

Measurement of Opaque Liquids with UV-Vis and ATR

Combining the Agilent Cary 60 UV-Vis spectrophotometer with an ATR fiber-optic probe



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Abstract

The use of an attenuated total reflection (ATR) probe in combination with an Agilent Cary 60 UV-Vis spectrophotometer is proven to be beneficial for quick and easy measurement of opaque liquids without dilution.

Introduction

Today, ATR is considered synonymous with FTIR spectroscopy, being the most widely used sampling technique. However, ATR can also be applied to UV-Vis spectroscopy, offering particular benefits for measuring opaque liquids with a fiber-optic probe.

Samples such as paints, inks, food colorings, sunscreens, and liquid medications are often highly pigmented, rendering them optically opaque. In some cases, measurement of these types of samples using a standard 10 mm path length cuvette may be possible, but only after several hundred-fold dilution, requiring significant solvent consumption and introducing the possibility of large dilution errors. In the case of emulsion samples such as paints and sunscreens, dilution is often not possible without the sample "splitting" and separating into its component parts.

ATR spectroscopy is an innovative sampling technique that overcomes these challenges. In ATR, incident light from the spectrometer is directed into a measurement crystal at an angle. Internal reflections within the crystal generate an evanescent wave at the crystal surface, allowing samples in direct contact with the crystal to interact with the standing wave and absorb some of the light energy.

In a UV-Vis fiber-optic ATR probe, a commonly used crystal is sapphire, with the incident light directed into the crystal to form an angle of 60° at the crystal surface. The crystal is designed to create three bounces at the surfaces (Figure 1). The "effective height" of the wave above the surface is a function of the relative refractive indices of the crystal and the sample medium, the angle of incidence, and the wavelength. The multiple bounces extend the effective path length. For this probe, the effective pathlength at 250 nm is approximately $3\text{ }\mu\text{m}$ and increases to approximately $13\text{ }\mu\text{m}$ at 1,000 nm.

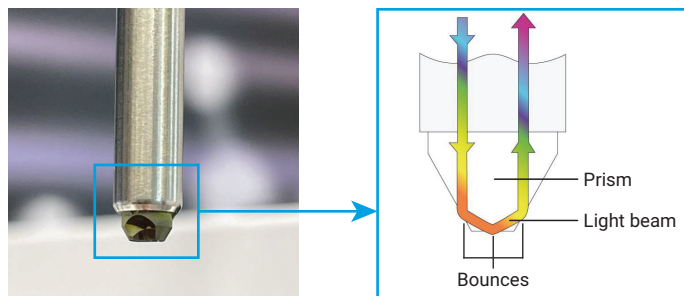


Figure 1. Schematic representation of the path of incident light within a sapphire crystal in an attenuated total reflection probe.

For a fiber-optic probe to deliver accurate and reliable results, it is important that the light is effectively guided from the spectrophotometer to the fiber-optic sampling head, then back to the instrument. The Cary 60 UV-Vis spectrophotometer (Figure 2) features a highly focused beam that enables efficient light coupling into the fiber. The powerful xenon flash lamp in the Cary 60 UV-Vis produces bright light pulses that ensure ample light intensity for fiber-optic measurements. The reliable xenon flash lamp also comes with a 10-year guaranty. In addition, the Cary 60's unique optical design makes it immune to distorting effects or interferences from room light, which is critical for remote fiber-optic measurements outside the sample compartment.



Figure 2. An Agilent Cary 60 UV-Vis spectrophotometer.

Experimental

Example data were collected using a Cary 60 UV-Vis spectrophotometer equipped with a fiber-optic interface and Hellma Katana XP 6, 3-bounce ATR probe (Hellma part number PK1KSSZUEASM210N). Spectra were collected by first measuring a baseline using the clean crystal, followed by sample measurement with the crystal immersed in the sample. The Agilent Cary WinUV software was used to collect the spectra as well as to process the measured spectra. All spectra were collected using the measurement conditions shown in Table 1.

Table 1. Measurement conditions.

Parameter	Value
Measurement Mode	Absorbance
Wavelength Range	800 to 250 nm
Spectral Bandwidth	1.5 nm
Signal Averaging Time	0.4 sec
Data Interval	2 nm
Scan Speed	150 nm/min

Paint

Bottles of red, yellow, and blue paint were measured directly by inserting the tip of the ATR probe into each sample as shown in Figure 3. Between samples, the probe tip was cleaned with a paper towel and water, and a new baseline was recorded with the newly-cleaned crystal.

