Analytical Science

A course (in 15 Chapters), developed as an Open Educational Resource, designed for use at 2nd year England & Wales undergraduate level and as a CPD training resource

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Description	This chapter considers the mathematical procedures used to obtain quantitative analytical measurements. It includes, units of measurement, use of calibration graphs, standard addition and internal standard procedures. It compares the uses of absolute and comparative techniques to obtain analytical data and reviews volumetric and gravimetric procedures.
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Chapter 4: Analytical Process Unit 6 – Measurement techniques

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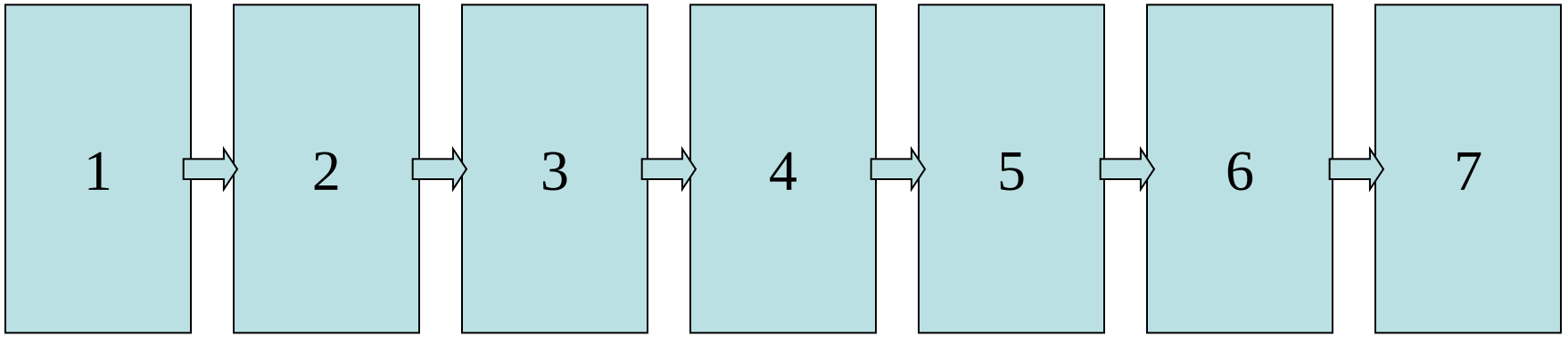
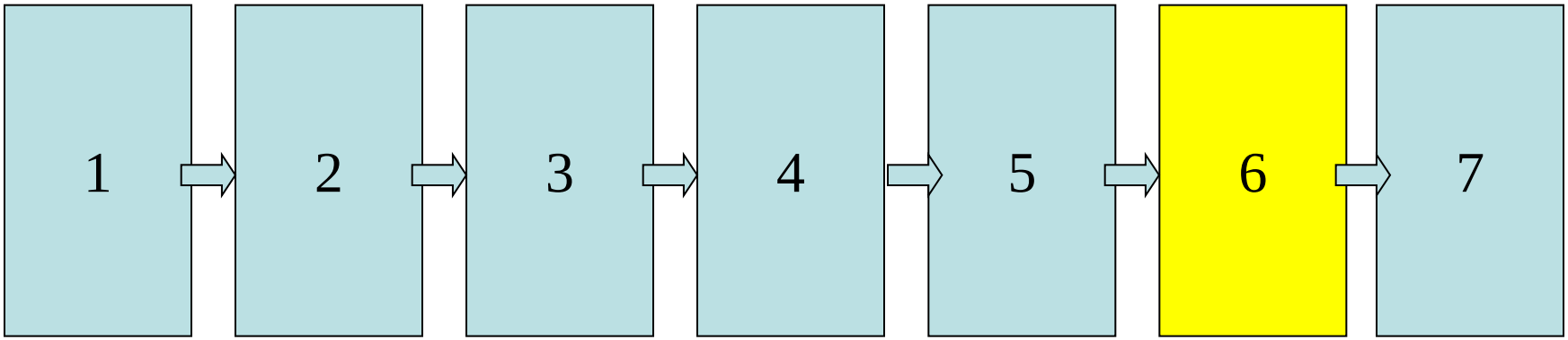
The analytical process model – revision slide

Any analysis may be considered as consisting of a maximum of seven unit processes. These are shown diagrammatically and descriptively below:



Unit 1. Consider the problem and decide on the objectives
Unit 2. Select procedure to achieve objectives
Unit 3. Sampling
Unit 4. Sample preparation
Unit 5. Separation and/or concentration
Unit 6. Measurement of target analytes
Unit 7. Evaluation of the data, have the objectives been met?

Process unit 6 – measurement of target analytes



Having agreed the information required from the analysis, taken the sample, prepared the sample and separated out any potential interferences, we eventually arrive at the stage of performing the analytical measurement. Many of the measurement techniques currently popular for performing this function are considered in chapters 7 - 13 of the teaching and learning programme. So in this section, consideration will be given to looking at the some of the generic aspects of quantitative measurement. A list of topics to be covered in this Process unit is given on the next slide.

Introduction

The terms – **Technique, Method** and **Procedure** are all frequently used to describe an analysis. However they do relate to individual aspects of an analysis and as such therefore should not be used indiscriminately.

Technique

A chemical or physical process by which a separation or a measurement is carried out. For example, solvent extraction, IR, UV & NMR spectroscopies, potentiometry, are all analytical techniques.

Method

A combination of techniques used to produce an analytical measurement. For example, to determine the trace mercury content of a fish liver sample, the sample will need to be digested and the mercury possibly concentrated before the final measurement is carried out.

Procedure

The set of detailed instructions to carry out an analysis. In statutory terms this is often referred to as a Standard Operating Procedure (SOP)

Categories of techniques

Analytical techniques have been and continue to be categorised into one of the following categories:

Classical;
Instrumental;
Chemical;
Physical;
Destructive;
Non-destructive.

In addition, it is often found that pairs of these categories are compared together, for example:

- **Classical** *versus* **Instrumental**
- **Chemical** *versus* **Physical**
- **Destructive** *versus* **Non-destructive**

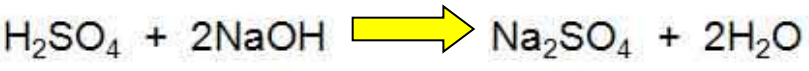
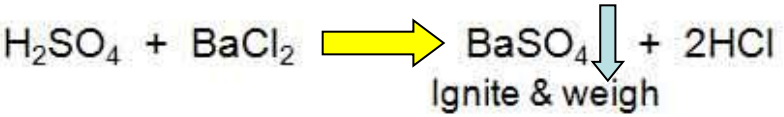
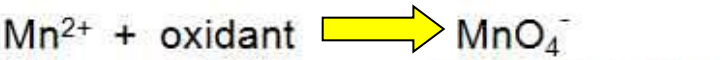
These will be explained in the following three slides, however it should become apparent that none of these descriptions are exclusive.

Classical *versus* Instrumental

Classical techniques	Instrumental techniques
This classification refers to those techniques used to obtain analytical information early in the 20th century. They are generally considered as: **Titrimetric** (volumetric) techniques; **Gravimetric** techniques.	The first instruments to be used to obtain analytical information first appeared early in the 20th century and were based upon spectral measurements recorded onto photographic plates. Nowadays the vast majority of analyses are carried out using sophisticated instrumentation. Most measurements are based upon principles of spectroscopy or electrochemistry, with a small group of techniques relying on measurements of weight or heat changes.

Comment: Although gravimetric techniques are generally classed as 'Classical', they required the use of an analytical balance to provide the final measurement. Would you class a balance as an instrument?

Chemical *versus* Physical

Chemical techniques	Physical techniques
Chemical techniques refer to those utilising a chemical reaction to create an analytical measurement. Examples include: **Titrimetric** analysis, for instance:  **Gravimetric** analysis, for instance:  Ignite & weigh **Colorimetric** analysis, for instance:  deep purple colour	Physical techniques refer to any analytical measurement based upon physical properties of the species (atoms, ions, compounds etc.). The techniques involved are the same as those categorised as 'Instrumental' and include: ▪ Spectroscopic principles of emission and absorption of radiation: ▪ Electrical measurements – current, voltage and resistance; ▪ Measurements of temperature, enthalpy and thermal conduction; ▪ Measurement of weight; ▪ Measurement of polarised light

Comment: Again, the difference between the two categories is blurred, in that gravimetric analyses require the use of a balance, colorimetric analyses require the use of a colorimeter or visible range spectrometer and titrimetric methods can use physical methods for detecting end points.

Destructive *versus* non-destructive

Destructive techniques	Non-destructive techniques
Refers to any measurement technique where it is not possible to recover the analyte after measurement. These include: ▪ Any measurement technique involving a chemical reaction; ▪ Any measurement techniques which involves a change of state (eg: atomic spectroscopic techniques); ▪ Any measurement technique that requires the sample to be incorporated into a matrix from which it is difficult to recover (eg: IR spectroscopy involving the use of 'Nujol' mulls, or X-ray techniques involving borax beads)	Refers to any measurement technique, where the sample may be recovered after the measurement has been made. These include: ▪ X-ray diffraction and some X-ray fluorescence techniques; ▪ Some spectroscopic techniques where the sample has only been dissolved in a suitable volatile solvent (eg: visible, UV & IR techniques). This can also apply to some chromatographic detectors; ▪ Simple 'dip-type' measurements (eg: pH and those measurements frequently carried out by medical personnel on urine samples) ▪ Measurements using changes in polarised light.

Units of measurement

Although in many areas of theoretical chemistry the use of **Molar** concentration units predominates, in industrial analytical situations, a combination of units are regularly used. These include units expressed in terms of:

- Molar, millimolar quantities;
- Molal solutions;
- Normality;
- Mass per volume quantities;
- Mass per mass quantities;
- Volume per volume quantities.

Mathematically these mass and volume quantities may be expressed in terms:

- Percentage (%);
- Quantity relationship to the l (dm^3) or the kg;
- Trace units expressed as ppm (parts per million), ppb (parts per billion) and ppt (parts per trillion).

The following slides considers each of these expressions of quantity in greater detail. It is also frequently necessary to be able to convert between units and this also will be described in these slides.

Expressing quantity in molar & molal terms

Definition of a molar (M) solution

A gram molecular mass of solute dissolved in water and diluted to 1 dm^3.
Units may be expressed as mol l^{-1}, mol dm^{-3}, 10^3 mol m^{-3}

Definition of a millimolar (mM)solution

A gram molecular mass of solute, divided by 1000, dissolved in water and diluted to 1 dm^3
Units generally expressed as mmol l^{-1}, mmol dm^{-3}

Definition of a molal (m) solution

A gram molecular mass of solute dissolved in 1000g of solvent.
This term is most frequently found in physiochemical measurements

Although the SI unit for concentration is mol m^{-3}, analysts are generally more practical people and therefore relate their units to volumes that can actually be handled.

Expressing quantity as Normality

Normality was a concentration term popular until around 50 years ago, however still persists in a small number of industries and of course may still be found in older copies of textbooks and the scientific literature. A one – **normal** (N) solution contains one **equivalent** per litre of solute.

Definition of equivalent

An equivalent, represents the mass of material containing Avogadro's number of reacting units. Reacting units can be protons or electrons.

Example (4.i)

A 1 **molar** solution of HCl is also a 1 **normal** solution as HCl has only a single proton that can react within an acid/base reaction. A 1 **molar** solution of H_2SO_4 on the other hand, will equal a 2 **normal** solution, as the acid has 2 available protons.

The ionic equation for a permanganate oxidation in acid solution is shown below:

$$MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O$$

This shows $5e^-$ being required to complete the reduction. Therefore a 1 **molar** solution of potassium permanganate is equivalent to a 5 **normal** solution. It is interesting to note that in many laboratories the standard permanganate solution is 0.02 M (0.1 N)

Expressing quantity in mass *per* volume (w/v) units

Units which are popular within this category are:

- **g/100 cm^3 (%)**
- **g/dm^3 (g/l)**
- **kg/m^3**
- **µg/cm^3, mg/dm^3, g/m^3 (ppm w/v)**
- **ng/cm^3, µg/dm^3, mg/m^3 (ppb w/v)**
- **ug/m^3 (ppt w/v)**

Note: In this programme a decision has been take to use units as described. Alternatively they can be presented as: (mass volume^{-3}) for instance:

mg dm^{-3}, µg cm^{-3}, g m^{-3}

The use of ppm and ppb units are popular in trace metal analysis, the choice being dependent upon the level of metal detected. Any quantity less than 0.01 ppm, (parts per million) would generally be expressed as a ppb (parts per billion) unit. These units are also used in air quality monitoring where atmospheric concentrations are expressed as ppt (parts per trillion) and ppb quantities

Note: mg = 10^{-3} g ; µg = 10^{-6} g ; ng = 10^{-9} g

It is possible to use a range of units to describe the concentration of a given solution. Example (4.ii) below shows how %, g/dm^3 and ppm could all be used quite logically to describe a pure dilute metal ion solution.

Example (4.ii)
:
To prepare a pure standard solution of gold, 1.000 g of Pure gold is dissolved in aqua regia and diluted to 1 dm^3. The concentration of the resultant solution may be expressed as:

0.1000 % (w/v);
1.000 g/dm^3;
1000 ppm (w/v)

Because, ppm, ppb & ppt are not well understood outside the scientific community, it is usual for concentrations To be expressed in simple weight per volume terms as illustrated in Figure (4.1). Note the use of litres instead of dm^3

Figure 4.1 - typical analysis of a sample of mineral water showing mineral concentrations in mg/l

Expressing quantity in mass *per* mass (w/w) units

Nutrition

Typical values	per 37.5g serving with 150ml of semi-skimmed milk	per 100g
Energy	836kJ 198kcal	1432kJ 338kcal
Protein	9.3g	11.5g
Carbohydrate	32.9g	68.4g
of which sugars	8.9g	4.4g
Fat	3.2g	2.0g
of which saturates	1.7g	0.6g
Fibre	3.8g	10.0g
Sodium	0.18g	0.26g
Niacin	5.9mg	15.3mg
% RDA	33	85
Thiamin (B1)	0.5mg	1.2mg
% RDA	36	85
Riboflavin (B2)	0.8mg	1.4mg
% RDA	49	85
Folic Acid	73µg	170µg
% RDA	36	85
Iron	4.5mg	11.9mg
% RDA	32	85

Figure 4.2 – nutritional information on a cereal packet

(RDA – recommended daily amount)

Units that are popular within this category:

- **g/100 g (% w/w);**
- **mg/100 g;**
- **g/kg;**
- **µg/g, mg/kg (ppm w/w);**
- **ng/g, µg/kg (ppb w/w)**

NUTRITION INFORMATION

Typical Values	Per 100g	Per 210g
Energy (kJ)	370	777
Energy (kcal)	87	183
Protein (g)	4.8	10.1
Carbohydrate (g)	16.1	33.8
Of which sugars (g)	5.9	12.4
Fat (g)	0.4	0.8
Of which saturates (g)	0.1	0.2
Fibre (g)	5.5	11.6
Sodium (g)	0.3	0.7
Salt Equivalent (g)	0.9	1.8

Figure 4.3 - nutritional composition of a sample of baked beans

These units are likely to be used for example in the following situations:

- Assay of mineral ores;
- Measurement of nutritional components in foodstuffs [see figures (4.2) & (4.3)]
- Specifying purity of commercial chemicals.

Expressing quantity in volume *per* volume (v/v) units

Units that are popular within this category:

- $cm^3/100\ cm^3$ (% v/v);
- cm^3/dm^3;
- cm^3/m^3 (ppm v/v);
- mm^3/m^3, $\mu l/m^3$ (ppb v/v)

The most popular use of the % terminology is in the expressing the ethanol content of alcoholic drinks. Popular brands of beer contain between 3.6 – 5.2 % (v/v) ethanol and a bottle of wine will typically contains between 11.0 – 14.5 % (v/v) ethanol.

Air quality monitoring websites frequently quote concentration levels of pollutant gases in the atmosphere in both $\mu g/m^3$ and **ppb** units. Although not specifically mentioned these **ppb** units relate to v/v comparisons.

Example of comparison figures:

95 $\mu g/m^3$ ≡ 49 ppb for NO_2 [quoted data]

$$95\ \mu g\ NO_2 \equiv (95 \times 10^{-6}) \div 46\ moles/m^3\ of\ NO_2$$
$$= 2.06 \times 10^{-6}\ moles/m^3$$
$$\equiv 2.06 \times 10^{-6} \times 24000\ cm^3/m^3\ of\ NO_2$$
$$= 0.049\ cm^3/m^3$$
$$= \mathbf{49\ ppb\ (v/v)\ NO_2}$$

Gram molecular volume is approximately 24,000 cm^3 at 20°C

Mol. Mass of NO_2 = 46

Conversion between units

It is frequently necessary to be able to convert concentrations expressed in one unit form into another unit form. Where molar or molal solutions are being converted to mass/volume units or *vice-versa,* it is necessary to know the relative atomic masses of the elements involved.
[**http://www.standnes.no/chemix/periodictable/realtive-atomic-mass.htm**]
Methods of achieving this conversion are best illustrated by examples as shown below and on the next slide.

Example (4.iii) – convert a 0.1 M solution of Fe^{3+} into % (w/v) of Fe

0.1 M Fe^{3+} ≡ 0.1 X 55.8 g/dm^3 = 5.58 g/dm^3 ≡ **0.558 g/100 cm^3 (% w/v)**

Example (4.iv) – what volume of 0.05 M $K_2Cr_2O_7$ is required to prepare 100 cm^3 of a standard Cr solution containing 1000 ppm (w/v) of Cr?

A **0.05 M $K_2Cr_2O_7$** solution contains 0.05 X 52.0 X 2 g/dm^3 of Cr = 5.20 g/dm^3

To prepare 100 cm^3 of a 1000 ppm solution requires 100 X 1000 µg of Cr = 0.1 g Cr

Volume of the 0.05 M solution required is therefore (0.1 ÷ 5.2) X 1000 cm^3 = **19.23 cm^3**

Example (4.v) – express a 1000 ppm (w/v) of K in molar units

1000 ppm = 1000 mg/dm^3 = 1 g/dm^3
The relative molar mass of K is 39.1 and thus:
1 g/dm^3 represents a 1 ÷ 39.1 M solution = **0.255 M**

Example (4.vi) – express an aqueous solution of 12.0 % (v/v) ethanol in both (w/v) and molar units.
Ethanol (C_2H_6O) has a molar mass of 46 and a density of 0.78 g/cm^3
12.0 % (v/v) = 12 cm^3/100cm^3 = 120 cm^3/dm^3 ethanol
≡ 120 X 0.78 g/dm^3 ethanol
= **93.6 g/dm^3** ethanol
≡ 93.6 ÷ 46 M
= **2.03 M**

Example (4.vii) – express a 50 ppb (v/v) concentration of SO_2 in air, measured at 20°C in ppt (w/v) [Note: 1 mole of SO_2 at 20°C occupies 24 dm^3]

50 ppb (v/v) = 50 mm^3/m^3 = 0.05 cm^3/m^3
≡ (0.05 ÷ 24000) moles/m^3 of SO_2
= 2.08 µmoles/m^3 of SO_2
= 2.08 X 64 µg/m^3
= **133 ppt (w/v)**

Quantitative methodologies

Quantitative methods and techniques may be described as being either:

- **Absolute** or
- **Comparative**

Definition of an absolute technique

Analytical techniques involving a chemical reaction which achieve stoichiometric completion in accordance with the chemical equation for that reaction

The techniques that come within this category are essentially those which earlier were described as classical, together with a few instrumental techniques where measurements are made solely on weight differences or the use of fundamental constants. Table (4.1) shown on the next slide lists those techniques that come within this category

Definition of a comparative technique

Comparative analytical techniques are those which require calibration against known standards, in order for accurate quantitative data to be obtained.

Absolute analytical techniques

Technique	Property measured
Gravimetry	Weight (mass)
Titrimetry (volumetric analysis)	Volume of liquid (titrant)
Gasometry	Volume of gas generated measured at STP
Coulometry	Charge transferred (current X time)
Electrogravimetry	Weight (mass)
Thermogravimetry	Weight (mass)

Table 4.1– property measured for individual techniques

With the exception of thermogravimetry, where sample sizes are small (few mg), the other techniques offer the potential of generating data to a high degree of accuracy and precision and are thus frequently used to produce the standards used to calibrate comparative techniques.

