

Rapid Kinetic and Spectroscopy instruments

SFM300&400 - μ volume operation (updated August 13, 2009)

This series of test was designed to demonstrate the operation of the SFM-300 or SFM-400 when using low total volume of protein samples.

INSTRUMENTS USED

Stopped-flow : SFM-300 equipped with FC-08 cuvette. Syringes 1 and 2 were the standard 10 mL syringes while the syringe N°3 was the small 1.9 mL syringe.

Spectrometer : MOS-250 in fluorescence mode.

- Excitation : 297 nm
- Emission 340 nm
- Band pass 10 nm
- 150 W Xe(Hg) lamp

TEST REACTION

Refolding of lysozyme. Henn egg lysozyme, (1 mg/mL) denatured in 6 M guanidine chloride was set in the instrument syringe N°3. Refolding reaction was initiated by diluting this solution in 50 mM Pi buffer pH 7.2.

Temperature was 20 °C

DESCRIPTION OF THE EXPERIMENTAL PROCEDURE

The syringe N°1 and N°2 of the SFM-300 where first loaded with the dilution buffer, all flow lines were washed and syringes were refilled with buffer.

Syringe N°3 was filled with air and that was used to flush all flow lines N°3.

0.5 mg of lysozyme were dissolved in **500 μ L** of 6 M GuHCl giving a concentration of 1 mg/mL of denatured enzyme.

a) Syringe N°3 filling

The 500 μ L sample solution were transferred in a 1 mL syringe installed (without plunger) in the syringe N°3 filling port.

The standard procedure of bubble evacuation was used to ensure that the content of syringe N°3 was free of air bubble

(in short : this consists in a few up and down movement of the drive syringe that allow bubbles to be evacuated through the filling port and replaced by the filling solution).

Once no more bubble were visible, syringe N°3 position was referenced (uppermost position of the piston). Content of the 1 mL syringe was then finally transferred (pumped) into syringe N°3 until the 1 mL syringe was empty *(a low drive speed was used for a better precision).*

At that time the syringe N°3 volume indicator on the MPS software showed 400 µL in S3.

The pumping action of S3 was then continued until the indicator showed 460 µL, this allowed a large fraction of the sample contained in the dead volume between the filling port and the S3 tip to be recovered.

b) Flow lines priming

The S3 valve was now connected to the “C” position.

The S3 motor was actuated upwards until S3 volume reading shows 350 µL.

c) Data collection

The instrument was programmed with the following sequence :

The sequence of shot was as followed :

Flow duration (ms)-----	12
S1 (buffer) (µL) -----	60
S2 (buffer) (µL) -----	60
S3 (Enzyme + 6 M Guanidine) (µL) -----	12

This sequence gives a 11 fold dilution of the enzyme solution, a total flow rate of 11 mL/s and a dead time of 1.5 ms.

Two dummy shots were used and the instrument was then ready for actual data collection.

The actual filling of the S3 syringe now allows 32 shots

NOTE : at that time a full series of experiment such as that described in Application Note #4 would have been possible.