

ARTIFACTS FROM ABSORPTION FLATTENING

There are two potential source of errors dealing with spectra of suspensions: scattering and absorption flattening effects. While for first proper hardware modifications may improve results, the second one is strictly related to sample nature.

You get absorption flattening, i.e. an underestimate of proper signal intensity, when sample is not homogeneous. A typical case is IR spectra of KBr pellets in which the sample is not properly and uniformly dispersed in the KBr matrix.

For ordinary solutions containing a very large number of independently moving chromophores, the relative concentration fluctuations are extremely small, and the Beer-Lambert law will be obeyed. In solutions of large macromolecules, aggregates or microparticles, each containing a large number of chromophores and thus having a high optical absorption, the number of freely moving particles may, however, be considerably smaller, and the local concentration fluctuations may become important...¹

This is true for any absorption spectroscopy technique. An early article² by Holzwarth, a man who strongly innovated CD technique (see T.R. 47), proposed a model to calculate correction factors for both normal UV-VIS absorption and CD spectra for suspensions of particles with a few idealized shapes.

To give an empirical idea we present here a much simpler and approximate example, assuming that our sample should have, in homogeneous conditions, an O.D. of 1, while the solvent has 0 O.D.

We consider now three cases: in which the light is free to pass freely through 50%, 10% and 1% of the cuvette:

For regular absorption we will have:

Case	Incoming photons	Outcoming photons	Correct O.D.	Measured O.D.	error
50%	100	$50 + 0.5 = 50.5^*$	1 Abs	0.296 Abs	-70%
10%	100	$10 + 6.97 = 16.97^{**}$	1 Abs	0.770 Abs	-23%
1%	100	$1 + 9.76 = 10.76^{***}$	1 Abs	0.968 Abs	-3%

* since 50 pass through and 50 are attenuated by 2 O.D.

** since 10 pass through and 90 are attenuated by 1.11 O.D.

*** since 1 pass through while 99 are attenuated by 1.01 O.D.

Let's now move to CD case, here we assume to measure CD as AC/DC on our PM detector kept at constant voltage:

Case	AC	correct DC	measured DC	Correct CD	Measured CD	error
50%	X	10	$50 + 0.5 = 50.5$	X/10	X/50.5	-80%
10%	X	10	$10 + 6.97$	X/10	X/16.97	-41%
1%	X	10	$1 + 9.76$	X/10	X/10.76	-7%

From above it's clear that absorption flattening may give a sizeable underestimation of the absorbance value, but also that error induced in CD measurement is even higher.

Not shown, but easy to calculate are the errors at different *true* absorbance levels, they increase with these.

Theoretical approaches and practical sampling procedures have been applied to compensate absorption flattening, but real compensation is a matter of art^{3 4}.

¹ Wittung P., Kajanus J., Kubista M., Malmstrom B.G., *FEBS Letters*, 352, 1994, 37

² Gordon D.J., Holzwarth G., *Arch. Biochem. Biophys.*, 142, 1971, 481

³ Mao D., Wallace B.A., *Biochem.*, 23, 1984, 2667

⁴ Wallace B.A., Kohl N., Teeter M.M., *Proc. Natl. Acad. Sci. USA*, 81, 1984, 1406