

An introduction to the use of visible and ultraviolet light for analytical measurements

5

Skills and concepts

This chapter will help you to understand:

- The wave-like properties of electromagnetic radiation.
- The wavelength ranges across the electromagnetic spectrum (such as the ultraviolet (UV) and visible region of the spectrum).
- The quantized nature of UV and visible light and how this causes absorption effects.
- How to use the Beer–Lambert law for the calculation of analyte concentrations within unknown samples.
- The electronic transitional basis for molecular fluorescence effects and how fluorescence may be exploited for analytical purposes.
- Why absorption is a unit-less quantity.
- Why measured absorptions should only be trusted in the range of 0–2.
- The operation of tungsten filament, hydrogen/deuterium lamps and the advantages each offer as light sources for UV–visible spectroscopy.
- The operation of: photo-tube, photo-multiplier, silicon photo-diode, and photo-voltaic cell based detectors and the relative advantages and disadvantages of each for use in UV–visible spectroscopy.
- The operation of single- and double-beam UV–visible spectrophotometers and their associated advantages and disadvantages.
- The wavelength ranges over which plastic, glass, and quartz cuvettes may be used.
- How to perform a number of practical colour-based complexation reactions for practical analyses.
- What is meant by an organic chromophore.
- What is meant by hypsochromic and bathochromic shifts and how these may be used to facilitate UV–visible determinations.

- How to relate intensities of fluorescent radiation to fluorescent analyte concentrations.
- How compounds act as fluorescent quenching agents.
- What is meant by optical activity and how this can be measured via polarimetry to determine the concentration of optically active compounds.
- How to relate the specific rotation of a compound and the measured optical rotation to the concentration of an optically active compound within an unknown sample.

5.1 An introduction to the use of visible and UV light and the electromagnetic spectrum

The interaction of light with different compounds offers many possibilities for performing both qualitative and quantitative measurements. Many chemical reactions generate vivid colours, which, as well as being fascinating, often provide sufficient information to perform an analysis. Colour changes may, however, be subtle or indeed difficult to distinguish due to the limitations our eyes have, which is especially true if the results are to be compared from one day to another. In these and many other similar situations, an instrument may be used to study the way in which light interacts with a sample.

Visible light forms part of the electromagnetic spectrum (Fig. 5.1) with γ -rays at one end of the spectrum having wavelengths of the order of about 10^{-14} m, and radio-waves at the other end having wavelengths of 3×10^3 m or greater. Our eyes detect or 'see' radiation in a fairly narrow part of the spectrum that spans wavelength from about 400 to 750 nm. Radiation falling within this region of the electromagnetic spectrum is therefore classified as the 'visible' part of the spectrum. Many instruments designed to measure and quantify the interaction of visible radiation with matter have the capability, however, for operating at wavelengths that extend into the UV range of the electromagnetic spectrum, which covers wavelengths from around 400 down to 180 nm or so. This region is known as the 'ultraviolet' region since it is beyond the range of our vision

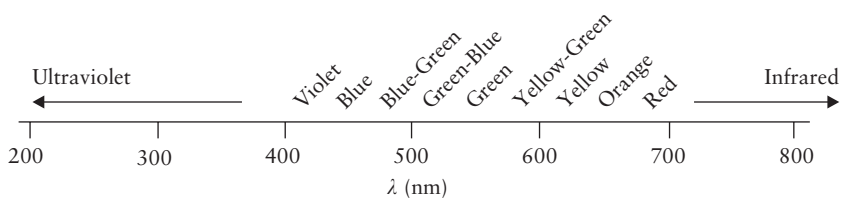


Figure 5.1 UV and visible region of the electromagnetic spectrum.

and extends from the violet region of the spectrum at the boundary of our vision.

The interaction of light with matter and the exploitation of colour is truly an everyday event that we often take for granted. We *perceive* each part of the visible electromagnetic spectrum as light of a different colour; these colours include red, orange, yellow, green, blue, indigo, and violet. The interaction of light with matter gives us the perception that objects are ‘coloured’. Colour plays a very important role in many aspects of our lives and, for example, we use coloured traffic lights to help with traffic safety, watch colour television sets, decorate our houses, and choose clothes based on their colours—to cite but a very few cases.

We often use light in everyday life to determine the *quality* of a number of goods such as food. We might, by experience, expect vegetables such as runner beans, to display a pleasant and reasonably homogeneous green coloration, which corresponds to a certain level of freshness. By the same argument, we are likely to avoid those which show signs of decay and rotting which we detect or ‘see’ as having unpleasant dark brown patches. We also know that drinking water should be colourless if held up to the light and that the water might not be good to drink if we can see suspended or dissolved materials that absorb light thereby discolouring the water. Both of these tests are qualitative in nature.

We also perform some rather rudimentary qualitative (or at least semi-quantitative) tests by eye. If we add milk to a cup of tea or coffee, we judge the correct amount to add based on the colour of the drink; we are using colour to estimate concentration. The judgement is obviously rather rudimentary, but the principle is clear. The use of instrumentation to perform far more accurate estimates of the intensity of the colour of a sample forms the basis of a *colorimetric measurement*.

Before we consider the details of any spectroscopic analysis, we should consider the reason why some compounds appear coloured. Our eyes detect the light that impacts on a retina. If green light reaches the eye, the eye perceives the colour green. If the eye perceives the colour red, then it is responding to red light entering the iris of the eye and striking the retina. The light may come from a light emitting source, be reflected from the surface of some object, or reach the eye having been transmitted through a transparent object that absorbs all other wavelengths than those that correspond to ‘red’ wavelengths.

When light impacts upon a material it may be reflected, be absorbed, or simply pass through unaffected in which case it is said to be *transmitted*. Many light sources are said to be ‘white’—that is, they emit radiation across the entire visible region of the electromagnetic spectrum. It is quite common for some wavelengths of light to be absorbed by a material, while others pass through unaffected. Our eyes detect and perceive the colour of the light which *is not* absorbed and, by default, is therefore

transmitted. If light is reflected from the surface of a material, the same argument applies. Some of the light striking the object will be absorbed. Our eyes, however, detect and therefore perceive the light that is reflected—that is, the component that is not absorbed.

If we perceive a fruit cordial drink to be orange in colour this is because the drink absorbs all of the visible electromagnetic spectrum except for the orange light (~600–650 nm), which is transmitted unaltered through the drink. Similarly, if we see an object such as a solid ball as orange, this is because most of the visible light striking the surface is absorbed, except for the orange light that is reflected. It should be remembered that a totally reflective surface is a mirror and conversely a totally absorbing surface appears matt black.

To conclude, the basis of absorption, transmission, and/or reflection of light is due to interactions of radiation with the molecules that go to make up a material and we consider these in greater detail throughout this chapter.

5.2 The quantization of light and electronic energy levels

In order to understand how light interacts with matter, we must first consider the atomic, and in particular, the electronic structure of molecules. Each atom consists of a positively charged nucleus that is surrounded by a series of electrons. Electrons travel around the nucleus in areas of space known as orbitals and possess differing energies. The choice of orbitals in which the electrons reside depend on their energetic states. The energetic states of orbitals and therefore of the electrons that reside in them correspond to a series of separate and well-defined energy levels and are said to be *quantized*. It therefore follows that the promotion of an electron from one energy level to another must correspond to a quantized energy change.

The promotion of a valence electron from one orbital to another involves the absorption of radiation, which is normally in the UV–visible range of the electromagnetic spectrum. Electrons are said to be promoted from a *ground state* to an *excited state*.

In some circumstances, light may be primarily considered to behave as a wave while in others it appears to possess *particle-like behaviour*. In one model, light may be considered to consist of a series of discrete ‘packets’ of energy or *photons*. The energetic state of each photon is quantized and is proportional to the frequency of the radiation, ν . The energy of the photon may be quantified from the product of the Planck constant, h , and the frequency, ν , Eqn (5.1):

$$E = h\nu \quad (5.1)$$

Since the frequency of the radiation is given by

$$\nu = \frac{c}{\lambda} \quad (5.2)$$

it follows that the energy of a photon may also be given by

$$E = \frac{hc}{\lambda} \quad (5.3)$$

If visible or UV light is to cause the promotion of a valence electron from the ground to an excited state, the energy of a photon must correspond exactly to the energy gap associated with the electronic transition.

Photons that are insufficiently energetic or indeed too energetic will not cause the electronic transition.

5.3 Absorption: when photons give up their energy

Photons of UV and visible light may sometimes impart their energy to materials by interaction with individual atoms or molecules. Energy is imparted to the atoms or molecules, causing the excitation of valence electrons. Molecules with excited electronic states represent an unstable state and will relax by allowing their electrons to fall to the ground state as soon as possible (ca. 10^{-16} s). The energy that is lost in a transition of this type is normally dissipated as heat. It follows that objects that are irradiated with light will often adsorb radiation and so be heated and we all know, for example, that objects placed in sunlight are warmed. There are circumstances, however, when some of the energy may be dissipated by heat while some is lost by the emission of a photon (light) of a lower wavelength. In this situation, the material emits light of a wavelength having been irradiated by light of another wavelength. This effect is known as *fluorescence* and we shall look at this phenomenon in Section 5.13.

