CHAPTER 6

Nonaqueous Titrimetry

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The first reports of the successful quantitative titration of organic acids and bases in a nonaqueous solvent appeared in the scientific literature about 1910.[1] Little notice was taken of this phenomenon however, until the

fundamental investigations carried out by Conant and Hall,[2,3] in 1927. It was during these studies on "super acids," that they observed: "Much important chemistry has been obscured by our staunch devotion to water." These workers reported the successful quantitative titration of a number of organic amines in glacial acetic acid using strong inorganic acids as titrants.

A short time later, several other workers in the field reported similar findings, but it was not until after World War II that the true value of acid-base titrations in nonaqueous solvents was fully appreciated. During the past 15 years there have been several hundred papers published in many languages on the analytical applications of this technique.

The importance of understanding the fundamentals of acid-base reactions prompted a French worker by the name of de Morveau to make the statement: "Tenir la definition des acides, c'est tenir la clé de la Chymie." For this reason, the Bronsted–Lowry theory and the Lewis concept, both of which are so vital to an understanding of nonaqueous titrimetry, will be examined in this chapter.

6.1 THEORETICAL CONSIDERATIONS

A. DEFINITIONS OF ACIDS AND BASES

Although the electrolytic theory of Svante Arrhenius was initially rejected when it was proposed in 1887, it was later hailed as the key to the knowledge of the behavior of acids and bases. Although this concept still serves a useful purpose today, it is regretable to report that unreasonable obedience to it was largely responsible for the lack of major advancements in this field for more than 30 years after its advent, and that it had a serious detrimental effect upon the teaching of the fundamentals of chemistry for a much longer period than that.

Arrhenius defined an acid as a substance that liberates hydrogen ions and a base as a substance that supplies hydroxyl ions on dissociation. Neutralization is therefore defined as the interaction of an acid and a base to produce a salt and water.

1. Bronsted–Lowry Theory

As a result of the need for a broader concept, Bronsted in Copenhagen and Lowry in London independently proposed parallel theories in 1923. According to the Bronsted–Lowry theory as it became known, an acid is a substance, charged or uncharged, capable of donating a proton, and a base is a substance, charged or uncharged, capable of accepting a proton from an acid. An acid HA dissociates to give a proton H^+ and its conjugate base A^-. Alternatively, a base B will unite with a proton to produce its conjugate acid, HB^+. Every base has its conjugate acid, just as an acid has its conjugate base.

Symbolically, the balanced reactions may be represented in the following manner:

$$\underset{\text{acid}}{HA} \rightleftharpoons \underset{\text{proton}}{H^+} + \underset{\text{conjugate base}}{A^-} \tag{6.1}$$

$$\underset{\text{base}}{B} + \underset{\text{proton}}{H^+} \rightleftharpoons \underset{\text{conjugate acid}}{HB^+} \tag{6.2}$$

The following examples illustrate that, according to this theory, an acid may be an electrically neutral molecule (HCl), a positively charged cation ($C_5H_5NH^+$), or a negatively charged anion ($H_2PO_4^-$). A base may be an electrically neutral molecule (C_5H_5N) or an anion (Cl^-).

Some examples of acids and bases are

acids	*bases*	
HCl	$\rightleftharpoons H^+ + Cl^-$	(6.3)
$C_5H_5NH^+$	$\rightleftharpoons H^+ + C_5H_5N$	(6.4)
$H_2PO_4^-$	$\rightleftharpoons H^+ + HPO_4^{2-}$	(6.5)

An acid can only exhibit its acidic properties in the presence of a base; conversely, a base can only function as such in the presence of an acid. The relative strengths of acids and bases are measured by the tendencies of these substances to give up or take on protons. HCl is a strong acid in water because it gives up its proton readily, whereas acetic acid is a weak acid since it relinquishes its proton to a small extent only. The strength of an acid or base varies with the solvent or environment. HCl behaves as a weak acid in glacial acetic acid and acetic acid is a strong acid in liquid ammonia. Consequently, the strength of an acid depends not only on its own inherent ability to release a proton, but also on the ability of the solvent to take up the proton from the acid. This latter situation is referred to as the basic strength of the solvent.

2. Solvents

Nonaqueous solvents may be classified as protophilic, protogenic, amphiprotic, and aprotic. A *protophilic*, or basic, solvent is one that is capable of accepting protons from the solute with the resulting formation of a solvated proton and the conjugate base of the acid. Such solvents are typified by acetone, the ethers (including dioxane), and the amines (including liquid ammonia):

$$\underset{\text{acid}}{HA} + \underset{\text{basic solvent}}{S} \rightleftharpoons \underset{\text{solvated proton}}{SH^+} + \underset{\text{conjugate base of acid}}{A^-} \tag{6.6}$$

The ease with which the acid donates its proton to the solvent will depend upon the basicity of the solvent. Strongly basic solvents such as ethylenediamine or liquid ammonia will tend to level all acids so that their strengths become indistinguishable. However, weakly basic solvents such as acetone behave as differentiating solvents for acids. The ease with which they receive the proton depends upon the inherent acidity of the acid.

