A Guide to Colorimetry

Sherwood Scientific Ltd
1 The Paddocks
Cherry Hinton Road
Cambridge
CB1 8DH
England

www.sherwood-scientific.com

Tel: +44 (0)1223 243444
Fax: +44 (0)1223 243300

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Group	I	II											III	IV	V	VI	VII	VIII
Period	1A																	8A
1	1 **H** 1.008	2A											3A	4A	5A	6A	7A	2 **He** 4.003
2	3 **Li** 6.939	4 **Be** 9.0122											5 **B** 10.811	6 **C** 12.011	7 **N** 14.007	8 **O** 15.999	9 **F** 18.998	10 **Ne** 20.183
3	11 **Na** 22.99	12 **Mg** 24.312	3B	4B	5B	6B	7B	[----------8B----------]			1B	2B	13 **Al** 26.982	14 **Si** 28.086	15 **P** 30.974	16 **S** 32.064	17 **Cl** 35.453	18 **Ar** 39.948
4	19 **K** 39.102	20 **Ca** 40.08	21 **Sc** 44.956	22 **Ti** 47.9	23 **V** 50.942	24 **Cr** 51.996	25 **Mn** 54.938	26 **Fe** 55.847	27 **Co** 58.933	28 **Ni** 58.71	29 **Cu** 63.546	30 **Zn** 65.37	31 **Ga** 69.72	32 **Ge** 72.59	33 **As** 74.922	34 **Se** 78.96	35 **Br** 79.904	36 **Kr** 83.8
5	37 **Rb** 85.47	38 **Sr** 87.52	39 **Y** 88.905	40 **Zr** 91.22	41 **Nb** 92.906	42 **Mo** 95.94	43 **Tc** [97]	44 **Ru** 101.07	45 **Rh** 102.91	46 **Pd** 106.4	47 **Ag** 107.87	48 **Cd** 112.4	49 **In** 114.82	50 **Sn** 118.69	51 **Sb** 121.75	52 **Te** 127.6	53 **I** 126.9	54 **Xe** 131.3
6	55 **Cs** 132.91	56 **Ba** 137.34	57* **La** 138.91	72 **Hf** 178.49	73 **Ta** 180.95	74 **W** 183.85	75 **Re** 186.2	76 **Os** 190.2	77 **Ir** 192.2	78 **Pt** 195.09	79 **Au** 196.97	80 **Hg** 200.59	81 **Ti** 204.37	82 **Pb** 207.19	83 **Bi** 208.98	84 **Po** 209	85 **At** 210	86 **Rn** 222
7	87 **Fr** 215	88 **Ra** 226.03	89** **Ac** 227.03	104 **Rt** [261]	105 **Db** [262]	106 **Sg** [266]	107 **Bh** [264]	108 **Hs** [269]	109 **Mt** [268]	110 [271]	111 [272]	112 [277]		114 [289]		116 [289]		

*** Lanthanides**	58 **Ce** 140.12	59 **Pr** 140.91	60 **Nd** 144.24	61 **Pm** 145	62 **Sm** 150.35	63 **Eu** 151.96	64 **Gd** 157.25	65 **Tb** 158.92	66 **Dy** 152.5	67 **Ho** 164.93	68 **Er** 167.26	69 **Tm** 168.93	70 **Yb** 173.04	71 **Lu** 174.97
**** Actinides**	90 **Th** 232.04	91 **Pa** 231	92 **U** 238	93 **Np** 237.05	94 **Pu** 239.05	95 **Am** 241.06	96 **Cm** 244.06	97 **Bk** 249.08	98 **Cf** 251	99 **Es** 252.08	100 **Fm** 257.1	101 **Md** 258.1	102 **No** 259.1	103 **Lr** 262.11

The ability to analyse and quantify colour in aqueous solutions and liquids using a colorimeter is something today's analyst takes for granted. As a result some of the fundamental principles of this routine technique are forgotten or overlooked when users of colorimeters come in contact with the technique for the first time. Also many experienced users do not always appreciate the power and versatility of the technique because they have always used it for the same routine tests day in day out.

So the aim of this booklet is to introduce the technique and the practical aspects of the instrumentation and to illustrate just how versatile today's colorimeter can be for the analysis of hundreds of ions, elements and compounds in solution.

CONTENTS

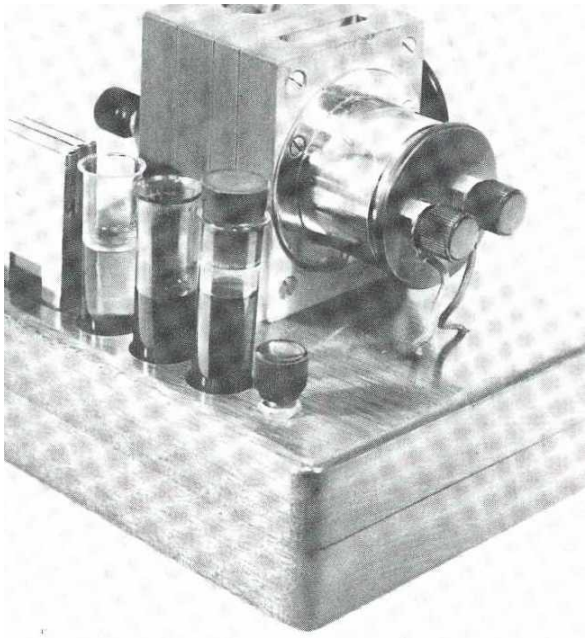
Introduction

Photometry is the general name given to the techniques which use the detection of light radiation and changes in radiation intensity. If the measured light is in the visible range of the Electromagnetic Spectrum then the term applied to the analysis is COLORIMETRY. The basis of the technique is that a beam of light from a light source (usually a tungsten lamp) passes through a cuvette or test tube containing the compound in solution, the intensity of light leaving the sample will be less than the light entering the cuvette. The loss of light or ABSORPTION is proportional to the concentration of the compound. The colour of the sample can be an inherent property of the solution or can be developed by the addition of suitable reagents. The absorption of the sample is compared with that of standards containing known amounts of the sample material thereby enabling the sample concentration to be estimated.

Colorimeters in the UK a History

Believe it or not the roots of colorimeter manufacture and development in the UK originated because of the Second World War - or rather the fear of it! In the late 1930's Mr Arthur Evans, son of a London dock worker had built for himself a successful import and export business. Whilst travelling in Europe in dark pre-war days he noted, with concern, that Germany had a monopoly in the manufacture of selenium photocells. These are small components which, when light falls on them, produce a very small electric current. Arthur Evans soon recognised that, in any conflict with Germany, the United Kingdom's imported supply of these would dry up He also realised that we would be in serious trouble due to the fact that photocells were, typically, found in such key wartime instruments as densitometers, used for the examination of photographs taken by the RAF over Germany and Europe. Alarmed at the thought of this he wrote to his local Member of Parliament - Winston Churchill. Winston, typically, wrote back and said to Arthur Evans 'Well, why don't you start making photocells!' And so, in 1938, Arthur Evans converted a barn at Potter Street, Harlow into a laboratory and started production with two employees. Little record remains of the wartime effort but we must surmise that the company's contribution was certainly important. Indeed, stringent government specifications stimulated the company to reach a quality level where users declared that these photocells were superior to German ones.

With the war over, Arthur Evans turned his thoughts to other uses for his photocells. His company, Evans Electroselenium Limited, and trading under the 'EEL' trademark (you will probably recognise the logo!), was approached by a leading medical researcher -Dr Rose of the Hammersmith Hospital, in London, to see if he could make a device which would enable the measurement of the intensity of a colour in a solution thereby indicating the concentration of an element in it; colorimetry was already a widely used analytical technique in medical, scientific and industrial laboratories but, until then, it had been performed by matching the solution under examination to known standards by eye. And so the first selenium photocell colorimeter (the model 2) was produced commercially and began appearing in laboratories all over the world (see fig 1). It provided both accurate and reliable results. The name EEL was thus established as the pioneer in colorimetry and the wealth and knowledge and expertise gained in making selenium photocells and colorimeters enabled the company to develop many more unique photoelectric instruments e.g. the famed model 100 flame photometer of which over 60,000 units were sold worldwide. (If you have not seen one, take a look in the Science Museum!)



In 1952, having been refused planning permission for expansion of his Harlow premises by the district council, Mr Evans purchased a site in Colchester Road Halstead, a market town in The Colne Valley, Essex.

After several milestones including the flotation of the company on the stock exchange in the sixties and huge continued growth Evans Electroselenium was purchased by Corning. Corning (the first company to produce pH sensitive glass for pH electrodes) were, and still are, a household name in medical and industrial laboratories alike.

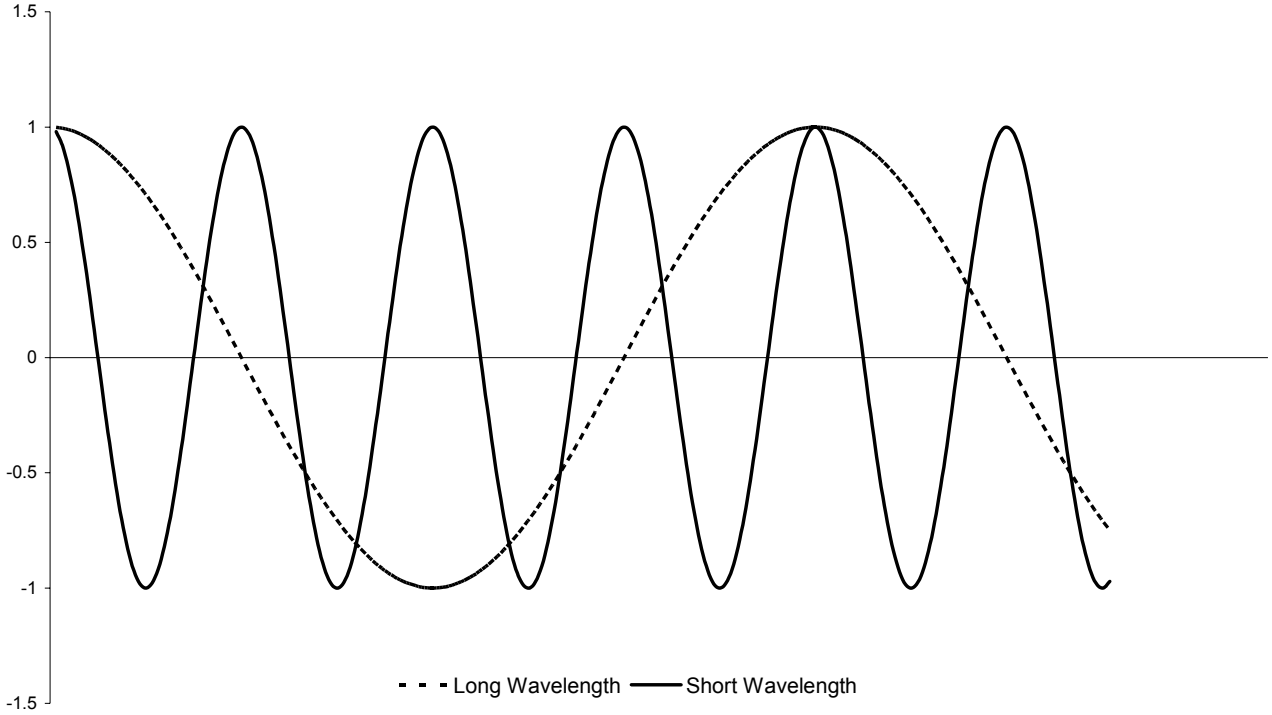
This partnership of experience, expertise and commitment led to the development of several new colorimetric products none of which now use selenium photocells.

With the formation of Ciba Corning Diagnostics, a company of obvious pedigree in the field of colorimetry, in 1985 the story has gathered yet further momentum…….

The Electromagnetic Spectrum

Before we can discuss the technique of colorimetry it is important to understand and to differentiate this technique and others which involve measuring electromagnetic radiation from various parts of the spectrum. The following table should clarify the types of radiation that constitute the electromagnetic spectrum. For those who are unfamiliar with the definition of wavelength and its units here is a brief guide:

Radiation may be considered as a wave. The wavelength is the distance between two successive peaks of that wave.