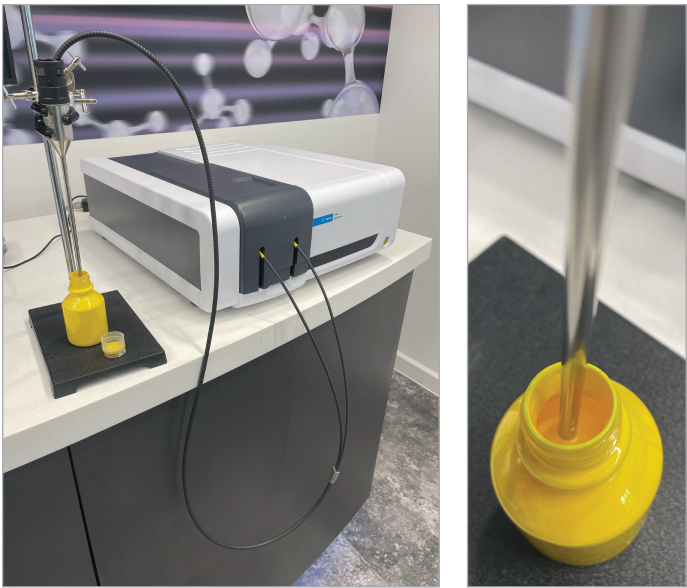


Figure 3. Yellow paint measured using an Agilent Cary 60 UV-Vis spectrophotometer with a Hellma Katana XP 6 ATR fiber-optic probe.

Using the yellow paint sample as an example, the spectrum shows a peak at 440 nm with an absorbance intensity of approximately 0.15 Abs. At this wavelength, the effective path length is approximately 6 μm . If the sample were measured in a standard 10 mm cuvette, the absorbance would be equivalent to approximately 250 Abs, a value well beyond the range of a spectrophotometer. Therefore, measuring this sample in a 10 mm cuvette would require roughly a 200-fold dilution.

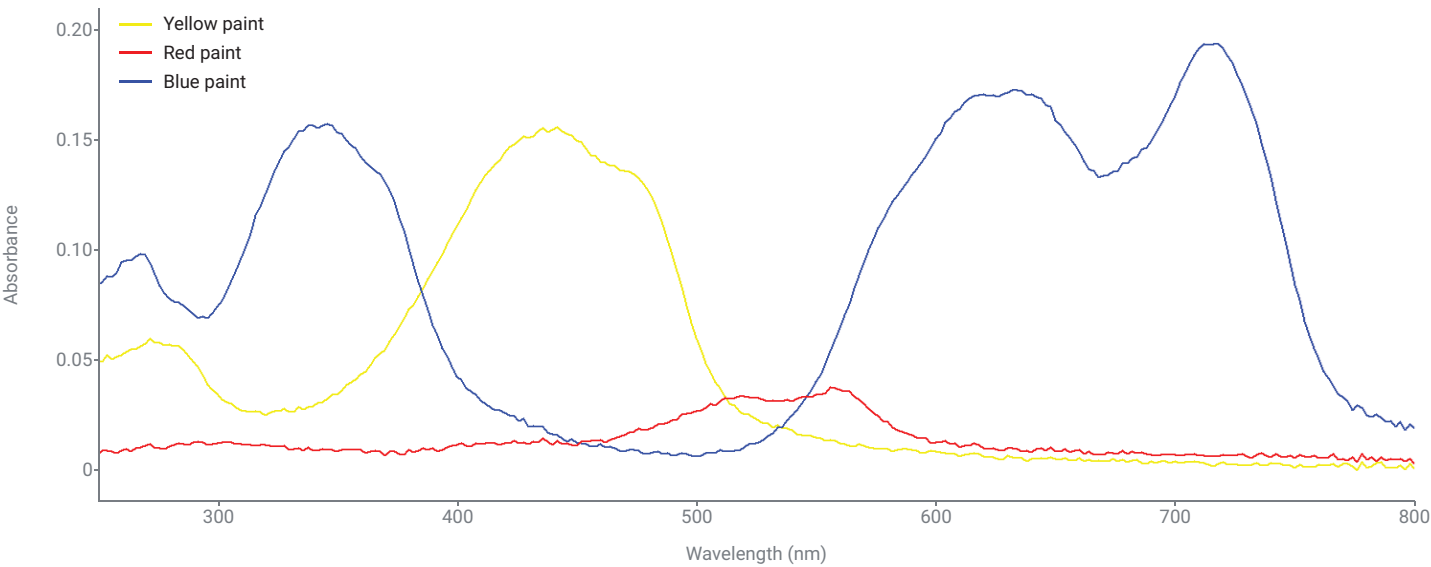


Figure 4. Absorbance spectra of the analyzed paint. The color of each trace corresponds to the color of the paint analyzed.

