3 Titrimetric and chemical analysis methods

KEYPOINTS

Principles

An analyte is chemically reacted with a standard solution of a reagent of precisely known concentration or with a concentration that can be precisely determined. The amount of a standard solution required to completely react with all of the sample is used to estimate the purity of the sample.

Applications

- Provide standard pharmacopoeial methods for the assay of unformulated drugs and excipients and some formulated drugs, e.g. those that lack a strong chromophore.
- Used for standardisations of raw materials and intermediates used in drug synthesis in industry. Suppliers of raw materials may provide these materials at a specified purity which has been assayed titrimetrically to a pharmacopoeial standard.
- Certain specialist titrations, such as the Karl Fischer titration used to estimate water content, are widely used in the pharmaceutical industry.

Advantages

- Capable of a higher degree of precision and accuracy than instrumental methods of analysis with precisions of *ca* ± 0.1% being achievable.
- The methods are generally robust.
- Analyses can be automated.
- Cheap to perform and do not require specialised apparatus.
- They are absolute methods and are not dependent on the calibration of an instrument.

Limitations

- Non selective.
- Time-consuming if not automated and require a greater level of operator skill than routine instrumental methods.
- Require large amounts of sample and reagents.
- Reactions of standard solutions with the analyte should be rapid and complete.

Introduction

Titrimetric methods are still widely used in pharmaceutical analysis because of their robustness, cheapness and capability for high precision. The only requirement of an analytical method that they lack is specificity. This chapter covers the theoretical basis of most of the commonly used methods; the practical aspects of titrations have been covered thoroughly by other textbooks.[1,2]

Instrumentation and reagents

Glassware

The manufacturers' tolerances for the volumes of a number of items of glassware are give in Chapter 1. The larger the volume measure the smaller the percentage the tolerance is of the nominal volume. Thus for a Grade A 1 ml pipette the volume is within ± 0.7% of the nominal volume whereas for the 5 ml pipette the volume is within ± 0.3% of the nominal volume. If greater accuracy than those guaranteed by the tolerances is required then the glassware has to be calibrated by repeated weighing of the volume water contained by the item of glassware. This exercise is also useful for judging how good one's ability to use a pipette is since weighing of the volumes of water dispensed correctly several times from the same pipette should give weights that agree closely.

Primary standards and standard solutions

Primary standards are stable chemical compounds that are available in high purity and which can be used to standardise the standard solutions used in titrations. Titrants such as sodium hydroxide or hydrochloric acid cannot be considered as primary standards since their purity is quite variable. So for instance sodium

hydroxide standard solution may be standardised against potassium hydrogen phthalate, which is available in high purity. The standardised sodium hydroxide solution (secondary standard) may then be used to standardise a standard solution of hydrochloric acid. Table 3.1 lists some commonly used primary standards and their uses.

Table 3.1 Primary standards and their uses

Primary standard	Uses
Potassium hydrogen phthalate	Standardisation of sodium hydroxide solution
Potassium hydrogen phthalate	Standardisation of acetous perchloric acid
Potassium iodate	Standardisation of sodium thiosulphate solution through generation of iodine
Anhydrous sodium carbonate	Standardisation of hydrochloric acid
Zinc metal	Standardisation of EDTA solution

Direct acid/base titrations in the aqueous phase

Strong acid/strong base titrations

Figure 3.1 shows the titration curve obtained from the titration of a strong acid with a strong base. The pH remains low until just before the equivalence point when it rises rapidly to a high value. In many titrations a coloured indicator is used although electrochemical methods of end-point detection are also used. An indicator is a weak acid or base that changes colour between its ionised and un-ionised forms; the useful range for an indicator is 1 pH either side of its p*K*a value. For example phenolphthalein (PP) p*K*a 9.4 (colour changes between pH 8.4 and pH 10.4) undergoes a structural rearrangement as a proton is removed from one of its phenol groups as the pH rises and this causes the colour change (Fig. 3.2). Methyl orange (MO) p*K*a 3.7 (colour changes between pH 2.7 and pH 4.7) undergoes a similar pH dependent structural change. Both these indicators fall within the range of the inflection of the strong acid strong base titration curve.

There are only a few direct strong acid strong base titrations carried out in pharmacopoeial assays.

Strong acid/strong base titrations are used in pharmacopoeial assays of: perchloric acid, hydrochloric acid, sulphuric acid and thiamine hydrochloride.

Weak acid/strong base and weak base/strong acid titrations

On addition of a small volume of the strong acid or strong base to a solution of the weak base or weak acid, the pH rises or falls rapidly to about 1 pH unit below or above the p*K*a value of the acid or base. Often a water miscible organic solvent such as ethanol is used to dissolve the analyte prior to addition of the aqueous titrant.

Figure 3.3 shows a plot of pH when 1 M NaOH is added to 25 ml of a 1 M solution of the weak acid aspirin.

In the case of aspirin, the choice of indicator is restricted by where the inflection in its titration curve lies; PP is suitable as an indicator whereas MO is not.

In the example of the titration of quinine with hydrochloric acid (Fig. 3.4), MO is a suitable indicator because it falls within the inflection of the titration curve whereas PP is not suitable.

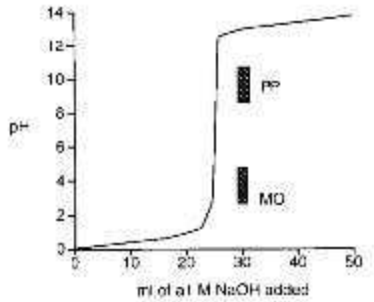


Fig. 3.1
The change in pH as 25 ml of 1 M HCl are titrated with 1 M NaOH.

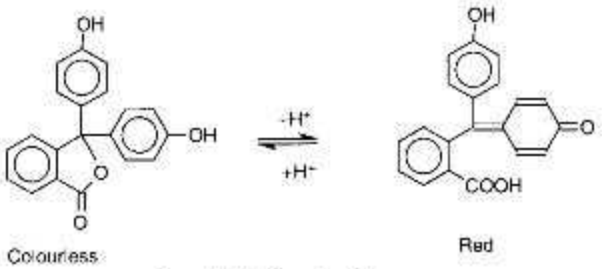


Fig. 3.2
The structural rearrangement responsible for the colour change in phenolphthalein.

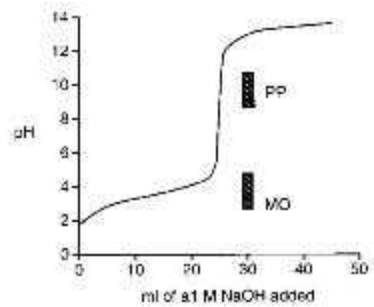


Fig. 3.3
Titration curve for 25 ml of a 1.0 M solution of aspirin (p*Ka* 3.5) titrated with 1.0 M NaOH.

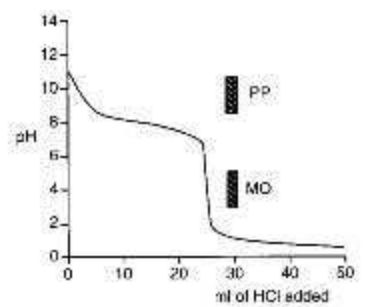


Fig. 3.4
25 ml of a 1.0 M solution of quinine (p*Ka* 8.05) titrated with 1.0 M hydrochloric acid.

Self-test 3.1

Which of these indicators could be used in the titration of aspirin and which could be used in the titration of quinine?

(i) Bromophenol blue p*K*a 4.0.
(ii) Methyl red p*K*a 5.1.
(iii) Cresol red p*K*a 8.3.
(iv) Chlorophenol blue p*K*a 6.0.