A *protogenic* solvent is a proton-donating compound which is represented

by acids such as formic, acetic, sulfuric, liquid HCl, and liquid HF. Such solvents exert a leveling effect on all bases dissolved in them. The basicity of weak bases would be enhanced to that of a strong base, and mixtures of weak and strong bases cannot be differentiated by titration in such solvent systems.

Amphiprotic solvents act as both proton donors and proton acceptors, and this class includes water, the alcohols, and glacial acetic acid. They are all capable of slight dissociation, and this property of glacial acetic acid, which is frequently used as a titration medium for weak bases, is illustrated by Eq. (6.7). In this instance, acetic acid is behaving as an acid. However,

$$CH_3COOH \rightleftharpoons H^+ + CH_3COO^- \tag{6.7}$$

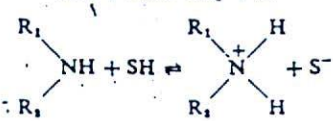
if a strong acid such as perchloric acid is dissolved in it, the acetic acid can function as a base and accept the protons donated by the perchloric acid to produce an "onium" ion:

$$HClO_4 + CH_3COOH \rightleftharpoons CH_3COOH_2^+ + ClO_4^- \tag{6.8}$$

This implies that acetic acid is a stronger base than the anion ClO_4^-. In such a solution then, the actual titrating species is the ion $CH_3COOH_2^+$ which readily donates its proton to a base.

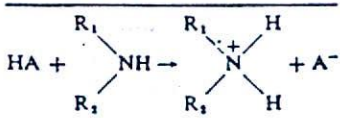
When a relatively weak base, such as ephedrine, is dissolved in glacial acetic acid, the solvent exerts a leveling effect and enhances the basicity of the ephedrine. It is this property of potentiating the weak basicity that permits the titration of weakly basic substances such as the alkaloids and antihistamines which are otherwise not titratable in water. The following general equations demonstrate the mechanism for the titration of a weak base in glacial acetic acid by acetous perchloric acid:

$$HA + SH \rightleftharpoons SH_2^+ + A^- \tag{6.9}$$



(6.10)

$$SH_2^+ + S^- \rightleftharpoons 2SH \tag{6.11}$$



(6.12)

Aprotic solvents such as the hydrocarbons, chloroform, and benzene are neutral in the sense that they neither accept nor donate protons. Since they are relatively chemically inert, they are useful for studying the reactions of acids and bases free of solvent effects. The absence of ionization in these solvents is illustrated by the behavior of picric acid in benzene. Such a solution is colorless, indicating that no dissociation has occurred, until ephedrine is added. The solution then becomes yellow, owing to the formation of the picrate ion. The presence of a base is required for picric acid to exhibit its acidic properties.

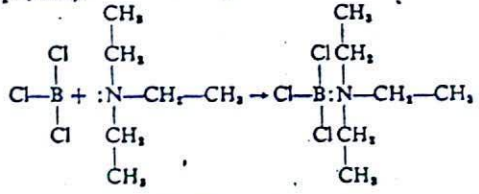
Dissociation is not a preliminary step required for neutralization. In fact, aprotic solvents are frequently added to the so-called ionizing solvents to supress solvolysis (a phenomenon comparable to hydrolyses in aqueous media) of the neutralization products and hence sharpen the end point. With this exception, aprotic solvents do not participate in any acid-base reaction; hence they serve only as carriers for the reaction.

The Lewis Concept

All the acids discussed thus far are classed as H-acids, and their behavior can be readily explained by the Bronsted-Lowry theory. Further examination showed that there are substances which can participate in an acid-base reaction and behave as acids yet possess no available proton. Lewis realized that a broader, more inclusive concept was required. His contribution to the understanding of acids and bases was the establishment of the electronic theory.

It was visualized that all acids have the one common property of being acceptor molecules, whereas bases are donor molecules. A base has one or more lone electron pairs which may be used in coordinate bond formation, and an acid is capable of accepting one or more electron pairs from a base. Neutralization, therefore, is the formation of a coordinate covalent bond between the acid and the base. This definition is applied to explain acid-base phenomena in either the presence or absence of a proton.

When triethylamine neutralizes boron trichloride in chlorobenzene, or even in the absence of any solvent, boron trichloride is behaving as an acid. It accepts an electron pair from the triethylamine to complete the boron atom octet. On the other hand, triethylamine is fulfilling the definition of a base according to Eq. (6.13).



(6.13)

In addition to boron trichloride, other substances such as aluminum chloride and stannic chloride are capable of behaving as acids even though they possess no proton. They are referred to as Lewis acids or L-acids. It is worthy of note that Lewis bases are identical with those of the Bronsted–Lowry theory.

6.2 TITRATION OF WEAK BASES

A. TITRANTS

Solutions of perchloric acid in either glacial acetic acid or dioxane are used almost exclusively for the titration of bases in nonaqueous titrimetry.

