Stopped-flow in cryogenic conditions

I – Introduction

The SFM-series of stopped-flows are temperature regulated using a circulating water bath. In their standard configuration, the stopped-flows may be operated from -20°C to +85°C, offering more than a 100 degrees temperature range to the user. However, some applications require lower temperatures to slow down the reactions enough to enable the observation of the intermediates and final products optically.

This application note describes the use of the CS-90°C accessory which extends the temperature range of the SFM to -90°C allowing for these lower temperature regime reactions to be observed. The reaction between 2,4 dinitrophenyl acetate (DNPA) and sodium methoxide in organic solvents is used to illustrate the use of this accessory in an SFM equipped with the appropriate O-rings.

II– Experimental set-up

The CS-90°C includes a cryo-bath and heater. The cooling is done by circulating liquid N2 in a coil immersed in cryo-solvent (1-propanol or oil). The last mixer (or the two last mixers if a double mixing set-up is needed) is embedded in a cryo-mixing chamber.

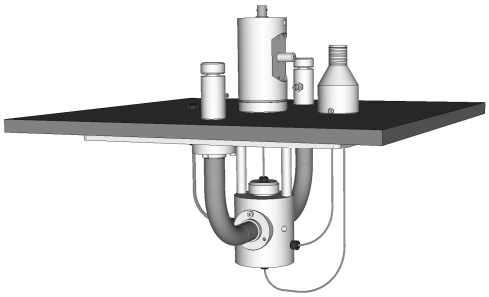

Fig. 1 : cryo-mixing chamber (below the plate)

The cryo-chamber includes a 1 cm light path cuvette which is submerged in the cryo-bath. The SFM is attached to the cryo-bath and a special umbilical connector transfers solution from the SFM driving syringes to the immersed cryo-chamber. 200µl of each reactant is incubated in HPLC tubings in the cryo-bath before the injection.

Given the design, only a few seconds are needed for the reactants to reach the desired temperature allowing for a series of shots to be easily programmed and completed under the control of the software without any further manipulation on the part by the user. A diode array detector (400µs per spectrum) and a combined Deuterium/Halogen light source are connected to the observation cuvette using two thermally protected optical fibers.

Fig. 2 : stopped-flow attached to CS-90°C

The output of the coil needs to be connected to a fume-hood or an extraction system so nitrogen in the gas phase can be evacuated. To achieve an experimental temperature, the user only needs to set the desired temperature. The cooling of the bath starts by opening the liquid N2 valve, when temperature of the bath goes below set temperature, the heater starts to create a temperature equilibrium defined by the user. The N2 valve is then closed. A temperature probe is inserted close to the observation cuvette allowing the user to verify the temperature in the cuvette.

III–Reaction in ethanol

Syringes 1 and 2 of a SFM-4000 are loaded with ethanol so they can be used for absorbance reference measurements. Syringe 3 is loaded with sodium methoxide 0.05 M (10% v/v methanol/ethanol) prepared from commercial 0.5M methoxide solution in methanol. Solution 4 is loaded with 60µM 2,4 dinitrophenyl acetate in ethanol.

The reaction is initiated by mixing 100 µl of the reagents at 12 ml/s providing a 2.5 ms dead time. For each temperature a 3 dimensions data file is saved that can be used for global analysis and identification of intermediates.

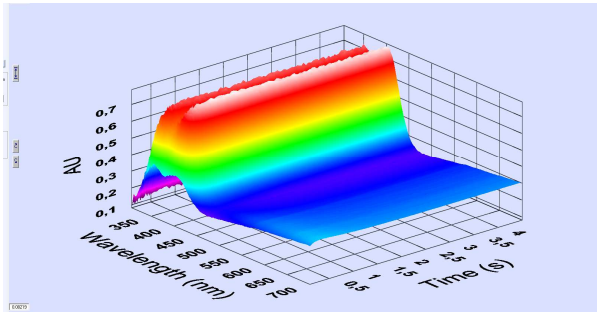

Fig. 3 : spectrum vs time (reaction at -12°C in ethanol)

The reaction is followed from +11°C to -55°C. At each temperature, the trace is analyzed at 400 nm and fitted using a single exponential model using Biokine.

The rate constants measured are summarized in table 1.

