6

Volumetric analysis of drugs

This chapter will deal with *volumetric analysis*, that is analysis carried out by the accurate measurement of volumes. To measure volumes accurately, use must be made of volumetric glassware. There are three pieces of volumetric glassware that are fundamental to successful volumetric analysis. These are the *volumetric flask*, the *pipette* and the *burette*, and each will be described below (see Figure 6.1). It should be stated, however, that no amount of reading about these pieces of apparatus (no matter how eloquently written!) is sufficient to educate a student. Analytical pharmaceutical chemistry is first and foremost a practical subject, and the laboratory is the best place to get to grips with the techniques required for consistent, reproducible analysis.

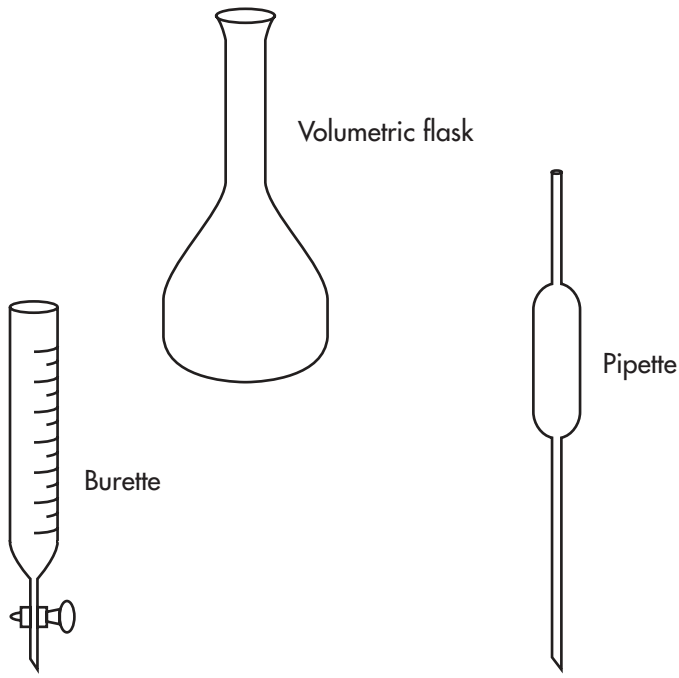


Figure 6.1 A volumetric flask, a pipette and a burette.

Volumetric flask

A volumetric flask is used to prepare accurate volumes of solution. These flasks are pear-shaped with long, thin necks that allow the operator to dilute accurately to the mark with solvent. Volumetric flasks are available in all sizes from 1 mL up to 10 litres, but the most common sizes are 20, 50 and 100 mL. When selecting which size of flask to use, a compromise should be reached between the desire to use a small-volume flask and so save on expensive reagent, and the desire to use a large-volume flask to minimise dilution errors. The usual procedure is to pipette in a known volume of concentrated solution, add solvent until just short of the mark, shake or invert the flask to mix the contents and then make up to the mark, as accurately as possible, with a Pasteur pipette. Volumetric flasks should be used for all accurate dilutions. Use of measuring cylinders or (even worse) beakers to dilute solutions should be avoided.

Pipette

Pipettes are used to transfer accurate volumes of solution from a container (usually a beaker) to a reaction flask for dilution or assay, usually in conjunction with pipette fillers. They are not drinking straws and should never be placed in the mouth, or used to 'mouth pipette' solutions. This practice is both dangerous and unhygienic. There are two main types of pipettes.

Transfer (or delivery) pipettes

Pipettes of this type possess only one graduation mark and are used for delivery of that single volume of solution. Common sizes are 10, 20 and 50 mL. These pipettes are filled to a little above the mark by use of a pipette pump or a bulb. The pump is removed and the solution is allowed to run out until the mark is reached, the flow of solution being controlled all the way by use of the index finger over the end of the pipette. Most transfer pipettes are calibrated to allow a small volume of solution to remain in the tip of the pipette once it has been drained and no attempt should be made to 'blow' this drop out of the bottom of the pipette.

Pipettes of this type are used in all analytical chemistry procedures. Care must be taken when inserting the pipette into the pipette filler. If the pipette is held by the bulb and pushed into the filler, the shaft of the pipette can break and the operator can be injured. When inserting pipettes into pipette fillers, the pipette must always be held close to the end to prevent this all too common accident occurring.

Graduated pipettes

Graduated pipettes are calibrated to allow a single piece of glassware to deliver a range of volumes: common sizes are 1 mL and 10 mL. These pipettes are considerably less accurate than transfer pipettes, and there is no place for them in an analytical chemistry laboratory. If very small volumes need to be transferred, use should be made of accurate glass syringes (e.g. a 'Hamilton' syringe) or an automatic micropipette.

Burettes

Burettes are used to deliver variable volumes of reagent accurately. The most useful size is the 50 mL burette. These burettes are calibrated in units of 0.1 mL, but students should be encouraged to read to the nearest 0.05 mL. Once students have achieved some skill in titration techniques, they will be able to read the burette to the nearest 0.02 mL. This will involve splitting each 0.1 mL graduation into five – i.e. 0.02, 0.04, 0.06, 0.08 and 0.1 mL.

All of the volumetric glassware described above is designed for use at ambient room temperature and should never be used for hot liquids or placed in hot ovens and the like to dry.

Units of concentration

Before we consider topics such as the design of an assay, calculation of drug purity, and so on, it is useful to revise the units and terms chemists use for amount of substance and concentration. The fundamental unit of quantity or amount of substance used in chemistry is the *mole*. The mole is the amount of a substance (either elements or compounds) that contains the same number of atoms or molecules as there are in 12.0000 g of carbon-12. This number is known as the Avogadro number (after Amedeo Avogadro, an Italian chemist) or Avogadro's constant, and has the value 6.02×10^{23}. When this amount of substance is dissolved in solvent (usually water) and made up to 1 litre, a 1 molar (1 M) solution is produced. In a similar way, if one mole of substance were made up to 2 litres of solvent, a 0.5 M solution would result, and so on. The litre is not the SI unit of volume but, along with the millilitre (mL), is still used in the *British Pharmacopoeia*.

In pharmaceutical analysis laboratories, concentration is usually expressed as (for example) 1 M (1.026) or 0.5 M (0.998). The nominal concentration is given as molarity, while the number in brackets refers to the *factor* (*f*) of the solution. The factor of a volumetric solution tells you by how much the given solution differs from the nominal, or desired strength.

