

QUANTITATIVE ANALYSIS OF METHYLENE BLUE DYE BY VISIBLE ATR SPECTROSCOPY

INTRODUCTION

ATR measurements are not commonly done in the visible. Historically, the choice of optically transparent materials in that spectral region was limited to materials of relatively low refractive index, like fused silica and sapphire, which was problematic at best.

For the ATR measurements, the incident angle, θ , must exceed the critical angle, θ_c , where the critical angle is defined as:

$$\theta_c = \sin^{-1} \frac{n_2}{n_1}$$

where n_1 is the refractive index of the ATR crystal and n_2 is the refractive index of the sample. Since many organic and aqueous materials have a refractive index

of approximately 1.5, this meant that the only reasonable configuration used a sapphire ATR crystal at an incident angle of 60° or higher. Since the real part of the refractive index changes through the absorption bands, there was still a chance that the critical angle criteria would not be met, producing in dispersion in the resulting spectrum.

The other limiting factor in the utility of ATR in the visible is the pathlength or effective thickness. Most liquid samples suitable for study in the visible are readily examined in transmission using cuvettes with pathlengths of 5-10 mm. ATR pathlengths are much shorter; the effective thickness is on order of micrometers rather than millimeters. So ATR is only likely to be a usable choice if the sample is too strongly absorbing or too concentrated to study easily with the standard cuvettes available.

In the 1990s, diamond ATR accessories became more commonly used in the infrared. With its higher refractive index and wide spectral range, this opened up the possibility of extending ATR measurements into the visible and the FIR. For strongly absorbing liquids, like dyes, diamond ATR may be a reasonable alternative to transmission measurements by cuvettes. Strongly absorbing

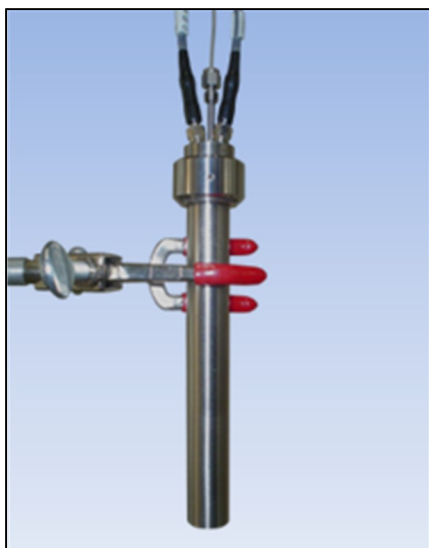


Figure 1. The Harrick Diamond ATR Probe.

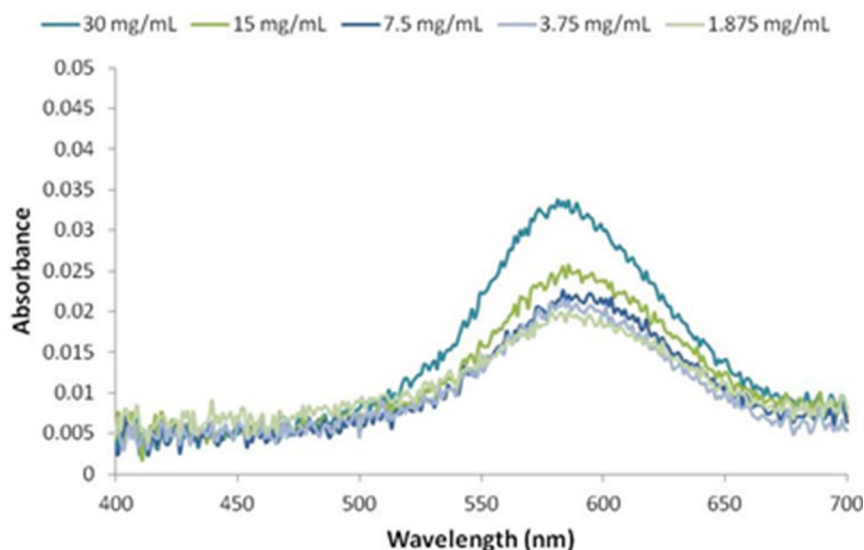


Figure 2. Absorbance of methylene blue solutions of various concentrations.

