Exmoor Environmental Services

An Introduction to the Analysis of Metals by Flame Atomic Absorption

by

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1. Basic Theory

Introduction

Atomic absorption exploits the fact that metal *atoms* can be excited by certain wavelengths of radiation. Thus, if a water sample contains compounds of metal *M* the atoms of which are excited by radiation having a wavelength λ_M, then if that sample is irradiated with radiation having a wavelength λ_M, a certain amount of that radiation will be absorbed. How much is absorbed will depend on the concentration of *M* in the sample. By measuring the intensity of the incident radiation before and after it has passed through the sample it is possible to determine the concentration of *M*.

Now, in the environment most metals exist as ions which means that before analysis can take place they must be converted into atoms. The ions can be atomised by feeding them into a flame where the high temperature and collisions between the ions and combustion products provide sufficient energy for this to take place. Flame Atomic Absorption Spectroscopy (FAAS), is the technique that uses this means of atomisation and the flame is generated by burning acetylene with either air or, for hotter flames, nitrous oxide as the oxidant.

The technique is used almost exclusively with liquid samples which, in the case of environmental work, means samples in solution. Again, the bulk of such samples will be aqueous solutions, although some situations require that metals be extracted into organic solvents. Whatever the liquid, it enters the spectrometer by being drawn up through a capillary tube into a chamber where it is mixed with the acetylene and oxidant gases. The chamber is fitted with baffles to ensure that mixing is complete. The mixture then passes to the burner and into the flame where atomisation takes place and the atoms subsequently interact with the incident radiation.

The success of the technique depends on a number of factors which will be covered in the following discussions which will concern the technique of FAAS in general.

Atoms and Energy

Any atom contains a large number of orbitals that can contain electrons. Each orbital is associated with a specific amount of energy, which increases with the distance from the nucleus. In the normal atom, the electrons are located in those orbitals having the lowest energies. This is the *ground state* of the atom. An electron can be promoted to a higher energy orbital provided it is given sufficient energy and if this happens the atom is said to be excited (Figure 1.1).

When it is in its ground state an electron has an amount of energy E_0. To be promoted to a higher energy level having an amount of energy E_1, the electron needs to absorb an amount of energy (ΔE) equal to the difference between E_0 and E_1, that is:

$$\Delta E = E_1 - E_0 \qquad \textit{(Equation 1.1)}$$

The source of that energy can be radiation, heat or electrical discharge. However, the excited state is not stable and decays within nanoseconds (10^{-9} s) emitting the absorbed energy as radiation. Thus:

$$\underset{\text{Ground state atom}}{M(g)} + \text{energy} \rightarrow \underset{\text{Excited state atom}}{M^*(g)} \rightarrow \underset{\text{Ground state atom}}{M(g)} + \underset{\text{Radiation}}{h\nu}$$

From this, it follows that radiation having an energy equal to ΔE has sufficient energy to promote the electron again, so causing the atom to become excited.

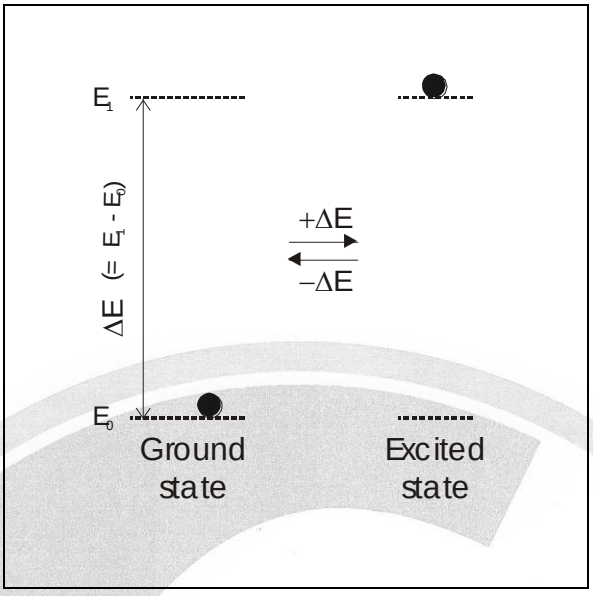

Figure 1.1: The transition of an electron between its ground and excited states

Now, the atomic energy levels are not the same for every element, therefore each metal has its own, individual set of energy levels. As a result, many of the wavelengths of radiation that will excite the atoms of one element will not excite the atoms of another element. This forms the basis of FAAS, since, by atomising metal ions in a flame, which is being irradiated with light having wavelengths that will only excite the atoms of a specific metal, it becomes possible to measure the concentration of that one metal in a mixture of several metals.

