2.1 Revision

The following questions cover the important concepts that you should have understood in the first year instrumentation subject.

1. **What are the wavelength ranges for the ultraviolet and visible regions of the spectrum?**

2. **What molecular or structural features give rise to absorption of ultraviolet/visible (UV/VIS) radiation in organic species?** Give an example of an organic compound that would not absorb UV/VIS radiation.

3. **What molecular or structural features give rise to absorption of ultraviolet/visible (UV/VIS) radiation in ionic species?** Give an example of an ionic species that would not absorb UV/VIS radiation.

4. **What solvent and cell materials would be suitable for the following scan?**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Region</th>
<th>Solvent</th>
<th>Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) copper sulfate</td>
<td>visible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) copper sulfate</td>
<td>UV/visible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) methylbenzene</td>
<td>UV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(d) non-polar yellow dye</td>
<td>visible</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.2 Absorbing species

**Organic compounds**

As you are aware, double & triple bonds and non-bonded electrons on atoms such as N, O and the halogens are the structural features that cause absorption in the UV/VIS region. Actually, this statement isn’t strictly true!

In fact, all compounds absorb UV radiation, it’s just that the compounds without the multiple bonds and non-bonded electrons absorb below the “dividing line” of 200 nm. Why that figure you might ask? A purely practical one: measurements in the far UV (as < 200 nm is known) require a vacuum, since the components of air absorb strongly. This makes for a less convenient, more complex device and lots of interferences for quantitative analysis. So when we talk about UV spectra, we are actually referring to near UV.

While we are on the topic of air absorbing UV, it raises a seeming contradiction to our general rule about what absorbs in the (near) UV: after all, what are the structural features of nitrogen and oxygen gases? Double (O=O) or triple (N≡N) bond and lots of non-bonded electrons (4 on each oxygen, 2 on each nitrogen). So, really air should absorb well above 200 nm, but fortunately it doesn’t (and don’t ask me why, because I don’t know).

For a compound to absorb strongly above 200 nm, it requires a number of multiple bonds in conjugation (alternating single-multiple) and/or a number of non-bonded electron atoms.

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**EXAMPLE 2.1**

*The following compounds show the effect of the presence or absence of absorbing groups (number given is the wavelength of maximum absorption).*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>210</td>
</tr>
<tr>
<td>Oxybenzene</td>
<td>256</td>
</tr>
<tr>
<td>Anisole</td>
<td>282</td>
</tr>
<tr>
<td>Phenol</td>
<td>312</td>
</tr>
<tr>
<td>Toluene</td>
<td>260</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>265</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>270</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>280</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>300</td>
</tr>
</tbody>
</table>

These are all obviously absorbing in the UV region only. For an organic compound to absorb in the visible region (even if the peak isn’t past 400 nm), it will need numerous conjugated double bonds and non-bonded electrons. Figure 2.1 shows the structure of methyl orange to illustrate this.

![Methyl Orange Structure](image)

**FIGURE 2.1 The structure of methyl orange (the yellow form in neutral solution)**
**Inorganic compounds**

Some simple metal ions absorb in the ultraviolet or visible region, because they have valence electrons, eg Cu$^{2+}$ and Ni$^{2+}$, but usually what absorption there is, is not particularly strong, and therefore, not useful for quantitative analysis.

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**EXERCISE 2.1**

*Why would weak absorption by a chemical species, eg Cu$^{2+}$, make it not useful for quantitative analysis?*

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Polyatomic ions, such as permanganate and dichromate, get their much stronger absorbance from a combination of factors: multiple bonds and non-bonded electrons. In the majority of cases, complexes of metal ions and ligands are needed for intense absorption, and the ligands are known as colour-forming reagents.

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**2.3 Cells and solvents**

The basic rule for the sample “container” – cell and solvent – is that it (they) should not absorb more than 0.2 at wavelengths of interest.

**Cells**

The three standard cells for UV/VIS are, of course, quartz, glass and plastic. All three are suitable for the visible region and into the UV down to 350 nm, but in practice, plastic is used because of its cheapness, unless there is an organic solvent being used, in which case, glass becomes the choice. Below 350 nm, quartz is the only choice.

