CHAPTER 9

Miscellaneous Methods

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In this chapter are discussed a series of unrelated techniques. In general, each technique is specific in purpose and application. They represent important and in many cases classical solutions to definite problems in pharmaceutical analysis.

9.1 AQUAMETRY

A. SCOPE

Aquametry can be defined as the quantitative determination of water. The importance of such determinations becomes evident when it is realized that many samples contain water as a solvent, as absorbed water, or as water of crystallization. Therefore, most determinations must be on a dried sample basis, or any water present must be quantitatively determined and used as a correction. When dealing with expensive materials and even with large quantities of less costly materials, water can be considered an adulterant. Quantitative specifications for the amount of water present must be established to ensure true value. Physical properties of a material are modified by its water content, and many processes such as the pharmaceutical procedures of granulation, tablet formation, and coating operations are also affected by water content.

Many chemical reactions involve water as either a reactant or product in stoichiometric relationships. Such processes can be followed or a reactant can be assayed by the quantitative determination of the water involved. Reactions (9.1) through (9.7), of pharmaceutical significance, illustrate where the quantitative determination of water has been used for such purposes.[1]

Esterification reactions

$$ROH + CH_3COOH \xrightarrow{BF_3} CH_3COOR + H_2O \quad (9.1)$$

$$ROH + Cl_3CHCOOH \longrightarrow Cl_3CHCOOR + H_2O \quad (9.2)$$

$$RCOOH + CH_3OH \xrightarrow{BF_3} RCOOCH_3 + H_2O \quad (9.3)$$

Acid anhydride analysis

$$(RCO)_2O + H_2O \xrightarrow[\text{pyridine}]{NaI} 2RCOOH \quad (9.4)$$

Carbonyl analysis with hydroxylamine hydrochloride

$$RCOR' + H_2NOH{\cdot}HCl \rightarrow RR'C{=}NOH + HCl + H_2O \quad (9.5)$$

Schiff reaction for amines

$$RNH_2 + C_6H_5CHO \rightarrow C_6H_5CH{=}NR + H_2O \quad (9.6)$$

The hydrolysis of a nitrile

$$RCN + H_2O \rightarrow RCONH_2 \quad (9.7)$$

B. PHYSICAL METHODS FOR WATER DETERMINATION

1. Thermal Methods

The technique of loss in weight upon drying is obviously a method for water determination. It is, however, just as obvious that such methods may

also involve losses resulting from other volatile materials or from decomposition. Therefore, both the *British Pharmacopoeia* and *United States Pharmacopeia* recognize such measurements under the general term "loss on drying."

These measurements can be made more specific for water by limiting decomposition effects at lower temperature, that is, drying accomplished at reduced pressures. Also, interference by other volatile materials can often be controlled by a process of measuring the increase in weight of an absorbent

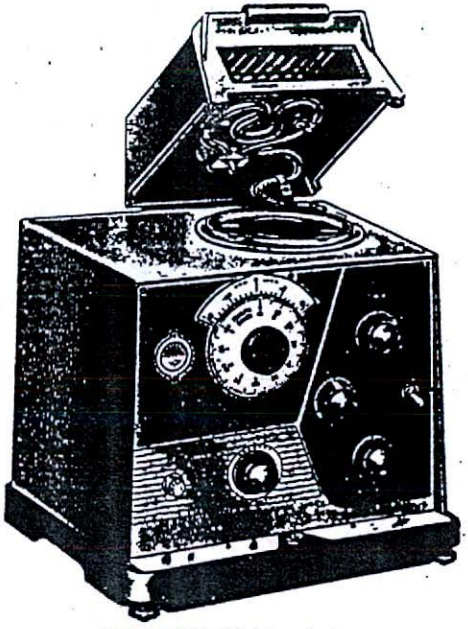


FIGURE 9.1: Moisture balance.

selective for water. Absorption agents which have been classically used for this purpose include anhydrous magnesium perchlorate (Dehydrite), barium oxide, calcium sulfate (Drierite), phosphorus pentoxide, and calcium chloride. An inert gas is allowed to carry the water lost from a known quantity of sample to the absorbent whose gain in weight is then determined. The importance of such measurements should not be underestimated. For example, the use of such absorbent trains for the determination of water is basic to the highly precise microdetermination of hydrogen in combustion analysis of organic compounds.

For the most part "loss-in-weight" measurements are made with the aid of the usual laboratory drying ovens and analytical balances. However, for extensive series of determinations the more specialized moisture balances (Fig. 9.1) are commercially available. The inclusion of a heated drying

chamber and the ability to read directly the percentage loss in weight are features of these specialized balances.

The technique of thermogravimetric analysis (TGA) offers an even more sophisticated approach to the study of loss-in-weight measurements.[1] TGA involves the continuous weighing of a sample as it is heated at a constantly increasing rate. The resultant curve of the change in weight versus temperature may have analytical chemical applications which includes the determination of moisture.

Differential thermal analysis (DTA) is another thermal technique that has among its applications[2] the determination of moisture. In DTA, the temperature of a sample is compared with the temperature of a reference material and this difference in temperature is recorded as a function of the change in temperature.

2. Azeotropic Distillation

Since 1900, moisture determination based upon distillation procedures have found extensive use. The development of these methods has been reviewed[3] and particularly noteworthy is the advent of the Dean and Stark trap[4] (Fig. 9.2), which makes the determination continuous and greatly increases its efficiency.

The usual procedure is to add a water-immiscible solvent to the material containing moisture and in this manner to co-distill any water present. Since the vapor pressure of a boiling liquid is equal to the sum of the partial pressures of each component in a mixture, there is a significant contribution due to any mositure present. Recondensation of the vapors results in separation of water from the immiscible solvent, making it available for volumetric measurement. The hydrocarbons benzene, toluene, and xylene are the solvents usually used in this determination. These water-immiscible solvents

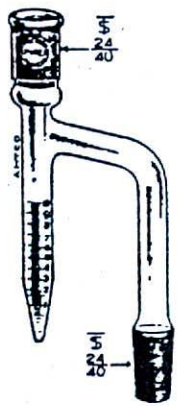


FIGURE 9.2: Dean and Stark moisture trap.

will usually be miscible with most other volatile components present and correct for this potential error. These solvents with a specific gravity less than one have the added advantage of allowing the water to form a layer at the bottom of the Dean and Stark trap, where it can be measured directly. The excess solvent above this layer will automatically return to the boiling flask.

Azeotropic distillation determinations of moisture have extensive applications because of their simplicity, economy, efficiency, and accuracy. The method is especially successful for moisture determinations in bulk materials, such as plant parts, and for aqueous solutions, such as medicinal soap solutions. The main disadvantage to the procedure is that relatively large samples are required, making the technique unsuitable for trace amounts of water in expensive pharmaceutical materials.

3. Other Physical Methods

a. Spectrophotometric. Various physical constants have been used for water determinations by comparison to values for mixtures of known composition. For example, the *United States Pharmacopeia* has an alcoholometric table consisting of a series of alcohol-water mixtures with their corresponding specific gravities. Although primarily designed to determine alcohol concentrations, the tables, of course, can also be used for water determinations provided that the unknown consists only of a mixture of alcohol and water. Refractive index measurements have been used in a like manner with the same limitation.

Spectrophotometric methods are available for aquametry and are of particular interest when only small amounts of material are available for analysis. It has been demonstrated that water absorbs at numerous places throughout the electromagnetic spectrum, and practical applications have been made of this fact for the quantitative determination of water.

Water absorbs in the 2.7-μ region of the infrared, owing to OH stretching vibrations, whereas there is also less intense absorption near 6.2 μ, because of bending vibrations of the molecule.[5] The exact wavelength depends upon the degree of association of the water molecules which in turn is dependent upon both the concentration of water and the system in which it is being measured. Actual analysis is usually based upon the intensity of 2.7-μ bands of unknowns as compared to known concentrations. It is also important to determine whether the system under investigation is free of other absorption which may interfere with the water bands. That is, these bands are not specific for water so that OH, NH, and in some cases CH stretching vibrations may interfere with the determination.

