Fundamentals of modern UV-visible spectroscopy

Presentation Materials

The Electromagnetic Spectrum

$$E = h\nu \qquad \nu = c / \lambda$$

Electronic Transitions in Formaldehyde

Electronic Transitions and Spectra of Atoms

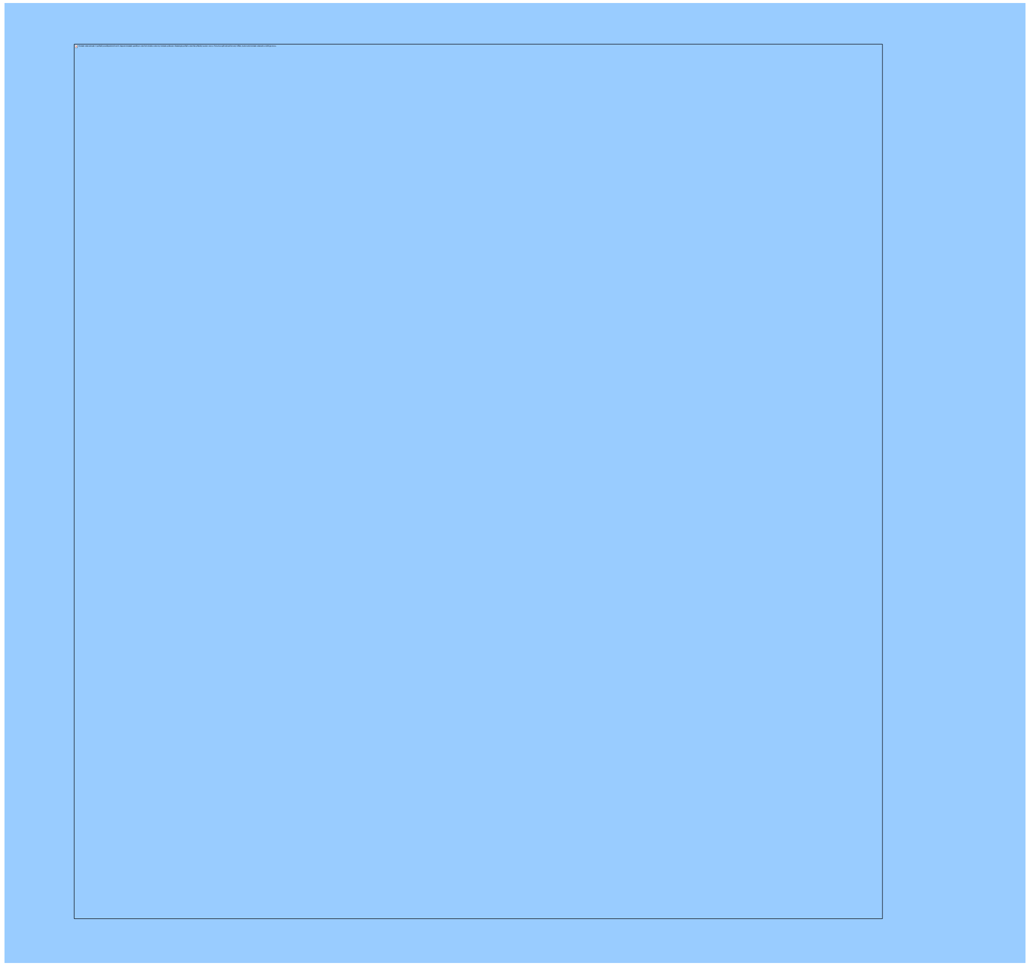
Electronic Transitions and UV-visible Spectra in Molecules

Derivative Spectra of a Gaussian Absorbance Band

Absorbance: $A = f(\lambda)$

1st Derivative: $\dfrac{dA}{d\lambda} = f^{'}(\lambda)$

2nd Derivative: $\dfrac{d^2 A}{d\lambda^2} = f^{''}(\lambda)$



Fundamentals of modern UV-visible spectroscopy

Resolution Enhancement

- Overlay of 2 Gaussian bands with a NBW of 40 nm separated by 30 nm

- Separated by 4th derivative

Transmission and Color

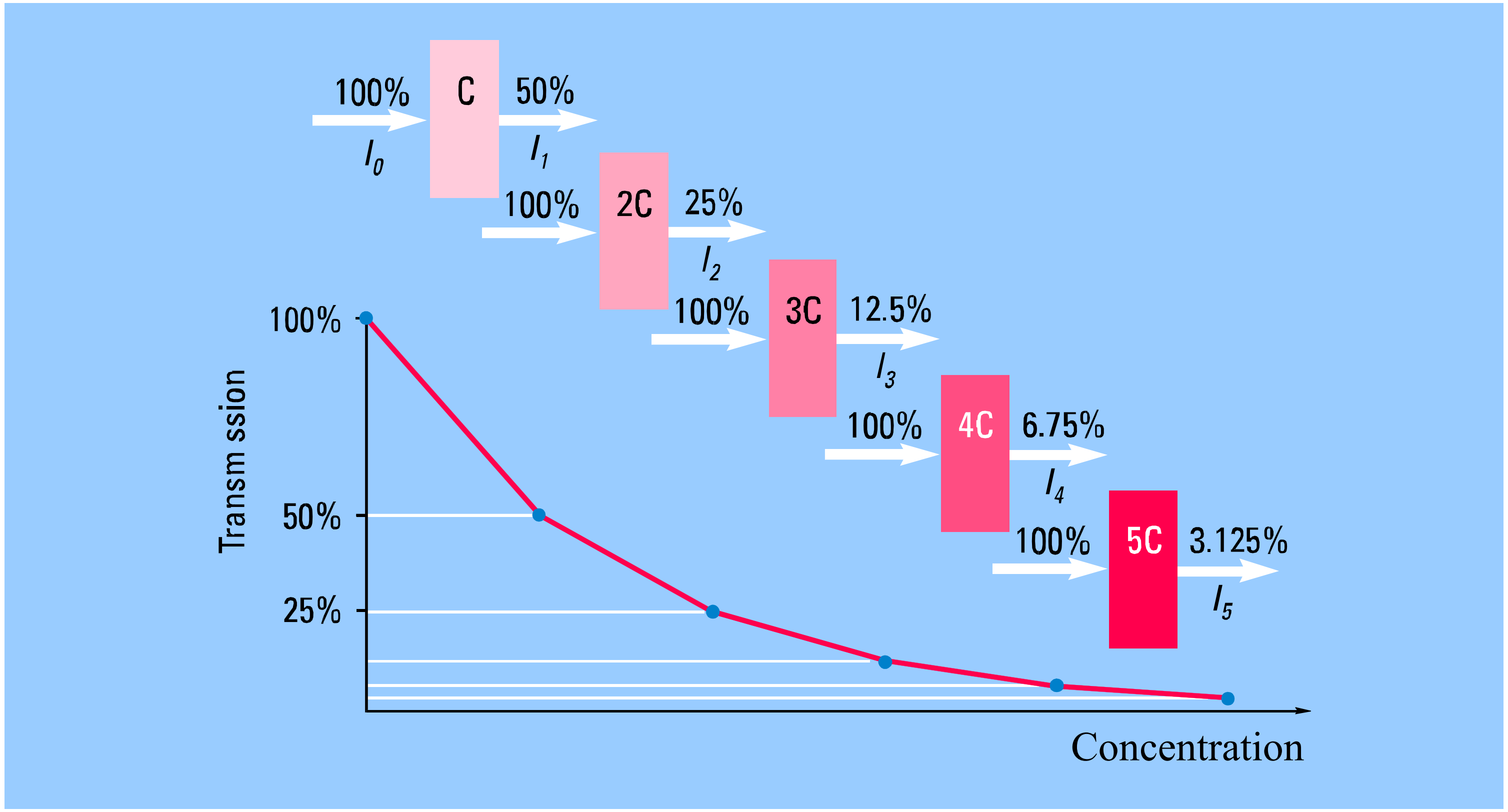
The human eye sees the complementary color to that which is absorbed