The wavelengths in the table are expressed in nanometers (nm) these are related to metres thus:

1 nanometre = 10^{-9} metres

Portion of the spectrum	Wavelength Range (nm)
Radio waves	2x 10^{12} - 10^{9}
Micro waves	10^{9} - 300,000
Infrared rays (IR)	300,000 - 3000

Near infrared	3000 - 780
Visible rays	780 - 380
Near ultra violet rays(UV)	380 - 200
Far ultra violet rays	200 - 50
X-rays	50 - 10^{-1}
(Gamma) X-rays	10^{-1} – 10^{-3}
Cosmic rays	10^{-3} and less

Most of us are more familiar with the visible, UV and IR segments of the spectrum so let's look at these regions more closely and label them according to the kind of measurements made in the respective regions.

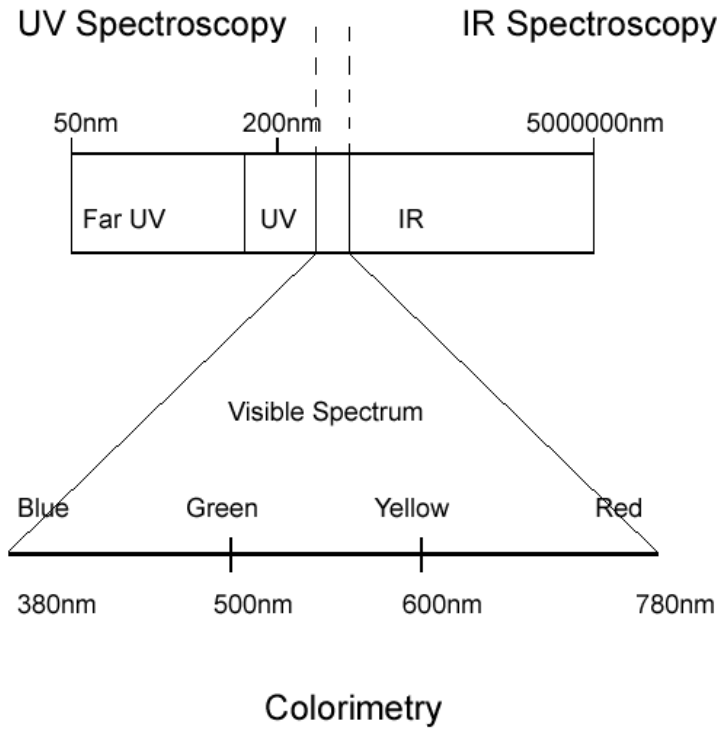


Fig 3

Principles of Colorimetry

Colorimetry is just one of the types of photometric analysis techniques i.e. it is a light measuring analytical procedure. Colorimetric measurements are made using a white light source which is passed through a colour filter or alternative wavelength selection device. This incident light then passes through a cuvette containing a chemical compound in solution. The intensity of the light leaving the sample will be less than the light entering the cuvette. The loss of light or absorption is proportional to the concentration of the compound. Colorimetry however only applies to measurements made in the visible region of the electromagnetic spectrum e.g. (380 - 780 nm). The extent to which light is absorbed by a sample is dependant upon many factors. The main general contributors are the wavelength of the incident light and the colour of the solution. Each compound in solution has a typical (and usually unique) absorption spectrum, an example is shown in fig. 4.

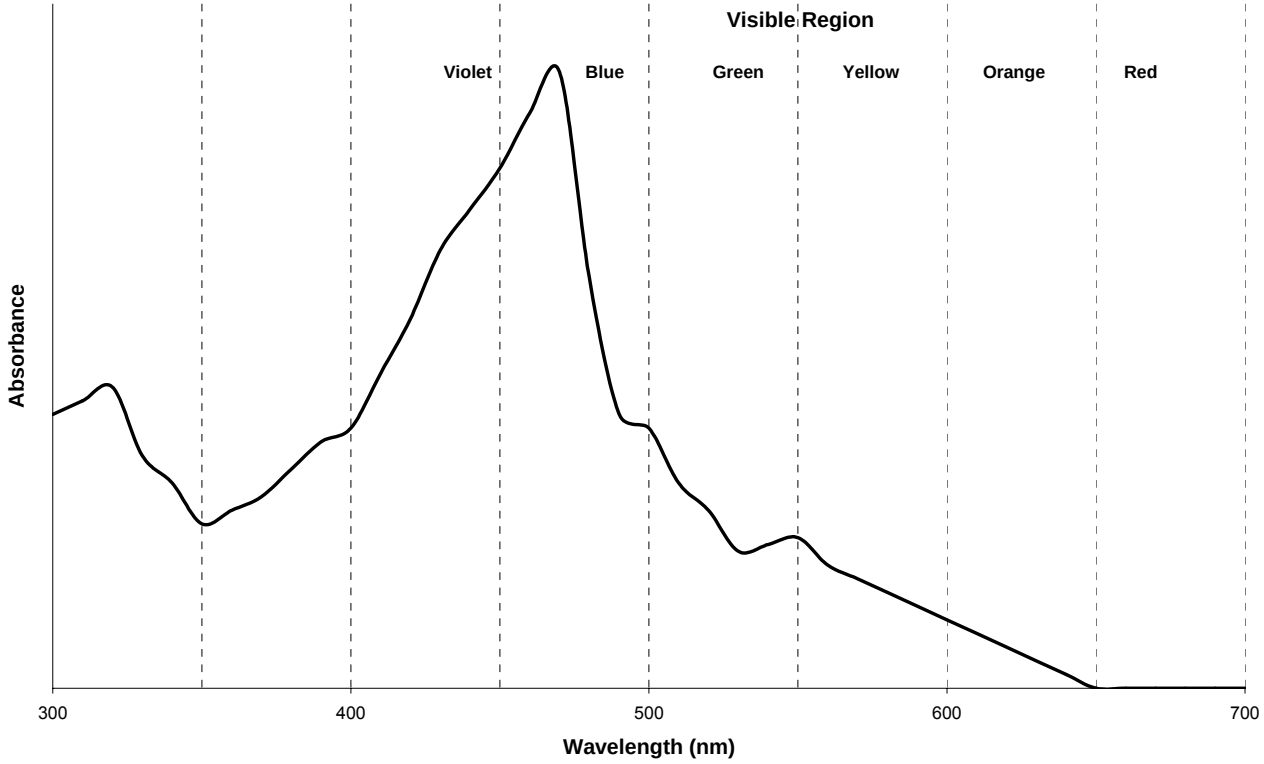


FIG 4

The spectrum is basically a pattern of the amount of absorption at each of the individual wavelengths in the spectrum.

In most cases the spectrum will have a peak i.e. a wavelength at which absorption is at a maximum. This is often referred to as the λ max for the compound in question. If the absorption is being quantified it is essential that it is measured as close as possible to the max. Sensitivity is reduced at any other wavelength.

From the example our sample has a max at about 460 nm in the blue part of the spectrum. So what colour will it appear to be?

Well the answer is **yellow!**

Confused? Well here is the explanation:

Inert materials whether solid or liquid appear coloured due to the way they modify light illuminating the object. Thus different objects absorb some wavelengths and reflect others.

If white light passes through a yellow solution, it absorbs all colours except yellow.

Similarly, a book cover appears red since it absorbs all colours except red.

If a solution is clear and colourless it has not absorbed any visible radiation and therefore all the white light is transmitted i.e. it is transparent.

See the example of the spectral distribution curve of the solution in figure 5. The solution absorbs blue light strongly, has a λ max at 460nm and therefore appears yellow.

If the concentration of the yellow solution is reduced to half, the two solutions will give the curves shown.

Therefore for greatest sensitivity and linearity it is essential to limit the measuring wavelength to the area of highest absorption.

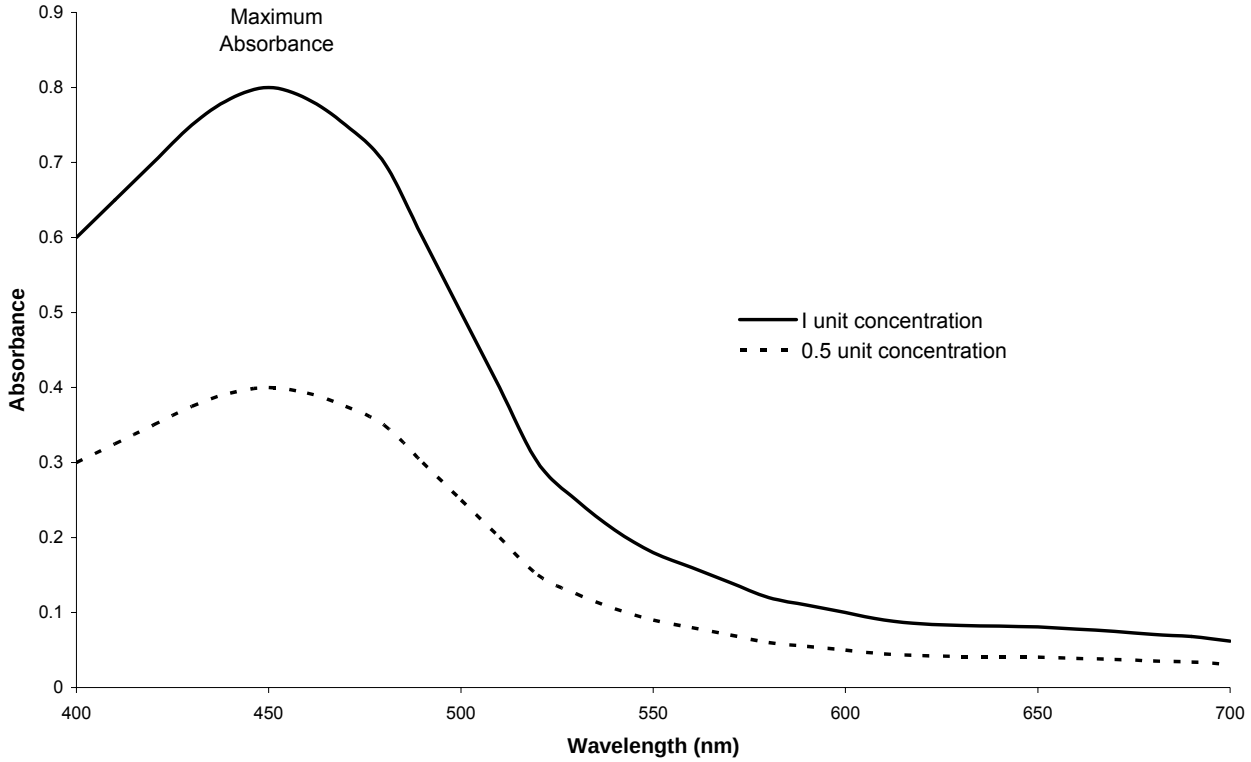


Fig 5

Selecting the measuring wavelength

Figure 5 shows that the correct wavelength at which to measure a solution is the one which gives greatest absorption. The wavelength or colour filter that will produce the maximum absorbance can be selected in two ways:

1. Taking readings throughout the spectrum on a typical standard and establishing the peak wavelength of the solution.

2. Choosing a filter of complementary colour to the standard solution. Figure 6 shows the basic relationship between colours.

The complementary colour is the one opposite on the circle, e.g. if the sample solution is red a bluish green filter is selected for analysis.

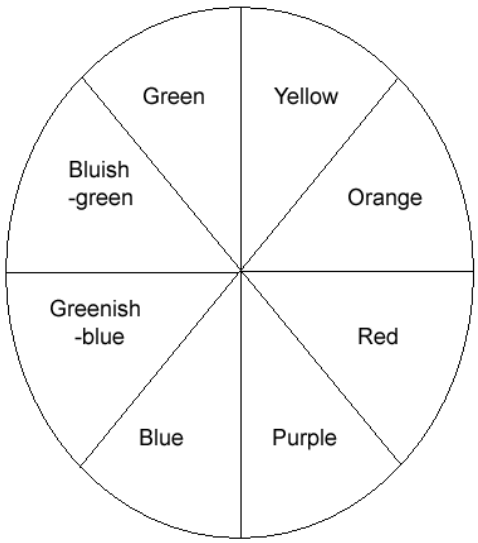


Fig 6

So that is the basis of colorimetry - what about the instruments themselves?

