

Fwd: CD spectrometer: important information

Mark Foster [foster.281@osu.edu]

Sent: Tuesday, October 05, 2010 5:23 PM**To:** Eric Danhart [danhart.1@osu.edu]; David Smith [smith.5551@osu.edu]; BRANDON CROWE [crowe.184@osu.edu]; Adhytia Putra [putra.1@osu.edu]; Sri Vidya Oruganti [oruganti.1@osu.edu]; Elihu Ihms [elihuihms@gmail.com]; Mary Anne Refaei [refaei.1@osu.edu]; Ian Kleckner [kleckner.5@osu.edu]; Tim Wang [wang.999@osu.edu]; Ian Smith [smith.5888@osu.edu]; MARK FOSTER [foster.281@osu.edu]**Attachments:** CD calibration.pdf (72 KB) ; ATT00001.htm (7 KB)

Begin forwarded message:

From: Thomas J Magliery PhD <magliery@chemistry.ohio-state.edu>**Date:** October 5, 2010 2:36:12 PM EDT**To:** faculty@chemistry.ohio-state.edu, brooks.8@osu.edu, breitenberger.1@osu.edu, "Michael K. Chan" <chan@chemistry.ohio-state.edu>, "Donald H. Dean" <dean.10@osu.edu>, "Mark P. Foster" <foster.281@osu.edu>, Venkat Gopalan <gopalan.5@osu.edu>, 'Karin Musier-Forsyth' <musier@chemistry.ohio-state.edu>, Jennifer Ottesen <ottesen.1@osu.edu>, Zucui Suo <suo.3@osu.edu>, "Richard P. Swenson" <swenson.1@osu.edu>, wang.892@osu.edu, Justin Wu <wu.473@osu.edu>, Dongping Zhong <dongping@mps.ohio-state.edu>, behrman.1@osu.edu, marzluf.1@osu.edu, James Hopper <hopper.65@osu.edu>, Jane Jackman <jackman.14@osu.edu>, Charles Bell <bell.489@osu.edu>, Ralf Bundschuh <bundschuh@mps.ohio-state.edu>, means.1@osu.edu, Ross Dalbey <dalbey@chemistry.ohio-state.edu>, mpoirier@mps.ohio-state.edu
Cc: Besik Kankia <bkankia@chemistry.ohio-state.edu>, lab group <maglierygroup@chemistry.ohio-state.edu>**Subject: CD spectrometer: important information**

This message is for users of the Chemistry circular dichroism spectrometer. If your lab doesn't use CD, please disregard.

Attached you will find a spectrum of d-CSA and a reference spectrum. You will notice that it is basically featureless at shorter than 225 nm (although well-calibrated at longer wavelengths). Also note that the dynavoltages between 210-225 nm aren't especially alarming, despite there being no meaningful signal in the range. For some time, we have been aware that there were sensitivity issues with the Aviv CD for beta-sheet proteins, especially. However, recently we found that in UV-opaque samples the dynavoltages were not being reported by the machine as especially high (like 350-450, where >500 is typically taken as too little light to be of significance).

Here is the take-home message. Any spectrum with features between 205-225 nm taken in the last 6-12 mo. is probably suspect. Difference spectra (such as thermal melts) for helical proteins may be OK, and spectra for nucleic acids (with longer wavelength features) are probably fine. Spectra for beta-sheet proteins are probably not meaningful.

Because of this and other problems, we recently purchased a new CD, funded by the Chemistry department, Karin Musier-Forsyth's BICF funds, and my start-up funds. It took over two months for the order to pass through sole source approval, but Jasco was able to ship it almost immediately. The new spectrometer will be installed tomorrow in 0041 MP. I will forward information shortly on training for that instrument.

Besik and I are trying to determine if we can get the Aviv repaired.

However, the main optical mirror is no longer available, and its deterioration is almost certainly the main reason for the lack of far UV sensitivity. There may also be a problem with the D/A converter, however, since the dynavoltages are not being reported correctly. The first time we inquired, Aviv told us that it would be better to replace the instrument than to repair it. Until further notice, I recommend that you do not use the Chemistry Aviv CD spectrometer for proteins or other molecules with features at shorter than 225 nm. I also recommend that you check samples with a UV-VIS spectrometer for $A < 1$ (at the CD path length) at the wavelength of interest even if it's > 225 nm.