5.4 Absorption: how much radiation is absorbed?

Let us consider a simple transparent cell through which an incident beam of light of intensity, I_0 is passed; Fig. 5.2. Some of the light is absorbed and therefore a transmitted beam of light of a lower intensity, I , emerges from the cell. Light is a form of energy and therefore the intensity of a light beam is a measurement of power (energy per unit of time) and therefore

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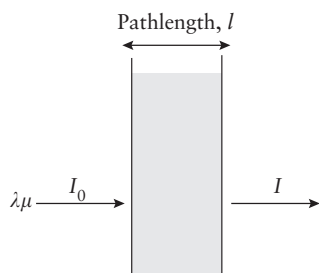


Figure 5.2 Absorption of light by an analyte sample within a cuvette.

has the units of J s^{-1} or watts (W). The absorbance, A , is defined as the \log_{10} of the ratio of the incident to transmitted light beams, Eqn (5.4):

$$A = \log_{10}\left(\frac{I_0}{I}\right) \quad (5.4)$$

Since I_0 and I are both measured in units of power (W), their units cancel each other out and so **absorption is a unit-less quantity**. The implications of absorption being defined from a logarithmic expression are worth noting.

If I and I_0 are equal, then it follows that the intensity of the emergent beam is equal to the incident beam and that I_0/I equals 1. The \log_{10} of 1 is 0, which corresponds to an absorbance of zero. Now, an absorbance of 1 means that (I_0/I) equals 10 (i.e. 10/1) and that 90% of the light is being absorbed. Similarly, an absorbance of 2 means that I_0/I equals 100; in this case 99% of the incident light is absorbed and only 1% is transmitted. It is therefore normally only feasible with most instrumentation to *measure* absorbance on a scale of 0 though to 2, since absorbances of greater than 2 correspond to an immeasurably small fraction of the incident radiation being transmitted.

Absorbance is measured by comparing the intensity of an incident and transmitted light beam through a sample. The intensity of the light beams may be detected by devices such as a photo-multiplier tubes. The ratio of the incident and transmitted light beam intensities may then be used to calculate the absorbance of the sample.

5.5 The Beer–Lambert (or absorption) law

The collision of a photon of suitable energy with the appropriate molecule results in absorption of light. It follows that if a greater number of molecules are placed in the path of the light beam, then there will be a greater chance of a collision occurring and therefore of absorption. Our everyday experience knows this to be true. Think of a glass of blackcurrant cordial that we look through; the drink appears purple in colour. We know that certain wavelengths are absorbed while others are transmitted and pass through easily and it is this that gives the drink its characteristic colour. Now if we hold another similar glass of the drink behind the first, the colour appears twice as intense. We know that less light is able to pass through the two glasses than the one alone. It is clear that the probability of a collision between a photon and a light absorbing molecule has increased. By the same argument, we know that if we dilute the drink with

water, its colour intensity will decrease and this is because we have decreased the concentration of the molecular absorbing species. In this situation, we have decreased the probability of a photon colliding with a light absorbing molecule.

Let us imagine a simple transparent cell or *cuvette* (Fig. 5.2) containing a solution that absorbs radiation of a particular wavelength.

From the previous discussion it follows that the absorption will be proportional to the concentration of the absorbing molecular species and also to the path length through which the light has to travel.

The absorption or **Beer–Lambert** law relates the absorption of most molecular species to the concentration, c_n , the path length, l , and the molar absorptivity, ε , Eqn (5.5):

$$A = \varepsilon c_n l \quad (5.5)$$

ε is sometimes known as the *extinction coefficient*. The Beer–Lambert law is sometimes expressed in terms of transmittance, T , where $T = 1/A$. Unfortunately, there are differing ways of defining the units of ε and l . ε is most commonly described in terms of $\text{mol dm}^{-3} \text{cm}^{-1}$ in which case, l , the path length must be quoted in terms of cm. Some reference books, however, will quote ε values in terms of $\text{mol dm}^{-3} \text{m}^{-1}$ in which case the path length, l , must also be quoted in terms of, m . A path length of 1 cm is normally chosen to simplify the calculation of the absorbance or molar absorptivity. The Beer–Lambert law is one of the most widely used relationships within analytical chemistry and is at the heart of the majority of UV and visible quantitative analyses.

The Beer–Lambert law holds for the majority of compounds over a wide range of experimental conditions. It should be noted, however, that the absorption of light is highly wavelength specific. We have already seen that radiation of a specific wavelength must be supplied if an electronic excitation is to occur. It should be remembered that this corresponds to the absorption of light.

Molar absorptivities are therefore quoted for individual compounds at a specific wavelength and pathlength.

The majority of compounds will only absorb radiation at specific wavelengths and it is this that gives rise to their colour. If the compound is coloured then there will be at least one and even a number of differing wavelengths which show maximal absorbances.

A plot of *absorbance* versus *wavelength* is known as a **UV–visible spectrum** and may be measured by a **UV–visible spectrophotometer**. Most UV–visible spectrophotometers will allow the scanning of a wavelength range. The UV-visible spectrum of a potassium permanganate (KMnO_4)

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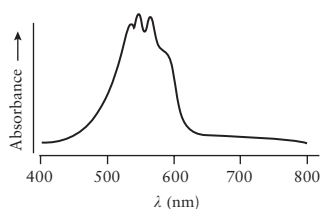


Figure 5.3 Absorbance spectra for KMnO_4 in water.

solution is shown in Fig. 5.3. KMnO_4 absorbs strongly across a wide range of wavelengths but has little absorption in the red and blue regions of the visible spectrum. Light corresponding to these colours passes through the solution with relative ease and is thus transmitted. The mixing of blue and red transmitted light is seen as purple, which is how our eyes perceive the solution to be coloured. The wavelength of maximum absorption is known as the λ_{max} and is usually used as the wavelength which is quoted within data values for the molar absorptivity, ϵ (Section 5.4).

Since the absorption of light is so wavelength specific, it is crucial that the wavelength of as well characterized and indeed as narrow a wavelength range as possible is used to irradiate the sample under study.

5.6 The nature and use of UV and visible absorptions—chromophores

The UV–visible spectrum of a compound may often be used to identify its presence within a sample. Many compounds and especially those which are highly coloured, absorb radiation over characteristic and often comparatively narrow wavelength ranges. Differing molecular groupings give rise to the absorption of light at characteristic wavelengths and are known as *chromophores*. Chromophores are functional molecular groups that cause compounds to be coloured—that is, to absorb radiation at particular wavelengths.

Chromophores are moieties that possess electrons which may be readily promoted by the absorption of UV or visible light. Many highly coloured compounds contain either a *transition metal ion* or a number of *unsaturated* carbon–carbon bonds. Very small changes in structure may often, however, give rise to extremely different absorption phenomena. It is changes of this type which often form the basis of many pH sensitive indicators such as phenolphthalein (Fig. 5.4). Within environments of ($\text{pH} > 8.1$), phenolphthalein resides in a de-protonated (quinoid) structure that absorbs light across a broad band of the visible spectrum; in this state the indicator appears red. If the pH falls below ~ 8.1 , the structure becomes protonated by conversion of the two carbonyl ($\text{C}=\text{O}$) groups to hydroxyl groups ($\text{C}-\text{OH}$) and this corresponds to a colourless state. The hydroxyl moieties are more electron withdrawing towards the aromatic carbon ring than the electron rich $\text{C}=\text{O}$ bond. The subtle change in the structure of the chromophores causes the absorption band to shift to shorter wavelengths.

Moieties are functional groups within molecules that impart certain features—in the case of chromophores these cause absorption in the UV–visible range and so impart colour.

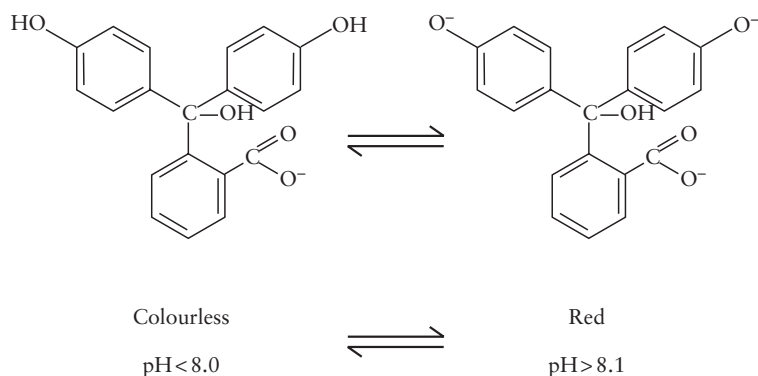


Figure 5.4 Structural and colour-change properties of phenolphthalein with pH.

5.7 Light sources and monochromators

5.7.1 Light sources

The irradiation of a sample for UV or visible spectroscopy requires a light source with a constant output intensity. The light source should also be sufficiently intense as to allow sufficient transmitted radiation to be detected when the absorption falls within a range of 0–2. The sample should at any one time be irradiated with as *monochromatic* a source of radiation as possible (i.e. as narrow a wavelength range as possible). A monochromator is the most commonly used device to select a wavelength of light for the irradiation of a sample.

The radiant power of many light sources increases essentially exponentially with the applied voltage and, consequently, even very small fluctuations in the applied electrical supply voltage may cause significant variations in the intensity of the incident radiation and it is therefore common practice to employ voltage regulators in the light source power line.

Tungsten filament lamps

The majority of UV and visible spectrophotometers employ tungsten filament lamps, Fig. 5.5(a), to supply radiation in the wavelength range of around 320–2500 nm, which covers most of the visible part of the spectrum. The energy output characteristics with respect to wavelength are shown in Fig. 5.5(b). It can be seen that the power output of lamps such as these dramatically decreases as the UV region is approached. The lower limit is in fact primarily due to the absorption of the glass casing of the lamp. The normal operating temperature of tungsten filament lamps may be in the range of 2900–3000 K.

