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A FEW PRACTICAL ASPECTS FOR STOPPED-FLOW CD

This argument has been already discussed previously (see T.R. N° 10, 34 and 40), but it pays to raise a few practical aspects in order to optimize the experimental set-up. When you deal with a Jasco spectrometer and a Bio-Logic SFM you get proper instructions for each separate module, but nearly nothing about the combined operation.

1. If you want to start a new experiment you can benefit from a definite plus of the Bio-Logic SFM: the possibility to remove easily the observation cell and to replace it with a conventional rectangular cuvette (semi-micro type 4mm width are quite OK*)
This will allow you to measure separately CD and absorption spectra of your sample and of your reagents, in order to select proper concentrations, measuring wavelength and so**.

** this facility may be important for other experiments, since you can use the SFM as a normal micro/semi-microcell holder. This is particularly beneficial when low volume/high path conditions are required, a good example is the scan of aromatic region of proteins …..*

*** a compromise in wavelength selection may be used when absorption is sloping up dramatically (for example in protein case α-helix may be more easily followed at 225-230 rather than at 222nm).*

2. best operating mode is typically keeping PM tube at constant value while monitoring separately CD and DC signal. Corrected CD intensity can be recalculated after run by the ratio of CD with DC, while DC signal itself is proportional to transmittance. This approach however calls for a bit of care, since it pays to load in advance manually the SF cell with sample in order to set-up the PM tube HT voltage to give a DC signal as close as possible to 1V to avoid saturation while keeping an ample measuring range.

3. using the original Jasco software parameters should be optimized as follows:

a- a larger bandpass will call for less HT voltage and so less noise. So use larger bandpass allowed by your sample spectra (as measured in point 1), you may also accept compromises keeping it larger than normal*.

b- number of acquired data point will limit the speed of transfer of data to the PC (you must await full data transfer to Spectra Manager™ before starting next shot), so do not oversample**.

c- first shot is always bad: make a preliminary shot if you are using automatic accumulation

d- last shot may be bad too, so accumulate 1 time less than the number of shots allowed by SFM software

** with prism monochromator you cannot gain much in the UV, for example on Jasco Js you get 3mm slits (maximum) at 214nm selecting 3nm bandpass.*

*** PC RAM memory may limit further the transfer speed. Using old PCs pls close other graphic windows in advance.*

4. while a qualified engineer has installed the unit, it pays from time to time to check alignment. A simple way is to run baseline and spectra of a reference sample (for example 0.06% aqueous solution of ammonium-d-camphorsulfonate) in a 1 cm cell:
-placed in standard sample compartment
-positioned in place of the stopped flow cell
and
-filling the stopped flow cell with same solution
In case of strange results (a certain spectral distortion/loss of intensity can be accepted), pls open sample compartment (to shut-off the PM tube), remove the SF cell, set wavelength to 540nm with 2nm slits, and inspect with a small piece of paper proper alignment of the green sample beam in the SF head.
You can even remove the PM tube and check alignment/proper focussing with the cell fitted.

5. last ……, obvious, but essential remark: keep the SF unit clean, particularly wash it fully after the use, do not let salts dry inside, remove the SF cell when not in use and keep it in a safe place.

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