application note

Cintra 40—Exceptional Performance for Difficult UV-Visible Applications

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Introduction

There are a number of instrument parameters, which are important in UV-Visible Spectroscopy. These most important of these are Resolution, Stray Light, Photometric Noise, Photometric Drift and Photometric Linearity as these are key to enabling difficult applications to be performed.

This application note explains the significance of these important parameters and how the Cintra 40 performs in relation to these.

The data for this study was generated using the Cintra 40 UV-Visible Spectrometer together with the powerful Spectral Software.

Resolution

The size of the instrument slit width or bandpass has a direct influence on resolution.

The Cintra 40 has a variable slit width from 0.1 nm to 2.0 nm in 0.1 nm increments. The advantage of this is that if high resolution is required, (the ability to separate two very close peaks), as is often required in life science applications, then the bandpass can be set to 0.1 or 0.2 nm.

If high resolution is not required but, more light throughput is required, then the bandpass can be opened to 2 nm.

To illustrate the effect of various slit widths on resolution, one drop of benzene (0.03 mL) was put into a 100 mm path length cylindrical cell (approximately 130 mL capacity). The resultant benzene vapour was scanned from 220 to 280 nm with slit widths of 2.0, 1.0, 0.5 and 0.1 nm.

Figures 1 to 4 show the variation in the benzene vapour scan at various slit widths.

With a 2 nm slit width, eight broad peaks were produced. As the slit width was decreased each broad band was resolved into further peaks. A slit width of 0.1 nm results in over thirty five very sharp peaks for the benzene vapour scan.

Each scan shown has been autoscaled so that the resultant scan fills the graphics page. As indicated by the Absorbance scale on each scan that as the slit width is decreased the absorbance actually increases.

The maximum absorbance using the 2.0, 1.0, 0.5 and 0.1 nm slit width was 1.30, 1.60, 1.80 and 4.60 Absorbance units respectively.

By reducing the slitwidth both the resolution and the sensitivity increases.

Stray Light

Stray light is any light that has a different wavelength from that supplied by the monochromator. It may also be