The first solution, above, is slightly stronger than 1 M, since the factor is greater than 1.000. The second solution is slightly weaker than half molar, as the factor is less than 1.000. It follows that a solution with a factor of 1.000 is of precisely the stated molarity.

If the absolute molarity of the solution is required, it can easily be found by multiplying the factor and the nominal molarity. For instance, in the examples above, the first solution has an absolute molarity of 1 M × 1.026 = 1.026 M, which as predicted above is slightly stronger than 1 M. Similarly, the second solution has an absolute molarity of 0.499 M (i.e. 0.5 M × 0.998). It follows from this that the factor of a solution is simply the ratio

$$\frac{\text{Actual concentration}}{\text{Desired or nominal concentration}}$$

Factors are used in volumetric analysis because they simplify calculations (a laudable aim, in any subject). Consider the first solution above: the strength of the solution is 1 M (1.026). If 10 mL of this solution were removed, by pipette, transferred to a 100 mL volumetric flask, and made up to volume with water, the resulting solution would have a concentration of 0.1 M (1.026). The original solution has been diluted tenfold, but the factor of the new solution remains as 1.026. This illustrates an important principle, namely, that once a factor has been determined for a volumetric solution, subsequent dilution or reaction will not affect it (although see later for an exception to this).

Once the factor for a solution is known (i.e. once the solution has been *standardised*), multiplication of the experimentally determined volume by the factor will yield what the volume would have been if the solution had been precisely the nominal molarity (i.e. if the factor had been 1.000). In practice, very few volumetric solutions are factor 1.000; this is due, in the main, to the time that would be taken to weigh out a sample to four decimal places. Volumetric solutions are usually prepared by weighing out approximately the desired weight of sample, then standardising the resulting solution against a solution of known concentration.

All volumetric solutions used in pharmaceutical analysis are prepared from a *primary standard*. This is a compound that can be obtained in a very high level of purity (>99.9%). Examples of compounds used as primary standards include sodium carbonate (Na_2CO_3) and potassium hydrogen phthalate ($C_8H_5O_4K$). Compounds such as these can be weighed accurately, to four or even six decimal places, and made up to volume in a volumetric flask to give a solution of known molarity. Solutions that are prepared by standardisation against a primary standard are referred to as secondary standards. A solution standardised against a secondary standard is termed

a tertiary standard, and so on. This process cannot continue indefinitely, however, as errors creep in with every assay, and the results become less reliable the farther the solution gets from the initial primary standard.

Worked example

A primary standard solution of Na_2CO_3 was prepared and used to standardise a solution of H_2SO_4 of unknown concentration. 25.0 mL of 1 M (f = 1.000) Na_2CO_3 was added by pipette to a conical flask and 24.60 mL of H_2SO_4 was required for neutralisation. Calculate the factor of the H_2SO_4 solution.

From the reaction

$$Na_2CO_3 + H_2SO_4 \longrightarrow Na_2SO_4 + CO_2 + H_2O$$

it can be seen that 1 mole of sodium carbonate reacts with 1 mole of sulfuric acid. Then

1 mole $Na_2CO_3 \equiv$ 1 mole H_2SO_4

1000 mL 1 M $Na_2CO_3 \equiv$ 1000 mL 1 M H_2SO_4

1 mL 1 M $Na_2CO_3 \equiv$ 1 mL 1 M H_2SO_4

Since both solutions are 1 M, the concentrations effectively cancel out to leave the relationship

(volume × factor) of $Na_2CO_3 \equiv$ (volume × factor) of H_2SO_4

or, to put it another way,

(25 mL × $f(Na_2CO_3)$) ≡ (24.60 mL × $f(H_2SO_4)$)

(25 mL × 1.000) ≡ (24.60 mL × $f(H_2SO_4)$)

and $f(H_2SO_4)$ is given by 25 × (1.000/24.6), so that

$f(H_2SO_4)$ = 1.016

A moment's thought will confirm that the correct answer has been achieved. The only calculation error that could be made in this simple example is to get the factor upside-down (a so called 'inverted factor'). But, in the reaction, 25 mL of a f = 1.000 solution of Na_2CO_3 was neutralised by *less than* 25 mL of the acid. The acid must clearly be stronger than f = 1.000 if it required only 24.60 mL to neutralise the 25 mL of sodium carbonate. A check of this type should be carried out after every volumetric calculation. It is quick and easy to do and, to paraphrase the great Robert Burns, 'It wad frae monie a blunder free us, An' foolish notion'.

Concentration of active ingredients

Although, in chemistry, all concentrations are expressed in molarity, pharmacists and pharmaceutical analysts have to contend with the medical profession, which tends to prescribe drugs not in molarities but in units of mass per volume or weight per millilitre. The most common way to express the concentration of active drug in a medicine is in terms of mass or volume of active ingredient per 100 grams or millilitres of medicine. This can be expressed in four ways, of which the first is the most common.

'Percentage weight in volume' (% w/v) is the number of grams of drug in 100 mL of final product. This term is used for the concentrations of solutions, suspensions, etc. where the active ingredient is a solid; for example, 5% dextrose infusion is 5 g of dextrose in 100 mL of final solution.

- 'Percentage volume in volume' (% v/v) is the number of millilitres of drug in 100 mL of final product. This version is found in medicines where the active drug and the final product are both liquids. This terminology should be familiar to students since the strength of alcoholic drinks is usually expressed in this way. A single malt whisky is 40% by volume alcohol. This means that for every 100 mL of 'Glen Fusel' you drink you consume 40 mL of ethanol. Most beers are approximately 5% by volume alcohol. Thus, for every 100 mL of beer consumed, the drinker has taken in 5 mL of ethanol. (A pint is approximately 568 mL.)
- 'Percentage weight in weight' (% w/w) is the number of grams of drug in 100 g of final product. This term is encountered most often in solid dosage preparations such as powders, and semi-solid preparations such as creams and ointments, e.g. 1% salicylic acid ointment.
- 'Percentage volume in weight' (% v/w) is the number of millilitres of drug in 100 g of final product. This usage is quite rare and is only encountered in ointments and creams where the active ingredient is a liquid, e.g. 1% glycerol ointment.

Design of an assay

Before a substance is analysed, or assayed, the experiment must be designed and planned. Initially, students will be told what to do in the analysis laboratory, but they must quickly begin to plan assays and experiments for themselves. The procedures to be followed when designing an assay are outlined below.

1. Identify functional groups on the molecule that can react rapidly and quantitatively (i.e. the reaction should proceed almost 100% to the products; to put it another way, the chosen reaction should have a high equilibrium constant, K).
2. Work out the stoichiometric ratio, i.e. the number of moles of each compound reacting.

