

**ABOUT LD (Linear Dichroism)**

When we talk of Linear Dichroism, we all know that it's the difference in absorption of light linearly polarized parallel and perpendicular to an oriented status.

LD measurements are easily obtained on a CD spectropolarimeter if:

-equipped with pertinent accessory (a doubled frequency lock-in amplifier) matched with an LD program for the piezoelectric modulator (to operate as ½ wave rather than ¼ wave retarder)

-in a pure optical way inserting an Oxley prism or a Fresnel rhomb in your CD path

The usual problem in LD is to get proper sample orientation (films can be stretched, molecules can be absorbed on polymer films; magnetic or electrical field, squeezed gel, are the most common orientation systems), but most popular system in case of biological samples is flow orientation.

Flow orientation methods for biological applications (DNA ...):

The classical approach is the Couette flow cell described by Wada¹. The solution containing the sample is subjected to a constant gradient over the annular gap between an inner cylinder (rotating at high speed) and a fixed outer cylinder. Both cylinders are made of quartz to freely pass observation light. This accessory was available many years ago from Shimadzu (model QV-12 for their QV-50 single beam spectrophotometer). Total sample volume required is 3 ml. Several clones have been reproduced later on and we know that in the future a similar device, as published by Rodger², may become commercial.

With these devices the shear rate can be calculated as follows:

$$G \text{ (sec-1)} = dv/dR = 2\pi Rv/[60(R_o - R_i)]$$

Where:

v is the rpm speed of the rotating cylinder

R_o is the inner radius of the external cylinder

R_i is the outer radius of the inner cylinder

Assuming (as from the original commercial accessory) R_o=15 mm and R_i=14.5 mm and a maximum speed of 1000 rpm, we can calculate G up to over 3000/sec at maximum speed.

The simplest alternative is to use a short path flow-through cell with a recirculating, high flow rate pump.

Shear rate in a flow cell can be calculated as:

$$G \text{ (sec-1)} = \text{flow speed} / \text{cell path}$$

Where:

flow speed is expressed in mm/sec

cell path in mm

So for example if we use a 0.1mm path cell with internal width of 8 mm, when we pump our solution at 20 l/min we will have a shear rate of over 4000/sec at maximum flow rate.

Total volume required by this approach can be kept down to about 2 ml.

The second approach has the obvious disadvantage of a much shorter optical path (.1 versus 1 mm), but usually sensitivity is not the major concern here, while a shorter path may be a must in far UV measurements (here too rare in this case).

Last, for LD you cannot miss the excellent Norden³⁴ presentations.

¹ A. Wada, S. Kozawa, *J. Polym.Sci. Part A:Polym.Phys.* 2, 1964, 853

² A. Rodger, *Linear dichroism, Meth.Enzymology* Vol 226, 1993, 232,

³ B. Norden, M. Kubista, T. Kurucsev, *Linear dichroism spectroscopy of nucleic acids, Q.RevBiophysics*,25,1992,51

⁴ B. Norden, *Applications of Linear Dichroism Spectroscopy, Appl. Spectrosc. Rev.*, 14, 1978, 157