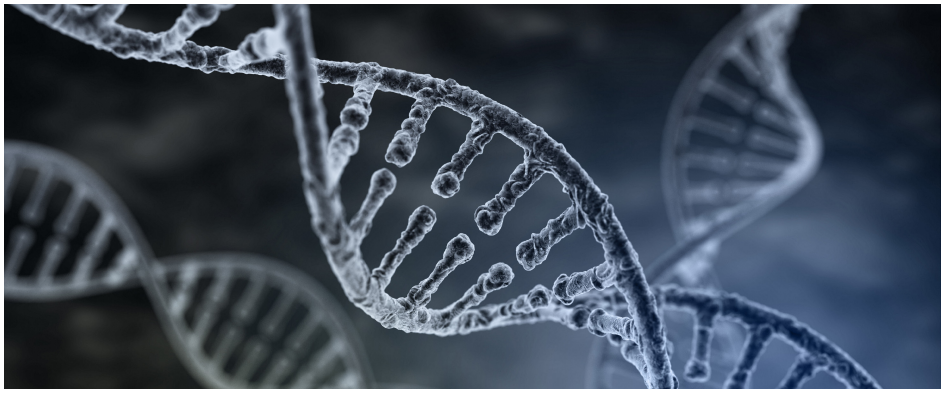
Technical Overview

Characterization of Biomolecules using High-Performance UV-Vis Spectrophotometry

Accelerating research into oligonucleotides and proteins with the Agilent Cary 3500 UV-Vis



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Introduction

Molecular biology research plays a vital role in understanding biochemical and molecular processes within the cells of living organisms. These processes involve the interaction of lipids, carbohydrates, proteins, and nucleic acids. Biomolecule characterization is important in understanding the development and growth of living organisms, disease mechanisms, and accelerating therapeutic developments. There are several examples of biomolecules, including oligonucleotides and proteins, that are of interest to researchers and are used as therapeutics.

Oligonucleotides are short pieces of DNA that are designed to base pair with a strand of DNA or RNA. There is increasing focus on oligonucleotides due to advancements in the precise and reproducible synthesis of oligonucleotide sequences.[1] Oligonucleotides have been used extensively in drug discovery, in research, and in diagnostic test kits such as polymerase chain reaction (PCR) and microarray-based assays. Oligonucleotides have also been used in vaccine development against viruses as they can be developed quickly and can be specific against the genetic sequence of the targeted virus or viruses.

Proteins are another example of biomolecules that have become more frequently used as a therapeutic over the last 25 years.[2] The first protein therapeutic was insulin, which was developed to treat diabetes. Protein therapeutics can be monoclonal antibodies that influence the immune response, or enzymes that can act as catalysts and messengers in cellular processes.

To understand the function of biomolecules, various techniques are used to analyze and characterize them. While UV-Vis spectroscopy is a well-established technique suited to biomolecule characterization studies, the Agilent Cary 3500 UV-Vis spectrophotometer (Figure 1) includes many features and benefits that will accelerate biomolecule research.

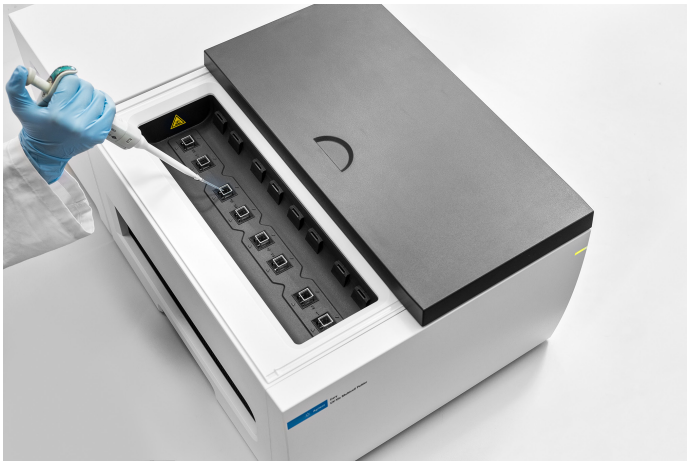

Figure 1. The Agilent Cary 3500 UV-Vis multicell spectrophotometer can be used for up to four temperature experiments across eight cuvette positions, simultaneously.