The near-infrared region of the spectra also shows a series of absorption bands for water and, even though they are less intense than the 2.7-band, they have been advanced for the determination of water.[6] The 1.9-band in the

near infrared is of particular interest. It is not only the most intense of the near-infrared water bands, but also is fairly specific for water. For example, the water content of a series of glycerol samples has been determined[7] without interference by the hydroxyl groups of glycerol.

Although water is transparent in the normal ultraviolet range and, indeed, is an excellent solvent in this region, it does exhibit absorption in the far ultraviolet below 180 mμ. Water absorption in this region has been used for the determination of trace quantities in various gases.

Nuclear magnetic resonance (NMR) spectroscopy has also been applied to the quantitative determination of water. The free water in highly complexed organic systems such as solid foods has been successfully determined.[8] Although relatively large samples in the order of 1 to 15 g of sample were required, the technique is rapid and nondestructive.

b. Gas-Chromatographic Methods. Recently, gas-chromatographic procedures have been introduced for water determinations. For example, a column consisting of 10% carbowax on Fluoropak 80 has proved successful for the analysis of water extracted by methanol from a variety of food products.[9] Gas-chromatograph procedures should become increasingly important for aquametry because of their potential for specificity, efficiency, and ease of adaptation to routine and automatic analysis.

c. Electrochemical Methods. The principle electromethods for water determination are (1) conductivity or resistance measurements, (2) dielectric methods, and (3) coulometric methods. Whereas general instruments can be used to make these measurements, there are also available a series of more compact "water analyzers" based upon these electromethods and designed specifically for routine water determinations.

Conductivity measurements appear to be the most generally applicable of the electromethods. The flow of current at a constant voltage between two electrodes in a system to be evaluated will increase with an increase in electrolyte concentration. In systems where only a limited amount of water is present, it is often possible to make conductivity readings directly on the system. An increase in conductivity with an increase in water content is then dependent upon an increase in extraction of the electrolytes naturally present. In general, however, indirect methods are easier to control. That is, the conductivity measurement is made after an electrolyte is added to saturate the water extracted by organic solvents. Such systems have been reviewed and an alcohol-acetone-water-sodium chloride system has been found to show increased conductivity with increased water content.[10]

An electrical condenser with a charge q at potential V has a capacitance $C = q/V$. If C_0 is the capacitance of this condenser in air and C_ε is its capacitance filled with a material of dielectric constant, ε, then ε can be defined as $\varepsilon = C_\varepsilon/C_0$. Water has a dielectric constant of about 80, which is 40 times greater than the hydrocarbons, more than three times that of alcohol and, in

general, greater than most common materials. Therefore, small changes in water content can be detected by changes in the dielectric constant of a system. The measurements can be made by placing the material per se or an extract of the material between the plates of a condenser. Dioxane, which is miscible with water and has a low dielectric constant of 2, makes a satisfactory extracting solvent, provided it is selective for water in the system under investigation. The measurement of dielectric constants has recently been reviewed,[11] and there is also a discussion available on the application of such measurements to water assays.[12]

Coulometric measurements based upon the quantity of electricity required for the electrolysis of water have also been applied to the quantitative determination of water in various samples, especially in the vapor form. Water analysis instruments are available based upon a measurement of the electrolysis current required to completely electrolyze water present in gases in concentration of 1 to 1000 ppm.[13] Although these measurements are made in the gaseous form, they also include analysis after vaporization of liquids or after liquids or solids have been swept by an inert gas. These instruments are of particular interest because they can be made to measure water in a flow of vapor on a continuous basis.

C. CHEMICAL METHODS FOR WATER DETERMINATION

1. Karl Fischer Procedure

a. Chemistry of the Reaction. Among the numerous chemical procedures that have been developed for moisture determinations, a method first suggested by Karl Fischer,[14] in 1935, remains the most generally applicable procedure. The reader is referred to the classic comprehensive text by Mitchell and Smith[1] for a detailed description of this technique and its application.

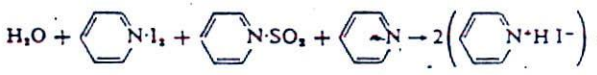
A typical Karl Fischer reagent as indicated by the USP is a mixture of 125 g of iodine, 170 ml of pyridine, 670 ml of methanol, and 100 ml of liquid sulfur dioxide. One milliliter of this reagent, when freshly prepared, will react with about 5 mg of water. The reagent is commercially available but can be prepared with good success in the laboratory. When so prepared, it is general practice to increase the stability of the reagent by adding sulfur dioxide to a stock solution of the other components the day before actual use.

In the presence of water, iodine will be reduced and the sulfur dioxide oxidized in the following manner:

$$H_2O + I_2 + SO_2 \rightleftharpoons 2HI + SO_3 \tag{9.8}$$

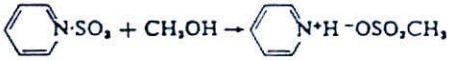
The reversibility of reaction is prevented by the presence of pyridine which reacts with the sulfur trioxide produced. The pyridine sulfur trioxide compound, an inner salt, reacts, in turn, with the methanol present to form the

pyridine salt of methylsulfate. Thus, the over-all reaction is best considered in the following terms:



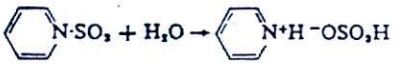
(9.9)

and



(9.10)

The primary reaction (9.9) occurs rapidly and permits the direct titration of any available water with the reagent. It is also accompanied by a color change from pale yellow to dark brown, which can be used to indicate the end point. The pyridine-sulfur trioxide inner salt in Eq. (9.9) would react with a second molecule of water if methanol were absent. That is,



(9.11)

Reagents modified without methanol in this manner, however, have not proved successful in actual practice.

As a result of problems in stability due to side reactions and reaction with traces of moisture, the Karl Fischer reagent must be standardized directly before use. This can be accomplished by titration against a standard water-in-methanol solution. The USP outlines a concentration of 2 ml of water added to 1000 ml of methanol. The reagent, of course, can also be standardized directly against weighed quantities of water, or hydrated salts can be employed as weighed standards. The USP lists sodium tartrate ($Na_2C_4H_4O_6 \cdot 2H_2O$) as its primary standard.

Karl Fischer determinations either can be performed by direct titrations or an excess of reagent can be added and the excess back titrated with a standard water-in-methanol solution. For a direct titration, methanol is added to dissolve the sample or aid in the penetration of an insoluble material. In back-titration procedures, the reagent alone often serves this purpose.

b. Equipment. A titration system protected from moisture of the air, similar to those used in many nonaqueous titration procedures, is required. Figure 9.3 illustrates a typical set-up. The use of a second burette for the water-in-methanol solution, although not absolutely necessary, is helpful for standardization and back-titration procedures. Modified smaller equipment is helpful for microprocedures.

c. End-Point Detection. The color change accompanying the Karl Fischer reaction is suitable for the detection of the end point in many titrations, and when a spectrophotometric technique is employed,[16] the color change may even be applied to microtitrations. However, electrometric methods have decided advantages where samples are colored, automatic titrators are employed and, in general, are used for most microdeterminations.