Normalities of 0.1 to 0.05 are most commonly employed; under certain conditions, however, it is possible to use titrants as dilute as 0.001 *N*.

In glacial acetic acid, the titrating species becomes the "onium" species, $CH_3COOH_2^+$, as typified by Eq. (6.8) and is prepared as follows. For a 0.1 *N* solution, dissolve approximately 8.4 ml of 70 to 72% perchloric acid in about 200 ml of glacial acetic acid and mix well. Add slowly, with adequate mixing, sufficient glacial acetic acid to prepare 1 liter of solution.

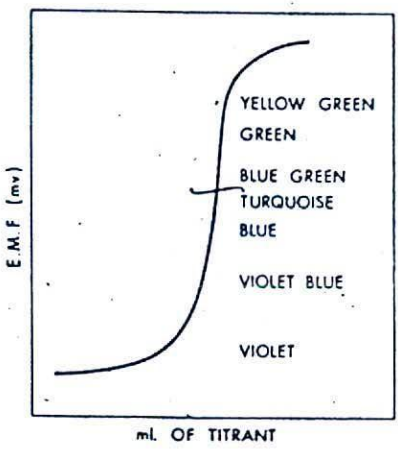


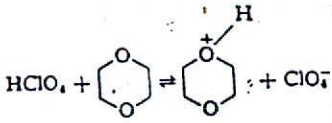
FIGURE 6.1: Standardization of acetous perchloric acid

The titrant is usually standardized against an accurately weighed quantity of primary standard potassium acid phthalate which is sufficient to result in a 20 to 30 ml titration. The standard is dissolved in 50 ml of glacial acetic acid and the titration followed either potentiometrically or with 1 drop of 0.5% crystal violet indicator in glacial acetic acid. Visually, the end point is reached when the indicator exhibits a turquoise color. This is usually a 1-drop change and occurs when all trace of the violet color has disappeared and just before the green becomes clearly evident. A blank is performed on the solvent system. Figure 6.1 traces the color changes of the indicator on a potentiometric curve.

Many chemists recommend the addition of a small quantity of acetic anhydride to neutralize the trace amount of water present in the titrant. In our opinion, this is a dangerous and unnecessary step. If 70 to 72% perchloric acid is used, the prepared titrant contains such a minute amount of water as to have no effect upon the end point; therefore it is unnecessary to remove it. On the other hand, by adding acetic anhydride, one runs the risk of having an excess of it remain in the titrant. The result of this is the likely

acetylation of any primary or secondary amines present in the titration vessel, and thus a low recovery would be obtained.

Solutions of perchloric acid in dioxane may be standardized in the same manner as those of acetous perchloric acid. It is reasonable to suppose that the titrating species for this titrant is either mono- or diprotonated dioxane



(6.14)

It is worthy of note that one must employ a high quality dioxane otherwise the titrant will darken considerably with a loss of titer. A slight yellowish tinge which usually develops in solutions, even when high quality dioxane is used, has no effect upon normality. Dioxane may be purified by passing it through a carboxylic acid ion exchange resin or shaking it with asbestos and then filtering.

B. APPARATUS

1. Burettes

Those burettes having a teflon stopcock are most suitable, since the necessity of lubricating the stopcock is eliminated. The low surface tension of the organic solvents which serve as carriers for the titrant results in the formation of smaller drops on the burette tip. This permits a higher degree of accuracy and precision than is possible with aqueous titrants. It is common, therefore, to employ burettes having a volume as low as 1 ml and frequently not higher than 10 ml.

2. Titration Vessels

Erlenmeyer flasks or beakers may be used with equal versatility, since it is not necessary to protect the titration from the atmosphere.

C. SOLVENTS

Although it is not reasonable to attempt to list all the solvents and their combinations that have been employed, it is of value to mention some of the more common ones.

Glacial acetic acid alone or combined with an aprotic solvent has been utilized more than any other solvent combination. Other solvents worthy of mention are chloroform, acetonitrile, acetone, benzene, chlorobenzene, acetic anhydride, and various combinations of these. In addition, one group of workers has extensively used glycol-hydrocarbon mixtures.[4]

D. INDICATORS

A number of dyes have been recommended for use by various investigators, and Stock and Purdy[5] published a valuable summary in 1959. However, the

most common indicators are crystal violet, 0.5% w/v in glacial acetic acid; methyl red, 0.1% w/v in anhydrous methanol; and oracet blue B, 0.5% w/v in glacial acetic acid. Regardless of the indicator employed, each time it is used in the titration of a new basic substance, a comparative test must be made with a potentiometer to determine whether the indicator color change coincides with the potentiometric end point.