Tungsten–halogen lamps contain small quantities of iodine, and the lamp is enclosed within quartz (as opposed to glass) housings. The halogen gas allows the temperature of the lamp to be raised to about 3500 K

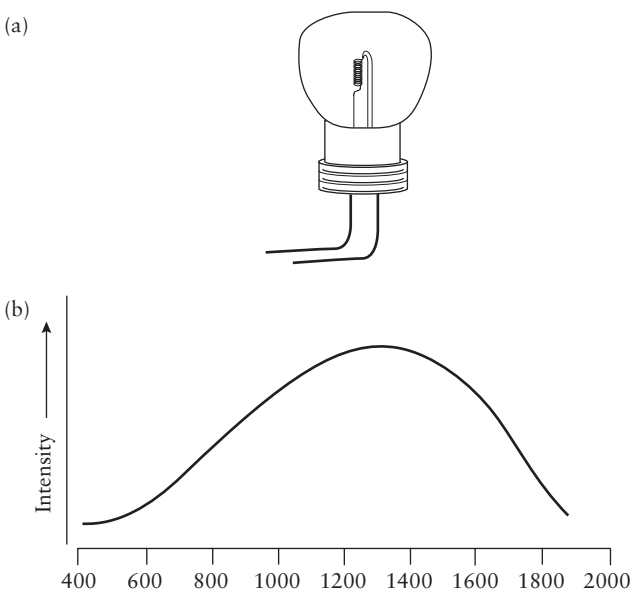


Figure 5.5 (a) Tungsten filament lamp and (b) its emission spectrum output.

and this permits the intensity of the output radiation to be increased—as well extending the output down to ~ 190 nm, which extends well into the UV region. The quartz envelope in turn allows the transmission of the UV radiation (glass, in contrast, would absorb and therefore block UV light). Despite the higher operating temperatures, tungsten–halogen lamps typically have operating lifetimes that are double those of standard tungsten filament lamps. The lifetime of both of these lamps is limited due to the sublimation of the tungsten, W, from the filament. If trace amounts of iodine are introduced then sublimed tungsten molecules react with iodine to give WI_2 molecules which diffuse back to the hot filament, where they decompose to re-deposit metallic tungsten once more. Tungsten–halogen lamps are more expensive to manufacture, although their greater longevity and performance normally more than justify their cost.

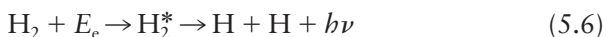
It is extremely important that tissue paper or gloves are used to handle tungsten filament or tungsten–halogen lamps, since even tiny traces of grease from skin may cause tiny fractures of the glass or quartz casings at the extreme operating temperatures, reached and this shortens the lifetime of the lamp.

Hydrogen and deuterium lamps

Many instruments employ a tungsten filament lamp for the visible and fringe UV–visible ranges in conjunction with a hydrogen or deuterium lamp to produce high-intensity UV radiation.

The electrical excitation of hydrogen or deuterium at low pressure produces a continuous spectrum of UV radiation. Hydrogen (or deuterium)

gas may be excited by electrical energy to produce two hydrogen atoms with the release of a photon of energy, Eqn (5.6):



The total excitation energy input, E_e , must be distributed between the two hydrogen atoms and the photon. The energy distribution between the two atoms is random. If two hydrogen atoms of low energy are produced, it follows that the photon will be highly energetic. Conversely, if the hydrogen (or deuterium) atoms are highly energetic the photon of light emitted will be of a correspondingly lower energy value. The result is that deuterium or hydrogen lamps both give uniquely uniform outputs in intensity of radiation over a wavelength range of 160–375 nm.

At longer wavelengths, the lamps may produce emission lines that are superimposed on the otherwise stable output. Many spectrophotometers employ a tungsten or tungsten–halogen lamp for supplying radiation of wavelengths above 360 nm and a hydrogen or deuterium lamp for wavelengths below this value.

5.7.2 Monochromators

All of the light sources used within UV–visible spectrophotometers simultaneously produce a spectrum of radiation across a wide range of wavelengths. Photo-multiplier tubes and other radiation detectors are indiscriminatory in detecting radiation of different wavelengths. It therefore follows that if a spectrum is to be measured then some method must be employed to select as narrow a wavelength range as possible to irradiate the sample with. It is important to note that it is impossible to truly select one wavelength alone and that is why we say we will select as narrow a wavelength range as possible. The wavelength of the radiation source will tend to follow a Gaussian distribution around a mean value of wavelength known as the *nominal wavelength*. The *effective bandwidth* is defined as the wavelength range which corresponds to the half peak height width of the wavelength distribution profile; Fig. 5.6.

There are many different designs for the construction of monochromators: low specification instruments sometimes use *optical filters* to select wavelengths with a bandwidth of around 30 nm or so. It is far more common, however, to employ a *monochromator* for the wavelength selection.

Monochromators use a series of lenses, mirrors, slits, windows together with either prisms and/or diffraction gratings to isolate a narrow band of wavelengths. There are many designs for monochromators. However, we shall only consider in detail here two of the most popular designs that are based on *diffracting prisms* and *reflection gratings*.

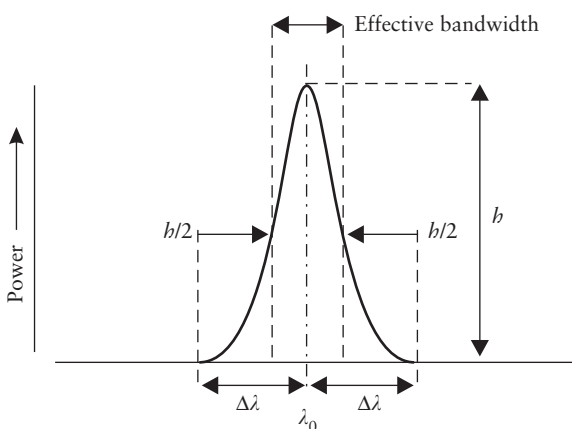


Figure 5.6 Nominal wavelength λ_0 and the effective bandwidth.

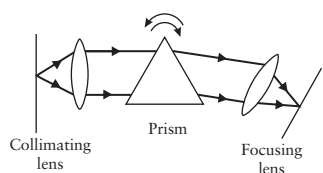


Figure 5.7 Simplified schematic of a prism monochromator.

Monochromators based on diffraction prisms

Monochromators of all types tend to be encased in discrete housings within the spectrophotometer to prevent the ingress of dust and other contaminants. In diffraction prism monochromators, the ‘white’ light enters the monochromator via an *entrance slit* before being collimated by a *collimating lens*; Fig. 5.7. The light then passes through a *diffracting prism* that disperses the light into its component wavelengths. The light is then focused by another lens towards an exit slit, which is situated at the focal plane. The prism is rotated by means of a stage and a stepper motor to select radiation with different frequencies to pass through the exit slit.

Monochromators based on reflection grating

While the light path through a reflection prism based monochromator might initially appear to be rather different to that of a diffraction grating, the principle is fairly similar.

White light passes through an entrance slit and is focussed towards a reflection grating via a concave mirror; Fig. 5.8. The reflection grating disperses the light into its component wavelengths and reflects the light onto a second concave mirror. The grating is mounted on a stage which may be rotated via a stepper motor. Light may then be reflected and focussed by this concave mirror towards the exit slit. As the grating is rotated different wavelengths can be selected.

5.8 The detection of light—photon detectors

If the absorption of an analyte is to be determined, the intensity of the transmitted light must be monitored and there are several ways in which this can be achieved. The intensity of electromagnetic radiation may be

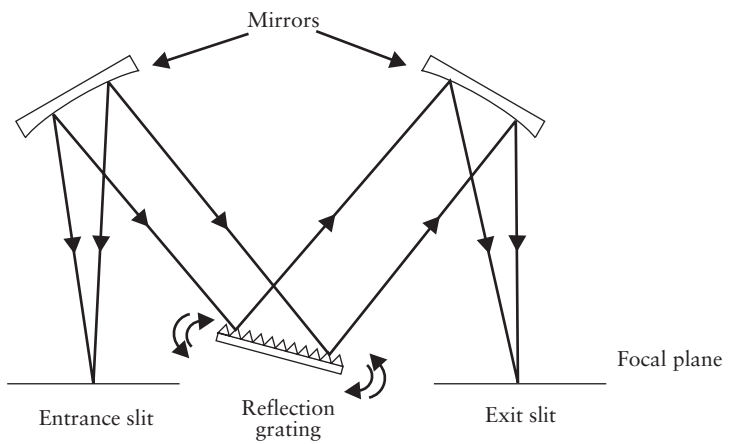


Figure 5.8 Simplified schematic of grating monochromator.

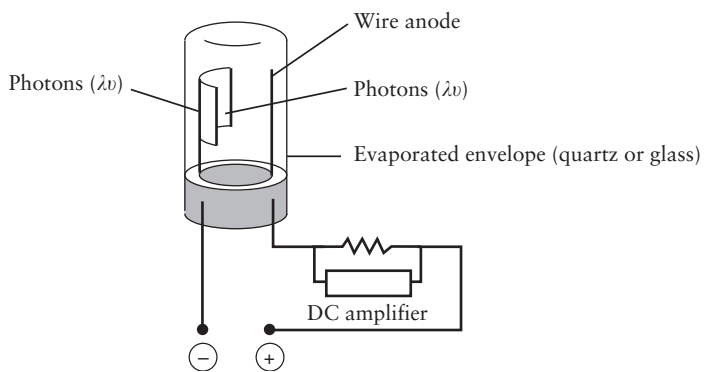


Figure 5.9 Photo-tube and monitoring circuit.

measured by: (a) the photo-emission of electrons; (b) the electronic excitation of valance electrons following the absorption of electromagnetic radiation; or (c) the measurement of the heat imparted to a material as a result of the absorption of electromagnetic radiation.

Within the UV–visible range the most popular approaches are based on photo-emission and/or electronic transition principles, with both of these techniques essentially counting photons and hence monitoring the intensity of the light. Four types of detector based on these approaches are commonly employed within UV–visible instrumentation namely; photo-tubes, photo-multiplier tubes, silicon photo-diodes, and photo-voltaic cells, each of which are described in the following sections.