The Colorimeter

Any colorimeter whether simple or complex consists of five basic components:

1. Light source

2. Wavelength selector (filter)

3. Sample container

4. Photo detector

5. Display

These are configured in the following fashion:-

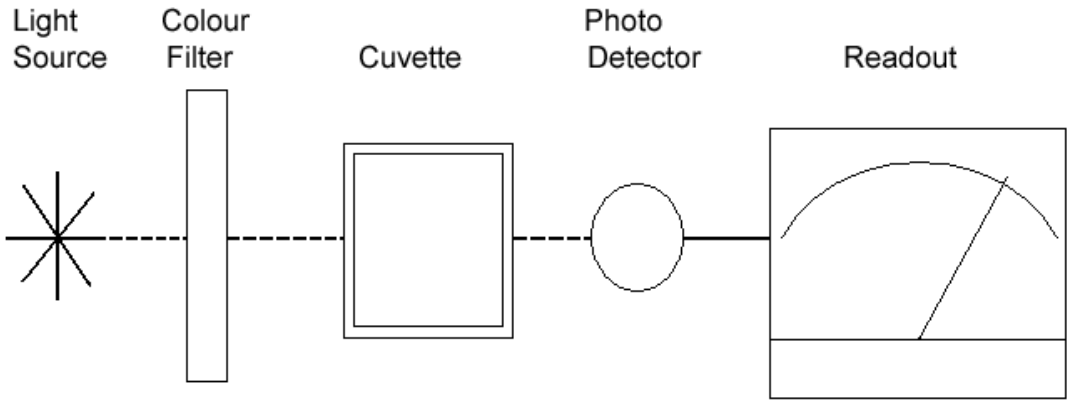


Fig 7

The Light Source

The light source to be used for successful colorimetry must produce energy at sufficient intensity throughout the whole visible spectrum e.g. 380 - 780nm. Fortunately this is achieved readily by using a tungsten lamp. This as we all know (since we use it in the home) provides adequate light to enable us to see all the visible range. The following diagram illustrates the output of a tungsten lamp over the visible spectrum.

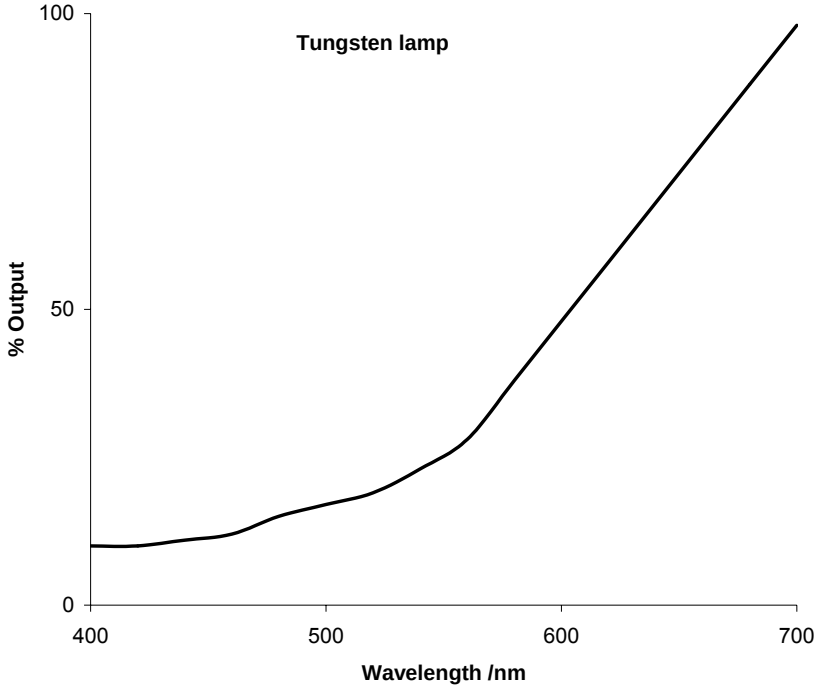


Fig 8

Note that the energy decreases towards the near ultra violet but it is still sufficiently powerful to energise a photodetector down to around 350 nm.

To increase the intensity of light into the near UV range a tungsten halogen lamp may be used, this consists of a tungsten filament in a quartz envelope which also contains traces of a halogen such as iodine.

Tungsten lamps are of a type which use black body radiation e.g. dependent solely on its temperature. There is another type which uses radiation produced at specific energies e.g. the excitation of atoms such as a gas discharge lamp.

Here an electric current is passed through a gas at low pressure resulting in molecular excitation and the emission of light at very specific wavelengths. The mercury lamp is a good example of the gas discharge lamp which produces strong emission in the UV and blue parts of the spectrum (see fig 9).

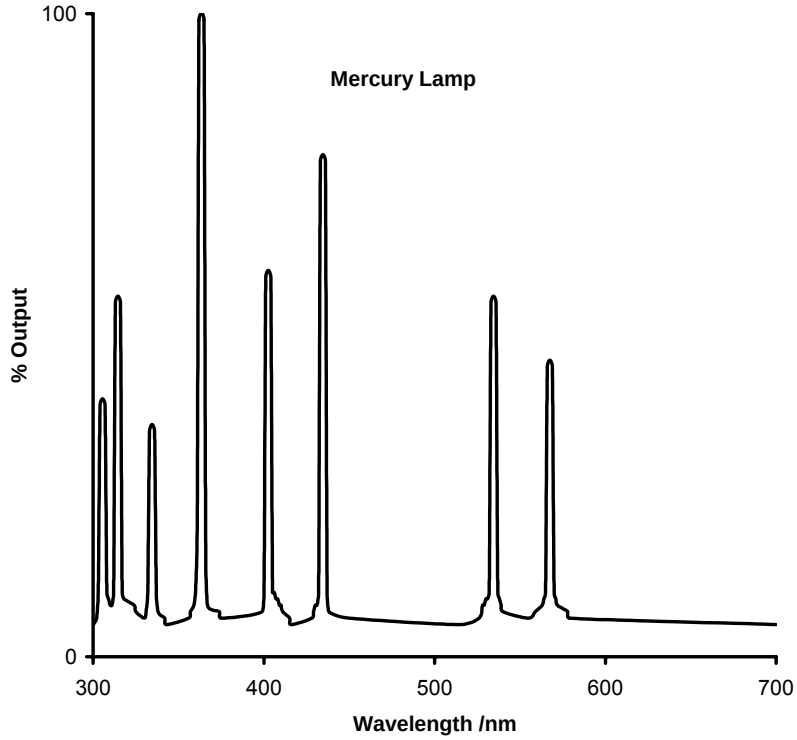


Fig 9

The major disadvantage of the mercury lamp is that it can only be used at specific wavelengths.

For continuous output in the ultra violet range deuterium lamps need to be used and though expensive and with a comparatively short life do have an effective output down to 190 nm (see fig 10).

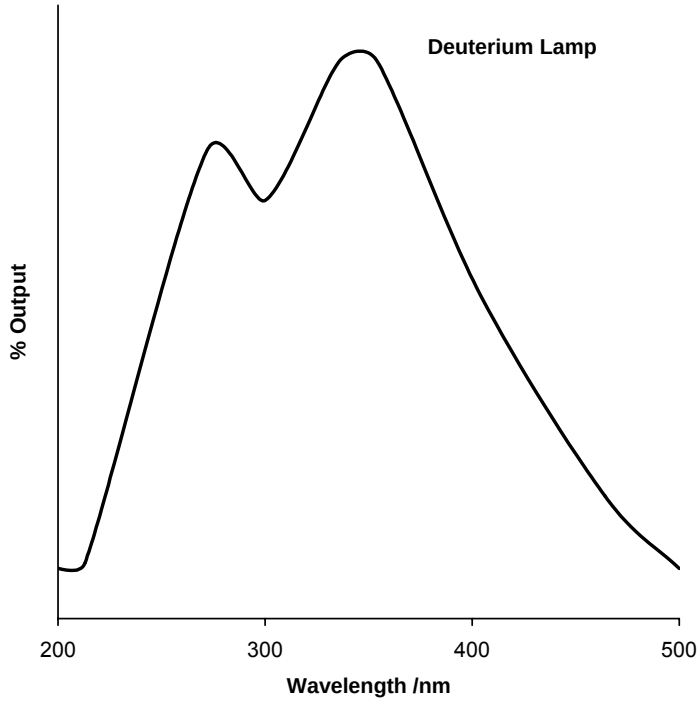


Fig 10

Wavelength Selection

There are several options open to the manufacturer of a colorimeter when deciding how to select the wavelength i.e. produce mono chromatic radiation (one wavelength band) from polychromatic radiation (white light).

These basic options are:

1. Gelatin filters
2. Interference filters
3. Grating monochromators
4. Prisms

Gelatin Filters

These are low cost selection devices which produce or transmit a wide band of radiation usually± 20 nm.

Fortunately most colorimetric analyses have a wide absorption band which allows excellent results to be obtained from a simple colorimeter.

The most common type of filter is constructed by sandwiching a thin layer of dyed gelatin of the desired colour between two thin glass plates.

There are two drawbacks which can be encountered using gelatin filters:

1. They have a wide bandpass which can lead to non linearity in standard curves (see page 45)
2. They absorb approximately 30-40% of all incident radiation thereby reducing energy throughput to the detector. However these filters are eminently suitable for most general applications.

Glass Filters

Coloured glass filters are now more or less historical selection devices in colorimeters and have very wide bandpasses often up to 150nm. Specific wavelengths can however be achieved by using a combination of glass filters.

Wavelength Coverage

To ensure all wavelengths in the visible spectrum are catered for approximately 8 Gelatin filters are required. A typical range of filters will have the following transmission curves.

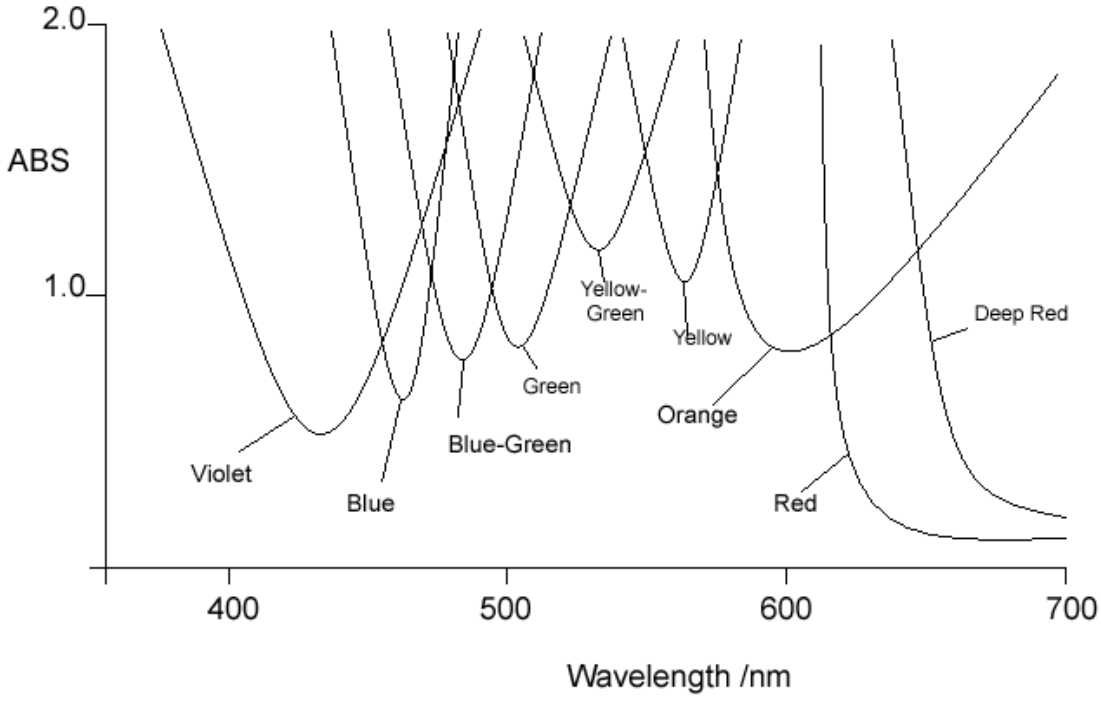


Fig 11

Filters are specified by their peak transmission wavelengths

Interference Filters

These are used to select wavelengths more accurately by providing a narrow bandpass typically of around 10nm. The interference filter also only absorbs approximately 10% of the incident radiation over the whole spectrum thereby allowing light of higher intensity to reach the detector.