Biomolecule characterization by UV-Vis spectroscopy

UV-Vis spectroscopy is an important tool for the characterization of proteins and nucleic acids. This technique provides information about the quantity (e.g., concentration) and quality, stability (e.g., thermal melts and buffer suitability), and biochemical activity of biomolecules. Six uses of the technique are outlined in this technical overview.

Stability studies

UV-Vis spectroscopy can be used to study the stability of proteins and nucleotides. Changes in absorbance can be monitored over time, temperature, or buffer conditions such as salt concentration, or the presence of chemical denaturants (e.g., urea). UV-Vis provides valuable information about degradation, aggregation, or structural changes of biomolecules.

Protein aggregation

Since proteins absorb light near 280 nm (due to the presence of the aromatic amino acids tryptophan, tyrosine, and phenylalanine) and not at 350 nm, UV-Vis spectroscopy can be used to study protein aggregation by measuring scattered light at approximately 350 nm. Protein aggregation studies are useful for investigating protein therapeutics, as protein function is tightly linked to structure. UV-Vis spectroscopy is a simple and fast analytical solution that provides critical data in this area of research.

Comparative studies

UV-Vis spectroscopy is a fast technique, making it useful for comparative studies. In addition to providing absorption spectra, a second derivative of the spectra can be performed to analyze features with more resolution. As second derivative methods can resolve complex protein spectra, they provide a sensitive measure of changes in the local microenvironment of the aromatic side chains (tryptophan, tyrosine, and phenylalanine) in the protein structure. Through comparison of the respective spectra, conformational changes of proteins that occur due to changing the buffer, pH, polarity, or presence of different excipients can be detected.

Thermal melts (T_m)

Thermal stability is another important metric for both proteins and nucleic acids. For nucleic acids, as the temperature increases, double-stranded nucleotides become denatured into single strands, and the temperature at which this denaturing happens is mainly dependent on the nucleotide composition and sequence. Under controlled conditions, UV-Vis spectroscopy can be used to measure absorbance while gradually changing the temperature. Temperature-ramping experiments can be done by monitoring a single wavelength or a full range wavelength scan. Thermal melt data can be used for oligonucleotide identity confirmation, quality control purposes, investigating protein-nucleic acid interactions, and screening for thermostable oligonucleotides.

Quantification and quality control

UV-Vis is widely used for the quantification of nucleotides and proteins. Nucleotides absorb light at 260 nm, so monitoring absorption at other wavelengths can indicate the presence of contaminants. For example, the 260/280 and 260/230 ratios can be used to determine the purity of an isolated nucleic acid and to identify phenol or other organic or protein contaminants. For the quantification of protein, UV-Vis spectroscopy can be used for both pure proteins and mixtures through use of UV-active dyes such as the Bradford assay. Detecting impurities in proteins/nucleotides is an important step in quality-controlled environments to ensure that downstream processes will be successful. UV-Vis spectroscopy is a quick, easy, nondestructive analytical technique, making it ideal for the quantification of both proteins and nucleotides.

Enzyme activity

UV-Vis spectroscopy is useful for investigating enzyme reactions. The UV-Vis measures changes in absorbance over time as the reaction progresses. For example, if the substrate or cofactor absorbs light, the decrease in absorbance can be monitored over time as the substrate is consumed in the reaction. Conversely, a product of the reaction may absorb light, and the level of absorption will increase over time as the reaction proceeds.

The absorbance data can be used to determine the reaction rate constant and catalyst efficiency, and to characterize reaction inhibitors. Enzymatic activity studies can be done by performing repeated wavelength scans and monitoring changes in absorbance. A simpler way to perform these studies is by measuring the absorbance change at a single wavelength.

Advantages of the Cary 3500 UV-Vis for the characterization of biomolecules

The Cary 3500 UV-Vis is a double-beam spectrophotometer that offers unique measurement capabilities for liquid analysis applications in pharma and biopharma studies. The instrument can be fitted with various UV-Vis sample measurement modules to suit the application.

The Cary 3500 Multicell and Compact UV-Vis modules provide measurement solutions for cuvette-based applications, and so are suitable for molecular biology research studies. The modules can accommodate up to eight and two cuvettes, respectively, including small-volume cuvettes that are suitable for the analysis of low-volume samples.

