Unique Benefits of the Small Beam Geometry of the Agilent Cary 60 UV-Visible Spectrophotometer

Technical note

Author

Dr. Ursula Tems

Agilent Technologies
Mulgrave, Australia

Introduction

The Agilent Cary 60 UV-Vis spectrophotometer has a patented optical design based on a Xenon flash lamp that produces a highly focused beam (approximately 1.5 mm x 1 mm) where the cell holder is located in the sample compartment. The key benefit of this design is that all of the available light is going through the sample, improving performance and sensitivity, and reducing noise. The geometry of the optical design also allows a range of specialized accessories and cuvettes to be used that offer unique measurement capabilities.

This technical note describes the most common accessories and applications that are made achievable with the unique design of the Agilent Cary 60 UV-Vis spectrophotometer.

Measurement of very low sample volumes

As more laboratories move to a high throughput workflow for routine analysis and quality control applications, the requirement to be able to rapidly and easily measure small volumes has increased. The Cary 60 UV-Vis spectrophotometer can be used with an ultra-microvolume cuvette that can measure sample volumes between 2 and 10 µL. The performance of the ultra-microvolume cuvette with the Cary 60 is excellent due to the highly focused beam, that ensures maximum light throughput.

The ultra-microvolume cuvette sits in the cell holder like a typical 3 mL cuvette, and requires no special installation procedure (Figure 1). With this simple installation, the Cary 60 provides a flexible solution, from small volume analysis through to larger volumes. This enables a wide range of sample concentrations to be measured, all with excellent photometric performance.

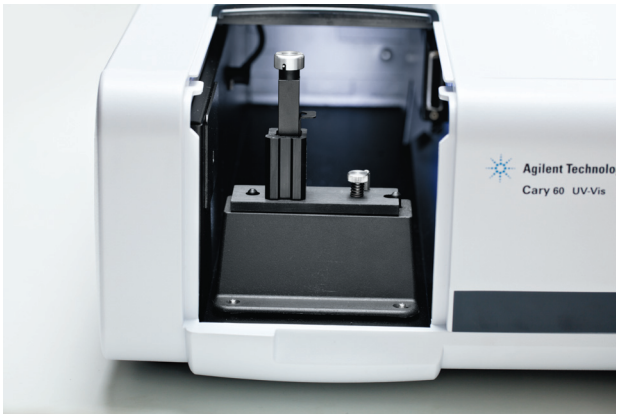

Figure 1. The ultra-microvolume cuvette in the cell holder of the Agilent Cary 60 sample compartment.

The highly focused beam of the Cary 60 allows a wide range of sample concentrations to be measured. Up to 2.3 Abs can be measured using a 1.0 mm cap (Figure 2). This is equivalent to 23 Abs when using a 10 mm pathlength cell, removing the need for sample dilutions before measurements are taken, saving time and reducing error. For more information refer to Data Sheet #5990-7944EN.

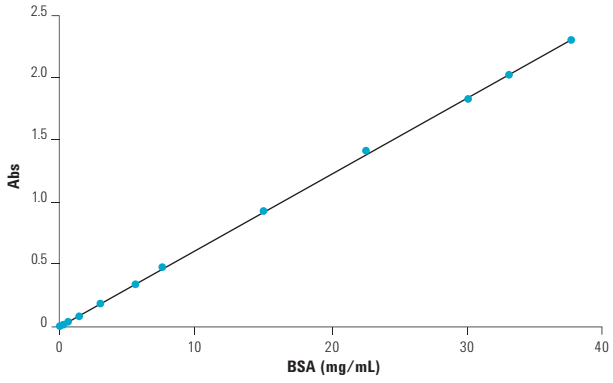

Figure 2. The wide linear absorbance range of the Cary 60 with the ultra-microvolume cuvette means a wide range of sample concentrations can be measured as shown here for Bovine Serum Albumin (BSA).

Measurement of micro-liter sample volumes in micro and submicro cuvettes

The highly focused beam geometry of the Cary 60 was designed for performing small volume measurements, with the aim of reducing the minimum required sample volume and reducing reagent cost – this is particularly important when samples are expensive or difficult to purify.

The ability to measure a sample in a submicro cuvette with the same sensitivity as a 3.5 mL standard cuvette is a benefit of the Cary 60 UV-Vis spectrophotometer's optical design, which focuses the maximum amount of light through the sample. Submicro cuvettes have volumes in the range of 10 to 135 µL and are ideal when: sample volumes are limited; for highly concentrated samples, or for highly absorbing solvents.

Submicro cuvettes offer low volume, short path length and excellent heat transfer for temperature controlled experiments. The low volume is achieved masking the walls of the cuvette, reducing the available area for the spectrophotometer's light beam to pass through (Figure 3). In the Cary 60, even the smallest 2.0 x 2.0 mm aperture submicro cuvette can be used in the confidence that the maximum light will be passing through the sample. For smaller volume cuvettes (<10 µL), the Cary 60 still provides the highest quality data.