3. Convert the number of moles of sample to a weight, and the number of moles of titrant to a volume.
4. Calculate the weight of sample that will react with 1 mL of the titrant. This figure is called the *equivalent relationship* or sometimes *the equivalent* and is the most important part of the calculation.
5. Carry out the assay, at least in duplicate. If agreement is not achieved with two results, the assay should be repeated until concordant results are obtained.
6. Calculate the weight of active drug in the sample, and express the answer as percentage weight in weight (% w/w) of sample weighed. This answer represents the percentage purity of the drug and should be compared with the *British Pharmacopoeia* (BP) limits to see whether the sample complies with the requirements of the BP. The *British Pharmacopoeia* lays down purity criteria and limits within which a sample must lie to be of BP quality. Both determinations must fall within the BP limits to be acceptable. If one result falls within the BP limits and the duplicate result does not, then the sample does not comply with the BP limits, and should not be used.

In addition to the limits of purity, the *British Pharmacopoeia* contains a wealth of information about the substance in question. The *British Pharmacopoeia* is a legally enforceable document produced every four or five years by the Pharmacopoeia Commission and lists the criteria for the purity of drugs and medicines used in the UK and Commonwealth. Each substance in the *British Pharmacopoeia* is given a specific *monograph*, which lists the chemical structure of the compound (if known), the definition and statement of BP limits (quoted to one decimal place), a description of its characteristics (colour, solubility, etc.), some tests for identification of a sample of the material and limit tests for impurities (usually a colour test that compares the levels of an impurity with the maximum permitted limit allowed by the BP for that impurity). Limit tests are often used when the BP assay is not *stability indicating*, i.e. does not differentiate between the drug and its major decomposition product. The monograph ends with the official BP assay for determination of purity. Formulated medicines may have, in addition to a specific monograph, a *general monograph*, which applies to that class of medicine. For example Aspirin Tablets BP will have to comply with all of the monograph for Aspirin BP as well as the general monograph for *tablets*. Similarly, Chloramphenicol Eye Drops BP must comply with the general monograph on *eye drops* for sterility, etc. in addition to the requirements for the purity of chloramphenicol.

To illustrate these points, we can consider the assay of citric acid. Citric acid is a natural product found in citrus fruits (lemons, oranges, limes, etc.) and is used in pharmaceutical formulations as a buffer and a preservative. Its structure is shown in Figure 6.2.

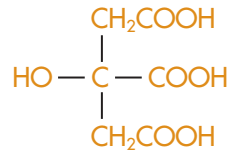
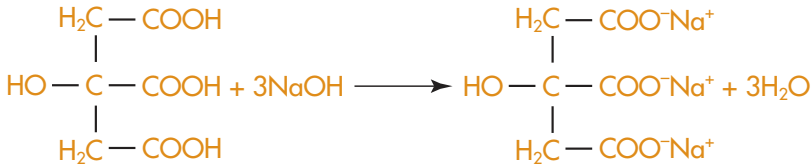


Figure 6.2 The structure of citric acid.

Examination of the structure of citric acid reveals three carboxylic acid groups; these should react quantitatively with a strong alkali, such as sodium hydroxide. So the reaction equation is



Therefore,

1 mole citric acid ≡ 3 moles NaOH

and

192.1 g citric acid ≡ 3 litres 1 M NaOH

or

192.1 g citric acid ≡ 3000 mL 1 M NaOH

Therefore,

(192.1/3000) g citric acid ≡ 1 mL 1 M NaOH

or

0.06403 g citric acid ≡ 1 mL 1 M NaOH

The equation in bold type is the equivalent relationship and tells us that for every 1 mL of titrant added, we can expect to react slightly more than 64 mg of citric acid. Note also that the equivalent is derived for a precisely 1 M solution, i.e. $f = 1.000$.

This reaction was carried out using phenolphthalein as an indicator and the following data were obtained:

Weight of citric acid = 1.5268 g

Volume of 1 M NaOH ($f = 0.998$) required 23.95 mL

The volume of titrant used in the assay must now be modified to give what the volume would have been if a factor 1.000 solution had been used. This is achieved by multiplying the experimental volume by the factor, so that

23.95 ml of titrant ($f = 0.998$) ≡

(23.95 × 0.998) ml 1 M NaOH ($f = 1.000$)

Since, from the equivalent,

1 mL 1 M NaOH ($f = 1.000$) ≡ 0.06403 g citric acid

then the weight of citric acid in the sample is given by

(23.95 × 0.998 × 0.06403) g

However, 1.5268 g was weighed, so the content of citric acid is given by

$$\frac{23.95 \times 0.998 \times 0.06403}{1.5268} = 1.0024$$

This figure is usually expressed as a percentage, to give the percentage purity of citric acid as 100.2% w/w.

A duplicate determination is now carried out and the answer is compared to 100.2% w/w. Agreement is usually considered to be not more than 0.5% error between duplicates. Once duplicate determinations have been carried out, and agreement is obtained, the answers may be averaged and the *British Pharmacopoeia* consulted to see whether the sample complies. Not every sample assayed will comply; there may be impurities present if, for example, the sample was old or had been adulterated. However, an analyst who has obtained duplicate results, in good agreement, should be confident to state that the sample does not comply with the BP limits.

Practical points

Weighing by difference

In all accurate pharmaceutical analyses, samples are weighed by difference: that is, the weight of sample added to the flask is determined by subtraction of consecutive weighings of the sample container. The procedure adopted is as follows.

1. Twice the desired amount of sample is weighed roughly on a top pan balance (i.e. if a procedure requires a sample weight of 1.5 g, then for duplicate determinations 2 × 1.5 g = 3.0 g will be required).

2. The sample container and contents are weighed accurately on an analytical balance, to four, or sometimes six, decimal places.
3. Some of the sample is transferred to the reaction flask and the sample container is re-weighed. Care should be taken not to touch the sample with the fingers, a spatula, or anything else for that matter. The difference in weight between steps 2 and 3 represents the weight of sample transferred.
4. This process is repeated until the desired weight has been transferred. If more than the desired weight of sample is transferred, the sample should be discarded and the whole procedure begun again. *On no account should excess sample be returned to the original container*. The *British Pharmacopoeia* allows discretion of ±10% on the stated sample weights.