Answers: Aspirin: (iii) and (iv). Quinine: (i) and (ii)

Some acids or bases can donate or accept more than one proton, i.e. 1 mole of analyte is equivalent to more than 1 mole of titrant. If the p*K*a values of any acidic or basic groups differ by more than *ca* 4, then the compound will have more than one inflection in its titration curve. Sodium carbonate is a salt of carbonic acid and it can accept two protons. The p*K*a values of carbonate and bicarbonate are sufficiently different (p*K*a 10.32 and 6.38) for there to be two inflections in the titration curve. The two stages in the titration are:

1 $CO_3^{2-} + H^+ \longrightarrow HCO_3^-$
2 $HCO_3^- + H^+ \longrightarrow H_2CO_3$

In a titration of sodium carbonate, the first inflection is indicated by PP and the whole titration by MO (Fig. 3.5).

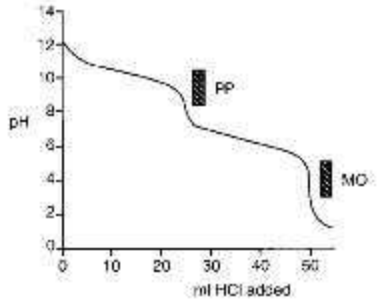


Fig. 3.5
Titration of 1 M sodium carbonate with 1 M HCl.

Self-test 3.2

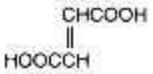
A sample containing 25.14 g of neutral salts, glucose and a sodium carbonate/bicarbonate buffer was dissolved in 100 ml of water. A 25 ml aliquot of the resultant solution required 20.35 ml of 0.0987 M HCl when titrated to the PP end-point. A second 25 ml aliquot was titrated to the MO end point and required 56.75 ml of the acid. Calculate the percentage of Na_2CO_3 (MW 106) and $NaHCO_3$ (MW 84) in the sample.

Answers: 3.39% and 2.12%, respectively

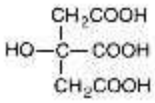
Self-test 3.3

How many inflections do the following substances have in their titration curves when titrated with a strong base? Draw the predominant forms of these substances which would exist at pH 14.

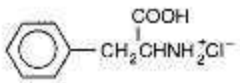
Oxalic acid
pKa 1.19, 4.21



Fumaric acid
pKa 3.03, 4.47



Citric acid
pKa 3.06, 4.74, 5.4



Phenylalanine
pKa 1.83, 9.13

Answers: oxalic acid 1, fumaric acid 1, citric acid 1, phenylalanine 2

Weak acid/strong base titration is used in the pharmacopoeial assays of: benzoic acid, citric acid, chlorambucil injection, mustine injection, nicotinic acid tablets and undecanoic acid.

Indirect titrations in the aqueous phase

These can be of the strong acid/strong base, weak acid/strong base or weak base/strong acid type. The more common examples are weak acid/strong base.

Estimation of esters by back titration

Excess of sodium hydroxide is added to the ester. The following reaction occurs:

$$RCOOR' + XSNaOH \longrightarrow RCOONa + R'OH$$

The XSNaOH is back titrated with HCl using PP as an indicator.

This procedure is used in pharmacopoeial assays of: benzyl benzoate, dimethyl phthalate, ethyl oleate, methyl salicylate, cetostearyl alcohol, emulsifying wax, castor oil, arachis oil, cod liver oil, coconut oil.

Saponification value

The assay of fixed oils provides a special case of ester hydrolysis since they are triesters of glycerol. The saponification value for a fixed oil is the number of mg of KOH equivalent to 1 g of oil. A high value means rancidity, a low value possible adulteration with mineral oil. Almost all edible oils have a saponification value between 188 and 196. Hydrolysis of the fixed oil is carried out with ethanolic KOH.

This procedure is used in the pharmacopoeial assays of: castor oil, cod liver oil, cotton seed oil, almond oil and sesame seed oil.

Acid values are also determined for fixed oils. The acid value for a substance is the number of mg of KOH required to neutralise 1 g of the test substance when it is

Calculation example 3.1

The following data were obtained for a sample of cod liver oil:

Weight of oil taken for analysis = 2.398 g

Ethanolic KOH (MW 56.1) used in determination = 0.986 M

Amount of ethanolic KOH used for hydrolysis and in blank titration = 25 ml

Amount of 0.470 M HCl required to neutralise excess KOH = 35.2 ml

Amount of 0.470 M HCl required in the titration of blank = 52.3 ml

Calculation

Amount of KOH used initially = $52.3 \times 0.47 = 24.6$ mmole

Amount of HCl required to neutralise excess KOH = $35.20 \times 0.470 = 16.5$ mmole

Amount of KOH used in hydrolysis = 24.6 – 16.5 = 8.1 mmole × MW = mg

Amount of KOH used in the hydrolysis = $8.1 \times 56.1 = 454.0$ mg

Amount of KOH/g of fixed oil used in the hydrolysis = 454/2.398 = 189.3 mg

Therefore saponification value = 189.3.

Self-test 3.4

Calculate the saponification value of a sample of castor oil from the following data:

- Weight of oil taken for analysis = 2.535 g
- Ethanolic KOH used in the hydrolysis = 1.03 M
- Amount of KOH used in hydrolysis = 25 ml
- Amount of 0.514 M HCl required to neutralise excess KOH = 34.2 ml
- Amount of 0.514 M HCl required in the titration of blank = 50.2 ml

Answer: 182

titrated with 0.1 M ethanolic KOH to a PP end-point. This value is quoted for many fixed oils in order to eliminate rancid oils, which contain large amounts of free fatty acid. Typically acid values for fixed oils are in the range of 1–2.

Estimation of alcohols and hydroxyl values by reaction with acetic anhydride (AA)

Alcohols can be determined by reaction with excess AA (Fig. 3.6). This is a useful titrimetric method because the alcohol group is difficult to estimate by any other means.

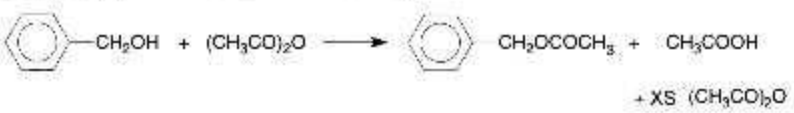


Fig. 3.6 Estimation of benzyl alcohol by reaction with acetic anhydride.

The excess AA and acetic acid may be backtitrated with NaOH using PP as an indicator.

In a related assay, a hydroxyl value is determined for a fixed oil. A 1:3 mixture of AA in pyridine is used in the determination; the pyridine is present as a catalyst. The hydroxyl value may be defined as:

The number of mg of KOH required to neutralise a blank titration of the reagents – the number of mg KOH required to neutralise excess AA + acetic acid after reaction with 1 g of the test substance.

Calculation example 3.2

The following data were obtained for a sample of castor oil:

Weight of castor oil taken for analysis = 1.648 g

Volume of acetic anhydride used for the reaction = 5 ml

Molarity of ethanolic KOH used to neutralise the excess AA + acetic acid = 0.505 M

Volume of ethanolic KOH required to titrate 5 ml of reagent = 53.5 ml

Volume of ethanolic KOH required to neutralise excess AA + acetic acid after reaction with the castor oil = 44.6 ml.

Number of mmoles of KOH used in the blank titration = $53.5 \times 0.505 = 27.0$

Number of mg of KOH used in the titration of the blank = $27.0 \times 56.1 = 1515$

Number of mmoles of KOH used in titration of AA + acetic acid = $44.6 \times 0.505 = 22.5$

Number of mg KOH used in titration of excess AA + acetic acid = $22.5 \times 56.1 = 1262$

Hydroxyl value = 1515-1262/1.648 = 154.