In practice, the flame is irradiated with radiation having the wavelengths capable of exciting the analyte atoms and its intensity is measured. Sample is then introduced into the flame and the intensity of the radiation is measured again. From the two readings it is possible to calculate the ***absorbance*** (see Beer's Law and Absorbance below) and thence, the concentration of analyte in the sample.

Radiation and Energy

Electromagnetic radiation is the means by which energy is transported from one place to another. This radiation consists of a waves of energy and a wave is characterised by its wavelength, λ. and its frequency, ν.

Wavelength, frequency and energy are inter-related so that the higher the energy the shorter the wavelength and the higher the frequency; the number of waves generated in one second. The relationships are as follows:

$$\Delta E = h.c/\lambda \qquad \textit{(Equation 1.2)}$$

where h = Planck's constant (6.626×10^{-34})
c = the speed of light in a vacuum (2.998×10^{8} m.s^{-1})

or, since $c/\lambda = \nu$:

$$\Delta E = h.\nu \qquad \textit{(Equation 1.3)}$$

Beer's Law and Absorbance

If a beam of radiation is incident on a sample containing an absorbing species, the intensity of that radiation will decrease, both as the path length through the sample gets longer and as the concentration of the absorbing species increases. This is a general statement of Beer's Law (or, more accurately, Beer Lampert's Law). More specifically, the Law can be expressed as follows:

$$I_t = I_0.10^{-act} \qquad \textit{(Equation 1.4a)}$$

or:

$$I_t/I_0 = 10^{-act} \qquad \textit{(Equation 1.4b)}$$

where I_0 = the intensity of the incident radiation
I_t = the intensity of the radiation that emerges from the sample, the transmitted radiation
c = the concentration of the absorbing species
t = the path length through the sample
a = a constant for a given wavelength at a given temperature

Taking common logarithms:

$$\log_{10}(I_t/I_0) = -a.c.t \qquad \textit{(Equation 1.4c)}$$

To get rid of the negative term on the right hand side of the equation, take the reciprocal:

$$\log_{10}(I_0/I_t) = a.c.t \qquad \textit{(Equation 1.4d)}$$

Redefine $\log_{10}(I_0/I_t)$ as ***Absorbance***, A, so that:

$$A = \log_{10}(I_0/I_t) = a.c.t \qquad \textit{(Equation 1.4e)}$$

From this it can be seen that when there is no absorption, $I_0 = I_t$, so $I_0/I_t = 1$ and, since the logarithm of 1 is 0, $A = 0$. As the amount of radiation absorbed increases so too does A as I_t decreases and, when I_t becomes infinitely small, A, becomes infinitely large. In practical spectroscopy, the values of A normally fall between 0 and 1 and, maybe, 2. By default, many systems are set to take measurements between 0 and 1 absorbance units, although the upper limit can be increased if necessary.

In most spectroscopic techniques measurements are taken on samples contained in cells of constant size so that t is kept constant. This is true even of FAAS as the path length through the flame is virtually constant. Therefore, if we let $a.t = k$, a combined constant, we have:

$$A = k.c \qquad \textit{(Equation 1.4f)}$$

This equation is of the general type $y = m.x + b$, which, when y is plotted against x, gives a straight line with a slope m and an intercept on the y-axis at b. In this case, if A is plotted against c, the result should be a straight line graph with a slope equal to k and an intercept of zero (Figure 1.2).

