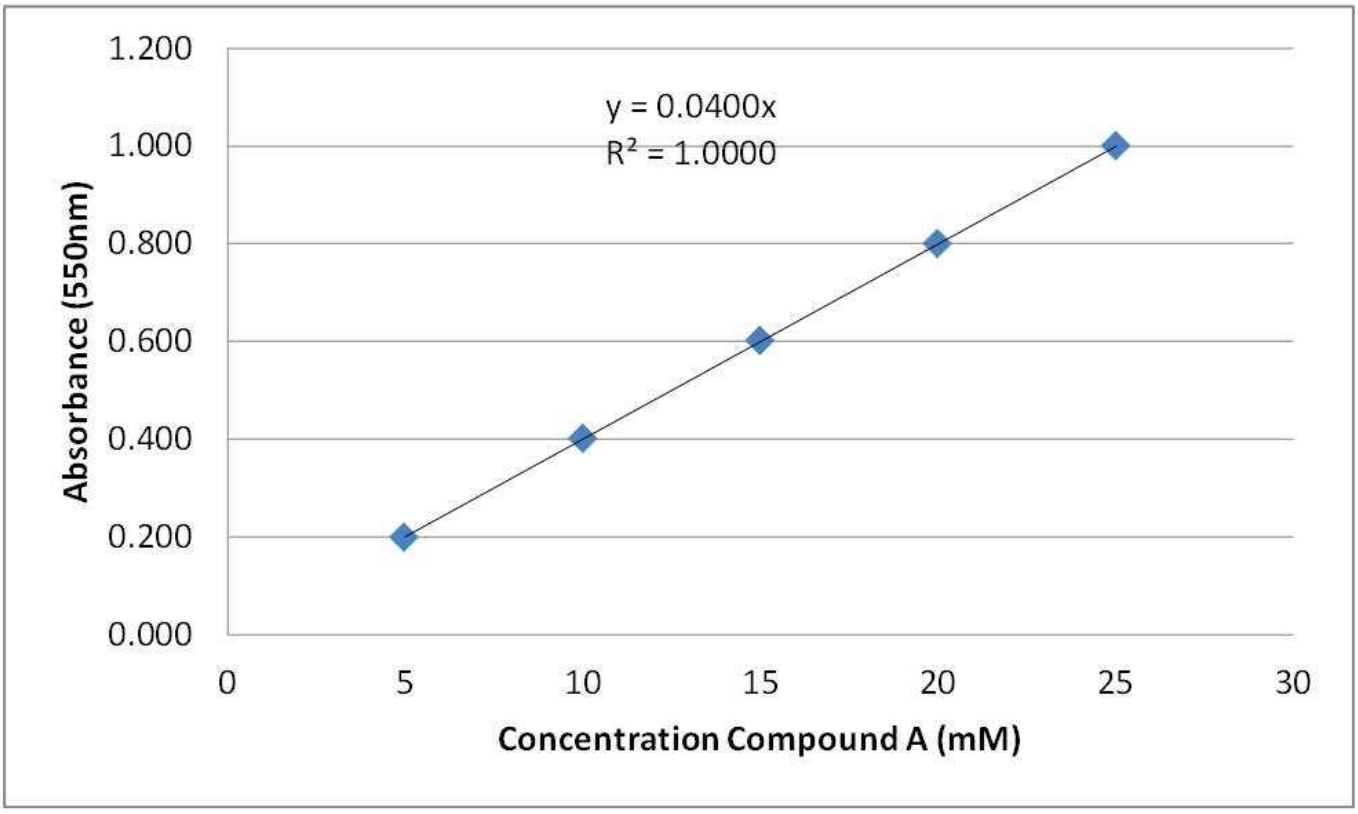
MOLAR ABSORPTIVITY

DETERMINING MOLAR ABSORPTIVITY COEFFICIENTS.

Suppose that we made a standard curve where the data obeys Beer's Law and fits a straight line. We should be able to calculate the molar absorptivity coefficient (ε).



Since A=εbc and the graph is of A vs C (mM)

A = (εb) c the slope is therefore equal to (εb). We get the slope from the y=0.400x .

In the above: slope = 0.0400 mM^{-1} = εb where b is usually 1 cm (the pathlength of a cuvette)

Therefore we can calculate the value of ε:

ε = (slope / b) = (0.0400 mM^{-1}/ 1cm) = 0.0400 $mM^{-1}cm^{-1}$ or 0.0400 $\frac{L}{mmoles\,cm}$

ε is most often written in $M^{-1}cm^{-1}$

so $\varepsilon = 0.0400\frac{L}{mmoles\,cm}\ x\ \frac{1000 mmoles}{mole} = 40.0\frac{L}{moles\,cm} = 40.0\ M^{-1}\,cm^{-1}$