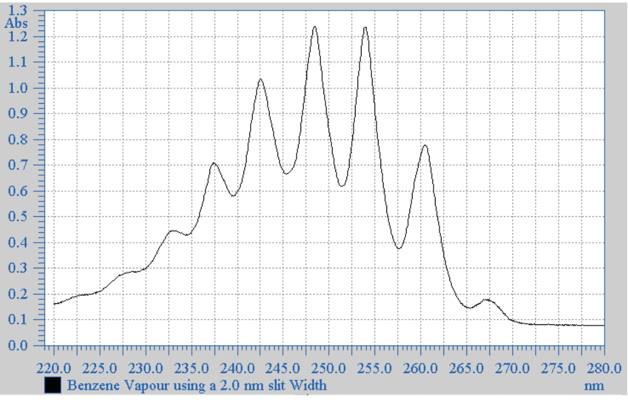

Figure 1:*Benzene Vapour Scan using a 2.0 nm slit width*

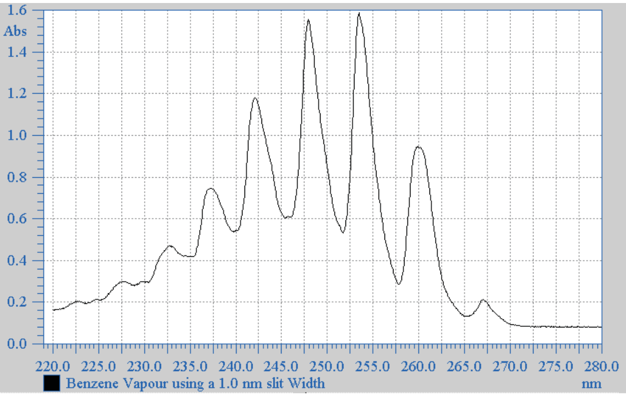

Figure 2:*Benzene Vapour Scan using a 1.0 nm slit width*

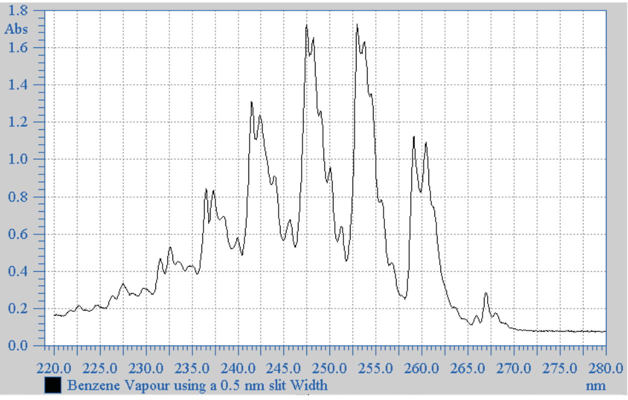

Figure 3:*Benzene Vapour Scan using a 0.5 nm slit width*

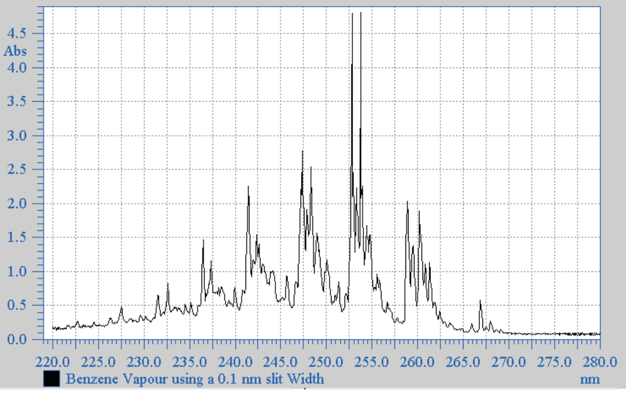

Figure 4:*Benzene Vapour Scan using a 0.11 nm slit width*

light, which reaches the detector without having passed through the sample.

The most effective way to reduce stray light is by the use of a double monochromator system in which two monochromators are connected in series. A double monochromator system can be quite expensive due to the very complicated construction but this arrangement is the most effective way to reduce stray light. A double monochromator system enables a stray light reduction of about 100 times lower compared to a single monochromator system.

Table 1 shows how even low levels of stray light coming from the monochromator can cause large errors in measurement in certain regions of the spectrum and can result in the production of false absorption bands.

Stray light effects of this sort are likely at the limits of operation of any of the spectrometer components including detectors, sources, and gratings. Under these conditions monochromatic light is at a low level or is producing a very small detector response. Any stray light, which is present, may produce a relatively large effect on the detector. The usual overall effect is to produce absorbance readings, which are lower than the true values.

As an example, a sample has an absorbance of 2 (which corresponds to 1% transmittance) at a wavelength of λ_0. If the sample beam contains 0.1% stray light then the light, which reaches the detector, will contain 1% of the λ_0 light plus the 0.1% of the stray light.

The transmittance will therefore be 1 + 0.1 = 1.1%.

A= log 100/T

A= log 100 - log T

A = 2 - log (1.1) = 2 - 0.41393 =1.95861

As the nominal absorbance should 2 then the error will be 2 -1.95861 = 0.04139 Abs.

The relative error caused by the stray light will greater than 0.04139/2 x 100 = 2.1%.

Similarly, calculating the relative error for the other values in Table 1, the % error ranges from 0% (stray light of 0.00015 @ 1 Abs) to 67% error (stray light of 1% @ 6 Abs).

Note: That as the absorbance increases, the error increases for all stray light levels. The lower the stray light, the lower the error is for a given absorbance.

Stray Light	1 %	0.1 %	0.01 %	0.001 %	0.0001 %
	Error (Δ Abs)	Error (Δ Abs)	Error (Δ Abs)	Error (Δ Abs)	Error (Δ Abs)
1 Abs	0.04139	0.00432	0.00043	0.00004	0.00000
2 Abs	0.30103	0.04139	0.00432	0.00043	0.00004
3 Abs	1.04139	0.30103	0.04139	0.00432	0.00043
4 Abs	2.00432	1.04139	0.30103	0.04139	0.00432
5 Abs	3.00043	2.00432	1.04139	0.30103	0.04139

Table 1: *Absorbance Error at various nominal absorbance readings and varying stray light levles*

Figure 5 illustrates the effect of stray light on the linearity of the plot. The lower the stray light the more linear the response is.