An amperometric detection of the Karl Fischer titration end point has been popular since 1943, when Wernimont and Hopkinson[16] first applied the bi-platinum electrode system, developed[17] in 1926, to the reaction. Dead-stop, kick-off, polarization and biamperometric endpoint have all been used as names for the procedure. The system required for this end point is relatively

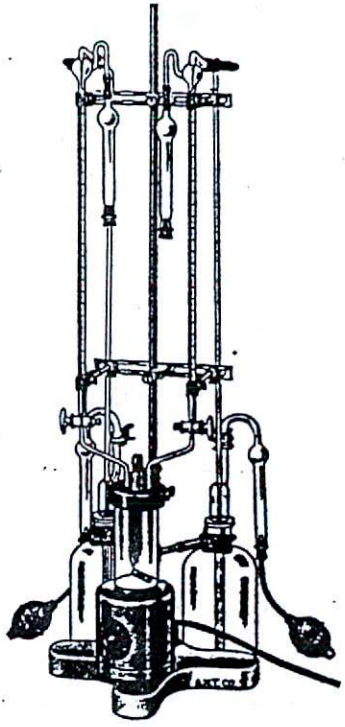


FIGURE 9.3: Karl Fischer titration apparatus.

simple. The BP suggests a 1.5- or 2-volt dry cell across a variable resistance of about 2000 ohms, which is in series with the two platinum electrodes and a microammeter. Under these conditions of constant low voltage with the direct titration procedure, there is a small constant residual flow of current until the end point is reached, accompanied by a large increase in current. Thus, when water is titrated with Karl Fischer reagent, there is a "kick-off" or major deflection of the microammeter to indicate the end point as the first drop of excess Karl Fischer reagent enters the titration flask. Conversely, when a back titration is employed, there is a sudden drop in current or a "dead-stop" end point occurs. It should be noted, however, that with micro-titrations this change is somewhat more gradual, and the current as a function of added titrant should be plotted for exact location of the end point.

Up to the end point in the direct titration of water with Karl Fischer reagent, there are iodide ions present but no free iodine. At the potential used, the system is irreversible and the electrodes are polarized, that is, they have an impressed potential with little flow of current.[18] However, as free iodine enters the system there is a reversible iodine-iodide couple established with depolarization of the electrodes and an increase in the flow of current.

The Karl Fischer end point can also be determined potentiometrically with the two platinum electrodes polarized at a constant current. Under these conditions, a curve similar to the amperometric titration results. Before the end point, there is a small residual potential established between the two electrodes, whereas at the end point there is a large increase in potential with the introduction of the free iodine. The principles of potentiometric titrations at a constant current have been outlined by Lingane.[19] Its application to the Karl Fischer procedure is increasing, based upon the availability of pH meters with the required polarizing circuit and on the fact that this procedure lends itself to recording and automatic titrations.

d. Limitations. The Karl Fischer reagent is highly specific for water with remarkably few interfering materials being reported. Compounds which react with either iodine or iodide, however, will interfere with the reaction. For example, ascorbic acid will be oxidized by the iodine present in the reagent, whereas quinone is reduced by the iodide produced during the reactions. The fact that a compound will react with iodine or iodide does not necessarily exclude its presence in a successful determination. Mercaptans are oxidized to disulfides by iodine. However, this interference can be prevented by the formation of a sulfide through an additional reaction of the mercaptan with an unsaturated hydrocarbon.[1]

Some carbonyl compounds will also interfere with the Karl Fischer determination. Active carbonyl compounds under the conditions of the Karl Fischer determination react with methanol with the formation of acetals or ketals and the liberation of water.

$$R_2CO + 2CH_3OH \rightarrow R_2C(OCH_3)_2 + H_2O \qquad (9.12)$$

This reaction again can be prevented by a prior reaction with hydrocyanic acid[1] to form a cyanohydrin, which in turn does not react with Karl Fischer reagent.

2. OTHER CHEMICAL PROCEDURES

a. Inorganic Reagents. Although the Karl Fischer reaction is the most popular and versatile of the chemical procedures for moisture determinations, other chemical procedures also have proved of value. Calcium carbide reacts rapidly and quantitatively with water in the manner of Eq. (9.13). The

$$CaC_2 + 2H_2O \rightarrow C_2H_2 + Ca(OH)_2 \qquad (9.13)$$

reaction has been used for the assay of water by measuring the loss in weight of the system or the amount of acetylene produced. Although the acetylene procedure can be determined gasometrically, a number of other determinations are also suitable.

The reactions of calcium hydride and, to a lesser extent lithium aluminum hydride, have been applied to water measurements in methods similar to those applied to calcium carbide:

$$CaH_2 + 2H_2O \rightarrow 2H_2 + Ca(OH)_2 \quad (9.14)$$

$$LiAlH_4 + 4H_2O \rightarrow 4H_2 + LiOH + Al(OH)_3 \quad (9.15)$$

Although the hydride methods are not specific for water, because of the liberation of hydrogen by active hydrogen compounds, in general, they represent efficient methods where applicable.

Magnesium nitride has also been advocated as a reagent for water determinations:

$$Mg_3N_2 + 6H_2O \rightarrow 3Mg(OH)_2 + 2NH_3 \quad (9.16)$$

The ammonia liberated can be determined by reaction with excess standard hydrochloric acid and back titration with standard base in a manner similar to the liberation of ammonia in the Kjeldahl determination.

The following reaction of sodamide with water can be used in the same manner:

$$NaNH_2 + H_2O \rightarrow NaOH + NH_3 \quad (9.17)$$

b. Organic Reagents. A number of organic reactions involving water have been of merit in aquametry. Grignard reagents can be used in a gasometric method analogous to the inorganic reagents:

$$CH_3MgI + H_2O \rightarrow CH_4 + MgI(OH) \quad (9.18)$$

This method is again limited to systems where water is the only active hydrogen compound.

The hydrolyses of various organic reagents have also been advocated for water determinations. Acetyl chloride reacts rapidly with water, but because of its high volatility, it is usually used in combination with pyridine in the following manner:

$$C_5H_5N{\cdot}OCCH_3\ Cl^- + H_2O \rightarrow C_5H_5N{\cdot}H\ Cl^- + CH_3COOH \quad (9.19)$$

An excess of acetylpyridinium chloride is used in this reaction and the excess can be reacted with dry methanol.

$$C_5H_5N{\cdot}OCCH_3\ Cl^- + CH_3OH \rightarrow C_5H_5N{\cdot}H\ Cl^- + CH_3COOCH_3 \quad (9.20)$$

The moles of water present are equal to the molar increase in acidity, owing to the formation of the acetic acid in Eq. (9.19). This increase in acidity is determined by titration with standard base after reactions (9.19) and (9.20) with comparison to a blank determination of the reagent. This method is relatively free of interferences, for example, limited amounts of alcohols and amines react with the reagent, but only have the effect of decreasing its strength.

Higher molecular weight nonvolatile acid chlorides have also been used. The hydrochloride liberated is collected and titrated as a measure of the water present.

$$RCOCl + H_2O \rightarrow RCOOH + HCl \tag{9.21}$$

Anhydrides, especially acetic anhydride, although slower than the corresponding chlorides in their reaction with water, have been used in the presence of catalysts for aquametry:

$$(RCO)_2O + H_2O \rightarrow 2RCOOH \tag{9.22}$$

In these procedures, an excess of reagent is used, and the difference in the acidity of the sample reaction mixture as compared to a reagent blank is a measure of the water present. The acidity is usually determined by titration with standard sodium methoxide solutions.

Recently, an interesting procedure based upon the methanesulfonic acid-catalyzed hydrolysis of the dimethyl acetal of acetone has been proposed for the determination of trace amounts of water[20]:

$$H_2O + CH_3C(OCH_3)_2CH_3 \xrightarrow{H^+} CH_3COCH_3 + 2CH_3OH \tag{9.23}$$

The acetone produced becomes an assay of the water present and is measured on the basis of the carbonyl absorption at 5.7 μ in the infrared. Because of the acid conditions required for quantitative results, the presence of basic materials represents an interference and the presence of any carbonyl compounds in the system would at the least reduce the sensitivity of the method. Care also must be observed with the reagent because of its instability in presence of heat or water.

c. Colorimetric Procedures. Colorimetric determinations of water have been proposed, varying from those which depend upon the general reaction discussed above to those which involve specific reagents for yielding color reactions with water.