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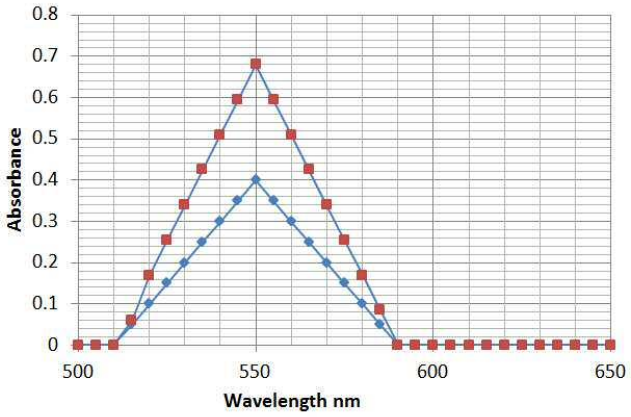
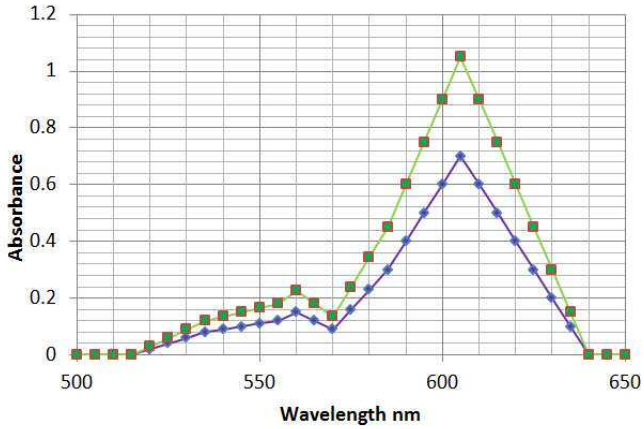
TWO COMPOUNDS ABSORBING AT THE SAME WAVELENGTH.

Suppose we took a scan of a sample containing 10.0 mM Compound A (blue dots) and a scan of a sample containing 17.0 mM Compound A (red dots).



We can see that this compound has a maximum wavelength at 550 nm (λ_{max}). The higher the concentration, the greater the absorbance at 550nm. It does not absorb light above 600 nm at either concentration.

($\varepsilon_{A,550nm}$ = 0.0400 L mmoles^{-1} cm^{-1})
($\varepsilon_{A,605nm}$ = 0.000 L mmoles^{-1} cm^{-1})



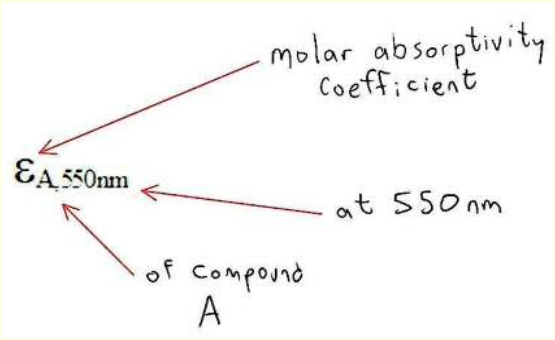
Suppose we took a scan of 10.0 mM Compound B (blue) and a scan of 15.0 mM Compound B (green).

We can see that Compound B has a maximum wavelength (λ_{max}) of 605 nm. It does absorb light below 600 nm, in the range of Compound A. The higher the concentration, the greater the absorbance at 550nm and 605nm.

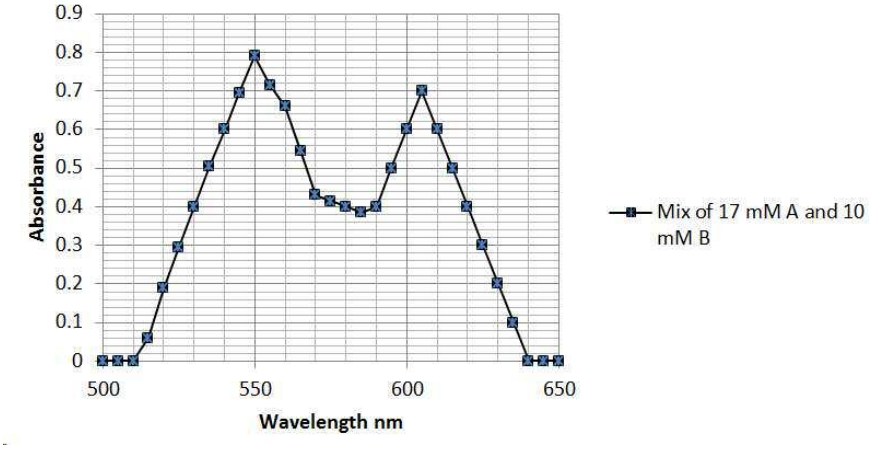
($\varepsilon_{B,605nm}$ = 0.0700 L mmoles^{-1} cm^{-1})
($\varepsilon_{B,550nm}$ = 0.0110 L mmoles^{-1} cm^{-1})

** note usually a scan would have a curved appearance, the above scans are made up numbers for demonstration.

What does $\varepsilon_{A,550nm}$ mean?



What happens if we have a mix of 17.0 mM A and 10.0 mM B in the same cuvette? How do we calculate concentration of A and B using Beer's Law?



