Spectrophotometry: An Analytical Tool

The process of light being absorbed by a solution of concentration 2 with sample I_I_0 where I_0 > I

As concentration increased, less light was transmitted (more light absorbed).

Some terminology

- I – intensity where I_0 is initial intensity
- T – transmission or %T = 100 x T (absorption: Abs = 1 – T or %Abs = 100 - %T)
- A – absorbance
  \[ A = -\log T = -\log I/ I_0 \]

Beer’s Law

\[ A = abc \]

where a - molar absorptivity, b - pathlength, and c - molar concentration

See the Beer’s Law Simulator

Analyze at what wavelength?

Scan visible wavelengths from 400 – 650 nm (detector range) to produce an absorption spectrum (A vs. \( \lambda \))

The BLANK

- The blank contains all substances except the analyte.
- Is used to set the absorbance to zero:
  \[ A_{blank} = 0 \]
- This removes any absorption of light due to these substances and the cell.
- All measured absorbance is due to the analyte.
The components of a Spec-20D

Light source - white light of constant intensity

Grating - separates white light into various colors

Sample - rotating the grating changes the wavelength going through the sample

Slits - when blank is the sample, I₀ is determined, otherwise I is measured

Phototube - detects light & measures intensity

What does the absorbed light (electromagnetic radiation) do to the molecule?

- high energy UV - ionizes electrons
- low energy UV and visible - promotes electrons to higher energy orbitals (absorption of visible light leads to a colored solution)
- IR - causes molecules to vibrate (more later)

Rotating the grating changes the wavelength going through the sample

UV/visible light absorption

Valence electrons

- In organic molecules, electronic transitions to higher energy molecular orbitals - double bonds: π → π*
- In transition metals, hydrated ions as Cu²⁺ have splitting of d orbital energies and electronic transitions - weak absorption
- In complexed transition metals, charge transfer of electrons from metal to ligand as Cu(NH₃)₄²⁺ - strong absorption

Uses of visible spectrophotometry

- Analysis of unknowns using Beer's Law calibration curve (Been there, done that!)
- Absorbance vs. time graphs for kinetics
- Single-point calibration for an equilibrium constant determination
- Spectrophotometric titrations - a way to follow a reaction if at least one substance is colored - sudden or sharp change in absorbance at equivalence point, a piece-wise function

Kinetics of Crystal Violet Reaction

CV⁺ + OH⁻ → CV-OH

Follow concentration of crystal violet over time as it reacts by measuring its absorbance.

How will absorbance change with time?

For a absorbance vs. time plot, how will you determine the rate of the reaction?
Single-point calibration

- Standard with measured absorbance
  \[ A_{\text{std}} = abc_{\text{std}} \]
- Unknown with measured absorbance
  \[ A_{\text{unk}} = abc_{\text{unk}} \]

Ratio the two equations

\[ \frac{A_{\text{unk}}}{A_{\text{std}}} = \frac{abc_{\text{unk}}}{abc_{\text{std}}} \]

- Solve for \( c_{\text{unk}} \)

Equilibrium Constant Determination

\[ \text{Fe}^{3+} + \text{SCN}^- = \text{Fe(SCN)}^{2+} \]

Color: colorless → colorless → orange

\[ K = \frac{(\text{Fe(SCN)})^{2+}}{(\text{Fe}^{3+})(\text{SCN}^-)} \]

Using the reactants, shift reaction based on Le Chatelier’s principle.

\[ (\text{Fe(SCN)})^{2+} + \text{SCN}^- = \text{Fe(SCN)}_2^{2+} \]

We start with a high concentration of \( \text{Fe}^{3+} \) and lower its value by dilution.

When calibration curves go bad!

- The linear Beer’s Law relationship starts to show curvature at high concentrations

- Single-point calibration assumes a linear calibration curve

Spectrophotometric titration

- Let’s consider the analysis of hydrogen peroxide with potassium permanganate in an acidic solution.
- The potassium permanganate or \( \text{MnO}_4^- \) is the only colored substance in the reaction. (It can serve as its own indicator.)
- How would the absorbance change as titrant was added?

\[ 5\text{H}_2\text{O}_2 + 2\text{MnO}_4^- + 6\text{H}^+ \rightarrow 5\text{O}_2 (g) + 2\text{Mn}^{2+} + 8\text{H}_2\text{O} \]

Notice you do not need to have a data point at the equivalence point. Equivalence point located by extrapolation of the two lines.

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