The production of acetylene by the reaction of calcium carbide with water [Eq. (9.13)] has been determined colorimetrically. The acetylene is passed through ammoniacal cupric sulfate with the formation of red-colored cuprous acetylide.

The liberation of ammonia by magnesium nitride [Eq. (9.16)] has also been determined colorimetrically using Nessler's reagent of alkaline mercuric potassium iodide.

As both cobaltous chloride and bromide undergo pronounced color changes upon hydration, they have served as the basis of several colorimetric determinations. Water is extracted with a suitable solvent and allowed to hydrate the cobalt salt so that the change in color is a measure of the water present.

The intensity of the red-orange color of lithium cupric chloride solutions can be related to the amount of water present in certain ketones, esters, and

ethers.[21] Also, the color intensity of potassium dichromate in water-alcohol mixture has been found to increase with the water content.[22]

D. SUMMARY

The above discussion of aquametry should indicate to the reader the variety of excellent methods available and that the choice for the most suitable method depends upon the system under investigation. Traditionally, pharmaceutical analysis has depended upon "loss-in-weight," azeotropic distillation, and the Karl Fischer procedures. However, there is every indication that the newer spectrophotometric, gas-chromatographic, and electrometric methods will become increasingly important.

Finally, it should be noted that aquametry is still an active area for the development of new methods. The current literature continues to describe an increasing number of modified and new techniques for the quantitative determination of water. An indication of this growth can be gained by a comparison of the excellent reviews of aquametry by Mitchell et al. in 1948[1] and 1961.[23]

9.2 GASOMETRIC ANALYSIS

A. GENERAL CONCEPTS

1. Gas Laws

Reactions involving the use or liberation of a gas can be followed quantitatively by precise measurement of the amount of gas involved. Rather than follow such reactions on the basis of a change in mass of the gas involved, it is the usual practice to measure a change in volume or pressure. That is, measurements are made upon the basis of Avogadro's hypothesis and upon the Boyle and Gay-Lussac relationships for ideal gases. For most analytical applications, deviations for ideal gas behavior are not significant. However, where attraction between molecules causes deviation from ideal behavior, this deviation can be determined and correction factors applied to the analysis.

According to Avogadro's hypothesis, under equal conditions of temperature, pressure, and volume, all ideal gases contain the same number of molecules. Boyle showed that the pressure of a gas of constant mass and at a constant temperature was inversely proportional to its volume. Gay-Lussac showed that a volume of a constant mass of gas at a constant pressure was directly proportional to its temperature. These relationships of temperature, pressure, and volume can be written as one relationship, the ideal gas law;

$$PV = nRT \tag{9.24}$$

where n is the number of moles, T is expressed in the absolute temperature scale, and R is the gas constant. Thus, at a given temperature the number of moles of a gas is proportional to the pressure of the gas at a constant volume or is proportional to the volume of the gas at a constant pressure. Therefore, gasometric methods can be based upon either the measurement of pressure, *manometric methods*, or of volume, *volumetric methods*.

In making these measurements on mixtures of gases or when corrections are to be applied for interfering vapors, two other ideal gas laws are of importance. Dalton's law states that the pressure of a given volume of gas is the sum of the partial pressures of its components at that volume and temperature:

$$P = p_1 + p_2 + p_3 + \cdots \qquad (9.25)$$

Leduc's rule applies to volume measurement of mixtures. The volume of a gas mixture at a given pressure is the sum of the volumes that would be occupied by each component at the given pressure and at the same temperature:

$$V = v_1 + v_2 + v_3 + \cdots \qquad (9.26)$$

2. Apparatus

The various techniques and approaches available for the measurement of pressure and volume of gases have been reviewed.[24,25] Pressure is usually measured by means of manometers, mechanical gauges, and electrical measurements. A manometer in its simplest form consists of a U-shaped tube filled with a liquid, usually mercury, and connected at one end to the system to be measured. If an absolute measurement of pressure is to be made, the other end of the manometer is closed so that essentially a vacuum exists between the closed end and the level of liquid. If a relative-pressure measurement is to be made, this second end is exposed to the atmosphere. In either form, pressure measurements are made on the basis of the difference in the height of the two columns of liquid.

An example of a mechanical gauge is the Bourbon gauge usually found as part of the reducing valve system for cylinders of gas. A curved piece of metal tubing is fixed at one end and is attached to an indicator needle at the other end. An increase in pressure causes the tubing to straighten and to indicate the pressure on a calibrated dial.

Electrical measurements of pressure are usually applied to reduced-pressure systems. The Pirani gauge, for example, depends upon the thermal conductivity of a low-pressure system being related to the pressure of the gas. The temperature and, therefore, the electrical resistance of a heated wire in such a system will depend on this change in thermal conductivity.

Volume measurements are of two basic types: Either the rate of flow of gas is measured as a function of time or the volume of gas in a container of known volume is measured. As the amount of gas involved in analytical

procedures is usually relatively small, most analytical gasometric methods are volumetric measurements and involve the use of gas burettes. These burettes are similar to those used for measurements of liquids with the exception that both ends have provisions for connection to a gas train and both ends are usually equipped with multiple-way stopcocks.

The usual train required for a volumetric gasometric technique will consist of one or more reaction vessels or gas pipettes and a gas burette connected

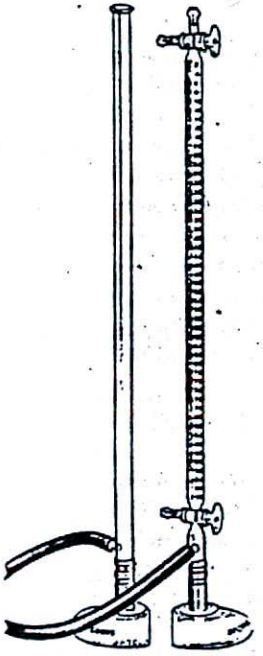


FIGURE 9.4: Volumetric gasometric apparatus.

directly to a leveling device. The leveling device consists of a reservoir containing a liquid, usually mercury so that its height may be adjusted to control the volume of gas in the burette. Figure 9.4 is an illustration of the basic equipment required for volumetric gasometric procedures.

B. PURITY DETERMINATIONS

Gasometric procedures are outlined in the official compendia as assay procedures for the medicinally important gases. In a given volume of gas, either the principal component or the most likely contaminant is removed and the residue measured. Oxygen can be absorbed by the strong reducing agent, cuprous ammino chloride. The reducing agent is formed by the reaction of a

coil of copper wire in ammonium chloride-ammonium hydroxide solution so that, in the presence of oxygen, Eq. (9.27) can occur. Thus, a known

$$4NH_4Cl + 8CuNH_3Cl + 8NH_3 + 2H_2O + O_2 \rightarrow 4CuCl{\cdot}CuCl_2{\cdot}4NH_3 + 4NH_4OH \quad (9.27)$$

volume of oxygen as measured in a gas burette is forced into a reaction vessel containing the reducing reagent and after reaction any gas remaining is remeasured in the gas burette. On this basis, the USP permits a residue of not more than 1%. The same procedure is used for an assay of oxygen as a contaminant in nitrogen, with USP requirements allowing reaction of no more than 1% of the total volume.

The official assay procedure for carbon dioxide involves the absorption of a measured volume of the gas by a concentrated solution of potassium hydroxide. To meet USP standards, a residue of no more than 1% of unabsorbed gas can be present. A similar procedure is used for the determination of the purity of cyclopropane by absorption in sulfuric acid. The gasometric assay for nitrous oxide depends upon the measurement of the volume of gas not condensed by a liquid nitrogen bath; a limit of not more than 3% uncondensable gas is required by the USP.

