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HOW TO CALIBRATE SHORT CELLS PATHLENGTH

In a well known paper[1] Curtis Johnson sorted out main source of errors analyzing circular dichroism spectra of proteins. In between other aspects he pointed out the need to check accurately the cell pathlength.
This may be a crucial issue when dealing with the short paths necessary to scan the low UV range.

For example Hellma[2] is giving the following light path tolerances:

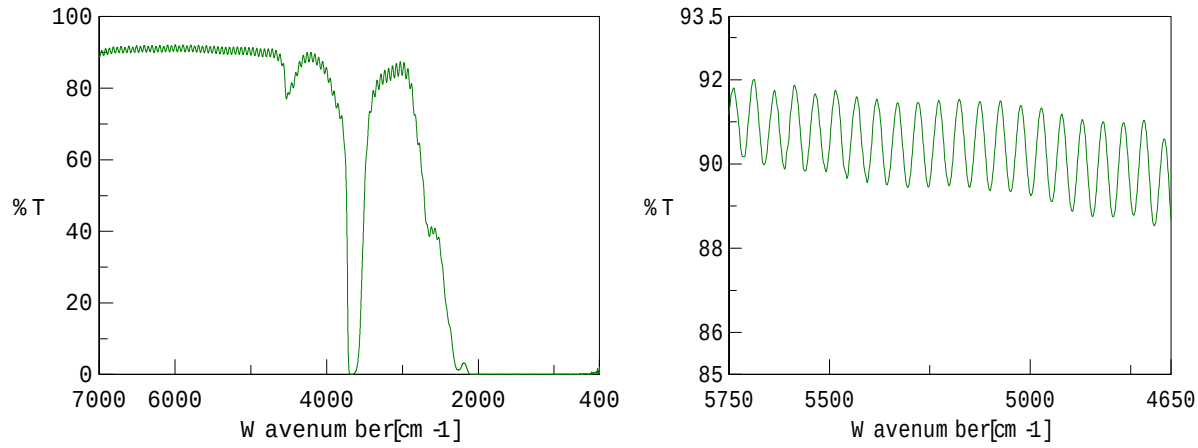
± 0.003mm	for paths between 0.01 and 0.05mm
± 0.005mm	"	"	"	0.1 and 0.2mm
± 0.01mm	"	"	"	0.5 and 20mm

You may verify exact path using dichromate solutions, but high concentrations are necessary and you must rely on your sample preparation in addition to the accuracy of the spectrophotometer you are using for (which often will not accept cylindrical cells ….).
Fortunately there is a physical way to do the job: measure cell path by interference fringes[3].
For 0.01 to 1mm paths this is easily carried on using an IR spectrometer.

$$d = (n/2) \times 1/(\nu_1 - \nu_2)$$

Where n is the number of interference fringes between ν_1 and ν_2 (in cm^{-1}) and d is the path in cm units.

Figure below shows the entire and a zoomed portion of the IR spectra of an empty Hellma 121.000 quartz cell of 0.1mm nominal path, run on a Jasco FT/IR-420.



Here we used the film thickness software provided with this IR (as in most of current FT/IR units), so it was easy to calculate the exact path of 97.835 μm, i.e. over 2% less than what stated. It's expected that shorter path cells will show even larger relative errors.

[1] Hennessey J.P., Johnson W.C.Jr., *Anal. Biochem.*, 125, 177 (1982)
[2] Hellma Worldwide Catalogue, 66/97-1
[3] Smith D.C., Miller E.C., *J. Opt. Soc. Am.*, 34, 130 (1944)

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