To be completely accurate the acid value for the fixed oil should be added to the hydroxyl value since any free acid in the oil will titrate along with the excess reagents giving a small overestimate. The acid value for castor oil is about 2.0 giving a hydroxyl value for the above sample of 156.

Reaction with acetic anhydride is used in pharmacopoeial assays of: benzyl alcohol and dienestrol and determination of hydroxyl values of castor oil, cetosteryl alcohol and cetomacrogol.

Non-aqueous titrations

Theory

Non-aqueous titration is the most common titrimetric procedure used in pharmacopoeial assays and serves a double purpose, as it is suitable for the titration of very weak acids and bases and provides a solvent in which organic compounds are soluble. The most commonly used procedure is the titration of organic bases with perchloric acid in acetic acid. These assays sometimes take some perfecting in terms of being able to judge precisely the end-point.

The theory is very briefly as follows: Water behaves both as a weak acid and a weak base thus in an aqueous environment it can compete effectively with very weak acids and bases with regard to proton donation and acceptance as shown in Figure 3.7.

The effect of this is that the inflection in the titration curves for very weak acids and very weak bases is small because they approach the pH limits in water of 14 and 0 respectively thus making end-point detection more difficult. A general rule is that

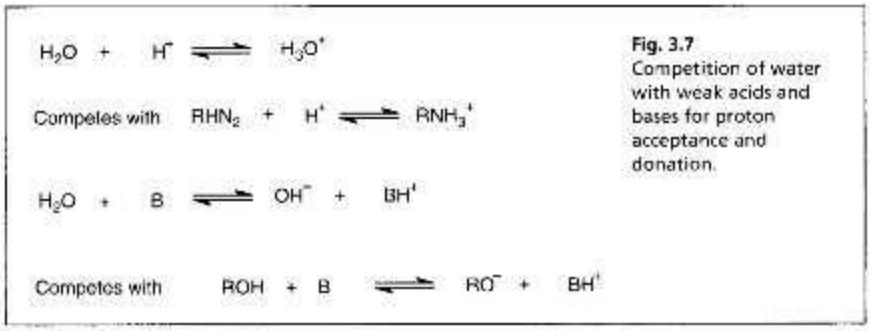


Fig. 3.7
Competition of water with weak acids and bases for proton acceptance and donation.

bases with p*Ka* < 7 or acids with p*Ka* > 7 cannot be determined accurately in aqueous solution. Various organic solvents may be used to replace water since they compete less effectively with the analyte for proton donation or acceptance.

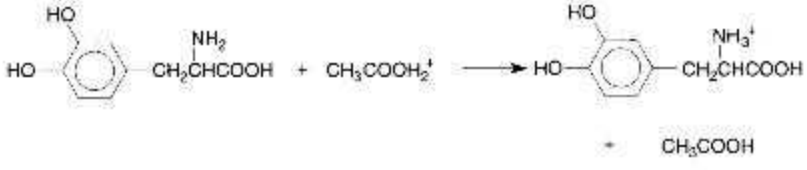
Non-aqueous titration of weak bases

Acetic acid is a very weak proton acceptor and thus does not compete effectively with weak bases for protons. Only very strong acids will protonate acetic acid appreciably according to the equation shown below:

$$CH_3COOH + HA \rightleftharpoons CH_3COOH_2^+ + A$$

Perchloric acid is the strongest of the common acids in acetic acid solution and the titration medium usually used for non-aqueous titration of bases is perchloric acid in acetic acid. Addition of acetic anhydride, which hydrolyses to acetic acid, is used to remove water from aqueous perchloric acid. Weak bases compete very effectively with acetic acid for protons. Oracet blue, quinalidine red and crystal violet (very weak bases) are used as indicators in this type of titration. A typical analysis is shown in Figure 3.8 for LDOPA.

Fig. 3.8
Analysis of DOPA by non-aqueous titration.



When the base is in the form of a salt of a weak acid, removal of an anionic counter ion prior to titration is not necessary, e.g. for salts of bases with weak acids such as tartrate, acetate or succinate. However, when a base is in the form of a chloride or bromide salt, the counter ion has to be removed prior to titration. This is achieved by addition of mercuric acetate; the liberated acetate is then titrated with acetous perchloric acid. This is illustrated in Figure 3.9 for the example of phenylephrine.HCl.

Non-aqueous titration with acetous perchloric acid is used in the pharmacopoeial assays of: adrenaline, metronidazole, codeine, chlorhexidine acetate, chlorpromazine.HCl, amitriptyline.HCl, propranolol.HCl, lignocaine.HCl and quaternary amine salts such as neostigmine bromide and pancuronium bromide.

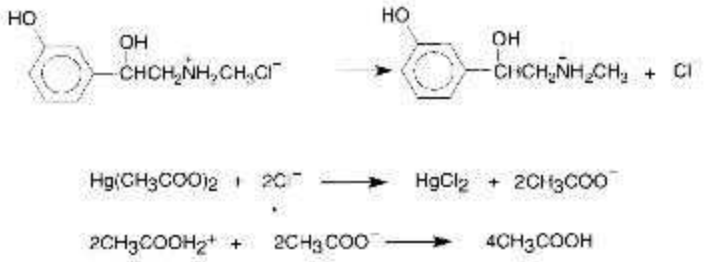


Fig. 3.9
The analysis of phenylephrine hydrochloride by non-aqueous titration.

Non-aqueous titration of weak acids

For the non-aqueous titration of weak acids a solvent such as an alcohol or an aprotic, solvent is used that does not compete strongly with the weak acid for proton donation. Typical titrants are lithium methoxide in methanol or tetrabutyl ammonium hydroxide in dimethylformamide. End-point detection may be carried out with thymol blue as an indicator or potentiometrically (see p. 65).

Non-aqueous titration of acidic groups is carried out in pharmacopoeial assays of: barbiturates, uracils and sulphonamides.

Argentimetric titrations

Argentimetric titrations are based on the reaction:

$$AgNO_3 + Cl \longrightarrow AgCl(s) + NO_3^-$$

Potassium chromate may be used as an indicator producing a red colour with excess Ag^+ ion. More widely applicable is the method of back titration. Excess $AgNO_3$ is added to the sample containing chloride or bromide ions. The excess $AgNO_3$ is then titrated with ammonium thiocyanate and ammonium ferrous sulphate is used as an indicator of excess SCN^-.

$$AgNO_3 + NH_4SCN \longrightarrow AgSCN(s) + NH_4NO_3$$

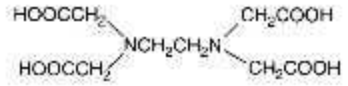
Before the back titration can be carried out, the precipitated AgCl has to be filtered off or coated with diethylphthalate to prevent SCN^- causing dissociation of AgCl. Organically combined chlorine has to be liberated by hydrolysis with sodium hydroxide prior to titration. A halogen attached to an aromatic ring cannot be liberated by hydrolysis and aromatic halides have to be burnt in an oxygen flask in order to release the halogen for titration.

Argentimetric titration is used in pharmacopoeial assays of: sodium chloride and potassium chloride tablets, thiamine hydrochloride, mustine chloride and carbromal.

Complexiметric titrations

These titrations are used in the estimation of metal salts. Ethylenediamine tetraacetic acid (EDTA) shown in Figure 3.10 is the usual titrant used. It forms stable 1:1 complexes with all metals except alkali metals such as sodium and potassium. The alkaline earth metals such as calcium and magnesium form complexes which are unstable at low pH values and are titrated in ammonium chloride buffer at pH 10. The general equation for the titration is:

$$M^{n+} + Na_2EDTA \longrightarrow (MEDTA)^{n-4} + 2H^+$$



Ethylenediamine tetracetic acid

Fig. 3.10
EDTA.

