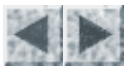
Table of pK_a and pI values

- The pK_a values and the isoelectronic point, pI, are given below for the 20 α-amino acids.
- pKa_1= α-carboxyl group, pK_{a2} = α-ammonium ion, and pK_{a3} = side chain group.

Amino acid	pKa_1	pKa_2	pKa_3	pI
Glycine	2.34	9.60	---	5.97
Alanine	2.34	9.69	---	6.00
Valine	2.32	9.62	---	5.96
Leucine	2.36	9.60	---	5.98
Isoleucine	2.36	9.60	---	6.02
Methionine	2.28	9.21	---	5.74
Proline	1.99	10.60	---	6.30
Phenylalanine	1.83	9.13	---	5.48
Tryptophan	2.83	9.39	---	5.89
Asparagine	2.02	8.80	---	5.41
Glutamine	2.17	9.13	---	5.65
Serine	2.21	9.15	---	5.68
Threonine	2.09	9.10	---	5.60
Tyrosine	2.20	9.11	---	5.66
Cysteine	1.96	8.18	---	5.07
Aspartic acid	1.88	9.60	3.65	2.77
Glutamic acid	2.19	9.67	4.25	3.22
Lysine	2.18	8.95	10.53	9.74
Arginine	2.17	9.04	12.48	10.76
Histidine	1.82	9.17	6.00	7.59



Chapter 27: Amino Acids, Peptides and Proteins

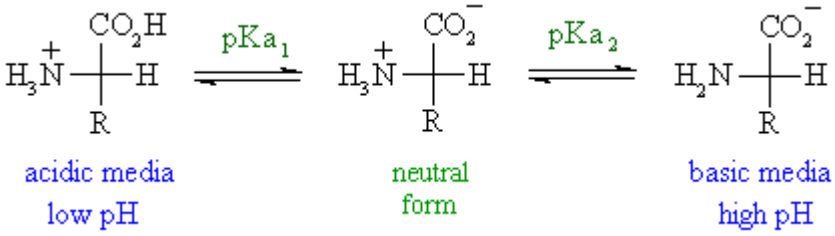
Isoelectronic point, pI

- The **isoelectronic point** or **isoionic point** is the pH at which the amino acid does not migrate in an electric field.
- This means it is the pH at which the amino acid is neutral, i.e. the zwitterion form is dominant.
- A table of pK_a and pI values can be found on the next page.
- The pI is given by the average of the pK_as that involve the zwitterion, i.e. that give the boundaries to its existence.

There are 3 cases to consider....

- **neutral side chains**

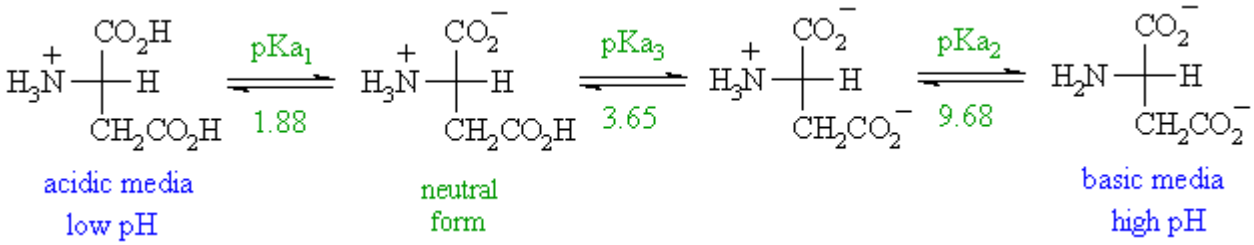
These amino acids are characterised by two pK_as : pK_a1 and pK_a2 for the carboxylic acid and the amine respectively.
The isoelectronic point will be halfway between, or the average of, these two pK_as, i.e. **$pI = 1/2 (pK_{a1} + pKa_2)$**. This is most readily appreciated when you realise that at very acidic pH (below pK_a1) the amino acid will have an overall +ve charge and at very basic pH (above pK_a2) the amino acid will have an overall -ve charge. For the simplest amino acid, glycine, pKa1= 2.34 and pK_a2 = 9.6, pI = 5.97.



The other two cases introduce other ionisable groups in the side chain "**R**" described by a third acid dissociation constant, pK_a3

- **acidic side chains**

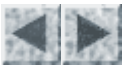
The pI will be at a lower pH because the acidic side chain introduces an "extra" negative charge. So the neutral form exists under more **acidic** conditions when the extra -ve has been neutralised. For example, for aspartic acid shown below, the neutral form is dominant between pH 1.88 and 3.65, pI is halfway between these two values, i.e. **$pI = 1/2 (pK_{a1} + pK_{a3})$**, so pI = 2.77.