Importance of chemical equilibrium in absolute analytical techniques

All absolute techniques are based upon that the assumption that **the chemical reaction on which the analysis is based, achieves stoichiometric completion In accordance with the equation that defines the reaction**. A general reaction may be expressed by the following equation:

$$aA + bR \rightleftharpoons cC + dD$$

Where: A represents the Analyte, R the Reagent and C & D the Products

As this equation represents an equilibrium between **Reactants & Products**, the forward and back reactions may be expressed as individual rate constants:

$$Rate_{forward} = k_f [A]^a [R]^b \text{ and } Rate_{backward} = k_b [C]^c [D]^d$$

Where: K_f & k_b are the rate constants and [ ] represent molar concentrations

For a system at equilibrium, $Rate_{forward} = Rate_{backward}$

Thus, the equilibrium constant (K) for the reaction may be defined as:

$$\mathbf{K = \frac{k_f}{k_b} = \frac{[C[^c\,[D]^d}{[A]^a\,[R]^r}} \qquad \text{Equation (4.1)}$$

Continued on next slide

The value of ‘K’ can be calculated empirically by measuring the concentrations of A, R, C, D at equilibrium. **The larger the rate constant for the forward reaction relative to the backward reaction, the larger will be the equilibrium constant and the farther to the right the reaction will be at equilibrium**. This is the situation required for a reaction said to achieve **stoichiometric completion in accordance with the chemical equation for that reaction**.

It should be recognised that the value of ‘K’ is dependent upon the individual rate constants for the forward and backward reactions and that these are dependent upon:

- Temperature and/or pressure of the reaction;
- Presence or absence of catalysts;

It is necessary to point out that even though a particular reaction may normally have a high value of ‘K’, the presence of high concentrations of product species in the sample, could prevent the reaction moving stoichiometrically to completion.

Note: a more comprehensive discussion of equilibrium may be found in any Textbook of Physical Chemistry and in most textbooks on general Analytical Chemistry. There are also many WEB references including: [http://en.wikipedia.org/wiki/Chemical_equilibrium]

Titrimetry (volumetric analysis)

There are four types of volumetric analysis, the differences being based upon the type of chemical reaction involved:

- **Acid/base –** can be used to analyse both inorganic and organic acids and bases, by titration with a titrant of opposite character (acid titrated with a base and *vice-versa*). This mode of titration can be carried out in a range of solvent matrices (such as water, low molecular weight alcohols, acetone) and thus allows organic acids and bases which are insoluble or have limited solubility in water, to be analysed by titration.
- **Precipitation –** the titrant reacts with the analyte to produce an insoluble or at least, a sparingly soluble reaction product, which precipitates. This is then filtered, dried and weighed.
- **Redox (reduction/oxidation) –** the reaction involves titration of an oxidising agent with a reducing agent or *vice-versa.* The reaction involves a transfer of electrons, however for the reaction to go to completion in accordance with the equation, requires there to be sufficient difference in the standard electrode potentials for the analyte and titrant species. See Chapter xxx of this teaching and learning programme.
- **Complexometric –** refers to reactions whereby the titrant forms a water soluble complex with the analyte. Although the reaction can also be carried out in reverse, this is not normally the case. The most popular complexometric reagents (chelating agents) are based on alkyl amine tetra-acetic acids of which ETDA (ethylenediamine tetra-acetic acid) is the most common.

Detection of end-points in Titrimetric (volumetric) reactions

In any form of titration there is the need to be able to detect the point of equivalence (normally referred to as the end-point) in the chemical reaction. In all of the types of titration reactions referred to on the previous slide there are two ways for end-points to be detected:

- By use of a chemical indicator; ★
- By use of a physical indicator.

Chemical indicators change colour at the end-point of the titration reaction, however specific indicators are required to monitor specific chemical reactions.

Physical detection on the other hand, is more universal and is based upon detecting a change in a physical property of either the **analyte** or of the **titrant** or both. Physical properties which have been used include:

- Cell potential (potentiometry); ★
- Current flow (amperometry);
- Solution conductance;
- Solution absorbance;
- Temperature changes in exothermic or endothermic reactions.

Note: only those starred in red will be considered in this Chapter of the teaching and learning programme

The use of chemical (visual) indicators

In many instances in titrimetry, visual indicators function in a similar way to the type of reaction being carried out. For instance:

- Acid/base indicators are themselves weak acids;
- Redox indicators undergo redox transformations;
- Complexometric indicators also complex with the analyte metal ions.

In all cases where visual indicators are used, only very small amounts of the indicator are added to the analyte solution. The reasons for this are:

- So as not to contaminate the sample solution significantly;
- So as not to lead to insolubility problems. Many are complex organic molecules that only have limited solubility in aqueous solutions;
- So as not to make the colour changes, difficult to observe.

Continued on the next slide

Visual indicators for use in acid/base titrations

These are all weak acids which change colour significantly on ionisation. If '**HIn**' represents the acid form of an indicator, then on ionisation it produces the base form '**In^-**', with the equilibrium constant (acid dissociation constant) for the reaction being expressed as $\mathbf{K_a}$. Thus

$$HIn \rightleftharpoons H^+ + In^- \qquad \text{and} \qquad K_a = \frac{[H^+]\,[In^-]}{[HIn]} \qquad \text{Equation (4.2)}$$

Rearranging equation (1.2) and taking negative logs of both sides gives:

$$\mathbf{pH = pK_a + \log \frac{[In^-]}{[HIn]}} \qquad \text{Equation (4.3)}$$

As the titration proceeds the ratio of the acid and base forms of the indicator change in accordance with the changing pH, **the most significant change in colour being seen when the relative concentrations of the acid : base forms change from 10:1 in favour of one to 10:1 in favour of the other**. Putting these relative concentrations into equation (4.3) gives:

$pH = pK_a + \log 1/10$ and $pH = pK_a + \log 10/1$

$= \mathbf{pK_a - 1}$ $\mathbf{= pK_a + 1}$

Continued on the next slide

From the previous slide we can see that the indicator changes colour over around 2 pH units. Thus for the end-point in the titration to be detected accurately, requires that this change matches the pH at the equivalence point for the acid or base being titrated. It is necessary therefore, to have some idea of where the pH equivalence point is likely to occur so that an appropriate choice of indicator may be made. The Table (4.2) below shows some coloured indicators for use in acid/base titrimetric reactions.

Table (4.2) – some coloured acid/base indicators

Indicator	Low pH color	Transition pH range	High pH color
Gentian violet (Methyl violet)	yellow	0.0–2.0	blue-violet
Leucomalachite green (first transition)	yellow	0.0–2.0	green
Leucomalachite green (second transition)	green	11.6–14	colorless
Thymol blue (first transition)	red	1.2–2.8	yellow
Thymol blue (second transition)	yellow	8.0–9.6	blue
Methyl yellow	red	2.9–4.0	yellow
Bromophenol blue	yellow	3.0–4.6	purple
Congo red	blue-violet	3.0–5.0	red
Methyl orange	red	3.1–4.4	orange
Bromocresol green	yellow	3.8–5.4	blue-green
Methyl red	red	4.4–6.2	yellow
Methyl red / Bromocresol green	red	4.5–5.2	green
Azolitmin	red	4.5–8.3	blue
Bromocresol purple	yellow	5.2–6.8	purple
Bromothymol blue	yellow	6.0–7.6	blue
Phenol red	yellow	6.8–8.4	red
Neutral red	red	6.8–8.0	yellow
Naphtholphthalein	colorless to reddish	7.3–8.7	greenish to blue
Cresol Red	yellow	7.2–8.8	reddish-purple
Phenolphthalein	colorless	8.3–10.0	fuchsia
Thymolphthalein	colorless	9.3–10.5	blue
Alizarine Yellow R	yellow	10.2–12.0	red

http://en.wikipedia.org/wiki/Acid-base_indicator

Figures (4.4) and (4.5) shown below and repeated on the next slide, show two acid/base titration reactions for both typically strong (hydrochloric) and weak (acetic) acids.

The end-point for the hydrochloric acid titration is at a pH of 7.0 and that for acetic acid at 8.7. The appropriate visual indicators would thus be those which respectively change colour over respective ranges of (6 – 8) and (7.7 – 9.7).

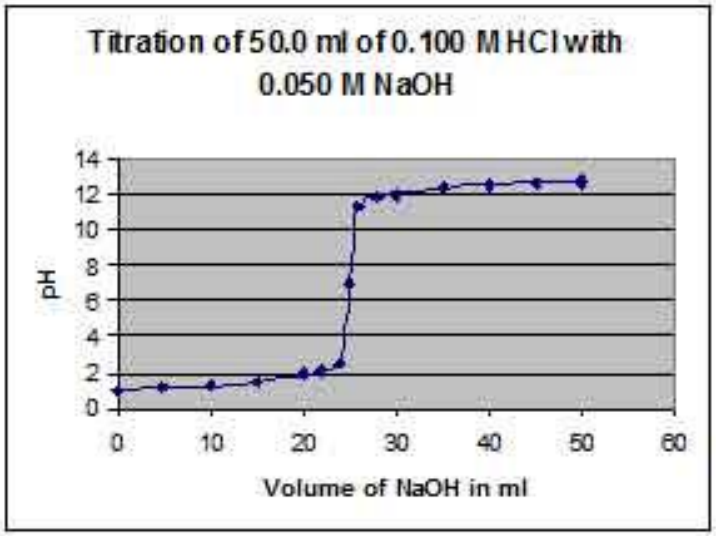


Figure (4.4) – strong acid/base titration

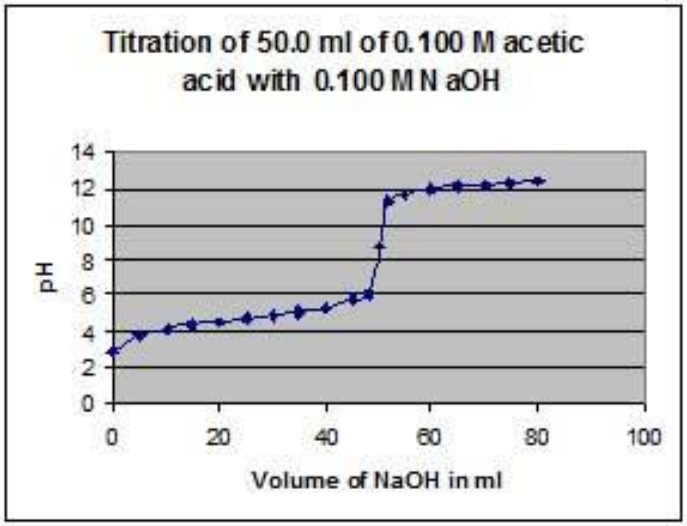


Figure (4.5) – weak acid/base titration

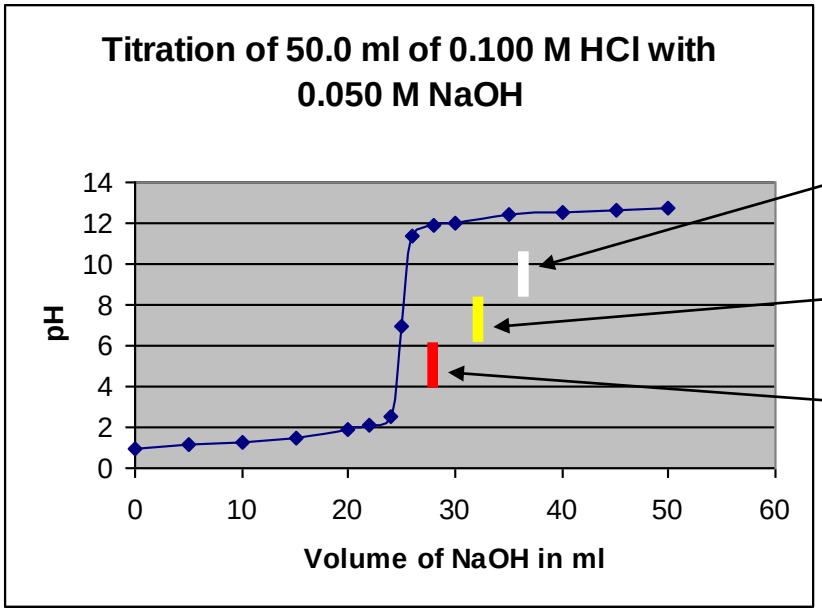


Figure 4.6

Phenolphthalein

Bromthymol blue

Methyl red

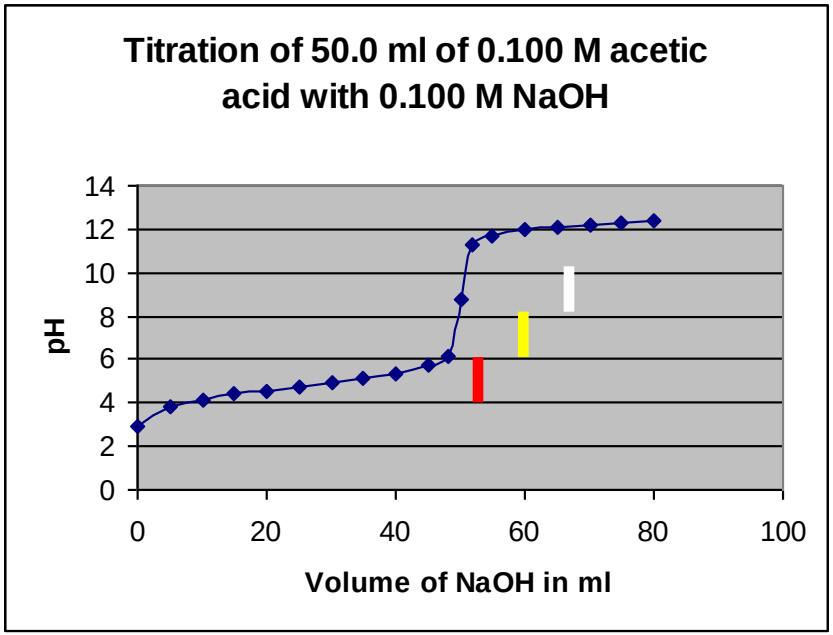


Figure 4.7

Comment on the data

Bromothymol blue, which changes from yellow to blue between pH (6 -8) would appear to be the best choice for the titration illustrated in figure (4.6), however is likely to give a slightly low results for that illustrated in figure (4.7). Phenolphthalein is the indicator of choice for the titration illustrated in Figure (4.7). However, whereas all three indicators could probably be used for the strong acid titration, methyl red would be inappropriate for the titration of the weak acid.

Visual indicators for use in Redox titrations

These can be placed in three categories:

- Those where the titrant is itself strongly coloured, for instance the MnO_4^- ion. Here, the slightest excess of this ion, gives the solution a purple colour.
- A few substances indicate the presence of specific oxidised or reduced species, for instance starch which forms a deep blue colour in the presence of the I^{3-} ion.
- General redox indicators such as Ferroin.

The general redox indicators undergo their own redox transitions, thereby effecting a colour change. Equation (4.4) below represents the general half-reaction for an indicator of this type:

$$In_{ox} + ne^- \rightleftharpoons In_{red} \qquad \text{Equation (4.4)}$$

Whereas the important criteria for the indicator in acid/base titrations was its pK_a value, in redox titrimetry, it is the standard electrode potential (E^0) that is important

Continued on the next slide

The Nernst equation for the indicator reaction can be expressed as shown in equation (4.5):

$$E = E^0 + \frac{0.059}{n} \log \frac{[In_{ox}]}{[In_{red}]} \text{ volts} \qquad \text{Equation (4.5)}$$

At equilibrium, the concentrations of the oxidised and reduced forms of the indicator are equal and thus equation may now be expressed as:

$$\mathbf{E = E^0 \pm \frac{0.059}{n} \text{ volts}} \qquad \text{Equation (4.6)}$$

Where: E represents the voltage range over which the colour change will occur;
n represents the number of electrons transferred in the half reaction.

Note: tables of redox indicators is shown as table (4.3) on the next slide

Again, a range of indicators is required to reflect the range of redox titrations that can be carried out, the correct choice of indicator being that which changes colour over the same region of cell potentials as that of the analyte *versus* titrant reaction.

pH independent redox indicators

Indicator	E^0, V	Color of Ox form	Color of Red form
2,2'-Bipyridine (Ru complex)	+1.33 V	colorless	yellow
Nitrophenanthroline (Fe complex)	+1.25 V	cyan	red
N-Phenylanthranilic acid	+1.08 V	violet-red	colorless
1,10-Phenanthroline (Fe complex)	+1.06 V	cyan	red
N-Ethoxychrysoidine	+1.00 V	red	yellow
2,2`-Bipyridine (Fe complex)	+0.97 V	cyan	red
5,6-Dimethylphenanthroline (Fe complex)	+0.97 V	yellow-green	red
o-Dianisidine	+0.85 V	red	colorless
Sodium diphenylamine sulfonate	+0.84 V	red-violet	colorless
Diphenylbenzidine	+0.76 V	violet	colorless
Diphenylamine	+0.76 V	violet	colorless
Viologen	+1.0 V	blue	colorless

pH dependent redox indicators

Indicator	E^0, V at pH=0	E^0, V at pH=7	Color of Ox form	Color of Red form
Sodium 2,6-Dibromophenol-indophenol or Sodium 2,6-Dichlorophenol-indophenol	+0.64 V	+0.22 V	blue	colorless
Sodium o-Cresol indophenol	+0.62 V	+0.19 V	blue	colorless
Thionine (syn. Lauth's violet)	+0.56 V	+0.06 V	violet	colorless
Methylene blue	+0.53 V	+0.01 V	blue	colorless
Indigotetrasulfonic acid	+0.37 V	-0.05 V	blue	colorless
Indigotrisulfonic acid	+0.33 V	-0.08 V	blue	colorless
Indigocarmine (syn. Indigodisulfonic acid	+0.29 V	-0.13 V	blue	colorless
Indigomono sulfonic acid	+0.26 V	-0.16 V	blue	colorless
Phenosafranin	+0.28 V	-0.25 V	red	colorless
Safranin T	+0.24 V	-0.29 V	red-violet	colorless
Neutral red	+0.24 V	-0.33 V	red	colorless

Table 4.3 – redox indicators

http://en.wikipedia.org/wiki/Redox_indicator

Visual indicators for complexometric titrations

Most indicators for complexometric titrations are organic dyes that form stable complexes with metal ions and thus compete for the metal ion with the complexing reagent. Given that this form of titration is used normally to measure metal ion concentrations in the sample solution, the indicators are frequently referred to as **metallochromic indicators.**

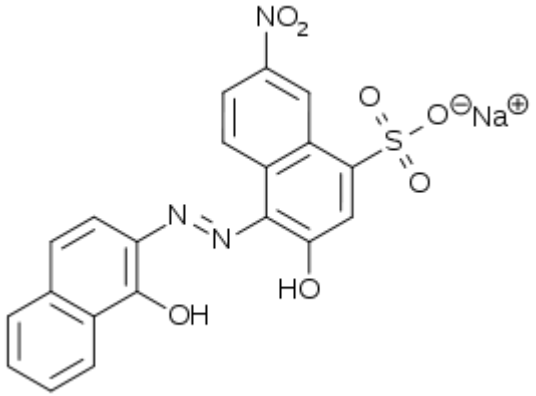


Figure 4.8 – Eriochrome Black T

Eriochrome Black T is a typical indicator frequently used for measurement 'water hardness' in mineral and drinking waters. The indicator contains 3 ionisable protons and thus may be represented as H_3In.

The indicator has a lower formation constant (ie; forms a weaker complex) with usable metal ions and when added to the analyte solution containing Mg^{2+} and Ca^{2+} will react immediately with one of the analyte metal ions to produce a red coloured complex. Titrating this solution with the complexing titrant (eg EDTA), only releases the bound metal ion to react with the titrant when all other metal has been consumed, which is of course the end point in the titration. The indicator then reverts to it's original blue colour according to equation (4.7)

$$\underset{\text{(Red)}}{MgIn^-} + H_2Y^{2-} \rightarrow \underset{\text{(blue)}}{MgY^{2-}} + HIn^{2-} + H^+ \qquad \text{Equation (4.7)}$$

In all complexometric titrations, the pH of the sample solution being analysed must be buffered both in order for the indicator to function, and also, as shown in equation (4.7), to help draw the reaction to completion by consuming the H^+ generated as a by-product of the reaction.

Table (4.4) below shows some useful metallochromic indicators

Indicator	pH range	Analyte application
Eriochrome Black T	7.5 – 10.5	Ba, Ca, Mg, Zn
Eriochrome Blue-black R	8 – 12	Ca, Mg, Zn, Cu
Murexide	6 – 13	Ca, Ni, Cu
PAN	2 – 11	Cd, Cu, Zn
Salicylic acid	2 – 3	Fe

Table (4.4) – list of some important metallochromic indicators

Visual indicators in precipitation titrimetry

In precipitation titrimetry, all of the best know examples involve the use of Ag^{+} as the titrant and there are only three recommended visual indicators methods:

- Volhards;
- Mohr;
- Fajans (adsorption indicators);

Of these, Volhards and Fajans are the most important.