Food coloring

The measured spectrum of royal blue food coloring (Figure 5) is shown in Figure 6. This sample exhibits an intense absorbance band measuring 1.27 Abs at 650 nm. At this wavelength, the effective path length is calculated to be approximately 8.5 μm . This corresponds to a theoretical absorbance of 1,500 Abs if measured in a standard 10 mm cuvette, requiring a minimum 1,000-fold dilution to bring the sample within range of most commercial UV-Vis instruments.



Figure 5. Royal blue food coloring.

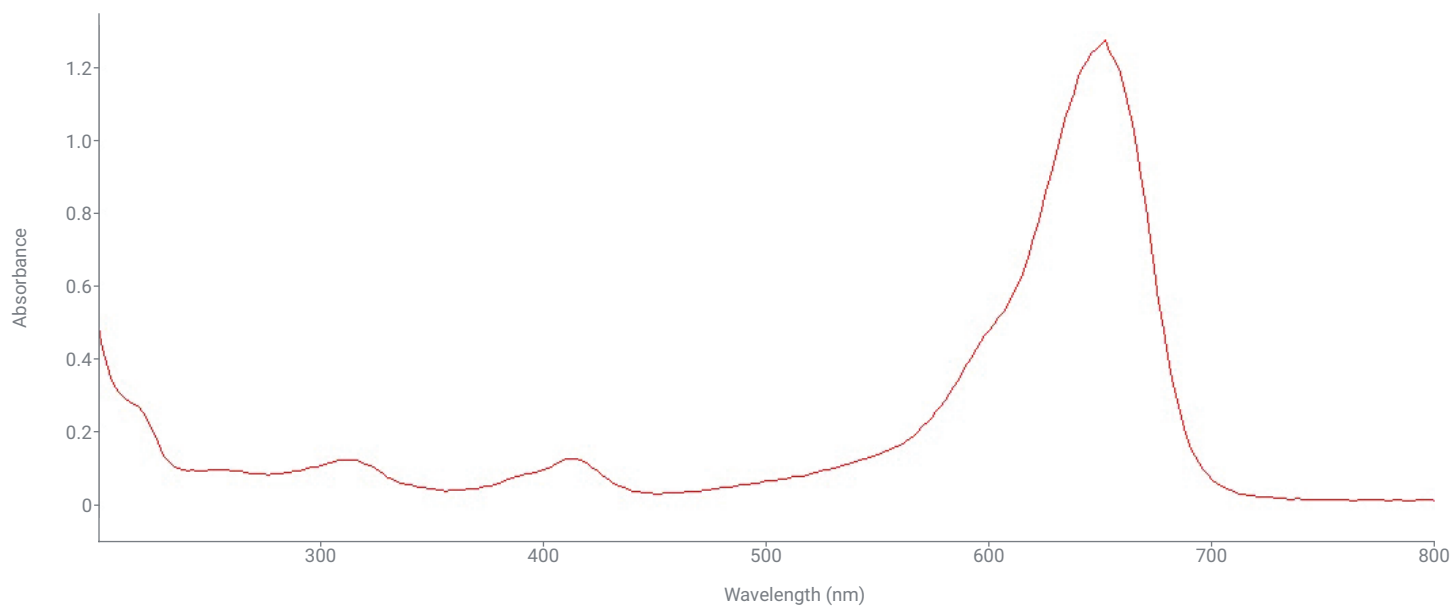


Figure 6. Absorbance spectrum measured for royal blue food coloring.

Sunscreen

Sunscreens contain mineral-based ingredients that offer skin protection against (harmful) UVA and (very harmful) UVB rays by reflecting them away from the skin. The spectra in Figure 7 show measurements made on three different bottles of the same brand of sunscreen, each having a different protection factor rating.

The absorbance level decreases as the protection factor increases, which is expected because higher-factor formulations are designed to reflect more light.

Mathematically converting the absorbance spectra to reflectance spectra makes the trend easier to visualize. The image in Figure 8 clearly shows that the reflectivity increases as the protection factor increases. The UVA, UVB, and UVC regions are displayed for information only.

