Absorption and emission of radiation
Rhodamine 6G (Basic Red 1)

Red dye in solution, solvent dependent green or yellow fluorescence

Absorbs at $\lambda_{\text{max}}$ 524 nm, fluoresces under green light
Absorbance and emission spectra of rhodamine G in ethanol
Peak Width of Electronic Spectra of Molecules

Electronic transition from ground vibrational and electronic state to one of several closely spaced vibrational energy levels in the excited electronic state.

Gives broad absorptions in electronic spectra.

Electronic ground state

Electronic excited state

Vibrational (bond stretches) energy levels of excited electronic state

Vibrational (bond stretches) energy levels of ground electronic state

Energy/Wavelength

Distance (Arbitrary Units)
Absorbance and Emission Spectra of Cresyl Violet

![Absorbance and Emission Spectra of Cresyl Violet](image-url)
Blood Stains

Amido black

Crowle’s stain

Hungarian red

Coomassie Blue
**Leuco (colourless) dyes**

- Methylene blue
- Leucomethylene blue

Leuco (colourless) dyes are reduced to their coloured forms, such as methylene blue and leucomethylene blue, through the action of NADPH, O2, and H2O2.
Transmittance, \( T = \text{fraction of incident radiation passing through the sample} \)

Transmittance \( T = \frac{I}{I_0} \quad 0 < T < 1 \)
Absorbance is a measure of the amount of radiation absorbed

Absorbance, \( A = \log_{10} \left( \frac{I_0}{I} \right) = - \log_{10} (T) \)

The Beer - Lambert Law (Beer - Bouguer - Lambert Law)
- Beer’s Law

(Lambert 1760 - intensity of radiation passing through a sample decreases with pathlength
Beer 1852 - Intensity of radiation passing through a sample decreases with concentration)
Beer’s Law - fundamental equation for analytical spectroscopy

Amount of monochromatic radiation absorbed by a sample

\[ A = \varepsilon c d \]

\( A \) = absorbance (dimensionless)
\( c \) = concentration, \( M \) (moles per litre)
\( \varepsilon \) = molar absorptivity \( (\text{cm}^{-1} \text{M}^{-1}) \) at a particular wavelength
  - varies with wavelength
\( d \) = pathlength through sample (cm)
The drug tolbutamine (Formula Weight = 270) has a molar absorptivity, \( \varepsilon \), of 703 cm\(^{-1}\)M\(^{-1}\) at 262 nm (UV). One tablet is dissolved in water and diluted to a volume of 2 L. If the solution exhibits an absorbance, \( A \), of 0.687 in a 1 cm cell at 262 nm, how many grams of tolbutamine are contained in the tablet?
\[ A = 0.687, \ \varepsilon = 703 \ \text{cm}^{-1} M^{-1}, \ d = 1 \ \text{cm} \]

\[ A = \varepsilon \ cd \]

substituting for \( A, \ \varepsilon \) and \( d \),

\[ 0.687 = 703 \ c \]

solving for \( c \),

\[ c = \frac{0.687}{703} = 9.772 \times 10^{-4} \ M \]

This gives the concentration per L. The tablet was dissolved in 2 L.

in 2 L, \( 1.954 \times 10^{-3} \) moles of tolbutamine

i.e., \( 1.954 \times 10^{-3} \times 270 = 0.527 \) g per tablet
Deviations from Beer’s Law

Applies well in *dilute* solutions

Deviations may be due to *Chemical* or *Instrumental* effects

(i) *Chemical Deviations from Beer’s Law*

(a) deviations at higher concentrations of sample

* interactions between solute and solvent can become more important
* refractive index of solvent (and, hence, \( \varepsilon \)) changes
Deviations from Beer’s Law

Chemical Deviations from Beer’s Law

(b) concentration dependent chemical reactions or equilibria cause deviations

e.g., dissociation of an acid:

\[ \text{HA} \iff \text{H}^+ + \text{A}^- \]
Deviations from Beer’s Law

Chemical Deviations from Beer’s Law

(b - cont) complex equilibria of metal ions

\[
[Ni(H_2O)_6]^{2+} + en \iff [Ni(H_2O)_4en]^{2+} + 2\,H_2O \quad K_1 = 10^{7.5}
\]

\[
[Ni(H_2O)_4en]^{2+} + en \iff [Ni(H_2O)_2(en)_2]^{2+} + 2\,H_2O \quad K_2 = 10^{6.3}
\]

\[
[Ni(H_2O)_2(en)_2]^{2+} + en \iff [Ni(en)_3]^{2+} + 2\,H_2O \quad K_3 = 10^{4.3}
\]

Unless an excess of ligand or metal is present, a concentration-dependent mixture of compounds will exist.
Deviations from Beer’s Law

**Chemical Deviations from Beer’s Law**

(c) the presence of a dimer/monomer equilibrium

(d) the presence of interfering species in the sample

E.g., it is convenient to determine $[\text{Br}_2]$ by measuring $A$ at 410 nm.