Absorbance and Complementary Colors

Transmittance and Concentration
The Bouguer-Lambert Law

$$T = I / I_0 = e^{-Const \cdot Pathlength}$$

Transmittance and Path Length
Beer's Law



$$T = I / I_0 = e^{-Const \cdot Concentration}$$

The Beer-Bouguer-Lambert Law

$$A = -\log T = -\log\left(I / I_0\right) = \log\left(I_0 / I\right) = \varepsilon \cdot b \cdot c$$

Two-Component Mixture

Example of a two-component mixture with little spectral overlap

Two-Component Mixture

Example of a two-component mixture with significant spectral overlap

Influence of 10% Random Error

Influence on the calculated concentrations

- Little spectral overlap: 10% Error
- Significant spectral overlap: Depends on similarity, can be much higher (e.g. 100%)

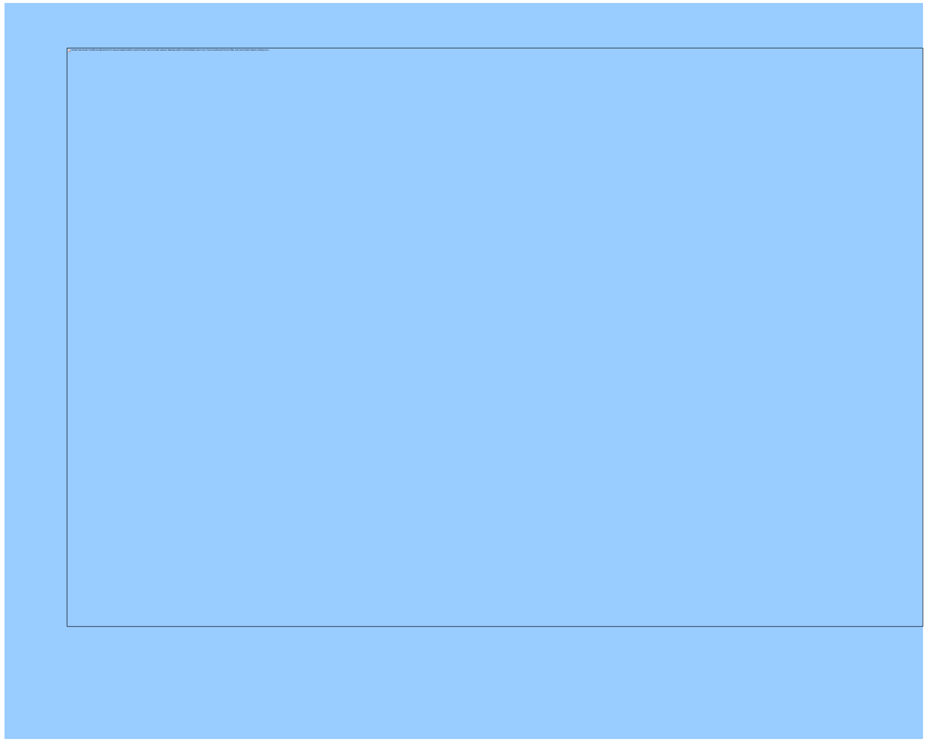
Absorption Spectra of Hemoglobin Derivatives

Intensity Spectrum of the Deuterium Arc Lamp

- Good intensity in UV range
- Useful intensity in visible range
- Low noise
- Intensity decreases over lifetime

Intensity Spectrum of the Tungsten-Halogen Lamp

- Weak intensity in UV range
- Good intensity in visible range
- Very low noise
- Low drift



Intensity Spectrum of the Xenon Lamp

- High intensity in UV range
- High intensity in visible range
- Medium noise

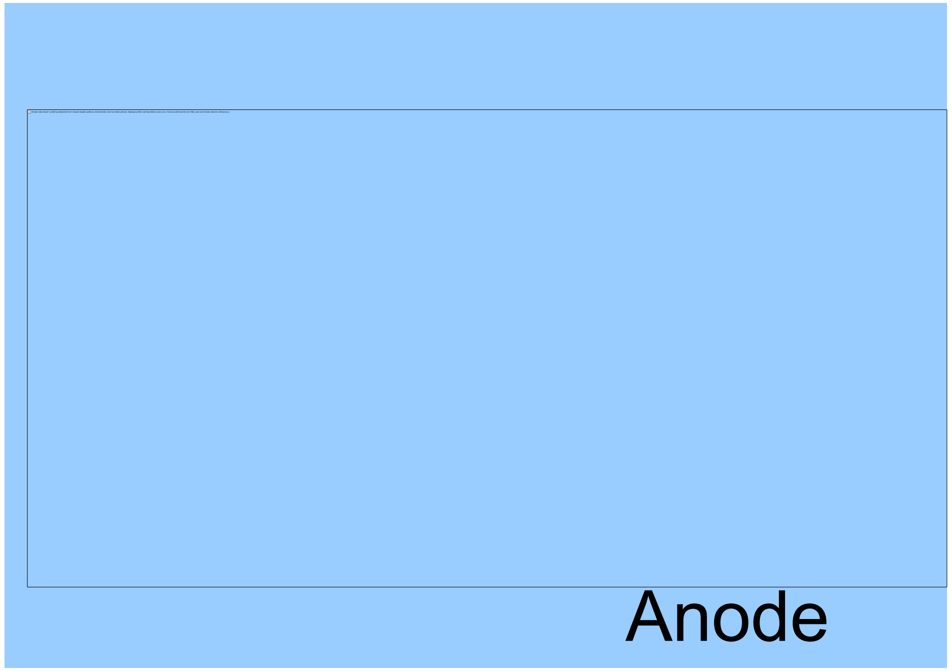
Dispersion Devices

- Non-linear dispersion
- Temperature sensitive

- Linear Dispersion
- Different orders

Photomultiplier Tube Detector

- High sensitivity at low light levels
- Cathode material determines spectral sensitivity
- Good signal/noise
- Shock sensitive



