

Spectrophotometry and Beer's Law

How do chemists measure concentration with light, and what is Beer's law?

●●○ Intermediate

Analytical Chemistry

Last reviewed 4 October 2026

Before you start: it helps to understand [Molarity and Solution Stoichiometry](#) and [Significant Figures and Measurement](#).

What is it?

A coloured solution looks coloured because it **absorbs** some wavelengths of light and lets the rest through. The more concentrated the solution, the more light it absorbs.

Spectrophotometry measures this absorption to find the **concentration** of a substance, quickly and with very small samples.

A **spectrophotometer** shines light of one chosen wavelength through the sample and measures how much comes out:

- I_0 is the intensity of light **entering** the sample, and I the intensity **leaving** it.
- **Transmittance:** $T = \frac{I}{I_0}$ (between 0 and 1), or as a percentage, $\%T = T \times 100 \%$.
- **Absorbance:** $A = -\log T = \log \frac{I_0}{I}$

Absorbance and transmittance are ratios, so they have **no units**. $A = 0$ means no light is absorbed; $A = 1$ means only 10 % gets through; $A = 2$, only 1 %.

🔑 KEY IDEA

Beer's law (the Beer-Lambert law): absorbance is directly proportional to concentration.

$$A = \epsilon bc$$

where ϵ is the **molar absorptivity** ($\text{L mol}^{-1} \text{cm}^{-1}$, a constant for that substance at that wavelength), b is the **path length** of the cell (cm, usually 1.00 cm) and c is the **concentration** (mol/L). Double the concentration, double the absorbance.

Why does it matter?

- **Medicine.** Blood tests for glucose, cholesterol and haemoglobin use colour reactions read by a spectrophotometer.

- **Environment.** Nitrate, phosphate and iron in drinking water and rivers are measured this way.
- **Biology and industry.** DNA and protein concentrations, food colourings and the progress of reactions are all followed by absorbance.

How does it work?

1. The spectrophotometer

Light from a **lamp** passes through a **monochromator** (a grating), which selects one wavelength. That light passes through the **sample** in a cuvette of path length b and reaches a **detector**, which reports T or A . The wavelength chosen is usually the one at which the substance absorbs most strongly, $\lambda_{\max}$: there the measurement is most sensitive, and small errors in the wavelength setting matter least.

2. Checking the units in Beer's law

$$A = \epsilon \times b \times c$$

$$\text{units: } (\text{L mol}^{-1} \text{ cm}^{-1})$$

$$\times \text{cm} \times (\text{mol L}^{-1})$$

All the units cancel, so A has no unit, as it should.

3. The calibration curve

In practice, ϵ is rarely taken from a table. Instead:

1. Zero the instrument with a **blank**: the solvent and reagents, without the substance being measured. This removes absorption by everything else.
2. Measure a series of **standards** of known concentration.
3. Plot absorbance against concentration and draw the best-fit straight line (by least squares, using a calculator or spreadsheet).
4. Measure the **unknown** in **replicate** (at least in triplicate), find the **mean** absorbance and its **standard deviation**, and read the concentration from the line.

The unknown should lie **within** the range of the standards. If its absorbance is too high, dilute it by a known factor and measure again.

THINK OF IT LIKE THIS

Think of sunglasses. One pair of lenses dims the light by a certain fraction; two pairs stacked dim it again by the same fraction. A thicker or darker filter (a longer path length or a higher concentration) lets through less light. Absorbance is the scale that turns this “fraction of a fraction” behaviour into a simple straight line.

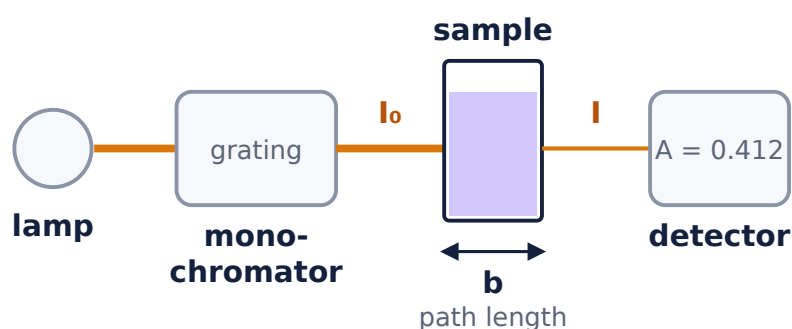
🔍 MORE PRECISELY

Beer's law holds best for **dilute** solutions and absorbances up to about 1. At high concentrations, molecules interact with one another, and at very high absorbances so little light reaches the detector that stray light causes errors; the calibration line then curves. Beer's law also assumes **monochromatic** light, which is why the monochromator matters. The **slope** of the calibration line is the sensitivity, and the intercept should be close to zero if the blank was correct.

Visualise it

Inside a spectrophotometer

One wavelength passes through the sample.