C. MEASUREMENT OF GASEOUS PRODUCTS OR REACTANTS

There are many important examples of the measurement of a liberated gas as the basis for quantitative analytical procedures. An outstanding procedure of biological significance is the Van Slyke method for the analysis of α-amino acids. This procedure, in which the amino acid is nitrosated with the subsequent liberation of nitrogen, is discussed in Section 9.3.

The gasometric determination of nitrogen is also used in the classical Dumas microdetermination of the nitrogen content of organic compounds. Nitrogen and nitrogen oxides are produced by the combustion of the sample mixed with copper oxide and heated in an atmosphere of carbon dioxide in a closed system. The gases produced are passed over heated copper to reduce the oxides to nitrogen and total nitrogen content is measured in a gas burette over 50% potassium hydroxide solution.

Two general types of gasometric procedures can be used for the determination of active hydrogens. The Grignard reagent, methyl magnesium iodide, reacts with hydrogen attached to oxygen, nitrogen, sulfur, or unsaturated carbon atoms with the liberation of methane:

$$RH + CH_3MgI \rightarrow CH_4 + RMgI \quad (9.28)$$

In a similar manner, lithium aluminum hydride reacts with active hydrogen compounds with the liberation of hydrogen:

$$4RH + LiAlH_4 \rightarrow 4H_2 + LiR + Al(R)_3 \quad (9.29)$$

The gasometric determination of this liberated hydrogen is again used as a measure of the number of active hydrogens.

Alkyl nitrites yield nitric oxide upon reaction with potassium iodide in the presence of acid. The alkyl nitrites, amyl nitrite, octyl nitrite, and ethyl nitrite, which are used as vasodilators, are assayed in this manner:

$$2RONO + 2KI + H_2SO_4 \rightarrow 2NO + 2ROH + K_2SO_4 + I_2 \qquad (9.30)$$

The nitric oxide liberated in these assays is measured in gas burettes in which saturated salt solutions are used as the adjusting liquid.

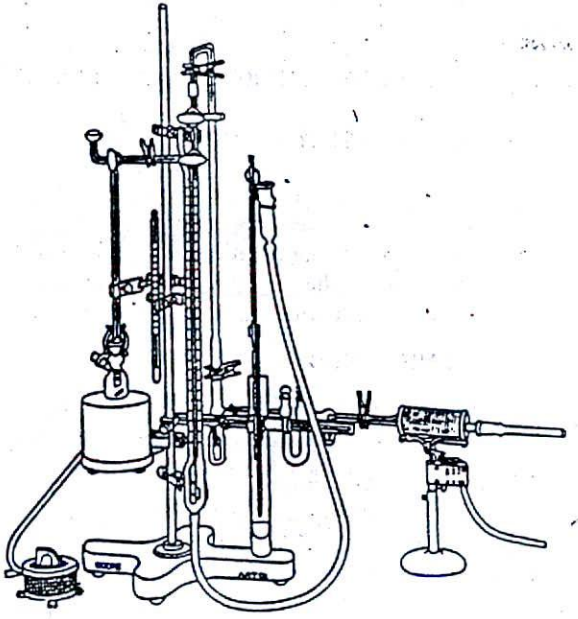


FIGURE 9.5: Microhydrogenation apparatus.

The outstanding example of the measurement of the consumption of a gas in assay procedures is the catalytic hydrogenation of alkene functions.

$$R_2C{=}CR_2 + H_2 \xrightarrow[\text{catalyst}]{} R_2CHCHR_2 \qquad (9.31)$$

This reaction, either when used for the quantitative determination of unsaturation or when used as part of a structure proof of a compound, is usually run on a microbasis. Figure 9.5 illustrates a typical microhydrogenation apparatus in which the volume of hydrogen consumed is measured by a gas burette. Apparatus is also available for the measurement of hydrogenation on the basis of the change in pressure.

The choice of proper catalyst is important and involves the following considerations. The more active catalysts will permit quantitative results in a reasonable period of time at room temperature and atmospheric pressure, which are the conditions easiest to control for reproducible results. However, too active a catalyst will result in a loss in selectivity and the reduction of other functional groups, in addition to alkene unsaturation. The preparation and use of platinum, palladium, and Raney nickel catalysts suitable for microquantitative hydrogenation has been described in the literature.[26] It should be noted that, in contrast to most catalytic processes, relatively large amounts of catalyst are required, and the amount of catalyst may even exceed the amount of alkene to be analyzed.

9.3 SODIUM NITRITE; NITROSATION PROCEDURES

A. CHEMISTRY OF NITROSATION

Nitrosation reactions in which nitrous acid reacts with amines are of analytical significance. The stability of nitrous acid is poor and, therefore, it is usually prepared in situ by the addition of sodium nitrite to an acidic solution. The final products in the nitrosation of amines vary with the class of amine. Primary amines react with the liberation of nitrogen:

$$RNH_2 + HNO_2 \rightarrow ROH + N_2 + H_2O \tag{9.32}$$

Although it is postulated that this reaction proceeds through a diazonium salt intermediate, it is only with aromatic amines that such salts can be isolated and then only at low temperatures:

$$ArNH_2 + HNO_2 \xrightarrow[0\text{–}5^\circ]{} ArN_2^+ + OH^- + H_2O \tag{9.33}$$

At higher temperatures, these diazonium salts will decompose rapidly with the liberation of nitrogen. Thus, the summation of reactions (9.33) and

$$ArN_2^+ + OH^- \rightarrow ArOH + N_2 \tag{9.34}$$

(9.34) at even room temperatures again yields reaction (9.32). However, preservation of aryl diazonium salts in cold solution is of both preparative and analytical value. The coupling reaction of diazonium salts with aromatic amines and phenols with the formation of colored azo compounds is also an important analytical reaction:

$$ArN_2^+ + Ar'H \rightarrow ArN{=}NAr' + H^+ \tag{9.35}$$

In reaction (9.35), Ar' must have a strong electron-donating group such as a phenolic or amino group. The coupling occurs *para* to this substituent group, and where the *para* position is already substituted, *ortho* coupling occurs. As the coupling reaction is an electrophilic-substitution reaction, it

may also occur with the carbanions associated with active hydrogen compounds. Secondary aromatic and aliphatic amines also react with nitrous acid but yield N-nitrosoamines:

$$R_2NH + HNO_2 \rightarrow R_2NNO + H_2O \qquad (9.36)$$

It is interesting to note that the mechanism of amine nitrosation has been extensively studied,[27] and that the initial step with primary amines [reactions (9.32) and (9.33)] is also the formation of an N-nitrosoamine intermediate.

Tertiary amines only react with nitrous acid to form salts, or aromatic tertiary compounds may undergo nonquantitative side reactions such as replacement of a ring hydrogen and the formation of C-nitroso compounds.

The nitrosations of analytical importance to be discussed in this chapter are given in the following three subsections.

1. Nitrosations with Nitrogen Liberation

The Van Slyke method[28] for α-amino acids is the classical example of the measurement of nitrogen liberated from a primary amine following reaction with nitrous acid [Eq. (9.32)]. The amino acid is allowed to react with excess nitrous acid produced from sodium nitrite and acetic acid. Before the liberated nitrogen can be measured, the oxides of nitrogen formed from the decomposition of nitrous acid must be removed by treatment with an alkaline permanganate reagent. A closed system is required for the analysis and must include a reaction chamber with stirring, provision for the permanganate treatment, and a gas burette. Figure 9.6 is an illustration of a commercially available Van Slyke microapparatus.

It is important to realize that, although the Van Slyke procedure results in the rapid liberation of nitrogen from primary α-amino acids at room temperature, this is not a general rule for other primary amines. The reaction must be run at higher temperatures, and for most amines this introduces both side reactions and interferences by compounds other than primary amines. Not only other nitrogen compounds, but also such compounds as phenols and active methylene compounds react with nitrous acid to yield gaseous products.[29]

An interesting modification of the Van Slyke procedure, especially for the general microanalyses of primary amines, is the use of a simplified reaction flask and the introduction of the gas produced into a gas-chromatograph column.[30] By this process, the nitrogen produced can be separated from nitrogen oxides on a molecular sieve column and quantitatively related to the primary amine under investigation.