Approximate titre calculation

The end point of a titration should not come as a surprise to the analyst. Before a single drop of titrant has been added, an estimate of the endpoint volume should be carried out. For a simple forward titration, like the citric acid example above, the approximate titre is given by

$$\frac{\text{Sample weight}}{\text{Equivalent weight}} = x \text{ mL}$$

This calculation makes two assumptions, neither of which is actually valid, namely that the factor of the titrant to be used in the assay is 1.000, and that the sample is 100% pure. Neither of these assumptions will be true, but the factor will be close to 1.000 and the purity will, usually, be close to 100%, so the estimate is worth doing. The approximate titre calculation is also the first sign the analyst has that things are going wrong in the assay. If the approximate titre is estimated as (say) 18 mL, alarm bells should begin to ring if no end point has been reached after approximately 20 mL. The stated sample weights in the BP are usually chosen to give titres between 20 and 25 mL. This is because analysts are, by nature, lazy and do not want to have to refill a 50 mL burette during a titration!

Use of molarities in calculation

Students often prefer to perform simple calculations, like the direct titration of citric acid, using absolute molarities of titrant instead of deriving the equivalent and making use of factors. The procedure adopted is to convert the volume of titrant required to a number of moles and, from the balanced chemical equation, relate this to the number of moles of reactant used in the assay. This number is then converted into a weight and the

purity is obtained by dividing this calculated weight by the mass of sample weighed out.

Using the figures above:

Volume required = 23.95 mL of 1 M (0.998) NaOH

= 23.95 mL 0.998 M NaOH

= (23.95/1000) × 0.998 moles of NaOH = 0.0239 moles NaOH.

Since 1 mole of citric acid = 3 moles of NaOH,

Number of moles of citric acid reacted = 1 × (0.0239/3)

= 0.007967 moles citric acid

Since M_r = 192.1 g,

Mass of citric acid reacted = 192.1 × 0.007967 = 1.53053 g

But 1.5268 g was weighed, so the content of citric acid is given by

$$\frac{1.53053}{1.5268} \times 100 = 100.2\% \text{ w/w}$$

which is the same answer as obtained above.

Sources of error can be introduced in each conversion from volume to moles and back to weight, although for simple examples such as the one above it does not really matter which method of calculation is employed as long as the correct answer for the purity of citric acid is obtained. However, for more complicated calculations, involving the use of back and blank titrations, this author believes that factors and equivalents simplify volumetric analysis and they will be used for that reason (rather than any reason of dogma) in the remainder of this book.

Choice of indicators

The end point of the titration is detected by the use of a suitable indicator. These indicators are themselves weak acids or bases whose colour in solution depends on their degree of ionisation. In practice, the endpoint pH is estimated (see Chapter 1, p. 22), and an indicator that changes colour at this pH chosen. For convenience, a table of common indicators and their pH ranges is shown in Figure 6.3.

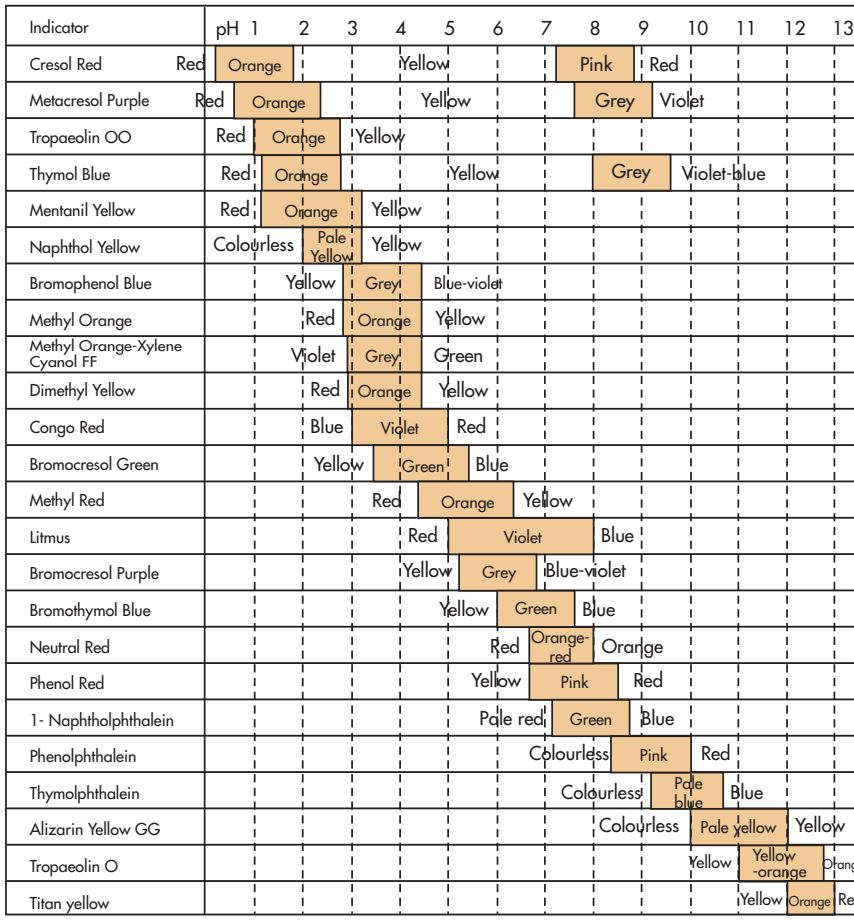


Figure 6.3 A table of the pH ranges of indicators.

Back and blank titrations

In the example above, a reaction was chosen that was quick to carry out and was quantitative, i.e. it went to completion. In many pharmaceutical analyses this is not the case and a *back titration* has to be carried out.

Back titrations are often combined with *blank titrations*, particularly if there is some loss of reagent during the assay (e.g. as a result of splashing or vigorous boiling) or the concentration of a volumetric reagent changes during the assay. A back titration involves addition of a known excess of reagent to the sample (this drives the reaction to completion) and titration of the unreacted excess of reagent with a suitable titrant. The volume that

reacted with the sample is determined by simple subtraction. For example, if 50.0 mL of reagent were added to the sample and the back titre was 30.0 mL then, clearly, 20.0 mL of reagent has reacted with the sample.

In a blank titration, the assay is carried out, then repeated without any sample being present. This appears, at first sight, to be a perfect waste of time, but determinations of this type allow the analyst to measure any changes that occur to the reagent during the course of the assay. If the procedure involves heating and subsequent cooling of the sample (e.g. to allow the sample to dissolve), some of the volumetric reagent may be lost either by evaporation or mechanically due to splashing or bubbling. The blank determination must be identical to the test determination in every way except, of course, that there is no sample in the blank. This means that heating times, dilutions, etc. must all be duplicated exactly.