However, whereas in the acid/base, redox and complexometric procedures, the visual indicators have properties similar to the reactants, this is not the case in precipitation titrimetry.

Fajans method

This method is recommended for the determination of Cl^-, Br^- and I^-, by titration with Ag^+ to create the very sparingly soluble silver halides

$Cl^- + Ag^+ \rightarrow AgCl \downarrow$

The indicator used is an anionic dye – **dichlorofluoroscein**. Before the end point the white precipitate of AgCl has a negative charge and thus cannot absorb the dye. However immediately after the end point is reached, the precipitate adsorbs the excess of Ag^+, giving it a positive surface charge. This can then absorb the indicator to produce a pink coloured precipitate, an indication of the end-point. Table (4.5) lists some popular adsorption indicators.

Table 4.5 – some popular adsorption indicators

Indicator	Titration	Solution
Fluorescein	Cl^- with Ag^+	pH 7-8
Dichlorofluorescein	Cl^- with Ag^+	pH 4
Eosin	Br^-, I^-, SCN^- with Ag^+	pH 2
Thorin	SO_4^{2-} with Ba^{2+}	pH 1.5-3.5
Bromophenol blue	Hg^{2+} with Cl^-	0.1 M solution of titrant
Orthochrome T	Pb^{2+} with CrO_4^{2-}	Neutral, 0.02 M solution of titrant

Volhards method

This method has much wider application allowing analyses involving titrations with Ag^+ to be used for 10 or more anionic analytes. In the majority of cases a back-titration is involved whereby an excess of Ag^+ is added to the analyte and this excess is then back-titrated with KCNS (potassium thiocyanate), using Fe^{3+} as the indicator. Equations (4.8) - (4.10) Define the reactions involved in the analysis of the orthophosphate ion (PO_4^{3-})

$3Ag^+ + PO_4^{3-} \rightarrow Ag_3(PO_4) \downarrow + Ag^+(xs)$ Equation (4.8) Forward titration

$Ag^+(xs) + SCN^- \rightarrow AgSCN \downarrow$ Equation (4.9) Back titration

$SCN^- + Fe^{3+} \rightarrow Fe(SCN)^{2+}$ Equation (4.10) Indicator reaction

The end point is detected when the first excess of the thiocyanate ion allows it to complex With the Fe^{3+} producing a deep red colouration.

Anions that may be analysed by using this method are:

AsO^{3-}, Cl^-, Br^-, I^-, CO_3^{2}-, $C_2O_4^{2-}$, CrO_4^{2-}, PO_4^{3-} and S^{2-}

SCN^- can be titrated directly with Ag+ without the need for a back titration.

Potentiometric indicators

Note: the theory underlying the technique of potentiometry is covered in Chapter 9 of this teaching and learning programme.

Titrations carried out using potentiometric indicators are normally referred to as **potentiometric titrations**. This form of titration may be applied across all of the types of titration reaction shown on slide xxx, provided a suitable electrode is available that can detect either the **analyte** or the **titrant**. Table (4.6) lists the measured species in this form of titration and the electrodes normally employed to perform the measurement.

Titration type	What is measured	Type of electrode
Acid/base	$[H^+]$	Glass electrode
Redox	Ratio of $\frac{[\text{oxidised}]}{[\text{reduced}]}$	Inert metal wire electrode – normally Pt or Au
Complexometric	[specific metal ion]	Ion-selective electrode
Precipitation	$[Ag^+]$	Silver wire electrode

Table 4.6 - comparison of potentiometric titrations

Continued on the next slide

The instrumental components required in order to perform a potentiometric titration are:

- Source of titrant and mode of delivery;
- Titration vessel;
- Electrochemical cell comprising an indicator and a reference electrode;
- Mechanical stirrer;
- Millivoltmeter which is set to display pH for acid/base reactions;
- Computer controlled read-out device for use with an auto burette

These are combined together as illustrated in figure (4.9)

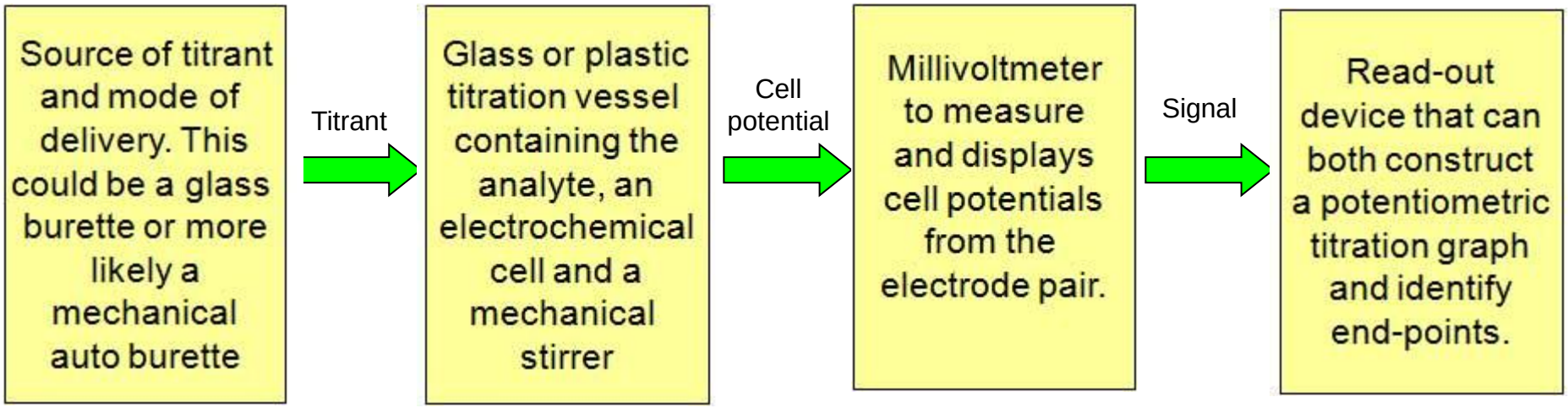


Figure (4.9) - potentiometric titration set-up

Introduction to the theory underlying potentiometric indicators

The cell potential registered during a potentiometric titration can be expressed as:

$$E_{cell} = E_{indicator(in)} - E_{reference(ref)} \text{ Volts} \qquad \text{Equation (4.11)}$$

The potential of the indicator electrode can be expressed by the Nernst equation:

$$E_{indicator} = E^0 \pm \frac{0.059}{n} \log \frac{[oxid]}{[red]} \text{ Volts} \qquad \text{Equation (4.12)}$$

Where: E^0 represents the standard electrode potential for this half-cell
n is the number of electrons transferred in the redox reaction

For analyte ions where the oxidised or reduced form of the species are in their standard state (metal or gas for instance), this simplifies to equation (4.13) as either:

$$E_{in} = E^0 + 0.059/n \log [cation] \quad \text{or}$$
$$E_{in} = E^0 - 0.059/n \log [anion] \quad \text{Volts@}20^oC \qquad \text{Equation (4.13)}$$

As the reference electrode chosen for the cell, is assumed to maintain a constant potential throughout the experiment, equation (4.14) may now be expressed as:

$$\mathbf{E_{cell}} = \{E^0 \pm 0.059/n \log [ion] - E_{ref}\}$$
$$= \mathbf{\{const. \pm 0.059/n \log [ion]\} \text{ Volts}} \qquad \text{Equation (4.14)}$$

Thus $\mathbf{E_{cell} \alpha \log [ion]}$ as all other terms are constant

Continued on next slide

Whatever the chemical reaction are involved in the titration, all potentiometric titrations produce 'S' shaped graphs of the types shown in figure (4.10 A&B)

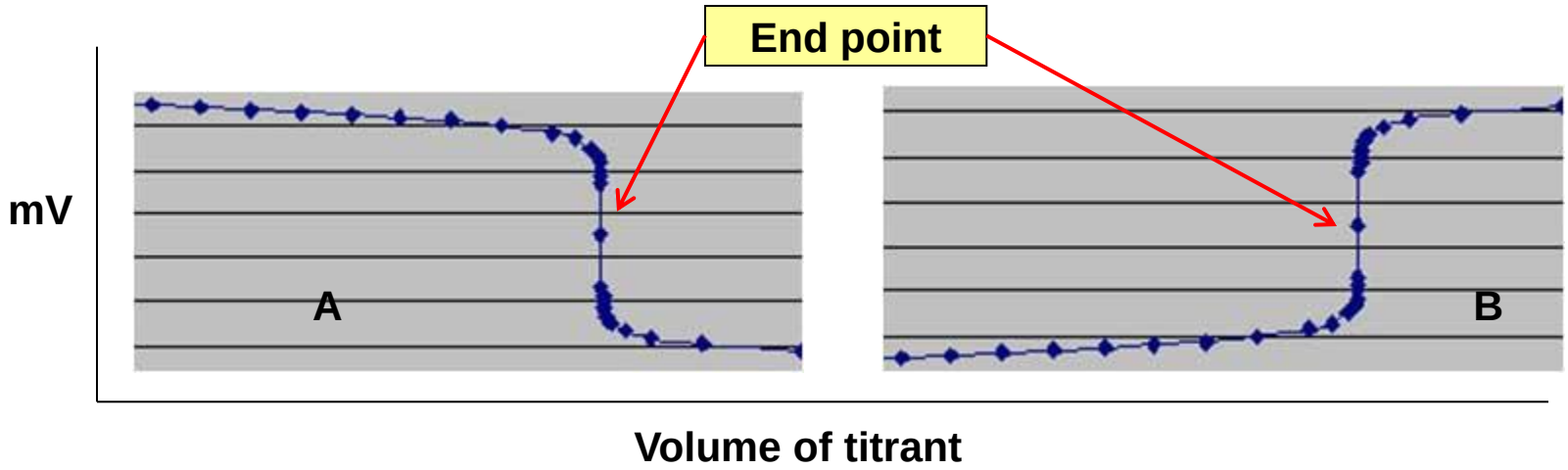
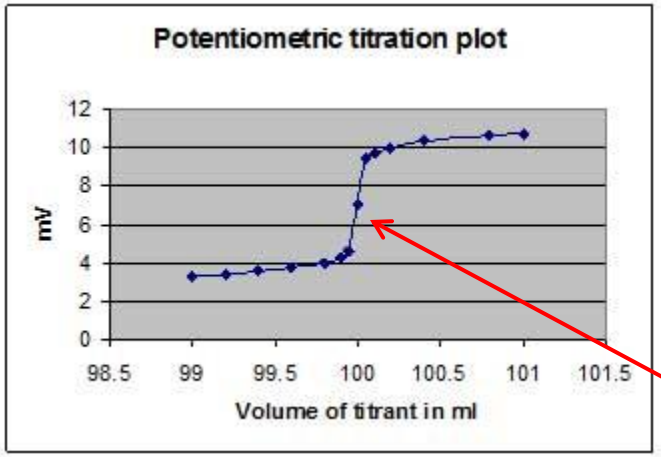


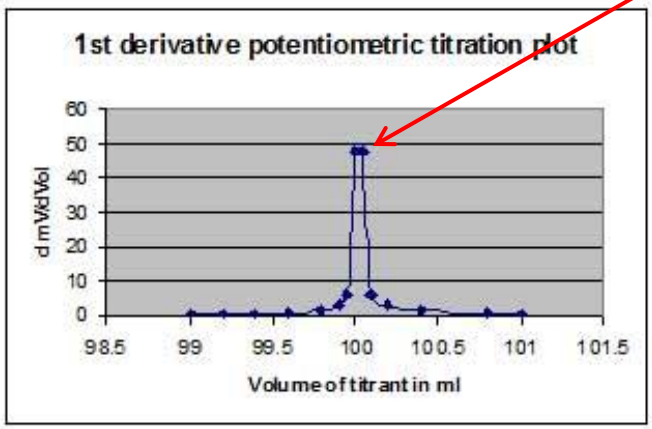
Figure 4.10 - typical potentiometric titration graphs

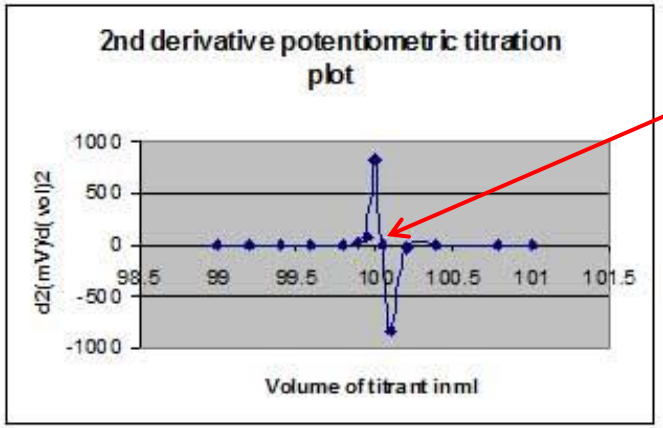
One of the main advantages of potentiometric titrimetry, is the ability of the system to be automated, not only to produce titration graphs as illustrated in figure (4.10), but to calculate and display titration end-points as well. The calculation of end-point location is achieved by use of 1st or 2nd mathematical derivative calculations. These are:

$\frac{\mathbf{d(mV)}}{\mathbf{d(vol)}}$ *versus* **volume of titrant** or $\frac{\mathbf{d^2(mV)}}{\mathbf{d(vol)^2}}$ *versus* **volume of titrant**

Graphs in these formats are shown on the next slide







End point

End point

Figure (4.11) - potentiometric titration plot and 1st and 2nd derivative plots

Potentiometric titration plots are characterised by showing significant changes in slope [d(mV)/d(Vol)] in the immediate vicinity of the end-point. This feature can be utilised to detect the maximum value in a plot of this first derivative *versus* volume of titrant. By going one stage further and calculating the second mathematical derivative, the resultant plot passes through zero at the end point. This can be detected by a computer controlled titrator and displayed as the end-point. Illustrations of these plots are shown in figure (4.11). A typical auto-titrator is shown as figure (4.12) on the next slide

Figure 4.12 shows a typical automatic potentiometric titration instrument, capable of allowing 12 samples of the same type to be analysed sequentially.

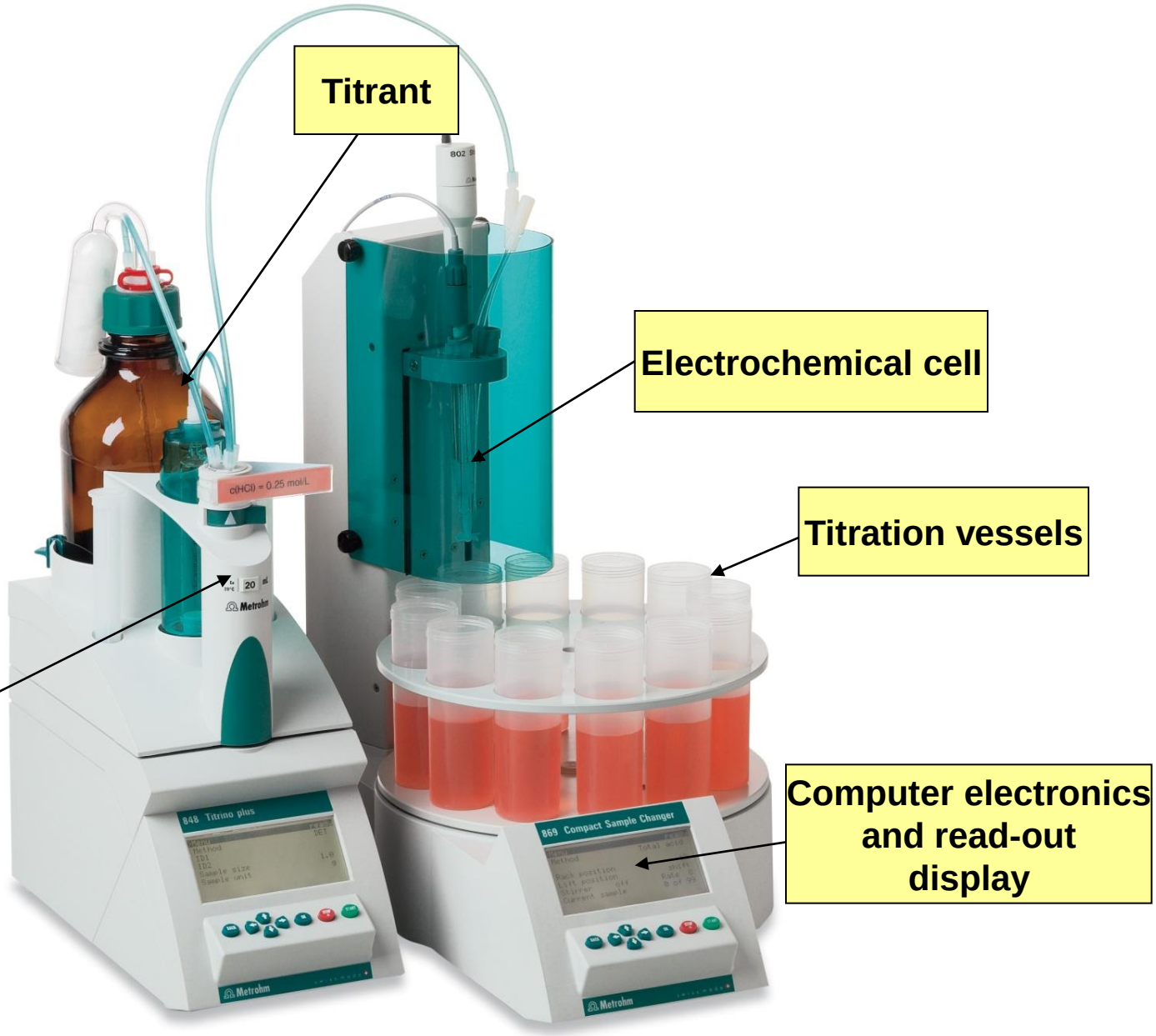


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Figure 4.12 - typical potentiometric auto-titrator

Advantages of potentiometric over visual indicators

There are number of advantages offered by potentiometric indicators over visual indicators to follow the progress of titrimetric reactions and detect end-points. These are:

- Ability to function is highly coloured solutions;
- Ability to find multiple end-points when samples contain more than one titratable species. For instance, a sample containing both weak and strong acids or polyprotic acids (eg: orthophosphoric acid H_3PO_4) where there is A significant difference between the K_a values of the titratable protons. **See example (4.viii) on the next slide**
- Offers opportunities for automation for both detection of end-points and for the analysis of multiple samples dispensed from auto-samplers.

Example (4.viii) – titration of orthophosphoric acid solution with standardised NaOH

The 3 protons are all titratable, however only the first two will be detectable potentiometrically, as the K_a value of the 3rd proton is too low to be detectable.

$$H_3PO_{4(s)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + H_2PO_4^-{}_{(aq)} \qquad K_{a1} = 7.5\times10^{-3}$$ - 1st end point

$$H_2PO_4^-{}_{(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + HPO_4^{2-}{}_{(aq)} \qquad K_{a2} = 6.2\times10^{-8}$$ - 2nd end point

$$HPO_4^{2-}{}_{(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + PO_4^{3-}{}_{(aq)} \qquad K_{a3} = 2.14\times10^{-13}$$

Figure (4.13), shows a typical potentiometric titration plot for a polyprotic acid. For orthophosphoric acid on its own, the volume of titrant required for the second end point should be exactly double that to the first.

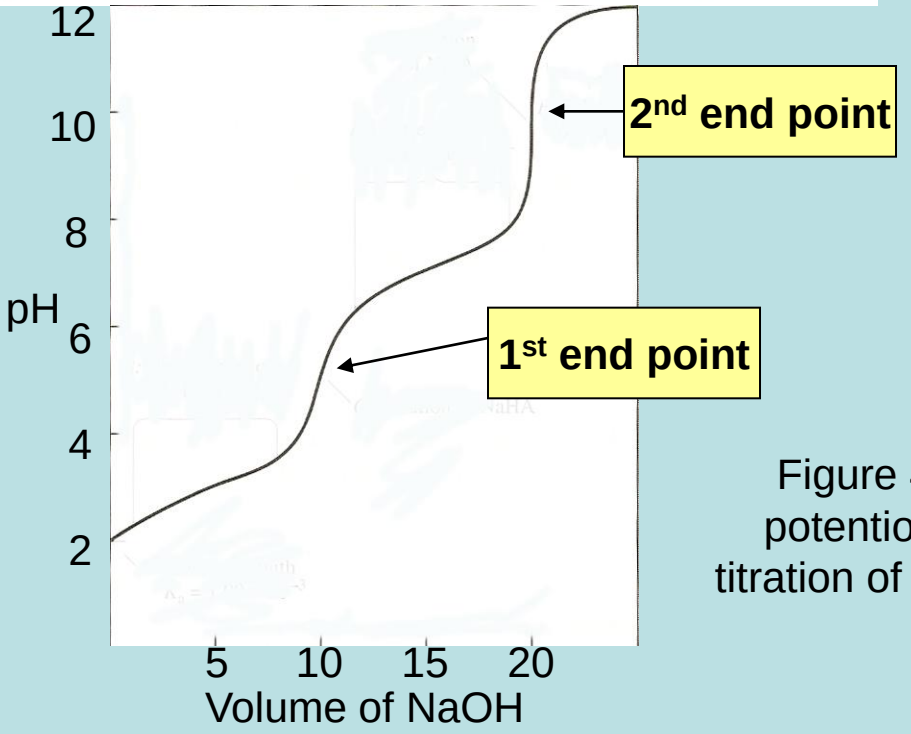


Figure 4.13 – typical potentiometric plot for titration of a polyprotic acid

Volumetric calculations

On slides 9 – 11 the ways of expressing concentration in terms of molarity and normality were reviewed. As normality is used only infrequently and then in special circumstances, all volumetric calculations will be considered in terms of molar units of concentration.