The end-point of the reaction is detected using an indicator dye. The dye is added to the metal solution at the start of the titration, and forms a coloured complex with a small amount of the metal. The first drop of excess EDTA causes this complex to break up resulting in a colour change.

Titration with EDTA is used in the pharmacopoeial assays of: bismuthsubcarbonate, calcium acetate, calcium chloride, calcium gluconate, magnesium carbonate, magnesium hydroxide, magnesium trisilicate, bacitracin zinc, zinc chloride and zinc undecanoate.

Insoluble metal salts are estimated by back titration; the sample is heated with excess EDTA to form the soluble EDTA complex of the metal and then the excess EDTA is titrated with salt solutions containing Mg^{2+} or Zn^{2+} of known concentration.

Back titration with EDTA is used in the pharmacopoeial assays of: aluminium glycinate, aluminium hydroxide, aluminium sulphate, calcium hydrogen phosphate.

Redox titrations

Redox titrations are based on the transfer of electrons between the titrant and the analyte. These types of titrations are usually followed by potentiometry, although dyes which change colour when oxidised by excess titrant may be used.

Theory

Reduction potential is a measure of how thermodynamically favourable it is for a compound to gain electrons. A high *positive* value for a reduction potential indicates that a compound is readily reduced and consequently is a strong oxidising agent, i.e. it removes electrons from substances with lower reduction potentials. The oxidised and reduced form of a substance are known as a redox pair. Table 3.2 lists the standard reduction potentials for some typical redox pairs.

Table 3.2 Standard reduction potential (E_s) for some redox pairs relative to the standard hydrogen electrode potential 0

			E_s
$Ce^{4+} + e$	→	Ce^{3+}	1.61 V
$MnO_4^- + Se + 8H^+$	→	$Mn^{2+} + 4H_2O$	1.51 V
$Cl_2 + 2\,e$	→	$2Cl$	1.36 V
$Br_2 + 2\,e$	→	$2Br$	1.065 V
$Fe^{3+} + e$	→	Fe^{2+}	0.771 V
$I_2 + 2\,e$	→	$2I$	0.536 V
$AgCl + e$	→	$Ag + Cl$	0.223 V
$2H^+ + 2\,e$	→	H_2	0 V
$Fe^{2+} + 2\,e$	→	Fe	−0.440 V
$Ca^{2+} + 2\,e$	→	Ca	−2.888 V

A substance with a higher reduction potential will oxidise one with a lower reduction potential. The difference in potential between two substances is the reaction potential and is approximately the potential difference which would be

measured if the substances comprised two halves of an electrical cell. For example Cl_2 will oxidise Br^- according to the following equation:

$$Cl_2 + 2Br^- \longrightarrow 2Cl^- + Br_2$$

Taking values from Table 3.2 the reaction potential is given by:
1.36 – 1.065 = 0.29 V

For the reaction:

$$Ca + Cl_2 \longrightarrow CaCl_2$$

The reaction potential is given by: 1.36 (2.888) = 4.248 V (i.e. a large difference and calcium burns in chlorine).

Self-test 3.5

Complete the equations where reaction is possible and indicate the reaction potential.

(i) $I_2 + 2\,Cl^- \longrightarrow$
(ii) $Br_2 + 2\,I^- \longrightarrow$
(iii) $Ce^{4+} + Fe^{2+} \longrightarrow$
(iv) $I_2 + Fe \longrightarrow$
(v) $Fe^{3+} + AgCl \longrightarrow$

Answers: (i) No reaction; (ii) 0.529 V; (iii) 0.839 V; (iv) 0.976 V; (v) No reaction (Ag is already in its Ag$^+$ form)

In the above examples we have ignored the effect of concentration of oxidant and reductant on E_o values; in fact E (the observed electrode potential) is stable over a wide range of concentrations. The E-value for a solution containing a redox pair is governed by the Nernst equation:

$$E = E_o + 2RT/nF \ln [Ox]/[Red]$$

where [Ox] is the concentration of the oxidised form of a particular substance and [Red] is the concentration of the reduced form of a particular substance.

F = Faraday's constant

n = number of electrons transferred in the reaction

By substituting a value for the constant terms this equation can also be written as:

$$E = E_o + 0.0591/n \log [Ox]/[Red]$$

Where n is the number of electrons transferred during the reaction. It is clear that E is approximately equal to Eo except when there is a large difference between [Ox] and [Red].

Self-test 3.6

Calculate the E for the following redox pair when Mn^{3+} = 0.5 M and Mn^{2+} = 0.01 M (E_o Mn^{3+}/Mn^{2+} = 1.51 V).

Answer: 1.61 V

The titration curve for Fe^{2+} against Ce^{4+} is shown in Figure 3.11. This curve shown is for a titration carried out with a standard hydrogen electrode as the reference electrode.

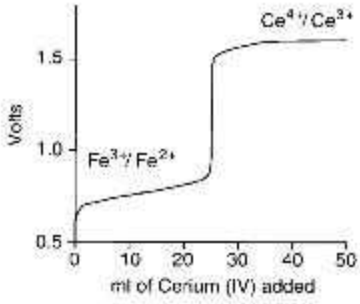


Fig. 3.11
Titration of 25 ml of 1 M Fe^{2+} with 1 M Ce^{4+}.

Where a reference electrode has a reduction potential > 0, then the predicted reading of the potential for a redox pair is obtained by subtracting the reduction potential for the reference electrode, e.g. for an Ag/AgCl reference electrode 0.223 V is subtracted.

Self-test 3.7

Using the values in Table 3.1, what would be the approximate potential measured for the Fe^{3+}/Fe^{2+} redox pair present in the first part of the titration shown in Figure 3.11 measured against:

(i) A standard hydrogen electrode.
(ii) An Ag/AgCl electrode?
Similarly what would the approximate potential be for the Ce^{4+}/Ce^{3+} redox pair on the plateau after the end-point measured against?
(iii) A standard hydrogen electrode.
(iv) An Ag/AgCl electrode.

Answers: (i) 0.77 V, (ii) 0.55 V, (iii) 1.61 V, (iv) 1.39 V

In carrying out redox titrations, standard Ag/AgCl or Hg/Hg_2Cl_2 electrodes are used as a reference in conjunction with an inert redox electrode, e.g platinum, which takes its potential from the particular redox pair in the solution in which it is immersed.

Redox titration is used in pharmacopoeial assays of: ferrous salts, hydrogen peroxide, sodium perborate and benzoyl peroxide by titration with $KMnO_4$. In the case of $KMnO_4$ titrations the end-point may be detected when the purple colour of the permanganate persists.

Iodometric titrations

There are a number of types of iodometric assay.

Direct titrations

Iodine is a moderately strong oxidising agent (See Table 3.1). During oxidation iodine is reduced as follows:

$$I_2 + 2e \rightleftharpoons 2I$$

It will oxidise substances with lower reduction potentials, e.g. the titration of ascorbic acid is carried out as shown in Figure 3.12.

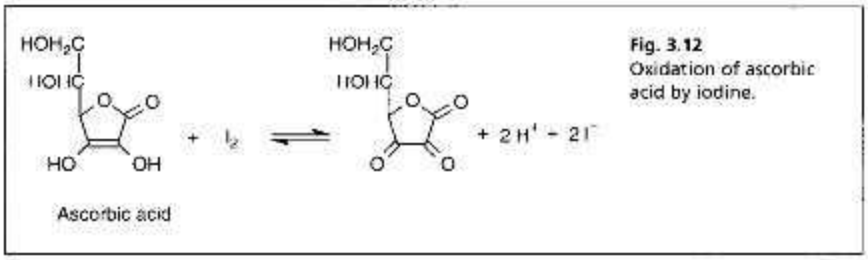


Fig. 3.12
Oxidation of ascorbic acid by iodine.

