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 \( \lambda \) through a plane parallel slab of material that is normal to the beam (4). 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.

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

\[
T = \frac{I}{I_0} \]

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 \( (\varepsilon) \).

\[
T = \frac{I}{I_0} = e^{-\varepsilon cl} \]

Absorbance \( (A) \) is the negative log of transmission. This mathematical transformation is used to make the relationship between concentration and absorbance linear:

\[
A = -\log \left( \frac{I}{I_0} \right) = \varepsilon cl \]

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

### Conclusions
In an absorbance experiment, light is attenuated not only by the chromophore, but also by reflections from the interface between 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 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” can also remove errors in the experiment, the equipment, and the batch of reagents (such as cuvettes of unknown pathlength).

### References

### Table I: Absorption wavelength bands of assorted chromophores

<table>
<thead>
<tr>
<th>Chromophore</th>
<th>Maximum Absorption (in nm)</th>
<th>Absorbance Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenanthrene</td>
<td>251, 292</td>
<td>66,000, 14,000</td>
</tr>
<tr>
<td>Naphthacene</td>
<td>272, 473</td>
<td>180,000, 12,500</td>
</tr>
<tr>
<td>Pentacene</td>
<td>310, 585</td>
<td>300,000, 12,000</td>
</tr>
<tr>
<td>Pyridine</td>
<td>174, 195, 257</td>
<td>80,000, 6000, 1700</td>
</tr>
<tr>
<td>Quinoline</td>
<td>227, 270, 314</td>
<td>37,000, 3600, 2750</td>
</tr>
<tr>
<td>Isoquinoline</td>
<td>218, 266, 317</td>
<td>80,000, 4000, 3500</td>
</tr>
</tbody>
</table>