The theory of operation of an interference filter is fairly complicated but has been simplified below.

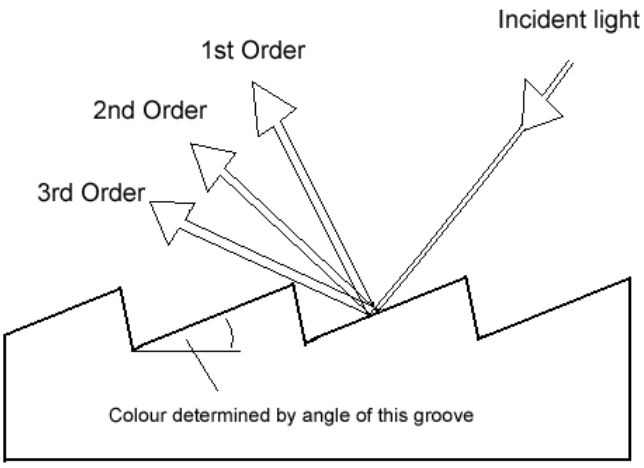
An interference filter comprises of several highly reflecting but partially transmitting films of silver separated by thin layers of transparent dielectric material (often magnesium fluoride (MgF_2). This is also referred to as an MD or metallic dielectric filter). When white (polychromatic) light passes through the dielectric layers multiple reflections appear between the semi-transparent mirrors. However some energy from the light beams passes straight through the filter. It is this wavelength which is desired for analysis. If the dielectric layer thickness is altered slightly the resultant wavelength is changed.

The Grating Monochromator

Diffraction gratings provide an alternative means of producing monochromatic light.

A grating comprises a huge number of parallel grooves etched closely together on a highly polished surface such as steel, glass or quartz.

The typical diffraction grating has 500 to 600 lines/mm whilst research grade instruments contain blazed holographic gratings with 1200 to 2000 lines/mm. When light falls on a grating the various wavelengths of white light are deflected at different angles see figure 12.



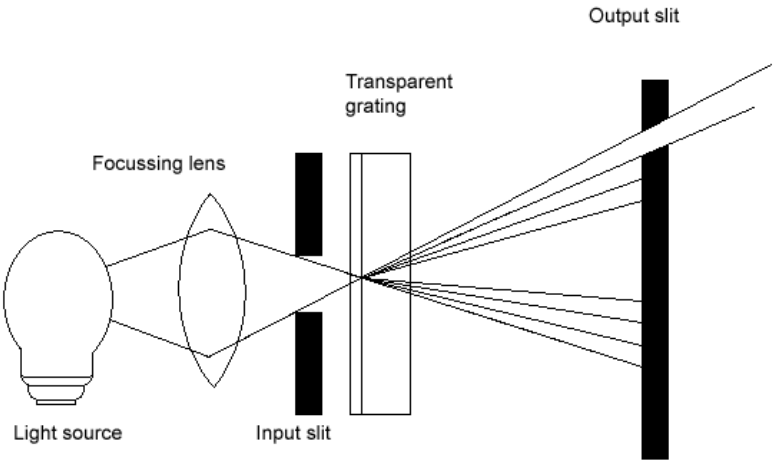
Diffraction using a reflection grating

Fig 12

If the dispersed beam is then refocused on an exit slit, the wavelength can then be selected by moving the exit slit.

The positioning of the exit slit is obviously crucial but more so due to the generation of several spectra of different orders from the grating this is shown in figure 12.

The gratings shown in previous diagrams are of the reflection type however many spectrophotometers utilise a transmission grating which is show in figure 13.



Transmission grating

Fig 13

Grating Mountings

There are several ways in which a grating is fixed to its mounting probably the most common is The Ebert mounting where the grating is rotated around its central axis.

Others include Littrow mounts, Czerny turner, crossed Czerny turner and monk-Gillieson types.

Some spectrophotometers however keep the slits and grating stationary and create wavelength selection via the rotation of a mirror.

Prisms

Prisms are not now generally used in today's colorimeters or spectrophotometers but historically are extremely important. A prism separates white light into its components see figure 14. The section of the spectrum required can be selected by rotation of the prism.

Several factors are important when assessing the prisms monochromatic powers. The spread of the spectrum depends mainly on the dispersive power, nature of the prism and the angle (a) at its apex (see fig 14)

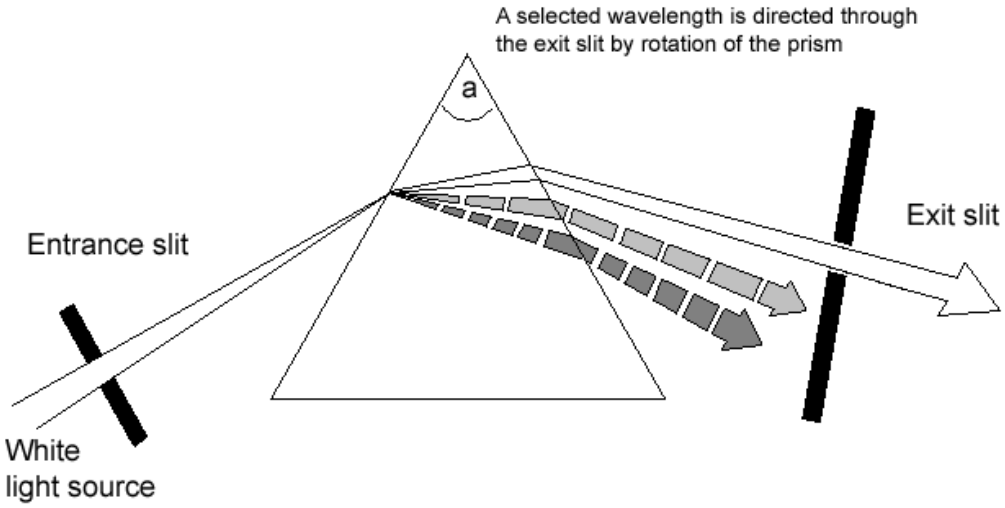


Fig 14

Prisms used in colorimeters are made from glass and are operable from around 350 nm to 800 nm. Should the analysis require work in the UV region then a quartz prism is required as glass absorbs radiation strongly in the ultra violet region.

Bandpass and Spectral Distribution

In colorimetry, it is most convenient to express the spectral properties of filters, solution, light sources and photodetectors by means of a graph of wavelength to optical density (or % output for light sources).

This type of graph is known as a spectral distribution curve, Fig 15 could illustrate a typical colour filter which is specified in terms of two main aspects.

1. Peak wavelength

2. Bandpass which is the wavelength range at half the height of the curve

The bandpass is a crucial factor when the quality of the colorimeter is being evaluated. Generally the smaller is the better. Fortunately most determinations have a wide absorption band allowing excellent results to be obtained. Wide bandpass can however be a major cause of non compliance with the Beer Lambert Law.

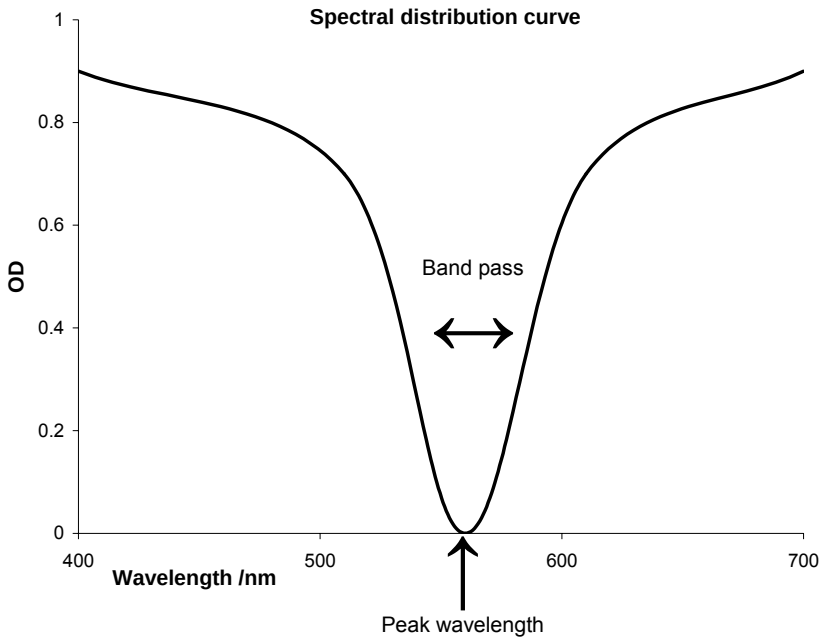


Fig 15

Sample Container

Colorimetric analysis is performed on solutions and as such the sample can only be analysed within a container. The container must therefore be (or made to be) transparent at the wavelength at which the sample is being measured.

Sample containers are offered in a variety of forms depending on whether the important factor is sample size, speed of measurement or accuracy.

For colorimetric work all containers should be made of glass or clear plastic. However if analysis is to be made in the ultra violet region of the spectrum then quartz vessels must be used as glass absorbs UV radiation.

The types of container are listed below.

Test Tubes

These are usually glass and are relatively low cost. They are supplied in matched sets, i.e. each tube has exactly the same absorbance characteristics, this ensures that once a blank has been measured every sample absorbance readout will be due to the sample only no matter which of the tubes is used.

Various diameter tubes are offered so that the sensitivity of measurement can be varied i.e. if a sample is concentrated (high absorbance values) then measurement in a small test tube is suitable as the light has to travel a smaller distance through the sample leading to lower absorbance values. However if dilute samples are to be analysed it is necessary to increase sensitivity by offering more sample to the light hence larger test tubes are used.

Cuvettes

These are basically fused glass cells either rectangular or square with an exact path length (usually 10 or 20 mm). Cuvettes for colorimetry can be made from either plastic or glass, however, if samples are to be analysed in the UV region of the spectrum then quartz cuvettes are required.

Cuvettes generally offer precision and allow measurement to be reported with a known path length. The most common colorimetric measurements are made in 10mm x 10mm cuvettes. These cuvettes are usually 45mm high and hold 4 ml of sample solution.

When smaller sample volumes need to be analysed in situations where the sample is in limited supply e.g. biological fluids then 10 x 10 mm cuvettes are also usable but do have wider glass side panels which provide an inner filling. This enables the sample to be analysed in cuvettes even though volumes are limited.

Cuvettes of larger path lengths e.g. 40, 50 and 100 mm are available.

Pour In - Suck Out Cells

For routine work it is extremely convenient to have a built in sample tube with the outlet connected to a vacuum.

Solutions are poured into the container and after measurement are sucked out the tube to waste. This technique has several advantages:

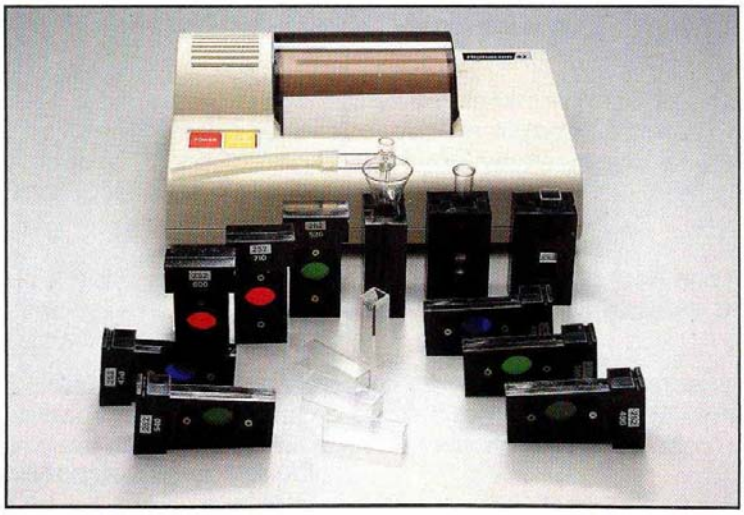
1. A high throughput of samples is achieved.
2. All measurements are made in the same tube eliminating the need for matched tubes.
3. Blank measurement is made only once and by nature of the method is now 100% accurate for all tubes.
4. Vacuum can be achieved by a water pump extremely cheaply

Flow Cells

When on line work is required flow cells are ideal. Most flow cells have a small total volume but maintain the 10 mm path length. This ensures fast response and good sensitivity. Several factors however should be taken into account:

1. A colorimeter with a recorder output is required to record the data.
2. Air bubbles must be eliminated at all costs.
3. Sample tubes must remain free from kinks etc. to ensure consistent flow.
4. All connections must be secured by clips or wire fittings

A representation of the typical accessories is shown below.



e.g. It should be noted several others are available e.g. water jacketed cuvettes for heated sample applications.