E. ELECTRODES

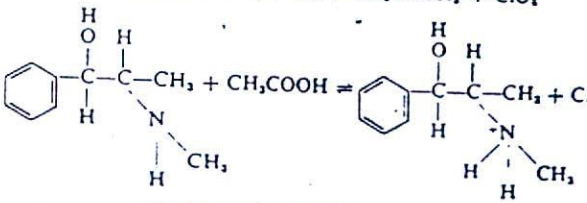
The usual combination of reference calomel and glass indicator electrodes has found the widest applicability. Some investigators have advocated the replacement of the aqueous saturated potassium chloride solution in the calomel electrode with a saturated solution of potassium or lithium chloride in glacial acetic acid or anhydrous methanol. Although this may have some utility in the titration of very weak bases, in general it is unnecessary and does little to enhance the potentiometric end point.

Another combination which has found some utility is that of a glass electrode with one of silver-silver chloride. However, the number and combinations of electrodes is beyond the scope of this chapter. For further information, the reader is referred to a useful summary by Stock and Purdy.[6]

F. PRACTICAL EXAMPLES

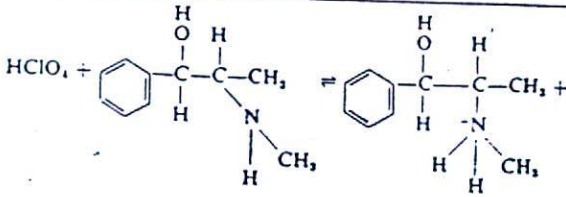
1. Titration of ephedrine alkaloid in glacial acetic acid by acetous perchloric acid is illustrated by Eq. (6.15) to (6.18). Equation (6.15) represents the preparation of the titrant, whereas (Eq. 6.16) depicts the protonation of the alkaloid when dissolved in glacial acetic acid. The next equation shows the addition of the titrating species, and the final one is a summation of the entire reaction.

$$HClO_4 + CH_3COOH \rightleftharpoons CH_3COOH_2^+ + ClO_4^- \qquad (6.15)$$



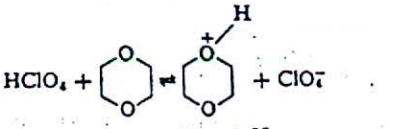
(6.16)

$$CH_3COOH_2^+ + CH_3COO^- \rightleftharpoons 2CH_3COOH \qquad (6.17)$$

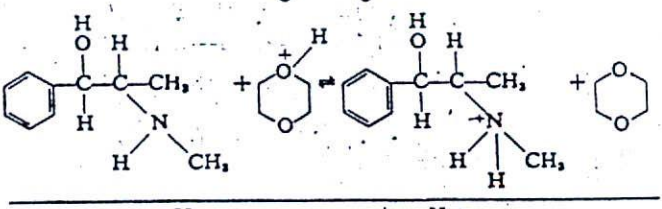


(6.18)

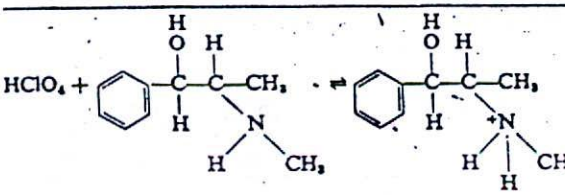
2. It would be instructional to follow the equations of the reactions involving the titration of ephedrine alkaloid in the aprotic solvent, chloroform. Although acetous perchloric acid will serve equally well, perchloric acid in dioxane was chosen for this illustration:



(6.19)



(6.20)



(6.21)

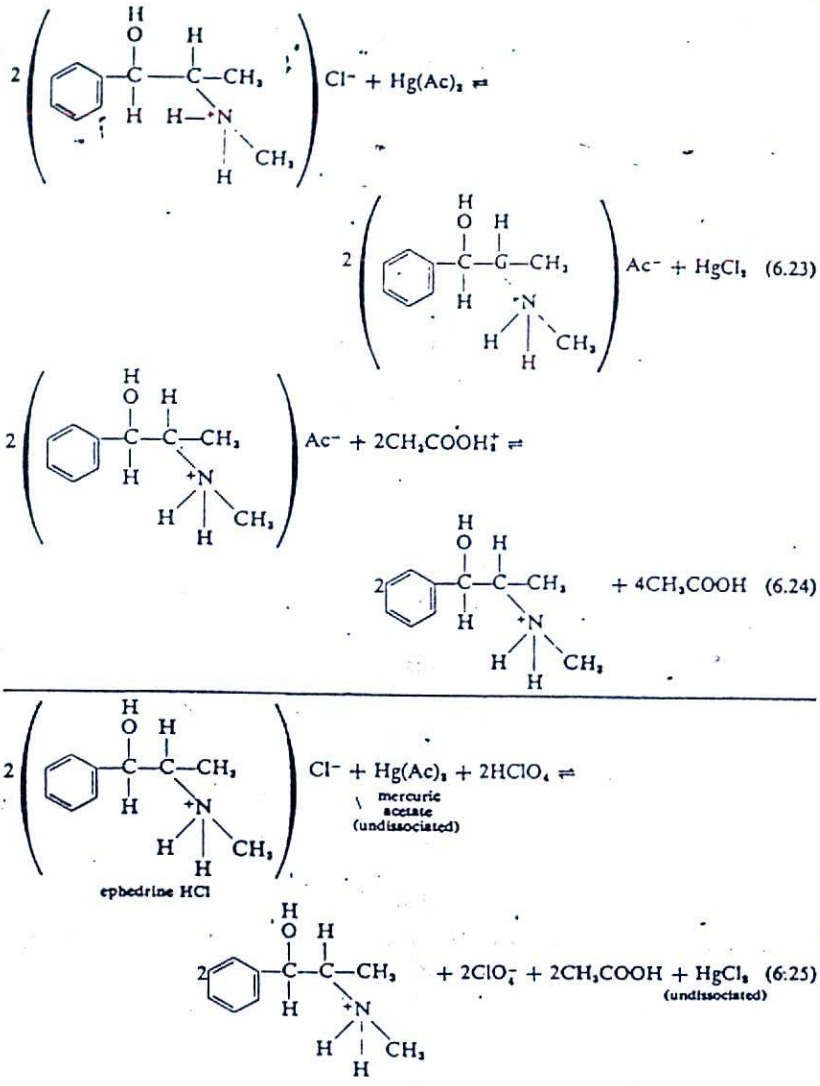
From the foregoing equations, it is apparent that aprotic solvents such as chloroform, carbon tetrachloride, and benzene do not participate in the reaction. Indeed, these solvents are equally useful media for the titration of organic acids.