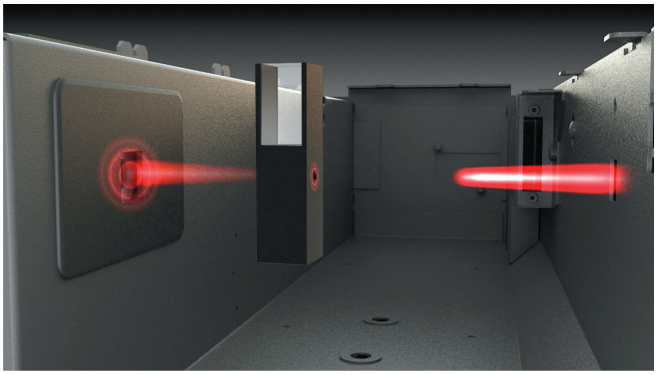

Figure 3. A submicro cuvette with a 2.0 x 2.0 mm aperture can be used for volumes between 40 and 135 µL. They are ideal for use with the Cary 60, as the beam image is only 1.0 x 1.5 mm (approximately) at the focal point in the sample compartment

Remote Measurement of Samples

The Cary 60's optical design provides both a highly focused beam and also room light immunity, making it ideal for use with fiber optics. The fiber optic probe is connected to the Cary 60 using a coupler, through which light is directed from the instrument through the probe, to the sample, then back to the detector. The data quality from fiber optic measurements is dependent on the efficiency of the coupler. The more light from the instrument that can be captured and sent through the probe the more sensitive is the measurement.

The Cary 60's highly focused beam means that coupling is highly efficient, and there is no compromise of data quality when using fiber optic accessories. Room light immunity makes the Cary 60 ideally suited to accessories such as fiber optics, where measurements are taken outside of the instrument (Figures 4a and 4b). There is no need to shield the tip of the probe from ambient lighting, and measurements can be taken with confidence. The suitability of the Cary 60 for fiber optics is a direct result of superior design.

Figure 4a. Measuring samples with the standard fiber optic probe and the Cary 60. Take the instrument to the sample.

Figure 4b. Measuring down to 140 µL in 0.5 mL microcentrifuge tubes using the fiber optic micro probe and the Cary 60.

The most obvious advantage of using fiber optics is that cuvettes are no longer required. There is no need to purchase or clean expensive quartz cuvettes nor hundreds of disposable plastic cuvettes. This naturally improves workflow and decreases the cost per analysis. It also allows the instrument to be taken to the sample – when measurements need to be taken in a fume hood for safety reasons, or when samples simply do not fit in the sample compartment. It is also a non-destructive technique, meaning that the sample remains available for downstream processes. Reducing the need for sample preparation increases laboratory productivity. See application notes #5990-7863EN and #5990-7945EN for more information about the advantages of using fiber optic probes for liquid sample analysis.

The Cary 60 extends this performance to also include remote measurements of solid samples using fiber optics and the remote diffuse reflectance accessory (DRA). Fast, accurate measurements of solid samples can be performed up to 1.5 m from the instrument. The optical fibers channel light out of the instrument and focus a 1.5 mm light patch onto the sample, allowing reflectance of very small solid samples to be measured. The remote DRA has a 0/30° beam geometry, which allows diffuse reflectance to be measured by a detector mounted within the accessory. A video camera, embedded in the remote DRA, assists with the accurate placement of the accessory on the sample.

For more information about the accessory, refer to application note # 5991-1430EN. The Cary 60 with remote DRA is idea for use when the sample must not be damaged or when samples cannot be placed in the instrument sample compartment (Figure 5).

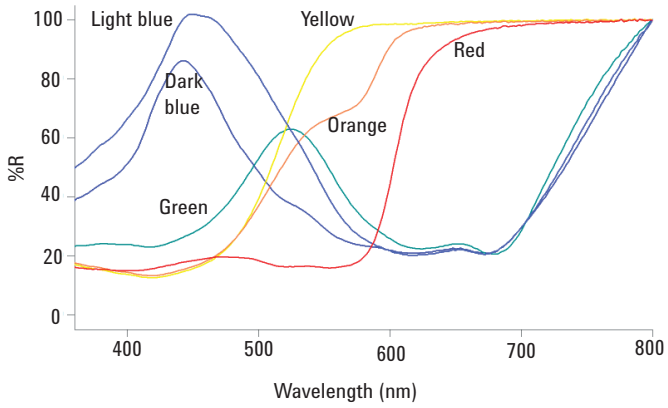

Figure 5. Reflectance measurements of color samples collected using the remote DRA accessory with the Cary 60.

Summary

The small, highly focused beam image of the Cary 60 was specifically designed for excellent coupling efficiency and high light throughput, which provides excellent sensitivity and allows for a wide range of measurements. The Cary 60 is able to perform measurements using ultra-microvolume cuvettes, microcells, and fiber optics—including measurements of large and solid samples outside of the instrument sample compartment. This ensures that most applications can be performed on the one instrument. The unique optical design of the Cary 60 is ideally suited for a wide range of sampling methods and high performance accessories.

www.agilent.com

Published August 15, 2014
Publication number: 5991-3852EN

Agilent Technologies