5.8.1 Photo-tubes

A photo-tube consists of an evacuated tube with a quartz window, behind which is placed a large cathode; Fig. 5.9. The cathode is coated with a layer of photo-emission material such as a metal oxide or alkali metal.

A smaller wire anode is situated in front of a cathode and a polarizing potential of 90 V placed across them. Photons enter the tube via the quartz window and strike the anode. The photo-current that is collected in this way may then be related to the intensity of the incoming light.

5.8.2 Photo-multiplier tubes

Photo-multiplier tubes operate in essentially the same way as photo-tubes, but produce a cascade of photo-emitted electrons via a series of accelerating and electron emitting electrodes. Photons again enter an evacuated tube by means of a quartz window; Fig. 5.10. The photons strike the cathode, which again causes the emission of electrons, which in this case are accelerated towards the first of a series of *dynodes* that are polarized at +90 V relative to the cathode. The electrons strike the dynode, which causes a series of further electrons to be emitted, which are then accelerated towards another dynode that is polarized at +90 V relative to the first dynode. The process continues till the electron cascade is finally collected at the collecting electrode. This process, known as the *cascade effect*, causes some 10^6 – 10^7 electrons to be collected for every photon entering the tube.

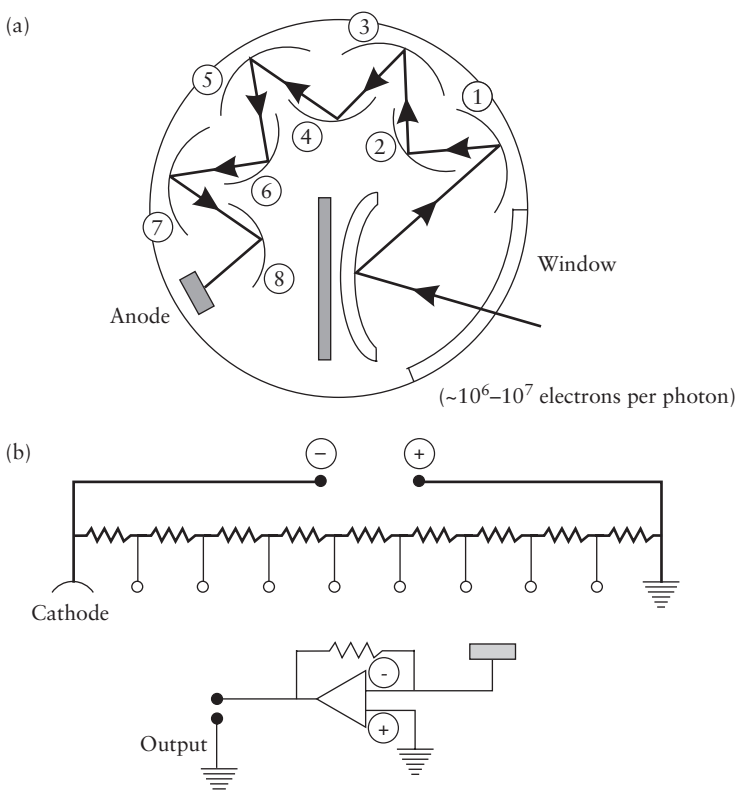


Figure 5.10 (a) Photo-multiplier and (b) associated circuitry.

5.8.3 Silicon photo-diodes

Photo-diodes comprise specially constructed silicon in which the conductivity may be modulated by UV or visible light illumination. Silicon, a Group IV element is a semiconductor, and as such, its conductivity is less than that of a metal but greater than that of an insulator. Each silicon atom is covalently linked within a covalent lattice super-structure to four of its neighbours. Thermal agitation at room temperature allows an occasional electron to leave a silicon atom to move within the lattice. The unoccupied position is known as a *hole*, which effectively represents a positive charge. Conduction occurs by the movement of electrons and holes in opposite directions. The conductivity may be greatly increased by the addition of trace amounts of either Group III or Group V elements. The addition of Group III elements produces a so-called p-type semiconductor that is rich in holes. The addition of a Group V element produces an n-type semiconductor rich in electrons.

If a piece of n-type silicon is joined to a piece of p-type silicon, a so called **p–n junction** diode is formed. These p–n junctions conduct electricity if polarized in one direction, (called the *forward bias*) but block the passage of current if they are *polarized with a negative bias*. The forward biasing of a p–n junction involves the *n* region being negatively polarized and the *p* region being positively polarized. An excess of electrons are made available to the n-type semiconductor. Similarly, electrons are drawn away from the p-type semiconductor, which creates more holes in this region. Holes and electrons neutralize each other in the vicinity of the p–n junction and conduction is permitted as more electrons and holes are effectively made available. By contrast, if the p–n junction diode is reverse biased, then both the holes and electrons move away from the p–n junction region to form a so called *depletion layer*, which now becomes non-conductive; in this arrangement the p–n diode impedes the passage of current.

Silicon photo-diodes are specially adapted p–n junction diodes with optically transparent windows to allow the illumination of the p–n junction region by UV or visible light. Photons that pass through the window and are absorbed in the vicinity of the p–n junction may, if sufficiently energetic, cause the excitation of electrons to form holes and free electrons; Fig. 5.11. The generation of electrons and holes in the depletion layer causes a significant increase in the conductivity of the diode, which is used to measure the intensity of the incident radiation.

5.8.4 Photo-voltaic cells

Photo-voltaic cells are the simplest but least sensitive type of cell used for the detection of visible light. They are insensitive to—and therefore cannot be

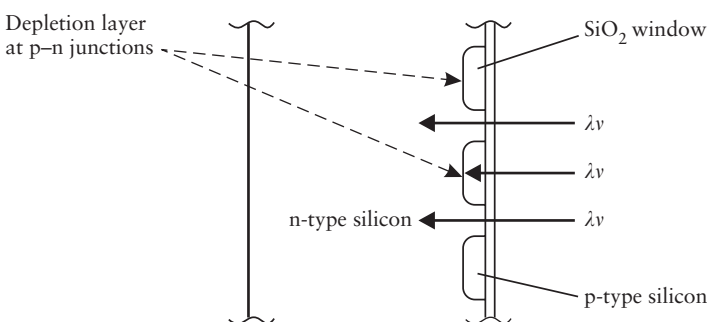


Figure 5.11 Schematic of silicon photo-diode array.

used for the detection of—UV light. Photo-voltaic cells also suffer from fatigue, that is, their response fades with constant illumination. Despite these disadvantages, photo-voltaic cells are used in simpler instrumentation due to their simplicity and the lack of need for an external power supply.

Most photo-voltaic cells consist of a copper or iron electrode coated with a semiconducting material such as copper(I) oxide or selenium, which, in turn, is coated with a film of gold, silver, or lead sufficiently thin so as to be optically transparent. This metallic film is polarized with respect to the copper or iron electrode acts both as an optical window and as a second electrode. Light that reaches the semiconductor causes the formation of electrons and holes which migrate away from each other and towards the two electrodes. If the electrodes are connected to a low resistance circuit, the current that flows may be related to the intensity of the incident light.

5.9 Spectrometers, spectrophotometers, and UV-visible cells

There is often some confusion as to the difference between a spectrometer and a spectrophotometer.

A *spectrometer* is a monochromator equipped with a fixed slit at the focal plane. A spectrometer equipped with a phototransducer is known as a *spectrophotometer*. In turn, a spectrophotometer that is capable of measuring the absorbance across a range of wavelengths by linearly varying the incident wavelength is known as a *scanning spectrophotometer*.

5.9.1 Single-beam spectrophotometers

As the name suggests, *single-beam spectrophotometers* use a single light beam in order to irradiate the cell in an arrangement as depicted in Fig. 5.12. The light enters the sample and a phototransducer monitors the

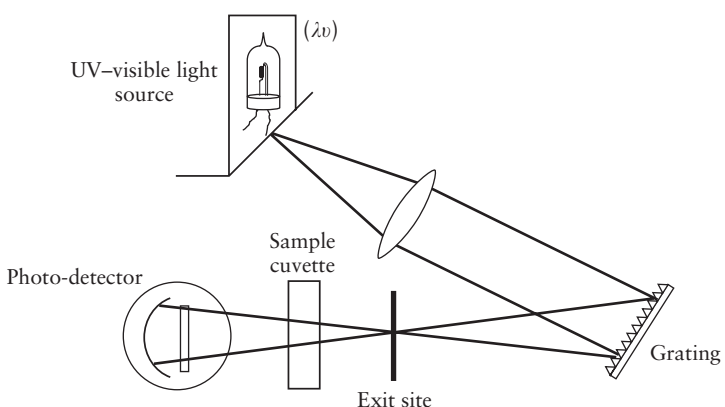


Figure 5.12 Schematic of a single-beam spectrophotometer.

transmitted radiation as it emerges from the sample. There is a problem, however, with this simple arrangement that must be addressed. The cell and the solvent in which the sample is dissolved will both absorb radiation at every wavelength to a finite extent. We wish to record the absorbance spectrum of the analyte and not the spectrum superimposed upon a background. Instruments of this type necessitate the measuring of a baseline spectrum. The baseline spectrum is normally recorded by placing a cell filled with the appropriate solvent (minus analyte) into the spectrophotometer and recording a *baseline spectrum*. The baseline spectrum is then subtracted from all subsequent spectrums for samples containing the analyte; the spectrum that is produced now corresponds to the absorption of the analyte alone and is sometimes known as a *normalized spectrum*. Most modern spectrophotometers, however, now store baselines within the memory of a computer and perform this function electronically.