The argument over whether dioxane is monoprotonated or diprotonated by the perchloric acid is purely academic. In no way does this alter the 1:1 relationship between the perchloric acid and the alkaloid, ephedrine.

3. Halogen salts cannot be titrated directly by acetous perchloric acid because there is not sufficient difference in the inherent proton attracting capabilities of the halide and perchlorate anions in glacial acetic acid. Therefore, the reaction will not proceed to completion. The most satisfactory means of overcoming this difficulty was provided by Pifer and Wollish.[7] It depends upon the fact that mercuric salts of the halogen acids or of acetic acid are all undissociated in glacial acetic acid and therefore are untitratable in that solvent medium. Consequently, addition of mercuric acetate to an amine halide in acetic acid removes the halide and replaces it with an equivalent amount of acetate ion which is readily protonated. Equations (6.22) to (6.25) illustrate the reactions involved in the titration of an amine halide in glacial acetic acid by acetous perchloric acid. Ephedrine hydrochloride serves as the

$$2HClO_4 + 2CH_3COOH \rightleftharpoons 2CH_3COOH_2^+ + 2ClO_4^- \qquad (6.22)$$

prototype. Similar treatment is required for quarternary ammonium halides prior to titration with perchloric acid.



6.3 TITRATION OF WEAK ACIDS

A. TITRANTS

In contrast to the fact that only two titrants are generally employed for bases, there are several available for the titration of acids.

1. Methoxides of the Alkali Metals

These are the most commonly used and are prepared by dissolving the appropriate amount of alkali metal in a mixture of benzene and methanol.

Preparation of a 0.1 *N* Solution. To a mixture of 40 ml of methanol and 50 ml of dry benzene in an Erlenmeyer flask with a loose cover, add about 4 g of potassium, or 2.3 g of sodium, or 0.6 g of lithium. The metals must be freshly cut and added slowly. When the metal has dissolved, add sufficient methanol to produce a clear solution, then add dry benzene slowly with continuous shaking until the solution again appears cloudy. Repeat the addition of methanol followed by benzene until 1 liter of solution has been produced, using only the minimum amount of methanol to ensure a clear solution. Store solution in sodium-free glass, and protect it from atmospheric carbon dioxide.

All these titrants are usually standardized against primary reference standard benzoic acid according to the following procedure. A sufficient quantity of benzoic acid to give a titration of 20 to 30 ml is accurately weighed in an Erlenmeyer flask and dissolved in 25 ml of dimethylformamide. Four drops of 0.5% thymol blue indicator in anhydrous methanol is added, and the titration carried on until the indicator reaches a full blue color. A blank is performed on the solvent system to account for the acidic impurities in the dimethylformamide.

2. Tetrabutyl Ammonium Hydroxide

This titrant has been prepared in both methanol-benzene and isopropanol. The following procedure will utilize the former solvent combination. Dissolve 40 g of tetrabutylammonium iodide in 90 ml of absolute methanol and add 20 g of finely ground, purified silver oxide. Shake the stoppered flask vigorously for 1 hr. Centrifuge a few milliliters of the mixture and test the supernatant for iodide. If the test is positive, add a further 2 g of silver oxide and continue shaking for an additional 30 min. Once the mixture is free of iodide, filter it through a sintered-glass funnel of medium porosity. Rinse the reaction vessel, funnel with three 50-ml portions of dry benzene, and add the washings to the filtrate. Dilute the mixture to 1 liter with dry benzene and flush the solution for 5 min with carbon dioxide-free nitrogen. Store in a reservoir which protects the titrant from moisture and carbon dioxide. Standardization is performed in the same manner as for the alkali methoxides.