When performing a titration, it is necessary for the strength of the titrant to be known beforehand and for this to be expressed usually in Molar units (mol/dm3 or mmol/dm3). It is also necessary to be able to construct a balanced equation for the reaction, showing the relative numbers of reacting species. In considering this topic, only ionic equations will be used. For example:

$Cl^- + Ag^+ \rightarrow AgCl\downarrow$ [a 1 : 1 reaction]

$CO_3^{2-} + 2H^+ \rightarrow CO_2\uparrow + H_2O$ [a 1 : 2 reaction]

$MnO_4^- + 5Fe^{2+} + 8H^+ \rightarrow Mn^{2+} + 5Fe^{3+} + 4H_2O$ [a 1: 5 reaction]

Note: in the above equations the analyte is shown in red and the titrant in blue

When performing a titration to calculate the molarity of the analyte solution (M_A), three pieces of experimental information will be available. These are:

- Volume of analyte solution taken for analysis (V_A);
- Volume of titrant required (V_T);
- Molarity of titrant (M_T).

If the general equation for the reaction is presented as:

a Analyte(A) + t Titrant(T) ⟹ Products

Where 'a' and 't' represent the number of moles of analyte and titrant respectively in the equation:

Then: Moles of A = Moles of T X a/t Equation (4.15)

which can be rewritten as:

$M_A \times V_A = M_T \times V_T \times a/t$ Equation (4.16)

$$\textbf{Thus } M_A = \frac{M_T \times V_T \times a}{V_A \times t}$$ Equation (4.17)

Note:
If the molarity of the Analyte solution is known, then the equation will need to be rearranged in order to calculate the molarity of the titrant

Example (4.ix)

1.3300 g of pure dry sodium carbonate was dissolved in pure water and diluted to 250 cm^3 in a calibrated flask. 25.00 cm^3 of this sample solution was then titrated with dilute HCl, 25.25 cm^3 being required. Calculate the molarity of the HCl

The balanced ionic equation for the reaction is:

$$CO_3^{2-} + 2H^+ \Longrightarrow CO_2 + H_2O$$

To calculate the molarity of the titrant using equation (1.17), the equation first needs to be rearranged from:

$$M_A = \frac{M_T \times V_T \times a}{V_A \times t} \quad \text{to} \quad M_T = \frac{M_A \times V_A \times t}{V_T \times a} \quad \text{Equation (4.18)}$$

Na_2CO_3 has a molar mass of 106. The strength of the standard sodium carbonate solution is therefore:

$$1.3300/106 \times 1000/250 = 0.0502 \text{ mol/dm}^3$$

The molarity of the HCl titrant may now be calculated by using equation (4.18)

$$\mathbf{M_{HCL} = \frac{0.0502 \times 25.00 \times 2}{25.25 \times 1} = 0.0994 \text{ mol/dm}^3}$$

Example (4.x)

The permanganate ion (MnO_4^-) reacts with the oxalate ion $(C_2O_4)^{2-}$ under acid conditions to produce carbon dioxide and water. This reaction can be used to standardise a solution of potassium permanganate or analyse for soluble oxalates. The equation for the reaction is:

$$5C_2O_4^{2-} + 2MnO_4^- + 16H^+ \Rightarrow 5CO_2 + 2Mn^{2+} + 8H_2O \qquad \text{Equation (4.19)}$$

1.875 g of impure dry disodium oxalate (molar mass of 134) was dissolved in water and diluted to 250 cm^3 in a calibrated flask. 25.00 cm^3 of this solution was pipetted into a conical flask and after adding the same volume of 2M sulphuric acid and heating to 60^oC, was titrated with 0.020 mol/dm^3 permanganate solution, 24.40. cm^3 being required. Calculate The % purity of the sample.

By using equation (4.19), the molarity of the oxalate solution may be calculated.

$$M_{oxalate} = \frac{0.020 \text{ X } 24.40 \text{ X } 5}{25.00 \text{ X } 2} = 0.0488 \text{ mol/dm}^3$$

250 cm^3 of a 1 mol/dm^3 solution of disodium oxalate requires 134/4 g of the salt
250 cm^3 of a 0.0488 mol/dm^3 solution thus requires (0.0488/1 X 134/4) g of the salt

% of disodium oxalate in the sample analysed was thus:

0.0488 X 134/4 X 100/1.875 = 87.2%

An alternative approach to volumetric calculations

The generic equation (4.17) shown on slide 47 is appropriate for molarity calculations when the analyte is a solution. However as illustrated in example (4.ix) on slide 47, it is not totally appropriate when the analyte is a solid. In this situation, an alternative approach is recommended using simple proportion calculations. The values quoted in example (4.x) will be used to illustrate this alternative approach.

The equation for the reaction between the oxalate ion and the permanganate ion is:

$$5C_2O_4^{2-} + 2MnO_4^- + 16H^+ \Rightarrow 5CO_2 + 2Mn^{2+} + 8H_2O$$ Thus:

2 moles of permanganate $\equiv$ 5 moles of oxalate, which may be represented as:

2000 cm^3 of 1.0 mol/dm^3 permanganate $\equiv$ (5 X 134)g of disodium oxalate

Volume containing 2 moles

Mass of 5 moles

Thus 24.40 cm^3 of 0.02 mol/dm^3 permanganate $\equiv \frac{24.40}{2000} \times \frac{0.02}{1.0} \times 5 \times 134$ g

Ratio volumes and multiply by equivalent mass

$= \mathbf{0.1635\ g}$

Only 25 cm^3 of the 250 cm^3 of sample was titrated

Purity of the sample analysed $= \frac{\mathbf{0.1635 \times 10}}{\mathbf{1.875}} \times \mathbf{100} = \mathbf{87.2\%}$

Continued on next slide

The general principles for performing a volumetric calculation in this way are:

- Derive the balanced equation for the reaction;
- Express the **mole quantities of titrant : analyte** as **[volume (V_1) @ 1 mol/dm^3] : mass (m_s)** respectively;
- By simple proportion, relate the **volume of titrant required to reach the end point (V_2) and it's molar concentration (x),** to the **standard volume and molarity** given above and multiply this by the total molar mass as it appears in the equation. Thus;

$$\textbf{Mass of analyte titrated} = \frac{V_2}{V_1} \times \frac{x}{1} \times m_s \text{ g} \qquad \text{Equation (4.20)}$$

Note: the mass of analyte titrated is given in grams as the other terms in equation (4.20) are both ratios.

Many examples of volumetric calculations may be found in textbooks on 'Analytical Chemistry'. The learner is recommended to seek out one of these standard texts and attempt some of the calculations.

Gravimetric analysis

Gravimetric analysis usually involves the selective separation of the analyte by precipitation followed by the non-selective measurement of the weight of the resultant precipitate. Given that weight is a totally non-selective measurement tool, the chemistry used to create the precipitate must be ultra selective.

Along with volumetric analysis it provides an accuracy and precision of around 1 part in 1000, which is probably 10 - 50 fold better than that achievable with most instrumental techniques.

However, the technique requires relatively large quantities of the analyte to be present, and thus is recommended only for the analysis of major constituents of a sample. It also suffers from two major disadvantages:

- The technique is generally extremely time consuming to carry out;
- Requires a very high level of manipulative skill.

Table 4.7 shown on the next slide gives a comparison of a range of analytical techniques in terms of a number of parameters

Technique	Approx. Range Mol/dm^3	Approx. Precision (%)	Selectivity	Speed	Cost	Main applications
Gravimetry	0.1 – 0.01	0.1	Poor to moderate	Slow	Low	Inorganic
Titrimetry	0.1 – 10^{-4}	0.1 – 1	Poor to moderate	Moderate	Low	Inorganic/ organic
Potentiometry	0.1 – 10^{-6}	2	Good	Fast	Low	Inorganic
Electrogravimetry & Coulometry	0.1 – 10^{-4}	0.01 – 2	Moderate	Slow to moderate	Moderate	Inorganic/ Organic
Voltammetry	10^{-3} – 10^{-10}	2 – 5	Good	Moderate	Moderate	Inorganic/ Organic
Spectrophotometry	10^{-3} – 10^{-6}	2	Good to moderate	Fast to moderate	Low to moderate	Inorganic/ Organic
Fluorimetry	10^{-6} – 10^{-9}	2 – 5	Moderate	Moderate	Moderate	Organic
Atomic spectroscopy	10^{-3} – 10^{-9}	2 – 10	Good	Fast	Moderate to high	Inorganic Multi-element
Chromatography	10^{-3} – 10^{-9}	2 – 5	Good	Fast to moderate	Moderate to high	Organic multi-component
Kinetic methods	10^{-2} – 10^{-10}	2 - 10	Good to moderate	Fast to moderate	Moderate	Inorganic/ Organic/ Enzymes

Table 4.7 – Comparison of Analytical Techniques

Steps in a gravimetric analysis

Although in theory the technique could be applicable to both inorganic and organic analytes, in practice it is only ever recommended for the analysis of inorganic constituents. If the sample is a solid, then the first stage in this type of analysis is one of dissolution. (**see slides 11 - 14 in the 'Sample Preparation' unit process**). The steps then required may be summarised as:

- **Preparation of the sample solution** (dissolution, adjustment of pH, addition of reagents to aid selectivity);
- **Precipitation of the analyte** by addition of a suitable reagent;
- **Digestion of the sample**, generally by heating on a boiling water bath, to create larger size and purer crystals. Following digestion, the solution will need to cool to room temperature;
- **Preparation of the crucible** by cleaning and then drying under the conditions to be required eventually by the sample. For instance, if the precipitate requires drying at 110^{o}C, then the crucible must also be prepared at this temperature. Weighing of the crucible;
- **Filtration of the precipitate** through an ashless filter paper (for ignition) or through a sintered glass crucible of appropriate pore size;
- **Washing of the precipitate** until all adsorbed impurities and excess reagents have been removed;
- **Drying or ignition**. Most precipitates just require drying to remove water or solvent molecules. In a few instances, precipitates require ignition at high temperature;
- **Weighing of the crucible** and its contents to constant weight;
- **Calculation** of the analyte concentration in the prepared sample solution.

Further information on this technique see: at http://en.wikipedia.org/wiki/Gravimetric_analysis

Examples of gravimetric analysis

Table 4.8 on the next slide gives some examples of gravimetric procedures for analysis of a selected range of anions

Given the two major practical disadvantages (see slide xx) of the technique, it tends to be applied only when no alternative procedure is available or when high accuracy and precision are required. Probably the most important use of the technique is for the analysis of anions such as sulphate (SO_4^{2-}), chloride (Cl^-) or orthophosphate (PO_4^{3-}). Now whereas there are good alternative methods available for the analysis of chloride and orthophosphate, there are few methods available for sulphate. Thus gravimetric analysis continues to be the preferred technique.

Gravimetric procedure for the analysis of sulphate

- Precipitate $BaSO_4$ by addition of an excess of Ba^{2+} [Ba^{2+} + SO_4^{2-} ⟹ $BaSO_4$]
- Following digestion, filter the white precipitate through an ashless filter paper and wash free of other reagents.
- Transfer the filter paper to a weighed silica or porcelain crucible, place a lid partially on the crucible and gently heat over a flame to char the paper, without allowing it to catch fire.
- Once most of the paper has been removed, transfer the crucible to a muffle furnace (see figure (xx) on slide xxx) and gradually raise the temperature of the furnace to 850^{o}C. When all of the carbon has been removed, transfer the crucible to a desiccator and weigh when cool. Return to the furnace until a constant weight is achieved. This precipitate will be pure BaSO4.
- Calculate the quantity of sulphate in the solution analysed.

Analyte	**Precipitant**	**Precipitate and weighed compound if different**
Cyanide	Silver nitrate	AgCN
Iodide	Silver nitrate	AgI
Bromide	Silver nitrate	AgBr
Chloride	Silver nitrate	AgCl
Chlorate	$FeSO_4/AgNO_3$	AgCl
Thiocyanate	$SO_2/CuSO_4$	CuSCN
Sulphate	Barium chloride	$BaSO_4$
Phosphate	Mg^{2+} and aq. NH_3	$Mg(NH_4)PO_4.6H_2O$ and Weighed as $Mg_2P_2O_7$

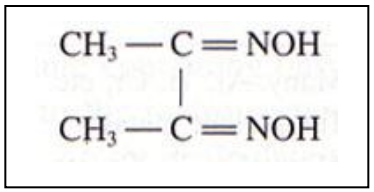
Table 4.8– Examples of gravimetric methods for anion determination

Use of organic reagents in gravimetric analysis

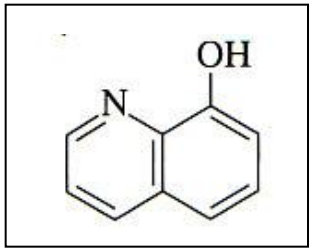
There are a number of organic reagents that are applicable to gravimetric analysis. All are used for the analysis of metal ions.

Two example reagents are illustrated below:

- Dimethylglyoxime (DMG)



- 8-hydroxyquinoline (oxine)



In both of these reagents, they produce stoichiometric precipitates of very low solubility in water. Sintered glass crucibles are used for filtration with drying at between 110 – 130°C

Both of these reagents are **chelating agents** that can form uncharged **chelates** by reacting with metal ions under pH controlled conditions. The general equation for the reaction is:

$$M^{n+} + nHX \rightleftharpoons MX_n + nH^+$$

DMG is virtually specific for Ni^{2+}, whilst oxine is capable of reacting with a number of metal ions of which Al^{3+} and Mg^{2+} are the most important.

Calculations following gravimetric analysis

Example (4.xi)

0.1150 g of a nichrome alloy was dissolved in acid and the nickel content of the alloy precipitated as bright red nickel dimethylglyoxime. After digestion, filtering through a sintered glass crucible and drying to constant weight, the precipitate was found to weigh 0.3397 g. Calculate the % of nickel in the nichrome alloy. The equation for the reaction is:

$Ni^{2+} + 2\,(DMG) \rightarrow Ni\,(DMG)_2 + 2H^+$

The empirical formula of nickel dimethylglyoxine is: $Ni\,(C_4H_7O_2N_2)_2$ and has a molar mass of 288.9

Nickel has an atomic mass of 58.7 and thus the weight of Ni in the Ni (DMG) is:

$$0.3397 \times \frac{58.7}{288.9} = 0.0690\,g$$

% of Ni in the nichrome alloy is thus: $\frac{0.0690}{0.1150} \times 100\,\% = \mathbf{60.0\,\%\ w/w}$

Example (4.xii)

A sample of crude oil containing sulphur (1.513 g) is quantitatively combusted such that all of the sulphur is oxidised to sulphate. This resultant sulphate is precipitated as barium sulphate and after careful filtering and ignition, the resultant white precipitate is weighed as BaSO4, 0.5163 g being found to be present. Calculate the % of sulphur in the oil sample analysed.

The equation for the reaction is: $Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4$

The formula mass of $BaSO_4$ is 233.40 and the atomic mass of sulphur is 32.07

Thus the mass of sulphur in the precipitated $BaSO_4$ is:

$$0.5163 \times \frac{32.07}{233.4} = 0.0709\ g$$

The % of sulphur in the oil was therefore:

$$\frac{0.0709}{1.513} \times 100\ \% = 4.69\ \%\ w/w$$

Advantages & disadvantages of gravimetric analysis

Advantages	Disadvantages
▪ The most accurate and precise technique available to the analytical scientist for quantitative measurement ▪ By combining the analyte with a heavy metallic element or a large organic molecule exerts an amplification effect on the mass of precipitate to be measured.	▪ Often very slow and time consuming ▪ Requires a high degree of manual skill and dexterity ▪ Only suitable when macro quantities of analyte are available – not suitable for trace analysis

Comparative analytical techniques

Virtually all of the popular instrumental analytical techniques come within this category, where a single property of the analyte (solid, liquid or solution) is to be measured. The property to be measured may:

- relate to the solution or solid as a whole, for instance the measurement of viscosity or particle size;
- relate to a single component present in the sample, for instance, the determination of the tin content in a mineral ore, by X-ray fluorescence spectrometry;
- be inherent in the sample to be analysed, for instance, the determination of ethanol in a sample of blood using gas-liquid chromatography;
- be created *via* a suitable chemical reaction, for instance, the colorimetric determination of the chromium content of steel following oxidation of the chromium to the highly coloured dichromate ion ($Cr_2O_7^{2-}$)

In all of these instances, the instrument will produce a signal which will relate to the property being measured. However, to analytically quantify that signal, requires for the **instrument used** to be calibrated using standard substances.

In all comparative techniques, there is a mathematical relationship that expresses the measured physical parameter/signal (Y) as a function of the analyte concentration (C) . [equation (4.21)]

$Y = f\ C$ Equation (4.21) [*f* expresses the relationship between (Y) & (C)

In many instances there is a linear relationship between (Y) & (C) as illustrated by the calibration graph shown in figure (4.14)

The graph shown in figure (4.14) is generally expressed as:

$Y = m\ C$ Equation (4.22)

where 'm' is the slope of the graph, which can be calculated by dividing A/B, each of which is measured in **units from the graph**. The value of 'm' is also the **sensitivity** of that analytical method [**signal/unit concentration**]. In some analytical techniques, more complex relationships exist, such as polynominals. For example equation (4.23):

$Y = m\ C + n\ C^2$ Equation (4.23)

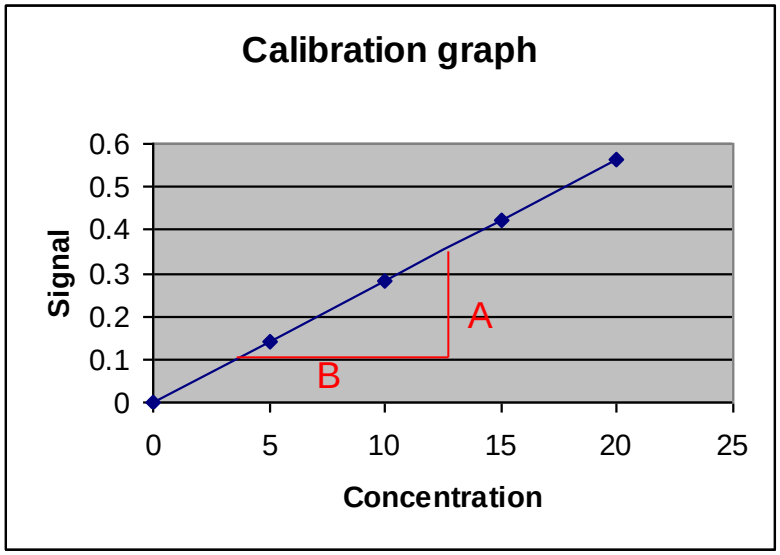


Figure 4.14 - signal : concentration relationship

Calibration techniques

There are numerous ways for calibrating an instrument in order to generate accurate analytical data, some of which find most use in particular analytical procedures. The most popular calibration methods are:

- Use of calibration curves;
- Method of standard addition;
- Use of internal standards.

All of these calibration methods, require the availability of standard substance or solutions at accurately known purity or concentration. Popular sources of standards are:

- Pure metals (eg: Cu, Ag, Au, Zn etc)
- Pure organic compounds
- Pure primary standards (eg: Na_2CO_3, $AgNO_3$, NaCl)
- 1000 mg/dm^3 standard solutions of cations and anions available from commercial suppliers
- Commercial mixtures of trace organics for environmental analysis (eg: mixtures of herbicides and pesticides for chromatographic analysis)

Preparation of standard solutions

In many instances, having obtained a standard substance or solution, it will be necessary to prepare ‘in-house’ standard solutions of known concentration from these pure substances or commercial solutions.

The next three slides give examples of how this may be achieved.