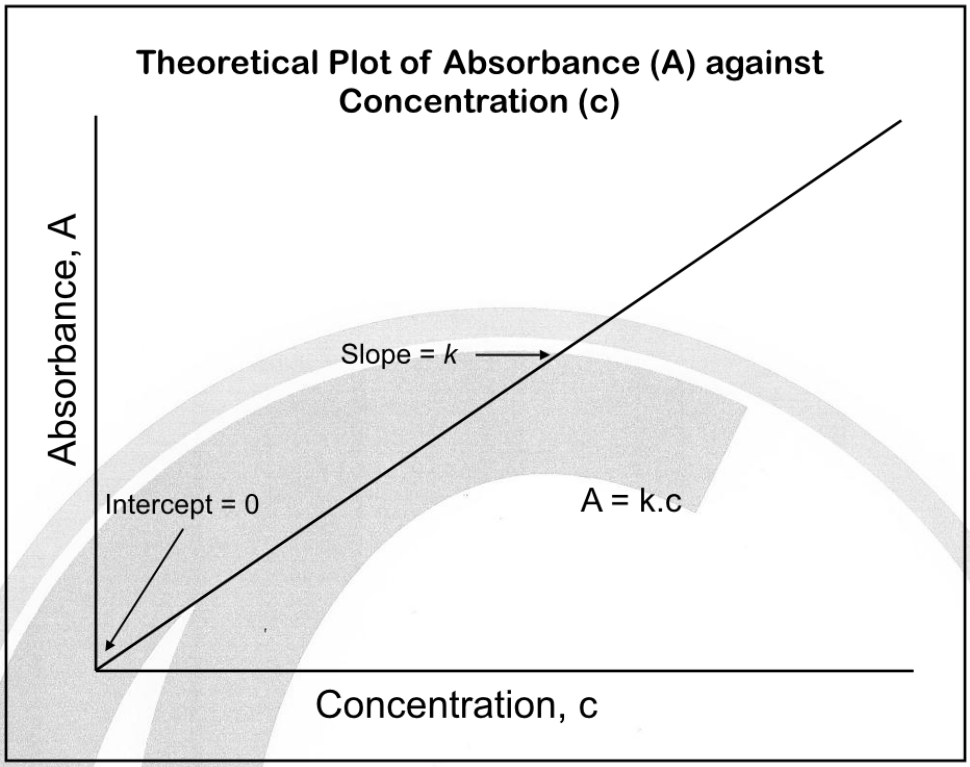

Figure 1.2: The variation of Absorbance with concentration as predicted by Beer's Law

In practice, this relationship does not hold for all concentrations of analyte and the graph becomes a curve flattening towards the *x-axis* (Figure 1.3).

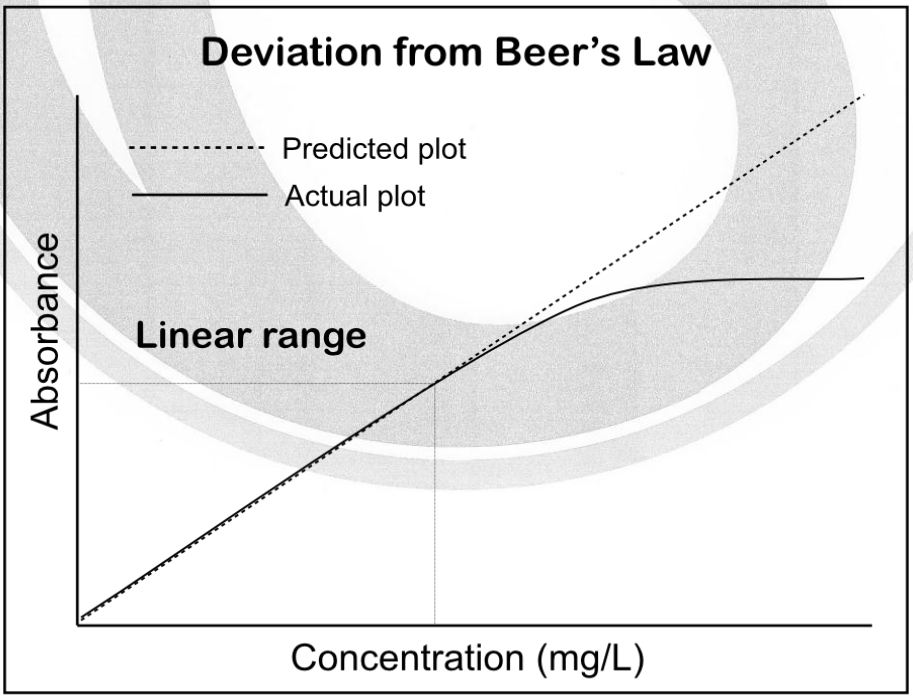

Figure 1.3: Plot of predicted absorbance and measured absorbance against concentration