3. Potassium Hydroxide in Methanol

This is a conveniently prepared titrant made by dissolving the appropriate amount of potassium hydroxide in anhydrous methanol to produce a 0.1 *N* solution. Standardization is performed against reference standard benzoic acid, as previously outlined.

This titrant is not so powerful as the two types mentioned before but is still satisfactory for the titration of many substances. Its chief disadvantage is that reaction with each acidic functional group produces a mole of water which would reduce the sensitivity in a large titration.

$$R{-}\overset{\overset{O}{\|}}{C}{-}OH + OH^- \rightleftharpoons R{-}\overset{\overset{O}{\|}}{C}{-}O^- + H_2O \qquad (6.26)$$

B. APPARATUS

Burettes

Since the titrant must be protected from the atmosphere if one is to obtain the highest degree of precision, it is preferable to store it in a burette with a reservoir sufficiently large to contain 1 liter. The reservoir is flushed out with

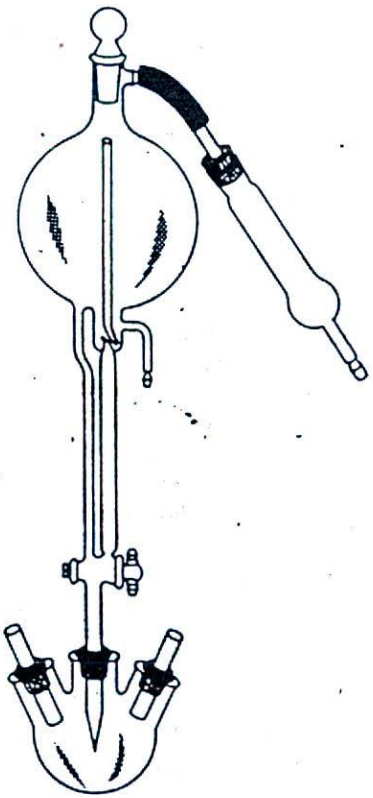


FIGURE 6.2: Apparatus for the titration of weak acids

nitrogen, and a layer of nitrogen is laid over the titrant. A suitable apparatus is illustrated in Fig. 6.2. Teflon stopcocks are highly desirable.

Titration Vessels

A three-necked flask would be ideal, because it provides an inlet and outlet for the nitrogen atmosphere as well as an opening to admit the burette tip. An Erlenmeyer flask equipped with a rubber stopper which has been drilled to permit passage of the burette tip is satisfactory. A groove must be notched in the stopper to provide an air vent.

In our laboratory we have used a seal of aluminum foil which has a small hole for the burette tip, with equal success. There are a number of devices, including a thin rubber membrane which provide adequate protection from the atmosphere.

In all instances, an electromagnetic stirring apparatus is essential.

C. SOLVENTS

The solvents most commonly employed in the titration of weak acids are dimethylformamide, *n*-butylamine, pyridine, ethylenediamine, acetone, chloroform, and morpholine.

D. INDICATORS

Thymol blue 0.5% w/v in anhydrous methanol and 0.2% w/v of azo violet in benzene are the most commonly used indicators. Others of lesser importance are *p*-hydroxyazobenzene, 0.2% w/v in benzene; *o*-nitroaniline, 0.15% w/v in benzene; and quinaldine red, 0.1% w/v in ethanol.

E. ELECTRODES

In weakly basic solvents such as dimethylformamide and pyridine, the conventional glass calomel electrode combination has found wide application. Some workers[8] have recommended that the aqueous saturated potassium chloride solution be replaced by a methanolic solution.

For strongly basic solutions, an antimony-antimony electrode combination has been utilized with considerable success.[9,10]

F. PRACTICAL EXAMPLES

The titration of benzoic acid in *n*-butylamine by sodium methoxide is illustrated by Eqs. (6.27) to (6.29).

$$C_6H_5COOH + CH_3(CH_2)_3NH_2 \rightleftharpoons CH_3(CH_2)_3\overset{+}{N}H_3 + C_6H_5COO^- \quad (6.27)$$

$$CH_3(CH_2)_3\overset{+}{N}H_3 + CH_3O^- \rightleftharpoons CH_3OH + CH_3(CH_2)_3NH_2 \quad (6.28)$$

$$C_6H_5COOH + CH_3O^- \rightleftharpoons CH_3OH + C_6H_5COO^- \quad (6.29)$$

In aprotic solvents such as chloroform, it is obvious that the solvent plays no part in the reaction. The titration is performed by the direct withdrawal of the proton from benzoic acid by the methoxide.

Acetone, a weakly protophilic solvent in the presence of a strong acid, undoubtedly behaves as an aprotic solvent in the presence of weak organic acids. In fact, it has been employed successfully as a differentiating solvent by Fritz and Yamamura.[11]

6.4 SCOPE OF NONAQUEOUS TITRATIONS

Titrations in nonaqueous solvents may be performed as readily as those in aqueous media. The technique may be applied to any substance capable of behaving as an acid or a base, provided a suitable solvent can be found. Many compounds, which are too weakly basic or acidic to give detectable end points in aqueous media, can be titrated readily in a solvent which can either enhance their weakly basic or acid properties or, at least, does not compete with the substance for the titrant. End points may also be enhanced by the addition of an aprotic solvent which depresses the solvolysis of the reaction product.[12] In addition, individual substances of multicomponent mixtures of acids[11,13–18] and bases[19,20] can be determined by performing a differentiating titration in a nonleveling solvent.