Photo Detectors

Detectors for colorimeters basically convert the resultant light beam once it has passed through the sample compartment into an electrical signal. There are several types and there's nowhere better to start than the good old selenium photocell:

Selenium Photocell

These are the simplest type of photocell and are particularly useful as they respond well in the visible spectrum and do not require any power supplies. Photocells operate by converting photons of light into electron charges within the cell, this develops a potential across a plate caused by change in the electronic structure of a selenium layer. The output of the cell is proportional to the light intensity.

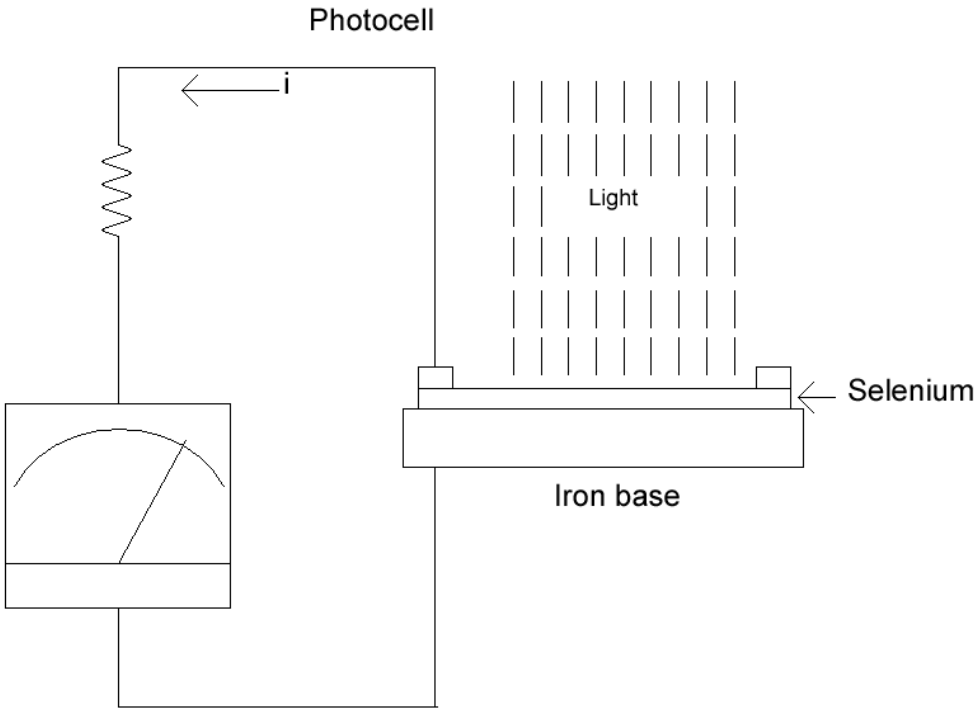


Fig 16

The selenium photocell is now largely historical.

The Photo Tube

The main feature is low cost with high sensitivity in the UV and visible regions. A diagram can be seen below.

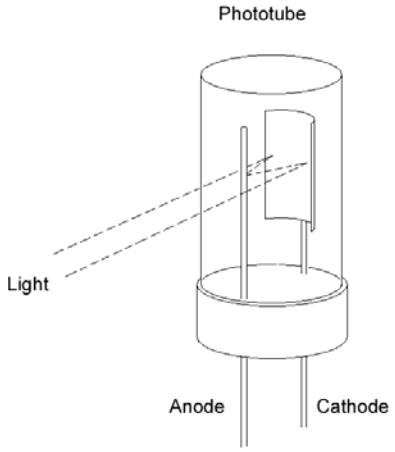


Fig 17

The construction is basically a glass bulb partly coated internally with a photosensitive material (caesium or potassium oxide). Within the gas filled tube is an anode maintained at high voltage. When a photon hits the photo cathode, electrons are liberated and flow towards the anode. The current this generates is amplified and the result obtained.

Phototubes give substantially more output than photocells and allow operation with lower light/ energy levels thus lower bandpass filters.

The Silicon Photocell

A silicon photocell generates an electric voltage when a photon of light hits its semi conductive surface. Sensitivity is normally lower in the UV than the visible region of the spectrum.

Photomultiplier Tubes (PMT)

A photo multiplier is a combination of a photo tube and a high gain amplifier. The sensitivity is widely variable by adjusting the applied voltage.

The PMT consists of a photo cathode and a number of dynode plates. Each time an electron hits a dynode plate several are emitted. This results in multi stage amplification of the original signal.

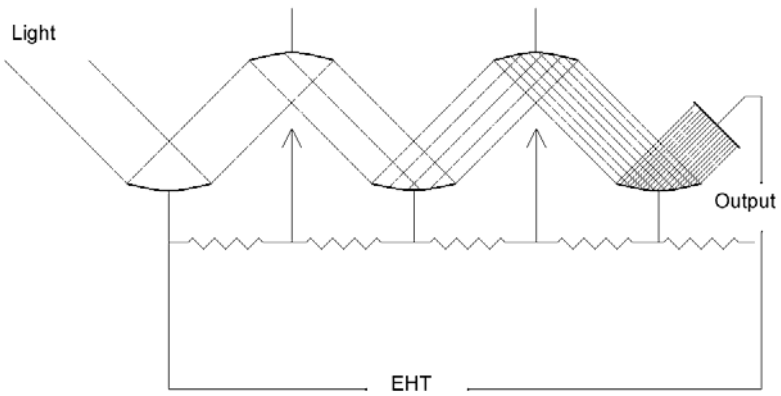


Fig 18

Extremely low sensitivity can be achieved with a PMT and this tends therefore to be the choice in high grade spectrophotometers.

Display

Readings are presented in analogue or digital form. Historically the analogue display was most important prior to the advent of the LCD (Liquid Crystal Display) or LED (Light Emitting Diode).

Today the LCD has become the most popular and is undoubtedly far easier to read with greater precision than the analogue readout.

Analogue meters are calibrated in absorbance (or optical density) with a supplementary % transmission scale.

Once a zero or blank has been set the reading is taken from the position of a needle on a mirror backed scale. The absorbance result has to be read from a logarithmic display see fig 19. As you can see this provides precise results at low absorbance values but does lead to difficulties when higher values are displayed.

Digital displays however indicate absorbance values to identical resolution whatever the magnitude of the data.

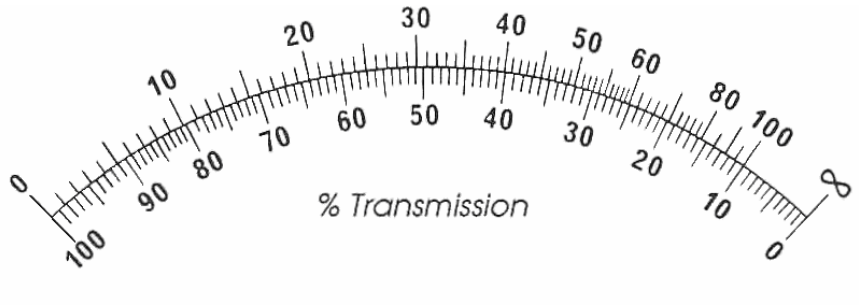


Fig 19

The digital display can also be read with the greater precision and without introducing interpretation errors. It is important to note the relationship between absorbance concentration and transmittance and so a full treatise on this can be found in the next section.

Advantages and disadvantages of analogue and digital displays

Digital	**Analogue**
Advantages	
Easy to use	Easy to see rate of change
Resolution is identical through the range	Shows the ABS / % transmittance relationship graphically
Choice of readout of ±0.01 ABU to±0.001 ABU	Can read ABS and % transmittance simultaneously
Can be transferred to a printer	Traditional in education
Readout is objective i.e. not open to interpretation errors	Easy to read even in bright sunlight
Can read up to 2 ABU on colorimeters (often displays are read up to 5 ABU on research spectrophotometers	

State of the art	
Can read concentration in direct units on concentration colorimeters	
Disadvantages	
Not easy to read in direct sunlight or bright lighting.	Poor resolution above 0.3 ABU
Does not illustrate relationship of %T and absorbance	Readout is subjective i.e. depends where you stand in relation to the needle
Difficult to see rate of change	Scale can only be read to 1 ABU
Can only read absorbance or transmittance - not both simultaneously	Logarithmic display is difficult to understand
	Not state of the art
	Resolution is not the same over the scale
	Cannot read in concentration

Colorimetry Theory

Before the analyst attempts to perform colorimetric analysis it is important to understand the theoretical aspects of the technique. The relationship between concentration and the light absorbed is the basis of theoretical consideration:

Concentration and Light Absorbance

The seemingly obvious way of taking readings on a colorimeter is to measure % transmission and adjust the 'blank' to 100%.

For example, consider a situation where a blank is measured followed by three standard solutions having concentrations of 1, 2 and 3 units respectively. Ideally, a colorimeter should be giving concentration readings directly, but consider the above solutions when analysed. The solution with a concentration of 1 unit reduces the light to 50% therefore, the solution with a concentration of 2 units will reduce the light to 25% and the solution with a concentration of 3 units will reduce the light to 12.5%.

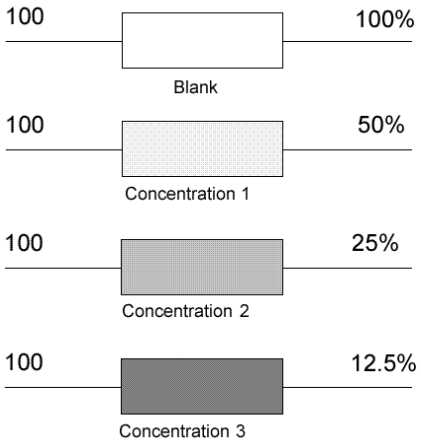


Fig 20

Therefore if the colorimeter is calibrated using a % transmission scale, the following graph is produced.

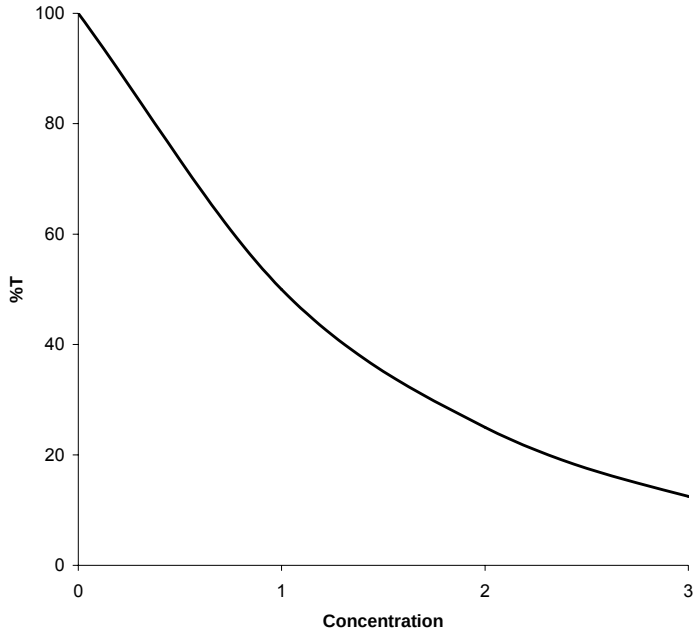


Fig 21

The calibration in %T has the drawbacks of being nonlinear and readings decreasing with increasing concentration.

Bonguer first investigated this type of relationship for changes in thickness of solid materials. His work was followed by Lambert and Beer in 1852, who extended the studies to solutions. All three investigators contributed what is universally known as The Beer Lambert Law.