The Photodiode Detector

- Wide dynamic range
- Very good signal/noise at high light levels
- Solid-state device

Schematic Diagram of a Photodiode Array

- Same characteristics as photodiodes
- Solid-state device
- Fast read-out cycles

Conventional Spectrophotometer

Schematic of a conventional single-beam spectrophotometer

Diode-Array Spectrophotometer

Schematic of a diode-array spectrophotometer

Diode-Array Spectrophotometer

Optical diagram of the HP 8453 diode-array spectrophotometer

Conventional Spectrophotometer

Optical system of a double-beam spectrophotometer

Diode-Array Spectrophotometer

Optical system of the HP 8450A diode-array spectrophotometer

Conventional Spectrophotometer

Optical system of a split-beam spectrophotometer

Definition of Resolution

Spectral resolution is a measure of the ability of an instrument to differentiate between two adjacent wavelengths

Instrumental Spectral Bandwidth

The SBW is defined as the width, at half the maximum intensity, of the band of light leaving the monochromator

Natural Spectral Bandwidth

The NBW is the width of the sample absorption band at half the absorption maximum

Effect of SBW on Band Shape

The SBW/NBW ratio should be 0.1 or better to yield an absorbance measurement with an accuracy of 99.5% or better

Effect of Digital Sampling

The sampling interval used to digitize the spectrum for computer evaluation and storage also effects resolution

Wavelength Resettability

Influence of wavelength resettability on measurements at the maximum and slope of an absorption band

Effect of Stray Light

Effect of various levels of stray light on measured absorbance compared with actual absorbance

Theoretical Absorbance Error

The total error at any absorbance is the sum of the errors due to stray light and noise (photon noise and electronic noise)

Effect of Drift

Drift is a potential cause of photometric error and results from variations between the measurement of I_0 and I

Transmission Characteristics of Cell Materials

Note that all materials exhibit at least approximately 10% loss in transmittance at all wavelengths

Cell Types I

Open-topped rectangular standard cell (a)
and apertured cell (b) for limited sample volume

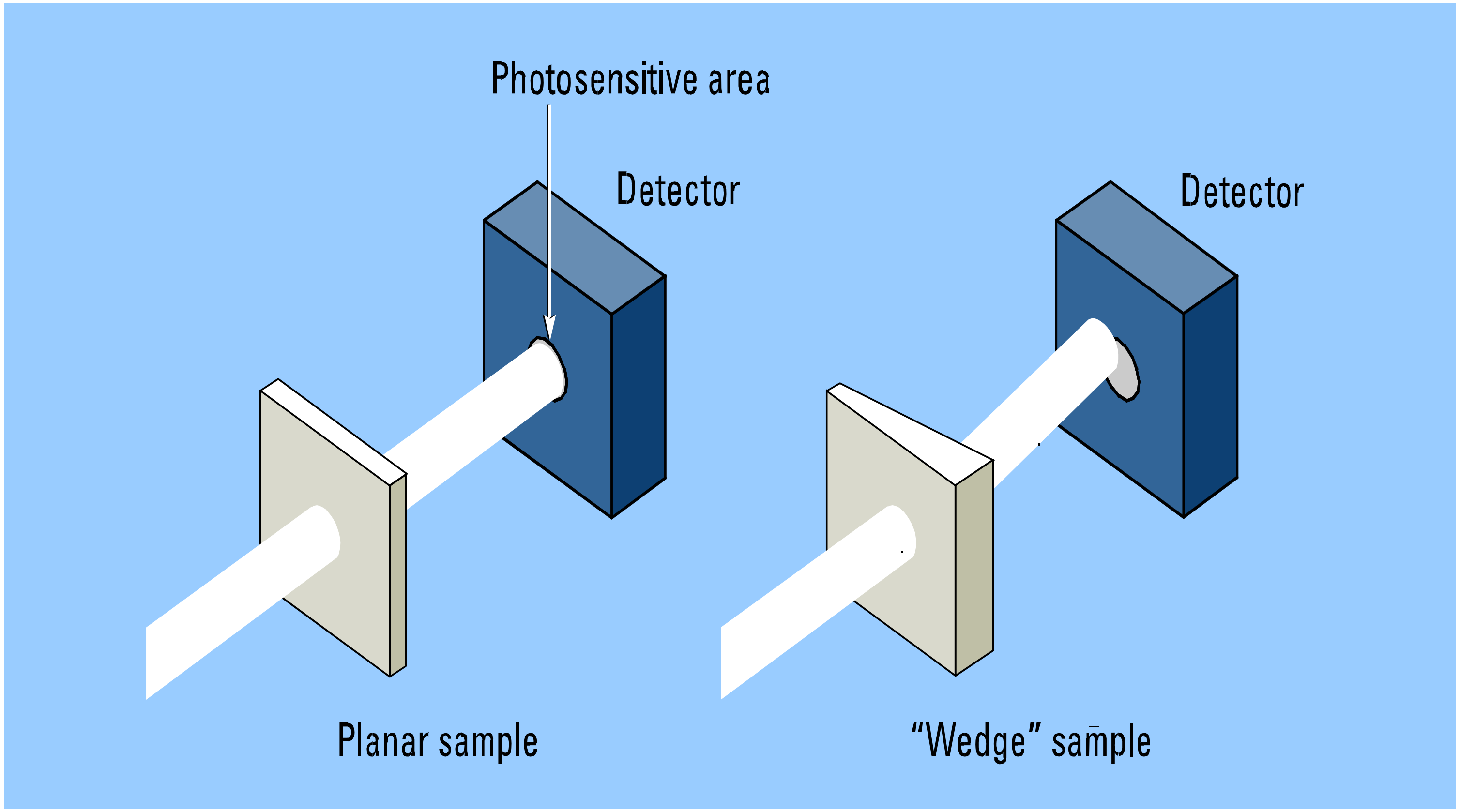
Cell Types II

Micro cell (a) for very small volumes
and flow-through cell (b) for automated applications

