

## Rapid Kinetic and Spectroscopy instruments

### $\mu$ SFM20, SFM300&400 - high dilutions and variable ratio mixing – part I (Updated August 13, 2009)

The possibility to obtain variable mixing ratio by a simple programming of the instrument (i.e. without changing the syringes) is one of the major advantages of the Bio-Logic stopped-flow instruments. The microprocessor control of the stepping motors giving 6400 steps per revolution of the motor gives a smooth and quasi continuous movement of the syringe over a very large range of flow rate.

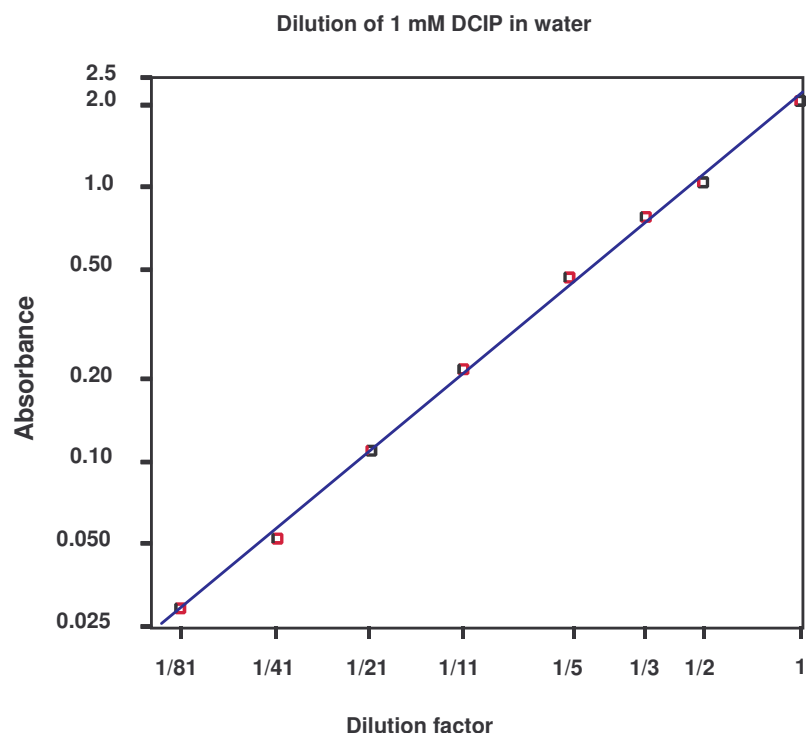
A few examples of significant experiments to test and apply this property are described below.

Experiments uses dichlorophenol-indophenol (DCIP) and ascorbic acid (AA)

#### TEST N°1

Dilution factors of 1/50 or higher can be obtained without syringe exchange. This is demonstrated in the next experiment where we mixed 1mM DCIP in one syringe with buffer in another. This can be syringe 1 and 2 in the  $\mu$ SFM-20 and any couple of syringes in multi-syringe machines such as SFM-3, SFM-300, SFM-4 or SFM-400.

There is no reaction to follow in this case, the only goal of this experiment is to test final absorbance after mixing.



The results shown in the **figure 1** indicate a satisfactory linear relationship between the absorbance measured after mixing in the stopped-flow and the final DCIP concentration which was calculated according to the ratio of the volumes delivered by the two syringes. A maximum of 5 % deviation is observed for this series of experiments.

Figure 1

## TEST N°2

This uses method uses at least three syringes and thus can be utilized for the SFM-3, SFM-300, SFM-4 or SFM-400 machines. This permits variation of the concentration of one substrate without changing the concentration of the other.

Contents of the syringes (for a three syringe instrument) :

Syringe 1 : AA (20 mM)

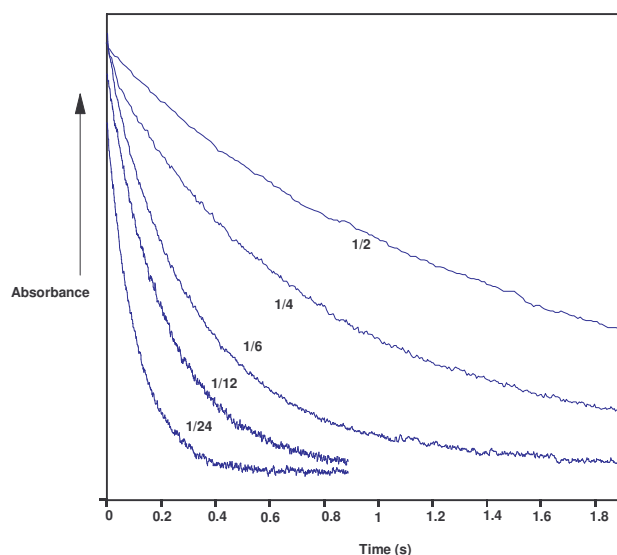
Syringe 2 : Buffer

Syringe 3 : DCIP (100  $\mu$ M)

During the drive sequence the three syringes are programmed to deliver flow-rate equal to  $\Phi_1$ ,  $\Phi_2$  and  $\Phi_3$  respectively.

The stopped-flow instrument is programmed so that the flow-rate of DCIP,  $V_3$ , is constant, as is  $(\Phi_1 + \Phi_2) / \Phi_3$ .  $(\Phi_1 + \Phi_2) / \Phi_3$  is then constant and equal to 1/1, giving a constant final DCIP concentration 1/2 that of the DCIP in syringe 3. Final concentration of DCIP was 50  $\mu$ M.

In a series of experiments  $\Phi_1$  and  $\Phi_2$  are varied, giving a changing final concentration of ascorbic acid proportional to  $\Phi_1 / (\Phi_1 + \Phi_2 + \Phi_3)$ . By this method, a high dilution ratio of one component (here AA) can be obtained without changing the concentration of the other (DCIP).