The range of concentrations over which Beer's Law is obeyed is known as the ***Linear Range*** and it will vary with both analyte and analytical conditions. Despite this deviation, most analytical, spectroscopic techniques use Beer's Law to determine the concentration of analyte in a sample. For analysis by spectroscopic techniques, including AAS, the traditional method has been to prepare from three to five standard solutions, with concentrations spanning the linear range, and to present them to the instrument in turn. The absorbance of each solution is noted and plotted against concentration to give a calibration graph. The sample is then presented to the instrument and the absorbance noted. Providing it falls within the range of standards, the concentration of analyte in the sample can be read directly off the calibration graph.

With modern instruments, much of this is now handled by computer software. The computer is programmed to note the concentrations of the standard solutions and their absorbances. It then performs regression analysis on the results and prepares a calibration graph for the analyst to check. Noting the absorbance of the sample, the programme will then calculate the concentration of analyte. Such is the sophistication of the software, it can even handle curved calibration graphs; although it is better to arrange for sample responses to fall within the linear range, if possible. This will minimise the errors involved in accurately fitting curves to data; a notoriously difficult task. In this respect, computer software can misinterpret the data. Often the message comes up that the software has detected an 'S' shaped distribution. This is incorrect as the data points are following a straightforward curve but the computer programme has been fooled by the lie of the points at the lower concentrations of analyte. The programme then performs a third or fourth order polynomial calculation rather than the normal non-linear regression. This will affect the values calculated for the concentration of analyte in the samples. The problem arises when more than two standards are used to calibrate the instrument. Therefore, if a non-linear calibration is chosen for the evaluation of analytical results, the instrument should be calibrated using just two standards, the second standard being 3x the concentration of the first, unless curvature is severe. If the curvature is severe then three standards will be needed, the first having a concentration at the top of the linear range. The other two standards should then be 3x and 6x the concentration of the first. For a more extended discussion of this topic see Analytical Methods for Atomic Spectrometry.

2. The Atomic Absorption Spectrophotometer

Flame AAS

The instrumentation involved in atomic absorption is basically very simple and it comprises a:

- radiation source
- deuterium lamp
- burner
- monochromator
- photo multiplier tube to measure the radiation coming from the radiation source
- series of mirrors to direct the beam of radiation to the various components
- means of displaying the results of an analysis

In addition to the above there is a nebuliser that converts the liquid sample into a fine spray and causes it to become intimately mixed with the acetylene-oxidant mixture prior to its entering the flame. For details, see the references at the end of this document.

In practice, radiation from the radiation source is directed through the flame, into which the sample is being aspirated. From here it passes on to the monochromator which selects the wavelength of interest and directs it on to the photo multiplier tube, where its intensity is measured. The absorbance and concentration of analyte are then calculated and the results displayed.

On older models of spectrophotometer, it is often the case that only the absorbance is displayed. When using such instruments, it is necessary to manually record the concentrations and absorbances of the standard solutions, plot a graph of absorbance against concentration and interpolate the absorbance of the samples. If the plot is linear then it is possible to use a computer programme such as Excel, or a dedicated statistics package, to perform linear regression analysis on the calibration results and use the results of this to calculate the concentrations of the samples.

Modern instruments are generally controlled by computers and so all the data generated during an analysis are manipulated by the software. As a result, the analyst, having made all the usual preparations for an analysis, has to enter the name of the analyte, the method to be used in the analysis, the concentrations of the calibration standards and sample details, including their positions in the auto analyser tray, into the computer. Then, the standards and samples are aspirated in turn into the flame of the spectrophotometer. The computer processes the signals, performs the computations and sends the results of the analysis to a printer. Modern system can even handle non-linear calibration plots so that analysts are not necessarily restricted to working with concentrations which fall only in the linear range of the curve.