The best way to illustrate the procedures adopted for back and blank titrations is to consider an example, the determination of chalk, or calcium carbonate, $CaCO_3$. Chalk is used as an antacid and indigestion remedy, particularly in children, and is official in the *British Pharmacopoeia* as the powder and the mixture (Paediatric Chalk BP).

The official assay is by the addition of a known excess of hydrochloric acid and back titration of the unreacted excess with sodium hydroxide. A blank determination is carried out since the sample is heated and cooled. The calculation will be carried out initially as a back titration without a blank and then compared with the answer obtained when the blank is taken into account. The calculation should be studied closely as there are subtle differences between the back and blank calculations.

The chemical reactions taking place are as follows.

$$CaCO_3 + 2HCl \text{ (in excess)} \longrightarrow CaCl_2 + CO_2 + H_2O$$

Then

$$2HCl \text{ (unreacted excess)} + 2NaOH \longrightarrow 2NaCl + 2H_2O$$

The relative molecular mass of chalk is 100.1, so that

$$1 \text{ mole } CaCO_3 \equiv 2 \text{ moles HCl} \equiv 2 \text{ moles NaOH}$$

Therefore,

$$100.1 \text{ g } CaCO_3 \equiv 2000 \text{ mL } 1 \text{ M HCl}$$
$$\equiv 2000 \text{ mL } 1 \text{ M NaOH}$$

and

$$0.05005 \text{ g } CaCO_3 \equiv 1 \text{ mL } 1 \text{ M HCl}$$
$$\equiv 1 \text{ mL } 1 \text{ M NaOH}$$

In the experiment, approximately 1.5 g of sample was weighed and added to 100 mL of water in a conical flask and 50.0 mL of 1 M hydrochloric acid was added by pipette. The mixture was boiled gently for 2 minutes and cooled and the unreacted HCl was titrated with 1 M NaOH using methyl orange as indicator. The entire procedure was repeated omitting the sample and the % w/w $CaCO_3$ in the sample was determined.

Results

Weight of chalk = 1.5961 g

Volume of 1 M (f = 0.996) HCl = 50.00 mL

Volume of 1 M (f = 1.012) NaOH = 18.50 mL

Since neither volumetric solution is factor 1.000, the experimental volumes must be modified by the factor to obtain the factor 1.000 volumes.

Volume of HCl available = (50.0×0.996)

Volume of NaOH in excess = (18.50×1.012)

Therefore, the volume reacting with chalk is given by

$$(50.0 \times 0.996) - (18.50 \times 1.012) = 31.08 \text{ mL}$$

From the equivalent,

1 mL 1 M HCl or NaOH ≡ 0.05005 g $CaCO_3$

Therefore,

$$31.08 \text{ mL } 1 \text{ M solution} \equiv (31.08 \times 0.05005) \text{ g } CaCO_3 = 1.5554 \text{ g } CaCO_3$$

However, 1.5931 g of sample was weighed. Therefore, the percentage of calcium carbonate is

$$\frac{1.5554}{1.5931} \times 100 = 97.6\% \text{ w/w}$$

Using these same data, the calculation can be repeated, but this time taking account of the blank determination. If an assay requires a blank, then the concentration of the reagent (hydrochloric acid in this case) must change in the course of the assay; therefore, the volume and factor of the

hydrochloric acid will not appear anywhere in the calculation.

Volume of 1 M NaOH ($f = 1.012$) in blank titration = 49.65 mL

In this case, the volume of 1 M NaOH reacting with chalk is given by

(Volume of blank titration − volume of back titration)
× factor of NaOH

The NaOH factor is used because both of these volumes are NaOH volumes. That is,

(49.65 − 18.5) × 1.012 mL 1 M NaOH

Since from the equivalent

1 mL 1 M NaOH ≡ 0.05005 g $CaCO_3$

then the weight of calcium carbonate in the sample is

(49.65 − 18.50) × 1.012 × 0.05005 g = 1.5778 g $CaCO_3$

However, 1.5931 g of chalk was weighed, so the percentage purity of calcium carbonate is

$$\frac{1.5778}{1.5931} \times 100 = 99.0\% \text{ w/w}$$

The calculation involving the blank should be more accurate than the back titration on its own since the NaOH has, in effect, been standardised during the course of the assay.

These two procedures should be studied closely since there is a subtle difference in calculation. In the back titration, the volume of acid was multiplied by the factor of the acid, and the volume of base was multiplied by the factor of the base. In the blank titration, neither the volume nor the factor of the reagent added in excess is required and the volume of titrant equivalent to the chalk is given by the expression (blank volume − test volume) × factor of titrant.

Assay of unit-dose medicines

Unit-dose medicines are preparations that contain doses designed to be taken separately. Examples of this type of preparation include tablets, capsules, suppositories or pessaries. To determine the purity of unit-dose medicines, the calculations outlined above need to be modified, in order to determine how much drug is present in each individual dosage form. The

purity of the bulk powder sample is not so important. The drug content is expressed as a percentage of how much drug should be present and is called the *percentage of the stated amount*. The *British Pharmacopoeia* uses this calculation to express the purity of all unit-dose medicines.

An example of this type of calculation is the assay of Lithium Carbonate Tablets BP. Lithium carbonate is used as an antidepressant in 250 mg and 400 mg strengths. The BP assay is to weigh and powder 20 tablets. Add a quantity of the powder containing 1 g of lithium carbonate to 100 mL of water; add 50 mL of 1 M hydrochloric acid and boil for 1 minute to remove carbon dioxide. Cool and titrate the excess acid with 1 M sodium hydroxide solution using methyl orange as indicator. The assay is then repeated omitting the sample.

The reactions taking place are as follows.

$$Li_2CO_3 + 2HCl \longrightarrow 2LiCl + H_2O + CO_2$$

$$2HCl + 2NaOH \longrightarrow 2NaCl + 2H_2O$$

Therefore, since the relative molecular mass of Li_2CO_3 is 73.9,

$$73.9 \text{ g } Li_2CO_3 \equiv 2000 \text{ mL } 1 \text{ M NaOH}$$

$$0.03695 \text{ g } Li_2CO_3 \equiv 1 \text{ mL } 1 \text{ M NaOH}$$

The assay was carried out and the following results were obtained.

Weight of 20 tablets = 3.7279 g

Weight of powder for assay = 0.4707 g

Volume of 1 M NaOH (f = 1.006) added (blank) = 48.75 mL

Volume of 1 M NaOH (f = 1.006) added (test) = 21.35 mL

The weight of 20 tablets is 3.7279 g; therefore, the average weight of one tablet is 3.7279/20 = 0.1864 g.