Example 4.xiii – preparing a standard solution from a pure metal or from a commercial 1000 ppm standard

Example 4.xiv – preparing a standard solution from a pure salt

Example 4.xv – preparing a standard solution from a volatile organic compound

Example (4.xiii) – prepare 100 cm^3 of a 100 ppm (w/v) Ag^+ solution.
The options are to prepare the standard from either **pure Ag metal** or from a **1000 ± 2 ppm Standard solution**

The total mass of silver in the 100 cm^3 of standard solution will be 10.0 mg. Given that a 4 place analytical balance is only guaranteed to weigh to an accuracy of ± 0.1 mg, this means that the solution could be prepared with a possible error of ± 1%. If this level of accuracy is deemed acceptable, then the following procedure 1 would be used. Alternatively procedure 2 could be used whereby the 1000 ppm standard solution is simply diluted.

Procedure 1

"Weigh out 10.0 mg of pure silver (record the exact weight) and dissolve in a small quantity of pure concentrated nitric acid. Transfer the resultant solution to a 100 cm^3 calibrated flask and dilute to volume with pure water. Stopper, shake the flask and label with the exact concentration of silver ion".

Procedure 2

"Transfer by bulb pipette, or other calibrated transfer device, 10.0 cm^3 of the 1000 ppm standard solution into a 100 cm^3 calibrated flask, add about 0.5 cm^3 of nitric acid and dilute to volume with pure water. Stopper and shake the flask and label with the solution concentration." This procedure will produce a solution with an error of ± 0.2% and is to be recommended.

Example (4.xiv) – prepare 250 cm^3 of a standard solution containing 500 ppm (w/v) of Cl^-, from pure dry NaCl

500 ppm ≡ 500 mg/dm^3 Cl^-

Thus 250 cm^3 of this solution will contain 500 ÷ 4 mg of Cl^- = 125 mg of Cl^-

The relative molar mass of NaCl is 23 + 35.5 = 58.5

Thus the mass of NaCl required to make this solution is;

125 X (58.5 ÷ 35.5) mg = **206.0 mg**

Procedure

"Weigh out accurately about 206 mg of pure NaCl. Transfer quantitatively to a calibrated flask, dissolve in pure water and dilute to volume with the same. Stopper the flask, shake to mix and label with the exact concentration of Cl^-".

[Note: the exact concentration will not necessarily be 500 ppm, as it will be difficult to weigh 206.0 mg exactly. If for instance the weight of NaCl taken was 206.8 mg, then the solution should be labelled as 502 ppm [(206.8 ÷ 206) X 500 = 502]

Example (4.xv) – prepare 50 cm^3 of a 1% (w/v) solution of MEK (methyl ethyl ketone) in methanol for use in chromatographic analysis.

50 cm^3 of solution will contain 0.5 g of MEK. This solution may be prepared in one of two ways:

Procedure 1

Assuming that MEK has a density of 0.75 g/dm^3, the volume of MEK required to be equivalent to 0.5 g is: $(0.5 \div 0.75) = 0.67\ cm^3$.
"Thus, carefully transfer using a calibrated transfer pipette, 0.67 cm^3 of MEK into a flask containing some methanol. Stopper the flask and swirl gently to mix. Now dilute to volume with additional methanol, stopper, shake to mix and label with the concentration".

Procedure 2 (the recommended procedure)

Half fill a 50 cm^3 calibrated flask with methanol, stopper and weigh. Now add carefully to the methanol in this flask, 0.5 g of MEK. Stopper the flask and reweigh. Swirl to mix before diluting to volume with further methanol. Stopper the flask, shake to mix and label with the exact concentration based upon the weight of MEK added to the flask".

Note: Never add a volatile substance directly into an empty calibrated flask when preparing a standard solution, as part of it will be lost due to evaporation, when the solvent is added.

Random *versus* systematic errors

These two statistical terms **random errors** and **systematic errors** are being introduced at this stage, as the terms will be used throughout the discussion of calibration procedures. **Statistical analysis of data is covered in Chapter 5 of this teaching and learning programme, at which time the terms will be considered in greater detail.**

Definition of random error

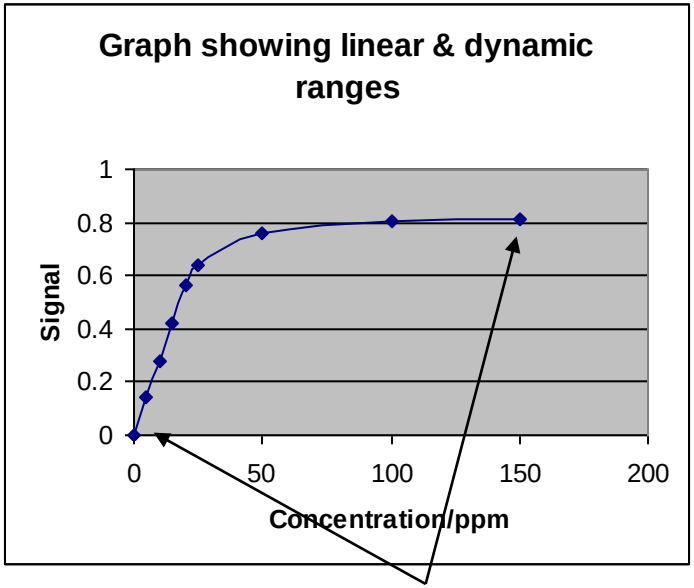
These are errors that arise from uncertainties in a measurement that are unknown and cannot be controlled. The result, is a scatter of replicate measurements that can only be assessed by statistical tests.

Definition of systematic error

These are measurable errors which in theory have a definite value and thus can be allowed for during an analysis.

Calibration using graphical plots

Unfortunately not all analytical techniques produce linear calibration graphs as illustrated in figure (4.15), over large concentration ranges. Although a non-linear graph can still be used for calibration purposes this often requires the preparation of more standard solutions so that the curvature of the graph may be accurately predicted. The loss of linearity is caused by the measurement system responding less sensitively at higher concentrations. This is illustrated in figure (4.15 A & B)



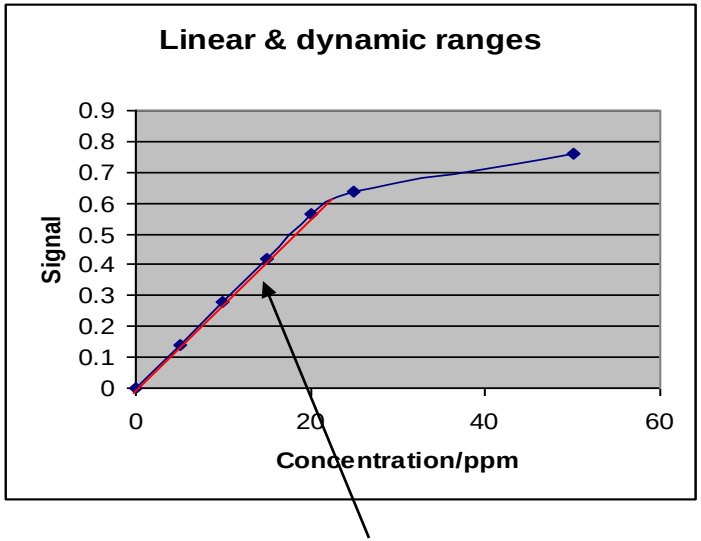


Figure (B) is an expanded version of figure (A). Both show linear and dynamic ranges within the calibration plots. In this example the linear range is up to 20 ppm and the dynamic range up to about 150 ppm. This represents the extent to which a continued increase in solution concentration Is matched by an increase in signal.

Dynamic range (A)

Linear range (B)

Figure (4.15 A & B) – linear and dynamic ranges

Equation (4.22) [**Y = m C**], is the equation for a linear calibration plot, where the calibration line goes through the origin (0,0) of the graph. This will only be the case if::

- There is no measurable signal from a reagent/reaction/instrument **blank**
- The **blank** signal has been subtracted from the standard signals, prior to plotting on the graph

Definition of a blank signal

A measurement made on a calibration sample, following an established procedure, but where the analyte has not been deliberately added. A constant blank value can be considered as a **systematic** error

The **general equation** for a linear calibration plot is shown in equation (4.24), where 'b' represents the blank signal.

Y = m C + b Equation (4.24)

Equation (4.24) is illustrated graphically in figure (4.16) and shows the calibration line intercepting with the 'Y' axis at the value of the **blank** signal

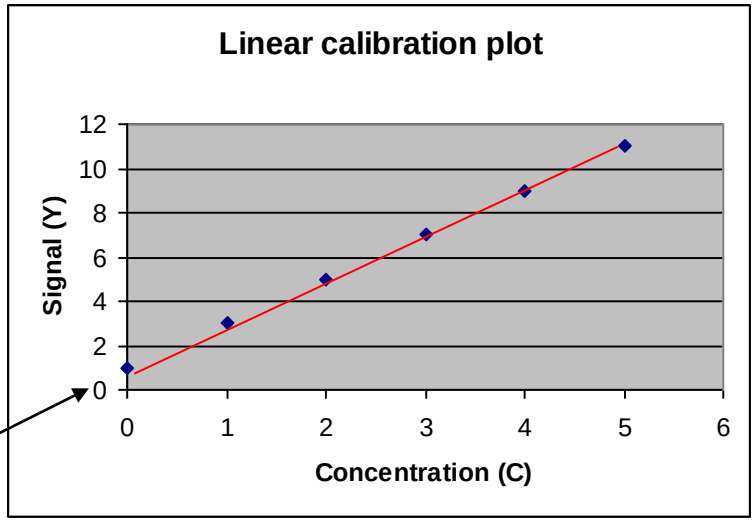


Figure 4.16 - calibration plot

In figure (4.16) we saw a calibration plot exhibiting a positive **blank** value. This may arise due to:

- The presence of the target analyte in any reagents used;
- Contamination of the standards during preparation;
- The instrument detector, not being sufficiently selective, so as to be able to distinguish the target analyte from other substances present in the samples and standards.

The assumption has to be made that within experimental error, the value of the blank signal remains constant throughout the experimental procedure.

Negative **blank** signals may also occur, but their presence is less frequent. Where this occurs, the negative value maybe added to all standard and sample so as to produce a calibration graph through the origin. The reason for this negative **blank** signal is less obvoius than for a positive one, however one possible scenario, would be in a spectrophotometric experiment, where a pair of cells was being used and the 'blank' cell was more absorptive that the sample cell.

Constructing the best straight line

In the calibration graph shown in figure (4.16), all of the data points appear to be exhibiting very little **random** error – all point sit on a straight line. However in real-life analytical procedures, some **random** error will occur with every measurement. In many cases, the size and variability of the **random** error is dependent upon:

- the skill of the analyst;
- the care taken by the analyst and
- the accuracy of the equipment used.

Random errors can be larger when very small volumes of liquids are being dispensed or transferred or very small amounts of analyte are being measured. Table (4.9) contains some calibration data for the colorimetric determination of formaldehyde in aqueous solution.

Concentration of formaldehyde in ppm (w/v)	Absorbance
0	0.080
5.0	0.340
10.0	0.635
15.0	0.885
20.0	1.165
25.0	1.420

A plot of this data and some calculations relating to this data are shown on the next four slides and figures (4.17) and (4.18)

Table (4.9)

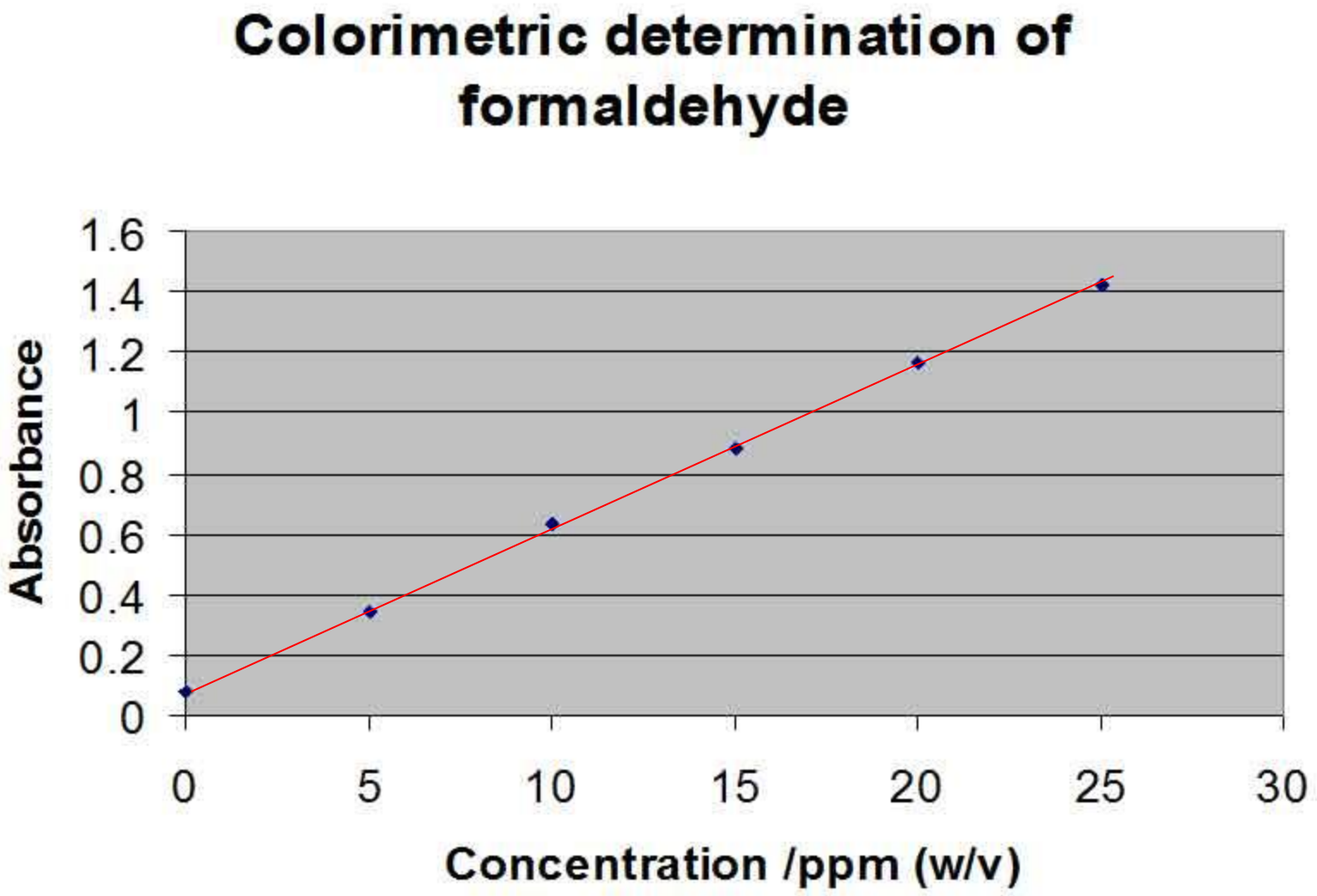

Colorimetric determination of formaldehyde
Absorbance
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
Concentration /ppm (w/v)

Figure (4.17)

The red line graph shown in figure (4.17) is a good interpretation of the best line fit for this set of data. However, how accurate is it, and moreover, can we do better? Most modern instruments have in-built software which allows not only the best straight line to be calculated from a measured set of data, but also the most accurate curved plot, for that data which does not appear to have a linear relationship. To calculate a best-fit linear plot, the **method of least squares** is used, whereby the the unknown variables 'm' and 'b' as shown in equation (4.24), are calculated.

For the data shown in table (4.9), the values calculate to be:

m = 0.0538 : b = 0.081

By using these values with the highest concentration standard (**25 ppm**), it is now possible to calculate the corresponding value of 'Y' and then to draw a line through this point and the blank value. For example:

Y = (0.0538 X 25) + 0.081
= **1.426**

This resultant graph is shown in figure (4.18) on the next slide.

Note: additional information on the method of least squares may be found at: http://en.wikipedia.org/wiki/Method_of_least_squares or in Chapter 5 of this teaching and Learning programme

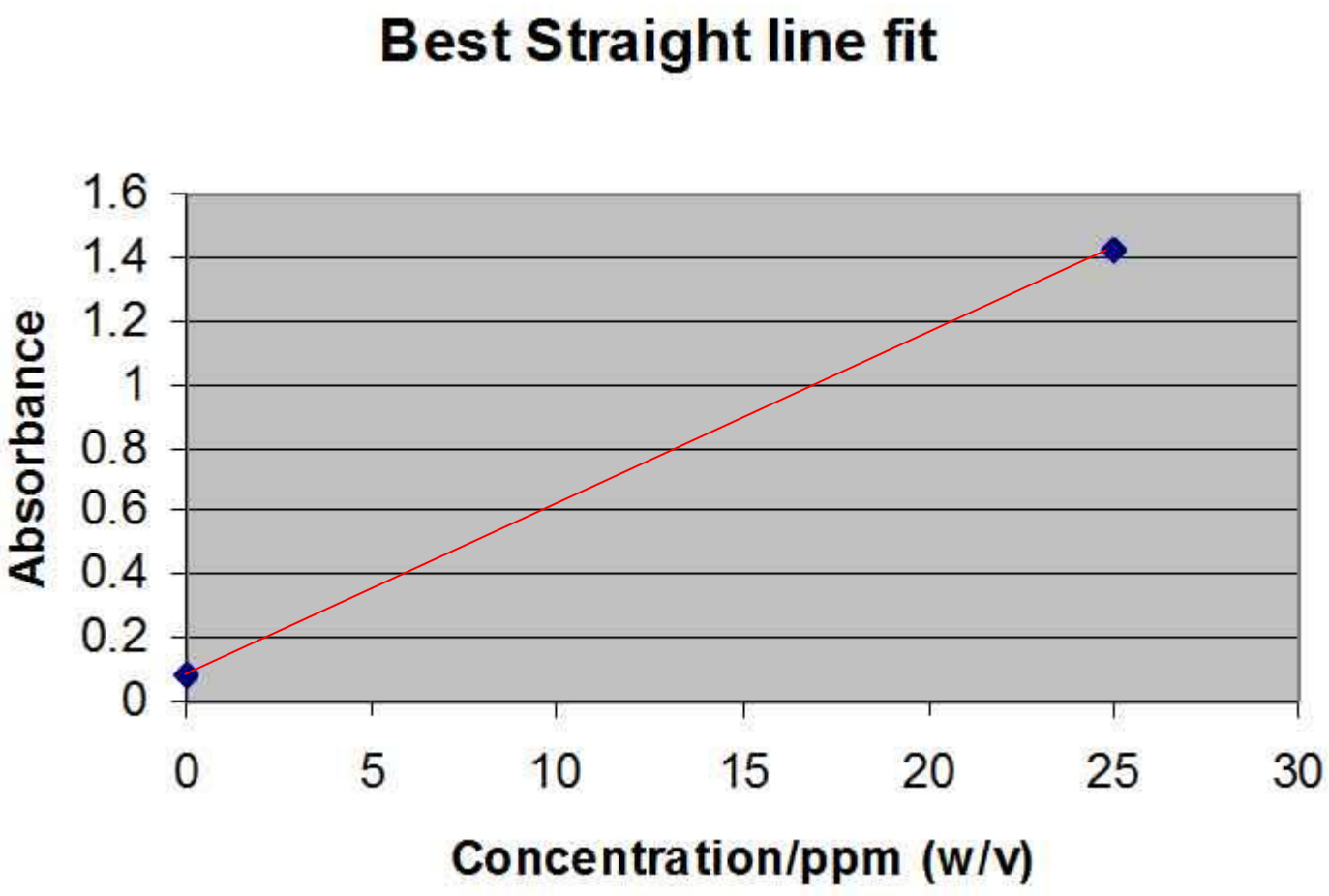


Figure 4.18 - best line fit

The graphs shown in figures (4.17) & (4.18) were plotted using Microsoft Excel, version 1997-2003

When employing the 'method of least squares', a number of assumptions are being made:

- That a linear relationship **does** exists between the signal (measured parameter) and the concentration of the analyte;
- That no significant error exists in the composition of the standards used to construct the graph (they have all been accurately prepared) and thus **all of the error lies in the values of the measured signal (Y).**
- That no pieces of data have been included in the data set, which appear to fall outside the normal statistical pattern. Any pieces of bad data should be rejected before performing the least squares calculation. Example (xx) below shows where this situation might arise.