2. Titrations with Sodium Nitrite

As was indicated in Eq. (9.33), primary aromatic amines can form diazonium salts in the cold, whereas secondary amino compounds will react with nitrous

acid to form N-nitroso groups as indicated in Eq. (9.36). In both reactions, the amine is dissolved in hydrochloric acid and the nitrosation performed by titration with standardized sodium nitrite solution. The end point of this titration was originally determined by withdrawing drops of the reaction mixture and testing with starch-iodide indicator solution or paper. In this

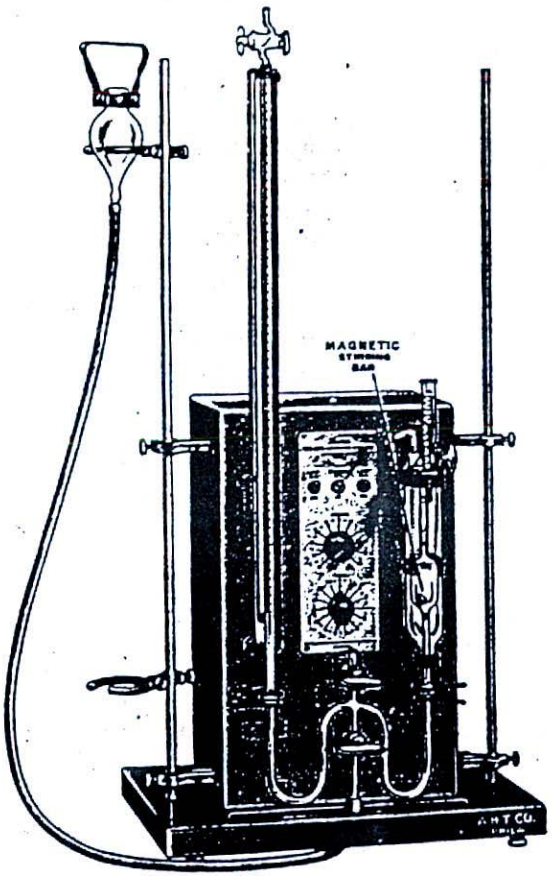


FIGURE 9.6: Van Slyke microapparatus.

manner, the first excess of unreacted nitrous acid would oxidize the potassium iodide of the indicator to free iodine and would result in a characteristic purple color of starch in the presence of iodine.

More elegant methods of determining the end point include the internal use of redox indicators, such as diphenylbenzidine disulfuric acid, and electrometric titrations. The reactions have been followed potentiometrically using a calomel-platinum system.[31]

A "dead-stop" or biamperometric procedure has also been suggested to detect the end point.[32] Upon using two platinum electrodes at a potential of 0.4 volt, there is no flow of current until an excess of nitrous acid is present.

The nitrosation of both the aromatic primary amines and the secondary amines may proceed slowly so that a catalyst of potassium bromide is often used. Because of the slowness of the reaction with certain amines, a back- or indirect-titration procedure has also been suggested.[33] An excess of standard sodium nitrite is added and allowed to react for a given time. The excess of nitrous acid present in the acidic solution is back titrated with a standardized solution of a fast reacting amine such as *p*-nitroaniline. In this procedure, there is the danger of a loss of nitrous acid as oxides of nitrogen during the initial reaction period. Nitric acid is added to control this decomposition by forcing the equilibrium of Eq. (9.37) to the left.

$$3HNO_2 \rightleftharpoons HNO_3 + 2NO + H_2O \qquad (9.37)$$

Also in the direct titration procedures it is important to limit this loss of nitrous acid. Therefore, by the use of efficient stirring and the slow addition of sodium nitrite solution, a large excess of nitrous acid is not allowed to build up. It has also been recommended that the burette tip be placed below the surface of the reaction mixture to limit the loss of nitrous acid.

An important pharmaceutical application of sodium nitrite titrations is the analysis of the sulfonamides by the diazotization of the primary aromatic amino group usually present in this class of drugs. Several sulfonamides require the formation of a primary amine prior to the diazotization step. For example, phthalylsulfathiazole and succinylsulfathiazole are hydrolyzed to yield primary aromatic amines and determined by the sodium nitrite titration procedure. *p*-Nitrosulfathiazole is first reduced to the amine and then diazotized.

Of course, the sodium nitrite procedure is not specific for sulfonamides and would apply to all primary aromatic amines as well as both aromatic and aliphatic secondary amines. In addition, although tertiary amines and certain other nitrogen compounds cannot be determined quantitatively, they do consume nitrous acid to an extent that causes an interference with the reaction. As was mentioned for the Van Slyke procedure, active methylene and phenolic compounds also represent potential interferences.

3. Diazonium Salt Reactions

The coupling reaction of diazonium salts as outlined in Eq. (9.35) can be used as the basis of various colorimetric assays. Either (1) the primary aromatic amine to be analyzed is diazotized and reacted with an excess of coupling reagent or (2) a diazotized amine reagent can be used to determine phenolic or aromatic amines. In either procedure, the azo compound formed is compared colorimetrically to a standard solution.

a. Reaction of a Diazonium Salt with a Coupling Reagent. The Bratton and Marshall[34] procedure for sulfonamides is an outstanding pharmaceutical example of this type of analysis. Those sulfonamides containing a primary aromatic moiety can be diazotized by treatment with sodium nitrite in dilute mineral acid. The excess nitrous acid is decomposed with sulfamic acid as illustrated in Eq. (9.38). The sulfonamide diazonium salt is coupled with

$$HSO_3NH_2 + HNO_2 \rightarrow N_2 + H_2SO_4 + H_2O \qquad (9.38)$$

excess N-(1-naphthyl)-ethylenediamine. The color of the azo dye produced is then compared to the dye formed by the same procedure from a known concentration of the sulfonamide.

The coupling diamine used in the Bratton and Marshall procedure was chosen from a series of possible other coupling reagents on the basis of (1) availablity in pure form, (2) the ability to couple in the same acid solution used to produce the diazonium salt, and (3) the solubility of the azo dye form in the acidic aqueous reaction mixture.

Essentially the same procedure has been used for the analysis of a series of local anesthetics containing primary aromatic amine groups.[35] The Bratton and Marshall method or allied procedures using phenolic or arylamine coupling reagents are generally applicable for the microanalysis of diazotizable aromatic primary amines with a precision in the order of 1 to 2%.

b. Diazonium Reagent Assays. A series of aromatic primary amines has been suggested for the preparation of diazonium salts which in turn can be used for the analysis of phenols and aromatic amines. The diazonium salt is prepared in the usual manner by addition of sodium nitrite in a cold acid solution. For solubility purposes, it is usually desirable to accomplish the coupling of phenols after adjustment to a basic pH, but the amines are more soluble in an acid reaction mixture. In general, the rate of coupling is greater in basic solution; however, the stability of the diazonium salt is decreased. The optimum pH condition for coupling a given compound, therefore, depends upon solubility, rate of reaction, and stability factors. Coupling assays are performed either as titration procedures with standardized diazonium solutions or, since the final product is highly colored, as colorimetric procedures.