The weight of lithium carbonate in the sample is

$$(48.75 - 21.35) \times 1.006 \times 0.03695 \text{ g } Li_2CO_3$$

$$= 1.0185 \text{ g } Li_2CO_3$$

The number of tablets assayed is given by

$$\frac{\text{Sample weight}}{\text{Average weight of one tablet}}$$

$$= \frac{0.4707}{0.1864}$$

$$= 2.53 \text{ tablets}$$

Therefore, 1.0185 g lithium carbonate was found in 2.53 tablets, so the weight of lithium carbonate in one tablet is 1.0185/2.53 = 0.4034 g.

The stated content of lithium carbonate is 400 mg per tablet, so the percentage stated amount is given by (0.4034/0.4) × 100 = 100.8%.

Non-aqueous titrations

Non-aqueous titrations are titrations carried out in the absence of water. They are particularly useful for the assay of drugs that are very weakly acidic or basic, so weak in fact that they will not ionise in aqueous conditions. Water, being an amphoteric compound, acts to suppress the ionisation of very weak acids and bases. All the apparatus and glassware for a non-aqueous titration must be scrupulously dry, as even a drop of water will ruin the whole assay. All glassware should be rinsed with distilled water, rinsed again with a volatile solvent such as acetone, then dried thoroughly in an oven or hot air dryer. It is also a good idea to remove all wash bottles from the laboratory. There is no sadder sight than to watch a student conscientiously carry out a non-aqueous titration and then spoil all the hard work by thoughtlessly adding water from a wash bottle.

Non-aqueous titrations are widely used in Volumes I and II of the *British Pharmacopoeia* for the assay of drug substances. A large number of drugs are either weakly acidic (such as barbiturates, phenytoin or sulfonamides), or weak bases (antihistamines, local anaesthetics, morphine, etc.). The weak acids are usually titrated with tetrabutylammonium hydroxide ($N(Bu^n)_4OH$) or potassium methoxide (CH_3OK) in dimethylformamide (DMF) as solvent. Weak bases are dissolved in glacial acetic acid and titrated with perchloric acid ($HClO_4$). When a strong acid, such as perchloric acid, is dissolved in a weaker acid, such as acetic acid, the acetic acid is forced to act as a base and accept a proton from the perchloric acid. This generates an *onium* ion, which functions, in the absence of water, as a super-strong acid, and it is this species that reacts with the basic drug.

The reactions occurring are as follows.

$$HClO_4 + CH_3COOH \longrightarrow CH_3COOH_2^+ + ClO_4^-$$

$$CH_3COOH_2^+ + \text{base} \longrightarrow CH_3COOH + \text{base } H^+$$

Overall, the reaction is

$$HClO_4 + \text{base} \longrightarrow \text{base } H^+ + ClO_4^-$$

That is, the perchloric acid acts as a monoprotic acid and 1 mole of perchloric acid is equivalent to 1 mole of basic drug. The derivation of the equivalent and the calculations required are the same as for their aqueous counterparts.

REDOX titrations

REDOX titrations are titrations that involve the processes of *oxidation* and *reduction*. These two processes always occur together and are of huge importance in chemistry. Everything from simple ionic reactions to the generation of energy within human mitochondria depends on these two processes.

- Oxidation is defined as the loss of hydrogen, or the gain of oxygen, or the *loss of electrons*.
- Reduction is defined as the gain of hydrogen, or the loss of oxygen, or the *gain of electrons*.

In a REDOX titration, the equation for the reaction is balanced not by counting the moles of atoms reacting but rather by counting the moles of electrons transferred in the process. This can be illustrated by considering the standardisation of the common reagent potassium permanganate solution with the primary standard, oxalic acid. This natural compound can be obtained in high purity and is well-known in pharmacognosy as the toxic constituent of rhubarb leaves.

The reactions occurring are as follows:

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

$$(COOH)_2 \longrightarrow 2CO_2 + 2H^+ + 2e^-$$

If the equation is balanced in terms of electrons:

$$2MnO_4^- \ (10e^-) \equiv 5(COOH)_2 \ (10e^-)$$

$$2000 \text{ mL } 1 \text{ M } MnO_4^- \equiv 5 \times 126.1 \text{ g oxalic acid}$$

$$1 \text{ mL } 0.02 \text{ M } MnO_4^- \equiv 0.006305 \text{ g oxalic acid}$$

Other REDOX reagents include iodine (I_2), either by itself in a forward titration or in a back titration with sodium thiosulfate ($Na_2S_2O_3$), and complex salts of the metal cerium (such as ammonium cerium sulfate,

$Ce(SO_4)2{\cdot}2(NH_4)_2SO_4{\cdot}2H_2O$). Salts of this type are complex by name as well as by formula, but in reality behave as

$$Ce^{4+} + e^- \longrightarrow Ce^{3+}$$

in solution. In the case of cerium, only one electron is transferred, and calculation of the equivalent relationship is very straightforward.

A good example of a back titration involving iodine and thiosulfate is the assay of resorcinol in Resorcinol Solution BP. Resorcinol is an antiseptic that was widely used in the past, although less so now. The assay of resorcinol involves a quantitative electrophilic aromatic substitution reaction using bromine as the reagent, as shown in Figure 6.4.

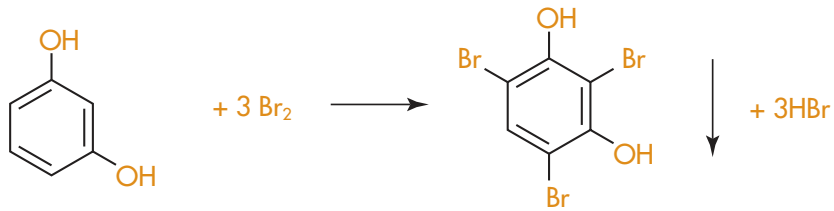


Figure 6.4 The reaction of resorcinol with bromine.