- **basic side chains**

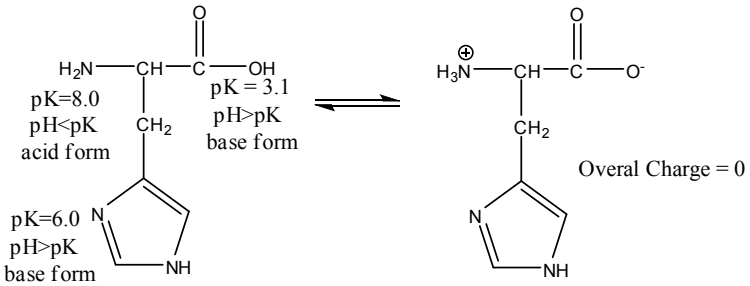
The pI will be at a higher pH because the basic side chain introduces an "extra" positive charge. So the neutral form exists under more **basic** conditions when the extra +ve has been neutralised. For example, for histidine, which was discussed on the previous page, the neutral form is dominant

between pH 6.00 and 9.17, pI is halfway between these two values, i.e. **$pI = 1/2\ (pK_{a2} + pK_{a3})$**, so pI = 7.59.

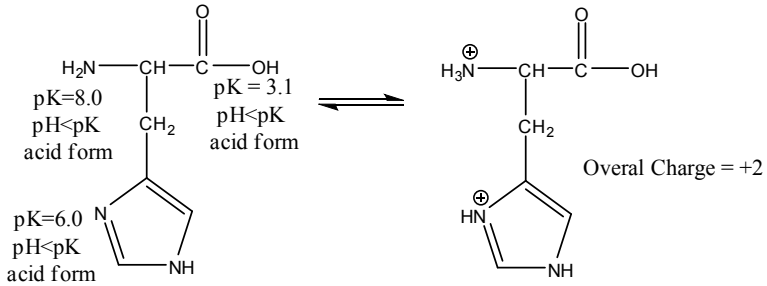


Predominant Forms of Amino Acids
Practice problems

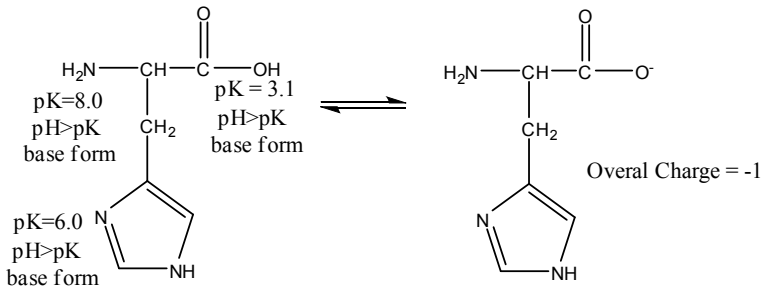
1. Draw histidine in its predominant form at pH = 7.0.



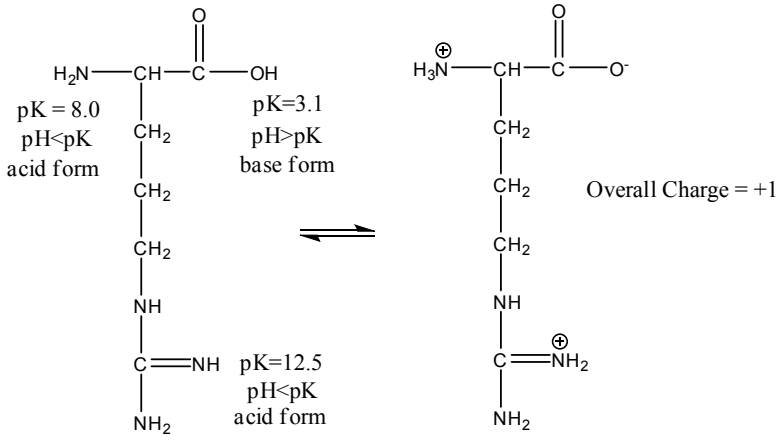
2. Draw histidine in its predominant form at pH = 2.0.



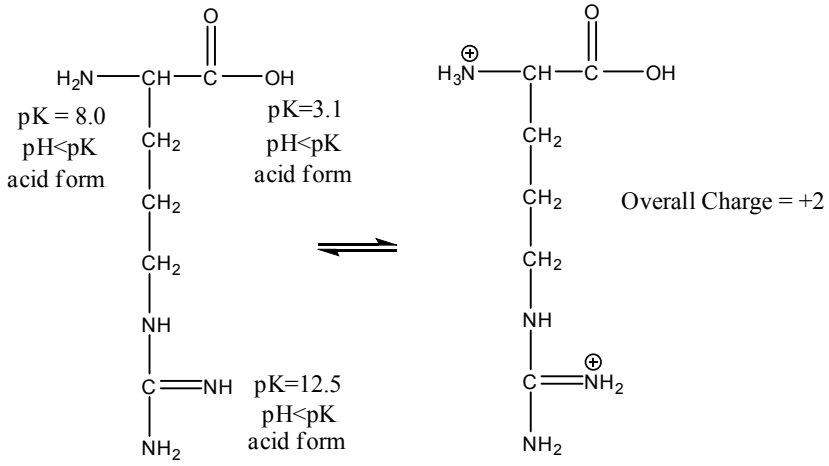
3. Draw histidine in its predominant form at pH = 10.0.