The Spectrometer Components

The major components of an atomic absorption spectrophotometer are the:

- Source of radiation
- Burner head
- Monochromator
- Radiation detector

Since these can vary from instrument to instrument, so the the following will be a general discussion only.

Radiation Source: The most commonly encountered source is the ***hollow cathode lamp*** (HCL) and this is what will be found in the Aanalyst 300. The HCL is the component which gives AAS its specificity since there is a specific lamp for every metal that can be analysed by this technique. Details of the construction of an HCL and how it works will be found in the references listed in the bibliography. The output from the lamp depends very much on the current that is applied to it and this in turn affects both the sensitivity and the precision of the measurements. Lowering the lamp current increases sensitivity but decreases the precision, since the system is more sensitive to the background noise as well as to the signal from the lamp. Conversely, increasing the lamp current reduces sensitivity but precision is improved. In practice, for many elements a lamp current is chosen which is a compromise and gives optimum sensitivity at an acceptable level of precision. The software normally imposes a default value which is one that is commonly used, but this can be changed to suit the needs of a particular analysis.

In addition to the HCL, a deuterium lamp will also be present. This is used to provide ***background correction*** in situations where absorbance is increased as a result of materials other than analyte being present in the flame and interfering. A full explanation of this interference is given in Beaty and Kerber and certain aspects are discussed in the next section, Preparing the Atomic Absorption Spectrophotometer.

Burner Head: This is a hollow titanium block that sits on the nebuliser chamber. There is a narrow slot in the top surface and this is either 10cm long of 5cm long depending on which oxidant is being used with the acetylene. The 10cm burner head is used for the air-acetylene flame and the 5cm head with nitrous oxide-acetylene flame. In normal practice, the radiation passes along the length of the flame to maximise the path length. However, since the flame is very narrow the beam of radiation must be focussed very accurately. So, the burner head has to be positioned with great care to maximise the chances of absorption.

The object of the flame is to atomise the metal ions as they emerge from the nebuliser. Since the degree of atomisation varies vertically within the flame, care must be taken to arrange the burner height so that the radiation passes through the region of greatest atomisation.

Monochromator: The beam of radiation enters the monochromator where it is separated into its constituent wavelengths by a diffraction grating. By changing the position of this grating it is possible to direct only the wavelength of interest to the radiation detector. This measures the intensity of the radiation. Thus, the monochromator enables the most appropriate wavelength to be selected for a particular analysis.

The radiation enters and leaves the monochromator through slits, the width of which can be varied to control the amount of light incident on the grating, and thence on to the radiation detector. Both the entrance slit and the exit slit should be the same width. As with the lamp current, the most common slit width for the analysis of a given metal is set by the computer. However, as in the case of the lamp current, it is possible for the analyst to alter the width to suit a particular situation.

Once again, a full discussion of the structure and function of the monochromator will be found in Beaty and Kerber.

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Radiation Detector: In atomic absorption spectroscopy, the radiation detector is a photo multiplier tube (PMT), details of which will be found both in Cresser and in Lajunen. It consists of a photo emitting cathode and a series of dynodes coated with a material that will emit electrons when bombarded with electrons. There is also a terminal anode to collect all of the electrons emitted by the dynodes. Since one electron hitting the surface of a dynode will cause several electrons to be emitted from that surface, the number of electrons emitted increases exponentially through the detector. As a result, an appreciable current is generated when all these electrons reach the terminal anode. The sensitivity of the photo multiplier can be varied by changing the potential difference across the electrodes, but, as with the other parameters, photo multiplier sensitivity is optimised by the controlling software and, unlike many of the other parameters, cannot normally be altered by the analyst.