Bromine is a volatile liquid at room temperature and pressure and so cannot be measured accurately by pipette. It is also an extremely corrosive compound, irritant to eyes, lungs and mucous membranes. To overcome these difficulties, the bromine required for reaction with the resorcinol is generated *in situ* by reaction of potassium bromate and potassium bromide in the presence of strong mineral acid.

$$KBrO_3 + 5KBr + 6HCl \longrightarrow 3Br_2 + 3H_2O + 6KCl$$

To ensure that the bromination reaction proceeds quantitatively to the right-hand side, an excess of bromine is generated and the volume of bromine that does not react with resorcinol is determined by back titration. Bromine cannot be titrated easily, so the excess bromine is determined by addition of an excess of potassium iodide and titration of the liberated iodine with sodium thiosulfate, to give sodium iodide and sodium tetrathionate.

$$Br_2 + 2KI \longrightarrow I_2 + 2KBr$$

$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

This assay is great fun to do because the whole titration is carried out using a special type of conical flask called an *iodine flask*. This type of flask

has a glass well around the stopper into which the titrant is added. The stopper is then gently rotated (but not removed!) to allow titrant to enter. The iodine flask is used for two reasons:

- to prevent the escape of volatile bromine reagent
- to allow the contents to be shaken vigorously as the end point is approached.

Some analysts choose to add a non-polar solvent such as chloroform to the reaction. The chloroform acts as a solvent for the iodine (which is not very soluble in water) and, by concentrating the colour in a small volume, increases the sensitivity of the assay. Often, a small amount of starch indicator is added (to the well of the flask) as the end point is approached. Starch forms a blue-black complex with iodine and the end point of the titration is reached when the blue colour in the chloroform has disappeared.

The calculation of the content of resorcinol in the solution is identical to the back titration method explained above for lithium carbonate. Consequently, the volume of added bromate is modified by the bromate factor and the thiosulfate titre volume is modified by the thiosulfate factor. A blank titration is not required for this assay since no heating or cooling of the reaction is involved.

Complexiметric titrations

Titrations of this type rely on the formation of complexes between metal ions and compounds capable of donating electrons to form stable, soluble complexes. Compounds of this type are called (not surprisingly) *complexing agents*, while complexing agents that form water-soluble complexes with metal ions are termed *sequestering agents*. The most commonly used agent of this sort is disodium edetate.

Disodium edetate has the structure shown in Figure 6.5 and ionises with the release of two H^+ ions. For this reason, complexiметric titrations involving disodium edetate require an alkaline pH and a buffer to ensure that the released protons do not lower the pH. The usual buffer is ammonia solution, which buffers to around pH 10. Careful choice of buffer conditions can allow the assay of several different metal ions in the same sample; for example, in the assay of Intraperitoneal Dialysis Solution BPC, both Ca^{2+} and Mg^{2+} are assayed by titration with 0.02 M disodium edetate.

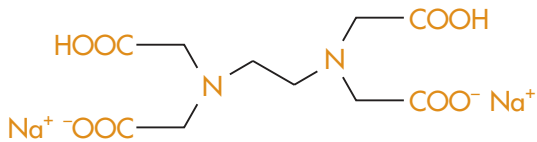


Figure 6.5 The structure of disodium edetate.

The concentration of metal ions in electrolyte preparations is often stated in millimoles per litre or sometimes millimoles per mL, where a millimole is simply one thousandth of a mole. This means that the method of deriving the equivalent relationship needs to be altered slightly from that previously stated. Using calcium ions as an example:

1 mole Ca^{2+} ions ≡ 1 mole disodium edetate

1 mole Ca^{2+} ions ≡ 1000 mL 1 M disodium edetate

1 millimole Ca^{2+} ions ≡ 1 mL 1 M disodium edetate

0.02 millimole Ca^{2+} ions ≡ 1 mL 0.02 M disodium edetate

This implies that for every 1 mL of titrant added from the burette, 0.02 millimoles of calcium will be complexed. The relationship is called a *millimolar equivalent*.

Older readers may remember the use of milliequivalents per litre as a means of describing electrolyte concentrations. Derivation of milliequivalents relies on calculation of the *equivalent weight* of the sample. For metal ions, the equivalent weight is found by dividing the relative atomic mass of the ion in question by its valency. In the case of monovalent ions such as Na^+ and K^+ this is straightforward, since the relative atomic mass and the equivalent weight are the same. For divalent ions such as Ca^{2+} and Mg^{2+} the equivalent weight is half the relative atomic mass, while for trivalent ions (e.g. Al^{3+}) the equivalent weight is a third of the relative atomic mass. The use of equivalent weights was discarded in pharmacy some years ago but, unfortunately, some physicians still prescribe injections and infusion solutions in terms of milliequivalents of ion per litre.

The indicators used in complexiметric titrations are usually themselves complexing agents, which form weak complexes with the metal ion when added initially. As the edetate solution is titrated, the weak complex is displaced by the stronger edetate complex to reveal the free colour of the indicator. The most commonly used indicator is known by the sinister name of *mordant black*. This indicator forms wine-red complexes with metal ions, but changes to a dark blue colour at the end point when the edetate has displaced all of the metal ions from the indicator complex.

Disodium edetate really is God's gift to undergraduates. It is a stable, water-soluble compound that gives sharp end points and, best of all, reacts with most metal ions in a 1 : 1 molar ratio irrespective of the valency of the ion. In this way, metal ions such as Zn^{2+}, Ca^{2+} and Al^{3+} can all be assayed in pharmaceutical samples.

Argentimetric titrations

As the name suggests, these assays all involve silver nitrate ($AgNO_3$). This salt is the only water-soluble salt of silver, so reaction of silver nitrate with any other salt will result in the production of a precipitate. Salts such as sodium chloride (NaCl) and potassium cyanide (KCN) can be assayed in this way.

$$AgNO_3 + NaCl \longrightarrow AgCl(ppt) + NaNO_3$$

$$AgNO_3 + KCN \longrightarrow AgCN(ppt) + KNO_3$$

The sample of salt is dissolved in water and titrated with standardised silver nitrate solution until all the silver salt has precipitated. Titrations of this type can be self-indicating, but usually an indicator is chosen that gives a coloured precipitate at the end point. In the assay of NaCl, potassium chromate is added to the solution; once all the NaCl has reacted, the first drop of $AgNO_3$ in excess results in the precipitation of red silver chromate, which changes the colour of the sample to brown-red.

Potassium cyanide and sodium cyanide are widely used industrial chemicals and notorious poisons, much favoured by writers of crime novels. Both compounds release hydrogen cyanide gas on exposure to strong acids and have LD_{50} values in rats of 10 mg kg^{-1}. The cyanide ion is rapidly absorbed into the body following ingestion or inhalation and binds to ferric iron (Fe^{3+}) in mitochondrial cytochrome oxidase (cytochrome aa_3), a component of the electron transport chain. Cyanide inhibits electron transport and oxidative phosphorylation and reduces the cellular redox potential, leading to anoxia and death. Treatment with hydroxocobalamin (a form of vitamin B_{12}) is effective if commenced quickly. The antidote works by exchanging a weakly bound hydroxy ligand with the cyanide ion to form the non-toxic cyanocobalamin.