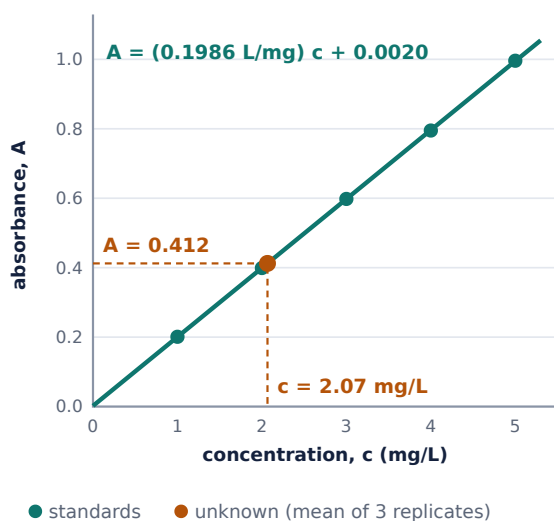
$$T = I \div I_0 \quad A = -\log T = \log(I_0 \div I)$$

Less light gets through (I below I_0): higher absorbance.

The sample absorbs part of the light: I is smaller than I_0 .

Calibration curve

Standards of known concentration; unknown read from the line



Standards give the line; the unknown's mean absorbance is read across and down.

Worked example

WORKED EXAMPLE: From transmittance to absorbance

Question: A solution transmits 25.0 % of the light. What is its absorbance?

Step 1 $T = \frac{25.0\%}{100\%} = 0.250$

Step 2 $A = -\log(0.250) = \mathbf{0.602}$ (no unit)

WORKED EXAMPLE: Using Beer's law

Question: A dye has $\epsilon = 1.20 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ at its λ_{max} . A solution of the dye in a 1.00 cm cell has an absorbance of 0.540. Find its concentration.

Step 1 Rearrange: $c = \frac{A}{\epsilon b}$

Step 2 Substitute:

$$\begin{aligned}\epsilon b &= \left(1.20 \times 10^4 \frac{\text{L}}{\text{mol cm}}\right) \\ &\quad \times (1.00 \text{ cm}) \\ &= 1.20 \times 10^4 \text{ L/mol} \\ c &= \frac{0.540}{1.20 \times 10^4 \text{ L/mol}} \\ &= 4.50 \times 10^{-5} \text{ mol/L}\end{aligned}$$

Step 3 The cm cancels, and $\frac{1}{\text{L/mol}} = \text{mol/L}$: $\mathbf{4.50 \times 10^{-5} \text{ mol/L}}$.

 WORKED EXAMPLE: An unknown from a calibration curve, measured in triplicate

Question: Standards of an iron complex (1.00 to 5.00 mg/L) give the best-fit line $A = (0.1986 \text{ L/mg}) c + 0.0020$. A water sample is measured three times: $A = 0.412, 0.416$ and 0.409 . Find the iron concentration, and comment on the precision.

Step 1 Mean absorbance: $\bar{A} = \frac{0.412 + 0.416 + 0.409}{3} = 0.4123$

Step 2 Standard deviation (sample, $n - 1$): $s = 0.0035$; relative standard deviation
 $= \frac{0.0035}{0.4123} \times 100 \% = 0.85 \%$

Step 3 Rearrange the line: $c = \frac{\bar{A} - 0.0020}{0.1986 \text{ L/mg}}$

Step 4 Substitute: $c = \frac{0.4123 - 0.0020}{0.1986 \text{ L/mg}} = 2.07 \text{ mg/L}$

Step 5 Report: $\bar{A} = 0.4123 \pm 0.0035$ ($n = 3$). An RSD below 1 % shows good precision; the result lies inside the range of the standards (1.00 to 5.00 mg/L), so the reading is reliable.

Common mistake

 COMMON MISTAKE: Using %T directly in the absorbance formula

Convert a percentage to a fraction first: for 25.0 %T, $A = -\log(0.250) = 0.602$, not $-\log(25.0) = -1.40$. An absorbance is never negative for a real sample.

 COMMON MISTAKE: Thinking transmittance is proportional to concentration

Only **absorbance** is proportional to concentration. Doubling the concentration doubles A , but squares T (for example, $T = 0.50$ becomes $T = 0.25$).

 COMMON MISTAKE: Reading far outside the calibration range

A result outside the standards relies on the line continuing straight, which may not be true at high absorbance. Dilute the sample by a known factor (and multiply the result by it), or add more standards.

NOTATION NOTE

- Molar absorptivity ε is also called the molar extinction coefficient; its units can be written $\text{L mol}^{-1} \text{cm}^{-1}$ or $\text{M}^{-1} \text{cm}^{-1}$.
- Some books write $A = abc$ with a the absorptivity in mass units (for example $\text{L g}^{-1} \text{cm}^{-1}$), when c is in g/L .
- The blank is sometimes called the reference solution.

Remember this

REMEMBER THIS

- $T = I/I_0$; $A = -\log T$. Both have no units.
- Beer's law: $A = \varepsilon bc$, with ε in $\text{L mol}^{-1} \text{cm}^{-1}$, b in cm , c in mol/L .
- Measure at λ_{max} , zero with a blank, and use a calibration curve of standards.
- Measure unknowns in replicate (at least triplicate) and report the mean $\pm$ standard deviation; stay inside the calibration range.

References

1. Brown, T. L.; LeMay, H. E., Jr.; Bursten, B. E.; Murphy, C. J.; Woodward, P. M.; Stoltzfus, M. W. Chemistry: The Central Science, 15th ed.; Pearson, 2022.

Practise this topic with flashcards and a quiz at chemistryclarity.com/chemistry/beer-lambert-law/