

Application note #17

## Precise control of flow rate

Precision control of flow rate is always essential in stopped-flow and quench-flow rapid kinetics experiments. It is most critical in multi-mixing experiments or when using pressure sensitive samples.

The best way to vary the ageing time in a multi-mixing experiment is to use a calibrated ageing line and vary the flow rate through the line from one shot to the next. A precise ageing time requires a precise flow rate. Pressure sensitive samples such as vesicles and membranes may require some variable flow rates to secure solutions during the pushing and stopping phase.

## Stepping motor technology provides users with wide range, precise and accurate control of flow rates in the $cuvette^{(1)}$ .

Some experiments require flow rates as high as possible to get the shortest dead times. Others may require lower flow rates such as FT-IR cells, or vesicle studies. It is essential to be able to vary the flow rate rapidly, precisely and without re-calibration. Thanks to our independent stepping motor technology, and Bio-Logic's unique software, users can increase or decrease total flow rate easily and have the estimated dead time calculated for each flow rate. A color coded window alerts the user of out of range conditions (mechanical limits, cavitation effects, laminar flow rate, etc.), so they know the instrument is used in safe conditions.

lixing ratio	Volume			Total flow rate
1 0	Total volume / shot	S1 0 μl	0 mL/s	
2 0		S2 0 μl	0 mL/s	16.00 mL/s
3 1	150 μl	S3 75 μl	8.00 mL/s	
4 1		S4 75 μl	8.00 mL/s	Default
	quisition	Sequence		
C At stop At 10 Configuration	ms before the stop	Ready		ted dead time : 1.0 ms
← At 10 Configuration	ms before the stop Content of sy	Ready	tial concentration	
At 10 Configuration yringe 1 10 m	ms before the stop Content of sy	Ready		
At 10 Configuration yringe 1 10 m	Content of sy	Ready		

Figure 1: Mixing sequence interface and flow rate control

Precision of the flow rate delivery can be demonstrated with an easy test. In absorbance mode, DCIP (70 $\mu$ M) is mixed with ascorbic acid (10mM) at flow rates from 1mL/s to 20mL/s. Observation is made using a 1 cm light path cuvette.

The reaction is pseudo-first order, and each kinetic can be fitted as a single exponential. The reaction gives a rate constant of 192s<sup>-1</sup> at all flow rates selected; however the amplitude of signal increases with shorter dead times. A plot of the signal amplitude versus the dead time is shown in figure 2.

(1): Pneumatic based systems use a stop syringe to stop the flow and speed of the ram is set by adjusting pressure of gas: the combination leads to overpressure and possible damage of sample. No direct control of flow rate is possible, the user can just guess the flow rate from result obtained.

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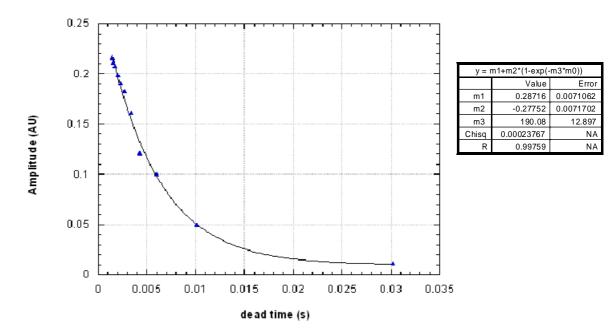


Figure 2: Amplitude =f(dead time) at different flow rates

The exponential fit gives a rate constant of is  $190 \pm 12 \text{ s}^{-1}$  so in perfect accordance with rates of the single samples measured.

This experiment demonstrates the excellent accuracy and precision of flow rates delivered by stepping motor technology, and the user-friendliness of Bio-Logic's Biokine software, as the full series of shots can be done in less than 5 minutes.

Please contact us if you have questions.

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