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3. Preparing the Atomic Absorption Spectrophotometer

Instrument set up

Before any analyses can be performed it is necessary to adjust the various instrumental components so that they are operating at their optimum effectiveness. From the previous discussion it will be obvious that the objects which need to be adjusted are the

- Lamp and lamp current
- Slit
- Wavelength
- Detector sensitivity
- Gas ratio
- Burner height and angle

When a method file for a particular element is opened, it will be found that default values have been set by the computer for the first five parameters. However, all but the detector sensitivity can be changed by the analyst should it be necessary. The most common reason for changing any of the settings is to alter the sensitivity or, in the case of the wavelength, to change the linear range of the calibration standards. For example, if a series of samples contains higher concentrations of analyte, some of which fall outside the linear range of the default wavelength, then it might be better to set a wavelength at which the linear range is greater. This will avoid diluting the samples, so introducing another potential source of error. Although for some models of AAS the burner height and location relative to the radiation beam are computer controlled, this is not always the case. Consequently, these parameters have to be optimised manually by the analyst.

One other decision that may have to be taken is whether to use background correction or not. This is generally decided by which wavelength is chosen for the analysis. If it is less than 250nm then it is advisable to have background correction on. This topic is discussed more fully below.

Lamp Current

It is important to realise that the lamp current can have a significant effect on both the sensitivity and precision of the instrument. As mentioned earlier, a decrease in current leads to an increased sensitivity but with a corresponding loss of precision, while an increase in lamp current leads to decreased sensitivity but greater precision. In most systems the computer is programmed to set the most commonly used value for the current. However, should greater or less sensitivity be required then it is possible to change the lamp current accordingly, the changes being made either in the appropriate method file or in the Lamps Window

Slit

Once it has travelled through the flame, radiation from the lamp passes to the monochromator via a slit and then exits to the detector through a second slit. Both the entrance and exit slits are set to be the same width. The width of these slits can be varied, so changing the amount of radiation which reaches the detector. The actual width set depends on the dispersion power of the monochromator which, in turn, depends on the number of grooves etched in the surface of the grating.

Once again, the computer sets a default value for every metal and, while it can be changed by the analyst, it is probably better to leave the default value unchanged for most analyses.

Wavelength, Sensitivity and Linear Response

(a) Sensitivity: The wavelength determines the sensitivity of the analytical procedure because not all emitted wavelengths have the same intensity. It follows that the more intense the radiation beam then the more sensitive will be the instrument's response to low concentrations of analyte. Conversely, the less intense the emitted beam then only the more concentrated solutions of analyte will be able to bring about a measurable absorbance.

Sensitivity and Characteristic Concentration

Sensitivity is actually defined as:

> "The concentration of analyte that will absorb 1% of the incident radiation, so giving an absorbance of 0.0044."

This absorbance can be calculated using Equation 1.4e. Thus, if the incident radiation has an intensity of 100% and if there is a drop of 1% in intensity as it passes through the analyte solution, then the intensity of the transmitted radiation is 99%. Now:

$$A = \log_{10}(I_0/I_t).$$

Therefore, when $I_0 = 100$ and $I_t = 99$

$$A = \log_{10}(100/99)$$

$$= 0.0044$$

The concentration of analyte that gives an absorbance of 0.0044 is known as the ***Characteristic Concentration***, and the smaller this concentration the greater is the sensitivity. For example, consider iron. The most intense emission occurs at 248.3nm and the characteristic concentration at this wavelength is 0.1mg/L. The next most intense emission occurs at 302.1nm but, at this wavelength, the characteristic concentration is 0.4mg/L. In other words, it takes a fourfold increase in concentration to give the same absorbance at 302.1nm. Thus, the sensitivity at this wavelength is only a quarter of that at 248.3nm.

(b) Linear Response: One consequence of the lower sensitivity is that the ***linear range*** usually increases; the linear range being that range of concentrations over which the absorbance increases linearly. With iron at 248.3nm, the wavelength at which sensitivity is greatest, the response is linear up to 6.0mg Fe/L. At the less sensitive wavelength of 302.1nm the response is linear up to 10.0mg Fe/L.

(c) General settings: In most systems the computer generally selects the wavelength which is emitted with the greatest intensity. This, then, is the most sensitive setting and the one with the shortest linear range. As mentioned before, it is possible to change this default setting to suit a particular situation. For example, if a sample has a higher than usual concentration of analyte then, rather than dilute the sample to bring the concentration within the linear range of the more sensitive wavelength, it would be better to change the wavelength to a less sensitive setting. Not only does this avoid including an additional source of error in the dilution step but it is likely to give an extended linear range as well.