Conc^{n} of formaldehyde in ppm (w/v)	Absorbance
0	0.080
5.0	0.340
10.0	0.635
15.0	0.885
20.0	1.417
25.0	1.420

This is the same set of data as shown in table (1.7) with one data point altered - that shown In RED. The absorbance is obviously too high, and indicates That the 20 ppm standard probably contains 25 ppm and thus has been prepared in error. This data point should therefore be excluded

When employing the 'method of least squares', a number of assumptions are being made:

- That a linear relationship **does** exists between the signal (measured parameter) and the concentration of the analyte;
- That no significant error exists in the composition of the standards used to construct the graph (they have all been accurately prepared) and thus **all of the error lies in the values of the measured signal (Y).**
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20.0	1.417
25.0	1.420

This is the same set of data as shown in table (4.9) with one data point altered - that shown In RED. The absorbance is obviously too high, and indicates That the 20 ppm standard probably contains 25 ppm and thus has been prepared in error. This data point should therefore be excluded

Example (4.xvi)

Separate solutions *versus* in-situ methodologies

There are two alternative methods for constructing calibration graphs:

- Preparation of separate calibration standards (solutions and solids);
- Generating calibrations using an ‘in-situ’ procedure.

Most calibrations are carried out by the use of separate standards. For instance, to analyse for the alcohol content in beer by gas-liquid chromatography, a calibration graph would be prepared, by analysing five standard solutions containing 0, 1.5, 3.0, 4.5 and 6.0 % (v/v) respectively of ethanol in water. The area underneath the ethanol peak on each chromatogram would be measured by the instrument’s integrator and these values then plotted as a function of solution concentration. Samples of beer may then be analysed under the same chromatographic conditions, with the area underneath the ethanol peak, being interpolated from the calibration graph, to give the concentrations of ethanol in the samples analysed.

Figure (4.19) on the next slide shows a typical set of data for this experiment

See Chapter 7 of this teaching & learning programme for more detail on gas-liquid chromatography

Peak area	Concentration % (v/v)
0	0
27456	1.50
54900	3.00
82380	4.50
109800	6.00
78045	Sample 1
70628	Sample 2
81692	Sample 3

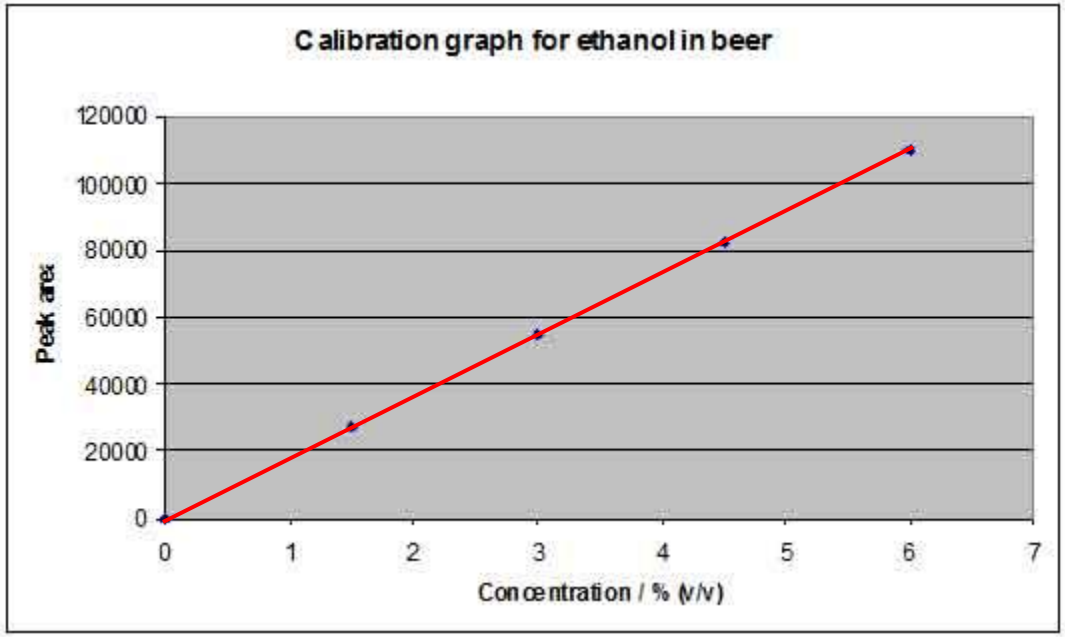

Figure (4.19) – calibration graph for the determination of ethanol in beer

Why not plot this data yourself and see if you can achieve the same results

The slope of the graph is 18300, and thus the concentration of ethanol in the three beer samples is:

Sample 1	4.26 %
Sample 2	3.86 %
Sample 3	4.46 %

In-situ calibration procedures

This technique for calibration, relates to a situation where a **single** matrix-matched solution is used for calibration and increasing concentrations of the analyte are introduced into it. The technique can only be used when:

- No significant level of analyte is consumed during the analytical measurement;
- No loss of solution occurs during the measurement process.

The measurement technique which most benefited from using in-situ calibration was **polarography** (an electrochemical technique which is now rarely used). However, in-situ calibration is still a valid procedure in certain circumstances, albeit generally offering less accuracy than the more traditional multi-standard approach, due to the very small volumes of standard added, in order to avoid significant dilution of the initial solution

Procedure

A measured volume of blank solution containing all components for matrix matching the sample, is added to the measurement cell and the blank measurement taken. A small volume (generally µl amounts) of a strong analyte standard [frequently 1000 ppm (w/v)] Is then added to that blank, mixed and another measurement made. This process Is repeated, until a calibration graph can be plotted of signal *versus* concentration. This solution is now replaced by the sample solution and a measurement obtained under exactly the same experimental conditions. The concentration of analyte in the sample can then be obtained by interpolation from the calibration graph.

Example of an in-situ experimental calculation

The example is from polarographic analysis, where the measured signal is μA (microamps). Assume that the initial volume of blank dilute acid solution was **20.0 cm^3,** and that the standard was a **1000 ppm (w/v)** solution of cadmium ion. Results are shown in Table (4.10) and figure (4.20)

Total volume of 1000 ppm standard added / μl	Conc^n. of analyte in solution in ppm (w/v)	Signal / μA
0	0	0
10	0.5	1.60
20	1.0	3.30
30	1.5	4.70
40	2.0	6.35
Sample	0.81	2.55

Table 4.10

Note: **10 μl** = 0.01 cm^3, and thus 10 μl of a 1000 ppm Cd^{2+} solution contains:
0.01 X 1000 μg of Cd^{2+}
= **10 μg of Cd^{2+}**

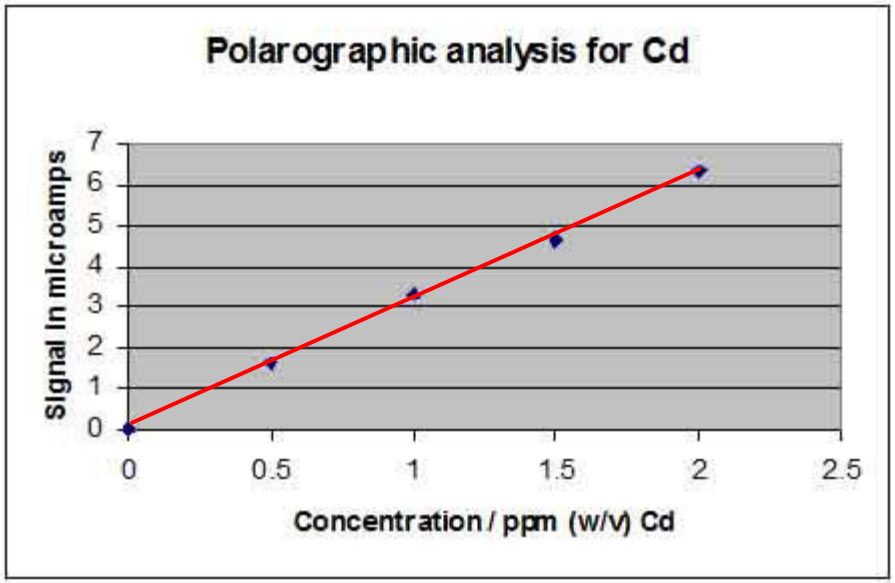

Figure 4.20

Abridged methods of calibration

Linear calibration plots as illustrated earlier in this section, require several calibration standards to be prepared in order to predict the calibration graph accurately. **The more calibration data points the more accurate the resultant analysis information**. Five calibration standards are normally considered as a good practical compromise, although statisticians would have you believe that ten or more are required. In terms of value for money however, five standards can only really be justified if a large number of similar samples are to be analysed for the target analyte. If you have only one or two samples to analyse then an abridged method of calibration is often adopted. There are three abridged methods which can be used, **provided that a linear relationship is known to exist between the measured signal and the analyte concentration**.

- Using a single standard;
- Using a single standard and a blank;
- Using two standards and a blank.

The first two of these procedures, can only be used where the analytical method exhibits only **random** error. The third method can be used where both **random** and **systematic** errors are thought to be present.

Using a single standard

This method can be applied where the blank signal is known to be zero or at least negligible.

A **single standard** containing all of the necessary reagents etc. is prepared at the **maximum concentration** where linearity is known to hold or in the case of measurements involving ABSORBANCE, to give an **absorbance value of between 0.4 to 0.5** (*the point of minimum error for this type of measurement)*.

There is no need to construct a calibration plot, as the calculation is based upon The principle of simple proportion:

$$\text{Concentration of analyte in sample} = \frac{Y_{sample}}{Y_{standard}} \text{ X Concentration of standard}$$

Advantage	Disadvantage
Quick and cost effective if only a few similar samples are to be analysed	The standard must be prepared to a very high degree of accuracy.

Using a single standard and a blank

This method can be adopted where a finite blank signal is known to exist, and there is no known systematic error.

Two solutions are prepared, one being the same as in the previous slide and one being reagent blank to which no analyte has been added.

Again, there is no need to construct a calibration plot, as the calculation is again based upon simple proportion:

$$\text{Concentration of analyte in sample} = \frac{Y_{sample} - Y_{blank}}{Y_{standard} - Y_{blank}} \times \text{Concentration of standard}$$

Advantage	Disadvantage
Quick and cost effective if only a few similar samples are to be analysed	The standard and blank solutions must be prepared to a very high degree of accuracy.

Using two standards and a blank

When the method is known to possess both a **systematic** error and a finite blank value, three calibration measurements must be made – two calibration standards and one blank. It is usual to choose the two standards to be at both ends of the calibration scale for the method. The results are plotted on a graph as illustrated in figure (4.21)

The sample signal is interpolated from the graph as shown in green

Advantage	Disadvantage
Cost effective if only a few similar samples are to be analysed.	The standard and blank solutions must be prepared to a very high degree of accuracy. Maybe preferable to produce a full calibration graph to increase reliability

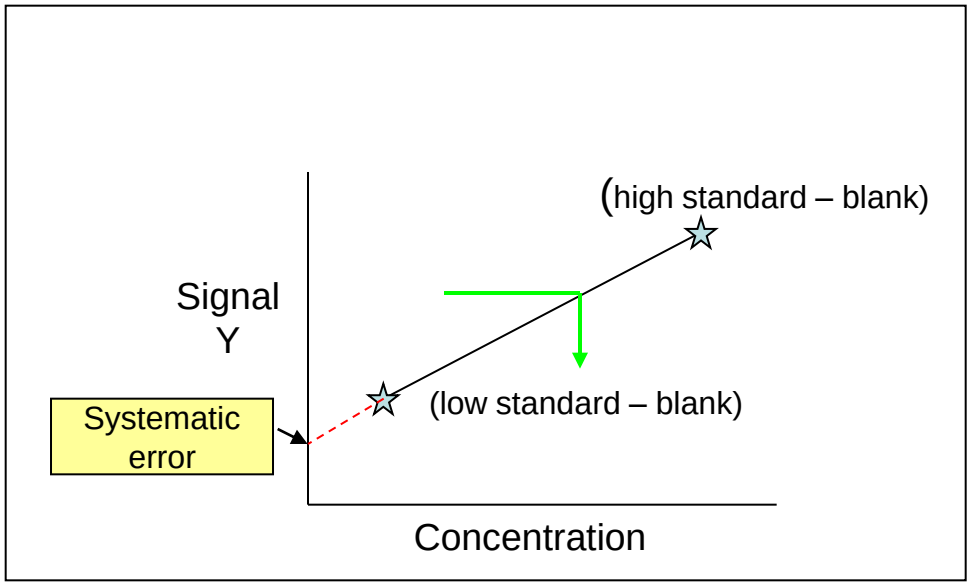

Figure 4.21 - calibration using two standards and a blank

Matrix matching of samples & standards

Standard solutions used for calibration purposes are frequently prepared by simple dilution of higher concentration standards. Many of these standards will have small quantities of reagents present (eg: dilute mineral acid), which in most cases do not cause interference with the measurement techniques employed. However, 'real' samples can have a variety of other substances present, some of which maybe unknown and which could cause problems with the method or technique used for analysis. Examples of where interferences could occur are:

- Differences in solution volatility or viscosity – both of these could have an effect of atomic spectroscopic techniques by altering neblization rates;
- Changes to the exact nature of the analyte (termed speciation), whereby for instance, the chemistry of the analyte is altered by reaction with components of the sample matrix to produce a complex product.

Assuming that the major components of the sample are known, then it is possible to prepare standards that contain similar quantities of these matrix components. This process is known as **matrix matching** and can allow for many interferent effects caused by matrix components.

Example (4.xvii) – determination of fluoride ion in water

This analysis can be carried out by a using a selective, fluoride ion-selective electrode as the measurement sensor (technique). This is a potentiometric method of analysis where the cell potential generated between the ion selective electrode and a reference electrode is related to the concentration of the fluoride ion (F^-) in the solution, providing the ionic strength of the solution remains constant. The relationship can be expressed as:

$$E = K - \frac{2.303\,RT}{F} \log [F^-]$$

As K, R, T and F are all constants, **E** (cell potential) **α log [F^-]** (fluoride ion concentration)

As the fluoride ion is capable of complexing with a number of other metal ions frequently found in natural and treated water samples (eg: Si^{4+}, Al^{3+} and Fe^{3+}), all of the samples and standards need to be mixed with in a special buffer (TISAB – total ionic strength adjustment buffer) which both stabilises the ionic strength, adjusts the pH and prevents the fluoride ion from complexing with these metal ions.

By matrix matching the samples and standards in this way, it is possible to use this technology for routine measurements of fluoride ion in water.

If the 'real' sample contains substantial quantities of unknown ingredients then it probably wise to use the alternative 'standard addition method' to solve the calibration situation. [**see next slide**]

Method of standard-addition

Matrix matching of samples and standards is feasible when the composition of a sample is reasonably well documented. However, there are occasions when samples of unknown composition need to be analysed for selected target analytes. In this situation, the method of standard-addition may be used, whereby the **calibration is carried out within the sample matrix**.

The complexity of the standard-addition method may vary from one analytical technique to another, however, the procedure adopted always involves the same pattern of events:

- Preparation of the sample that is to be analysed, by the addition of reagents and buffers etc. before measuring the chosen parameter (eg: absorbance, emission, current, potential) of the analyte targeted for analysis;
- Addition to this sample, of a known quantity of a standard analyte, mixing the the resultant solution and re-measuring the chosen parameter.

Assuming a linear relationship exists between signal and analyte concentration, the increase in measured signal, is related to the increase in solution concentration caused by the addition of the standard to the prepared sample solution.

Before considering in detail how the technique of standard-addition may be applied, three important points must be emphasised:

- A linear relationship must exist, between signal and analyte concentration;
- The sample solution must not contain any interfering substances that can masquerade as the analyte.
- Any 'blank' signal generated by the measuring technique, must be assumed to be constant for all measurements made and subtracted from the measured signal before the calculation is performed.

The method of standard-addition may be applied in two ways:

- By using the addition of a single portion of a standard analyte solution;
- By using multiple additions of a standard analyte solution, taking measurements after each addition and finally plotting a graph of the results, in order to calculate the concentration of analyte in the sample.

Dependent upon the analytical situation, it maybe possible to use an 'in-situ' procedure for the additions, however most application will use separately prepared solutions. **A comparison of the various procedures is shown on slide 101.**

The method of single standard-addition

The method involves the addition of an aliquot of a standard solution of the analyte to the sample solution, with measurements of the target parameter relating to the analyte being made before and after addition. The concentration of the analyte in the sample is calculated by the use of two simultaneous equations, (4.25 and 4.26).

$\mathbf{Y_0 = m\,C}$ Equation (4.25)

$\mathbf{Y_1 = m\,(C + C_s)}$ Equation (4.26)

Where:

Y_0 is the measured signal before the addition of the standard

Y_1 is the measured signal after the addition of the standard

m is the sensitivity of the method

C is the concentration of the analyte in the sample

C_s is the **increase** in concentration of the analyte due to standard-addition

The values of Y_0, Y_1 are measured and the value C_s is known. The sensitivity 'm' will be same in both measurements and thus can be eliminated, so that 'C' can then be calculated. **The calculation is shown on the next slide**

$Y_0 = m\,C$ Equation (4.25)

$Y_1 = m\,(C + C_s)$ Equation (4.26)

We know the values of Y_0, Y_1 and C_s. The sensitivity 'm' will be same in both measurements and thus can be eliminated, so that 'C' can be calculated.

Equation (4.25) may be rewritten as: $m = \frac{Y_0}{C}$

Therefore equation (4.26) can now be rewritten as:

$$Y_1 = \frac{Y_0}{C}(C + C_s) = Y_0 + \frac{Y_0\,C_s}{C}$$

Which may be expressed as: $(Y_1 - Y_0) = \frac{Y_0\,C_s}{C}$

Therefore $\mathbf{C = \frac{Y_0\,C_s}{(Y_1 - Y_0)}}$ **Equation (4.27)**

It is important to remember that C_s is the **increase** in concentration of the analyte in the solution being analysed and **NOT** the concentration of the analyte in the standard used.

For example:

1 cm^3 of a 1000 ppm (w/v) standard is added to the sample present in a 50 cm^3 flask. The whole is then diluted to volume. The value of Cs is therefore:
(1 ÷ 50) X 1000 ppm
= 20 ppm

Note: The units for 'C' are defined by those chosen for 'C_s'

Example (4.xviii) – a single standard-addition analysis

An analgesic preparation is to be analysed for it's caffeine content by hplc. Two tablets are selected randomly from a box of 16 tablets, crushed and the caffeine extracted with methanol using an ultrasonic procedure. The extract after filtration, is transferred to a 50.0 cm^3 calibrated flask and diluted to volume with methanol. Two 10.0 cm^3 aliquots of this prepared solution are pipetted into two 25.0 cm^3 calibrated flasks. To one of these flasks is now added 5.0 cm^3 of a standard caffeine solution containing 1.75 mg/cm^3 of caffeine. Both solution are now diluted to volume with a methanol/water mix. Using a validated method, the two solutions are analysed by hplc for caffeine and the following peak areas recorded. There was no peak area recorded for the solvent blank.

Sample	**976 mm^2**
Sample + standard	**1450 mm^2**

Calculate the average weight of caffeine in each of the tablets taken for analysis

From equation (1.7) on the previous slide:

$$C = \frac{Y_0 C_s}{(Y_1 - Y_0)} \ mg/cm^3$$

$C_s = (5 \div 25) \times 1.75 \ mg/cm^3 =$ **0.35 mg/cm^3**

Therefore **C** $= \frac{(976 \times 0.35)}{(1450 - 976)} = \frac{341.6}{474} =$ **0.72 mg/cm^3**

Concentration of caffeine in the prepared sample solution is thus $0.72 \times \frac{25}{10} =$ **1.8 mg/cm^3**

Weight of caffeine / tablet is thus: $(1.8 \times 50) \div 2 =$ **45 mg/tablet**

In-situ method of single standard-addition

The example shown on the previous slide, is an example of a single standard-addition procedure using separate solutions – with and without the standard.

The ‘in-situ’ procedure is simpler to carry out, however can only be applied where the measurement technique **consumes none or only negligible quantities of the prepared standard solution**. Also the calculation is more complicated if the volume of standard added, dilutes the sample significantly (say by more than 1%).

The equation that define the procedure and how it can be applied are shown on the next two slides.

Note: The concentration of the standard added in either of the two methods, should ideally increase the signal by a substantial level (between 50 – 150% of that generated without the standard), so long as the resultant total does not exceed the linearity of the analytical procedure.

