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# ARTIFACTS FROM SCATTERING

In a previous report about absorption flattening artifacts we mentioned the need to talk about potential errors coming scattering.
The conventional approach to *compensate* for forward scattering consists in the possibility to move the sample as close as possible to the photomultiplier tube window (38mm diam for the standard R-376) in order to increase the solid angle collection[1]. Changing distance (and so acceptance angle) between the PM tube and the detector you can somehow quantify the extent of the differential light scattering; this is also the base on which commercial accessories have been developed (for example the Jasco SCAT-441).
Another approach for the same target was the *fluoroscat* cell[2]: a specially shaped cell filled with a fluorescent solution surrounds the sample and captures all the transmitted and most of the scattered light. So the PM tube will measure the fluorescent light generated by both transmitted and scattered components.
A further way was proposed[3], in which fluorescent molecules are added directly to the sample.

But what's effect of scattering in spectral shapes?
This is not so clear despite the fact that a few theoretical papers have been presented[4 5 6].
In some recent papers you'll find that scattering is felt as the reason for presence of CD bands without any corresponding absorption[7] or change of relative intensities between bands[8].
Dealing with membrane proteins scattering is usually present and felt as responsible of spectral differences[9 10 11] even if more recently spectral changes have been attributed to differences in the dielectric constants of the surrounding hydrophobic environment[12].

Can we reduce scattering?
In several cases proper sample preparation may reduce the scattering ….. sample centrifugation, sonication or use of refractive index matching solvents may minimize the effect.

Can we measure scattering component?
Geometrical approaches are possible: see for example here enclosed a poster presented years ago at 7th CD conference in Mierki, Poland in Aug 1999: while UV operation may be limited (in any case it's necessary to use a very good quality lens) the device is very simple.


[1] Schneider A.S., Harmatz D., *Biochem.*, 15, 1976, 4158
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[3] Tinoco I.Jr., Maestre M.F., Bustamante C., *TIBS*, 8, 1983, 41
[4] Kokhanovsky A., *J. Opt. A: Pure Appl.Opt.* 4, 2002, 288
[5] Kokhanovsky A., *Int. J.. Electron. Commun. (AEU)*, 55, 2001, 240
[6] Vitkin A., Hoskinson E., *Opt. Eng.*, 39, 2000, 353
[7] Abe H., Nakanishi H., *Anal. Sciences*, 19, 2003, 171
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[10] Park K., Perczel A., Fasman G.D., *Protein. Sci.*, 1, 1992, 1032
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[12] Wallace B.A., Lees J.F., Orry A.J.W., Lobley A., Janes R.W., *Protein Science*, 12, 2003, 875



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# CD SPECTRA OF PORPHYRIN AGGREGATES USING A NEW ACCESSORY FOR SCATTERING SAMPLES

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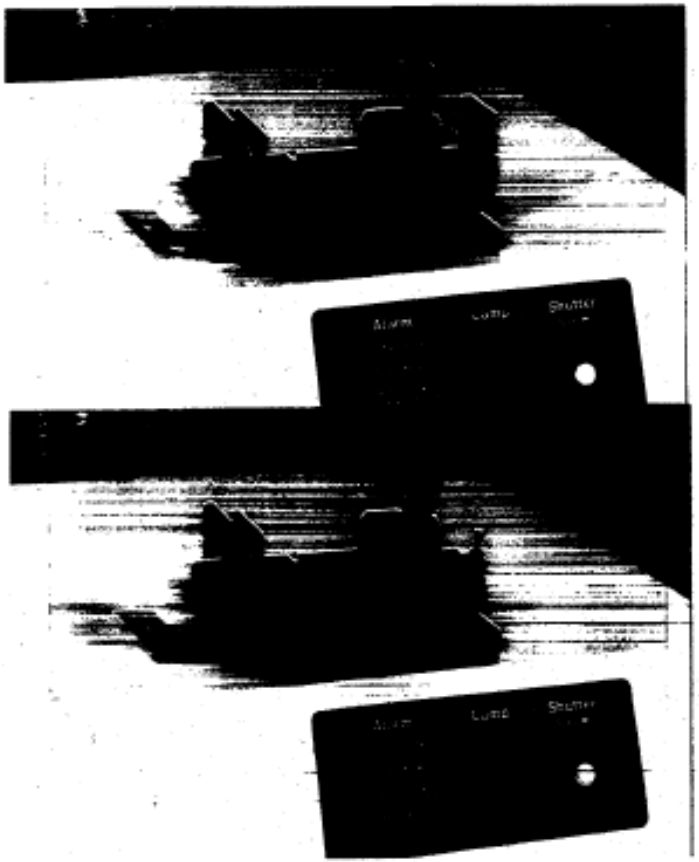
**The capability to obtain reliable CD spectra from scattering samples has always been a problem.**
**Usual approach is very empirical: if scattering is supposed to interfere, good operating practice suggests to collect different spectra while changing the position of the sample in the compartment ….. as close and as far as possible from the photomultiplier tube …..**
**This approach, which is particularly valuable when the CD spectropolarimeter has collimated light path through the sample compartment, allows to change somehow the collecting angle of the detector: spectral differences can be so attributed to scattering.**

