Flexar PDA Plus

Linearity and Stray Light Using the Flexar PDA Plus

Introduction

Stray light is generally defined as essentially any light that is detected by a detector that does not pass through a sample. This causes the detector to see an additional, unintended source of light. The causes of stray light are higher order diffraction, scattering from surfaces such as mirrors and gratings, and generally poor instrument design. Stray light causes a decrease in the true absorbance values, is particularly observable at high absorbance values and reduces the linearity range of the detector. Stray light is generally measured by using well defined ASTM methodology. The method used for the experiments reported here is ASTM E 3870-4, using a 10 g/L solution of sodium Iodide.

The Effect of Stray Light on Absorbance

Stray light causes an error in absorbance measurements. The absorbance error caused at various percentages of stray light is shown in Table 1. The reference (water or non-absorbing solution) is assumed to transmit 100 % of the source light to the detector. Since absorbance is a log function of the transmitted light, a sample having an absorbance of 2.0 will transmit only 1% of the available light relative to the reference. Therefore, the log of 100/1 is 2.0. Now, if we factor in stray light, this additional light will be seen by the detector both in the reference and in the sample measurement. As shown in Table 1, the additional light will cause negative deviations from the true absorbance and the deviation increases as the level of stray light increases. Considering that the Flexar™ PDA Plus™ detector stray light level is typically <0.03 % between 200 – 260 nm (average), the greatest absorbance error at an absorbance of 2.0 is expected to be less than 1%.

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Table 1. Calculating the effect of stray light levels on a sample with an absorbance value of 2.0.

% Stray Light	Sample Transmittance	Sample Transmittance + stray light (S)	Initial Transmittance (reference) (R)	Absorbance A = log(R/S)	% error at Abs = 2.0
0	1 %	1 %	100 %	2.000	0.0
0.01	1 %	1.01 %	100.01 %	1.996	0.2
0.05	1 %	1.05 %	100.05 %	1.979	1.1
1	1 %	2 %	101 %	1.703	14.9

Methodology

Three measurements are required to determine the amount of stray light in a photodiode array detector. These are: 1) energy or transmission measurement with the flow cell filled with water (Figure 1); 2) a measurement of (or a correction for) the detector's dark current with the deuterium light source turned off; and 3) a measurement with the flow cell filled with the test sample (in this case, 10 g/L of sodium iodide solution).

Detector linearity was measured at 273 nm using various amounts of caffeine dissolved in water. Linearity was checked for three injection volumes: 1, 2, and 3 µl. Separations were performed using a PerkinElmer® Brownlee™ SPP C18 column, 100 x 3 mm, packed with 2.7 µm particles using a mobile phase of 17 % acetonitrile in water at a flow rate of 0.7 ml/min at room temperature.

Results

The percentage of stray light in the PDA Plus detector was determined according to ASTM method E 387-04 by measuring the dark-signal corrected transmission of water and a solution containing 10 g/L sodium iodide in water. Figure 2 shows stray light calculated as a percentage of the transmission relative to water (fully transmitted light) per ASTM protocol.

Figure 3 shows the linearity plots and correlation coefficients for the three injection volumes of a dilution set of caffeine standards. All R^2 values were above 0.999.

Conclusion

As exemplified by the results, the overall design and optical layout of the Flexar PDA Plus photodiode array detector lends itself to very low stray light levels. As was demonstrated, the resulting average percentage of stray light is <0.03%. This, in turn, results in extremely good linearity to absorbance values above 2.0.
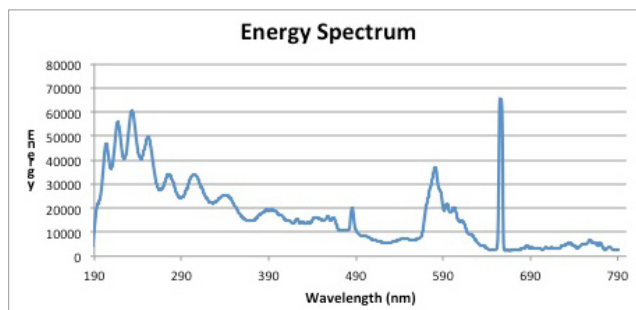

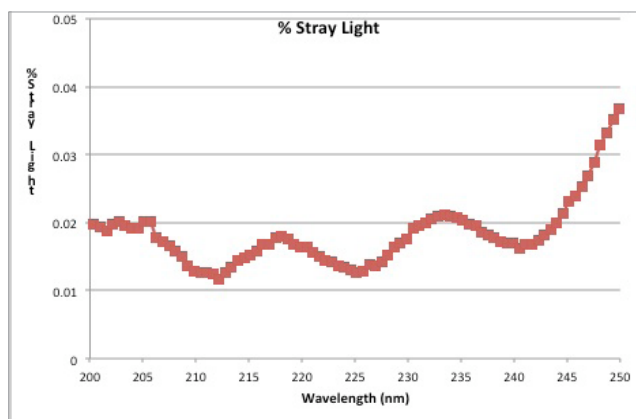*Figure 1.* Lamp energy spectrum of PDA Plus detector.

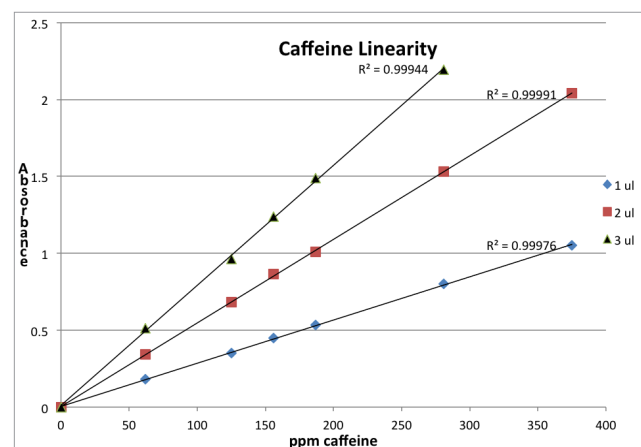*Figure 2.* Stray light measurement on PDA Plus detector.

Figure 3. Linearity plots on PDA Plus detector using various levels of caffeine at three injection volumes.

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