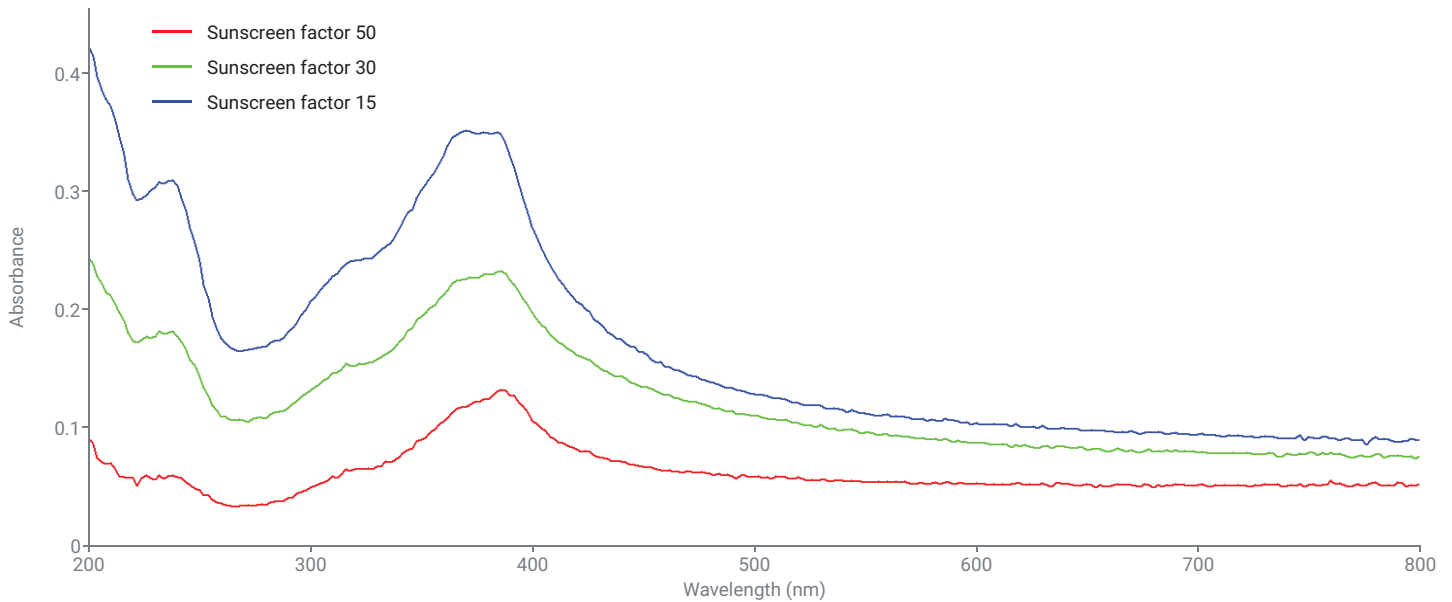


Figure 7. Absorbance spectra of three different bottles of sunscreen from the same brand, each with different protection factors.