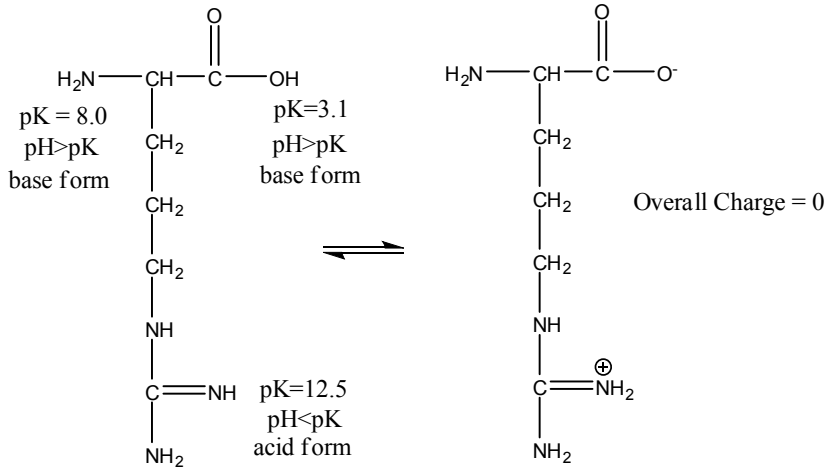
4. Draw arginine in its predominant form at pH = 7.0.



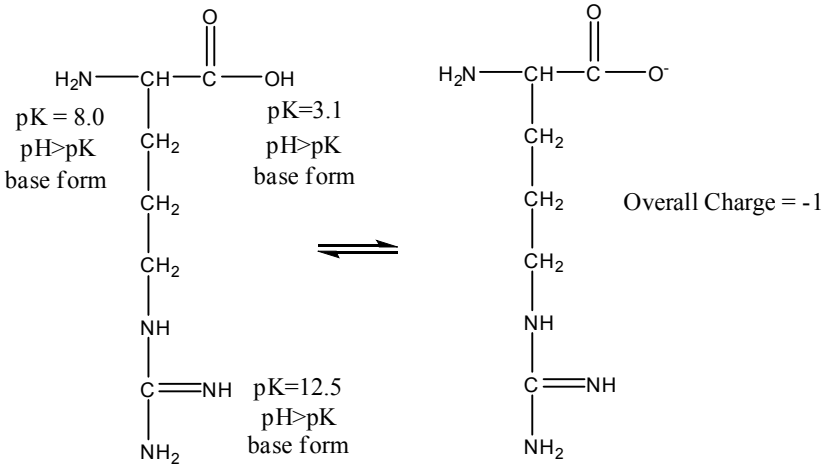
5. Draw arginine in its predominant form at pH = 2.0.



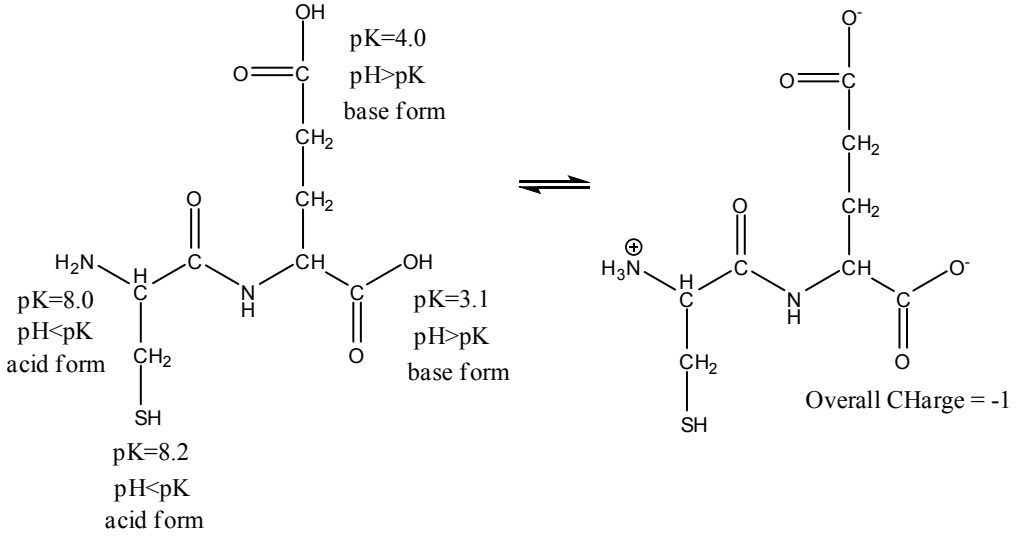
6. Draw arginine in its predominant form at pH = 10.0.