5.9.2 Double-beam spectrophotometers

Double-beam spectrophotometers, as shown schematically in Fig. 5.13, employ two light beams of equal intensity together with two photo-multipliers to record and subtract the baselines from spectra. One light beam irradiates the cell containing the analyte. The other irradiates a cuvette containing the appropriate solvent only. In essence, two spectra are recorded simultaneously as the wavelength of light is scanned through the desired range. The baseline spectrum is then subtracted from the spectrum corresponding to the analyte sample and a normalized UV-visible spectrum is obtained.

Several assumptions must be made if we use this approach. First, the two light beams must be of exactly the same intensity. Second, the two cells must possess exactly the same absorptivity, and for this reason, should be cells of the same make and type. Third, the solvent must be

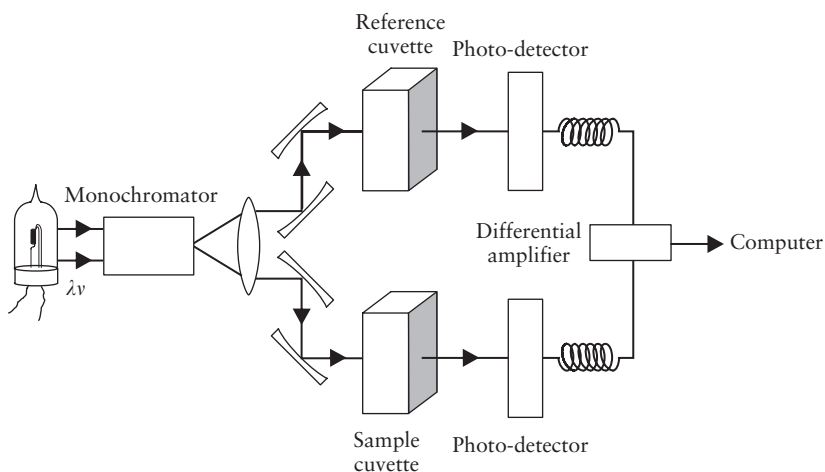


Figure 5.13 Schematic of a double-beam spectrophotometer.

exactly the same in each cuvette. Finally, it must be assumed that the photo-transducers exhibit exactly the same sensitivities.

5.9.3 The use of UV–visible cells and cuvettes

An optically transparent cell with a volume of a few cubic centimetres (known as a *cuvette*) is used to contain the analyte samples. The cuvette is normally designed with an internal cross-sectional area of 1×1 cm to give an optical path length of 1 cm, although cells of differing dimensions may be purchased for specialized applications. The cuvette normally has a height of several centimetres to allow easy insertion and removal from the spectrophotometer.

The cell is normally provided with two facing optically transparent faces and two frosted optically opaque faces. The opaque faces are designed to provide surfaces that may be handled without placing fingerprints on the faces through which the optical beam must pass.

Fingerprints, grease, or other trace contaminants may have a profound effect on the spectrum obtained, so it is imperative to keep the optically transparent faces of the cuvette as clean as possible. For this reason, cuvettes should always be polished with a piece of lens tissue every time they are handled.

For similar reasons, it is most important that if a spectrum is taken using a double-beam spectrophotometer, that matched optical cells are used. The optical properties of matched cells may be verified by running a baseline spectrum with the two cells in the path of each beam. If the cells are well matched and contain only solvent, a blank spectrum should be obtained, that is, a constant absorbance $A = 0$.

Cuvettes are typically made of one of one three main classes of material and these are:

1. optically transparent plastics;
2. optical grade glass;
3. fused silica or quartz cells.

Optically transparent plastics

For many purposes, an optically transparent plastic cuvette suffices for the measurement of a visible spectrum within the wavelength range of ~480–900 nm. Beyond these limits, the cuvette may begin to absorb radiation to a significant extent and should therefore not be used. The precise optical properties of cells may vary from one manufacturer to another, though the limits should be supplied with the cuvette. Plastic cuvettes are the least expensive type of cells that may be used for determinations in the visible region, although care should be taken that the cells do not become scratched, since this will render them unsuitable for further use.

Optically transparent glass cells

Many glasses have slightly wider optically transparent wavelength ranges of 400–750 nm than optical plastics. Glass has, moreover, the additional advantage of being far more resistant to scratching than plastic.

Fused silica and quartz cells

Fused silica and quartz cells offer the optimal optical properties available and allow UV and visible spectra to be taken throughout the full range of modern instrumental capabilities (approximately 190–750 nm). Fused silica and quartz cells are, unfortunately, very much more expensive than their glass counterparts and their use is primarily reserved for situations where the UV part of the spectrum must be recorded (i.e. 190–400 nm).

5.10 Qualitative UV–visible measurements

5.10.1 Qualitative applications of UV–visible spectroscopy

Comparison of the UV–visible spectra of the two structures of phenolphthalein would easily allow us to deduce whether the molecule is in the protonated or de-protonated form. It is clear therefore that UV–visible spectroscopy can be used both to *identify the presence* of a particular molecular species as well as for an analytical ‘fingerprinting’ technique.

Analyte samples, however, rarely contain a single absorbing species. The UV–visible spectrum of real samples is normally the summation of several molecular absorption spectra and the absorption at any one particular

wavelength will be equal to the sum of the individual absorptions of each component within the solution. For simple solutions, it is often possible to identify the presence of individual solutes from wavelength maxima of different absorption peaks, although it should be noted that the identification of compounds should normally be confirmed by infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), or melting point data.

5.10.2 The effect of different organic chromophores

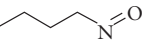

Organic moieties that act as chromophores possess electrons that may be easily excited and thereby promoted to higher energy levels via the absorption of light in the UV or visible ranges. Organic chromophores often contain one or more double or triple bonds and/or an aromatic ring. Many chromophores absorb radiation over a wavelength range of 20 nm or greater, which may cause the overlapping of one or more absorption peaks. The exact wavelength range over which the absorption occurs is also dependent upon the electron withdrawing/donating properties of the rest of the molecule, so it is impossible to identify with certainty the presence of a specific moiety in the same way that one can with IR spectroscopy (see Chapter 12).

A few examples of some of the most common chromophores are given in Table 5.1.

5.10.3 UV and visible light absorption by inorganic compounds

Many inorganic compounds absorb UV and/or visible radiation and possess absorption spectra with broad and frequently overlapping absorption spectra.

Table 5.1 Examples of UV or visible absorptions for a number of organic functional moieties acting as chromophores

Chromophore	Functional groups	Typical λ_{\max} values (nm)
Alkene	$-\text{CH}_2=\text{CH}_2-$	175–185
Alkyne	$-\text{C}\equiv\text{C}-$	175–195 and 220–30
Amines	$-\text{NH}_2$	195–200
Carbonyl	$-\text{CH}=\text{O}$	186 and 280
Nitro	$\text{R}-\text{NO}_2$ (Nitro-alkanes)	280
		
Nitroso	(Nitrosamines)	300 and 665
		
Aromatic	(Benzene)	200

Compounds of the first two transition series are among the most highly coloured inorganic compounds. Absorption by these compounds involves transitions of electrons between the unfilled and filled d-orbitals, and thus the wavelength at which absorption occurs depends on the atomic number, the oxidation state of the metal, and the ligand to which it is bonded. The detailed treatment of the colour chemistry of inorganic compounds is beyond the scope of this book although the interested reader is referred to other works.

5.10.4 Charge transfer UV and visible light absorption processes

Many inorganic and organic compounds absorb UV or visible radiation due to charge transfer processes, and are thus known as *charge transfer complexes*. ϵ_{\max} values are frequently in the order of $10\,000\text{ mol dm}^{-3}\text{ cm}^{-1}$ or more, which makes them both highly coloured and easy to quantify even at very low concentrations.

A charge transfer complex contains an electron donor group together with an acceptor group. Upon absorption of light, an electron is transferred from an orbital largely associated with the donor to an orbital largely associated with the acceptor grouping. This behaviour contrasts with the absorption of an organic chromophore in which electrons are associated within *shared* molecular orbitals.

In many examples, a metal ion acts as the electron acceptor. One of the most familiar examples of this type of absorption is potassium permanganate $\text{K}^+ [\text{KMnO}_4]^-$ that appears purple in aqueous solution.

5.10.5 The choice and effect of using different solvents

The overwhelming majority of UV or visible analyses demand that the analyte be dissolved within a solvent. The solvent must of course first and most importantly solvate the analyte so that it is distributed homogeneously in the path of the incident radiation beam. Water will often be the solvent of choice, however, many organic compounds require that an aprotic solvent such as acetonitrile or dimethylformamide (DMF) be used.

It should be remembered that the light must, pass through the solvent itself (i.e. be transmitted), although solvents are never perfectly optically transparent and in all cases exhibit their own absorptions. It is therefore crucially important to choose a solvent that allows the optimal transmission of the light throughout the wavelength region of interest. Water and many organic solvents appear to be colourless yet possess significant absorption spectra of their own in the UV range, which of course our eyes are insensitive to. In practice, it is as we approach the UV range that many of the most commonly encountered solvents begin to absorb significantly.

An aprotic solvent is one that does not contain protons due to dissociation of the solvent molecules. Aprotic solvents are almost always organic solvents. Dimethylformamide (DMF) is a good example.

126 5: Use of visible and UV light for analytical measurements**Table 5.2** Polar solvents

Solvent	Lowest wavelength beyond which solvent must not be used for analysis (nm)
Water	200
Ethanol	220
Diethyl ether	210
Acetonitrile	185

Table 5.3 Non-polar solvents

Solvent	Lowest wavelength beyond which solvent must not be used for analysis (nm)
Hexane	200
Cyclohexane	200
Benzene	280
Carbon tetrachloride	260
Dioxane	320

It is therefore especially important that consideration be given to the choice of solvent when absorption spectra below around 250 nm are required.