If the volume of the prepared solution on which the measurement is made is ‘V’, and the volume containing the standard is ‘v’, then the two equations needed to define the procedure are:

$$Y_0 = m\,C \qquad \text{Equation (4.28)}$$

$$Y_1 = m\left[C\left(\frac{V}{V+v}\right) + C_s\right] \qquad \text{Equation (4.29)}$$

$$= \frac{Y_0}{C}\left[C\left(\frac{V}{V+v}\right) + C_s\right] \qquad \text{Equation (4.30)}$$

Rearranging equation (4.30) produces the equation for the procedure which is:

$$\mathbf{C = \frac{Y_0\,C_s\,(V+v)}{Y_1\,(V+v) - Y_0\,V}} \qquad \text{Equation (4.31)}$$

An example to illustrate how it may be applied is shown on the next slide

Example (4.xix) - a single 'in-situ' standard addition

Compound A is to be determined by UV spectroscopy

25.0 cm^3 of the sample solution are pipetted into a small beaker, to which is added a small magnetic stirrer bar. Some of this solution is carefully transferred into a clean dry 1 cm silica cell and a measurement of absorbance made at 300 nm. The contents of the cell are then carefully returned to the beaker. To the sample is now added by pipette, 1.0 cm^3 of a 0.1 % (w/v) standard solution of the analyte. The solution is now mixed by stirring, before again carefully transferring some of the solution into the same silica cell. This process is carried out twice, the first portion being used to wash out the cell (returning the wash solution to the beaker) and the second used for measurement of absorbance at 300 nm. If the absorbances measured at 300 nm were respectively **0.513 and 0.875**, calculate the concentration of A in the sample solution.

We need first to calculate the increase in concentration of A caused by the addition of the standard (C_s)

$$\mathbf{C_s} = \frac{1}{25 + 1} \text{ X } 0.1\ \% = \mathbf{0.00385\ \%}$$

Using equation (1.29) we can now calculate the concentration of A in the sample solution

$$\mathbf{C} = \frac{Y_0\, C_s\, (V + v)}{Y_1\, (V + v) - Y_0\, V} = \frac{0.513 \text{ X } 0.00385 \text{ X } 26}{(0.875 \text{ X } 26) - (0.513 \text{ X } 25)} = \frac{0.0513}{22.75 - 12.82} = \mathbf{0.00517\ \%}$$

Note: Performing the calculation by using equation (1.26) and not allowing for the dilution of the sample due to the addition of the standard, gives a figure of **0.00567 %**

Method of multiple standard-addition

The main disadvantage of the single standard addition method is the reliance on the accuracy of the volume of standard solution added. An error introduced at this stage cannot be identified and thus produces an erroneous result. This potential error situation can be overcome by the use of multiple standard-addition whereby the individual errors are averaged out by use of a graphical procedure. The equations underlying this procedure are described below:

Equation (4.27) [$C = \frac{Y_0 C_s}{(Y_1 - Y_0)}$] can be rewritten as $C = \frac{Y_0 C_s}{\Delta Y}$

Which can then be rearranged as:

$$\Delta Y = \frac{Y_0 C_s}{C} \qquad \textbf{Equation (4.32)}$$

Thus there is a linear relationship between the total change in measured signal (ΔY) and the total increase in solution concentration of the analyte. The plot of equation (4.32) is shown in figure (4.22). The value of C (concentration of analyte in the sample), is calculated from the slope.

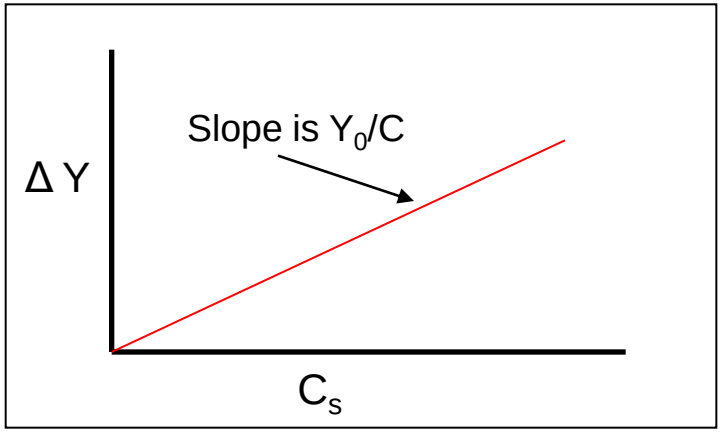

Figure (4.22)

Equation (4.27) can be written in a general form [equation (4.33)] to represent the successive standards added in the multiple standard-addition procedure

$$C = \frac{Y_0\, C_{sn}}{(Y_n - Y_0)} \qquad \text{Equation (4.33)}$$

Where C_{sn} and Y_n represent the increase in analyte concentration (or weight) and the resultant signal respectively at any stage during the multiple addition.

Equation (4.33) can be rearranged as:

$$Y_n = \frac{Y_0\, C_{sn}}{C} + Y_0 \qquad \text{Equation (4.34)}$$

This produces a graph of the type shown in figure (4.23), where Y_0 is the intercept on the signal axis. If in equation (4.34) we make $\mathbf{Y_n = 0}$ (the situation that exists at the start of the experiment), then the equation can be rearranged to show that under **this particular condition**:

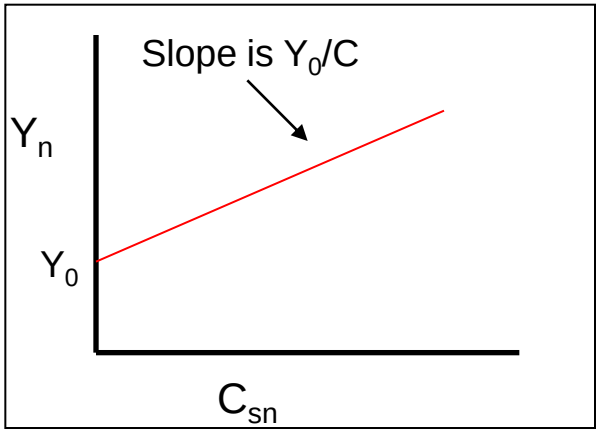

Figure (4.24)

$$\mathbf{C = -\, C_{sn}}$$

continued on the next slide

Thus in order to calculate the value of 'C' (concentration of analyte in the prepared sample solution), we need to redraw the graph such that the graph can be extrapolated back to the concentration (C_{sn}) axis. This is illustrated in figure (4.25)

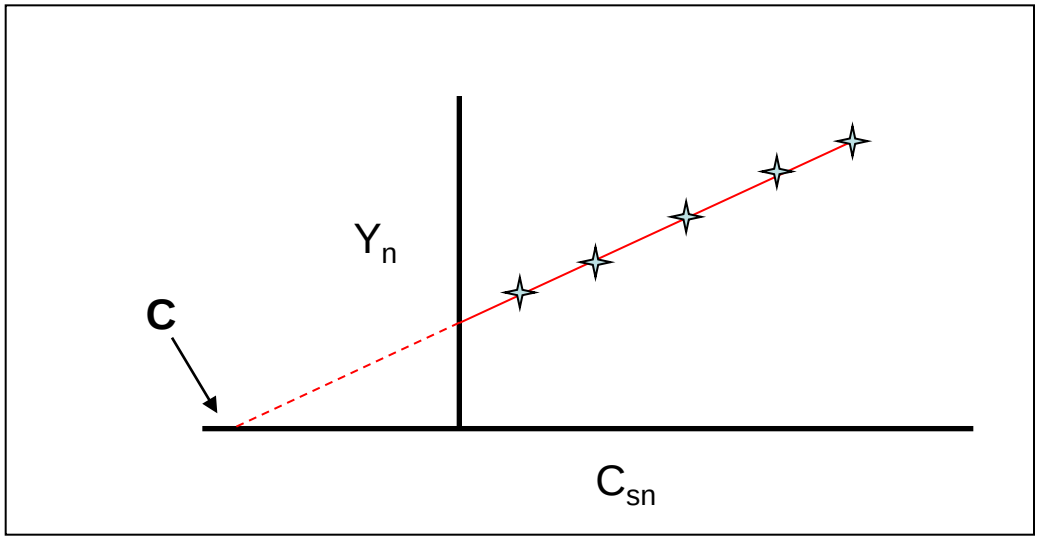

Figure (4.25)

In the examples shown to illustrate this procedure, 4 or 5 standard have been added to produce the graph. The number of additions to be made will depend upon the accuracy and precision required for the result. The higher the accuracy and precision required, the more standards that need to be added. The total concentration of analyte at the end of the procedure, should not exceed the known linearity for the analytical method.

An example to illustrate this procedure is shown on the next slide

Example (4.xx) – Determination of the iron content of a multi-vitamin tablet using a multiple standard-addition procedure

Fe^{2+} can react with 1,10 phenanthrolein to produce a deep red coloured complex, exhibiting a maximum absorbance at 508 nm. As the iron present in the tablet will be in the Fe^{3+} form and also that the iron standard used for calibration is also in the Fe^{3+} state, it is necessary to incorporate a reduction step using hydroquinone (HQ) into the procedure. The relevant equations for the reaction are:

$2Fe^{3+} + HQ = 2Fe^{2+} + Q + 2H^{+}$ [where Q indicates quinone]

$Fe^{2+} + 3\ Phen = \{[phen]_3\}\ Fe^{2+}$ [where phen represents 1,10 phenanthrolein]

1 vitamin tablet was dissolved in acid and diluted to 100 cm^3. This sample solution was then diluted 5 cm^3 to 100 cm^3 to produce the sample solution (A) for analysis.

10 cm^3 of A was pipetted into 5 X 100 cm^3 calibrated flasks. To the 5 flasks were added sequentially 0, 1.0, 2.0, 3.0, and 4.0 cm^3 of a standard solution containing 40 µg/cm^3 of Fe^{3+}. To each flask was added a buffering agent, hydroquinone solution and a phenanthrolein solution in that order, before diluting each flask to volume. A blank solution was also prepared containing no added iron or sample. After mixing the solutions and allowing them to stand to develop the coloured complex, the absorbance of each solution was measured at 508 nm. The results are shown in table (4.11). A graph of the results is shown as figure (4.26) on the next slide.

Solution identity	Wt. of Fe added in µg	Absorbance	Absorbance - blank
Blank	0	0.007	0
Sample (A)	0	0.212	0.205
Sample (A) + 1 cm3 of standard	40 µg	0.332	0.325
Sample (A) + 2 cm3 of standard	80 µg	0.457	0.450
Sample (A) + 3 cm3 of standard	120 µg	0.567	0.560
Sample (A) + 4 cm3 of standard	160 µg	0.692	0.685

Table (4.11)

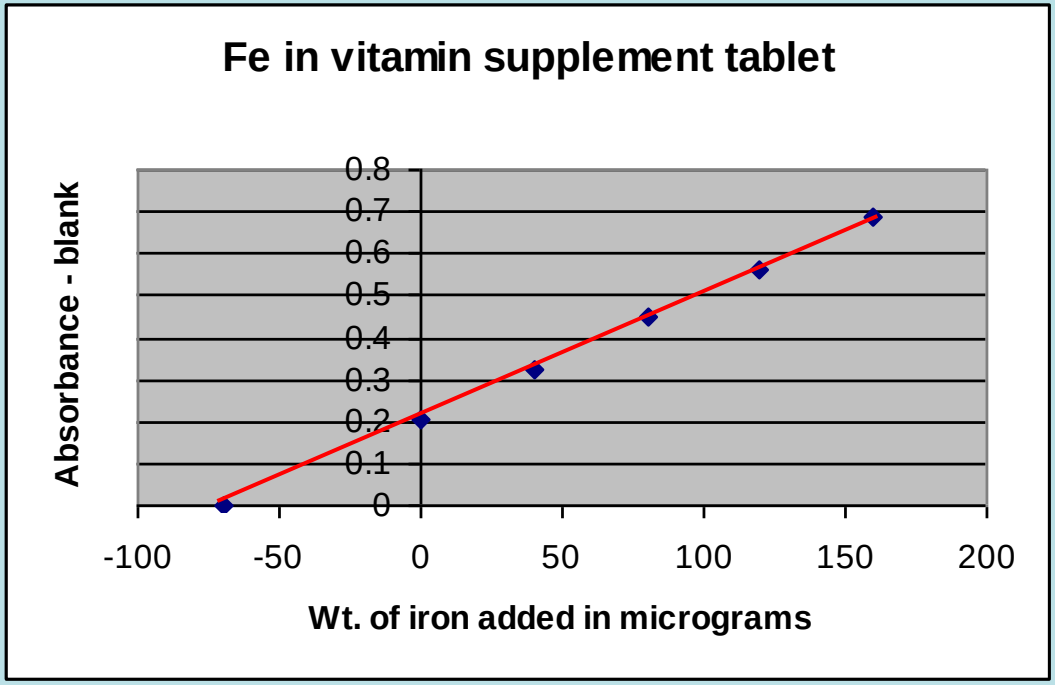

Figure (4.26)

Extrapolating the calibration line back to the weight axis, shows that the prepared solution (A) contained 70 µg /10 cm^3 of solution – that is 7 µg /cm^3.

As (A) was prepared by diluting the original sample solution 20 times, the concentration of iron in that original solution is thus:

7 µg /cm^3 X 20 = 140 µg /cm^3

As the total volume was 100 cm^3, 1 tablet contained:

140 X 100 µg **= 14 mg of iron**

Comparison of standard-addition procedures

Method	Advantages	Disadvantages
Single standard-addition	▪ Allows for automatic matrix matching of samples & standards and thus can compensate for some forms of solution interference; ▪ Economic for small numbers of samples.	▪ Not possible to identify human errors in diluting samples or adding standards; ▪ No mechanism to average out normal instrumental errors; ▪ Can only be applied where signal is known to be α analyte conc^{n}.
In-situ single standard-addition	As above plus: ▪ Only one sample needs to be prepared.	As above plus: ▪ Can only be used where no sample is consumed during the measurement process; ▪ Calculation is more complex.
Multiple standard-addition	▪ Mechanism to average out normal instrumental and experimental errors; ▪ Gross operator errors can be identified and allowed for; ▪ Allows for automatic matrix matching of samples & standards and thus can compensate for some forms of sample interference.	▪ More time consuming than the single procedure; ▪ Not economic for more than a few samples.

Table 4.12 – comparison of standard addition procedures

Comparison of graphical and standard-addition procedure

- It is very time consuming to produce a calibration graph when only one or two samples are to be analysed. The use of standard-addition is thus recommended in this situation.
- Components present in the sample may interfere with the measurement of the analyte in either an additive or a proportional way. Proportional effect may be compensated for by matrix-matching of samples and standards (which can be time-consuming) or by using standard-addition procedures.
- Calibration graphs produced from three or more standards, will be able to identify gross operator errors induced when preparing the standard solutions. This will also be the case with multiple standard-addition procedures.
- It is unlikely that other components of samples that can masquerade as the analyte in the measurement process, will be compensated for by the use of either of these procedures
- Whereas a 'blank' signal can be allowed for in a calibration graph, this is not the case with standard-addition procedures, where the blank will need to be subtracted from all measured signals before performing calculations.

The method of internal-standards

Equation (4.21) [**Y = *f* C**], shown on a earlier slide, expresses the generic relationship underlying the use of comparative analytical techniques. The term '*f*' will usually be a combination of a number of experimental parameters, all of which are assumed to be constant during the calibration and analysis. Some examples of these parameters are:

- In molecular spectroscopic techniques (eg: UV, visible & IR), the cells used for measurement are assumed to be of a constant pathlength. Also individual cells can show slight differences in transmittance of radiation.
- In some aspects of atomic spectroscopy, the function '*f*' is a combination of a number of terms, including terms related to the rate of aspiration and efficiency of nebulization of the solution, prior to measurement of either emission or absorption of radiation.
- In chromatographic analysis, amongst other parameters, the volume of the sample taken for analysis is assumed to be constant. Nowadays, fixed volume sample loops are used in order to control volumes for analysis. However in some older equipment, where manual injection of samples is required, it can be difficult to reproduce sample volumes accurately.

In all calibration procedures we assume:

- All of the stages in the analysis process are accurately controlled;
- All of the parameters that affect the physical measurements have been held constant;
- The quantity of sample taken for calibration and analysis was accurately known or was constant.

The method that can be adopted to overcome any of these potential unknowns is '**The Method of Internal Standards'**. An internal standard is another substance present in the sample when it is analysed, whose concentration or abundance (in the case of direct analysis of solid matrices) is measured in addition to that of the analyte. The measurement of analyte concentration or abundance is based upon the **ratio of measured signals (analyte : internal standard).**

Although in a few instances, the internal standard may already be present in the sample, (for instance as a major component at assumed constant composition), in the majority of cases where the method is employed, the internal standard is **added to the sample prior to analysis.**

The equations that define the usage of the internal standard method are shown on the next slide

To illustrate the procedure, consider an imaginary analytical technique, where the measured parameter 'Y', for the analyte, obeys the relationship shown in Equation (4.35)

$$Y = m\ k\ C \qquad \text{Equation (4.35)}$$

Where: **m** is the unique response given by the analyte at the detector – assumed to be constant;
k is a variable parameter whose exact value may not be known (eg volume);
C is the concentration of the analyte

Equation (4.35) differs from the simple linear equation used for normal calibration plots and standard addition (Y = m C), in that in those circumstances, 'k' is assumed to be constant and is in fact incorporated in to the **sensitivity** or slope term which 'm' then represents. An equation which relates very closely to that of (4.35), is the Beer-Lambert Law [equation (4.36)], which is applicable in quantitative spectroscopy.

$$\text{Absorbance} = \epsilon\ C\ L \qquad \text{Equation (4.36)}$$

Where: ϵ is the unique response term know generally as 'Absorptivity'
L is the pathlength of the cell holding the solution to be measured

Continued on the next slide

Assuming that ‘ϵ’ and ‘L’ are both constant, then:

Absorbance α Concentration

This can only strictly be the case, when a single cell is used for all measurements of samples and standards. If we are unable to assume this consistency then the use of an internal standard is to be recommended.

We can rewrite equation (4.35) for both the analyte and for the internal standard:

$$Y_1 = m_1 k_1 C_1 \qquad \text{Equation (4.37)}$$

$$Y_2 = m_2 K_2 C_2 \qquad \text{Equation (4.38)}$$

Where subscript (1) refers to the analyte and (2) relates to the internal standard

Dividing equations (4.37) by (4.38) gives:

$$\frac{Y_1}{Y_2} = \frac{m_1 k_1 C_1}{m_2 k_2 C_2} \qquad \text{Equation (4.39)}$$

Given that both measurements of ‘Y’ were made on the sample portion the analysis sample, the variable parameters k_1 and k_2 become equal

Continued on the next slide

Equation (4.39) thus may be simplified as:

$$\frac{Y_1}{Y_2} = \frac{m_1 C_1}{m_2 C_2} \qquad \text{Equation (4.40)}$$

Within equation (4.40), the ratio m_1/m_2 is termed the **response ratio** (R), and thus equation (4.40) may be rewritten as:

$$\frac{\mathbf{Y_1}}{\mathbf{Y_2}} = \mathbf{R}\,\frac{\mathbf{C_1}}{\mathbf{C_2}} \qquad \text{Equation (4.41)}$$

Equation (4.41) is the fundamental equation for use with the internal standard method. As with standard-addition, it can be used in both a **single** and a **multiple** mode. In both of these modes we need to rearrange equation (4.41) to give:

$$\mathbf{R} = \frac{\mathbf{C_2\,Y_1}}{\mathbf{C_1\,Y_2}} \qquad \text{Equation (4.42)} \quad \text{and} \quad \mathbf{C_1} = \frac{\mathbf{Y_1\,C_2}}{\mathbf{Y_2\,R}} \qquad \text{Equation (4.43)}$$

From an initial experiment using equation (4.42), the value of 'R' can be found and then used in equation (4.43) to calculate the concentration of analyte (C_1)

Method of single internal-standard

In this mode of usage, two solutions need to be prepared for the analysis of one or more analytes in a single sample. For simplicity, consider analysis for only a single analyte (A) using an internal standard (IS). The two solutions required are:

- The calibration solution containing known quantities of both an analyte standard and an internal standard;
- The sample solution containing the sample and the same quantity of internal standard that was chosen for the calibration.

Analysis of the 'calibration' solution will yield two signals 'Y_A' and 'Y_{IS}'. Thus knowing the concentration of analyte 'C_A' and of the internal standard 'C_{IS}', it is now possible using equation (4.42) to calculate 'R'.

Analysis of the 'sample solution' will again yield two signals 'Y_A' and 'Y_{IS}' and by using equation (4.43) it is now possible to calculate 'C_A', the value of 'R' being known.

Consider an example shown on the next slide.

Example (4.xxi) - Determination of the ethanol content of a sample of white wine by gas-liquid chromatography, using the method of single standard addition.

25.0 cm^3 of a sample of white wine were pipetted into a 100 cm^3 calibrated flask. To a further 100 cm^3 calibrated flask was added 5.0 cm^3 of absolute ethanol. To each of these solutions was then added 5.0 cm^3 of pure propanol-1 as an internal standard. The resultant solutions were then diluted to volume with pure water.

Nominal I µl volumes of each of these solutions were then injected sequentially on a gas chromatograph and the peak areas for the ethanol and propanol-1 peaks were measured. The results Are tabled below. Calculate the concentration of ethanol in the wine sample in terms of % (v/v).

