How to perform an Instrument ZERO and Baseline Correction

In SCAN Application

Preamble by Dr Mark Fisher:

The simple answer to your question of why do we zero the instrument is so we get the right answer. Now, to the details since someone will say, but you have a double beam instrument which accounts for differences. While that statement is true, it works for real time corrections in lamp energy changes. The major reason to zero the instrument is to totally balance the beams. No matter how carefully we try, the optics in the reference beam and the sample beam will never be perfectly balanced. By doing a zero, we tell the instrument that there is nothing present and when you see this response, you will call it zero. This also takes into account the offsets seen in the electronics. The op amps used will never have zero output unless there is complex feedback loops and even then a zero input may have a slight positive or negative output. Again, telling the instrument that this level of response is zero eliminates it and allows us to get the right answer.

Now, what is the difference between zero and baseline? A zero is what is done at a single wavelength. A baseline is a collection or “zeros” done at each wavelength in the scan. There is a natural baseline for any instrument and is related to the transfer function of the source, monochromator, and detector. That is why the natural baseline of the instrument is not flat. If you do a zero, it is done at only the starting wavelength. The chances of that zero being the same for all the wavelengths of the scan are less than slim, verging on none. That is why you must do a baseline and not a zero for a scan.

Next you might ask, “Why do we have both zero and baseline functions in Scan?” The answer to that question rests in how long has it been since a baseline was done? Over time, a baseline can drift, so that you have a non-zero value. However, the natural baseline has not changed. Thus, if you re-zero the instrument the entire baseline is moved up or down and you have again accounted for the deviations from zero.
Procedure;

In **SCAN** Application;

In **SETUP,**

- Click on the **Baseline Tab,**
Select Baseline correction.

Click OK

Insert a BLANK (solution without the sample) in BOTH the Front beam and the Rear beam.

Press ZERO (this performs the Instrument Zero at the Scan starting wavelength)
Next, Press **Baseline** (this will perform the scan of wavelengths, store the absorbance values for each Data Point, and call this the Baseline).

Once the Baseline is completed, you will see the word **BASELINE** in the top left box for the Absorbance readings.
Now remove the cuvette from the Front Beam and insert your **Sample**

Press **START** to begin the analysis.

The Result will have the baseline automatically ratio’ed from the Scan. (The correction factor is... ‘Scan÷Baseline’...)

David Grintuch

Customer Service Representative

Online Technical Support Team

Agilent Technologies