Effect of Refractive Index

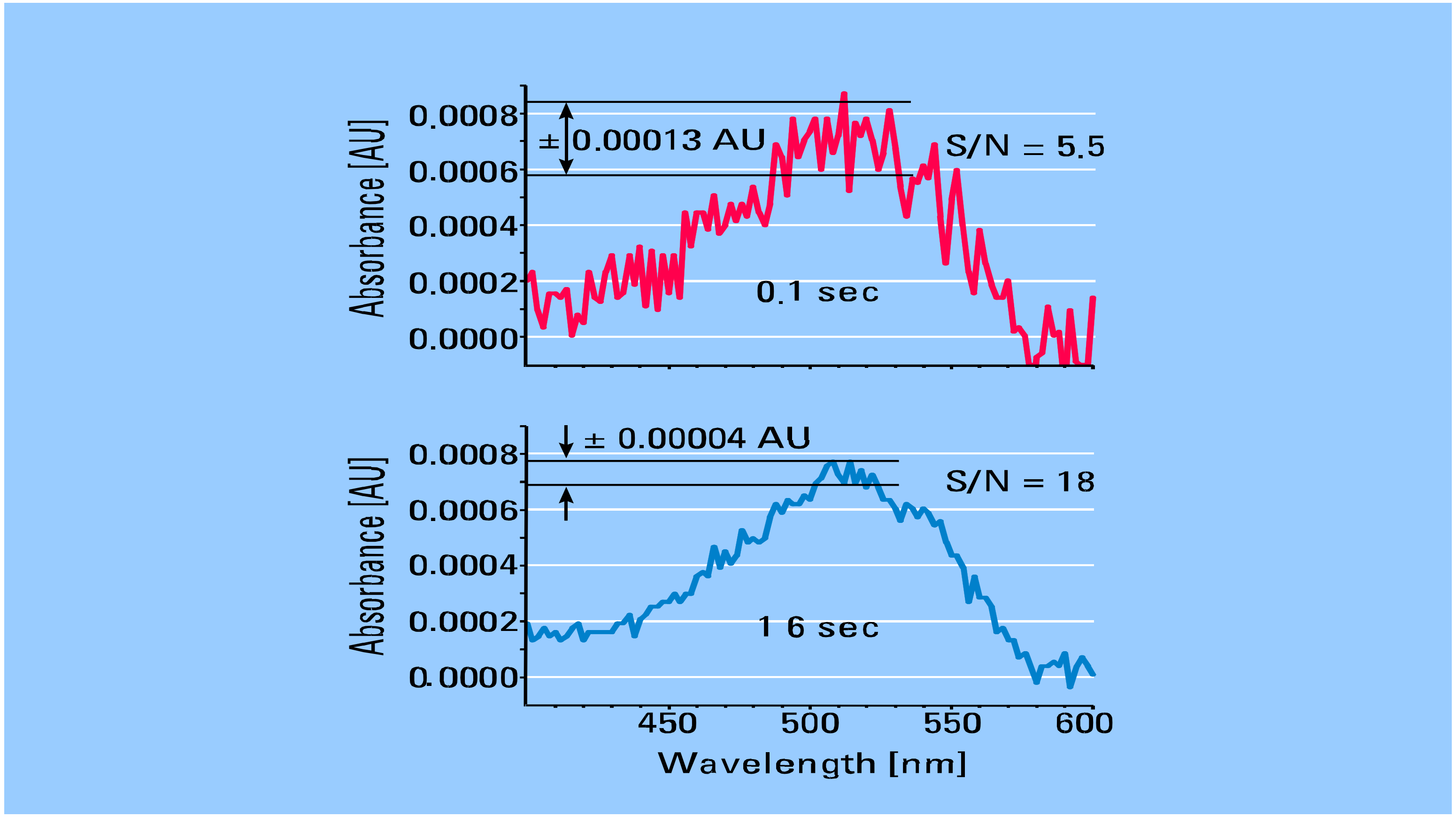
Changes in the refractive index of reference and sample measurement can cause wrong absorbance measurements

Non-planar Sample Geometry



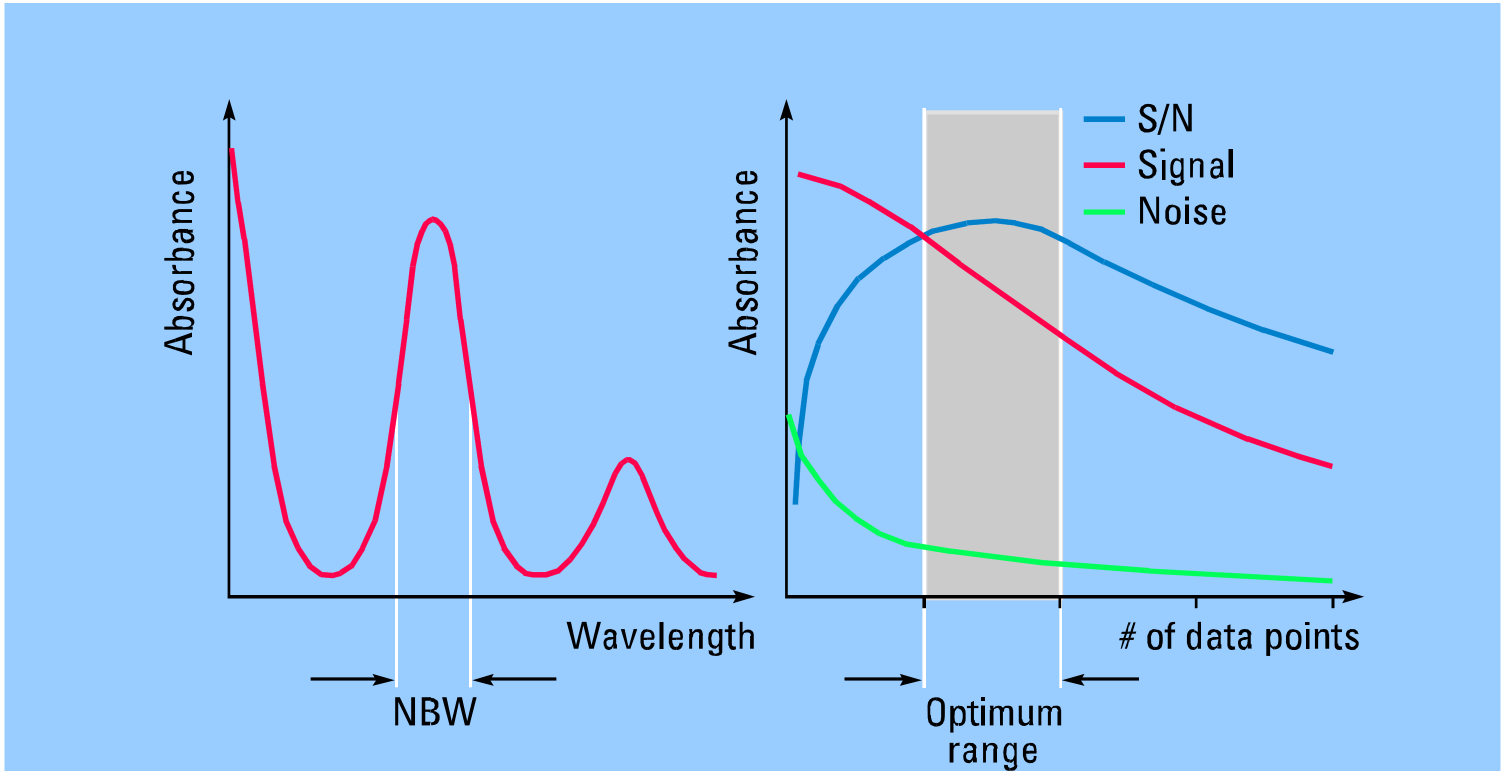
Some sample can act as an active optical component in the system and deviate or defocus the light beam