Resulting data are shown in the **figure 2**, where dilutions up to 1/24 were assayed.

Figure 2

Rate of DCIP decoloration  
Variation with AA concentration obtained by dilution

The rate constants measured using the Bio-Kine software show a satisfactory linear relationship as a function of AA concentration  $k$  (s<sup>-1</sup>) (**figure 3**). Numbers in parentheses represent the degree of dilution of AA.

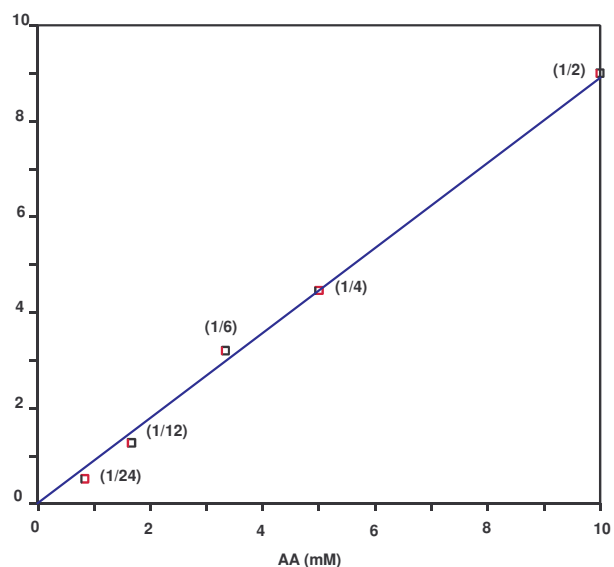


Figure 3

### TEST N°3

This is another example of dilution experiment using three syringes

This describes a variable ratio mixing experiment used to follow alcohol dehydrogenase activity at variable concentration of enzyme.

Buffer used was : 100 mM Tris-Cl, 1 mM EDTA, 5 g/l of semicarbazide-Cl and 25 mM ethanol.

Contents of the syringes :

S1 = Buffer

S2 = Buffer + 1 mg/ml alcohol dehydrogenase (ADH)

S3 = Buffer + 1 mM NAD

As described for the test N°2 above, each experiment is a mixture resulting from variable flow rates  $\Phi_1$ ,  $\Phi_2$  and  $\Phi_3$  delivered by the three syringes.

Ratio  $(\Phi_1 + \Phi_2) / \Phi_3$  was kept constant and equal to 1. Varying  $\Phi_1$  and  $\Phi_2$  give a final dilution of ADH equal to  $\Phi_2 / (\Phi_1 + \Phi_2 + \Phi_3)$ .

The advantage of this technique is that the final concentration of NAD is constant whatever the final ADH concentration. Ratio of 1/2 to 1/120 were used.

Absorbance was measured at 340 nm with a 5 mm cuvette and the rate of formation of NADH was observed by following the decrease of the transmitted light.

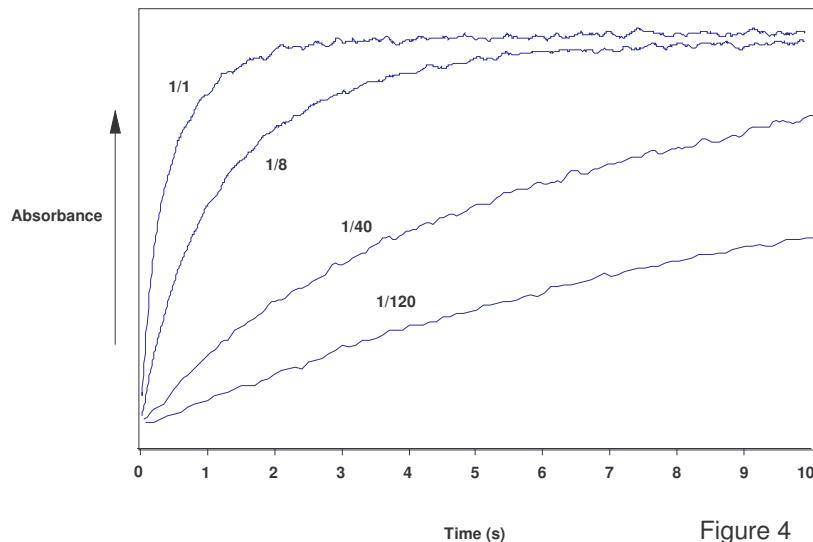


Figure 4 shows the traces obtained.

Figure 4

The initial rate of the reaction was measured for each of the traces. These rates are plotted as function of the dilution factor on a log-log scale (**figure 5**). This plot shows a reasonable alignment on a line of slope 1 indicating a linear relationship between the initial rate and the dilution factor.

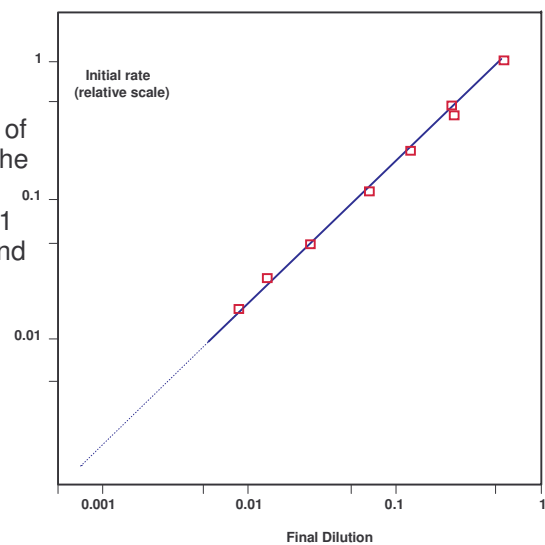


Figure 5