

Fluorescence room light immunity with the Agilent Cary Eclipse

Application Note

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Introduction

The Agilent Cary Eclipse is the first fluorescence spectrophotometer to have room light immunity. What this means is that sample size need no longer be a limitation as the instrument can be operated with the lid open. The front of the sample compartment is also removable, allowing full front and top access. This provides greater flexibility and increased sample throughput. This applicaton note demonstrates this room light immunity by measuring the fluorecence from a sample of Ovalene in PMMA (Polymethylmethacrylate) with the sample compartment opened and closed.

Discussion

The emission spectrum of Ovalene in PMMA was measured on the Cary Eclipse with the sample compartment opened and closed. The spectra were collected using an excitation wavelength of 342 nm, 5 nm excitation and emission slits, 0.5 nm data interval and an averaging time of 0.5 seconds. Figure 1 shows the two spectra overlaid. As can be seen, the peak shape and peak positions remain unchanged when running with either the sample compartment lid opened or closed.



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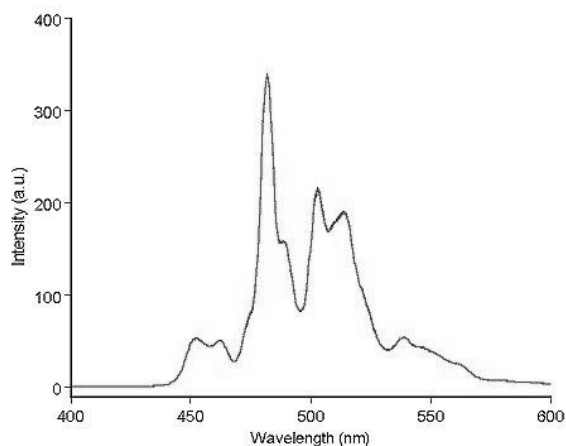


Figure 1: Fluorescence emission spectra of Ovalene in PMMA with sample compartment opened and closed

To illustrate this further, zooming in on the 482 nm peak, Figure 2, shows that both traces are overlaid perfectly.

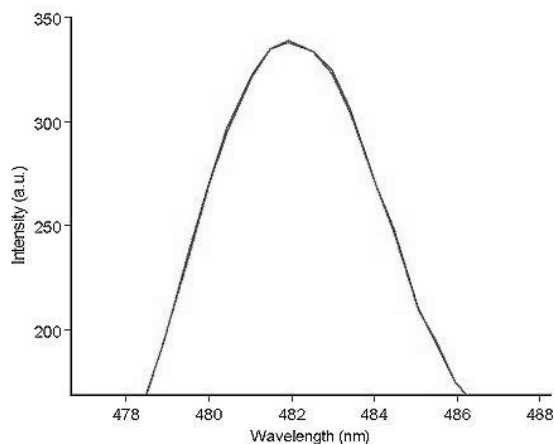


Figure 2: Zoomed in area of fluorescence emission spectra of Ovalene in PMMA

The room light immunity is achieved by using a Xenon flash lamp and sophisticated electronic signal processing. The high peak power of the source makes the Cary Eclipse approximately 10,000 times less susceptible to room light which means that the fluorescence signal produced per flash from the sample is much higher than the signal resulting from room light hitting the detector. Although the total signal measured

by the detector is the sum of the fluorescence from the sample and the room light, the room light contribution to this total signal is subtracted, leaving only the signal resulting from the fluorescing sample. This is achieved by measuring the room light signal just prior to flashing the lamp. The result is Room Light Immunity.

Not all fluorescence instruments that have flash lamps can operate with their sample compartment open. On the contrary, the Cary Eclipse is unique. The flash lamp has a peak power in excess of 75 kW and with its small arc size, provides a much brighter source, which is necessary to generate fluorescence well above room light levels. Also, the measurement taken just prior to flashing the lamp is much shorter than that of mains frequency, which means that the signal due to room light does not change within a single measurement. As a result the room light contribution is subtracted from the sample signal.

Operating with the sample compartment opened may increase the noise in the spectrum, which may be evident in weakly fluorescing samples. However, to overcome this, the signal to noise of the spectrum can be improved simple by just increasing the averaging time. The above example uses an averaging time of only 0.5 seconds, which is short enough to allow the scan to finish within a reasonable time.

Summary

Materials and reagents

The Agilent Cary Eclipse fluorescence spectrophotometer can operate in fluorescence mode with the sample compartment opened or closed. This provides the flexibility needed for measuring large or odd-shaped samples, and increases sample throughput by eliminating the need to open and close the sample compartment lid. Gone are the days of black cloths and inadequate light-sealing. With the Cary Eclipse room light need never be a problem again.

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