The Agilent Cary 60 eliminates photodegradation of aromatic markers used in UV-Vis spectrophotometric applications

Application Note

Chemicals

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Summary

The Agilent Cary 60 UV-Vis spectrophotometer is the new, improved successor to the revolutionary Cary 50 UV-Vis spectrophotometer. This instrument platform was evaluated for its potential to eliminate unwanted photobleaching of colorimetric markers used in common UV-Vis applications. In this short review we demonstrate how the unique, patented optics of the Cary 60 enable us to measure the absorbance of aromatic markers in a variety of applications with the confidence that no photo-bleaching will occur as a result of measuring the absorbance of the sample

Introduction

There are many photobleaching markers available for a broad range of applications using UV-Vis spectrophotometry. An example is methylene blue, one of a number of tricyclic heteroaromatic compounds that are used as markers. Methylene blue is particularly susceptible to photobleaching[1] and the standard extrinsic probe used in transient photodichroism experiments to monitor the rotational dynamics of many species, e.g., DNA[2].

There are many applications where photobleaching is undesirable, as the probe (or colorimetric agent) used is required to retain its spectrophotometric integrity[3]. This can prove to be a challenge using other UV-Vis instruments that inherently induce photobleaching. In this review we demonstrate how the Cary 60 UV-Vis induces no photobleaching in tricyclic heteroaromatic compounds by virtue of its unique optical design, utilizing xenon flash lamp technology.

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Apparatus and materials

Part Number	Description
G6860AA	Cary 60 UV-Vis with WinUV software and PC

Methods and results

A baseline (blank) was taken using 3 mL purified water in a disposable cuvette with the Cary WinUV 'Scan' software application. Subsequently, data from 10 superimposed scans were recorded, as shown in Figure 1, in order to: 1) identify the maximum absorbance wavelength for kinetics studies; and 2) check reproducibility of the Agilent Cary 60 UV-Vis spectrophotometer.

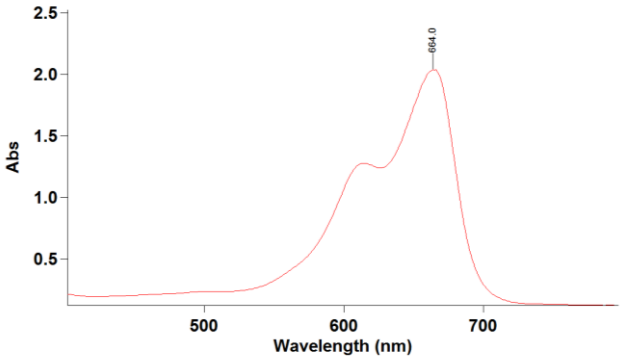

Figure 1. Ten superimposed wavelength scans of methylene blue (20 ppm) solution in water. As can be seen, the scans overlay perfectly confirming the accuracy of the Cary 60 UV-Vis and the absence of any photobleaching of the sample

Once the peak absorbance value was determined to be 664 nm (Figure 1), the instrument was set up to replicate the data given by Kok *et al*[1] except, in this instance, the exposure time was doubled from 10 min to 20 mins to further assess the effect of any photobleaching of methylene blue by the Agilent Cary 60. Results are shown in Figure 2.

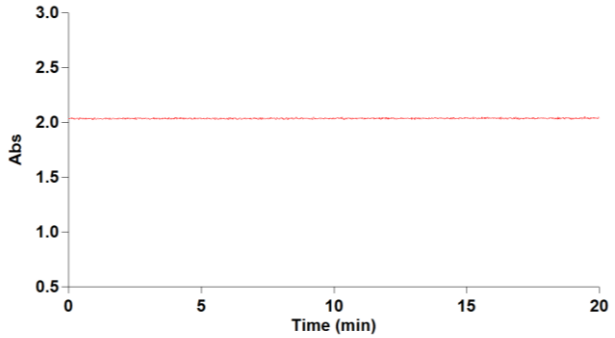

Figure 2. Continuous kinetics absorbance readings measured every 12.5 ms, of methylene blue (20 ppm) at 664 nm

Both Figures 1 and 2 demonstrate that there was no detectable photodegradation of methylene blue, despite continuous irradiation for up to 20 minutes. This is in contrast with what was seen by Kok *et al*[1], where significant photobleaching was observed in measuring methylene blue over time. This makes the Cary 60 UV-Vis the ideal spectrophotometer for studying systems where tricyclic heteroaromatic compounds are used as colorimetric indicators, or where photobleaching is an issue.

Conclusions

Data presented above demonstrate that the new Agilent Cary 60 UV-Vis does not induce photo-degradation of light-sensitive samples while taking a measurement by virtue of its unique xenon flash lamp design which ensures that results are always measurable, accurate and reproducible.

1. Kok, C. *et al* 2005. Study of Photobleaching Mechanism in Methylene Blue Sensitized Gelatin Using a Single Beam UV-Vis Fiber Optics Spectrophotometer. *Pertanika J. Sci. & Technol.* **2005**, 13(1), 23-30.
2. Fujimoto *et al* 1994. Fluorescence and Photobleaching Studies of Methylene Blue Binding to DNA. *J. Phys. Chem*. **1994**, 98, 6633-6643.
3. Comerford, J. 1998. Investigations of Photochemical Reactions using UV/VIS Spectroscopy. *Application UV-76*, February 1998 (www.agilent.com).

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