Please contact me or Besik with questions.

Tom

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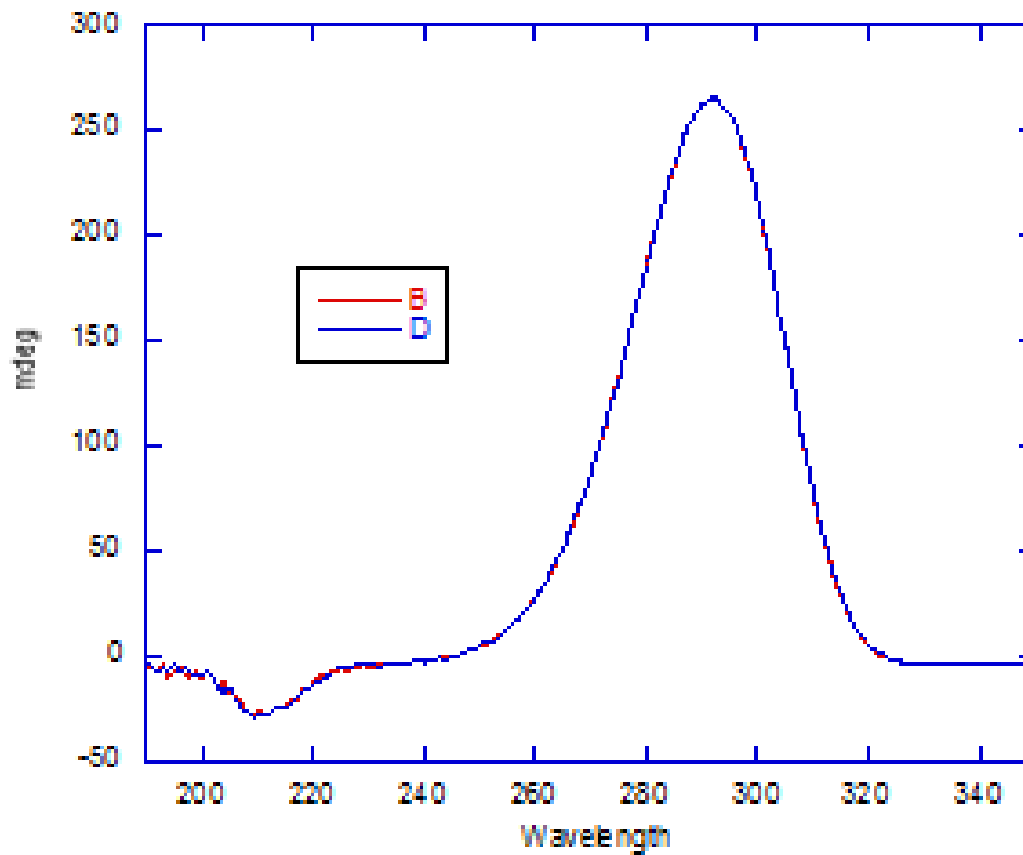
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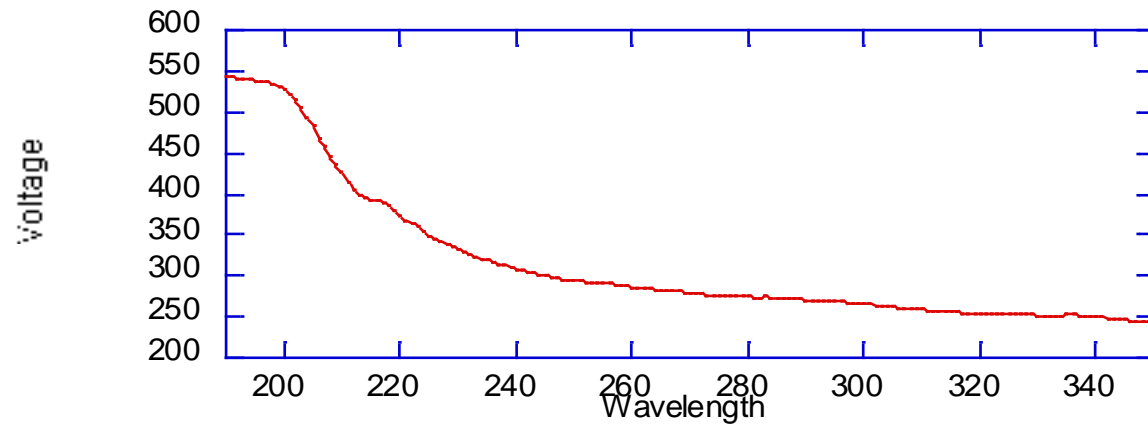
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d-CSA
 $A(285\text{nm}) = 0.1228$
 $l = 1 \text{ cm}$
expected:
+276 mdeg @ 290 nm
-414 mdeg @ 200 nm
wavelength scan on this
page, dynavoltage on next
page