If we used the Absorbance at 605nm and used Beer's Law to determine Compound B using ($\varepsilon_{B,605nm}$ = 0.0700 L mmoles^{-1} cm^{-1})

A = εbc
0.700 = (0.070 L mmole^{-1} cm^{-1}) * (1cm) * (c)

c = 10.00 mM = 10.0 mM

We could determine the concentration of B at 605nm with $\varepsilon_{B,605nm}$ alone because Compound A does not absorb at 605 nm. ($\varepsilon_{A,605nm}$ = 0.000 L mmoles^{-1} cm^{-1})

If we used the Absorbance at 550nm and used Beer's Law to determine Compound A using ($\varepsilon_{A,550nm}$ = 0.0400 L mmoles^{-1} cm^{-1})

A = εbc
0.790 = (0.040 L mmole^{-1} cm^{-1}) * (1cm) * c
c = 19.75 mM = 19.8 mM

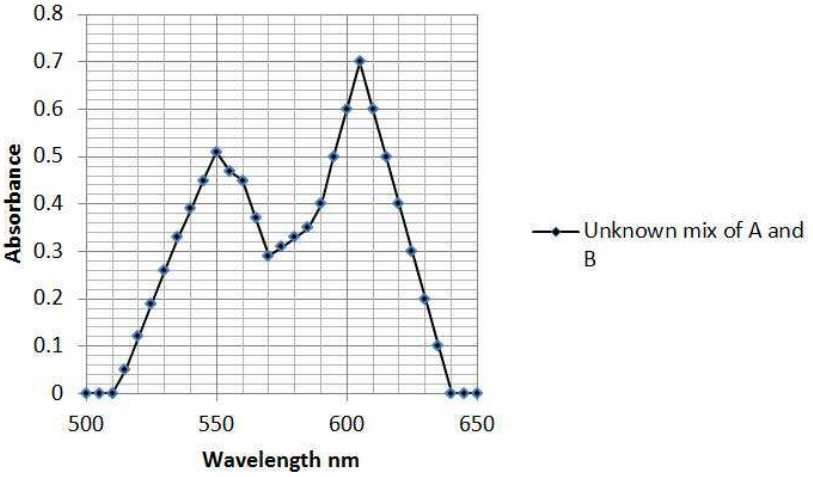
19.8 mM would be our answer which is close to the actual, but we have overestimated the real value of 17.0 mM. The reason is because Compound B also absorbs at this wavelength. ($\varepsilon_{B,550nm}$ = 0.0110 L mmoles^{-1} cm^{-1}).

In reality you must add Beer's Law together for compound A and compound B at 550nm.

A_{550nm} = $\varepsilon_{A,550nm}bc + \varepsilon_{B,550nm}bc$
= (0.0400 L mmole^{-1} cm^{-1} * 1cm * 17.0mM) + (0.0110 L mmole^{-1} cm^{-1} * 1cm * 10.0mM)
= 0.680 + 0.110
= 0.790

So what should you do to calculate the concentrations of Compound A and B when they are mixed??

Let's Try an Unknown



At 550 nm the mixed sample absorbs at 0.510. At 605 nm the mixed sample absorbs at 0.700. Since Compound A does not absorb at 605 nm ($\varepsilon_{A,605nm}$ = 0.000 L mmole^{-1} cm^{-1}), we should determine B's concentration first.

A_{605nm} = 0.700 = $\varepsilon_{A,605nm}bc_A + \varepsilon_{B,605nm}bc_B$

= 0.700 = (0.000 L mmole^{-1} cm^{-1} * 1cm * c_A) + (0.0700 L mmole^{-1} cm^{-1} * 1cm * c_B)

= 0.700 = 0 + (0.0700 L mmole^{-1} cm^{-1} * 1cm * c_B)

= 0.700/(0.0700 L mmole^{-1} cm^{-1} * 1cm) = c_B

c_B = 10.0 mM therefore concentration of Compound B is 10.0 mM

Compound A's concentration (c_A) is a little more difficult to determine, but since we now know B's concentration (c_B) to be 10.0 mM from the above calculation, we can determine A's next.

A_{550nm} = 0.510 = ($\varepsilon_{A,550nm}$ * b * c_A) + ($\varepsilon_{B,550nm}$ * b * c_B)

= 0.510 = (0.0400 L mmole^{-1} cm^{-1} *1cm*c_A) + (0.0110 L mmole^{-1} cm^{-1} *1cm*10.0mM)

= 0.510 = (0.0400 L mmole^{-1} cm^{-1} *1cm*c_A) + (0.110)

= 0.510-0.110 = (0.0400 L mmole^{-1} cm^{-1} *1cm*c_A)

= 0.400/(0.0400 L mmole^{-1} cm^{-1} *1cm) = c_A

C_A = 10.0 mM therefore concentration of Compound A is 10.0 mM.

Using multiple wavelengths in a Biochemical assay is fairly common.

- An example of this is in determining the purity of DNA. Samples are measured at 260nm and 280nm.
- Another example is the AlamarBlue assay which is used to determine cellular proliferation. This takes measurements at 570nm and 600nm.