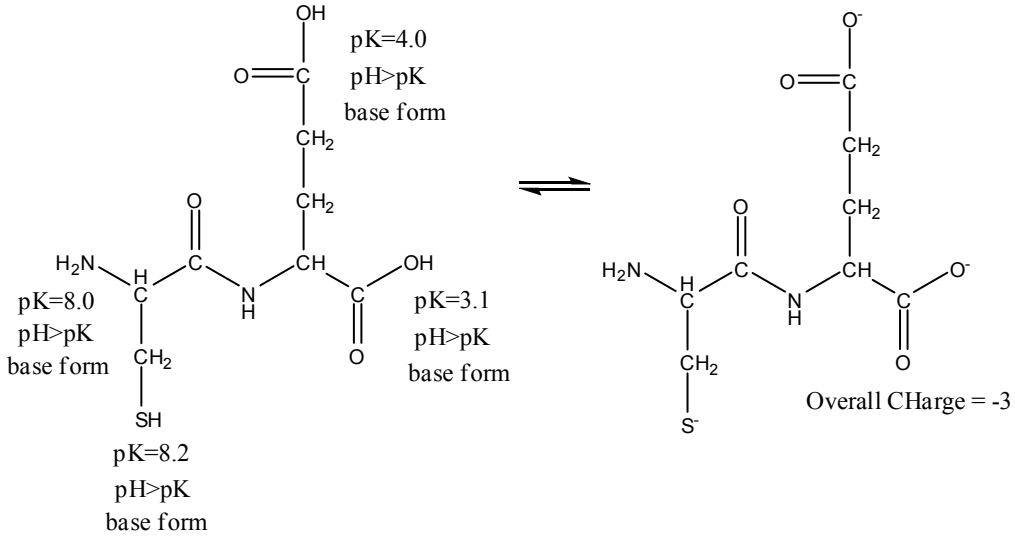
7. Draw arginine in its predominant form at pH = 13.0.



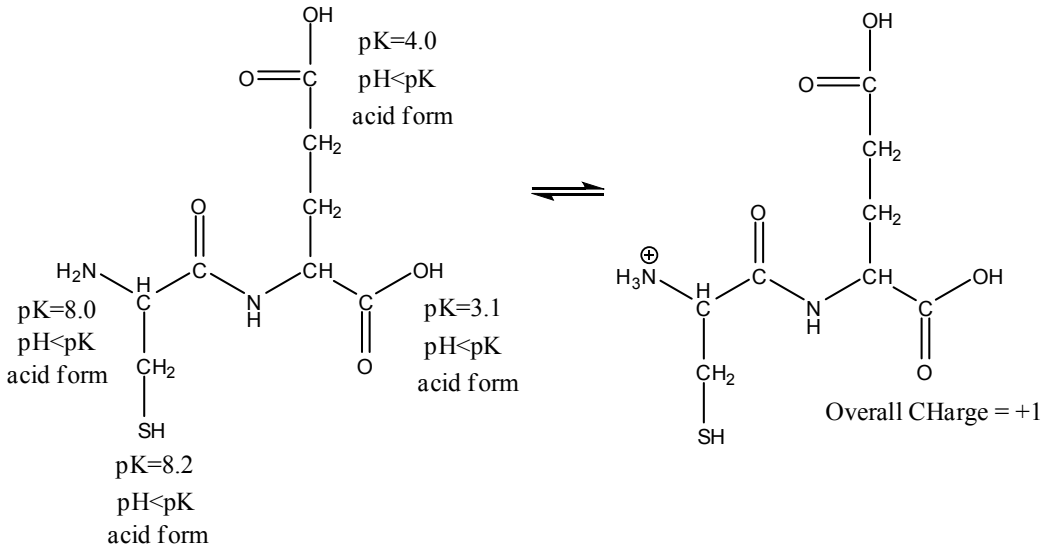
8. Draw Cys-Glu in its predominant form at pH = 7.0.



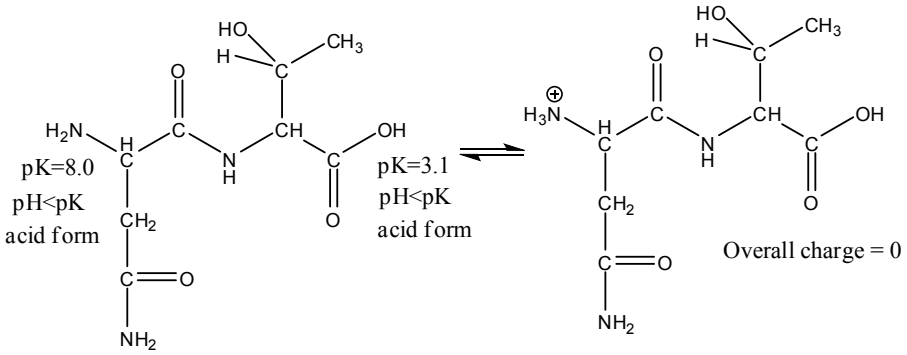
9. Draw Cys-Glu in its predominant form at pH = 10.0.