## ACCESSORY DESIGN

**The accessory consists in a focusing lens mounted in a rectangular cell holder.**
**The unit is placed toward (nearly in contact) the active surface of the photomultiplier tube. A pin at the focal point accepts either a slit or a light stopper.**
**Three types of measurement are possible:**

- **Direct transmitted CD (with nothing at the focal pin)**
- **Unscattered CD (with slit mounted)**
- **Scattered component (with stopper in)**



## MEASUREMENT CONDITIONS

### Samples:

**Induced Circular Dichroism (ICD) in the Soret region took place adding L and D phenylalanine (about 8x10-3M) to $H_2$TPPS and CuT4 (about 2x10-6M each) porphyrin in ultrapure water.**
**The obtained solutions are cloudy, indicating the formation of large aggregates. This fact stimulated our interest to sort out a way to evaluate if scattering may alter the resulting CD spectra.**
**Measurements were performed on a 10 mm path rectangular quartz cell.**

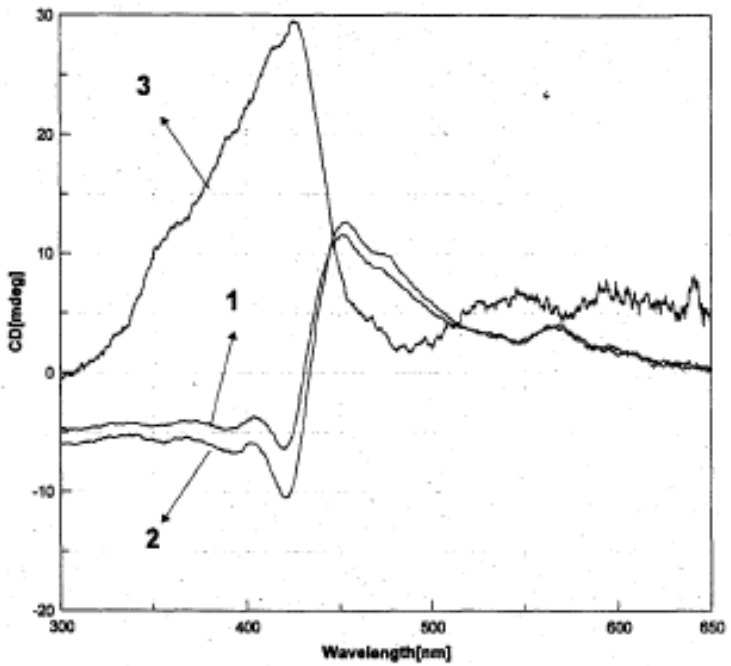
### Instrument:

**J-600 (Jasco Corporation Japan)**
**Nitrogen flow rate 3 ml/min**

### Scanning parameters:

**-single scan**
**-100 nm/min scanning speed**
**-2 nm SBW (constant bandpass mode)**
**-response time 1 sec**
**-data pitch 0.2 nm**
**-no baseline correction**

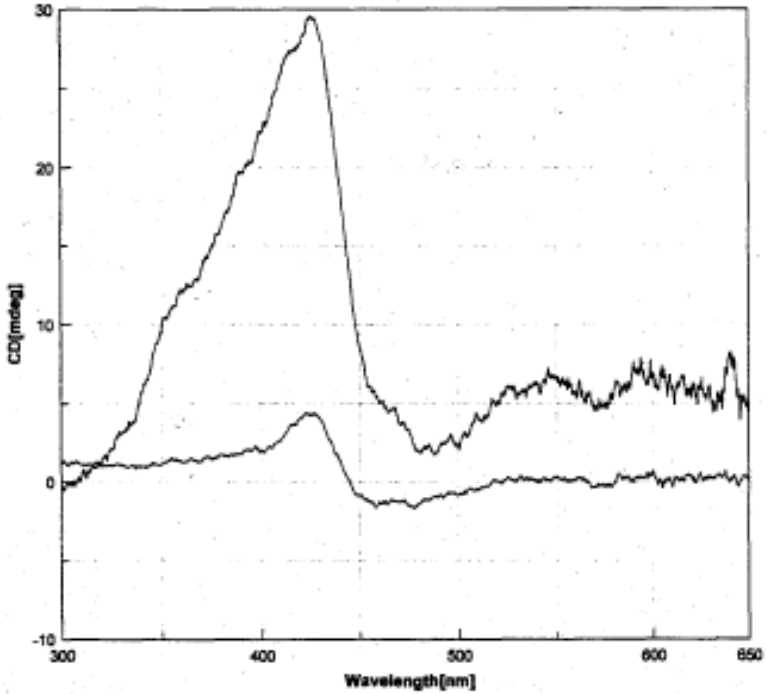
## PORPHYRIN BOUND WITH L PHENYLANALINE A




Three spectra are shown:

