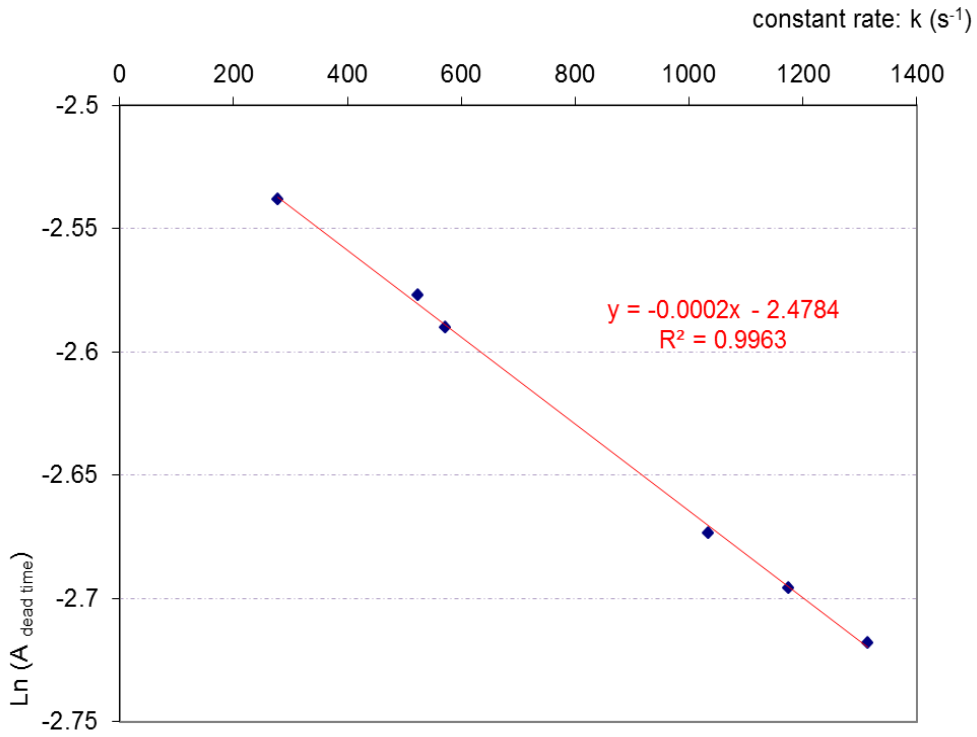


Demonstration:

100µM DCIP reductions by ascorbic acid, (ranging from 10mM to 50mM), were observed at 524 nm. Total flow rate is set to 20mL/s, which gives a theoretical dead time of 200µs for the micro-cuvette, and 700µs for the FC-08 with a mixing ratio of 1:1. The graphs $Ln(A_{t\ dead})= f(k)$ are displayed below.



Graphe1: dead time determination at 20°C using the microcuvette with a flow rate of 20mL/s

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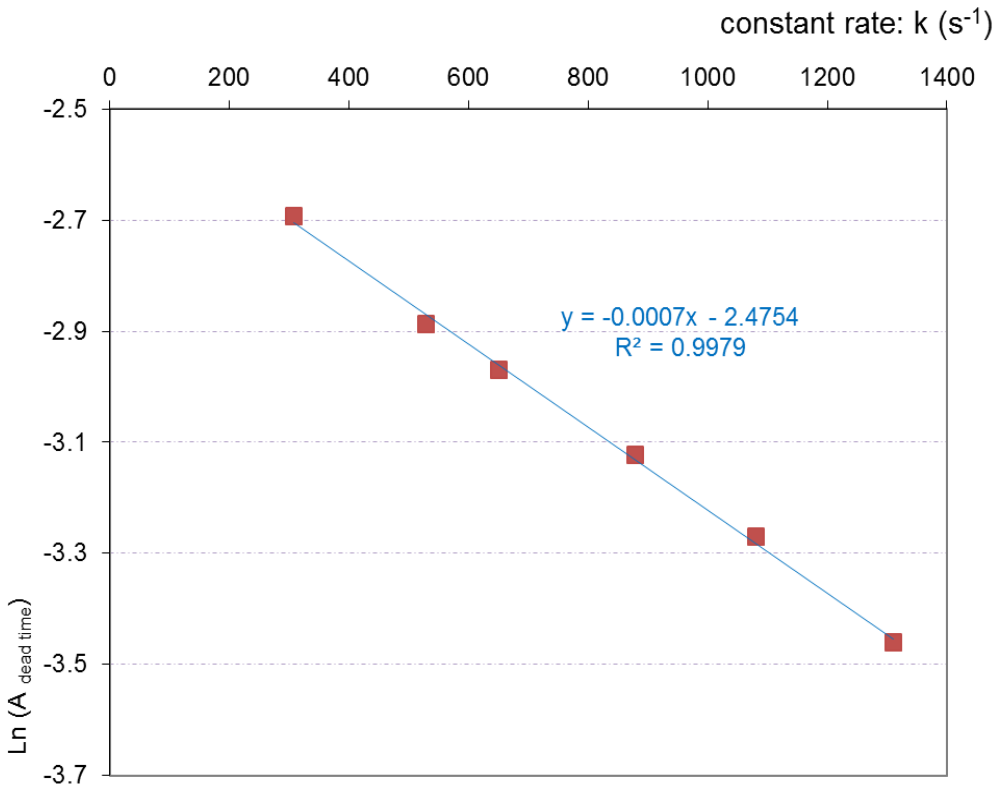

Graphe2: dead time determination at 20℃ using the FC-08 cuvette with a flow rate of 20mL/s

For the microcuvette and FC-08, experimental dead times found are 200µs and 746µs respectively. These dead times are the best results achieved by a commercially available system.

SFM x000 achieves sub-microseconds dead times. This constitutes a powerful and unequal tool for rapid kinetics investigations.

Please contact us for more information.

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