The iodine solution used is standardised against sodium thiosulphate (see later). In addition the end-point is detected using starch indicator, which produces a blue colouration with excess iodine.

Direct iodometric titration is used in pharmacopoeial assays of: ascorbic acid, sodium stilbigluconate, dimercaprol injection and acetarsol.

Iodine displacement titrations

These titrations involve displacement of iodine from iodide by a stronger oxidising agent followed by titration of the displaced iodine with sodium thiosulphate. For example, the available chlorine in bleach is estimated as follows:

$$Cl_2 + 2I^- \rightleftharpoons 2Cl^- + I_2$$

The displaced iodine is then titrated with thiosulphate according to the following equation:

$$2S_2O_3^{2-} + I_2 \rightleftharpoons S_4O_6^{2-} + 2I^-$$

A different approach is used in the estimation of phenols. Bromine is generated by reaction of potassium bromide with a defined volume of a standard solution of potassium bromate according to the following equation:

$$BrO_3^- + 5Br^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O$$

The bromine generated is then reacted with the phenol and 1 mole of phenol reacts with 3 moles of bromine (Fig. 3.13).

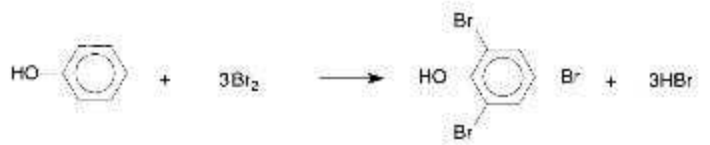


Fig. 3.13
Reaction of phenol with bromine.

Excess bromine is used and the bromine remaining after the above reaction is reacted with iodide as follows:

$$Br_2 + 2I^- \rightleftharpoons 2Br^- + I_2$$

The liberated iodine is then titrated with thiosulphate thus quantifying the excess bromine.

Iodine displacement titrations are used in pharmacopoeial assays of: liquefied phenol, methyl hydroxybenzoate, propyl hydroxybenzoate and phenidione.

Self-test 3.8

A sample of phenol glycerol injection was diluted with water and an aliquot was taken and reacted with excess bromine generated from potassium bromide and potassium bromate solutions. The excess bromine remaining after reaction was reacted with potassium iodide and the liberated iodine was titrated with sodium thiosulphate. A blank titration was carried out where the same quantity of bromine was generated as was used in the titration of the diluted injection, potassium iodide was then added and the liberated iodine was titrated with sodium thiosulphate. From the following data calculate the percentage of w/v of the phenol in the injection.
Weight of injection taken for analysis = 4.214 g.
The sample is diluted to 100 ml with water and then 25 ml of the solution is analysed.
The volume of 0.1015 M sodium thiosulphate required to titrate the XS bromine after reaction with the sample = 22.4 ml.
The volume of 0.1015 M sodium thiosulphate required to titrate the bromine blank = 48.9 ml.
Density of glycerol = 1.26.
The equations of the reactions are given above.

Answer: 5.04% w/v

Iodine-absorbing substances in penicillins

A major stability problem in penicillins is the hydrolysis of the lactam ring as shown in Figure 3.14. Penicillins with an open lactam ring are inactive as antibiotics since it is the reactive lactam ring which kills the bacteria.

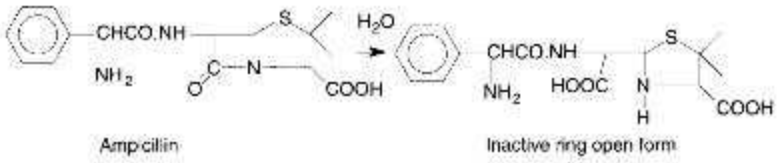


Fig. 3.14
Hydrolysis of the lactam ring in penicillins.

When the lactam ring is open it will react with iodine. 1 mole of the ring open form of penicillin will react with 8 equivalents of iodine, the intact lactam ring will not react. In this type of titration excess iodine solution is added to a sample of the penicillin and the iodine which is not consumed in the reaction is estimated by titration with sodium thiosulphate. The value obtained for the amount of hydrolysed penicillin in the sample should be no more than 5% of that obtained when all the penicillin in the same amount of sample is completely hydrolysed to the ring-opened form and then reacted with iodine. Most of the pharmacopoeial monographs for penicillins indicate that this test should be carried out.

Ion pair titrations

This type of titration is widely used in the cosmetics and detergents industry since it is very useful for estimating surfactants, which often cannot be analysed by spectrophotometric methods because they lack chromophores. There are two types of titration used.

Titrations using indicator dyes

A small amount of an anionic or cationic dye is added to an aqueous solution of the analyte, which is a lipophilic cationic or anionic compound. A small amount of coloured lipophilic ion pair is formed and this is extracted into a small amount of

chloroform, which becomes coloured by the ion pair. Titration of the lipophilic anion or cation is carried out with a lipophilic counter ion, e.g. benzethonium chloride or sodium dodecyl sulphate. At the end-point excess of the titrant breaks up the coloured complex in the chloroform layer.

Ion pair titration using a coloured indicator complex is used in pharmacopoeial assays of: dicyclamine elixir, procyclidine tablets, sodium dodecyl sulphate and cetrimide emulsifying ointment.

Titrations using iodide as a lipophilic anion

This procedure is more widely used in pharmacopoeial assays than the dye extraction procedure. Excess potassium iodide is added to an aqueous solution of the analyte, which is a lipophilic cation. A lipophilic ion pair is formed between the cation and the iodide ion and is then removed by extraction into an organic phase such as chloroform. The excess iodide remaining in the aqueous phase is then titrated in concentrated HCl (> 4 M) with potassium iodate. The iodate oxidises iodide to I^-, which immediately reacts with Cl^- to give ICl resulting in the following equation:

$$KIO_3 + 2KI + 6\,HCl \longrightarrow 3\,KCl + 3\,ICl + 3H_2O$$

A small amount of chloroform is used as an indicator and in the presence of the reaction mixture it becomes coloured purple by traces of iodine, which are present during titration. The purple colour disappears at the end-point when the conversion of all I^- and I_2 into ICl is complete.

Ion pair formation with iodide followed by titration of excess iodide with iodate is utilised in pharmacopoeial assays of: cetrimide, cetylpyridium bromide, domiphen bromide and benzalkonium chloride.

Diazotisation titrations

This type of titration is quite simple to carry out and is very useful for the analysis of sulphonamide antibiotics and aminobenzoic acid-derived local anaesthetics. Titration is carried out with acidified sodium nitrite causing the primary aromatic amine function to be converted to a diazonium salt shown in Figure 3.15 for sulphacetamide.

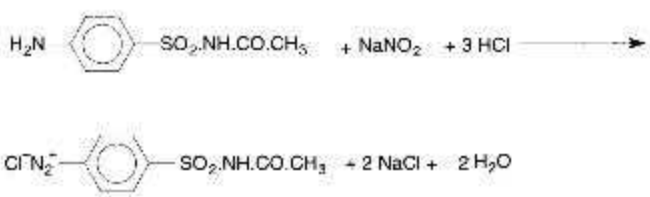


Fig. 3.15 Reaction of sulphacetamide with nitrous acid.

A small amount of iodide is included in the titration mixture. At the end-point the first drop of excess of nitrous acid converts iodide to iodine and this is detected using starch indicator.