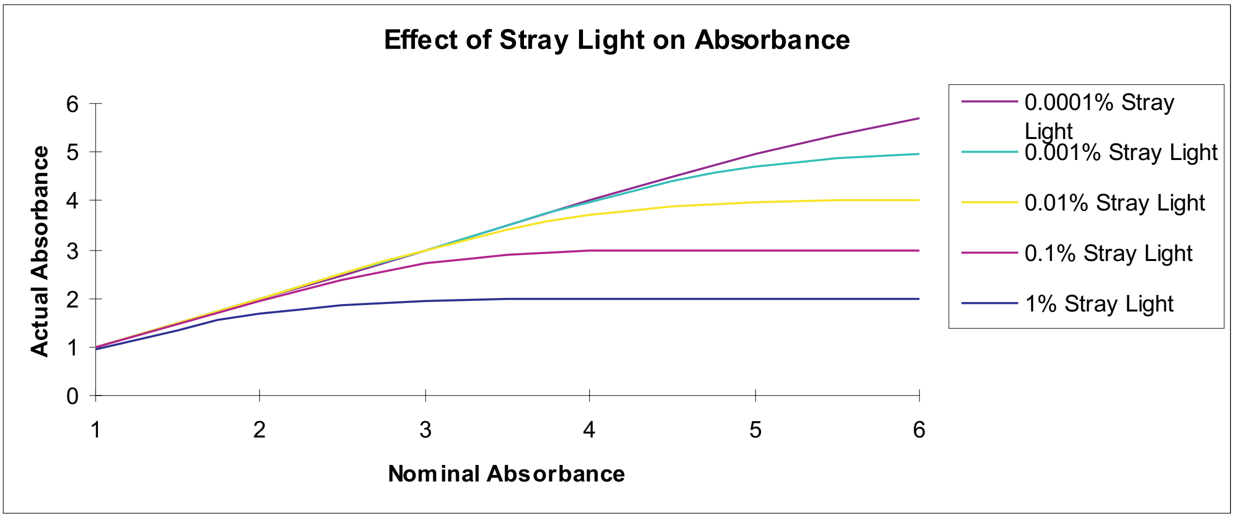

Figure 5: *Effect of Stray Light on Absorbance*

The Cintra 40 has typical stray light values of <0.0001%T at 220 nm and at 340 nm and hence will be linear up to at least 4 Abs and only slightly curved up to 6 Abs. An instrument with 0.1% stray light will not be linear to even to 1 Abs!

The Cintra 40 has a typical photometric noise of <0.00003 A RMS at 0 A and <0.00006 A RMS at 1 A. The very low photometric noise of the Cintra 40 results in samples which may have very small absorbances to be accurately measured as the actual sample absorbance will be able to be differentiated from the background. This differentiation of analyte and noise reading results in greater accuracy and precision of sample results.

Photometric Drift

The Cintra 40 has a typical Photometric drift of <0.0001 A/hr. So in 1 hour the maximum peak to peak drift will be less than 0.0001 A. This is very important in UV-Visible Applications especially in kinetics reactions. In kinetics reactions, Spectroscopists are interested in any variations in Absorbance versus time. Any variations in absorbance is directly correlated with a change in the enzyme substrate. In these experiments any change in Absorbance due to Instrument or thermal drifts will only contribute to experimental error.

With Absorbance changes barely detectable to the fourth significant figure, Spectroscopists using an instrument such as the Cintra 40 will have confidence that any absorbance changes are due to the reaction and not to the instrument drift.

Figure 6 graphically shows the excellent long term stability of the Cintra 40. The drift free optical performance of the instrument after more than 10 hours of operating time ensures that there is no need for the user to continually re-define the baseline. The optical stability of the Cintra 40 ensures reliability of results and allows for unattended operation of the instrument.