Effect of Integration Time



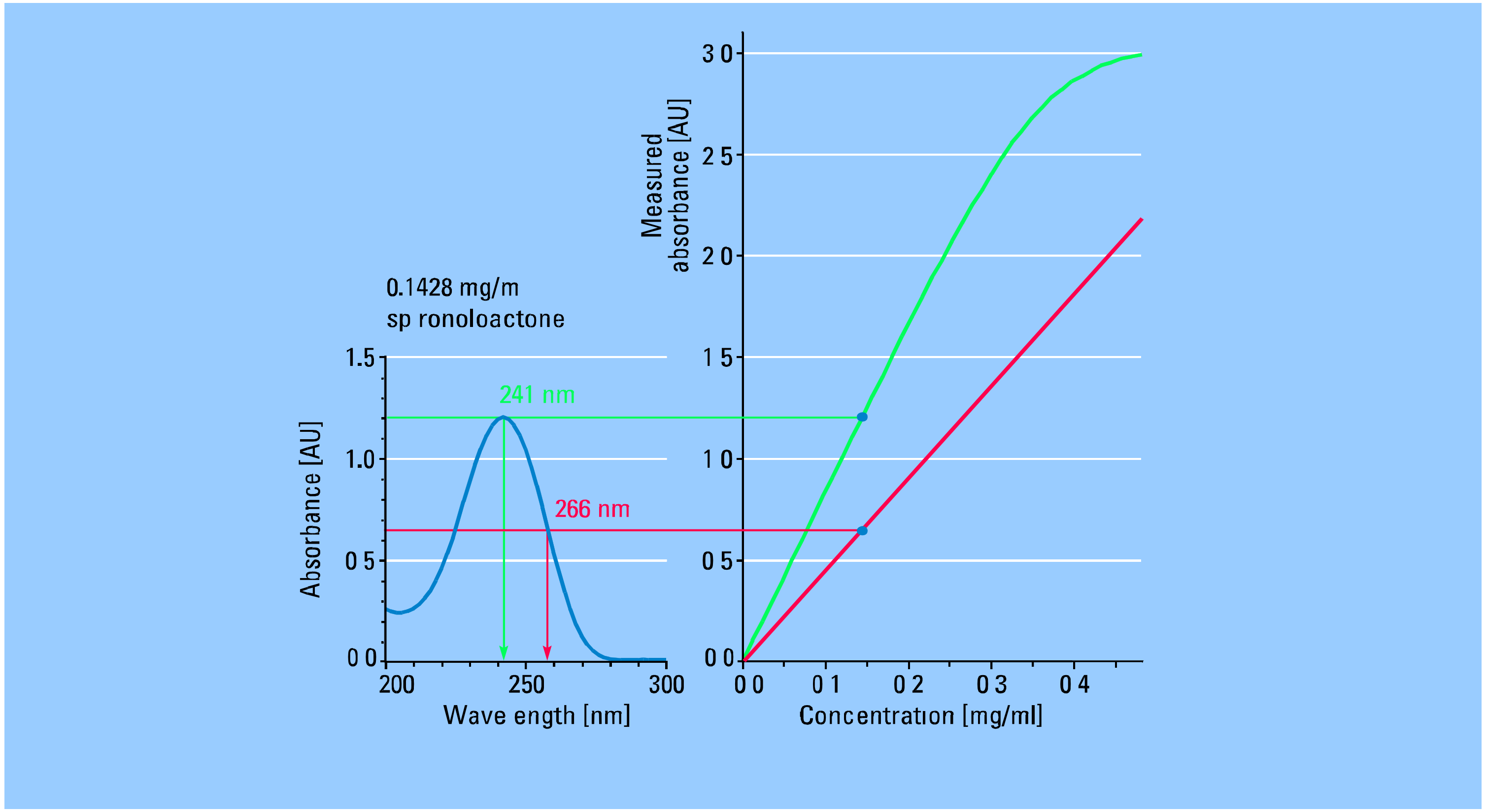
Averaging of data points reduces noise by the square root of the number of points averaged

Effect of Wavelength Averaging



- Wavelength averaging reduces also the noise (square root of data points)
- Amplitude of the signal is affected