The range of substances which can now be determined by volumetric analysis has been markedly extended by the application of nonaqueous techniques. There are many materials which are readily soluble in some suitable nonaqueous medium but are insoluble in water.

As previously mentioned, the use of indicators finds a widespread and ready application in nonaqueous titrimetry. However, where the color change of an indicator would be obscured by the presence of highly colored solutions, it is necessary to resort to potentiometry. Similarly, where the substance remains too weakly basic or weakly acidic despite the use of a favorable leveling solvent, it is necessary to perform a potentiometric titration.[21,22]

Nonaqueous titrimetry has found wide application in the realm of quantitative chemistry, and it is a most useful tool in the quality control of pharmaceuticals. Included in the many basic substances that lend themselves to titration by either acetous perchloric acid or perchloric acid in dioxane are ephedrine nasal sprays,[23] codeine phosphate in A:P.C. and C. tablets,[24] phenothiazine-type tranquillizers,[25–27] tetracycline antibiotics,[28] antihistamines,[27,29] and piperazine preparations.[30,31] In addition to these, the *British Pharmacopoeia*[32] makes considerable use of nonaqueous techniques to assay a variety of pharmaceutical preparations. This compendium as well as the *U.S.P.*[31] employs nonaqueous titrimetry in the monographs of many standard drugs, as a means of purity determination.

Many weak organic acids of medicinal interest have been analyzed by nonaqueous titrimetry. The technique has also found application to the pharmaceutical dosage forms. Vespe and Fritz[33] have utilized dimethylformamide as the solvent system for the titration of barbiturates and sulfonamides. Leavitt and Autian[34] employed combinations of benzene-isopropanol or benzene-chloroform as the solvent system for barbituric acids; Chatten[35] used chloroform-methanol (50:1) for the same group of drugs. Other organic medicinal agents which can be readily determined by nonaqueous techniques are dehydrocholic acid[36] and *p*-aminosalicylic acid.[37] Recoveries from the pharmaceutical dosage forms are highly satisfactory.

Harlow and co-workers have investigated the potentiometric titration of weak acids in inert solvents[38] as well as the resolution of acid mixtures[17] with quaternary ammonium titrants.

Blake and Siegel[39] have combined ion exchange and nonaqueous titrimetry in the assay of phenobarbital elixir. Such a technique further exemplifies the versatility of nonaqueous titrimetric procedures.

Chatten and Mainville[40] have investigated the influence of some 30 tablet excipients on the titration of acids and bases. They found that the judicious selection of solvents could virtually eliminate the adverse influence of excipients and permit quantitative assay of the active component.

6.5 ANALYTICAL EXPERIMENTS

A. AQUEOUS EPHEDRINE HYDROCHLORIDE SPRAY*

Transfer to a separatory funnel 10 ml of the preparation, accurately measured, completing the transfer with two 10-milliliter portions of water. Add 1 ml of 20% sodium hydroxide solution or a sufficient amount to render the system basic and completely extract the alkaloid with four 20-ml portions of chloroform. Wash the combined chloroform extract with 10 ml of water and re-extract the aqueous washings with a further 10 ml of chloroform which is added to the original extracts. After making certain that no water droplets have remained in the chloroform extracts, add 1 drop of 0.5% crystal violet indicator in glacial acetic acid, and titrate to a blue end point with either 0.1 *N* acetous perchloric acid or perchloric acid in dioxane. Each milliliter of 0.1 *N* perchloric acid is equivalent to 20.17 mg of ephedrine hydrochloride.

The above procedure is readily applicable to all aqueous ephedrine preparations regardless of the salt. It must be remembered, however, that when 1 mole of the sulfate is basified, it will release 2 moles of the alkaloid. Hence it will require twice the expected amount of titrant.

In addition, other nasal preparations such as phenylephrine hydrochloride or tuamine sulfate can be assayed with equal ease by this method.

* Reprinted with permission from *Drug Std.*, 22, 1 (1954).

B. CODEINE PHOSPHATE IN A.P.C. AND C. TABLETS*

Weigh and powder 20 tablets. To an accurately weighed portion representing approximately 32 mg of codeine phosphate add 5 g of phenol and 10 ml of chloroform, stir to dissolve, and filter through a pledget of cotton, using 5 ml of a solution of 5 g of phenol in 10 ml of chloroform for quantitative operation. Add 50 ml of acetonitrile to the filtrate and 2 drops of methyl red indicator (0.25% w/v in 2 g of phenol and 100 ml of chloroform). Titrate to a full deep red color with 0.05 *N* perchloric acid in dioxane. Each milliliter of titrant is equivalent to 20.32 mg of codeine phosphate hemihydrate.

