

## FDCD-2, A FEW SIDE COMMENTS

At the 8<sup>th</sup> International Conference on Circular Dichroism held in Sendai several people were talking about FDCD (see also TR 018).

### *Problems behind:*

If sample exhibits photoselection data are not reliable since large artifacts are present. These are caused mainly by the imperfect nature of the PEM modulator.

It's practically similar to the problem in getting proper CD signal in transmission mode when dealing with oriented samples.

A solution to this problem was proposed many years ago<sup>1</sup>. The set-up includes two photomultiplier tubes placed at 90° and 270°, this arrangement improves also sensitivity, since more emitted photons are collected.

At CD-2001 Jasco presented a poster illustrating correction in the conventional single PM tube design (placed at 90°) using a simple linear polarizer in horizontal position<sup>2</sup> in the emission path.

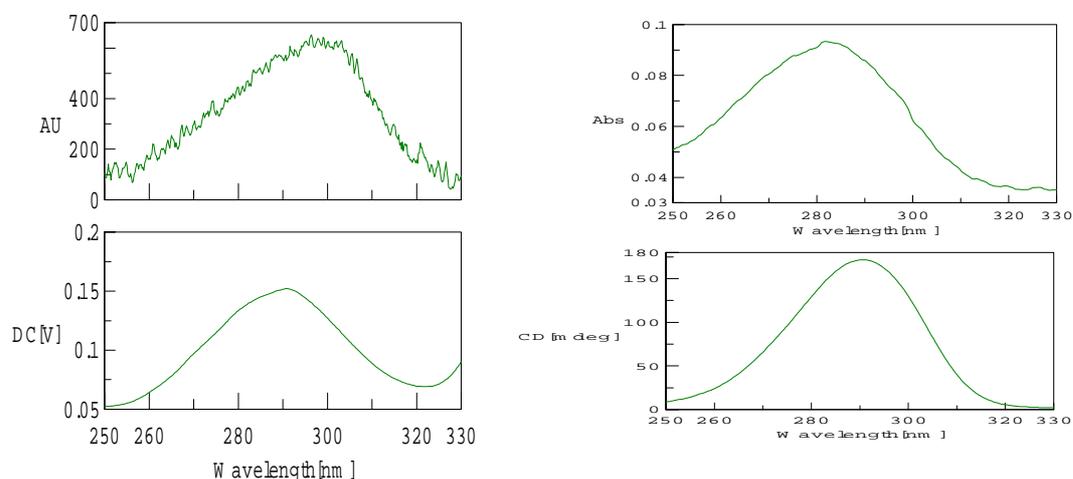
A polarizer however means a sizeable loss of sensitivity, so the approach pays only when required.

### *How to sort out if required?*

Best thing is to use a spectrometer with linear dichroism accessory, i.e. the capability to detect signal at twice the modulation frequency. In this mode you can detect (even simultaneously with small modifications) also the polarization of fluorescence P.

### *How to check basic performances?*

This is somehow another problem. To sort it out we tested FDCD data from a .055% aqueous solution (in 10 mm cell) of ammonium-d-camphorsulfonate (typical CD scale standard). Accessory used was home built, a 340nm long pass filter was in the emission path, 4nm SBW were used and high voltage on PM tube was fixed at 750V.



<sup>1</sup> Lobenstine E.W., Schaefer W.C., Turner D.H., *J.Am.Chem.Soc.*, 103, 1991, 4936-4940

<sup>2</sup> or nearly so, depending on the solid angle of the detection system, in this specific case best correction was achieved at 81° rather than 90°.

Figures above show in the left side FDCD (detected as FDCD/DC) and DC (i.e. total fluorescence) .  
On the right side you see the CD spectra and the absorption spectra of the same solution (absorption spectra calculated by HT→Abs conversion).

Both fluorescence and FDCD data are not *corrected* (fluorescence should have same shape of absorption, while FDCD should be same as CD), while correction of both data is in theory possible<sup>3</sup> it's not such a trivial job and probably it doesn't pay for the effort.

*What's best way to operate:*

On modern units (like the Jasco J-800) you can collect FDCD keeping the high voltage on PM tube in automatic or manual mode.

The other alternative is to operate at constant high voltage on PM tube and collect FDCD/DC on Ch1 and DC (total fluorescence) on Ch2.

But best accuracy in manual high voltage mode is obtained collecting FDCD on Ch1 and DC on Ch2.

Sample and solvent should be measured sequentially in this way, than you should subtract solvent from sample signals in order to get baseline corrected FDCD and DC, finally the FDCD file should be divided by the DC file in order to get the proper FDCD result.

This procedure too is rather boring, but it's necessary when you deal with weak signals.

*Conclusion:*

Technique seems easy: it's enough to place the PM tube at 90° port and to use a simple lens collection system + filter. But solvent baseline compensation and spectra correction are not trivial, if you add on top the potential photoselection problem of the sample, the matter becomes complicated.

Fortunately applications are mainly analytical, so correction is typically not necessary, while availability of LD accessory has to be strongly recommended to sort out potential photoselection problems and correct them (if necessary) with a suitable linear polarizer.

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<sup>3</sup> correction of fluorescence (DC) is necessary since lamp/monochromator intensity is not achromatic. Rhodamine B can be used or a second PM tube in the transmission path .....  
correction of FDCD should take into account the absorption of the sample .....