Increasing Dynamic Range

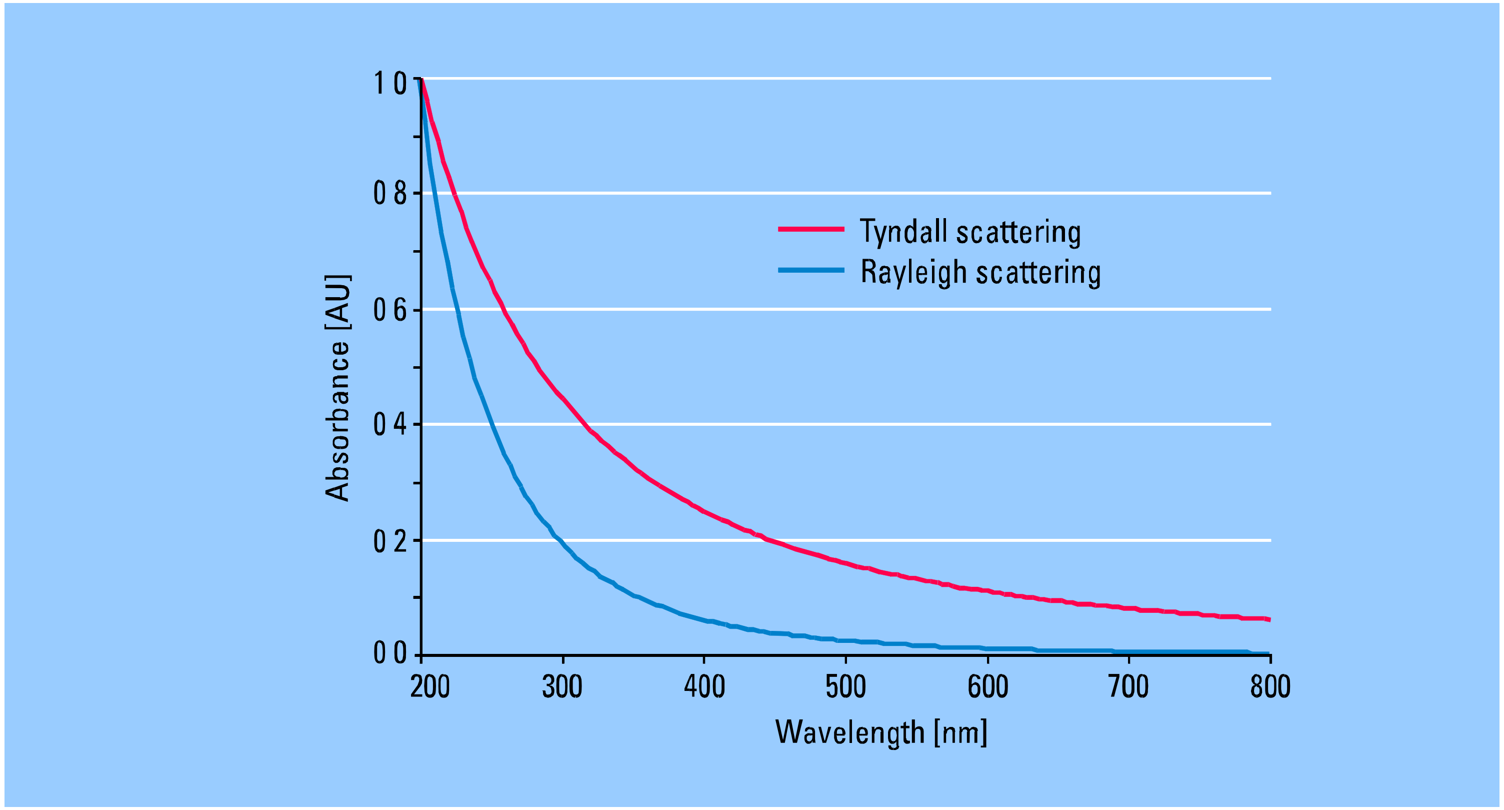


Selection of a wavelength in the slope of a absorption band can increase the dynamic range and avoid sample preparation like dilution

Scattering

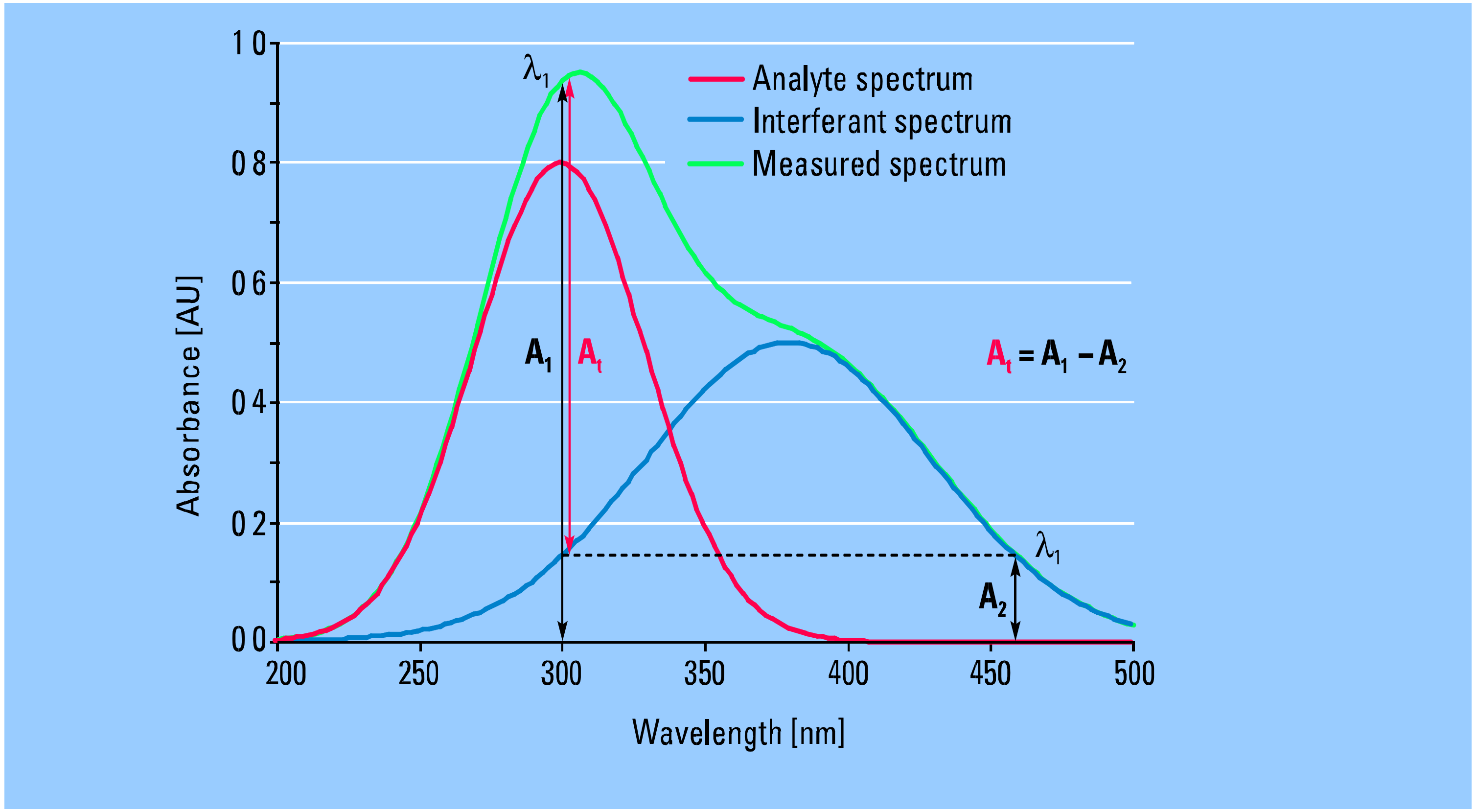
Scattering causes an apparent absorbance because less light reaches the detector