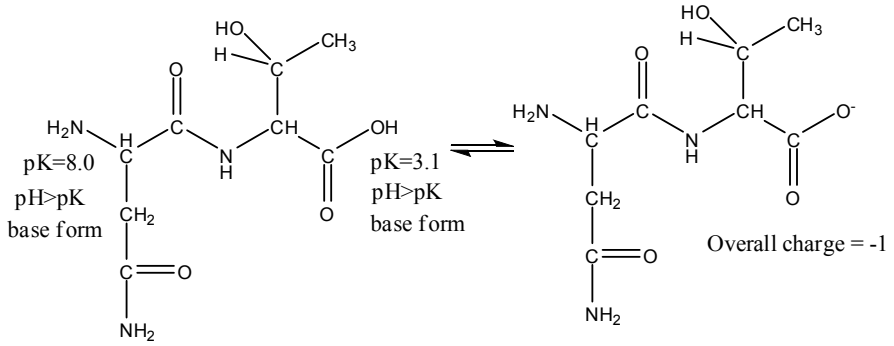
10. Draw Cys-Glu in its predominant form at pH = 2.5.



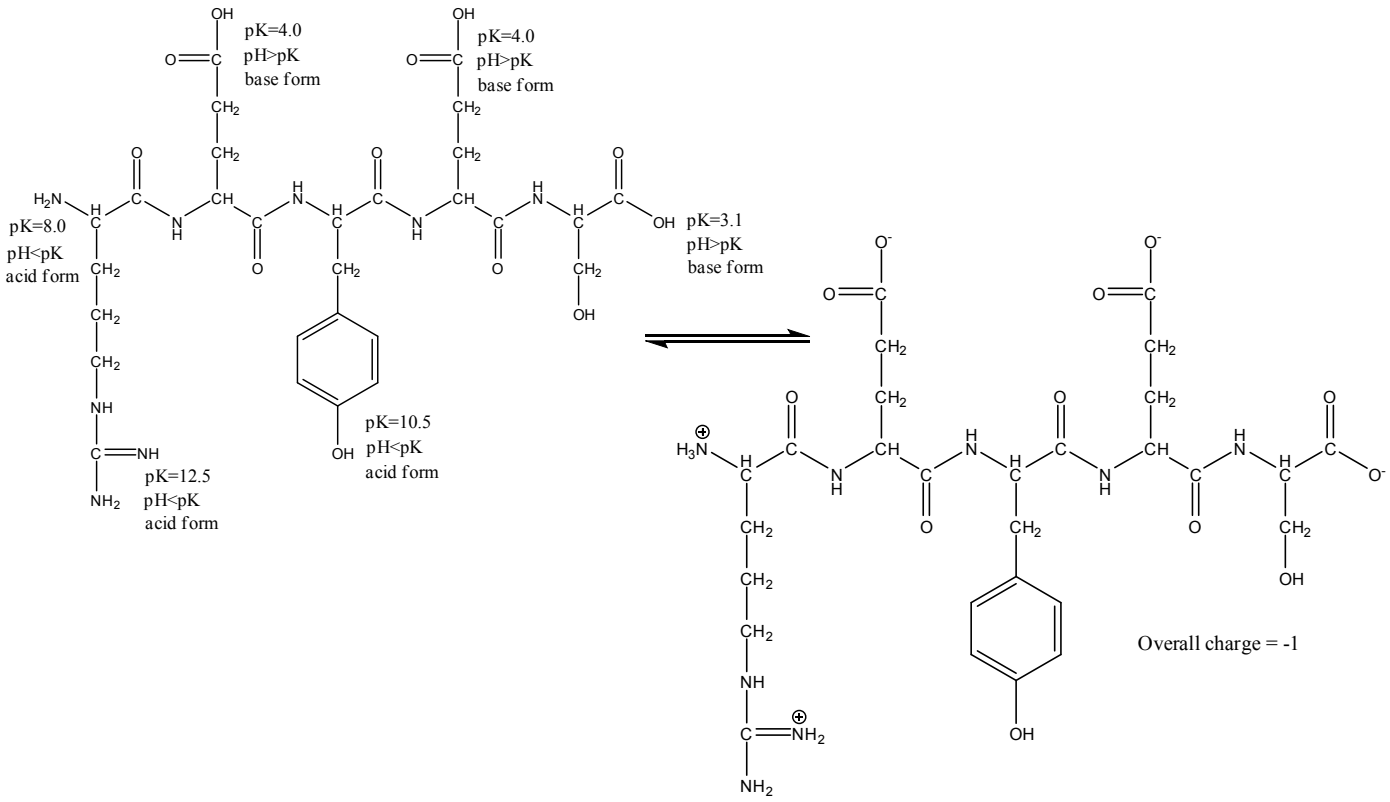
11. Draw Asn-Thr in its predominant form at pH = 1.0



12. Draw Asn-Thr in its predominant form at pH = 14.0



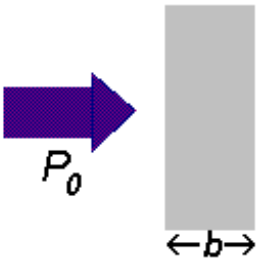
13. Draw the polypeptide R-E-Y-E-S (Arg-Glu-Tyr-Glu-Ser) in its predominant form at pH 7.0



Beer's Law

Introduction

Many compounds absorb ultraviolet (UV) or visible (Vis.) light. The diagram below shows a beam of monochromatic radiation of radiant power P_0, directed at a sample solution. Absorption takes place and the beam of radiation leaving the sample has radiant power P.