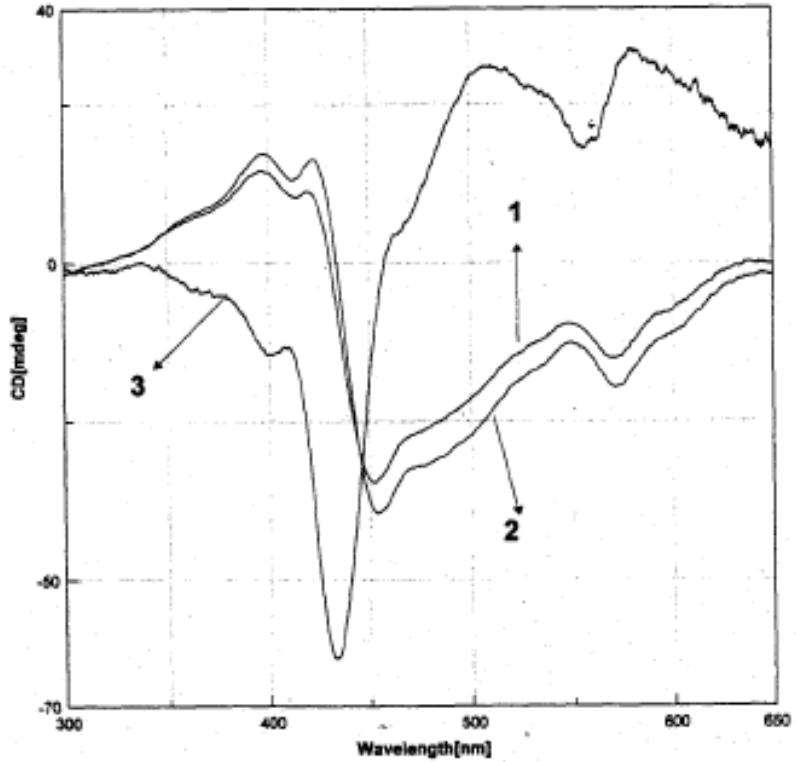
1 - total (no slit, no target)
2 - unscattered component (with slit)
3 - forward scattered component (with light target)

## PORPHYRIN BOUND WITH L PHENYLANALINE B




Measured scattered component versus the calculated one (difference between spectra 1 and 2). The % of scattering may be estimated around 5% with current actual sampling geometry.

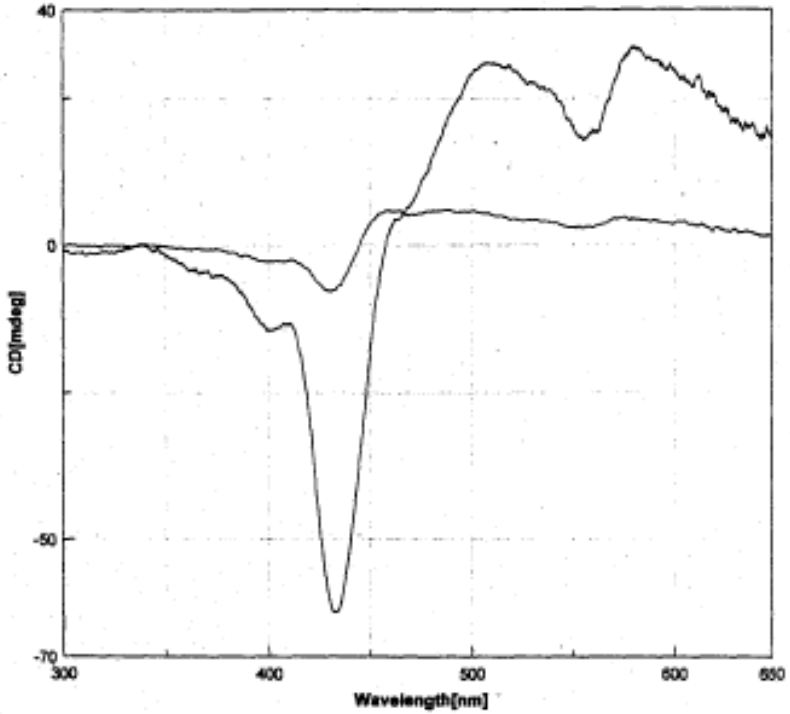
## PORPHYRIN BOUND WITH D PHENYLANALINE A




Three spectra are shown:

1 - total (no slit, no target)
2 - unscattered component (with slit)
3 - forward scattered component (with light target)

## PORPHYRIN BOUND WITH D PHENYLANALINE B




Measured scattered component versus the calculated one (difference between spectra 1 and 2). The % of scattering may be estimated around 6% with current actual sampling geometry.

# CONCLUSION

The simple accessory presented here seems to do its job properly.
Its main feature is the easy operation. All three measurements can be carried on without removing/touching the sample.

Trapping regularly transmitted light with a stopper calls here for the use of a focusing lens.
Lenses before the sample are potentially inducing birefringence artifacts, so attention is required for proper use.

We are currently investigating different, alternative, approaches:

-sliding photomultiplier tube assy, in which the PM tube can be moved in contact with the sample, getting very large solid angle collection.
-mild focusing lens before the PEM modulator to achieve relatively small beam spot on sample (easy to trap out and remove directly transmitted component)