The Gas Ratio

The ratio of acetylene to oxidant controls the temperature of the flame. As the ratio of acetylene to oxidant becomes smaller, combustion becomes more complete, the flame becomes bluer and less luminous and the temperature rises accordingly. Thus, a flame in which the acetylene-air ratio is 2:10 is hotter than a flame in which the ratio is 3:10. Another factor that changes with the gas ratio is the reducing property of the flame. With higher acetylene to air ratios combustion of the acetylene is not complete so that the products of combustion products include carbon and, more importantly, carbon monoxide. Thus, flames

in which there are high acetylene to air ratios are not only more smoky but they are reducing flames as well. As the proportion of acetylene becomes smaller so combustion becomes more complete and the flame becomes ***leaner***. Consequently, there is less and less carbon monoxide, with the result that the flame becomes less reducing and more oxidising.

Flame temperature is important in that it can affect the atomisation process. Metals such as sodium, potassium and calcium have relatively low ionisation potentials, which means that, at higher temperatures a greater proportion of the metal exists in the flame as ions. This creates a problem in that the flame needs to be hot enough to break completely the bonds between metals, such as calcium, and the other elements in their compounds. Indeed, metals like aluminium, which bond strongly to elements such as oxygen, need the even hotter acetylene-nitrous oxide flame to ensure complete dissociation. With increasing temperature, however, there is increased ionisation of the metal and a consequent loss in sensitivity. In practice, an easily ionisable salt is added to the samples and standards. This suppresses the ionisation of analyte atoms by increasing the concentration of electrons in the flame so pushing the equilibrium below (Equation 4) to the left.

$$M_{(g)} \rightleftharpoons M^{n+}_{(g)} + ne^- \qquad \textit{(Equation 3.1)}$$

Often the computer will set an acetylene:air ratio of 3:10 which gives a flame suitable for general analytical work. However, when optimising the instrumental settings for sensitivity then this ratio will probably have to be changed. Indeed, when a method specifies a lean, oxidising flame then a ratio of 2:10 is more appropriate.

The Burner Head Settings

The alignment of the burner head, radiation beam and radiation detector is critical. The beam, which must be centred on the detector, must pass through the flame, and it must pass through the correct region of the flame for maximum effect. The flame is wide, to provide a long path length, but it is very narrow. Therefore, the horizontal positioning of the burner and the beam has to be very precise, as Figure 3.1 illustrates.

To check the path of the radiation it is necessary, before igniting the flame, to switch on a lamp and place a white card over the circular exit to the burner compartment. The beam should pass through the centre of the exit. If it does, then it is properly aligned with the detector. Now place the card on the burner head at right angles to the beam, which should be centred over the slot. If it is not, then the burner head must be moved, using the horizontal and rotational adjustments, until the slot and beam path are aligned correctly.

If the beam is not aligned correctly with the detector, then it is likely that one of the mirrors, that send the radiation to where it should go, has moved. This is the most common cause for situation (d) in Figure 5. Again, if the burner and light beam are correctly aligned but radiation seems not to be reaching the detector then it is possible that a mirror between the exit to the burner compartment and the detector has moved and is misdirecting the beam.

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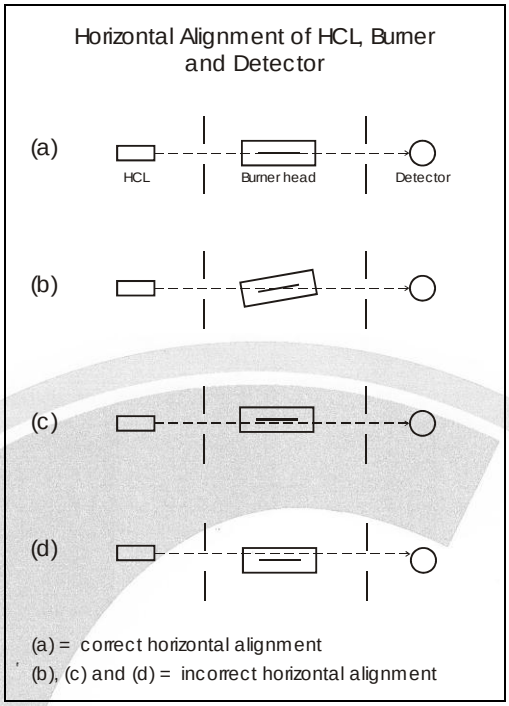