The amount of radiation absorbed may be measured in a number of ways:

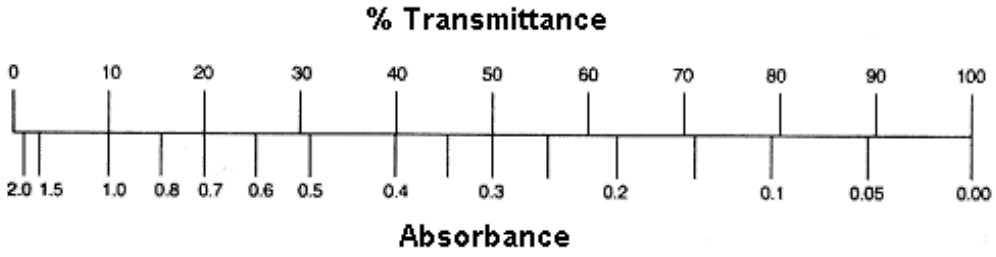
Transmittance, $T = P / P_0$
% Transmittance, $\%T = 100\ T$

Absorbance,

$$A = \log_{10} P_0 / P$$
$$A = \log_{10} 1 / T$$
$$A = \log_{10} 100 / \%T$$
$$A = 2 - \log_{10} \%T$$

The last equation, $A = 2 - \log_{10} \%T$, is worth remembering because it allows you to easily calculate absorbance from percentage transmittance data.

The relationship between absorbance and transmittance is illustrated in the following diagram:



So, if all the light passes through a solution *without* any absorption, then absorbance is zero, and percent transmittance is 100%. If all the light is absorbed, then percent transmittance is zero, and absorption is infinite.

The Beer-Lambert Law

Now let us look at the Beer-Lambert law and explore it's significance. This is important because people who use the law often don't understand it - even though the equation representing the law is so straightforward:

A=εbc

Where A is absorbance (no units, since $A = log_{10} P_0 / P$)

ε is the molar absorbtivity with units of L mol^{-1} cm^{-1}

b is the path length of the sample - that is, the path length of the cuvette in which the sample is contained. We will express this measurement in centimetres.

c is the concentration of the compound in solution, expressed in mol L^{-1}

The reason why we prefer to express the law with this equation is because absorbance is directly proportional to the other parameters, as long as the law is obeyed. We are not going to deal with deviations from the law.

Let's have a look at a few questions...

Question : Why do we prefer to express the Beer-Lambert law using absorbance as a measure of the absorption rather than %T ?

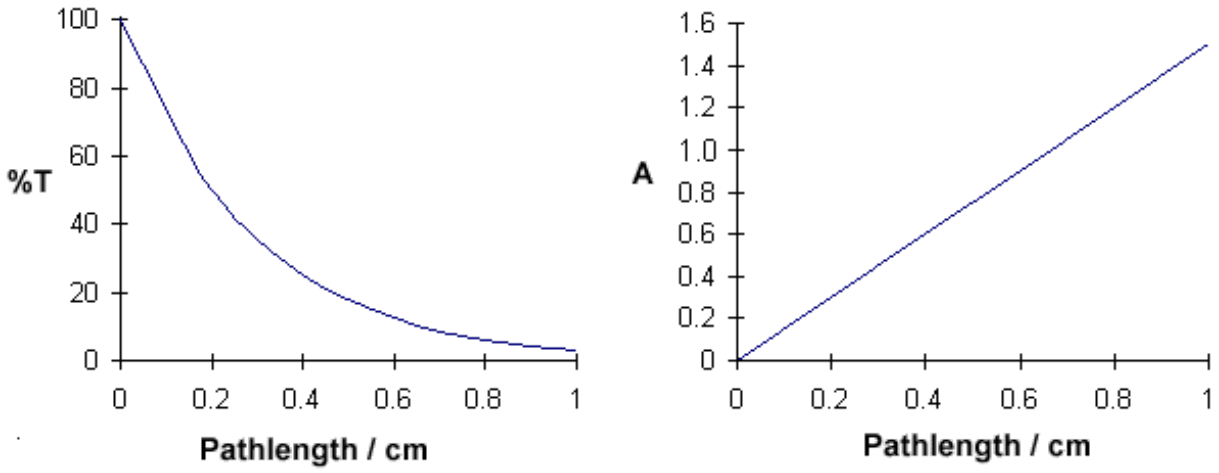
Answer : To begin, let's think about the equations...