However, in the presence of $\text{Br}^-$, $\text{Br}_3^-$ is formed.

\[
\text{Br}_2 + \text{Br}^- \rightarrow \text{Br}_3^-
\]

At 410 nm, $\varepsilon(\text{Br}_3^-)$ is $\sim 20 \varepsilon(\text{Br}_2)$
(ii) Instrumental Deviations from Beer’s Law

Generally less of a problem.
radiation is not entirely monochromatic - spread of wavelengths is used

- ok at an absorption maximum
- can be a problem on the side of an absorption - $\varepsilon$ changes rapidly with $\lambda$ - hard to determine concentration accurately
- stray radiation
- mismatched cells
- air bubbles (in solutions) affect path length

Generally calibrate against standard samples, rather than rely on values of $\varepsilon$.
Best to interpolate - not extrapolate from standards
Phosphorus in urine can be determined by treating with ammonium paramolybdate and then reducing the resultant phosphomolybdo complex with aminonaphtholsulfonic acid to give a characteristic molybdenum blue colour. A patient excreted 1270 mL urine in 24 hours. A 1.00 mL aliquot of the urine was treated with molybdate reagent and aminonaphthosulfonic acid and was diluted to a volume of 50 mL. A series of phosphate samples was similarly treated. The absorbance of the solutions at 690 nm, measured against a blank, were as follows:

<table>
<thead>
<tr>
<th>Solution (ppm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>0.205</td>
</tr>
<tr>
<td>2.00</td>
<td>0.410</td>
</tr>
<tr>
<td>3.00</td>
<td>0.615</td>
</tr>
<tr>
<td>4.00</td>
<td>0.820</td>
</tr>
<tr>
<td>urine sample</td>
<td>0.625</td>
</tr>
</tbody>
</table>

Calculate the number of grams of phosphorus excreted per day.
Note that Beer’s Law is valid over the concentration range examined.
The sample had a [P] of 3.05 ppm

It was prepared by diluting 1.00 mL to 50 mL.

Thus, the original 1 mL contained \((3.05 \times 50)/1000\) mg of P

Thus, in the 1270 mL excreted in 24 h, there will be 
\((3.05 \times 50 \times 1270)/1000\) mg = 193.6 mg = 0.19 g

i.e., 0.19 g of P are excreted in 24 h.
Thallium Poisoning
Sydney’s first Thallium murderer

1952 Mrs Yvonne Fletcher convicted
Sydney, 1948 -1953
“Aunt Thally” - Caroline Grills

victims:
fatal
1947 Christina Mickelson aged 87
1947 Angeline Thomas aged 80
1948 John Lundberg
Mary Ann Mickelson
poisoned
Eveline Lundberg & daughter

Caroline Grills arrested 11 May 1953, convicted October 1953
Sydney 1955

Balmain and Australian winger, Bobby Lulham, poisoned by his mother-in-law, Mrs Monty, at their Marrickville home.
The Pale Horse
Agatha Christie 1961
Thyrza Grey murders
> 8 victims using Tl

Tl₂SO₄ formerly used in control of rodents (e.g., Northern Gopher in Canada)
- sold in Australia as “Thalrat”
- no longer registered in USA for pesticide use
- lethal dose for adult human probably < 1g
Nurse in UK hospital reads “The Pale Horse” and realises that a child in her care has the symptoms of Thalium poisoning (accidental) - child cured
Graham Young

Convicted aged 14 in 1962 for the fatal poisoning of his stepmother and attempted poisoning of his father, sister and a school friend
In 1971, Young is released from prison and is employed as a storeman at the Hadland Chemical Company (photographic suppliers). He collects the tea for his workmates.
1971, head storeman, Bob Egle, suffers violent vomiting, pains in chest and back, loss of balance and wild delirium; dies in hospital, 19 July

19 November, 1971, Fred Biggs, Egle’s successor, dies after suffering the same symptoms
Two more Hadland employees, Jethro Batt and David Tilson, become seriously ill, others suffer similar symptoms.

Concerned that the illnesses might be workplace related, Hadland employ consultant toxicologist, Dr Iain Anderson and his team.

Young cannot resist airing his knowledge of Tl poisoning during a routine briefing.
Graham Young

Anderson alerts police

Egle’s ashes exhumed and analysed by AAS (first use of AAS on cremated remains)

9 mg of Tl, indicative of a large dose, detected in Egle’s ashes

August 1990 Young dies of a heart attack in Parkhurst Prison
Chinese student, Zhu Ling (21) falls mysteriously ill in Peking in 1994

Chemistry student dying in hospital

12/5/94, Zhu felt unexplained, persistent abdominal pain that was generally mild but with periods of episodic colicky pain.

12/8/94  Her hair began to fall out. She began to experience constipation, and she had delayed onset of her menses.

12/10/94  She became bald.

12/23/94  Hospitalized Tongren Hospital in Beijing.
Zhu Ling

Illness cannot be diagnosed

Friends use internet to publicise her plight - suggestions from Japan, USA, Europe of Tl poisoning - correct diagnosis

Zhu Ling now recovering

http://www.radsci.ucla.edu/telemed/zhuling/