Sulfanilic acid in the form of its diazonium salt has long been used for the analysis of phenols of biological significance.[36] *p*-Toluene- and *m*-nitrobenzene-diazonium chlorides have been suggested[37] as suitable reagents in a general coupling procedure for phenols, aromatic amines, and for certain active hydrogen compounds. The general applicability of diazonium coupling analysis can be extended to aromatic nitro compounds by first reducing them to amines. This colorimetric analysis has even been extended to biphenyl by nitrating this hydrocarbon followed by reduction to an amine which is then coupled with N-(1-naphthyl)-ethylenediamine.[38] There are many pharmaceutical examples of the diazonium colorimetric reagent procedures. Sulfonic

acid after diazotization has been used to produce an azo dye with antimalarial drugs.[39] Diazotized *p*-nitroaniline has been used for the colorimetric analysis of the phenolic aliphatic amine, phenylephrine.[40]

Diazonium salt coupling reactions are also of value for visualization of materials separated by paper and thin-layer chromatography. For example, primary aromatic amines have been detected on paper chromatograms by diazotization with a spray of sodium nitrite in dilute hydrochloric acid followed by coupling with N-ethyl-1-naphthlyamine to produce colored spots.[41] The reverse process has also been used as a detection aid. Thus, diazotized solution of amines such as sulfanilic acid, benzidene, and *p*-nitroaniline have been employed for the detection of phenols. However, it should be remembered in this application that these diazotized sprays will also react with aromatic amines and certain active hydrogen compounds.

9.4 MERCURIC ACETATE

The salt, mercuric acetate, will react quantitatively with several functional groups with liberation of acetic acid. The reaction is then followed by titration of the liberated acetic acid with standardized base. Determination of mercaptan groups and alkene unsaturation by this procedure are both of pharmaceutical interest.

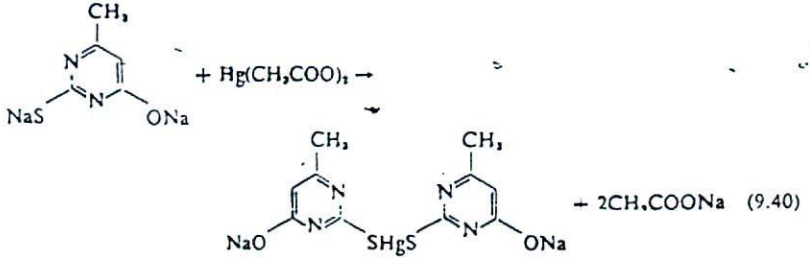
A. MERCAPTAN DETERMINATIONS

Mercaptans react with mercuric acetate to form sulfides and acetic acid. The acetic acid formed [Eq. (9.39)] can be titrated with standard base as a

$$2RSH + Hg(CH_3COO)_2 \rightarrow Hg(SR)_2 + 2CH_3COOH \qquad (9.39)$$

measure of mercaptan groups present.

An interesting variation of this procedure is the titrimetric determination of methylthiouracil as outlined in the BP. A weighed sample of the thiouracil is dissolved in an excess of sodium hydroxide and the resulting disodium salt is titrated in a buffered acetate system with a standardized solution of mercuric acetate:



(9.40)

The end point of this reaction is determined by the use of diphenylcarbazone as an indicator. Thus, when an excess of mercuric acetate has been added there is a formation of a mercury-diphenylcarbazone complex which is rose-violet in color.

B. DETERMINATION OF UNSATURATION

Mercuric acetate in the presence of methanol will form a methoxymercurate with certain olefins:

$$RCH{=}CHR' + Hg(CH_3COO)_2 + CH_3OH \rightarrow CH_3COOH + RCH(OCH_3)CHR'Hg(CH_3COO) \quad (9.41)$$

One method that has been suggested[42] to follow this reaction is to use an excess of standardized mecuric acetate. The excess can then be determined by a nonaqueous titration of mercuric acetate:

$$(CH_3COO)_2Hg + 2HCl \rightarrow HgCl_2 + 2CH_3COOH \quad (9.42)$$

Although the methoxymercurate product also reacts with nonaqueous hydrochloric acid, only 1 mole of hydrochloric acid is required to yield 1 mole of acetic acid. The difference in the amount of hydrochloric acid used for a blank as compared to the sample is then a measure of the unsaturation present.

Other mercuric salts such as the nitrate and sulfate as well as other methods of following the mercuric acetate reaction have been suggested for determination of olefinic unsaturation. This subject of the addition of metal salts for the determination of unsaturation has been covered as part of a general review of the determination of olefinic groups.[43]

9.5 KJELDAHL NITROGEN DETERMINATION

Kjeldahl[44] introduced in 1883 a method for the determination of nitrogen in organic compounds that has been widely used ever since. Although the procedure was originally designed for proteins, it has been found to be applicable even without modification to other nitrogen-containing compounds. Modifications in the procedure, especially as to the choice of catalysts and the addition of reducing agents, have permitted extension of the method to the analysis of most nitrogen-containing compounds. The proper choice of apparatus and method has also made possible the use of the Kjeldahl procedure on large to ultramicro samples. The various Kjeldahl procedures have been extensively reviewed.[45]

The initial step in the determination is the digestion of the nitrogen-containing sample in hot concentrated sulfuric acid, with the resulting formation of ammonium sulfate together with decomposition products as carbon dioxide and water.

The second step involves the addition of concentrated sodium hydroxide which neutralizes the excess sulfuric acid and liberates ammonia from the ammonium sulfate. The liberated ammonia in turn can be quantitatively distilled and determined by titrimetry. A typical determination becomes:

$$\text{nitrogen compound} + H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2O + CO_2 \quad (9.43)$$

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O \quad (9.44)$$

The percentage of nitrogen in the sample can then be calculated as follows:

$$\%N = \frac{100 \times 14.01 \times \text{eq. of ammonia formed}}{\text{wt. of sample in g}} \quad (9.45)$$

Most of the modifications in the method involve changes in the digestion procedure as contrasted to the use of only concentrated sulfuric acid as first suggested by Kjeldahl. Anhydrous sodium sulfate or more commonly powdered potassium sulfate is added to the sulfuric acid to allow for higher digestion temperatures. A concentration of 0.5 g of potassium sulfate per milliliter of sulfuric acid will raise the boiling point about 40° to 330°C and thereby ensure faster and more efficient digestion. The USP and NF procedures use copper sulfate as a catalyst in the digestive process; other experimental procedures call for selenium or mercury or various combinations of all three of these catalysts.

Nitrogen present in compounds such as osazones, oximes, hydrazines, azo, nitrates, and nitrites require prior reduction before proceeding with the Kjeldahl method. Although the addition of glucose serves as the reducing agent in many situations, hydriodic acid or thiosalicylic acid have been suggested as more general in their scope. The USP and NF procedures are modified for nitrates and nitrites by the addition of salicylic acid and sodium thiosulfate as reducing agents before digestion to ammonium sulfate.

Two general procedures are used for determining the ammonia liberated from the ammonium sulfate formed in the Kjeldahl procedure. The ammonia may be distilled directly into (1) an excess of standardized hydrochloric or sulfuric acid or (2) into a boric acid solution. Where an excess of hydrochloric or sulfuric acid is employed, the excess is then back titrated with standardized base. When boric acid is used, the trapped ammonia is titrated directly with standardized acid. Until recently, the USP and NF directed the use of boric acid only for a semimicroprocedure. However, boric acid is now used also in the macroprocedure, and a new indicator, methyl red-methylene blue, is employed for the titration of ammonia with standardized sulfuric acid.

The Kjeldahl experimental procedure requires great care in handling the concentrated acid and base involved. Caution is necessary upon dilution of the reaction mixture with water following digestion as well as when sodium hydroxide solution is added to the diluted digestion mixture. An excess of sodium hydroxide solution is added slowly down the side of the digestion flask held at an angle so as to form a layer of sodium hydroxide below that of

the diluted acidic reaction mixture. The acidic layer then serves to prevent any loss of ammonia until the digestion flask can be connected to the distillation apparatus. However, once this connection is made it is important to mix carefully, but thoroughly, the two layers before starting the distillation.