$$A=\varepsilon bc$$

$$\%T = 100\ P/P_0 = e^{-\varepsilon bc}$$

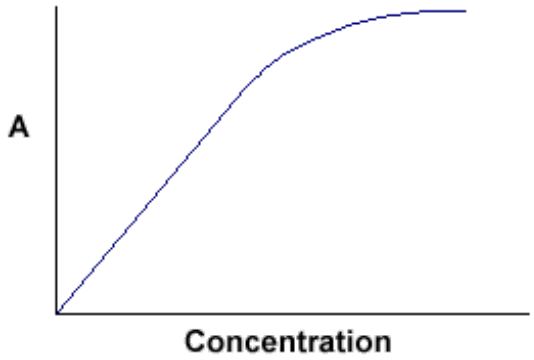
Now, suppose we have a solution of copper sulphate (which appears blue because it has an absorption maximum at 600 nm). We look at the way in which the intensity of the light (radiant power) changes as it passes through the solution in a 1 cm cuvette. We will look at the reduction every 0.2 cm as shown in the diagram below. **The Law says that the fraction of the light absorbed by each layer of solution is the same.** For our illustration, we will suppose that this fraction is 0.5 for each 0.2 cm "layer" and calculate the following data:

Path length / cm	0	0.2	0.4	0.6	0.8	1.0
%T	100	50	25	12.5	6.25	3.125
Absorbance	0	0.3	0.6	0.9	1.2	1.5



A = εbc tells us that absorbance depends on the total quantity of the absorbing compound in the light path through the cuvette. If we plot absorbance against concentration, we get a straight line passing through the

origin (0,0).



Note that the Law is not obeyed at high concentrations. This deviation from the Law is not dealt with here.

The linear relationship between concentration and absorbance is both simple and straightforward, which is why we prefer to express the Beer-Lambert law using absorbance as a measure of the absorption rather than %T.

Question : What is the significance of the molar absorbtivity, ε ?

Answer : To begin we will rearrange the equation $A = \varepsilon bc$:

$$\varepsilon = A / bc$$

In words, this relationship can be stated as "ε is a measure of the amount of light absorbed per unit concentration".

Molar absorbtivity is a constant for a particular substance, so if the concentration of the solution is halved so is the absorbance, which is exactly what you would expect.

Let us take a compound with a very high value of molar absorbtivity, say 100,000 L mol^{-1} cm^{-1}, which is in a solution in a 1 cm pathlength cuvette and gives an absorbance of 1.

$$\varepsilon = 1 / 1 \times c$$

Therefore, $c = 1 / 100{,}000 = 1 \times 10^{-5}$ mol L^{-1}

Now let us take a compound with a very low value of ε, say 20 L mol^{-1} cm^{-1} which is in solution in a 1 cm pathlength cuvette and gives an absorbance of 1.

$$\varepsilon = 1 / 1 \times c$$

Therefore, $c = 1 / 20 = 0.05$ mol L^{-1}

The answer is now obvious - a compound with a high molar absorbtivity is very effective at absorbing light (of the appropriate wavelength), and hence low concentrations of a compound with a high molar absorbtivity can be easily detected.

Question : What is the molar absorbtivity of Cu^{2+} ions in an aqueous solution of $CuSO_4$? It is either 20 or 100,000 L mol^{-1} cm^{-1}

Answer : I am guessing that you think the higher value is correct, because copper sulphate solutions you

have seen are usually a beautiful bright blue colour. However, the actual molar absorbtivity value is 20 L mol^{-1} cm^{-1} ! The bright blue colour is seen because the concentration of the solution is very high.

b-carotene is an organic compound found in vegatables and is responsible for the colour of carrots. It is found at exceedingly low concentrations. You may not be surprised to learn that the molar absorbtivity of b-carotene is 100,000 L mol^{-1} cm^{-1} !

Review your learning

You should now have a good understanding of the Beer-Lambert Law; the different ways in which we can report absorption, and how they relate to each other. You should also understand the importance of *molar absorbtivity*, and how this affects the *limit of detection* of a particular compound.

Back to index of topics	**Beer's Law**	Beer's Law - Quiz

Back to index of topics

Biosciences Homepage

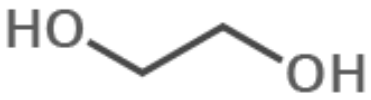
Sheffield Hallam University

THE CHEMISTRY OF WRITING INKS

It's back-to-school season, which means stocking up on office supplies. Billions of pens are manufactured every year, and a blend of chemicals dictates the color and flow of their ink.

BALLPOINT PEN MECHANISM

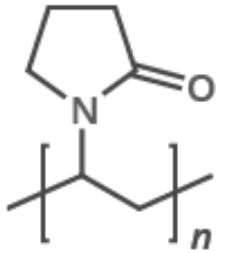
SOLVENTS



ETHYLENE GLYCOL

Solvents suspend or dissolve dyes and pigments in ink, allowing them to flow onto paper. In ballpoint pens, solvents are often glycols, such as ethylene glycol. Manufacturers also add lubricants to ensure that the metal ball doesn't stick.

BINDERS



POLYVINYLPYRROLIDONE

An example binder compound

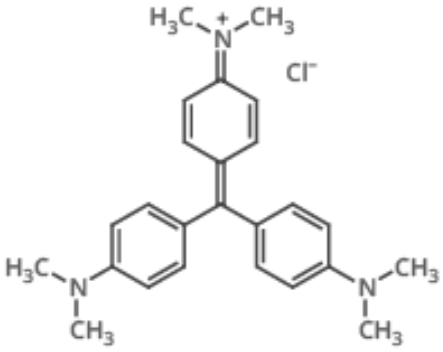
A variety of different binder compounds help carry an ink's dye or pigment and also help stick it to the surface of the paper.