A list of commonly used polar and non-polar solvents are shown in Tables 5.2 and 5.3, respectively.

It is essential that solvents of very high purity are always used (preferably high-performance liquid chromatography—HPLC grade) since many technical grade solvents such as ethanol and hexane contain contaminants of, for example, benzene that absorb at wavelengths below 280 nm.

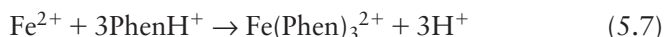
However suitable the choice solvent (and indeed however good its purity), it should be remembered that the solvent will always exhibit a finite absorption, which must be accounted for in all cases. A blank spectrum of the solvent must either be run as a baseline that can be subtracted from all subsequent spectra, or alternatively, if a double beam spectrophotometer is being used, then a twin cuvette containing the solvent and analyte sample may be used in tandem (see Section 5.9).

5.11 Colour-based complexation analyses

Many transition metal ions form highly coloured complexes. The colour can be exploited as the basis of simple and highly specific spectrophotometric

determinations. For example, solutions of aqueous iron(II) react with ortho-phenanthroline (1,10-phenanthroline) to form an orange-red complex that may be easily quantitatively determined spectrophotometrically. The extinction coefficient for this complex is $\sim 1.08 \times 10^4 \text{ mol dm}^{-3} \text{ cm}^{-1}$.

Aqueous *ortho*-phenanthroline acts as a weak base and dissociates in the presence of an acid to form phenanthroline ions, PhenH^+ , Eqn (5.7). At pH values of 3.5 or less phenanthroline ions react quantitatively with Fe^{2+} to form the $\text{Fe}(\text{Phen})_3^{2+}$ complex.



The iron content of any aqueous solution may be determined by the addition of excess reducing agent such as hydroquinone or hydroxylamine. The reducing agent ensures that all of the iron resides in the +2 oxidation state and so is ready to complex with the PhenH^+ ion.

The $\text{Fe}(\text{Phen})_3^{2+}$ complex exhibits a sharp absorption maximum (λ_{max}) at approximately 508 nm. The absorption of a series of standardised iron solutions should then be determined at concentrations which correspond to absorbances of around 0.1–1. A calibration graph may then be plotted, which should allow the concentration of an unknown aqueous iron containing sample to be determined.

Practical Methodology for an Iron Determination in an Unknown Sample

Standardized solutions of Fe^{2+} , hydroxylamine hydrochloride, *ortho*-phenanthroline and sodium acetate should first be prepared.

Fe²⁺ solution: Accurately weigh and dissolve approximately 0.07 g $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in a 1 dm³ volumetric flask. Add 2 ml of concentrated H_2SO_4 and dilute to the mark with de-ionized water.

Hydroxylamine hydrochloride solution: Dissolve 10 g $\text{H}_2\text{NOH} \cdot \text{HCl}$ in 100 ml of de-ionized water.

Ortho-phenanthroline solution: Dissolve 1.0 g of *ortho*-phenanthroline monohydrate in 1 dm³ of water; *this solution must be prepared freshly on a daily basis.*

Sodium acetate: Dissolve 166 g of $\text{NaOAc} \cdot 3\text{H}_2\text{O}$ in 1 dm³ of de-ionized water.

Procedure

A series of secondary iron standards (four to five should suffice here) need to be made using the stock

solution of Fe. This may be done by introducing 1 ml of the hydroxylamine, 10 ml of the sodium acetate, and 10 ml of the *ortho*-phenanthroline to each of the flasks. Iron solutions (5, 10, 15, 20 ml, ...) should then be added to each of the flasks, which should then be made up to the mark with dissolved water. A blank should also be prepared without the iron but containing each of the other reagents, that is, the sodium acetate, *ortho*-phenanthroline, and hydroxylamine hydrochloride.

We have already said that the wavelength of maximum absorption (λ_{max}) occurs at 550 nm. A UV-visible spectrum should, however, be run between the wavelengths of around 460–560 nm (± 50 nm of the expected λ_{max}) to determine as accurately as possible the λ_{max} (as determined by the machine you are to perform the analysis with). In this way you are: (a) ensuring that your experimental procedure is

providing results which are broadly in line with the literature; and (b) ensuring that you are maximizing the analytical sensitivity of your own experimental apparatus.

The absorptions recorded for each of the samples may then be plotted as a concentration calibration curve that should be seen to follow the Beer–Lambert law (i.e. following a good straight line fit). The absorption of the unknown sample may then be read off against the corresponding concentration. If the absorption of the unknown sample falls beyond the range of the calibration graph, then the sample should be diluted by a known factor (e.g. dilution by 2- or

10-fold) to bring the absorption of the sample to within the experimentally determined range. The concentration of the unknown sample is then simply given by multiplication of the concentration as given by the calibration curve by the appropriate factor. Dilution of known samples to bring the experimentally determined absorption values to within the calibration range is important since deviations from the Beer–Lambert law are often observed at higher concentrations as we shall see in Chapter 6. Moreover, we should not forget that as we have already seen within this chapter, instrumental errors increase dramatically at higher absorption values.

5.12 Bathochromic and hypsochromic shifts

If the λ_{\max} of an analyte overlaps with the absorption spectra of any of the reagents and/or any other chemical species that may be present within real analyte samples, then a quantitative spectrophotometric determination will certainly be complicated. One way around the problem would be to further react the complex with another reagent to form a *new* complex, with a λ_{\max} sufficiently far away from the absorption peaks of any interfering reagents and/or analytes.

A shift in the λ_{\max} from a shorter to a longer wavelength is known as a *bathochromic shift*.

A shift in the λ_{\max} from a longer wavelength to a shorter wavelength is known as a *hypsochromic shift*.

To understand how this is performed in practice, we need to consider a couple of examples:

Aqueous solutions containing tin may be spectrophotometrically determined by means of a bathochromic shift. Tin(IV) may be complexed with the dye catechol violet. The catechol violet–tin complex exhibits a strong absorption λ_{\max} at 555 nm; unfortunately, the catechol violet also possesses an absorption peak that completely overlaps the catechol violet tin absorption peak. Since the catechol violet has to be added in excess in order to ensure complete complexation of the tin and the tin–catechol violet complex cannot be easily separated from the excess catechol violet, a quantitative spectrophotometric determination of the tin–catechol violet complex is not possible.

The tin–catechol violet complex, may, however, be further reacted with a further ligand, cetyltrimethylammonium bromide (CTAB). CTAB

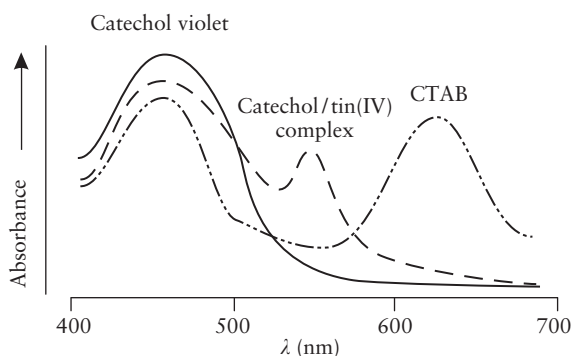


Figure 5.14 Bathochromic shift—showing shift in absorbance to longer wavelength.

does not exhibit any absorption maxima in the 400–700 nm wavelength range. This then forms a tin–catechol violet–CTAB complex, which exhibits two absorption maxima at approximately 555 and 662 nm. The addition of the CTAB ligand to the complex has also added a new absorption maximum, which now allows the quantitative determination of tin in the presence of both the catechol violet and CTAB reagents; Fig. 5.14.

Hypsochromic shifts may be used in a similar way to shift the absorption of a metal complex absorption to a shorter wavelength. For example ‘Reicandt’s dye’ in acetonitrile changes colour upon an uptake of water; the λ_{max} shifts from 520 to 600 nm with increasing water concentration and this can be used to estimate the water content of concrete.

5.13 Introduction to fluorescence and fluorometric determinations

So far we have considered how molecules absorb UV and visible radiation by the excitation of their valence electrons. The excited electrons are now energetically less stable than the ground state and must relax at some time. Upon relaxation, some or all of the energy gained by the capture of phonons upon the absorption of light must be lost, which is normally dissipated by heat. We know that an object will become heated if placed in a strong light source such as sunlight. In some cases, some of this excess energy is dissipated by the emission of a photon, that is, the emission of light.

Fluorescent reactions may be of great use to the analytical chemist since the emission of fluorescent radiation is highly specific to each individual compound. The intensity of the fluorescent radiation may, moreover, be directly related to the concentration of an analyte. In reality very few compounds fluoresce. A number of species may, however, be made to

The emission of photons (light) as a result of an electronic relaxation process is known as fluorescence.

fluoresce by attaching or ‘labelling’ the compound with a molecule that *does* fluoresce. We shall consider this approach of ‘fluorescent labelling’ in Chapter 6.

Let us consider further how photons are emitted by fluorescence. The energy which is imparted to the photons that comprise the fluorescent radiation is derived from photons that were originally captured by the fluorescent molecule. As we have seen, some of the excess excitation energy that is lost by an electronic relaxation process is lost by heat, and the remaining energy is lost by the emission of a photon. It follows that the energy of the emitted photons will be of a lower energy than the absorbed photons and, since energy is proportional to frequency, the frequency of the emitted radiation will be lower than that of absorbed radiation. If the frequency of the emitted radiation is lower than that of the absorbed radiation, it follows that the fluorescent radiation must be of a longer wavelength than that of the radiation which was originally absorbed.