Titration with nitrous acid is used in pharmacopoeial assays of the following: benzocaine, dapsone, primaquine, procainamide, procaine, sulphacetamide, sulphadoxine, sulphamethizole, sulphapyridine and sulphathiazole.

Potentiometric titrations

Potentiometric end-point detection

All of the titrations discussed in the preceding sections can be carried out using a suitable electrode to measure the potential of the solution as the titration progresses. The advantage of making potentiometric measurements in order to detect end-points is that the measurements can be made in solutions which are coloured, unlike indicator-based end-point detection, and give unambiguous end-points where indicator colour changes are not clear or sudden. The disadvantage of potentiometric titrations is that they are relatively slow since time has to be allowed for readings to stabilise, particularly near the end-point of the titration. However, potentiometric titrations can be automated and potentiometric end-point detection is used in automatic titrators where the titrant is pumped into the sample under microprocessor control. The electrode which is usually used to make the measurements in potentiometric titrations is the pH-sensitive glass indicator electrode. This electrode consists of a pH-sensitive glass membrane bulb which encloses a phosphate buffer solution containing potassium chloride solution and saturated with silver chloride. The solution is in contact with an internal reference electrode which consists of a silver wire. The circuit is completed by a second reference electrode, which in modern combination electrodes is a second silver/silver chloride electrode, which contacts the external solution via a porous junction (Fig. 3.16). The electrode monitors the variation in the potential difference which is largely caused by the interaction of hydrogen ions with the outer surface of the pH sensitive glass membrane.

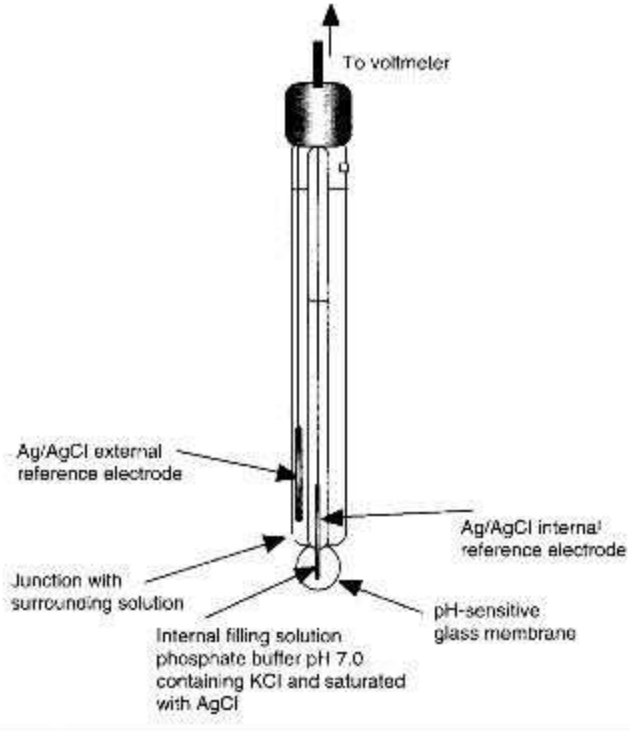


Fig. 3.16
pH sensitive glass electrode.

The potential which develops on the inner and outer glass surfaces of the electrode is due to the following equilibria:

$$H^+ + Gl^- \rightleftharpoons H^+Gl^-$$

Outer membrane

$$H^+ + Gl^- \rightleftharpoons H^+Gl^-$$

Inner membrane

The number of Gl^- sites on the outer membrane increases with decreasing $[H^+]$ and thus its potential becomes increasingly negative with respect to inner surface with increasing pH. The Nernst equation can be simplified and written in the following form for the glass electrode when the temperature is 20°C:

$$E = Ek - 0.0591pH.$$

where E is the measured potential in volts and Ek is a constant composed of the sum of the various potential differences within the electrode, which do not vary appreciably. The combination electrode is constructed so that its potential is *ca* 0 V at pH 7.0. It can be seen from the equation above that E changes by 59.1 mV for each pH unit.

Self-test 3.9

Assuming an indicator electrode is constructed so that $E = 0$ V at pH 7.0 calculate what its potential would be at: (i) pH 1; (ii) pH 14.

Answers: (i) +0.36 V; (ii) −0.41 V

When potentiometric titration is carried out, the volume of titrant added is plotted against the measured potential. Since the electrode takes time to equilibrate, the volume of titrant required to reach the end-point is first calculated and a volume of titrant is added to within *ca* 1 ml of the end-point. Then the titrant is added in 0.1 ml amounts until the steep inflection in the titration curve is passed. The end-point of the titration is the point where the slope of the titration curve is at its maximum. Thus if dE/dV is plotted for the titration, the maximum of the plot gives the end-point. The end-point can also be determined from the mid-point of the inflection in the titration curve or from the tabulated data. Figure 3.17 shows a curve for the titration of 2 mmoles of aspirin with 0.1 M NaOH. The end-point corresponds to the mid-point of the inflection or if the tabulated data is examined it can be be taken to be the mid-point between the two volumes where dE/dV is greatest, i.e. at 20.05 ml between 20 and 20.1 ml. The actual end-point for exactly 2 mmoles of aspirin titrated with 0.1 M NaOH should be 20 ml, addition of 0.1 ml aliquots towards the end-point means that the end-point is only accurate to within ± 0.05 ml.

Use of potentiometric titration to determine p*K*a values

Potentiometric titration provides the principal method for determining p*K*a values and it is best applied to substances with p*K*a values < 11. For example the p*K*a of benzoic acid can be determined as follows: A 0.01 M solution of benzoic acid (50 ml) is titrated with 0.1 M KOH. The KOH is added in 0.5 ml increments: it would be expected that 5 ml of 0.1 M KOH would be required to neutralise the

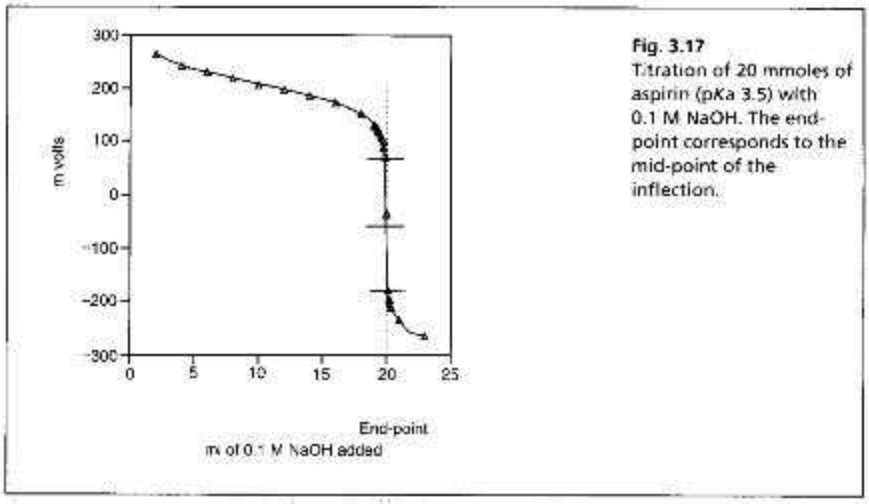


Fig. 3.17
Titration of 20 mmoles of aspirin (pKa 3.5) with 0.1 M NaOH. The end-point corresponds to the mid-point of the inflection.