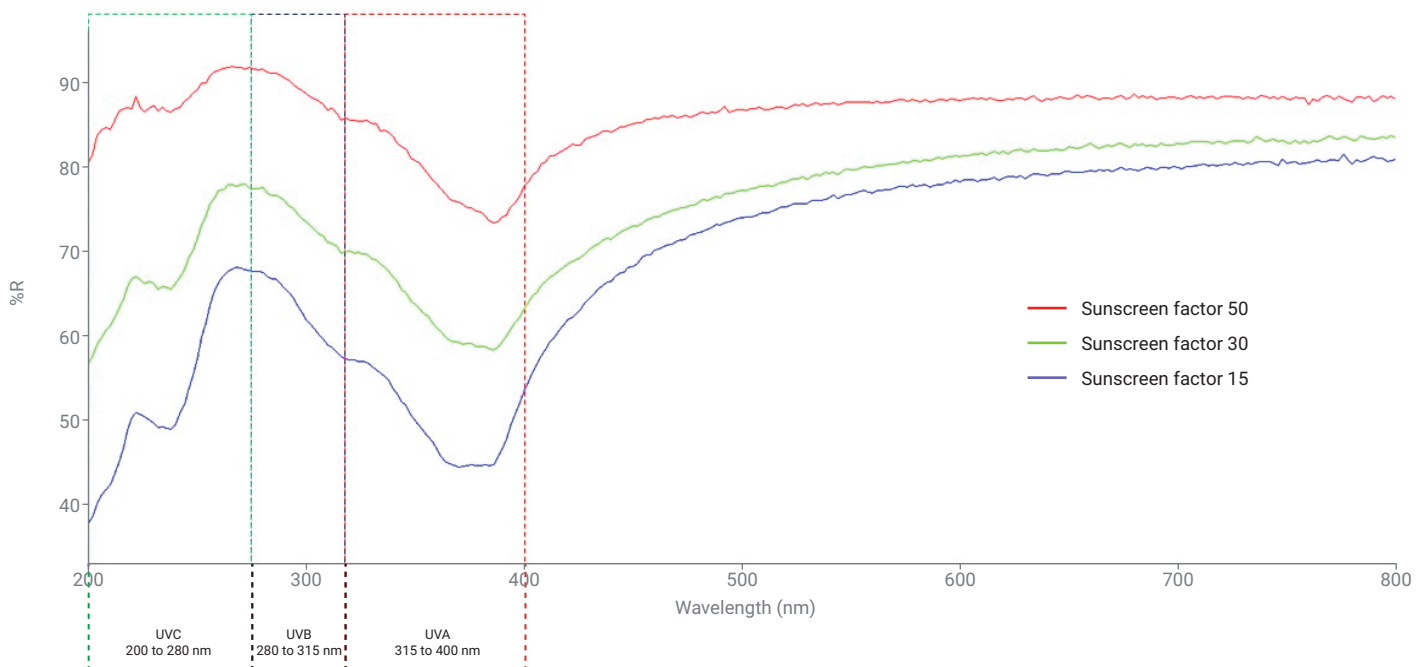


Figure 8. Reflectance spectra measured for three sunscreen products with different protection factors, illustrating an increase in reflectivity in correlation with increasing protection factor.

Reaction kinetics

The Cary 60 UV-Vis with an ATR probe can also be a useful tool for following the kinetics of reactions that involve an intense change in absorbance. Fluorenylmethoxycarbonyl (Fmoc) is a base-labile protecting group widely used in solid-phase peptide synthesis. It can be removed rapidly by the addition of piperidine, which reacts with Fmoc to form the byproduct dibenzofulvene, a polycyclic aromatic hydrocarbon that is a very strong UV absorber (Figure 9).

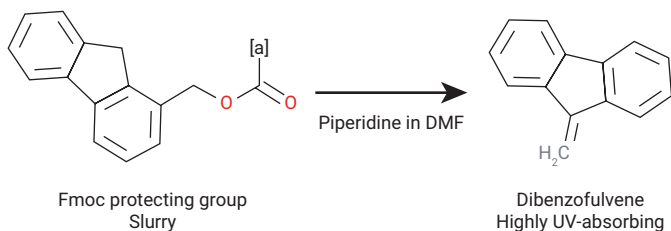


Figure 9. Reaction of fluorenylmethoxycarbonyl with piperidine to produce dibenzofulvene.

Figure 10 demonstrates how the Cary 60 UV-Vis with an ATR probe can be used to follow the decoupling reaction. The probe is inserted into the vessel containing a turbid slurry solution of Fmoc-terminated peptides bound to polystyrene resin beads in N,N-dimethylformamide (DMF) solvent. The data collection is started with the Cary 60 UV-Vis set to acquire absorbance readings every second at 280 nm. In this example, the reaction is initiated, after approximately 2.8 minutes, by adding a 20% piperidine in DMF solution. After 6 minutes, the reaction curve plateaus, indicating that the Fmoc is completely decoupled.

The overall absorbance increase is only 0.05 Abs, but if measured in a standard 10 mm path length cuvette, this would correspond to an increase of more than 140 Abs.