The intensity or power of the fluorescent radiation, P_f , will be proportional to the quantity or power of the light absorbed. The radiation power that is absorbed may be given by $(P_0 - P)$, where P_0 is the power of the incident radiation and P is the power of the radiation that is not absorbed—or, in other words, which is transmitted. Only some of the absorbed photons will give rise to the emission of fluorescent photons, since many electronic relaxations proceed solely as a result of molecular collisions and energy dissipation as heat. The *quantum efficiency*, ϕ , is a proportionality constant that describes the proportion of absorbed incident photons that give rise to the emission of fluorescent photons.

If we are to measure the intensity of the emitted fluorescence, then a detector must be placed to monitor the photons emitted by the fluorescing species. The detector can only detect the radiation that enters it and therefore only monitors the light that is emitted in one direction from the analyte sample. Photons are, however, emitted in all directions (i.e. 360°) and it is therefore necessary to include another proportionality factor so that we can make a quantitative estimate of the total fluorescent output. This factor, k' , is known as the *geometric factor*. In practice, the fluorescent detector is normally placed at an angle of 90° (Fig. 5.15) to the incident radiation beam, to help avoid any interference with the incident or transmitted light sources, even though the fluorescent radiation will be of a different wavelength and will be monitored using a monochromator in conjunction with the detector.

We are now in a position to relate the fluorescent power output, P_f , to these other parameters, Eqn (5.8):

$$P_f = \phi k'(P_0 - P) \quad (5.8)$$

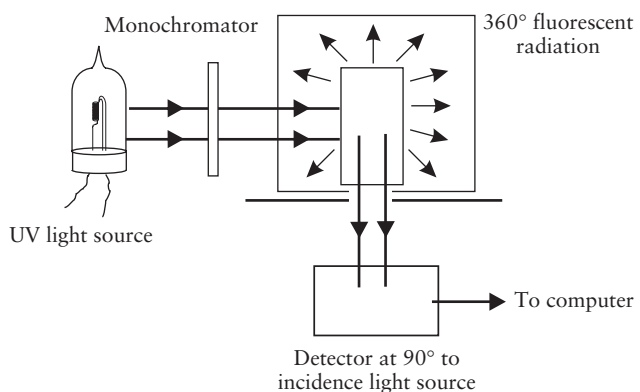


Figure 5.15 Schematic of a fluorescence detector.

The incident light absorbed ($P_0 - P$) is related to the concentration of the absorbing species, and if this is taken into account we can describe the fluorescent power output according to

$$P_f = \phi k' P_0 (1 - e^{-kcl}) \quad (5.9)$$

If one expands the exponential term to allow the substitution and expression of P_f in terms of \log_{10} we can estimate that

$$P_f = \phi k' \times 2.303 \epsilon cl \quad (5.10)$$

Eqn (5.10) is an approximation since it assumes that the absorbance is small. In practice, this equation is normally taken to be valid as long as ϵcl (i.e. the absorbance) is <0.05 , and in this case the error incurred will be $<5\%$.

If a fluorometric determination is to be performed, then we must normally determine: (a) the λ_{\max} corresponding to the absorption peaks of the compound; and (b) the λ_{\max} of the fluorescent emission spectrum. In practice, pinpointing the exact fluorometric emission λ_{\max} is slightly less crucial since fluorometric emissions occur across a broader range of wavelengths than absorption spectra. This is to be expected since the emitted light may be thought of as the residual energy that is not lost as a result of molecular collision and subsequent thermal dissipation. Electrons may relax in a number of separate (albeit quantized) steps so the fluorescent emission spectrum peaks tend to span a few tens of nanometres. The majority of modern spectrophotometers permit a continuous variation of both the incident radiation wavelength and fluorometric detection wavelengths although some simpler instruments rely on the interchanging of grating filters (which are normally supplied in 10 or 20 nm increments) if the output fluorescence wavelength is to be altered.

PRACTICAL EXAMPLE

Quinine is a naturally fluorescent molecule that is found in many proprietary soft drinks such as sparkling bitter lemon or tonic water. The quinine content of a drink may be determined as a simple demonstration of a fluorometric analysis.

Procedure:

Prepare the following solutions:

- (a) A total of 2 dm^3 of a $0.05 \text{ M H}_2\text{SO}_4$ solution.
- (b) A 1 ppm quinine sulphate standard. This may be prepared by weighing 0.1 g quinine sulphate (to within 0.5 mg), which should then be dissolved volumetrically in 1 dm^3 $0.05 \text{ M H}_2\text{SO}_4$. Ten millilitres of this solution should then be transferred to another 1 dm^3 flask and diluted to the mark with $0.05 \text{ M H}_2\text{SO}_4$. This latter solution will now contain 1 ppm quinine and should be prepared freshly and kept refrigerated in the dark between measurements since it is easily photo-oxidised.

Quinine fluoresces with an absorption maximum at approximately 450 nm and so the emission wavelength of the fluorimeter should be set to this or a similar wavelength. A calibration curve should be determined by analysing a series of standardized samples, which are made by diluting 10 ml and lower ($8, 6, 4,$ and 2 ml) of the stock 1 ppm dm^{-3} solution to 1 dm^3 with $0.05 \text{ M H}_2\text{SO}_4$.

The unknown sample may then be analysed by diluting the sample with $0.05 \text{ M H}_2\text{SO}_4$ to bring the fluorometric reading within the linear region of the calibration curve.

5.13.1 Fluorometric quenching

Fluorometric quenching is a term used to describe any process that suppresses the fluorescence of a molecular species. We have already seen that the fluorometric emission of light involves the relaxation of excited electrons together with the dissipation of heat as a result of molecular collisions. It follows that if more energy is lost as a result of molecular collisions, less energy is available for emission in the form of photons as light. The more molecular collisions that occur in the fluorescent mixture, the less fluorescence will occur. Any process that increases the frequency at which molecular collisions occur will therefore quench the fluorescence.

The majority of fluorescent reactions occur in solution, in which case molecular collisions between: (a) the fluorescent molecules; (b) the solvent molecules; and/or (c) any other solutes (including other fluorescent molecules) will all serve to quench the fluorescence to some extent.

Brownian motion will always be present and again any factor that increases Brownian motion and/or diffusion of solvent/solute molecules

will increase the frequency of molecular collisions and so increase the quenching of the fluorescent reaction.

Ions and/or other solutes may be added to the mixture to act as quenching agents; larger molecules will be involved with a greater number of molecular collisions than smaller solutes and it therefore follows, for example, that K^+ will act as a more effective quenching agent than Na^+ .

5.14 Polarimetry and optical rotations

Many inorganic and organic compounds possess the capability of *rotating the plane* of a source of polarized radiation. Materials that display this behaviour are said to be *optically active*. Some of the most popular examples include, for example, quartz as well as the mono- and disaccharide sugars such as glucose. If an observer looks towards the light source and the light is rotated towards the right (i.e. clockwise), then the direction of rotation is said to be *dextro* or (+) *rotatory*. Conversely, if the direction of rotation is towards the left (anticlockwise) then the direction of rotation is said to be *laevo* or (–) *rotatory*.

The extent of rotation depends on the *number* of atoms or molecules in the path of the light source and hence the concentration of a solution (c) and the path length (l). The degree of rotation also depends on the wavelength of the radiation (λ) and the temperature (t).

The *specific rotation*, $[\alpha]^t$ is defined as the extent of rotation in degrees of a plane of polarized light, α , at a specified wavelength through a 1 m pathlength of a solution of concentration, c mol dm^{–3} at temperature t , Eqn (5.11):

$$[\alpha]^t = \frac{\alpha}{lc} \quad (5.11)$$

A sodium vapour lamp is often chosen as the source of radiation, with an emission at 589.3 nm corresponding to the D line of a sodium emission.

Optical rotation may be measured most commonly by an *automated polarimeter*, a simplified schematic of which is shown in Fig. 5.16. The light must be: (a) monochromatic; (b) polarized; and (c) focused so that all of the light is travelling in the same direction. The light originates either from a white light source or, more commonly may be generated from a monochromatic source, for example, a sodium lamp. The light is then passed through a collimator to focus the light into a parallel beam, which, in turn, is passed through a calcite prism to produce the plane-polarized focused light beam. An auxiliary calcite prism is sometimes used to intercept or divide the incident radiation source into two beams of

For discussion of atomic emission lines, please refer to Chapter 7.

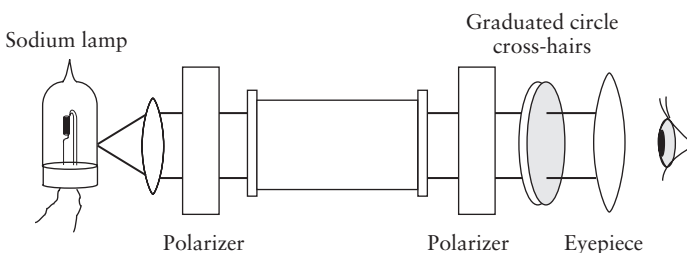


Figure 5.16 Schematic of a simple polarimeter.

equal intensity—*but* orientated a few degrees of rotation apart relative to each other.

The two light beams are passed through a glass tube of known length (normally 10 cm) and an ‘analyser’ is placed at the far end of the sample tube together with an eyepiece. The intention is to determine the angle through which a polarized light beam is rotated by the sample. There are a number of ways of achieving this. A polarizing filter *will fail to transmit any light* if this is placed at an angle of 90° to the plane of a polarized beam of light. The detector at the far end of the sample, however, may also be rotated; in this way the intensity of the light emerging from the sample may be monitored *at any angle*. The angle of rotation of the detector, which corresponds to a total blocking of the transmitted light, will therefore correspond to the angle through which the polarized light beam has been rotated. This method suits automated methods for determining the optical rotation of samples, since detectors readily detect signal minima or maxima, which in this case correspond to the output from a photomultiplier tube or other optical detector.

