

## **Correcting for Background Absorbance (Example)-UV-VIS ChemStation Software Rev.A.06.XX-A.08.XX**

This example uses two spectra of a yellow dye, one measured as a pure dye and the other in presence of scatter. It demonstrates the use of a single internal reference, three-point drop-line and derivatives to correct for a background absorbance.

Scatter causes a background absorbance, which depends on the wavelength. In the visible part of the spectrum, the background changes only slowly and is close to being constant; in the UV portion it increases rapidly with decreasing wavelength.

### **Single Internal Reference**

1. Clear all sample spectra and select a **New Method**
2. Load the data file stdexam1.sd as samples.
3. Switch to the **Fixed wavelength** task.
4. Set the **Use wavelength** to 414 nm, the wavelength of the absorbance maximum.
5. Select a **Single reference wavelength** for background correction at the wavelength 500 nm.

## Result:

#	Name	net Abs <414 nm>	Bkgd <500 nm>	Abs <414 nm>
1	Original dye	0.15898	0.00092	0.1599
2	Dye with scatter	0.16299	0.00987	0.17287
Difference:		2.5%		8.1%

The influence of the scatter on the reading at 414 nm is reduced by a factor of 3 by the single reference wavelength.

## Three-Point Drop Line

A three-point drop-line can be used to compensate for a background absorbance with a constant slope. In this example, the two reference wavelengths which define the slope, are both selected at higher wavelengths than the analytical wavelengths (commonly, a reference wavelength is selected on either side of the analytical wavelength).

6. Select **Three-point drop-line** for background correction at the wavelengths 500 nm and 800 nm.

The absorbance readings at 500 nm and 800 nm will define the slope of the background absorbance, which is used to extrapolate the background absorbance at 414 nm.

## Result:

#	Name	net Abs <414 nm>	Bkgd <500 nm>	Bkgd <800 nm>	Abs <414 nm>
1	Original dye	0.15874	0.00092	0.00008	0.1599
2	Dye with scatter	0.16106	0.00987	0.00311	0.17287
Difference:		1.5%			8.1%

The influence of the scatter on the reading at 414 nm is reduced by a factor of 5 by three-point drop-line.

### First Order Derivative

7. Set the **Use wavelength** to 444 nm, a wavelength in the slope of the absorbance band.
8. Select **none** for background correction and select **Derivative 1** as data type.

### Result

#	Name	d1(Abs) <444 nm>
1	Original dye	-6.7946E-3
2	Dye with scatter	-6.8162E-3
Difference:		0.3%

In this case, the influence of the scatter is almost completely removed.

A constant background absorbance over a large wavelength range can be eliminated using an internal reference or first order derivative.

A background absorbance with a constant (linear) slope over a large wavelength range can be eliminated by a three-point drop line or higher order derivatives.