The above procedure is equally applicable to many alkaloidal sulfates, phosphates, acetates, tartrates, and nitrates.[41] In addition it has been utilized in the assay of *d*-amphetamine sulfate tablets.[42]

C. TETRACYCLINE ANTIBIOTICS†

Weigh and powder 20 tablets or weigh the contents of 20 capsules. Place an accurately weighed sample equivalent to 50 mg of the drug in a 150-ml beaker. Add 25 ml of nitromethane containing 1 ml of formic acid and stir electromagnetically for 30 min. Filter through a Whatman No. 1 filter paper into a 150-ml beaker. Wash the residue with an additional 25 ml of nitromethane. Add 5 ml of benzene, 1 ml of 6% mercuric acetate in glacial acetic acid, and 0.1 ml of mixed indicator (0.1% w/v methylene blue and 0.2% w/v quinaldine red in anhydrous methanol). Titrate to a green end point with 0.05 *N* perchloric acid in dioxane. Where the formulation includes magnesium stearate, titrate to a blue end point before the addition of mercuric acetate, then add mercuric acetate and continue titration to a green end point. Each milliliter of 0.05 *N* perchloric acid is equivalent to 24.05 mg of tetracycline hydrochloride; 25.77 mg of chlortetracycline hydrochloride; 24.85 mg of oxytetracycline hydrochloride.

Note: The procedure is readily applicable to the pure antibiotics and has been modified for ointments, injections, and suppositories.[28]

D. PHENOBARBITAL ELIXIR‡,[39]

Transfer by pipette exactly 25 ml of phenobarbital elixir to a 150-ml beaker. Add about 25 ml of distilled water and pour the mixture into an anionic exchange resin Dowex 2 × 8, 50–100 mesh. The resin was previously prepared by washing successively with 100 ml of distilled water, 100 ml of 5% sodium hydroxide, and finally 200 ml of distilled water. The size of the column is 1 by 30 cm.

* Reprinted with permission from *J. Am. Pharm. Assoc. Sci. Ed.*, 43, 746 (1954).
† Reprinted with permission from *J. Am. Pharm. Assoc. Sci. Ed.*, 47, 548 (1958).
‡ Reprinted with permission from *J. Pharm. Sci.*, 51, 944 (1962).

As the last of the elixir is disappearing into the column, distilled water is added and the column is washed with 100 to 150 ml of water. The phenobarbital is eluted from the column with 50% acetic acid in 95% ethanol at the flow rate of 1 ml per min. The first 10 ml of eluate contains no phenobarbital and is discarded. The next 50 ml of eluate is collected and evaporated to dryness on a steam bath. Five millilters of distilled water is added, and the solution is again evaporated to dryness. The process is repeated once.

The residue is dissolved in 20 ml of dimethylformamide and titrated with 0.1 *N* sodium methoxide in benzene-methanol. Each milliliter of 0.1 *N* sodium methoxide is equivalent to 25.42 mg of sodium phenobarbital.

E. CHLOROTHIAZIDE DIURETICS*,[43]

Weigh and powder 20 tablets and place an accurately weighed sample equivalent to 250 mg of the diuretic in a flask. Dissolve the material in 80 ml of dimethylformamide by stirring electromagnetically and observing precautions against absorption of atmospheric carbon dioxide. Add 3 drops of *p*-nitrobenzene-azoresorcinol indicator (saturated benzene solution) and titrate with 0.1 *N* sodium methoxide to a blue color. Perform a blank determination on the solvent system.

Each milliliter of 0.1 *N* sodium methoxide is equivalent to 14.79 mg of chlorothiazide; 14.89 mg of dihydrochlorothiazide; 16.47 mg of flumethiazide; 16.57 mg of dihydroflumethiazide; and 21.07 mg of bendroflumethiazide.

QUESTIONS

Q6.1. Write all the equations involved in the titration of a weak base in benzene by perchloric acid in dioxane. What part does the solvent play? Explain.

Q6.2. Why is mercuric acetate necessary when halogen salts are titrated in nonaqueous media? Write all the equations involved when acetous perchloric acid is the titrant.

Q6.3. State why you would not recommend the titration of the weak base codeine phosphate in aqueous media. Support your reasons with equations.

Q6.4. State why you would not recommend the titration of a weak acid such as phenobarbital in aqueous media. Support your reasons with equations.

Q6.5. Sulfanilamide can be titrated in *n*-butylamine with sodium methoxide. Write the equations for all the reactions involved.

Q6.6. Benzoic acid is readily titratable in dimethylformamide. Write the equations for all the reactions involved.

Q6.7. What are the advantages of the Bronsted–Lowry theory over that of Arrhenius? Illustrate with equations.

Q6.8. Compare the Lewis concept of acids and bases with that of Bronsted and Lowry.

Q6.9. Assume that solubility is not a factor, and state what advantage an aprotic solvent possesses over an amphiprotic one.

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Q6.10. List several solvents that belong to each of the following classes: protophilic, protogenic, amphiprotic, and aprotic.

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