Scatter Spectra



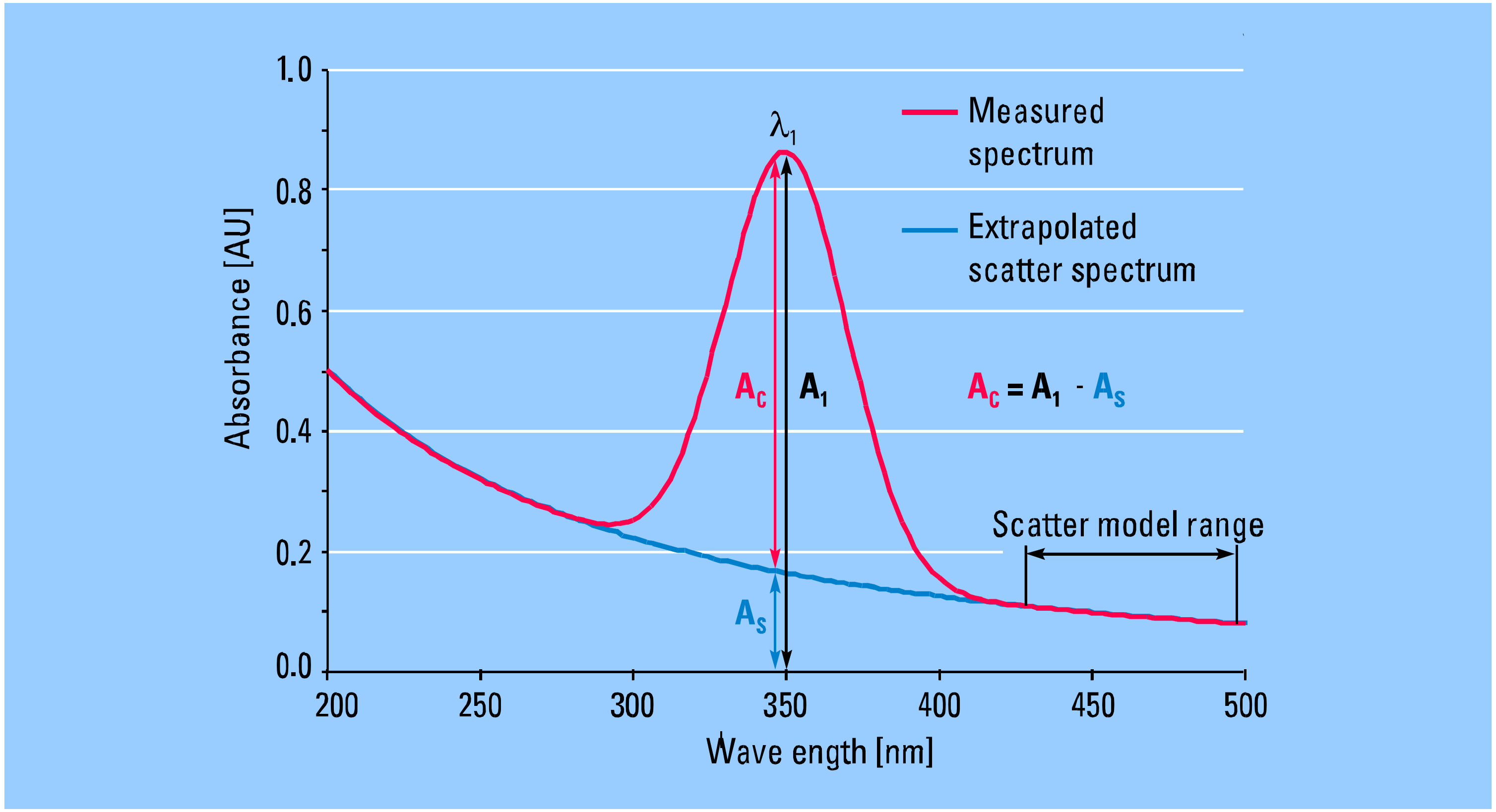
- Rayleigh scattering: Particles small relative to wavelength
- Tyndall scattering: Particles large relative to wavelength

Isoabsorbance Corrections



Absorbance at the reference wavelength must be equivalent to the interference at the analytical wavelength

Background Modeling



Background modeling can be done if the interference is due to a physical process

Internal Referencing

Corrects for constant background absorbance over a range

Three-Point Correction

- Uses two reference wavelengths
- Corrects for sloped linear background absorbance

Discrimination of Broad Bands

- Derivatives can eliminate background absorption
- Derivatives discriminate against broad absorbance bands

Scatter Correction by Derivative Spectroscopy

Scatter is discriminated like a broad-band absorbance band

Effect of Fluorescence

The emitted light of a fluorescing sample causes an error in the absorbance measurement