Many polarimeters rely, on the manual determination of the optical angle of rotation and if used correctly, provide results that compare favourably with many automated and therefore rather costly instruments. Manual polarimeters frequently use two light beams. A polarizing filter or prism is often placed directly in front of, and in the path of the sample at an angle of 90° to the plane of the incident light source. This prism splits the light beam into two separate beams that have a few degrees of rotation clockwise and anticlockwise, respectively, to the incident light. The two light beams are then passed through the sample and the transmitted light from both beams of light are then passed through a polarizing filter, which may be rotated. *It follows that there will be one angle at which the intensity of the radiation of the two light beams will be of very small but equal intensity.* The polarizing filter is rotated until the intensity of the two beams is equal. This angle corresponds to the *angle of optical rotation*. At this angle the light beams possess angles of rotation equidistantly spaced either side of the detector; the midpoint between these angles corresponds to the angle through which the sample has rotated the incident light beam. Two light beams are used to compare intensity, since our eyes more easily

match light intensities than determining the angle corresponding to the absolute extinction of the light as it emerges from the sample.

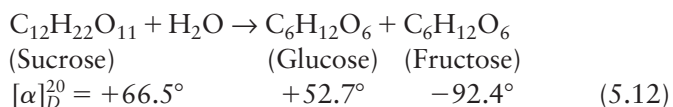
5.14.1 Practical determinations based on polarimetry

Many organic compounds contain asymmetric arrangements of atoms within a group that allow mirror images of the same molecule to exist; such compounds are said to possess *chirality*. Chiral and therefore optically active compounds are extremely important to many biological systems. Biological substrates often have asymmetric chiral centres and are acted upon by enzymes that only recognize the dextro- or laevo- rotatory forms. The dextro and laevo forms are known as rotamers. Many biological samples contain only one rotamer of a given compound and for this reason, many bio-analytes may be quantified by polarimetry.

Sucrose polarimetric determinations

The most widely used application for polarimetry is in the sugar industry for the determination of sucrose due to its huge commercial significance. Although Eqn (5.11) allows us to quantitatively determine solutions of sucrose in the absence of other optically active materials, the analysis becomes more difficult if other naturally occurring sugars are present. Practical determinations are normally to be undertaken within sugar solutions derived from plants (sugarbeet and cane) that contain a number of sugars other than sucrose.

Fortunately, sucrose (a disaccharide) may be hydrolysed in the presence of a dilute acid to yield glucose and fructose, Eqn (5.12). This hydrolytic splitting of the saccharide is known as an *inversion* reaction, and the resulting mixture of the two monosaccharides, glucose and fructose—as the *invert sugar*.



Glucose and fructose, however, have very different specific rotations from sucrose, Eqn (5.12). As the hydrolysis proceeds (i.e. the inversion reaction) the *specific rotation* for a solution initially containing only sucrose will change from +66.5 to -19.8° . The angle of rotation measured will of course be dependent on the concentration of sucrose and whether or not any other optically active compounds are present. The *change* in the angle of rotation will, however, depend on the concentration of sucrose in the sample. It therefore follows that if we note the change in the angle of rotation we can calculate the sucrose concentration.

Ten millilitres of concentrated HCl are typically added to every 100 ml of the sucrose sample and allowed to stand for at least 24 h at room temperature. The hydrolysis (or ‘inversion’) reaction can, however, be accelerated by heating the mixture at 70°C, and this should allow the reaction to reach completion within approximately 15 min.

Penicillin—penicillinase polarimetric determinations

The antibiotic *penicillin* is another example of an optically active biological compound that can be determined polarimetrically. The dextro-rotamer form is the biologically active form of the molecule and may be metabolized by the enzyme *penicillinase*. A solution of penicillin and penicillinase will therefore show a decreasing angle of optical rotation as penicillin is metabolised by the enzyme. The enzyme has a very slow turnover rate and is effectively saturated in all but the most dilute solutions. It follows that until almost all the penicillin is consumed, the rate of consumption of the substrate will be dependent on the concentration of the enzyme and not the penicillin and so the reaction will proceed at a near constant rate until all of the penicillin is consumed; the time for the reaction to reach completion may, however, be used to determine the concentration of the penicillin within the sample.

Conversely, the same reaction chemistry may be used to determine the concentration of a penicillinase antibiotic solution. In this situation, the time taken for the consumption of a known molar quantity of penicillin may be monitored polarimetrically and in this way, it is possible to calculate the concentration of penicillinase that is present in an unknown sample.

Exercises and problems

- 5.1.** Which electrons within molecules are normally involved in absorption of UV and visible radiation.
- 5.2.** A student performs a set of UV–visible determinations on a set of unknown samples for an analyte, a , with a known λ_{\max} . All of the student’s results give absorbances of between 2 and 3. Why may these results not be used for the quantitative determination of a , and what must the student do next?
- 5.3.** Explain why absorbance is a unit-less quantity.
- 5.4.** Why will many UV–visible spectrophotometer possess more than one lamp?
- 5.5.** Sodium chloride and potassium chloride both act as quenching agents for a fluorometric determination of quinine. Explain: (a) why this is the case; and (b) which salt will act as the most effective quenching agent.
- 5.6.** A cuvette with a path length of 1 cm and solution containing 8.96 ppm of a dye gives an absorbance of 0.8. Calculate the molar absorptivity of the dye.
- 5.7.** A compound of molecular weight 245 is found to have an absorptivity of $298 \text{ g dm}^{-3} \text{ cm}^{-1}$. Calculate its molar absorptivity, ϵ .

5.8. Three standard Fe^{2+} (aq) solutions are found to have absorbance values as shown below:

Conc. Fe^{2+} (mol dm ⁻³)	Absorbance
0.010	0.21
0.025	0.53
0.052	1.00

- (a) Show whether or not the absorbances recorded for these solutions obey the Beer–Lambert law.
- (b) An iron salt containing solution is found to exhibit an absorption of 0.2 at 510 nm following the addition of 1,10-phenanthroline (together with excess hydroxylamine) to form a coloured complex. The molar absorptivity, ϵ , for the iron–phenanthroline complex is $1.08 \times 10^4 \text{ mol dm}^{-3} \text{ cm}^{-1}$. Determine the concentration of iron within this solution.

5.9. A solution of the drug Tolbutamine is found to exhibit an absorbance of 0.85 in a 1 cm pathlength cuvette. The molecular weight of Tolbutamine is 270 and the molar absorptivity at 262 nm is $703 \text{ g}^{-1} \text{ dm}^{-3} \text{ cm}^{-1}$. What is the molar concentration of tolbutamine?

5.10. You have a compound which you know has λ_{max} at 215 and 244 nm and you wish to determine its ϵ value in a range of solvents. It is soluble in acetone, acetonitrile, benzene, carbon tetrachloride, dioxan, methanol, toluene, and water. Which of these solvents can you use for this experiment?

5.11. You have three dilute solutions of equal molarity of pentane, 1,3-pentadiene, and 1,4-pentadiene. How would you be able to tell them apart using UV spectrometry?

Summary

- Visible light forms part of the electromagnetic spectrum and extends from wavelengths of approximately 400 to 750 nm.
- Ultraviolet (UV) radiation (of wavelengths that may be exploited for analytical purposes) extends from wavelengths of approximately 180 to 400 nm.
- The energetic states of orbitals and their electrons are quantized.
- The promotion of a valence electron from one orbital to another involves absorption of radiation normally in the UV or visible range of wavelengths.
- The energy of electromagnetic radiation = $h\nu = hc/\lambda$.
- Fluorescence occurs when some of the energy of photons absorbed is dispersed by the emission of photons of lower wavelength than the incident radiation.
- The energy of adsorbed photons is normally dispersed as heat when electrons relax to their ground states.
- Absorbance is a unit-less quantity. $A = \log_{10} (I_0/I)$ where I_0 is the intensity of the incident radiation and I the intensity of the transmitted radiation.
- Fluorescence quenching is the term used to describe processes that suppress molecular fluorescence.
- The Beer–Lambert law describes the absorption of radiation by compounds with a molar absorption of ϵ , and a concentration, c , through a pathlength, l , and states that $A = \epsilon cl$.
- A number of different light sources may be used for UV–visible spectroscopy such as tungsten filament, hydrogen, and deuterium lamps.
- Monochromators are used for wavelength selection and are based on diffraction prisms or reflection gratings.
- A number of photon detectors are used with UV–visible spectrometers and include photo-tubes, photo-multiplier tubes, silicon photo-diodes, and photo-voltaic cells.

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14. UV–visible spectrometers are based either on a single- or double-beam formats. Single-beam spectrometers necessitate separate baselines to be determined with blank samples. Double-beam spectrometers, by contrast, allow two cells to be determined—one as a blank to establish the baseline and one for the sample itself.

15. Cuvettes may be made of quartz, glass, or plastic depending on the wavelength range over which spectra are to be run. Quartz cuvettes must always be used for wavelength ranges <300 nm.

16. A shift in λ_{\max} from a shorter to a longer wavelength is known as a bathochromic shift.

17. A shift in the λ_{\max} from a longer to a shorter wavelength is known as a hypsochromic shift.

18. Compounds that are capable of rotating the plane of polarized radiation are said to be optically active. Compounds that rotate the light in a clockwise (+) manner are said to be dextrorotatory. Those that rotate radiation in an anticlockwise (–) manner are said to be laevorotatory.

19. The specific rotation $[\alpha]^t$ is defined as the extent of rotation in degrees, that is,

$$[\alpha]^t = \frac{\alpha}{lc}$$

20. The study of optical rotation is known as plarimetry and may be used to quantify solutions of, for example, sugars such as sucrose or antibiotics such as penicillin.