Photometric Linearity

The dynamic range of any UV-Visible spectrometer is limited at high absorbance values by stray light and at low absorbance values by Photometric Noise.

The Photometric linearity will therefore be influenced by both of these parameters.

The Cintra 40 has a photometric linearity to at least 4.5 abs. Therefore any analyses will be linear up to at least 4.5 abs. The benefit of this is that any solutions up to 4.5 Abs do not require to be diluted to ensure that they are in the linear portion of the calibration curve. This both improves the accuracy and saves valuable laboratory time in not having to perform these tedious dilutions.

Conclusion

The Resolution, Stray Light, Photometric Noise, Photometric Drift and Photometric Linearity for the Cintra 40 has been discussed. The Cintra 40 has excellent specifications for these parameters due to the no-compromise double monochromator system used.

The Cintra 40 has the ability to analyse difficult samples accurately and precisely both in normal or very long time studies applications.

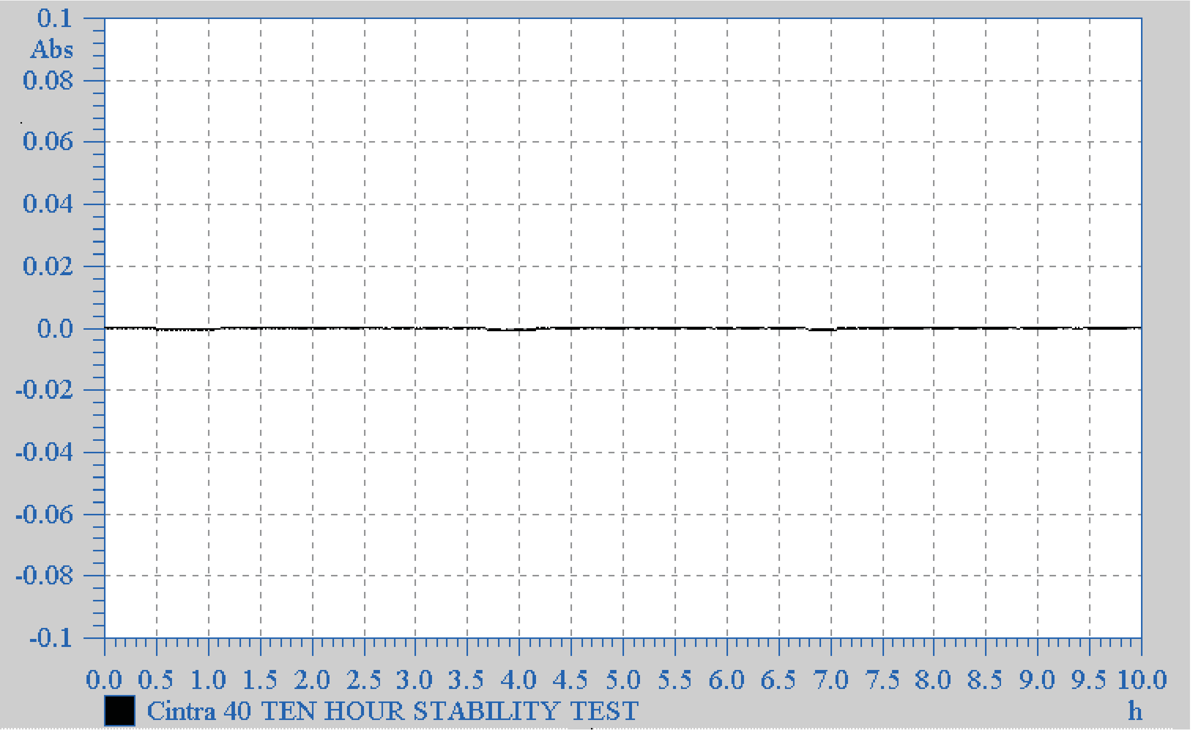

Figure 6: *Ten hour Absorbance versus Time plot*