**Solvents**

The wider range of solvents available makes for more flexibility, but the starting requirement for a solvent is that it dissolves the species of interest! All the solvents in Table 2.1 are colourless, so any of them are suitable for the visible region. In the UV, most organic compounds absorb to some extent, so it is a matter ensuring that the cutoff wavelength (see Table 2.1) is lower than the wavelength(s) of interest.

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**Table 2.1 Minimum wavelengths for common solvents (common names in brackets)**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>nm</th>
<th>Solvent</th>
<th>nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>205</td>
<td>ethanenitrile (acetonitrile)</td>
<td>210</td>
</tr>
<tr>
<td>cyclohexane</td>
<td>210</td>
<td>ethoxyethane (ethyl ether)</td>
<td>210</td>
</tr>
<tr>
<td>ethanol</td>
<td>210</td>
<td>hexane</td>
<td>220</td>
</tr>
<tr>
<td>methanol</td>
<td>210</td>
<td>dioxane</td>
<td>220</td>
</tr>
<tr>
<td>dichloromethane</td>
<td>235</td>
<td>trichloromethane (chloroform)</td>
<td>245</td>
</tr>
<tr>
<td>methylbenzene (toluene)</td>
<td>280</td>
<td>propanone (acetone)</td>
<td>330</td>
</tr>
</tbody>
</table>
EXERCISE 2.2

Choose a suitable solvent for the following analyses (wavelengths of interest in brackets).

<table>
<thead>
<tr>
<th>(a)</th>
<th>dimethylbenzene (250-300 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b)</td>
<td>sodium benzoate (250-320 nm)</td>
</tr>
<tr>
<td>(c)</td>
<td>aspirin (280-320 nm)</td>
</tr>
</tbody>
</table>

Some chemical companies sell a range of solvents known as Spectrograde, which are specifically designed for use in UV spectroscopy. They are not necessarily more pure than AR grade, but they are guaranteed not to have absorbing impurities. For example, AR grade hexane might be 99.9% pure, but the impurity could be benzene, whereas spectrograde hexane might only be 99% pure, but the impurity is heptane.

2.4 Radiation sources

Two radiation sources are required to cover the entire UV/VIS range:

- a deuterium discharge lamp for the UV
- a tungsten filament globe for the visible

Deuterium gas, when excited by low-voltage electrical energy produces an almost perfectly continuous spectrum in the UV region between 160 and 375 nm. At longer wavelengths, the lamp still produces some intensity, but the continuous spectrum is mixed with narrow emission lines, which cause problems with quantitative measurements.

Tungsten filament lamps are simply the familiar light bulb of domestic use. They work by being heated to incandescence, the temperature at which the solid emits visible radiation. Above this point the continuous radiation produced (known as blackbody radiation) has an intensity that is dependent on the temperature, rather than the chemical composition of the heated material. A tungsten filament at 3000K produces radiation over a range of 350-2500 nm, with the peak around 1000 nm. The intensity in the visible region is not uniform, and the lamp is not efficient, since only 15% of the intensity is in the visible region.

The output of the tungsten filament is dependent on the applied voltage, and, therefore, it is crucial that the power supply contains a voltage regulator to ensure a constant value. The significant levels of infrared radiation (heat) are often removed by a filter.

For a full spectrum covering both UV and visible regions, the instrument blocks off the source not required by use of a filter until the changeover point at 350 nm. There is inevitably a difference in intensity between the two sources at this wavelength, but design, gain adjustment and the background scan mean that there isn’t a bump in the spectrum.

2.5 Monochromators

Both prisms (in glass or quartz) or gratings can be used to disperse UV and visible radiation. Prisms have the disadvantage of requiring very high quality calibration and optics, hence diffraction gratings have become the most popular source of monochromation in UV/VIS spectrophotometers. Gratings have the advantage of being considerably less expensive and optically more efficient.
2.6 Detectors

These convert electromagnetic radiation into electrons and hence produce an output of current which can be interpreted as a measure of radiant energy reaching the detector surface. The devices most commonly employed in UV/VIS instruments are photomultiplier tubes (the most common) and photodiodes.

