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Why is fluorescence more sensitive than UV-VIS absorption spectroscopy?

Posted Jun 01, 2020

Answer

The reason why fluorescence is more sensitive than UV-Vis absorption is that they are measured in different ways. Absorbance is measured as the difference in intensity between light passing through the reference and the sample, whereas fluorescence is measured directly without any reference beam. In other words, fluorescence is measured over a dark background, while absorbance is measured over a bright background. It is relatively easy to detect low levels of light, but difficult to identify small differences in intensity. Therefore, fluorescence is more sensitive than UV-Vis absorption spectroscopy.

Additional resources

[iFluor™ 488 succinimidyl ester](#)

[Cyanine 5 monosuccinimidyl ester \[equivalent to Cy5® NHS ester\]](#)

[Lakowicz, J. R. \(Ed.\). \(2013\). Principles of fluorescence spectroscopy. Springer Science & Business Media.](#)

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acids and metabolites. It can also detect detailed information about the content and purity of a solution.

A [spectrophotometer](#) is designed to measure the amount of light absorbed or reflected by a specific compound and is an essential tool in any research laboratory. It is routinely used to determine the concentration of a particle in solution. Some spectrophotometers also can measure kinetic reactions.

Similarly, a spectrophotometer may have the added capability to measure [fluorescence](#). Fluorescence is the molecular absorption of light energy at one wavelength and its nearly instantaneous re-emission at another, longer wavelength. Some molecules fluoresce naturally, and others must be modified to fluoresce.

Common research applications for spectrophotometers include determining the concentration of DNA or proteins in a solution, measuring the rate of an enzyme-catalyzed reaction and monitoring growth curves of bacteria cells. Depending on the researcher's focus, any of these methods can be used to characterize the sample of interest.

As with any methodology, there are strengths and weakness to each. Here we present advantages and disadvantages of each method for the end user to consider when selecting an instrument.

Absorption (UV/Vis) spectrophotometer

Ultraviolet and visible (UV/Vis) spectrometers have been in general use for the past 35 years and are important analytical instruments in the laboratory. Here are some features to consider when selecting a UV/Vis spectrophotometer.

Advantages

- **Economic value:** These instruments offer an excellent value, providing robust operation and low cost of ownership. They require only minor attention to keep running smoothly. Monitoring overall cleanliness and lamp life are the bulk of the maintenance.
- **Ease of use:** Spectrophotometers are fairly straightforward instruments with very few moving parts; they are therefore relatively easy to use and maintain. In addition, many manufacturers provide intuitive software solutions for a satisfying user experience.
- **Noninvasive:** There is no direct manipulation of the material being measured, thus the integrity of the sample is not compromised.
- **Revealing contaminants:** Full-spectrum absorption readings detect additional peaks, revealing the presence of potential sample contaminants. This information may be useful for downstream applications in which the contaminants could be detrimental to the experiment.

Disadvantages

- **Selectivity:** Keep in mind that a UV/Vis spectrophotometer does not discriminate between the sample of interest and contaminants that absorb at the same wavelength. For example, all nucleic acids exhibit a peak at or around 260 nm; therefore, intact RNA and double-stranded DNA, as well as the degraded species of single-stranded DNA in a sample solution, contribute to total absorbance at 260 nm.
- **Stray light:** The detectors used in spectrophotometers are broadband, meaning they respond to all the light that reaches them. If there are impurities in the sample that reflect light, an erroneous reading may be recorded. Stray light also causes a decrease in absorbance and reduces the linearity range of the instrument.
- **Sample conditions:** Absorption results can be influenced by temperature, pH, impurities and contaminants. All of these factors can change the absorption properties of the sample, leading to inaccurate readings.
- **Low sensitivity:** The sensitivity of a spectrophotometer is often inadequate at low sample concentrations. Researchers may need to concentrate their sample, adding additional steps and time to their workflow.

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applies to different applications.

Advantages

- **Sensitivity:** The sensitivity of fluorescence detection is approximately 1,000 times greater than absorption spectrophotometric methods. This leads to greater limits of detection, while potentially using less sample material. This is important especially when working with precious or limited-quantity materials.
- **Specificity:** Only molecules that fluoresce are detected by this method, resulting in greater specificity compared with UV/Vis absorption.
- **Wide concentration range:** Fluorimetry generally can detect more than three to six log orders of concentration without sample dilution or modification of the sample.
- **Accurate results:** The sensitivity and specificity of fluorescence measurement leads to potentially more precise and accurate readings.

Disadvantages

- **Economic value:** The price of the instrument, as well as the assay reagents, can be a challenge to cost-conscious scientists. The light source and filters are key components of the instrument, providing sensitivity and selective detection, but these options also can affect purchase price. Price per assay or even per datapoint should be considered when evaluating the methodology.
- **Impurities:** Although fluorimeters are not affected by turbidity, light scatter may artificially increase the measured readings. On the other hand, if interfering substance or impurities absorb light, fluorescent readouts may be reduced.
- **Bubbles:** Bubbles in the sample preparation can result in erratic or fluctuating readings, as measurement depends on light cleanly exciting the sample's molecules.
- **pH:** Fluorescence can be affected by the pH of the sample solution, leading to inaccurate measurements. Using the same conditions for standards, blanks and samples helps eliminate this effect.

Three key points

Whether measuring nucleic acids, protein concentrations or enzyme activity, a spectrophotometer is your go-to instrument. There are many options to consider when selecting the type of instrument best for your laboratory to purchase. Three important considerations will guide you to select the appropriate method and instrument for your studies.

1. **Intended application and flexibility:** Although all spectrophotometers use a noninvasive methodology, spectrophotometers with additional fluorescence capabilities offer enhanced sensitivity and selectivity in their detection. Many manufacturers provide options to purchase a variety of spectrophotometers with various capabilities.
2. **Ease of use:** In most research labs, scientists using spectrophotometers have varying levels of experience. Companies are sensitive to providing intuitive software designed for convenient, easy interaction. For simplicity's sake, software may be contained within the unit itself. The software may include programming for frequently used applications and commonly used kits. Technical guidance also may be included in the software, with step-by-step explanations. Additionally, a USB-port option may be available to export data, eliminating the need to connect to an external computer. It is important for users to feel comfortable navigating their protocols on their spectrophotometer.
3. **Maintenance and reliability:** Although spectrophotometers are fairly simple instruments with limited moving parts, some maintenance is required to assure the most reliable and accurate measurements. Monitoring cleanliness and lamp life comprises most of the maintenance; doing so will keep your instrument running reliably. Be sure to check the manufacturer's warranty on parts and labor, and discuss with your sales representative the service options covered and available with the warranty.

As with any purchase for your lab, be sure to do your homework and understand your needs before purchasing. The spectrophotometer is an invaluable tool in any research lab. So explore your options, and then you can start taking your

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