Application Note

Keywords

- Beer-Lambert law
- Chromophore

Techniques

- Absolute absorbance
- Transmission

Applications

- Concentration of chromophores in solvents
- Chromophore linearity

Quantitative Absorbance Spectrophotometry

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For students and novice spectroscopy users, the fundamentals of absorbance spectrophotometry begin with an understanding of the Beer-Lambert Law. Whether measurements are qualitative or quantitative, Beer's Law rules the day.

Introduction

Absorbance spectrophotometry can be used as a qualitative tool to identify or "fingerprint" substances, and as a quantitative tool to measure the concentration of a colored substance (chromophore) in a transparent solvent. In some situations, the absolute absorbance, or extinction coefficient of the unknown substance is desired. In other situations, the concentration is related empirically to standard solutions of known concentration. In either case, the derivation of absorbance is called the Beer-Lambert Law (1,2,3).

Experimental Conditions

Absorbance is measured in a spectrophotometer by passing a collimated beam of light at wavelength λ through a plane parallel slab of material that is normal to the beam. For liquids, the sample is held in a cuvette. The light energy incident on the sample (I_0) is more intense than the light that makes it through the sample (I) because some of the energy is absorbed by the molecules in the sample.

Table I: Absorption Wavelength Bands of Assorted Chromophores		
Chromophore	Maximum Absorption (in nm)	Absorbance Intensity
Phenanthrene	251	66 000
	292	14 000
Naphthacene	272	180 000
	473	12 500
Pentacene	310	300 000
	585	12 000
Pyridine	174	80 000
	195	6 000
	257	1 700
Quinoline	227	37 000
	270	3 600
	314	2 750
Isoquinoline	218	80 000
	266	4 000
	317	3 500

Results

Transmission (T), expressed as a percentage, is the ratio:

$$T = I/I_0$$

Transmission ranges from 0% for an opaque sample to 100% for a transparent sample. If there are absorbing molecules in the optical path, the transmission will be <100%. The number of molecules that interact with the light will depend on the pathlength (l) and the concentration of the molecules (c). The attenuation will also depend on the ability of the molecule to absorb light at that wavelength, expressed as the extinction coefficient or molar absorptivity ε.

$$T = I/I_0 = e^{-\varepsilon lc}$$

Absorbance (A λ) is the negative $\log_{10}$ of transmission. This mathematical transformation is used to make the relationship between concentration and absorbance linear:

$$A\,\lambda = -\log (I/I_0) = \varepsilon cl$$

Absorbance ranges from 0 for perfectly transparent samples (T = 100%) to infinity for perfectly opaque samples (T = 0%). The value of ε depends on the units of c. Typically, c is in molarity (moles liter-1), l is measured in cm, and ε has units of absorbance (cm-1 liters mole-1).

Conclusion

In an absorbance experiment, light is attenuated not only by the chromophore, but also by reflections from the interfaces between the air and the sample and between the sample and the cuvette, and by absorbance by the solvent. These factors can be quantified separately, but are often removed by defining I_0 as the light passing through a sample blank or reference sample. The definition of the blank sample thus becomes an important qualifier of the measurement.

You can account for the absorbance of a blank solution either by measuring it and subtracting it from the sample absorbance measurements, or by setting the instrument to start at 100% T or 0 units of absorbance. This is much like setting the tare on a balance.

Many factors can affect the validity of Beer's Law. It is usual to check for the linearity of Beer's Law for a chromophore (see sampling of chromophores in Table I) by measuring the absorbance of a series of standards. This calibration exercise also can remove errors in the experiment, the equipment and the batch of reagents (such as cuvettes of unknown pathlength).

References

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