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dyes often leave residues in the cuvettes which are challenging to clean. Diamond, on the other hand, has a low coefficient of friction which makes cleaning simple and straightforward.

In this application note, we explore the use of a diamond ATR probe for in situ Vis analysis of liquids. A diamond probe is used to quantitatively measure highly concentrated standard solutions of the dye methylene blue and generate an accurate Beer's Law calibration curve.

EXPERIMENTAL

Methylene blue dye was obtained from Consolidated Chemical and Solvents, LLC. Standard solutions were prepared by dissolving 1.50 g of dye in 50 mL H₂O in a volumetric flask, and then making dilutions to prepare solutions of concentrations 30 mg/mL, 15 mg/mL, 7.5 mg/mL, 3.75 mg/mL and 1.875 mg/mL.

The spectroscopic measurements were carried out on a double beam UV-Vis-NIR spectrometer with the Harrick FiberMate2 installed in the sample compartment and connected by fiber optic cables to the Diamond Probe. The fibers used were 1.5m long silica fibers with a 1000um diameter core and an effective NA of 0.37.

Spectra were recorded over the range 400 nm – 700 nm with the spectrometer in double beam

mode with an attenuator (1.5 abs) in the reference beam, a 0.700 nm SBW and 1.100 nm data interval. This confirms that the sample follows Beer's Law, $A = \epsilon bc$, at

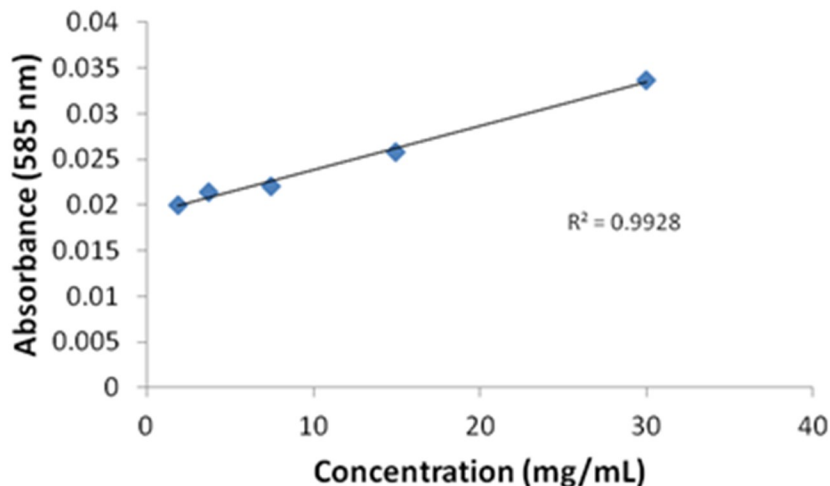


Figure 3. Calibration curve generated from absorbance of methylene blue at 585 nm.

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RESULTS AND DISCUSSION

The ATR UV-Vis spectra of the standard solutions are shown in Figure 2. The overall absorbance is somewhat low but there is a clear band with a maximum at 585 nm and clear distinctions between the intensities of the band at different concentrations. The absorbance at 585 nm was plotted against concentration to generate the calibration curve shown in Figure 3. The fit is clearly linear without much significant deviation, with a

correlation coefficient of 0.9928. Note that the spectrometer collection parameters could be further optimized for an improved signal to noise ratio.

CONCLUSIONS

ATR UV-Vis spectroscopy is a viable alternative to transmission spectroscopy which may be more convenient for in situ analysis of highly concentrated samples that are difficult to clean. The effectiveness of this method using the Harrick Diamond Probe is shown through the successful generation of an accurate calibration curve for the dye methylene blue.



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