Figure 3.1: Correct and incorrect horizontal alignments of burner and radiation beam

Note that, although situation (b) in Figure 3.1 is said to be incorrect, there may well be a time when the analyst will want to alter the angle of the burner head. By doing this he/she is shortening the path length of the radiation so reducing sensitivity. This manoeuvre, done consciously by one who understands what he/she is doing, sometimes enables samples with higher concentrations of analyte to be analysed without dilution.

The vertical position of the burner is just as important, as Figure 3.2 shows.

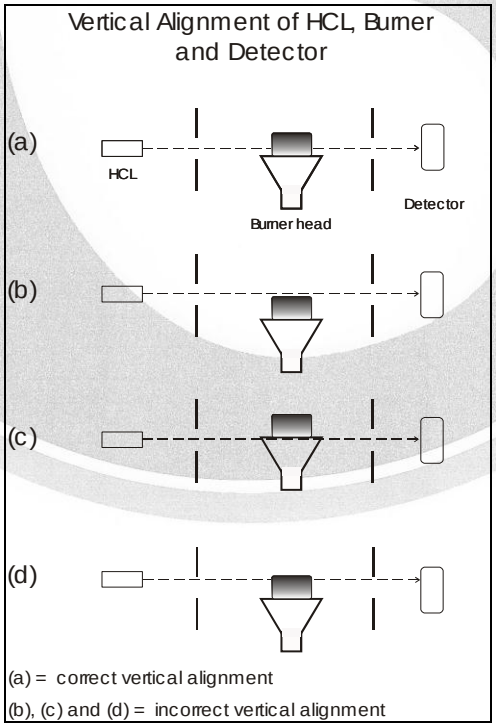

Figure 3.2: Correct and incorrect vertical alignments of burner and radiation beam

In (a), the beam is passing through the hottest part of the flame where the greatest degree of atomisation occurs. In the other situations this is not happening and so absorbance will be depressed. As with the horizontal alignment, situation (d) is most likely to be caused by a displaced mirror.

In practice, the burner height is checked by lighting the flame and aspirating a standard solution while irradiating with the appropriate radiation. The absorbance is observed while using the vertical adjustment to change the height of the burner. When the burner is at its optimum height, absorbance is at a maximum.

Radiation Scattering, Wavelength and Background Correction

Radiation can be subject to scattering as it passes through the flame. This occurs when high concentrations of non-absorbing species are present and physically get in the way of the radiation beam, deflecting it from away from the path to the detector. Therefore, less radiation reaches the detector, so giving the false impression of high absorbance and, consequently, overestimating the concentration of analyte present.

Another phenomenon that can give erroneously high absorbance readings is the presence of molecular species in the flame which absorb wavelengths close to that absorbed by the analyte atoms. While atomic absorption gives very sharp absorbance peaks, molecular absorption gives broad absorbance peaks extending over a large number of wavelengths. If these peaks should overlap with the analytical wavelength then the detector will record the absorption of both the analyte and the interfering molecules. Once again, the concentration of analyte will seem to be higher than it really is.

Not all wavelengths are affected equally and those that are affected most, at least by the scattering, are the wavelengths below 250nm. To overcome this, for analyses using wavelengths of 250nm or less, background correction is employed. The technique is discussed fully in Beaty and Kerber but, briefly, it involves passing a continuous spectrum of wavelengths from a deuterium lamp through the flame. The intensity of this beam is measured both before and after passing though the sample and the degree of scatter calculated. A correction factor is then applied to the absorbance recorded at the analytical wavelength, so providing a more accurate measure of the absorbance due to the analyte alone.

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