Limit tests

Limit tests are quantitative or semi-quantitative tests used in the *British Pharmacopoeia* to identify and control small quantities of impurity that

may be present in drug samples. A sample of the drug is reacted to produce a colour (usually) and the intensity of the colour is compared with that obtained from a known amount of standard drug. The colour obtained from the standard sample represents the absolute upper limit (hence the name of the technique) of impurity permitted in the sample of drug.

A typical example of a limit test is the test for salicylic acid in a sample of Aspirin BP. Salicylic acid is formed by hydrolysis of aspirin (or may be an impurity from the synthesis). The test involves comparing the violet colour produced when the sample is reacted with ferric chloride with that obtained from a standard salicylic acid solution.

The procedure is as follows.

> Dissolve 0.1 g of the sample in 5 mL of ethanol (96%) and add 15 mL of iced water and 0.05 mL of a 0.5% w/v solution of iron(III) chloride hexahydrate. After 1 minute the colour of the solution is not more intense than that of a solution prepared at the same time by adding a mixture of 4 mL of ethanol (96%), 0.1 mL of 5 M acetic acid, 15 mL of water and 0.05 mL of a 0.5% w/v solution of iron(III) chloride hexahydrate to 1 mL of a 0.0050% w/v solution of salicylic acid in ethanol (96%).

The absolute limit for salicylic acid in Aspirin BP is 500 ppm, as can be shown below.

> 1 mL of 0.0050% w/v solution of salicylic acid ≡ 0.1 g aspirin
>
> 1 mL of 0.005 g/100 mL solution of salicylic acid ≡ 0.1 g aspirin
>
> 1 mL of 0.00005 g/mL solution of salicylic acid ≡ 0.1 g aspirin
>
> 0.00005 g salicylic acid ≡ 0.1 g aspirin
>
> 50 μg salicylic acid ≡ 0.1 g aspirin
>
> 500 μg salicylic acid ≡ 1.0 g aspirin = 500 ppm

Problems

Q6.1 Lithium carbonate (Li_2CO_3, M_r = 73.9) is a drug widely used in the treatment of depression. The BP assay for lithium carbonate involves the addition of an excess of hydrochloric acid to a sample of the drug and back titration of the unreacted hydrochloric acid with sodium hydroxide.

(a) Explain why back titrations are sometimes used in volumetric analysis.

(b) Write balanced chemical equations for the reactions expressed above, and hence calculate the weight of lithium carbonate equivalent to 1 mL of 1 M HCl (the equivalent relationship).

(c) This assay was carried out and the following results were obtained

Weight of bottle + sample = 11.7707 g

Weight of bottle + residual sample = 10.7142 g

Volume of 1 M (f = 0.9989) HCl added = 50.00 mL

Burette readings, titrant 1 M (f = 1.012) NaOH:

Initial volume = 0.50 mL

Final volume = 21.55 mL

(i) Calculate the percentage weight in weight of lithium carbonate in the sample.

(ii) What is the significance of an answer greater than 100%?

(iii) Suggest an indicator for this assay, and explain your reasoning.

Q6.2 Methyldopa (Figure 6.6) is a drug useful in the treatment of hypertension. The BP assay for methyldopa is as follows.

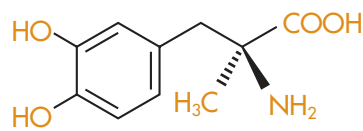


Figure 6.6 The structure of methyldopa, M_r = 211.2.

Weigh about 0.2 g of sample accurately and dissolve in a mixture of 15 mL of anhydrous formic acid, 30 mL of anhydrous acetic acid and 30 mL of 1,4-dioxan. Titrate with 0.1 M perchloric acid using crystal violet solution as indicator.

(a) State which technique of volumetric assay is used for methyldopa, and explain why titrations of this type are

sometimes required. What precautions should be observed for assays of this type.

(b) Describe, in detail, how the perchloric acid used in this assay may be standardised (no calculation required).

(c) The above assay was carried out and the following results were obtained. Derive the equivalent relationship for this assay and hence determine the purity of the sample of methyldopa.

Weight of sample taken = 0.2016 g

Volume of 0.1 M $HClO_4$ (f = 0.986) required = 9.64 mL

Q6.3 Vitamin C (ascorbic acid) is used in pharmaceutical formulation as an antioxidant and also has a medical use as a vitamin. Tablets of vitamin C may be assayed by titration with complex salts of cerium. The reactions occurring are as follows and are shown in Figure 6.7.

$$\text{vitamin C} + 2Ce^{4+} \longrightarrow \text{vitamin C (oxidised)} + 2Ce^{3+}$$

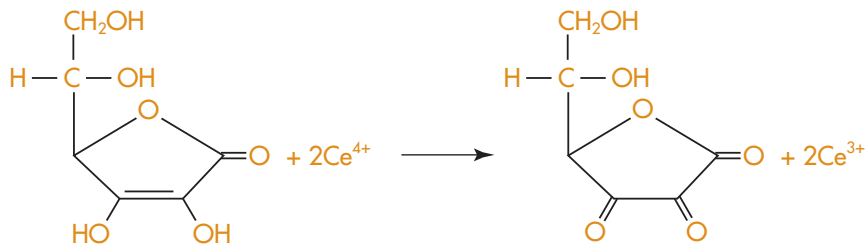


Figure 6.7 Reactions of ascorbic acid with cerium.

(a) What name is given to this type of titration?

(b) Ten 50 mg vitamin C tablets were weighed and powdered and an amount of powder equivalent to 0.15 g of ascorbic acid was dissolved as completely as possible in a mixture of 30 mL of water and 20 mL of 1 M sulfuric acid. This sample was then titrated with 0.1 M ammonium cerium sulfate (ACS) using ferroin sulfate solution as indicator. Given that the relative molecular mass of ascorbic acid is 176.12, derive the equivalent relationship for this assay and hence calculate the percentage of the stated amount of ascorbic acid in the tablets from the following data.

Weight of 10 tablets = 6.4319 g

Weight of sample = 2.0131 g

Volume of 0.1 M (f = 1.244) ACS required = 15.30 mL

(c) Ascorbic acid has pK_a values of 4.2 and 11.6. Assign the pK_a values to the structure of ascorbic acid, and explain why one acidic hydrogen is more than one million times more acidic than the other.

(Answers to problems can be found on pp. 261–262.)