This states that:

The light transmitted through a solution changes in an inverse logarithmic relationship to the sample concentration.

In order to take measurements both directly and linearly in terms of concentration, %T readings must be converted into an inverse logarithmic form which are called optical density units (OD) or absorbance (A).

The formula is:

$$OD = \log \frac{100}{\%T}$$

Therefore, for the given example, the relationship of OD to concentration is shown in the table below.

Concentration	%T	OD
0	100	0
1	50	0.3
2	25	0.6
3	12.5	0.9

A calibration curve of OD against concentration will be linear and directly proportional.

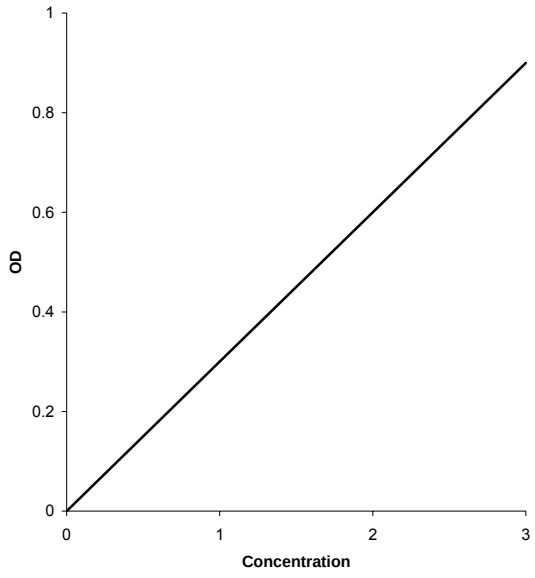


Fig 22

Optical density (absorbance) is used for colorimetric analysis so that readings relate directly to concentration. Similarly, optical density changes directly with sample path length. Thus we arrive at:

Abs =Ecl

A= Absorbance

E= Extinction coefficient or molar absorbtivity

c=Concentration

l= Path length

l is fixed by the path length of the cuvette and E is a constant for each species hence:

A α C

Absorbance is directly proportional to concentration.

Practical Considerations

The previous sections have dealt with the theory of measurement and should hold us in good stead to perform some real analysis. Be fore we do so it is very important to understand a few practical considerations:

1. Readings once made need to be linear and directly proportional with respect to concentration. Therefore quantitative analysis must be performed in absorbance or optical density units.

2. The wavelength or filter must be selected so that analysis is performed at λ max e.g. the wavelength at which maximum absorbance occurs.

   As mentioned previously this can be done by selecting a complementary colour filter or by measuring the absorbance of a standard throughout the wavelength range and noting the wavelength at which the highest reading was obtained.

   In practise when analyses are performed from a method sheet or booklet the λ max will be stated clearly.

3. Cuvettes or Test Tubes? The next stage is to select the vessels in which analysis is to be performed.

   The basic choice to be made is that between test tubes and cuvettes, the selection is often made however by what happens to be available at the time but there are good reasons for using either:

Advantages	
Cuvettes	**Test Tubes**
Fixed known path length	Samples can be analysed in the tube where the reaction took place
Essential for UV work (Quartz)	Low cost
Can select path length e.g. 10.. 20 and 40 mm	Ideal for large numbers of samples held in test tube holders
Face at 90° to path of light minimising scatter and reflection	Variety of tube sizes are available for varying sample volumes
Consistent quality making matching often unnecessary for colorimetry	Tubes are easier to clean
Two faces parallel with the light path	Plastic disposable tubes are available
Provides greater precision and accuracy for reseach or QC application	
Availability of low cost plastic disposable cuvettes	
Disadvantages	
Cuvettes	**Test Tubes**
Can be expensive	Optically are not easy to match i.e. inconsistent quality
Sample always has to be transferred into the cuvette prior to analysis	Round face causes reflection and scattering
Can be difficult to clean	Not usable for UV work (Quartz test tubes are not common)
Not viable to analyse many samples quickly due to expense of many cuvettes or the need to transfer sample at every step	Tubes are easily scratched and damaged during reaction, cleaning and using
	Not good for research grade analysis
	Easy to put finger marks on the tube which can be inserted in any position in the colorimeters

Please note that pour-in-suck-out and flow cells are available which can take the pain out of continuous and multi sample analysis.

4. After selecting the above we must now be sure that we have the three types of solution needed for analysis:

    a. The Blank

    This solution is used to set the instrument to read zero under the measurement conditions.

    Therefore by definition a blank is a solution that contains all the constituents of the determination except those which produce the colour.

    The most common blank is deionised water.

    b. Standards

    When the blank is set a calibration curve is constructed using a series of standard solutions.

    The definition of a standard is a solution containing an accurately prepared amount of the material being measured and mixed with the appropriate reagents to develop the colour.

    c. Samples

    These are measured following the standard and obviously contain an unknown amount of the material being quantified but in all other respects are the same as the standards.

Making Measurements

Once all of the above have been put in place the standardisation can take place.

A typical measurement routine is as follows:

1. Select the correct measurement wavelength.
2. Place the blank solution in the holder and set the absorbance display to zero,
3. Insert the lowest concentration standard in the instrument and note the absorbance readout.
4. Repeat 3 for all standards.

NB: For the standard curve to be appropriate at least three and preferably four standards should be measured.

5. Plot absorbance vs. concentration ensuring that the line passes through zero. See fig 23.
6. Measure the unknown and establish its concentration from reading off the calibration curve. See fig 23.

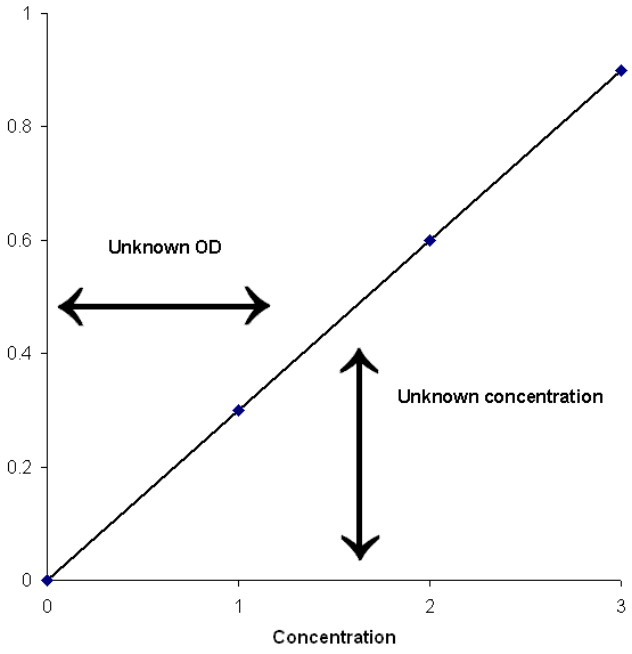


Fig 23

Variations

In practise the above procedure needs fine tuning to suit certain conditions. These variations are dealt with below and will be useful to note to produce ideal standard curves.

Symptom	**Cure**
Standards reading too high	Dilute all standards and samples or decrease the cell path length
Samples reading too high	Dilute samples or introduce more standards
Standards reading too low	Increase the path length low of the cell
Samples reading too low	Introduce lower low concentration standards or increase cell path length of standards and samples to improve sensitivity

Linear Calibration Curves

If the calibration curve is linear subsequent calibration need only be at a single point e.g. only one standard need be prepared

This means that the Beer Lambert Law is being obeyed.

It is therefore of interest to the user to perform all analysis on a linear calibration curve. This will eliminate the need to prepare large numbers or standards and elaborate calibration plots.

So, the dilution of solutions or the reduction of cell path length can often provide the much sought after linearity.

Summary

For linearity - Dilute the solution or reduce the path length

For sensitivity - Increase path length

Measuring without Standards

There are really two forms of measurement without standards, one acceptable and the other unacceptable.

If a standard curve is constructed and is linear there will be a factor by which absorbance can be multiplied to obtain concentration.

So, to obtain a factor, take the concentration of any standard, measure its corrected absorb ance (e.g. measured after zeroing with a blank) and perform the following calculation.

$$\frac{\text{Concentration}}{\text{Absorbance}} = \text{Factor}$$

This factor can then be used to perform concentration analysis thus:

1. Measure the blank and set to zero
2. Measure the absorbance of the sample
3. Multiply the absorbance by the factor to give concentration.

This method is legitimate and correct only if the standard curve obeys the Beer Lambert Law e.g the original standard curve from which the factor is taken must be linear.

The sample concentration must also fall within the linear range of the standard curve.

How not to do it!

Many people use the colorimeter as a colour comparator e.g. as a means to check the consistency and continuity of solution colour e.g. to ensure that the batch of malt whisky has the same colour as the previous batch etc.

In other words the colorimeter is a sophisticated eye.

If a solution, after performing a blank, has an absorbance of e.g. 0.34 ABU it can be compared with another on the same instrument, If that sample, however, was put in another instrument of the same type should the absorbance be 0.34 ABU?

The answer is **NO!**

It could be close but instruments differ and thereby introduce several factors which lead to absorbance values being different:

1. Filters - not all are the same, they may vary due to the age of the gelatin. The gelatin may be dried and cracked. This could lead to production of a different λ max and a different degree of light absorbed or scattered by the filter.
2. The bulb may be of a different age thereby producing different amounts of energy. This affects the signal to noise ratio and the incident intensity.

3. Detectors may be different ages and produce slightly different sensitivities.

In summary if any optical component is slightly different from that of another within a different unit the optical characteristics of both units will be different. Age is certainly a major contributory factor.

So without standardisation a colorimeter can be used as a colour comparator but should not be compared with other units of the same or different type due to the conditions above.

Over time however, its own optical characteristics will slowly change making long term comparisons invalid.

Deviation from the Beer Lambert Law

The perfect calibration curve is illustrated below. However more often than not premature non-linearity occurs.

1. Positive deviation
2. Perfect Beer Lambert curve
3. Negative

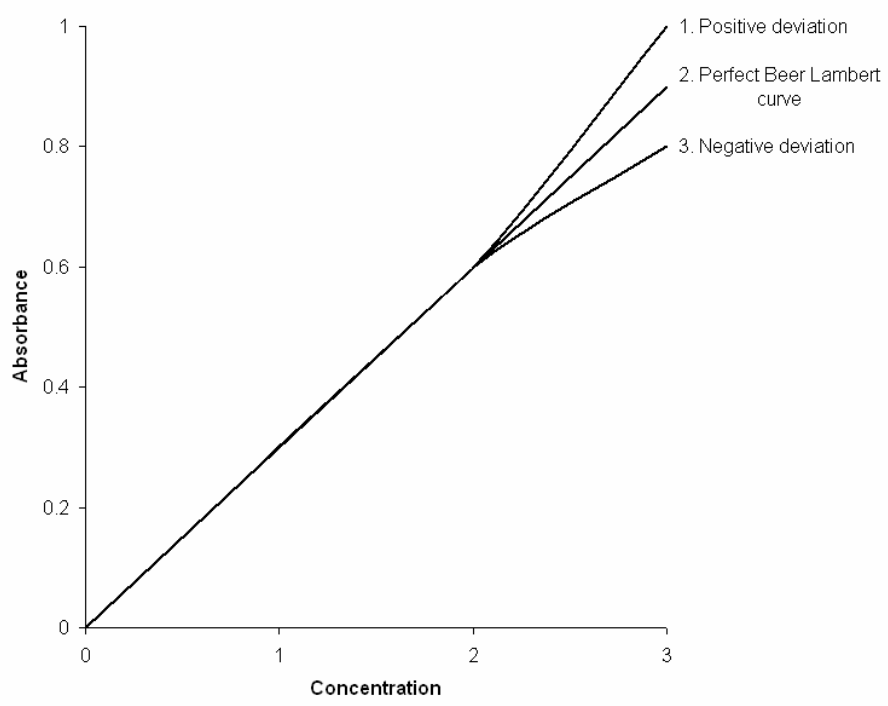


Fig 24

With a non-linear calibration curve there are several cures:

1. Dilute standards and samples
2. Reduce the path length of the cell
3. Prepare more standards to cover the non linearity

It may however be more appropriate to investigate the cause of this deviation.