Table 3.3 Potential difference values obtained for titration of 2 mmoles of aspirin (pKa 3.5) against 0.1 M NaOH

ml of 0.1 M NaOH added	potential mV
14	185
16	172
18	151
19	132
19.1	129
19.2	126
19.3	122
19.4	119
19.5	113
19.6	107
19.7	100
19.8	89
19.9	71
20	−44
20.1	−177
20.2	−195
20.3	−206
20.4	−212
21	−236
23	−266

benzoic acid. The pH of the titration is monitored with a glass electrode and the pH of the mixture after 2.5 ml of 0.1 M KOH has been added will equal the p*K*a value of benzoic acid since:

$$pKa = pH - \log \frac{[C_6H_5COOK]}{[C_6H_5COOH]}$$

The p*K*a value may be checked after addition of each 0.5 ml since the concentrations of acid and salt are known at each point on the titration curve. The slight increase in volume due to addition of the 0.1 M KOH may be ignored. For a base, the Henderson–Hasselbalch equation is written as given in Chapter 2, page 20.

Self-test 3.10

50 ml of a 0.01 M solution of the base diphenhydramine is titrated with 0.1 M HCl and the pH is monitored with a glass electrode. After 3 ml of 0.1 M HCl have been added the pH of the solution is 8.82. What is the pKa of diphenhydramine?

Answer: 8.6

Karl Fischer titration (coulometric end-point detection)

The Karl Fischer titration is a specialised type of coulometric titration. Coloumetry itself is a useful technique, but is not used as a mainstream technique for pharmaceutical analysis. Essentially coulometry is based on the electrolytic reduction of the analyte, i.e. the analyte is reduced by electrons supplied by a source of electrical power and the amount of charge passed in order to convert the analyte to its reduced form is equivalent to the amount of analyte present in solution. According to Faraday's Law, where one molecule of analyte reacts with one electron, 1 mole of analyte will react with 96 485 coulombs of electricity where coulombs = amps × s. If iodine is reduced by coulometry, the following reaction occurs:

$$I_2 + 2e \longrightarrow 2\,I$$

One mole of iodine will consume 2 × 96 485 coulombs of electricity. The Karl Fischer titration is widely used for the determination of water in pharmaceuticals. Quantitation in this case is not based on the total amount of current which flows through the solution but the reduction of iodine is simply used to indicate the end-point of the titration. The reagent consists of mixture of anhydrous methanol, anhydrous pyridine, iodine and sulphur dioxide. The equation for the reaction of water with the reagent looks complicated (see below)

$$H_2O + I_2 + SO_2 + 3C_5H_5N + CH_3OH \longrightarrow 2C_5H_5N.HI + C_5H_5NH.SO_4CH_3$$

but the essential thing to note is that the presence of water causes the conversion of iodine to iodide through its reduction by sulphur dioxide. When a titration is carried out the reagent is added to the sample and reacts with the water in it. A potential is applied across the solution containing the sample and no current flows until at the end-point, when all the water has been consumed. The presence of iodine in solution from addition of excess reagent allows current to flow through the solution in order to carry out the reduction of iodine shown in the equation above, thus providing the end-point detection.

The Karl Fischer titration is used to quantify water in pharmacopoeial assays of: ampicillin trihydrate, benzylpenicillin sodium, gentamycin sulphate, tetracosactrin and tobramycin.

Automation of wet chemical methods

Automatic titration (Fig. 3.18)

Titrations can be automated and controlled by a microprocessor. The titrant is delivered via an automatic burette and the end-point is detected potentiometrically

with a glass combination electrode. Alternatively, if ions other than hydrogen are being measured another ion-selective electrode may be used. The apparatus is microprocessor controlled and can be programmed to run in various modes:

(i) The rate of delivery of the titrant can be controlled according to rate of change of potential so it is added more slowly as the rate of change in potential increases, i.e. as the end-point is approached.
(ii) For titrations which take time to equilibrate as the titrant is added, the instrument can be programmed to delay after each incremental addition until the potential becomes stable.
(iii) The detection of the end-point can be pre-set at a fixed potential.

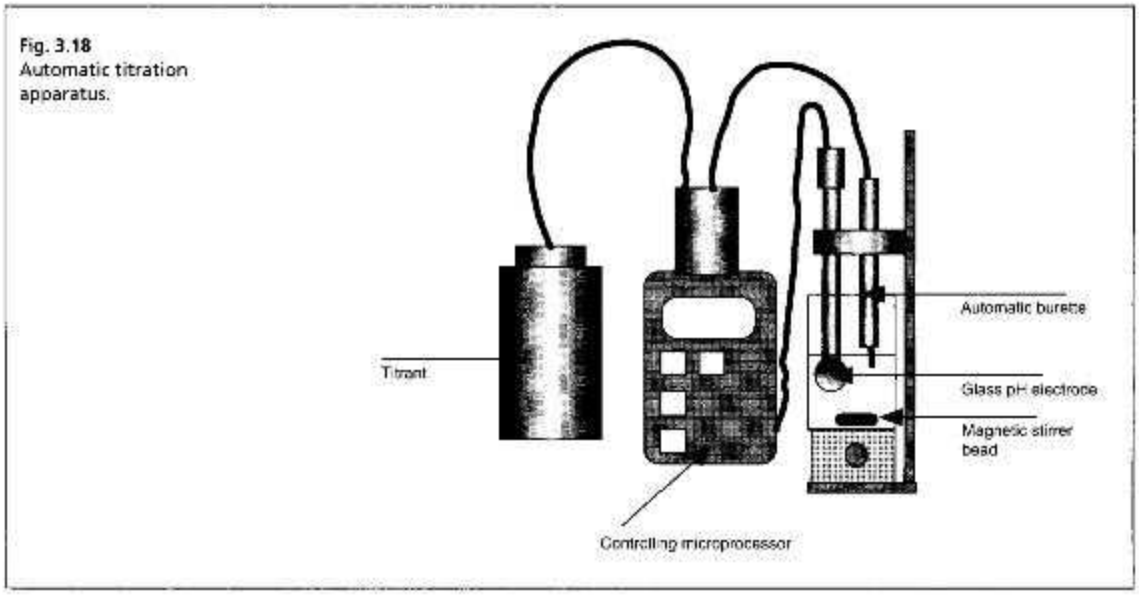


Fig. 3.18
Automatic titration apparatus.

The microprocessor control also enables the instrument to be set to calculate p*K*a values directly from the pH profile it obtains by titration of a sample. A sample changer can be incorporated so that several samples can be automatically titrated.

Flow injection analysis

Flow injection analysis (FIA) represents a refinement of wet chemical methods. The basis of the technique is that the sample is injected into a continuously flowing stream of reagent. The sample reacts with the reagent and this reaction is measured with a detector. The range of detectors available is the same as that which is used in conjunction with HPLC (Ch. 12, p. 248) except that there is no chromatographic separation involved. Thus the technique is not as selective as chromatographic methods and its selectivity is dependent on the specificity of the reaction between the analyte and the reagent or the property used for detecting it. A simple schematic diagram of a flow injection analysis system is shown in Figure 3.19. The basic set up may be modified to include several manifolds that allow the introduction of the sample followed by additional reagents. The advantages of the technique are its cheapness and rapidity.

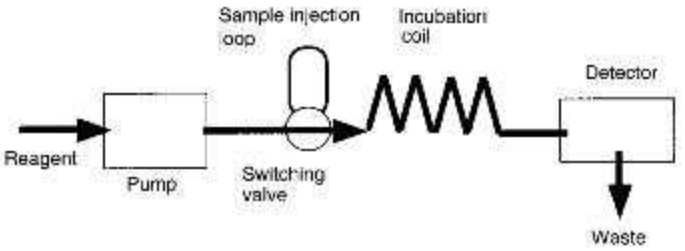


Fig. 3.19
A simple flow injection analysis system.

A precise volume of sample (1–100 μl) is injected and passed through the incubation coil, which is of sufficient length to allow the sample to disperse in the reagent but not long enough for the sample to become diluted so much that the integrity of the plug of sample is lost. The detector response is dependent on the degree of dispersion of the sample. A typical flow of the reagent + analyte is as shown in Figure 3.20.