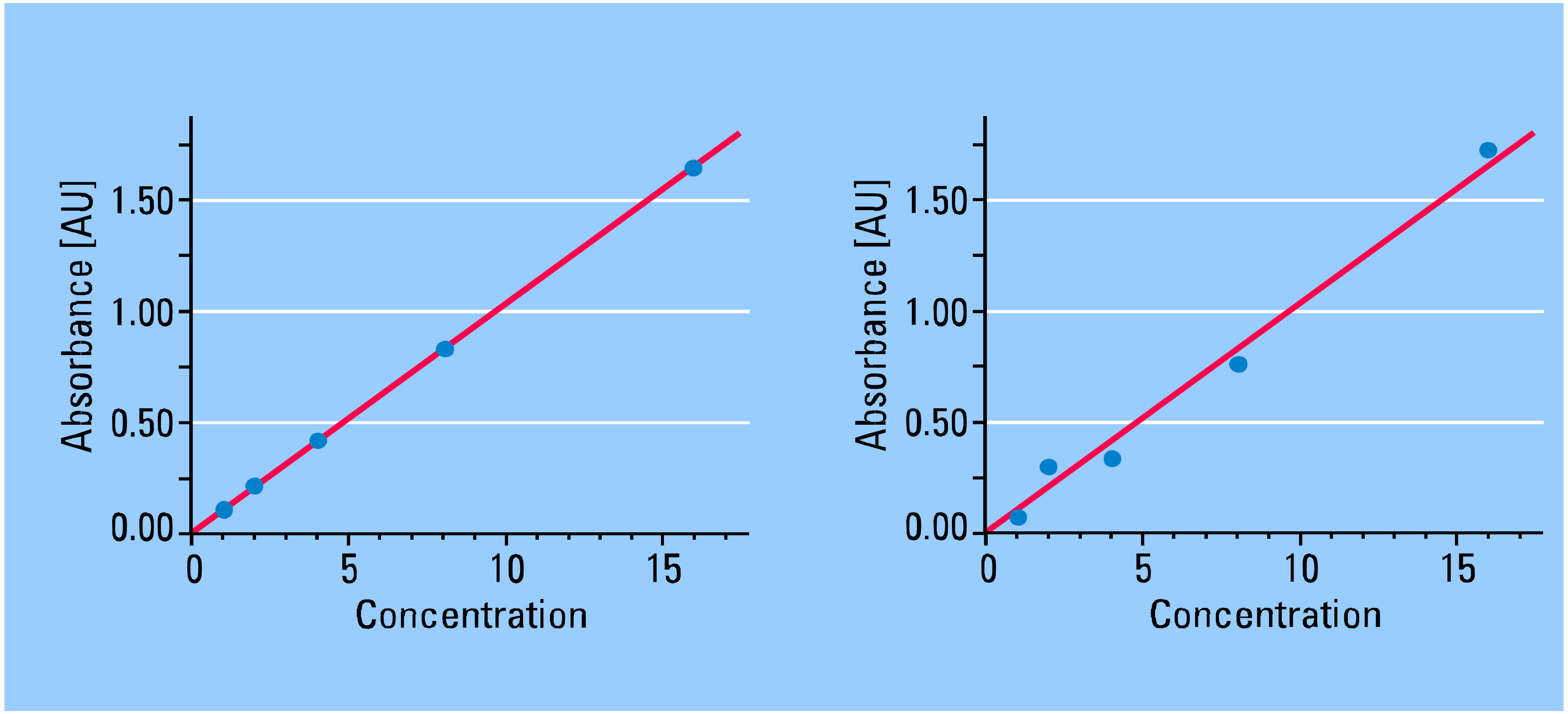
Acceptance Angles and Magnitude of Fluorescence Error

- Forward optics: Absorbance at the excitation wavelengths are too low
- Reversed optics: Absorbance at the emission wavelengths are too low

Inadequate Calibration

- Theoretically only one standard is required to calibrate
- In practice, deviations from Beer's law can cause wrong results

Calibration Data Sets



- Forward optics: Absorbance at the excitation wavelengths are too low
- Reversed optics: Absorbance at the emission wavelengths are too low

Wavelength(s) for Best Linearity

- A linear calibration curve is calculated at each wavelength
- The correlation coefficient gives an estimate on the linearity

Wavelength(s) for Best Accuracy

- The quantification results are calculated at each wavelength
- The calculated concentration are giving an estimate of the accuracy

Precision of an Analysis

Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings

Wavelength(s) for Best Sensitivity

- Calculation of relative standard deviation of the measured values at each wavelength
- The wavelength with lowest %RSD likely will yield the best sensitivity

Wavelength(s) for Best Selectivity

Selectivity is the ability of a method to quantify accurately and specifically the analyte or analytes in the presence of other compounds

Ideal Absorbance and Wavelength Standards

- An ideal absorbance standard would have a constant absorbance at all wavelengths
- An ideal wavelength standard would have very narrow, well-defined peaks

Ideal Stray Light Filter

An ideal stray light filter would transmit all wavelengths except the wavelength used to measure the stray light

Holmium Perchlorate Solution

The most common wavelength accuracy standard is a holmium perchlorate solution

Potassium Dichromate Solution

The photometric accuracy standard required by several pharmacopoeias is a potassium dichromate solution

Stray Light Standard Solutions

The most common stray light standard and the respectively used wavelengths

Toluene in Hexane (0.02% v/v)

The resolution is estimated by taking the ratio of the absorbance of the maximum near 269 nm and minimum near 266 nm

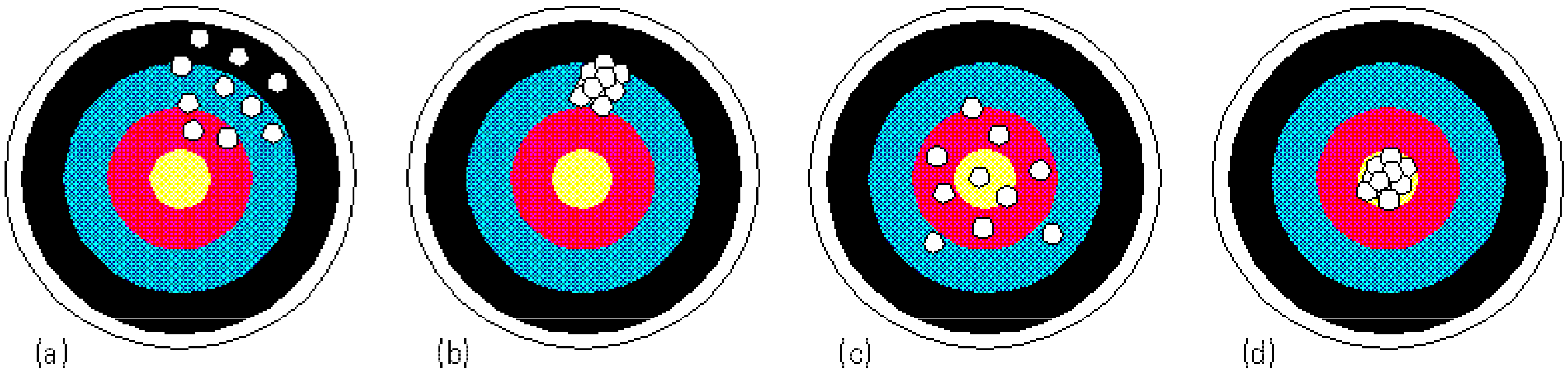
Confirmation Analysis

In confirmation analysis, the absorbance at one or more additional wavelengths are used to quantify a sample

Spectral Similarity

Comparative plots of similar and dissimilar spectra

Precision and Accuracy



(a)	(b)	(c)	(d)
Precision −	Precision +	Precision −	Precision +
Accuracy −	Accuracy −	Accuracy +	Accuracy +

Hydrolysis of Sultone