Causes of Errors

Bandwidth

In colorimetry the major cause of non-linearity is that the bandwidth is too wide or wider than the absorption peak of the test solutions.

Fig 25 shows the spectral distribution curves for a gelatin filter and an interference filter.

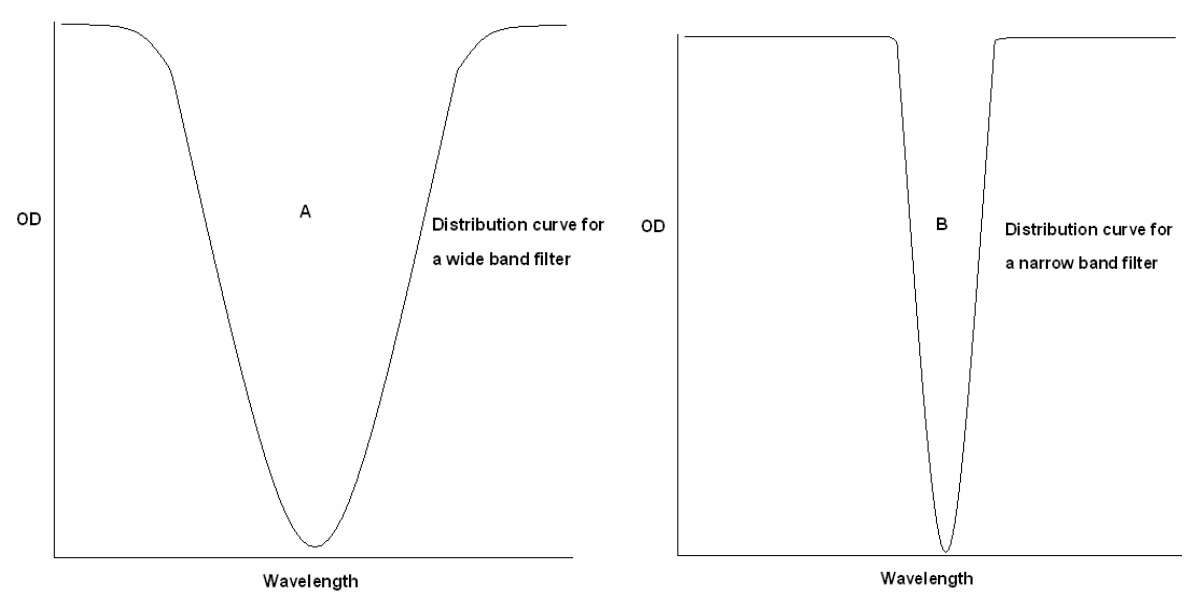


Fig 25

If a concentrated standard solution is then measured the effect of the filters can be seen in fig 26.

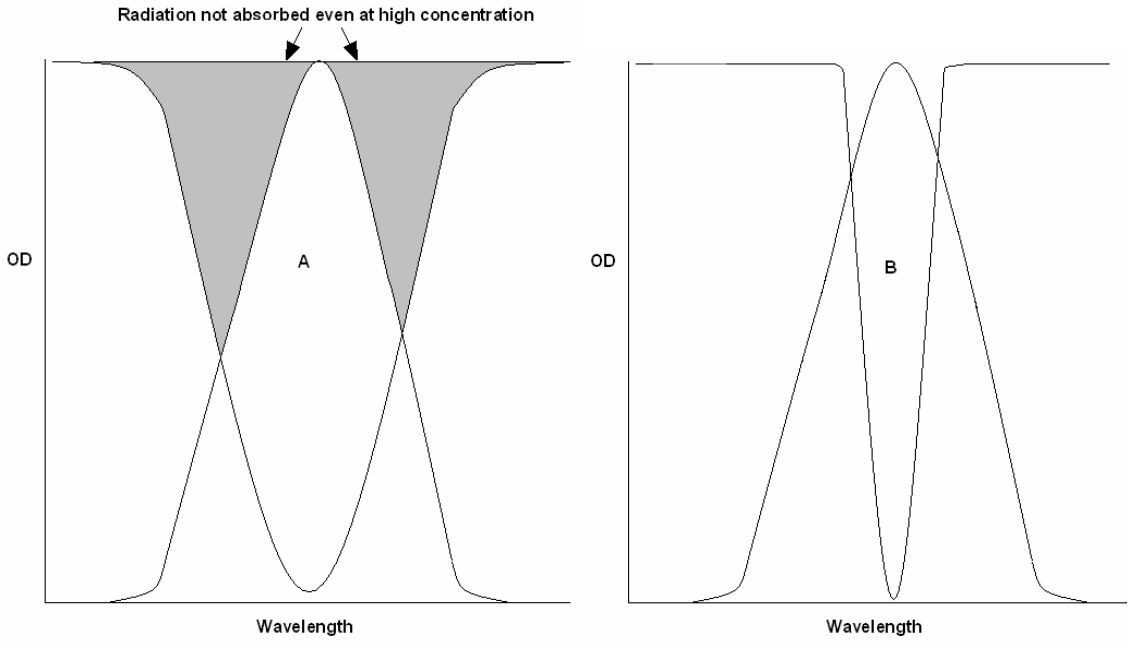


Fig 26

With the wide bandpass or gelatin filter no matter how concentrated the solution some radiation will remain. This will be detected by the photo detector and manifest itself as an increase in %T and therefore leads to a non-linear response

With the narrow band or interference filter there is no excess radiation transmitted by the filter thus its absorbance versus concentration plot will be linear. So if we are to minimise non linearity then the band width of the filter should be narrower than the absorption band width of the solution. Another common situation however is the turbidity in samples causing unexpectedly high results. This is because light is reflected by the suspended particles and is thus physically prevented from reaching the detector. Thus % transmittance is reduced and absorbance increased. The latter is eliminated completely by filtration or centrifugation of standards and samples when turbidity is suspected.

Stray light

Stray light creates exactly the same effect as a wide bandwidth filter when the sample solution has a narrow absorption band. Fundamentally any light falling onto the detector will manifest itself as on increase in transmittance and decrease in absorbance. Stray light can originate from light within the instrument caused by scratches, dust and any optical imperfections, or, from outside the instrument when it is incorrectly sealed.

Wavelength Selection

When measurements are not made at the λ. max then deviations occur from the perfect curve. The major effect is however an extreme loss of sensitivity leading to less accurate results.

Interference Effects

While many colorimetric determinations are simple and straightforward, several types of interference problems can arise.

Figure 27 shows a situation where two absorbing bands are present which cannot be resolved by a colour filter. If only one of the bands changes with concentration of the element being measured, misleading results will occur. The remedy is to select a narrower band filter or an instrument with a monochromator so that the bands can be separated.

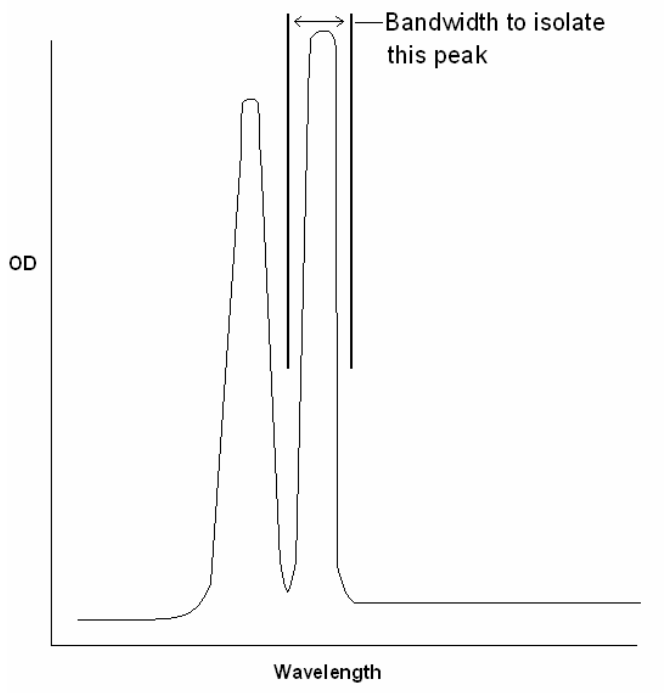


Fig 27

Other Factors

There are many other factors than can cause deviation the Beer Lambert Law including:

1. Chemical dissociation
2. Chemical association
3. Temperature changes
4. Presence of interferents
5. Side reactions
6. Differences in refractive index of tubes
7. Changes in pH

One interesting and common phenomenon is caused by particles in suspension which produce turbidity in the reaction mixture.

If a standard material contains an impurity that is insoluble then standards may have increased turbidity with concentration leading to a positive deviation from the Beer Lambert Law. See fig 24.

Hints and Tips

Colorimetry is a very versatile and wide ranging technique. However many fundamental mistakes are made during analysis which are easily prevented. Many hints are listed below to prevent such mistakes from occurring thereby making the analysis as accurate and painless as possible.

1. Handling of Cells
    a. Never handle the spectral faces of cuvettes or test tubes. Finger marks will cause higher absorbance values and gross inaccuracies. Glass or quartz cells if handled should be cleaned with ethanol or acetone with lens cleaning tissue.
    b. Never clean cells with anything other than lens cleaning tissue or soft tissue. Scratching of cells again causes increases in absorbance values and leads to inaccuracy.
    c. When using cells that are not disposable they should be rinsed thoroughly with deionised or distilled water after washing. If not, water marks will result which render the cells useless for immediate work.
    d. When using disposable cuvettes or tubes (plastic) they MUST BE DISPOSED OF after use. These cells scratch easily and do not therefore retain their original optical characteristics.
    e. Always store cuvettes and tubes in a place where they cannot be damaged. Any slight mark or scratch causes optical imperfection.
2. Wavelength Selection

    Always work at the λ max which should be predetermined before analysis.
3. Check that any samples are contained in cuvettes or tubes which are the same in terms of absorbance values to the containers used for the blank,
4. Always measure a blank first.

5. Ensure solutions are clear and free from contamination if not make fresh solutions or filter out the particulate matter.

6. Prior to analysis always standardise using one or a series of standard solutions.

7. Never expect a sample to read the same absorbance on all instruments - it wont! To gain comparative data standards must be used.

8. Follow analytical method sheets to the letter often the production of colour in samples takes time after a colour producing reagent is added. If the incubation time is not completed then low results occur.

9. When plotting standard curves ensure that the plot passes through the origin when a blank has been performed.

Examples of the Variety of Applications

1. Manganese in Aluminium Alloys
2. Iron in Aluminium and its Alloys
3. Nickel in Iron and Steel
4. Manganese Oxide in Slags and Similar Materials
5. Chromium in Cast Iron and Steel
6. Silicon in Steel
7. Titanium in Steel
8. Organic (KJELDAHL) Nitrogen
9. Nitrogenous Matter
10. Dissolved Oxygen using the “Winkler” technique
11. Anionic Detergents
12. Phosphates
13. Iron using Thioglycollic Acid
14. Chromium using 1,5 Diphenyl Carbazide
15. Phenol or “Tar Acid”
16. Phosphorus in Steel
17. Molybdenum Disulphide in Oils
18. Cobalt in Wax
19. Colloidal Graphite in Oils
20. Vanadium in Fuel Oil
21. Phosphorus in Copper Alloys
22. Analysis of Aluminium Alloys for Copper, Nickel,
23. Iron Manganese and Titanium
24. Titanium in Aluminium Alloy
25. Bismuth in Free Machining Aluminium Alloy
26. Chromium in Aluminium Alloys
27. Arsenic in Copper and Copper Alloys
28. Available Phosphorus in Soils
29. Betaine in Sugar
30. Green Dry Matter in Herbage
31. Boron in Plant Material
32. Boron, Water Soluble, in Soil
33. Carbohydrates,soluble, in Herbage
34. Chlorate in Soil
35. Cobalt in Plant Material
36. Copper in Plant Material
42. Molybdenum, Total, in Soil
43. Phosphorus, Extractable, in soil
44. Urea in Feeding Stuffs
45. Acid Phosphatase (Formaldehyde—Stable) in Plasma
46. Alkaline Phosphotase in Plasma
47. Bilirubin in Plasma Cholesterol in Plasma
48. Creatinine in Blood
49. Creatinine in Urine
50. Sugar in Blood
51. Carboxyhoemoglobin in Blood
52. Inorganic Phosphate in Blood or Plasma
53. Urea in Blood
54. Uric Acid in Blood
55. Uric Acid in Urine
56. Salicylates in Serum, Plasma or Whole Blood
57. Trace Determinations in Foodstuffs
58. Phosphates in Fruit Juices
59. Trace Determinations in Water and Effluents
60. Chloride in Water
61. Iron in Water

37. Iron in Plant Material
38. Manganese in Plant Material
39. Molybdenum in Plant Material
40. Phosphorus in Plant Material
41. Molybbdenum in soil

Application Methods

1. Iron in Water
2. Sugar in Blood
3. Phosphates
4. Phosphorus in Plant Material
5. Chromium in Aluminium Alloys

Measurement of Iron in Water

Equipment

1. Colorimeter
2. Platinum basin
3. Volumetric apparatus

Reagents

1. 0.4m hydrochloric acid
2. Standard stock iron solution (1mg/ml).
3. Working standard iron solutions (0- 4ug/ml of iron). Prepare 0.0, 0.8, 1.6, 2.4, 3.2, and 4.0ug/mL solutions in 0.4m HCl.
4. Phenanthroline solution. Dissolve 0.25g of 1,10 phenanthroline hydrate in 100ml of 25% v/v ethanol.
5. Quinol solution (1%).
6. Tri-sodium citrate solution (8%).
7. Concentrated HCL
8. 1m HCl.