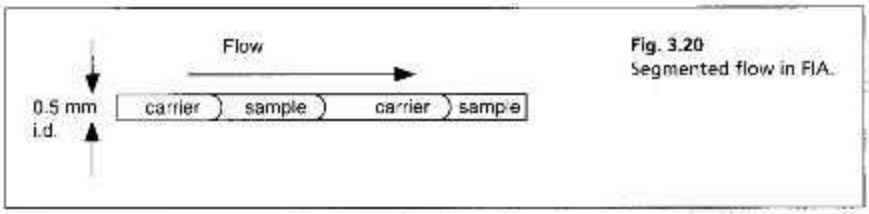


Fig. 3.20
Segmented flow in FIA.

The parameters which have to be optimised include:

(i) The length and internal diameter of the incubation coil
(ii) The flow rate
(iii) The volume of sample injected
(iv) The concentration of sample and reagents used.

Since a number of factors are involved in the optimisation, some time is required to develop the method. However, when set up, the method can replace titrations and replicate analyses can be conducted very quickly with minimal consumption of reagents.

As in chromatography, the ideal peak shape obtained in FIA should be Gaussian, although in practice the ideal shape may not have time to develop. The mathematics governing the dispersion processes have been developed thoroughly and the process is largely analogous to the dispersion occurring in capillary gas chromatography, where longitudinal diffusion is the major factor governing band broadening.

Figure 3.21 illustrates an application of FIA to the Karl Fischer titration. The consumption of the reagent by water is detected spectrophotometrically by monitoring the stream of reagent at 615 nm. The absorbance due to the iodine in the reagent is removed by its reaction with water, which causes formation of iodide and thus negative absorbance is measured.

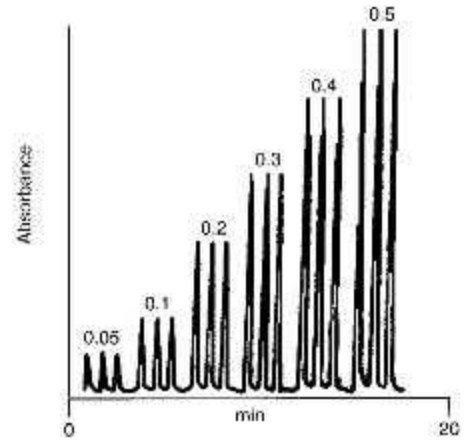


Fig. 3.21
Calibration of Karl Fischer FIA method for determination of residual in water in pharmaceuticals. The analyte was methanol containing 0.05, 0.1, 0.2, 0.4 and 0.5% w/v water.

Applications of FIA in pharmaceutical analysis

Determination of chloroxine

The antibiotic chloroxine was determined utilising the formation of a complex between the drug and Al^{3+} in an FIA system. The complex was determined by measurement of fluorescence with 399 nm as the excitation wavelength and 496 nm as the emission wavelength. In order to ensure solubility of the complex in the aqueous reagents a surfactant was included in the reagent mixture. The precision of the method was greater than that obtained using a laborious batch method for measuring samples manually using a fluorescence spectrophotometer.[3]

Determination of captopril

A FIA method for the determination of captopril was based on the oxidation of the thiol group in the molecule by Ce^{4+}. This reaction results in the emission of light (chemiluminescence), which can be measured. In this example the dye rhodamine G was used to enhance the emission of light by the reaction. The method developed was rapid and precise.[4]

Determination of non-steroidal anti-inflammatory drugs

Diclofenac sodium, famotidine and ketorolac were analysed utilising their formation of a coloured charge transfer complex with 2,4 dichloro-6-nitrophenol. The complexes were detected by UV/visible spectrophotometry at 450 nm. The method was not affected by the presence of common excipients in the formulations analysed. The precision and accuracy of the method was comparable to that of HPLC methods used to analyse the same samples.[5]

Determination of promethazine

The generation of a coloured product upon the oxidation of promethazine with Ce^{4+} was used in the development of a FIA method. Promethazine in tablet form could be analysed by this method with a precision of ± 1% and at a rate of 122 samples per h.[6] In a similar method promazine was oxidised by passing through a short column containing MnO_2 and then the oxidation product was measured.[7]

Determination of chlorocresol

Chlorocresol is a preservative commonly used in injections and its determination often involves the use of laborious extraction procedures in order to separate it from formulation components, followed by spectrophotometric measurement. A FIA method for chlorocresol was developed by utilising its reaction with nitrous acid to form a coloured nitro compound. The method was accurate to 99.5% of the true value of chlorocresol in a formulation and a precision of ± 1% was achieved.[8]

Limit test for heavy metals

Many pharmacopoeial monographs contain a limit test for heavy metals. Sometimes the metal is specified, e.g. lead, but often the test is more general. Pharmacopoeial tests often involve precipitation of the metals as their sulphides. A FIA method was developed based upon complex formation between heavy metals and diethyldithiocarbamate (DDC). Figure 3.22 shows the FIA system used for this analysis and illustrates how relatively simple components can be assembled to carry out a complex analytical task. The analysis was achieved by using a segmented flow system where alternate segments of buffer solution + reagent and carbon tetrachloride were produced. In the first extraction coil, the heavy metals in the sample are extracted as their complexes, along with some excess complexing agent, into carbon tetrachloride. In the second extraction coil, the excess reagent in the organic layer is back extracted by the borax solution, which is mixed into the carrier stream. The flow was then passed into a phase separator which only allowed the organic solvent to flow through to the detector.[9]

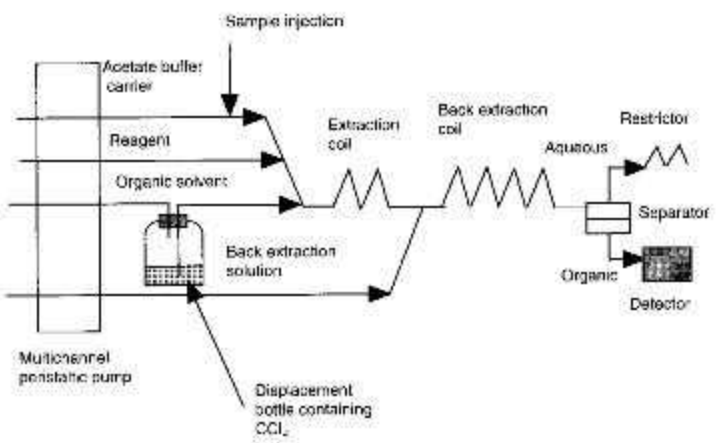


Fig. 3.22
FIA system used to analyse heavy metal residues by extraction of their complexes with DDC into carbon tetrachloride.

Use of segmented flow in determination of partition coefficients

A system similar to the one described above was used for determination of partition coefficients. A FIA system with segmented flow was devised so that the partitioning of a drug between aqueous buffer and chloroform could be measured. The aqueous and organic phases were separated using a phase separator. The system could be set up to measure the concentration of the drug in either the organic or the aqueous phase. Such a system enables rapid repeat determinations of partition coefficient at various pH values with minimal sample consumption.[10]

Automated dissolution testing

FIA was used to optimise sampling from a tablet dissolution apparatus in order to determine the rate of release of iron (II) from a sustained release formulation. The dissolution medium was automatically sampled at 30-minute intervals and the 100 μl aliquots of medium were mixed with the iron complexing agent ferrozine, diluted and then passed into a spectrophotometric detector. The system was microprocessor controlled thus enabling unattended sampling of the dissolution medium for a prolonged period.[11]

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