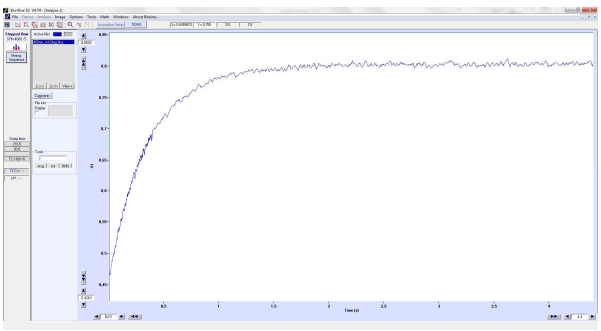

Fig. 4 : -12°C, reaction at 400 nm (reaction at -12°C in ethanol)

Temp (°C)	k (s-1)
11	13,4
2	8
-12	2,9
-26	1,07
-35	0,42
-44	0,202
-55	0,08

Table 1 : influence of °C on rate constant

The natural logarithm of the rate constants measured is plotted versus the inverse of temperature to check linearity of the Arrhenius plot.

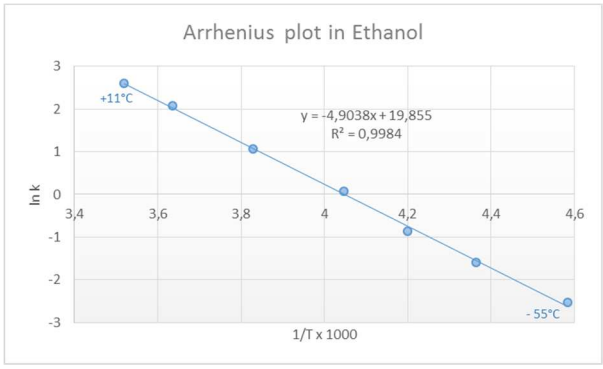

Fig. 5 : Arrhenius plot in Ethanol

Ln k varies linearly with 1/T demonstrating the accuracy of mixing system and performance of CS-90°C from +11°C to -55°C.

IV–Reaction in acetone

The same reaction is then performed using acetone instead of ethanol as the solvent because of viscosity issues with the temperature dependence of ethanol allowing for lower temperature to be achieved (alternatively slower injections could have been done to reduce viscosity effects)

Syringes 1 and 2 of a SFM-4000 are loaded with acetone so they can be used for absorbance reference measurements. Syringe 3 is loaded with sodium methoxide 0.05 M (10% v/v methanol/acetone) prepared from commercial 0.5M methoxide solution in methanol. Solution 4 is loaded with 60µM 2,4 dinitrophenyl acetate in acetone.

The reaction is initiated by mixing 100 µl of each reagent at 12 ml/s providing a 2.5 ms dead time. For each temperature a 3 dimensions data file is saved that can be used for global analysis and identification of intermediates.

The reaction is followed from -27°C to -80°C. At each temperature, the trace is analyzed at 417 nm and fitted using a single exponential model using Biokine.
The rate constants measured are summarized in table 2.

Temp (°C)	k (s-1)
-27	60
-40	17,7
-46	9,5
-58	2,76
-69	0,64
-78	0,16

Table 2 : influence of °C on rate constant

In acetone, the reaction of DNPA with sodium methoxide is about 60 times faster compared to ethanol which makes it a better model to check the fast mixing performances and temperature dependencies of the CS-90°C accessory coupled to a SFM.

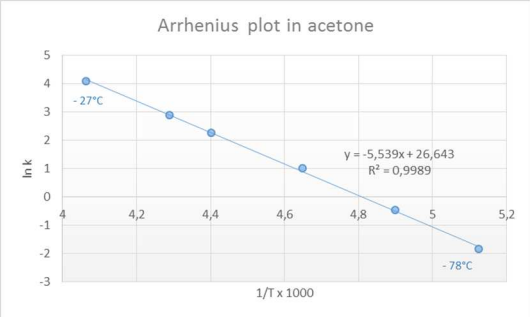

Fig. 6 : Arrhenius plot in acetone

Ln k varies linearly with 1/T demonstrating the accuracy of mixing system and performance of CS-90°C down to -78°C.

V–Conclusion

The CS-90°C accessory can extend the temperature range of SFM to -90°C and allows observation of reaction intermediates or of reactions that are normally too fast at standard temperatures. A complete Arrhenius plot can be done in half a day.
Furthermore, stopped-flow can be used over a 170°C range assuming a solvent covering this temperature range can be found.

VI–Alternative solution for the cold

Bio-Logic also offers a cryo-accessory compatible with the cryostat CC-905 from Huber GmbH. The mixing assembly and umbilical are similar to CS-90°C. The stopped-flow is then attached directly to the cryostat. Using an external cryostat the user set the target temperature on the cryostat and thus do not need to handle liquid N2 circulation.