



STOPPED-FLOW CD

Stopped-flow CD is an established and much wished technique. All CD manufacturers are offering stopped-flow options and a few dedicated instruments are available too. It'd pay to review here the stage of development to see where we are today.

Stopped-flow mixing cells (and related spectrophotometers) appeared commercially in the sixties: *Durrum* and in a lesser extent *Aminco* (both from US) dominated the market. Both systems were featuring pneumatically activated syringes to drive the reactants, a mixer, an observation cell and a stopping syringe. This last syringe actually stops the flow and triggers the detector electronics.

The "dead time" of the instrument, i.e. the capability to follow reactions from the early stage is affected by the efficiency of the mixer and the linear velocity of the reactants delivered by the syringes.

Stopped-flow devices had been used originally for absorption and later on for fluorescence applications, but soon people started to apply these to polarimetric and CD analysis.

Today different options are open for CD users:

First "cheap" option is the use of rapid kinetics stopped-flow cell shaped accessories as available from *Applied-Photophysics* and by *Hi-Tech* (both from UK). These devices have the monitoring cell fitting the standard cell compartment of commercial spectrometers and interconnection with the driving and the stopping syringe is by a flexible connection. Dead time is moderately long (over 10 msec) and mixing ratio may be changed fitting proper driver syringes. Syringe driving is by simple manual operation or by optional pneumatic actuator.

These devices are widely used for strong CD signals; their main limitation (apart from dead time) is the difficulty to automate multiple shot experiments. Averaging of experiments is a feature often mandatory in CD stopped-flow experiments, in order to get acceptable S/N.

CD manufacturers as *Jasco* and *Jobin-Yvon* offered (and in *Jasco* case are still offering) relatively low cost syringeless stopped-flow devices in which the reactants are fitted in reservoirs which are pressurised by nitrogen gas, opening an electrovalve after the sample cell forces the reactants through the mixer and the cell, closing quickly the electrovalve the flow is stopped and reaction is monitored. This approach is very suitable for CD applications since multiple experiments can be easily automated. The basic drawbacks are the undefined mixing ratio (actually dictated by the relative viscosity) and by the large sampling volume typically required.

So further improvements are possible integrating research grade stopped-flow cell with existing or dedicated CD spectropolarimeters:

-*Applied Photophysics* recently launched its π^*-180 spectrometer featuring a pneumatic driven two syringe system (expandable to 4 adding another actuator driving two extra syringes) matched with a dual grating monochromator. The unit has facility to measure CD, absorption and fluorescence

-*Hi-Tech SF-61* (here too pneumatically controlled, but with facility to automate experiments including syringes refilling) is being offered as stand alone absorption/fluorescence system, but it can be easily connected to commercial CD spectropolarimeters

-*Olis* (from US) is offering a rather classical stopped-flow cell which may be probably integrated in to their CD head

-*AVIV* (from US too) produces a dedicated stopped-flow accessory (syringes controlled by servo motors, not pneumatically) for its CD spectropolarimeter

-*Bio-Logic* (from France) developed since 15 years innovative stopped-flow cells with unique specifications:

- syringe drive controlled by stepping-motors
- easy interchange and wide selection of measuring cells
- choice of mixers

Recently an extensive cooperation program led to development of an integration of their cells and driving software with the *Jasco* CD spectropolarimeters.

It'd pay to give more details in order to understand where the advantages of the *Bio-Logic* are:

1. flow rate can be actively controlled using stepping motor drive, independently from actual syringe size
2. the system operates without stopping syringe, significantly reducing pressure built up in the system, potentially very dangerous in CD mode due to possible cell birefringence

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3. freedom to select the mixing ratio also within the same experiment, without hardware modifications

Today different versions of Bio-Logic stopped-flow cells are available:

μSFM-20

featuring two syringes, no valves, and relatively low cost.

This is the ideal solution for conventional stopped-flow experiments with minimum priming volume (so if you deal with very expensive samples and two syringes mixing is enough this is your first choice!)

SFM-300

With three syringes, two mixers, variable volume ageing volume between the two mixers and switching valves.

This is the ideal solution for multi-mixing experiments

SFM-400

Further expanded with 4 independently driven syringes, 3 mixers etc

For all models there is further an ample choice in the syringes used and in the observation cell. Cell is very easy to replace and offers free access from 4 sides, which allow easy multi-detection (typically CD, Absorption, Fluorescence ...) modes. For normal CD application (protein folding ...) we recommend the FC-20 with 2 mm path In both directions (transmission/CD and fluorescence).

Another available choice is the type of mixer: for standard CD application we would recommend HDS mixer type, since more suitable with very different viscosity solutions.

Only hardware modification to be considered is replacement of standard 150W Xe source with a Hg/Xe one of same power. This will typically improve S/N in your experiments. Replacement itself is trivial since lamps have exactly same shape.

The proper optical interfacing of the system has been achieved, there is no need to remove the stopped-flow cell or any accessory fitted into the CD equipment when passing from steady-state to stopped-flow mode or viceversa. Softwarewise the stopped-flow controller runs under Windows™ as the spectropolarimeter drive, so both can operate neatly at the same time. Normal data acquisition and processing is possible using the Jasco software, but with simple modifications you can also use the Bio-Logic Bio-Kine acquisition and/or processing software if needed/preferred.

In addition to stopped-flow capabilities the SFM allows extension to titration operation mode with inexpensive accessories. Titration experiments can be carried on directly replacing the stopped-flow cell with a static cell (thermostatted and stirred) and the housing allows insertion of other sensors such a micro pH electrode. Alternatively titration runs can be carried on in the standard sample compartment feeding and removing reactants using the stopped-flow syringes.

This facility is obviously possible only because syringes are stepping motor, not pneumatically controlled.

The addition of a Bio-Logic stopped-flow is therefore now not a simple add a box accessory, but it allows to create a consistent system. Jasco J-810 allows simultaneous collection of 4 data, typically in stopped-flow mode you use:

-CH1 for CD (CD/DC mode since best operation is carried on at constant high voltage on PM tube)

-CH-2 for DC (i.e. signal directly proportional to sample transmission)

-CH-3 for fluorescence

-CH-4 free or to be connected to pH or temperature sensors

The Bio-Logic SFMs fit well also J-700 series without any modification (but J-700 is limited to two channel acquisition, so if multiple parameters have to be detected it'd pay to consider the addition of their Bio-Kine 4 channel acquisition board).

With small modifications it's also possible to use the system with previous CD spectropolarimeters applying the necessary hardware work to get the measuring mode required. In this sense users who own an old unit somehow obsolete (at least softwarewise) may consider opportunity to build up a "new" fast kinetic dedicated unit around their hardware.

ECS is not selling instruments, but it can easily provide and install you all the necessary modifications.

For further information on Bio-Logic stopped flow you can explore their web site: www.bio-logic.fr (we suggest to give a look to the vast bibliography enclosed), but further information may be obtained also from your local Jasco office/representative.

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