of ΔA or ellipticity. For a given protein, different samples or mutants may be compared by calculating $\Delta A/(c \times l)$, with c in mg/ml.

Owing to the variety of units found in the literature and in software—e.g., mdeg and deg for ellipticity, and mm, cm or dm for path length—it is necessary to take care that experimental parameters are entered in the units requested by the machine software.

The Strategic Planning section deals with considerations regarding instrumentation and reagents for CD spectrometry. The Basic Protocol outlines the steps in recording a CD spectrum. The two support protocols explain the interpretation of CD spectra—Support Protocol 1 deals with near-UV spectra and Support Protocol 2 with far-UV spectra.

STRATEGIC PLANNING

CD Spectrometer

CD spectra are measured using a CD spectrometer, of which there are three different makes on the market (AVIV Associates, Jasco, and Applied Photophysics; see *SUPPLIERS APPENDIX*). The first two are classical spectrometers with some capability for studying fast reactions by CD, while the third is specifically designed for fast-reaction CD. These instruments are fitted for control, data collection, and data handling by computer. The principles of how they function are generally outlined in the manufacturers' manuals and a good general account is given in Bayley (1980). A supply of high-purity nitrogen is essential to displace oxygen in order to avoid degradation of the mirrors by ozone generated by the high-power xenon sources, as well as to reduce absorbance from oxygen bands below 200 nm (see discussion of Nitrogen Supply, below). A satisfactory means of thermostating cells is essential for work with proteins (see discussion of Cells, below), using a water-circulation system or the more convenient Peltier thermostat.

The CD spectrometer is usually required to work near the limits of sensitivity—e.g., reading ΔA values of $\leq 10^{-4}$ at a total absorbance of 1. Thus, particular care needs to be taken with cleanliness and orientation of cells and with settings of scan rate, time constant, and bandwidth. It is also important, especially when recording far-UV spectra, that the lamp is not old and that the mirrors are not clouded from radiation and traces of ozone. Because the spectrometer is a single-beam instrument, it is essential always to watch carefully for evidence of instrumental drift during measurements of sample and baseline.

Calibration of the Spectrometer

Depending on the make of the spectrometer, wavelength calibration may require the use of a mercury lamp and the services of an engineer, or the use of a holmium oxide solution with a simple computer-operated adjustment. It is unlikely to vary significantly during the lifetime of a xenon lamp. Calibration of ellipticity, on the other hand, must be carried out regularly—at least monthly, if the machine is in regular use—using a purified compound of known absorbance and ellipticity. D-(+)-10-camphorsulfonic acid (CSA) is the substance of choice (Fig. 7.6.7), but care must be taken to purify it from optically active impurities. Crystallization from ethyl acetate is recommended. CSA is hygroscopic; hence, although a stable monohydrate can be prepared (Yang et al., 1986), the concentration of the aqueous solution (~ 1 mg/ml) should be determined from $A_{285}^{1 \text{ mg/ml}} = 0.149$ using a high-quality spectrophotometer. Calibration of the spectrometer is carried out according to the maker's instructions, using $\theta = 33.5$ mdeg at 290.5 nm for a solution of 1.00 mg/ml anhydrous CSA in a 1-mm path-length cell. CSA exhibits a second peak at 192.5 nm where $\theta \approx -67.0$ mdeg for the same solution. If the value of the ratio of ellipticity at the two wavelengths falls below 2.0, this indicates a loss of performance of the spectrometer. Epiandrosterone,