When fitted with the eight-position Multicell, users have access to the simultaneous measurement capabilities of all eight channels. The multicell holder is built into the instrument and uses water-free, air-cooled Peltier devices to control the temperature of samples between 0 and 110 °C in four different temperature zones. An integrated in-cuvette temperature probe provides feedback to the Peltier block, enabling control of the temperature of the solutions during measurements, including the ability to apply fast ramp rates. The Compact system can also perform ambient and temperature-controlled experiments.

Alternatively, the Cary 3500 UV-Vis with Multicell can measure seven samples at the same time, and a reference, without compromising result accuracy. The capability to simultaneously measure at least three replicates of the sample through the instrument design represents valuable time saving for laboratories that require replicate measurements.

The 3500 is fully controlled using Agilent Cary UV Workstation software. The software is available with optional technical controls to securely acquire, process, report, and store data. These controls are vital for laboratories that must follow the compliance guidelines of FDA 21 CFR Part 11, EU Annex 11, GAMP5, as well as ISO/IEC 17025 and EPA 40 CFR Part 160.

A summary of the benefits of the Cary 3500 for the characterization of biomolecules is given in Figure 2.

Achieve more in less time
- Measure 8 channels simultaneously
- Perform a full wavelength range scan on all channels simultaneously

Reduce the frequency and cost of lamp replacement
- Long-lasting Xenon flash lamps with 10-year replacement warranty
- Remove daily warm-up burden
- Unique Xenon flash lamp source for high-quality data across the UV-Vis range

Measure samples at four different temperatures simultaneously
- Perfect solution for all temperature-related UV-Vis experiments, such as kinetic assay experiments

Deeper insight for biomolecules analysis
- Accurate quantification of proteins and oligonucleotides
- State-of-the-art technology for both constant and ramping temperature control

Fast oligonucleotide thermal melts
- Temperature can be ramped from 0.5 to 40 °C/min
- Improved accuracy, reproducibility, and confidence at any temperature ramp rate

Measure small volumes
- Measure small volumes (50 µL) using ultra-microvolume cuvettes
- Highly focused beam, no alignment required
- High repeatability

Powerful and flexible analytical software
- 50+ built-in calculations and customizable calculations
- Calculations can be saved within a method and applied automatically after samples are collected
- Optional OpenLab software offering technical control to securely acquire and store data

Achieve sustainability goals
- The system received My Green Lab's ACT (Accountability, Consistency, Transparency) label after independent audit and verification for its environmental impact throughout the product lifecycle

Figure 2. Features and benefits of the Agilent Cary 3500 UV-Vis spectrophotometer for the characterization of biomolecules.

Examples of Cary 3500 UV-Vis applications in biomolecule characterization

The Cary 3500 UV-Vis system has been used in different aspects of biomolecule characterization, including:

- Thermal denaturation of mature tRNAs.[3]
- Photoactivatable circular caged oligonucleotides thermal melt characterization.[4]
- Monitoring enzyme activity assays of recombinant EcSTH.[5]
- Quantification of DNAs.[6]
- Hydrodynamic diameter of the protein-AuNP conjugate measurements using dynamic light scattering.[7]
- Turbidity assay studies of liquid-liquid phase separation of the UBQLN2 protein.[8]

Conclusion

The Agilent Cary 3500 UV-Vis spectrophotometer is widely used for biomolecule characterization applications, such as thermal stability, quantification, and kinetics studies. Various research groups have used the system to advance their understanding of oligonucleotides and proteins.

The Cary 3500 UV-Vis with Multicell is especially useful for biomolecular applications as it reduces the time it takes to get results by monitoring eight channels simultaneously. Peltier technology ensures unmatched experimental reproducibility and enables four temperature-controlled experiments to be performed all at the same time.

The Agilent Cary UV Workstation instrument control software includes a range of operational qualification tests that are automated and align with global pharmacopeia requirements.

The distinctive design and performance capabilities of the Cary 3500 UV-Vis assist users in optimizing the experimental design of the application and a range of published studies provide confidence in the final results.

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For more information

- Cary 3500 Multicell UV-Vis Spectrophotometer
- Cary 3500 Compact UV-Vis Spectrophotometer
- Cary UV Workstation software
- Data Integrity Options for GMP Facilities for the Agilent Cary 3500 UV-Vis Spectrophotometer Series
- UV-Vis Spectroscopy & Spectrophotometer FAQs

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