Solution	Peak area for EtOH (mm2)	Peak area for PrOH (mm2)	Conc^n of EtOH % (v/v)	Conc^n of PrOH % (v/v)
Calibration	1567	1754	5.0	5.0
Sample	1056	1823	?	5.0

From equation (1.20) $R = \dfrac{C_{IS} \times Y_A}{C_A \times Y_{IS}} = \dfrac{5.0 \times 1567}{5.0 \times 1754} = 0.893$

From equation (1.21) $C_A = \dfrac{1056 \times 5.0}{1823 \times 0.893} = \dfrac{5280}{1573} = 3.36$ % (v/v)

Thus the concentration of ethanol in the wine sample analysed was $3.36 \times \dfrac{25}{100} =$ **13.4 % (v/v)**

Method of multiple internal-standard

As with the single standard-addition method, the use of a single internal standard procedure, has no means of averaging out experimental errors and thus relies heavily on the practical expertise of the analyst. The use of the multiple internal standard approach will overcome this potential error and thus give a more reliable result.

In the multiple internal standard procedure a series of standards are prepared containing varying quantities of analyte, but each containing a **fixed quantity** of the internal standard. Equation (4.41) can thus be modified, given that the term 'C_2' in the equation is now a constant term.

Thus $\frac{Y_1}{Y_2} = R \frac{C_1}{C_2}$ Equation (4.41) now becomes:

$\frac{Y_1}{Y_2} = R' C_1$ Equation (4.44) where [$R' = R/C_2$]

Thus the ratio of the two signals $\mathbf{(Y_1/Y_2)\ \alpha\ C_1}$ (the concentration of analyte) which should result in a linear calibration graph.

Example (4.xxii) - Determination of trace organic solvents in an aqueous effluent

An aqueous effluent is know to contain small quantities of both acetone (dimethyl ketone) and methyl-ethyl ketone (MEK). Ethanol, which separates chromatographically from both of these analytes on the chosen column, is selected as the internal standard.

To a set of five 100 cm^3 calibrated flasks, is added about 50 cm^3 of pure water together with increasing amounts of both acetone and MEK. The quantities added are shown on the table below To each flask is then added 0.50 cm^3 of pure Ethanol. All flasks are then diluted to volume with further water. To a further flask is added about 90 cm^3 of the effluent sample, to which is added 0.50 cm^3 of pure ethanol. The flask Is filled to volume with additional effluent sample. Each solution is then analysed by gas-liquid chromatography with the peaks due to acetone, MEK and ethanol all being measured. All results are tabulated below. Calculate the concentration of the two ketones in the effluent sample.

Solution	[EtOH] % (v/v)	[acetone] % (v/v)	[MEK] % (v/v)	Peak area for EtOH mm2	Peak area for acetone mm2	Peak area for MEK mm2
Blank	0	0	0	0	0	0
Standard 1	0.50	0.10	0.05	1500	240	165
Standard 2	0.50	0.20	0.10	1474	480	315
Standard 3	0.50	0.30	0.15	1540	730	498
Standard 4	0.50	0.40	0.20	1494	946	650
Effluent sample	0.50	?	?	1532	505	205

Table (4.13)

Calculation is continued on the next slide

From equation (4.44) it can be seen that the ratio of peak areas from the table of results has to be calculated before graphs can be plotted. This calculation and the subsequent graphs are shown below:

Solution	[EtOH] % (v/v)	[Acetone] % (v/v)	[MEK] % (v/v)	Peak area ratio acetone:EtOH	Peak area ratio MEK:EtOH
Standard 1	0.50	0.10	0.05	0.160	0.110
Standard 2	0.50	0.20	0.10	0.326	0.214
Standard 3	0.50	0.30	0.15	0.474	0.323
Standard 4	0.50	0.40	0.20	0.633	0.435
Effluent sample	0.50	?	?	0.330	0.134

Table (4.14)

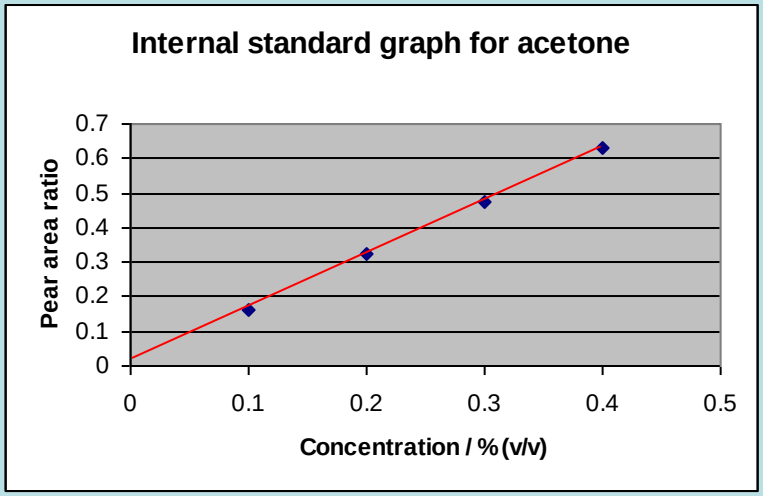

Figure (4.27)

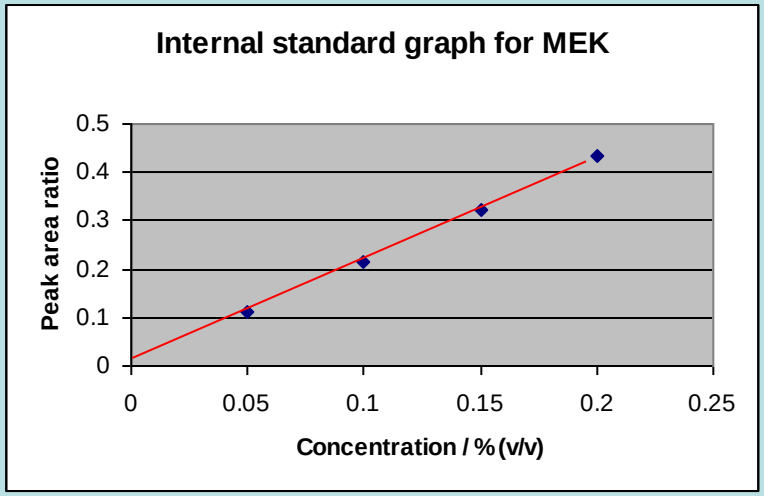

Figure (4.28)

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Note: calculation is completed on the next slide

Interpolating from the two graphs on the previous slide, the concentrations of acetone and MEK in the effluent are:

0.206 % (v/v) acetone and 0.062 % (v/v) MEK

These concentrations however must be modified slightly to account for the slight dilution that occurred During the preparation of the sample for analysis – sample diluted 99.5 cm^3 to 100 cm^3

Thus concentrations of the ketones in the effluent were respectively:

0.207 % and 0.062 %, which should probably be reported as:

0.21 % (v/v) acetone and 0.06 % (v/v) MEK

Examples (4.xxi) and (4.xxii) of the application of the internal standard method have highlighted some important points relating to the usage of the method. These are:

- Analysis by gas-liquid chromatography is the most frequent user of the method;
- Within this usage the choice of internal standard is crucial:
  - It must be miscible with the sample solution
  - It must be chromatographically separable from all other components in the sample
  - It should elute from the chromatographic column close to the analyte(s)
- A single internal standard can be used to measure a number of analytes in a sample matrix

Comparison of single and multiple internal-standard methods

Method	Advantages	Disadvantages
Single internal-standard	▪ Very rapid analysis once the response ratio has been determined ▪ Capable of analysing for a number of analytes present in the sample ▪ Capable of providing an analysis to ± 0.5 % of the true value.	▪ Response factor needs to be recalculated when experimental conditions change ▪ No mechanism for averaging out random errors ▪ No mechanism for identifying gross experimental errors
Multiple internal-standard	▪ Capable of averaging out random errors ▪ Capable of identifying gross experimental errors ▪ Capable of providing an analysis with a high degree of accuracy and precision ▪ Capable of analysing for a number of analytes present in the sample.	▪ Only really economic when a number of similar samples are to be analysed

Table 4.15 – comparison of internal-standard methods

Comparative analytical techniques - reflection

- All comparative techniques require calibration against known standards for quantitative measurement.
- Standards can be prepared from pure elements or their compounds, from commercially prepared solutions or standard mixtures.
- Calibration may be achieved by the use of calibration graphs, standard-addition or internal-standard procedures.
- Graphical procedures allow for the averaging out of random errors, however are not economic when only a very small number of samples is to be analysed.
- Matrix-matching of samples and standards is sometimes required in order to overcome proportional interference effects. This is best achieved by the use of in-situ calibration or standard-addition procedures.
- Internal-standards can be used to reduce some types of experimental error.

Question 4.1 Analytical techniques may be categorised in a number of ways. Discuss the possible categories explaining the reasons why none of them are perfect.. Distinguish between the terms 'Absolute' and 'Comparative' to categorise quantitative analytical methodologies.

Question 4.2 The end point in a potentiometric titration carried out by using an auto-titrator, is often found by plotting $d^2(mV)/d(vol)^2$ against volume of titrant. Explain how this method detects the end point in a typical acid-base titration. A solution of dilute HNO_3 is standardised by titration with a solution prepared from pure dry anhydrous potassium carbonate. Calculate the molarity of the HNO_3 from the following set of data:
Potassium carbonate solution: 25.00 cm^3 of a solution containing 3.4650 g /250 cm^3
Volume of HNO_3 required: 26.45 cm^3
Molar mass of K_2CO_3: 138.2

Question 4.3 Distinguish between the quantitative methodologies of 'Standard Addition' and 'Internal Standards', indicating where, how and why they are normally applied and the potential advantages that they offer over the construction of calibration graphs.

Question 4.4 Five tablets were selected from a batch and weighed (3.020) before being ground in a pestle and mortar. 0.4921 g of the resultant powder was weighed into a 50.0 cm^3 calibrated flask, to which was added methanol to volume. The flask was then stoppered and shaken to dissolve the paracetamol. Not all of the powder dissolved, as it contained additional inorganic excipients that were insoluble in methanol. Some of the resultant solution was centrifuged and 5.0 cm^3 aliquots of the supernatant liquid added to two 25.0 cm^3 calibrated flasks labelled flask 'A' and 'B'. Flask 'A' was diluted to volume with a 1:1 mixture of methanol and water. To flask 'B' was added 5.0 cm^3 of a standard paracetamol solution containing 3.800 g/dm^3 of pure paracetamol, before this was also diluted to volume using the same methanol/water mix. Both solutions were then analysed by hplc using the same volume loop injector and UV detection and the following peak area measurements were obtained. Calculate the average quantity of paracetamol in the tablets analysed in mg/tablet.
Flask 'A' 1354; Flask 'B' 1986.

Question 4.5 List the procedures used in question (4.4) that would add to the overall measurement uncertainty, and suggest how the reliability of the procedure as described could have been improved

Question 4.6 A sample of beer is analysed for its ethanol content by using propan-2-ol as an internal standard. The procedure adopted was as follows: To each of a series of 100.0 cm^3 calibrated flasks was added 3.0 cm^3 of propan-1-ol. To five of these flasks were added respectively 1.0, 2.0, 3.0, 4.0 and 5.0 cm^3 of absolute ethanol. All flasks were then diluted to volume with pure water. The 6th flask was filled to volume with the beer sample to be analysed. All of these solutions were then analysed by a glc method which allowed separation between the ethanol and the propan-1-ol peaks. The results obtained are tabulated below:

% v/v ethanol	Relative peak area of ethanol peak	Relative peak area of propan-1-ol peak
1.0	1501	4686
2.0	2816	4411
3.0	4714	4899
4.0	6018	4702
5.0	7002	4351
Beer sample	5703	4602

Calculate the % of ethanol in the beer sample analysed. Explain why propan-1-ol was chosen as the internal standard.

Outline answer to question number 4.1

The answer to this question may be found on slides 7 - 9, 19 - 20 and 61 - 62

Six terms have been routinely used to categorise analytical measurements. They are:

- Classical *versus* Instrumental
- Chemical versus Physical
- Destructive *versus* Non-destructive

Classical techniques are generally considered as titrimetric and gravimetric. Whilst the measurement of volumes can be carried out with basic glass apparatus, the measurement of accurate weights requires a sophisticated balance, which, while not necessarily being electronic, is indeed an instrument. Colorimetric techniques, involving the development of a coloured complex involve some initial chemistry, so is it correct to term these methods 'instrumental'? Chemical techniques are those using a chemical reaction prior to performing some final measurement. This includes titrimetric, gravimetric, colorimetric as well as some electrochemical techniques. In some of these cases, instruments and physical measurements are also involved. Physical techniques include any analytical measurement based upon the physical property of the species. However, titrimetric techniques can involve measuring a physical property (potentiometry). Although the definitions of destructive and non-destructive look to be cut and dried, the same analytical technique could appear in both categories – for instance IR and XRF spectroscopies

The terms 'absolute' and 'comparative' both relate to quantitative measurement. Absolute defines any technique involving a chemical reaction which achieves stoichiometric completion in accordance with the chemical equation for that reaction. Comparative includes all other techniques, all of which require calibration against known standards to produce accurate quantitative data.

Outline answer to question number 4.2

The answer to this question may be found on slides 40 -42 and 46 - 51

In any potentiometric titration, the end point is shown by the position of maximum slope on the graph of mV *versus* volume of titrant. This position can be difficult to measure accurately by eye and mechanical devices have been developed to aid the detection of this point. However the most satisfactory method is mathematically by using the first or better, second derivative plots – $[d^2(mv)/d(vol)^2]$ *versus* volume of titrant. A graph of this type of plot is illustrated in figure (4.11) shown on slide 42. On this plot the end point is given when the plot passes through the zero point on the differential axis.

Potassium carbonate reacts with nitric acid according to the following equation:

$$K_2CO_3 + 2\,HNO_3 \Rightarrow 2\,KNO_3 + CO_2 + H_2O$$

The 4 is used as the potassium carbonate was dissolved in 250 cm^3

The concentration of potassium carbonate solution is: $(3.465 \times 4) / 138.2 = 0.1003\ mol/dm^3$

By using equation (1.18) shown on slide 173, the concentration of the HNO_3 can be calculated

$$\text{Molarity of HNO3} = \frac{0.1003 \times 25.00 \times 2}{26.45 \times 1} = \mathbf{0.1896\ mol/dm^3}$$

Outline answer to question number 4.3

The answer to this question may be found on slides 88 - 115

Because of the similarity of titles, confusion sometimes exists between the quantitative methodologies of ‘standard addition’ and ‘internal standards’. Part of the confusion lay in the fact that they can both be used in both single and multiple modes. The main difference between the methods is that

- In ‘standard addition’, small quantities of pure target analyte are added to the sample matrix and measurements of the increase in signal strength caused by the addition of the standard are determined.
- With ‘internal standards’, small quantities of a substance other than the target analyte are added to the sample matrix and a measurement made of the signal ratio between that of the analyte and the internal standard.

In both methods, the assumption is made that there is a linear relationship between signal strength and concentration of the analyte, over the concentration range being examined.

Table (4.12) on slide 101 gives a comparison between the ways in which standard addition procedure may be used and table (4.15) on slide 114, does the same for internal standard procedures. Both tables list the advantages and disadvantages of the procedures on offer.

Although single standard addition and internal standard are the most economic way of utilising these methods, both must be carried out with extreme care, as there is no mechanism in the subsequent calculations for identifying gross errors or averaging out random errors.

Outline answer to question number 4.4

The equation for use in this calculation may be found as equation (4.27) on slide 91

Total weight of the 5 tablets was **3.020 g**
0.4921 g of powdered tablets dissolved and diluted to 50.0 cm3 (flask P)

5.0 cm^3 of solution (P) diluted to 25.0 cm^3 (solution A) - analysis by hplc to give peak area of **1354**

5.0 cm^3 of solution (P) + 5.0 cm^3 of standard paracetamol solution diluted to 25 cm^3 (solution B) – analysis by hplc to give peak area of **1986**

Increase in concentration of paracetamol in (B) - (C_s) -given by: $\dfrac{\text{wt of paracetamol added}}{25.0}$ g/cm^3

$$= \frac{5 \times 3.8}{1000} \times \frac{1}{25} \times \frac{1000}{1} = \frac{5 \times 3.8}{25} = \mathbf{0.76\ mg/cm^3}$$ [note change of units]

Concentration of paracetamol in flask (A) $= \dfrac{Y_0 \times C_s}{(Y_1 - Y_0)} = \dfrac{1354 \times 0.76}{1986 - 1354} = 1.63\ mg/cm^3$

Concentration in flask (A) = 1.63 mg/cm^3 = 1.63 X (25 / 5) = 8.15 mg/cm^3 in flask (P)

Total weight of paracetamol in flask (P) = 8.15 X 50 mg = 407.5 mg

Total paracetamol in the 5 tablets is thus given by: $\dfrac{407.5 \times 3.02}{0.4921} = 2500.8$ mg

Average weight of paracetamol per tablet = 2500.8 / 5 = 500.2 mg

Outline answer to question number 4.5

The answer to this question can be found on slides 224 and 50 – 51 in Chapter 5 of this teaching and learning programme

The procedures that would have influenced the overall measurement uncertainty in this procedure are:

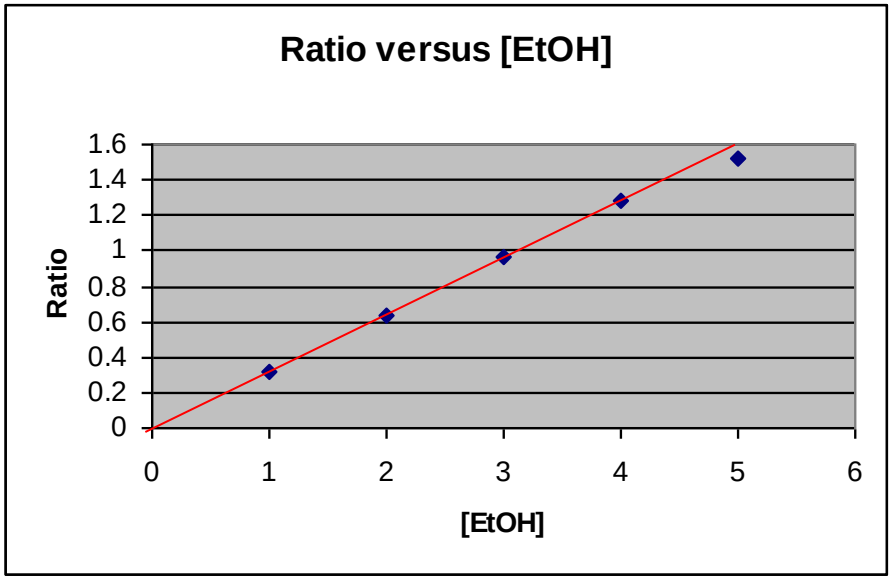
- The accuracy of the balance used to weigh both the tablets and the resultant powder
- How well the tablets were ground and mixed. Taking replicate samples of the powdered tablets would have increased the reliability of the analysis
- The accuracy of the volume transferred by the 5.0 cm^3 pipette
- The purity of the standard paracetamol (? 99.9 ± 0.2 %)
- The assumption that no methanol was lost by evaporation during centrifugation
- The repeatability of the loop injector used to introduce samples onto the hplc
- The accuracy of the integrator which measured the peak areas

The reliability of the analysis could have been improved by using multiple standard addition instead of single standard addition, on replicate samples of the powdered tablets. However this would have led to a considerable increase in the workload and thus has cost implications.

Outline answer to question number 4.6

The answer to this question can be found on slides 103 - 107 and 110 - 113
Each of the solutions analysed contained 3% by volume of propan-1-ol and varying amounts of ethanol. By using equation (4.44), the ratio of the areas of the ethanol:propan-1-ol peaks are plotted against the % (v/v) ethanol. The % ethanol in the sample of beer can then be interpolated from the resultant graph. Results are shown below:

% (v/v) ethanol	Ratio of peak areas
1.0	1501/4686 = 0.320
2.0	2816/4411 = 0.638
3.0	4714/4899 = 0.962
4.0	6018/4702 = 1.279
5.0	7002/4602 = 1.522
Sample	5703/4602 = 1.239



Interpolating the sample ratio from the graph gives an ethanol concentration in the beer sample analysed of 3.88 % (v/v). However, the beer was diluted 97 to 100 due to the addition of the 3 cm^3 of propan-1-ol. So real concentration is **3.88 X (100/97) = 4.0 % (v/v)**

Propan-1-ol was chosen as the internal standard because it was miscible with the sample solution, chromatographically separable from analyte and eluted close to the analyte peak