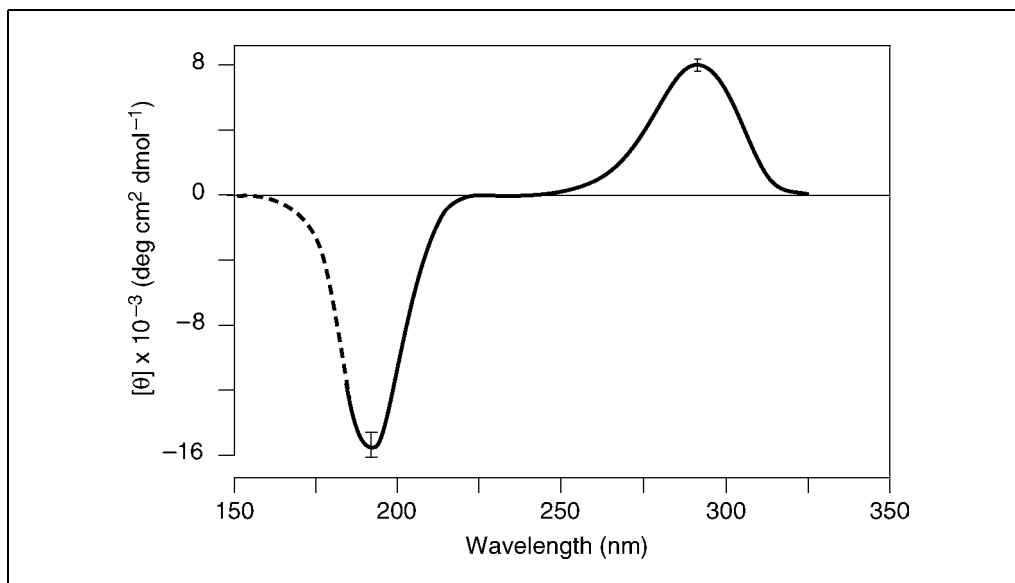


Figure 7.6.7 CD spectrum of D-(+)-10-camphorsulfonic acid (CSA) in water. The vertical bars represent variations of $\pm 1.5\%$ and $\pm 5\%$. The broken line represents the extrapolation of a gaussian band. Commercial CSA was twice recrystallized. (From Chen and Yang, 1977.)

at 304 nm, and D-(–)-pantoyllactone, at 219 nm, have also been used for calibration (Schmid, 1989). The peaks of the spectra scanned for calibration are sufficiently sharp to provide a check on the wavelength calibration.

Cells

Choice of cells

Quartz windows can exhibit dichroism, which is due to strain either remaining after the annealing process during manufacture or induced by distortion caused by heating or pressure. It is important for measurement of CD that the optical faces of the quartz QS cuvettes have low strain and exhibit low dichroism. In the near-UV region—where path lengths ≥ 5 mm are required—rectangular cells, in their standard, semimicro, or micro forms, are normally used. These are not only more economical with regard to protein than the cylindrical type, but allow mixing to be carried out in the cell. Testing a number of rectangular cells in the CD spectrometer usually yields one or two with low dichroism; it is also possible to purchase cells with certificates of low dichroism. Careful observation of the height of the light beam at the cell allows the minimum volume of solution to be used—usually < 1 ml in a 10×10 -mm cell. Because standard quartz (Suprasil) cells are constructed from two different types of glass, differential expansion of the faces with temperature leads to a significant change in cell dichroism. At least for temperature-dependence experiments, it is recommended to use cells with all four faces made from fused quartz. Semimicro cuvettes with 1-cm path length are available; these require ~ 300 μ l of solution.

For far-UV CD spectra, short-path-length cells—from 1 mm down to 0.01 mm—are necessary because of the higher absorbance and ellipticity in this region. Demountable cells, cylindrical or rectangular, of this size are easily cleaned, but require practice to fill without allowing evaporation and may be subject to solvent loss over long periods of time. Such leakage can be minimized by the use of Parafilm on the edges of the cells. Quartz-jacketed cylindrical cells with two filling holes, available from Hellma (see *SUPPLIERS APPENDIX*), provide the best economy of protein, temperature control, and reproducibility.