Double mixing Stopped-flow using interrupted flow mode

I – Introduction

SFM-3000 and SFM-4000 are the ideal systems for double mixing experiments taking full advantage of the precision of independent stepping motors. User can design easily a series of shots at different ageing times so reaction intermediates not visible with single mixing methods become observable.

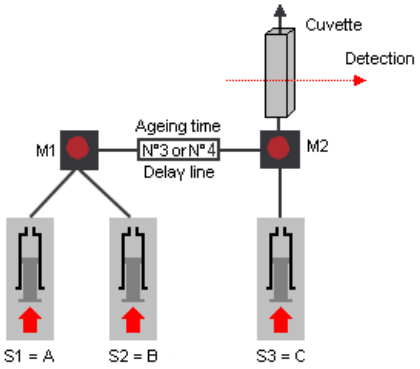

Fig. 1 : Double mixing set-up.

In a double mixing experiment, two reactants are mixed in mixer 1 and are allowed to age during an user-defined ageing time. After that content of the delay line is mixed with a third reactant so this second reaction can be observed using optical method. Depending on ageing time the mixing is done is continuous flow mode or in interrupted flow mode to cover ageing time range from 2 ms to several minutes.

II – Experiment

To illustrate double mixing the standard DCIP/ascorbic acid reaction can be used. In mixer 1 dichloro-indophenol (DCIP) is mixed with a low concentration of ascorbic acid (0.5 mM), the mixing is allowed to age for a define time in a 120 µl delay line before it is mixed with a higher concentration of ascorbic acid (5 mM). This second reaction is followed in absorbance mode using a TC-100/10 cuvette (1 cm light path).

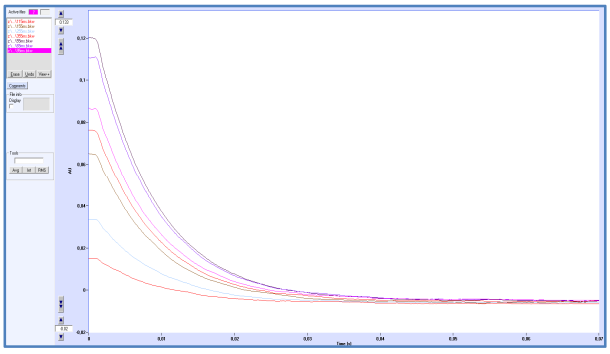

Fig. 2 : Transients obtained varying ageing time from 55ms to 600 ms.

Total flow rate is kept constant for all ageing times. Transient are recorded with ageing times from 55 ms to 600 ms as shown in figure 2. Each transient is fitted with a single exponential so its amplitude is measured and user can check observed rate constant is constant. As dead time is constant plotting the amplitude of transient versus time will show the first reaction and one can define the rate constant of first reaction

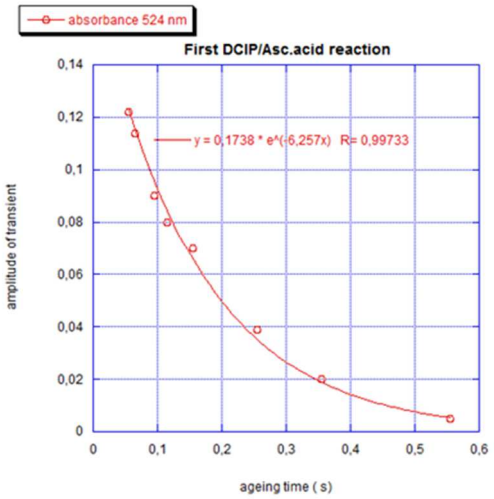

Fig. 3 : determining rate of first reaction

The plot of amplitude of transient versus ageing time is well fitted with a single exponential, rate of first reaction calculated is 6.2 s^{-1}.

To check if measurements is correct user can mix DCIP and ascorbic acid 0.5 mM from corresponding syringes making sure enough volume is pushed to fill both delay line and cuvette.

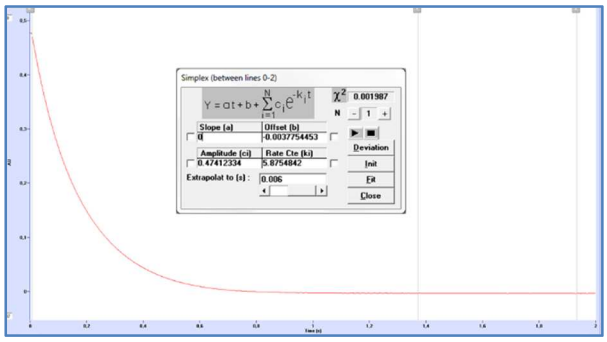

Fig. 4 : first mixing step

First mixing step is fitted with a single exponential and give a rate constant of 5.9 s^{-1} so in good agreement with rate constant determined in the double mixing mode. This demonstrates the performance of our SFM mixers.

III– Automation

Independent stepping motors offer extended capabilities to run such series of experiments automatically and make SFM instrument unique on the market.

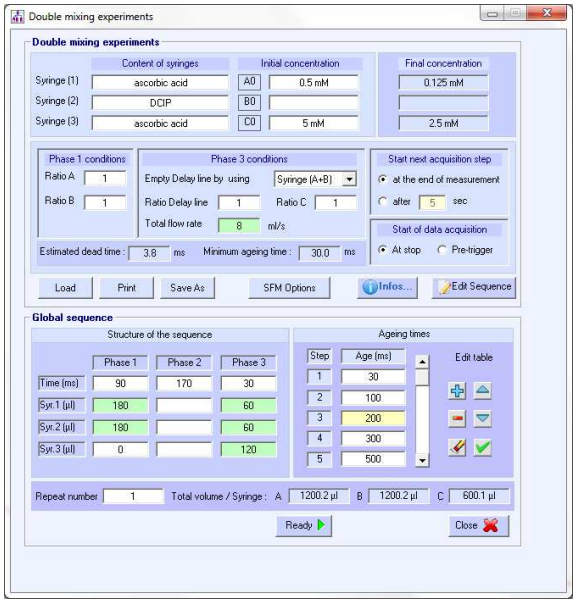

Fig. 5 : double mixing automatic mode

User needs to define a global mixing sequence according to delay line installed, cuvette in the observation head and mixing ratio applied, then he just needs to edit a list of ageing times he wants to perform. Software will automatically run the full series so user just needs to collect data.