Procedure

Transfer 100ml sample into a platinum basin. Evaporate to dryness. Moisten ash with 5 drops concentrated HCl. Evaporate to dryness on water bath. Add 4ml of 1m HCl. Warm. Transfer solution to 10ml centrifuge tube. Dilute to 10ml. If necessary, centrifuge and retain supernatant solution for iron determination. Carry out blank determination.

Preparation of standard graph

Pipette 5ml of each iron standard into 25ml flask. Add 1ml quinol solution. Mix. Add 3ml phenanthroline solution. Mix. Add 5ml sodium citrate solution. Dilute to 25ml and allow to stand for 4 hours. Measure the absorbance in a 40mm cell at 510nm. Construct a graph of absorbance vs iron concentration.

Sample measurement

Transfer 1ml sample solution into 25 ml flask. Dilute to 5ml with 0.4M HCl. Add quinol, phenanthroline, sodium citrate as for standards. Measure in 40mm cell at 510nm

Calculation of results

Read iron concentration from calibration graph. Allow for any sample dilution factor in calculation.

Sugar in Blood

Equipment

1. Colorimeter
2. 10mm test tubes and holder
3. Volumetric equipment

Reagent preparation

1. Copper sulphate (dissolve 13g in one litre of water).
2. Mixing reagent: Dissolve 50g sodium bicarbonate in 700ml water. Stir in 50g anhydrous sodium bicarbonate. Add solution of sodium potassium tartrate in 100ml water.

   Wash mixture into 1 litre flask. Make up to the mark with water.
3. Copper reagent (mix equal volumes of A and B daily).
4. Acid Reagent: Dissolve 35g molybdic acid + 5g sodium tungstate in 250ml 2M sodium hydroxide. Boil for 30 minutes. Add 100ml water + 125ml 89% phosphoric acid. Make up to 50ml.
5. Mix 320ml 3% sodium sulphate and 30ml 7% copper sulphate solutions
6. Sodium tungstate solution (10g/100ml)
7. Glucose stock standard: Dissolve 100mg anhydrous glucose in 0.3% benzoic acid solution. Make up to 100ml with water.

Calibration procedure

1. Prepare 40, 80, 120, and 160mg glucose 100ml blood standards by diluting 1, 2, 3 and 4ml aliquots of glucose stock standard (soln 7) to 100ml with 0.3% benzoic acid.
2. Pipette 0.5ml of each solution into separate test tubes. Place 0.5ml of distilled water into a fifth tube to act as a blank. Add 0.5ml copper reagent (soln 3) to each tube.
3. Place tubes in boiling water bath for 10 minutes.
4. Cool. Add 7.5ml phosphomolybdic acid reagent (soln 4) and 2.5ml water to each tube.

Sample procedure

Pipette 0.05ml whole blood into 1.85ml isotonic sodium sulphate copper sulphate solution (soln 5) in a test tube. Add 0.1ml sodium tungstate (soln 6). Shake well. Filter. To 0.5ml filtrate add 0.5ml copper reagent (soln 3). Boil for 10 minutes. Cool.

Method

1. Set up colorimeter at 700nm, set instrument to zero with blank solution.
2. Measure absorbance readings for standard solutions.
3. Plot a graph of absorbance versus concentration.
4. Measure sample absorbance.
5. Obtain concentration of sample from calibration graph in mg glucose/100ml blood

Phosphate Determination

The estimation of orthophosphates is carried out by adding acid ammonium molybdates to a dilute phosphate solution. The yellow phopho-molybdate formed is converted to a blue complex by the addition of a reducing agent such as amino-napthol sulphuric acid.

Equipment

1. Colorimeter
2. Volumetric equipment

Reagent Preparation

Ammonium Molybdates, Acid Solution: Add 310ml concentrated sulphuric acid to 400ml water, cool. Add a solution of 50g ammonium molybdate in 200ml water. Make up to 1 litre.

Amino Naphthol Sulphuric Acid Solution: Mix 0.125g of 1, 2,4 amino-naphthol sulphuric acid with 44ml of 15% sodium bisulphite solution in a dark bottle. Add a 20 sodium sulphite solution, dropwise until the solution is clear. Store the stock solution in a dark bottle, well stoppered and make freshly every two weeks. About 5 to 10ml will be required for each determination.

Sample procedure

1. 10ml of molybdate solution is added to 50ml of the sample, or a suitable dilution, containing not more than 40ppm PO_4.
2. Add 4ml amino-naphthol sulphuric acid solution. Make up to 100ml in flask
3. Allow to stand for EXACTLY ten minutes.

Standard procedure

Method

1. The intensity of colour produced is in proportion to the standing time and to the temperature of the reagents.
2. Interference will be given by the presence of chromium, iron and arsenate.
3. Estimation of poly-phosphates may be carried out by converting them to the ortho-phosphates.

Standard Preparation

1. Prepare 10, 20, 30 and 40ppm standard phosphate solutions.
2. To 50ml of standards, add 10ml molybdate solution and 4ml amino-napthol sulphuric acid solution.
3. Make up to 100ml in flask.
4. Allow to stand for exactly ten minutes.

Method

1. Set instrument at 700nm, set zero with blank solution.
2. Measure absorbance of standard solutions and plot graph of absorbance vs. concentration.
3. Measure sample solution. Use calibration graph to calculate unknown concentration of sample.

Phosphorous in Plant Material

Equipment

1. Colorimeter
2. Volumetric equipment

Reagents

1. Ammonium molybdate - ammonium metavanadate reagent.

   Add 25g ammonium molybdate and 1.25g ammonium metavanadate to 300ml water. Warm to dissolve. Cool. Dilute to 500ml. Filter if necessary.

2. 2 5M HCl.

3. Stock phosphorus solution (1mg/ml). Dissolve 0.879g dry potassium dihydrogen orthophosphate in water. Add 1ml concentrated HCl. Dilute to 200ml. Add 1 drop toluene to this solution.

4. Standard solutions (0 - 50 µg/ml Phosphorus). Prepare daily, solutions containing 0, 10, 20, 30, 40 and 50µg/mL.

5. Distilled water.

Method

1. Pipette 10ml each standard solution into 50ml volumetric flask.

2. Add 5ml 5M HCl, 5ml of ammonium reagent. Dilute to 50ml. Stand for 30 minutes.

3. Measure the absorbance in a 10mm cell at 400nm.

4. Construct a graph of absorbance vs. concentration of phosphorus.

5. Transfer 20ml aliquot of sample solution into 50ml volumetric flask.

6. Follow methodology from addition of hydrochloric acid, and ammonium reagent as detailed previously. (Step 2).

7. Again, measure absorbance of final solution at 400nm.

8. Use the calibration graph to calculate the amount of phosphorus in sample solution.

Chromium in Aluminium Alloys

Method

The method is based upon the measurement of the violet colour formed between chromate ion and diphenylcarbazide. The sample is dissolved in aqua regia, oxidised with ammonium persulphate and the manganese etc. precipitated by making alkaline with sodium hydroxide. The chromium is oxidised to chromate with sodium peroxide. An aliquot is then coloured with sulphuric acid and diphenylcarbazide. The colour density varies with the concentration of sulphuric acid, so this reagent addition should be precise.

Equipment

1. Colorimeter
2. Volumetric equipment

Reagents

1. Ammonium Persulphate
2. Sodium Chloride
3. Sodium Peroxide
4. Sodium Hydroxide
5. Sulphuric Acid
6. Diphenylcarbazide

Colour reagent

Dissolve 1gm of reagent (Diphenylcarbazide) in 75ml industrial methylated spirits (IMS). Add 6 drops of concentrated sulphuric acid, cool. Dilute to 100mls with IMS.

Sample procedure

1. Dissolve 0.5g drillings in 15ml nitric acid, hydrochloric acid, water (1:3:1 ratio) and 2.5mL concentrated sulphuric acid.
2. Boil till fumes of sulphur trioxide are liberated.
3. Add 100ml distilled water. Boil to dissolve the sulphates.
4. Oxidise with a few drops of nitric acid. Boil off any nitrous fumes.
5. Dilute to 150ml, cool, Add 15g ammonium persulphate. Boll for 10 minutes.
6. Add 1g sodium chloride. Boil to ensure complete reduction of manganese.
7. Cool and carefully add 25ml of 30% NaOH. Boil for 5 minutes.
8. Add 1g sodium peroxide. Boil for 10 minutes. Cool in running water.
9. Filter, washing with distilled water.
10. Transfer to 200ml volumetric flask. Dilute to the mark.
11. Pipette 20ml aliquot into 100ml flask. Add 8ml of 25% sulphuric acid. Dilute to 80-90ml. Add 4ml diphenyl-carbazide solution.
12. Dilute to 100ml.

Preparation of Standards

Dissolve 2.829g potassium dichromote in 200ml distilled water. Dilute to 1 litre in flask. Pipette 10ml solution into another 1l flask. Dilute to the mark.

Take three aliquots from this second solution and place into individual 100ml flasks, Colour according to the prescribed method. Suggested aliquots are:

2ml = 0.04% Cr

5ml = 0.1%Cr

10ml = 0.2%Cr

Method

1. Set colorimeter to 540nm.
2. Set instrument to zero with blank solution,
3. Measure absorbance of chromium standards.
4. Plot graph of absorbance vs. concentration.
5. Measure absorbance of sample.
6. From calibration graph, calculate concentration of sample, allowing for any dilution factor.

Appendix 1

To derive the relationship between Optical Density and Transmittance

$$OD = \log \frac{100}{\%T}$$

We must look at the intensity of the light falling on the sample and the intensity of the light transmitted by the sample.

If I_t = Intensify of light passing through the sample and

I_0=Intensify of light falling on the sample

then $\frac{I_t}{I_0}$ = fraction of light transmitted by the sample

This is called the Transmission T.

$$T = \frac{I_t}{I_0}$$

The reciprocal of Transmission is called opacity

$$\frac{1}{T} = \frac{I_0}{I_t} = \text{Opacity}$$

By definition $\text{Log} \frac{I_0}{I_t}$ = Opacity

It

Now if we assign a value of 100 to I_0

i.e. $I_0 = 100$

Then I_t = Percentage Transmission

=%T

By substituting these values in the derived relationships we get

$$\text{Optical Density} = \log \frac{100}{\%T}$$

Appendix 2

Summary of Terms

Optical Density Also called extinction (E) or absorbance (A). Defined as the logarithm of the ratio of the intensity of incident light to transmitted light.

$$OD = \log \frac{100}{\%T}$$

Transmission (T) Also called transmittance. Defined as the ratio of the intensity of transmitted light to incident light

$$T = \frac{I_t}{I_0}$$

Percentage Transmission (%T) Defined as the percentage of the incident light that is transmitted by the sample

Opacity Defined as the reciprocal of the transmission

$$\text{Opacity} = \frac{I}{T}$$