INK COLORANTS

Inks get their colors from pigments, which are insoluble compounds suspended in a solvent, or from dyes, which are soluble. Writing inks tend to use dyes because pigments can clog the pen tip.

Black inks use carbon black or a mixture of colored compounds. Blue ink usually gets its hue from triphenylmethane dyes, and red ink is often based on eosin dye.

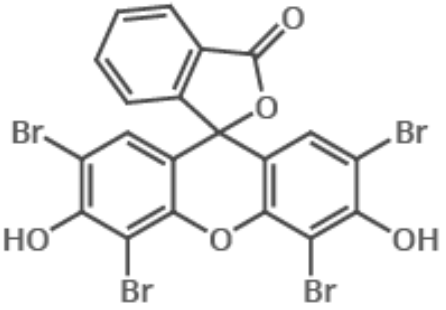
BLUE INKS



CRYSTAL VIOLET

Substituted triphenylmethane dye

RED INKS

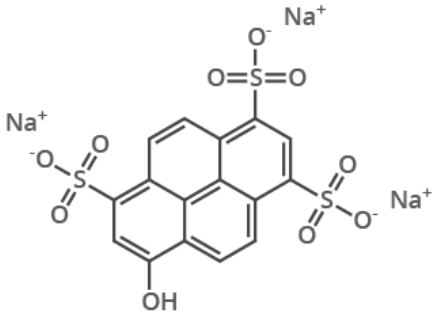


EOSIN Y

Used in dilute solution for red ink

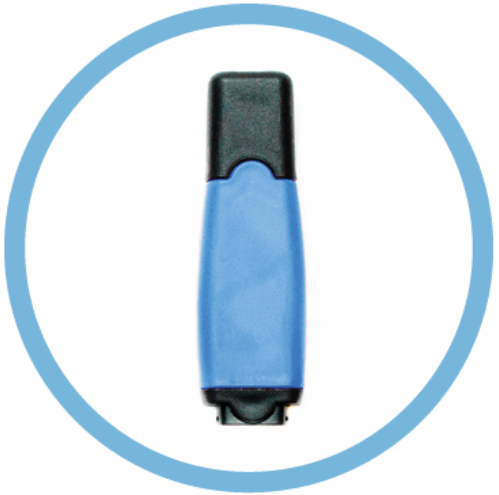
THE CHEMISTRY OF HIGHLIGHTER COLOURS

YELLOW

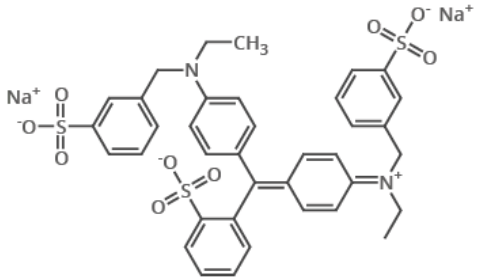


PYRANINE - SOLVENT GREEN 7
(Pyrene dye)

Pyranine, a pyrene dye, is the dye commonly used in yellow highlighters. Fluorescein can also be used. Mixing a pyrene dye with a triphenylmethane dye gives a green ink.



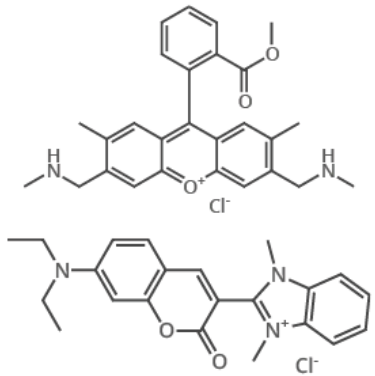
BLUE



ACID BLUE 9
(Triphenylmethane dye)

A triphenylmethane dye such as Acid Blue 9 is commonly used to achieve a blue ink colour; it is used in combination with a colour-brightening compound, e.g. an anionic stilbene derivative.

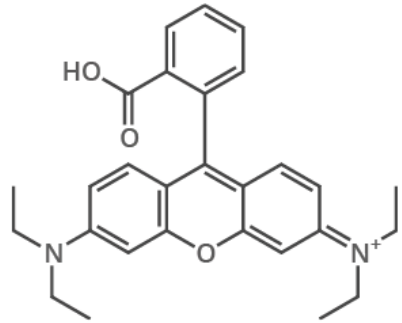
ORANGE



BASONYL RED 485 (TOP) & BASIC YELLOW 40
(Xanthene dye and Coumarin dye)

A mix of a xanthene dye and a coumarin dye is required to achieve an orange colour.

PINK



SOLVENT RED 49
(Rhodamine dye)

A rhodamine dye can impart a pink colour to the highlighter ink. A rhodamine dye can also be combined with a triphenylmethane dye in order to produce a purple-coloured highlighter.