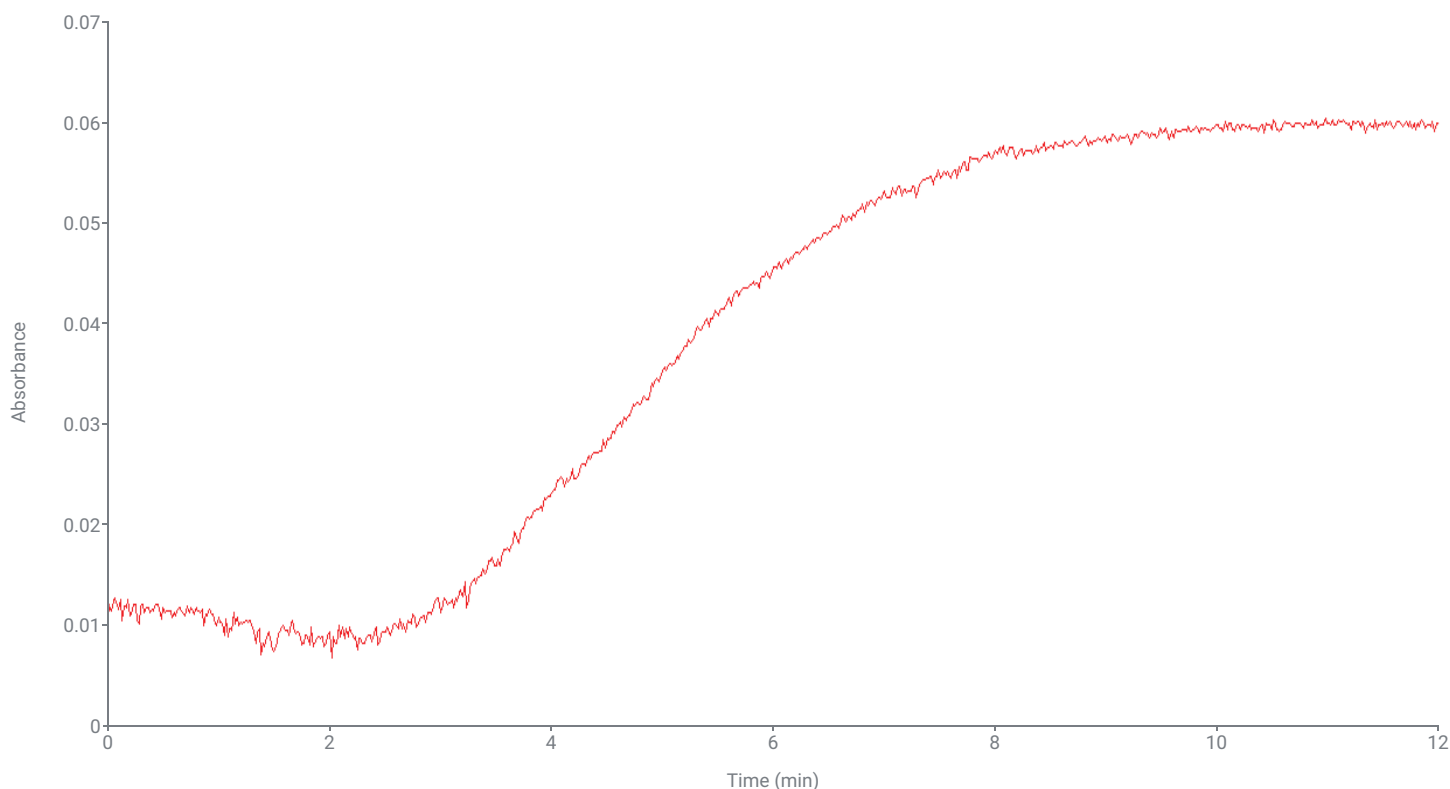


Figure 10. The reaction progress of the decoupling of Fmoc-terminated peptides from polystyrene resin beads.

The comparison of the measured absorbance values obtained using the Cary 60 UV-Vis with an ATR probe, and the theoretical absorbance readings if the measurements were conducted with a standard 10 mm cuvette, highlights the limitations of using a standard 10 mm cuvette. As shown in Table 2, the samples could not have been measured using a standard cuvette without performing a significant number of dilutions. Extra user handling can:

- Increase the risk of dilution errors
- Introduce contamination
- Lead to sample changes (e.g., separation of components)
- Affect reactions and reaction kinetics

Table 2. Comparison of the absorbance values of four different samples.

Sample/Application	Yellow Paint	Blue Food Coloring	Sunscreen SPF 15	Monitoring Fmoc Deprotection
Measured Absorbance ATR	0.15 Abs	1.27 Abs	0.35 Abs	0.05 Abs
Equivalent Abs to 10 mm Standard Cuvette	250 Abs	1,500 Abs	700 Abs	140 Abs
Required Dilution	Approx. 200 times	Approx. 1,000 times	Dilution not possible	Dilution not possible

Conclusion

This application note demonstrates how an Agilent Cary 60 UV-Vis spectrophotometer, when combined with a Hellma Katana XP 6 ATR probe, provides a practical and easy-to-use solution for measuring optically opaque liquids that would otherwise require extensive dilution, and for monitoring reactions with absorbance changes too large for conventional methods. The Cary 60 UV-Vis with the ATR probe combination is a powerful tool for a wide range of applications, including routine quality control, R&D studies, and research and teaching laboratories.

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