Photomultiplier tubes employ a photoemissive cathode which ejects electrons when a light particle strikes its surface. These then pass through a series of amplifying dynodes, which attract the electrons because of increasingly higher applied potentials. Dynodes produce several electrons for every one electron striking their surface. Finally the avalanche of electrons reach an anode. Hence, a small initial electron flow from the cathode produces a much greater electron flow at the anode. A small current known as the dark current flows even when no light is striking the detector. This arises from several sources, including the natural radioactive emission of β-particles (high energy electrons) from the glass envelope surrounding the tube. Figure 2.2 shows the design of a typical photomultiplier tube.

Photomultiplier tubes have a limited operating life due to breakdown of the photocathode, and cannot be exposed to the sunlight or bright room lighting. If this happens, a large semi-permanent dark current results, reducing the efficiency of the tube at low light intensities when in operation.

Photodiodes are semiconductor-based devices which conduct a current when electrons strike their surface. These devices have excellent stability (give good baselines), a good signal-to-noise ratio and are extremely reliable.

Photodiodes are becoming popular, joined in banks (or arrays), in multi-channel instruments known as diode array spectrophotometers. The advantages of such instruments have been discussed in Chapter 1. A typical UV/VIS diode array instrument can produce a complete 200-800 nm spectrum in 0.1 seconds. This is achieved by using an array of diodes as a detector, instead of conventional photomultiplier tubes. Each diode is responsible for detecting a small portion of the spectrum (1-2 nm per diode) and all diodes operate at the one time. Besides the speed advantages, these instruments do not drift in wavelength (their calibration is always perfect). They have a disadvantage, however, in that they cannot resolve with any greater detail than the width of the portion of the spectrum examined by each diode (1 or 2 nm). For most analysis this is not a problem as resolution greater than this level is rarely required.

FIGURE 2.2 A typical photomultiplier tube design (from www.physics.ubc.ca)
What You Need To Be Able To Do

- define important terminology
- describe characteristics of organic and inorganic species that allow them to absorb UV/VIS radiation
- choose appropriate cells and solvents for UV/VIS work
- list and describe common radiation sources used in UV/VIS spectrophotometers
- list and describe common detectors used in UV/VIS spectrophotometers

Revision Questions

1. Look up the structure of aspirin. It absorbs at around 275 nm. What structural components cause it to absorb radiation?

2. What solvent would be appropriate to record as much of the UV/VIS spectrum for (a) caffeine and (b) naphthalene. You should look up the structures of each of these to help with your answer.

3. Why are the emission lines of deuterium above 375 nm a limitation on the lamp’s use in the visible region?

4. Why is it important that the voltage to the tungsten filament is constant?

5. What is the meaning of “dark current” for detectors? What significance does it have?

6. What limits the resolution of a diode-array spectrophotometer? Why doesn’t it normally matter that the resolution is typically 2 nm?

Answers to these questions on following page.

Answers to class exercises can be found in the Powerpoint file provided on the website.
Answers to Revision Questions
To answer questions 1 & 2 requires you to find the structures for the compounds.

1. Absorption comes from 4 conjugated double bonds and multiple unbonded e’s on oxygen atoms.

2. Naphthalene is obviously non-polar, so hexane would be appropriate
   Caffeine is fairly polar, so water or ethanol would be OK.

3. See deuterium spectrum at Wikipedia. The sharp peaks at 500-700 would be very difficult to deal because very small changes in wavelength result in large differences in intensity. The gain would be a problem.

4. Intensity is directly related to voltage.

5. Output from detector when there is no radiation hitting it. It reduces the sensitivity of the detector, because it is more difficult to tell what is due to radiation and what is dark current.

6. The number of detectors. UV/VIS peaks are generally 30-50 nm wide, so a 2 nm bandpass is more than adequate.