The Kjeldahl determination is not only used to determine the percentage of nitrogen in various compounds and biological samples but it is widely employed to estimate the protein content of natural products. For example *The Extra Pharmacopoeia* lists a factor of 6.25 for multiplication of Kjeldahl percentage nitrogen to obtain percentage of an average protein present in a sample. The use of 6.25 is based upon the fact that the average protein contains 16% nitrogen. A factor of 6.38 is given to obtain the protein content of milk products while 5.7 is used for flour.

9.6 SODIUM TETRAPHENYLBORATE

A. INTRODUCTION

Wittig[46] first synthesized the tetraphenylborate (TPB) ion in the form of its lithium salt in 1947. He subsequently noted that the lithium and sodium salts are water soluble, and the rubidium, cesium, potassium, and ammonium compounds are water insoluble; he was able to introduce gravimetric procedures based upon these characteristics.[47] For example, an analysis of a soluble potassium salt would involve the precipitation of insoluble potassium TPB:

$$2Na^+ + 2(C_6H_5)_4B^- + 2K^+ + SO_4^{2-} \rightarrow 2KB(C_6H_5)_4\downarrow + 2Na^+ + SO_4^{2-} \qquad (9.46)$$

Wittig's investigations together with the early commercial availability of sodium TPB give rise to many analytical applications of TPB reactions. These applications have been reviewed,[48] and of particular pharmaceutical interest is the precipitation of amines. Most quaternary ammonium ions and protonated amines form insoluble TPB salts:

$$(R)_4N^+ + {}^-B(C_6H_5)_4 \xrightarrow[H^+]{} R_4NB(C_6H_5)_4\downarrow \qquad (9.47)$$

The scope of this reactivity with amines has been studied[49] and has been widely applied to both the identification and quantitative determination of amines.

B. METHODS

1. Gravimetric

a. Qualitative Applications. TPB procedures have found extensive application for the isolation and identification of amines in a manner analogous to the classical alkaloidal precipitating reagents. TPB derivatives are

highly satisfactory for this purpose because of their ease of isolation in good purity from dilute concentration of amines in complex mixtures. The reaction is extremely sensitive and for many compounds is superior to Mayer's and Dragendorff's reagents as a spot detection test for amines. The parent amine can often be recovered from its TPB salt by dissolving the salt in a suitable solvent, adjusting the pH, and extracting the amine. Recovery of amines in this manner is, therefore, a method for the concentration and purification of amines for their subsequent characterization and has been applied to alkaloid isolations.[50]

The physical characteristics of the TPB salts are also useful for identification purposes. The melting point, spectral and crystal characteristics, as well as the elemental analysis of TPB salts may be useful for the identification or characterization of the parent amine. There are numerous examples of this application of TPB salts and these include such pharmaceutical compounds as alkaloids,[50,51] local anesthetics,[52,53] antibiotics,[54] and sympathomimetic amines.[55]

b. Quantitative Procedures. Since the precipitated amine TPB salt often bears a stoichiometric relationship to the parent amine, quantitative procedures have been developed. Precipitation usually is conducted at a pH of 3 to 5 and from room temperature to 70°C. Care must be used in drying the precipitate to avoid decomposition. Pharmaceutical compounds, particularly alkaloids,[56] have been assayed in this manner.

2. Titrimetric Procedures

Quantitative TPB gravimetric methods may involve problems in obtaining precipitates of good filterability and in drying the precipitate to constant weight; they may also be time consuming. Therefore, there has been great interest in volumetric techniques. Argentometric titrations are based upon the greater solubility of TPB amines in acetone-water as compared to the silver TPB salt. The amine is precipitated as its TPB salt, washed free of excess reagent, dissolved in acetone, and then titrated with standardized silver nitrate solution.[57] A residual titration procedure in which an excess of standardized silver nitrate is used and the excess titrated with ammonium thiocyanate has also been suggested.[58] Potentiometric and "dead-stop" end-point detection methods have been applied to the argentometric titration of TPB salts.

TPB salts react with mercuric chloride to liberate hydrochloric and boric acids in the following reaction:

$$R_4NB(C_6H_5)_4 + 4HgCl_2 + 3H_2O \rightarrow R_4NCl + 4C_6H_5HgCl + 3HCl + H_3BO_3 \quad (9.48)$$

The hydrochloric acid liberated can be determined in the presence of the boric acid. The procedure[59] involves boiling an acetone solution of the TPB salt with an excess of mercuric chloride solution and a known amount of

standardized base. Potassium iodide is then added to form an inactive mercuric iodide complex and thereby prevent interference by the excess mercuric chloride. The excess standard base not neutralized by the liberated hydrochloric acid is then titrated with standard acid.

Nonaqueous titrations have been applied to TPB salts in polar organic solvents. For example, local anesthetic TPB salts were titrated in an acetone-acetic anhydride solution with perchloric acid.[52] Neither the TPB ion nor its decomposition products are acidic enough to interfere with the titration. Thus, the TPB ion does not enter into the titration and serves only as a method for isolating and concentrating the amine. The stoichiometry of the titration depends upon the basic groups of the parent amine.

Titration procedures for TPB salts have also been developed based upon end-point detection of quaternary amines-dye complex formation. The procedure was originally developed for the determination of potassium.[60] A known amount of standardized sodium TPB solution is added to the sample and is followed by titration of the excess of the TPB reagent in an aliquot of the filtrate. This aliquot is titrated with the quaternary amine, benzalkonium chloride, with bromophenol blue or a similar anionic dye as the indicator. Thus, the benzalkonium chloride reacts with the excess TPB ion and is prevented from forming a complex with the dye, with a resulting change in color, until the end point is reached. Variations of the potassium titration have also been applied to the analysis of quaternary amines.[61]

3. Spectrophotometric Determinations

Although spectral studies of TPB salts particularly in the infrared region are primarily of qualitative interest, they also can serve as quantitative procedures based upon the intensity of absorbance. The ultraviolet spectra of TPB in alcohol or acetonitrile solution are especially useful for the microdetermination of amines. The ultraviolet spectra of TPB salts are a summation of the spectra of parent amine and TPB ion. It follows that the molecular weight of an amine can be estimated by comparison of the absorptivity of its TPB salt to the molar absorptivity of the TPB ion.

4. Amperometric Titrimetric Procedures

Several indirect amperometric titration procedures involving titrations of an excess TPB reagent by polarographically active titrants have been proposed. The first direct TPB amperometric titration involved the determination of potassium and depended upon the reaction of TPB ion with positively charged mercury at a dropping mercury electrode.[62] The discovery of the electrochemical oxidation of TPB at a graphite electrode has been used for the amperometric titration of potassium[63] and also has been applied to the determination of sympathomimetic amines.[64]

9.7 EXPERIMENTAL PROCEDURES

Azeotropic Distillation Determination of Moisture

REFERENCE: *USP*, XVII, p. 925

To an accurately weighed sample which will yield from 2 to 4 ml of water in a 500 ml round-bottom flask is added about 200 ml of toluene. The flask is attached to a reflux condenser by means of a Dean–Stark trap. The mixture is heated to produce distillation at a rate of about 2 drops per sec until the majority of water has been removed. The rate of distillation is then increased to 4 drops per sec until no more water appears to be collecting in the trap. Any water held in the apparatus above the graduated scale of the trap is rinsed and brushed into the trap and the distillation is continued for at least 5 min more. After the contents of the trap have cooled to room temperature and the water phase has completely separated from the toluene, the volume of the lower water phase is read and the percentage of water in the original sample is calculated.

Karl Fischer Moisture Determination

REFERENCE: *NF*, XII, p. 515

In a titration assembly which prevents interference by atmospheric moisture, titrate 25 ml of anhydrous methanol to the end point with standardized Karl Fischer reagent. The volume of Karl Fischer reagent required to reach this end point is disregarded. Add the sample to be measured (containing 10 to 50 mg of water) and while the mixture is stirring vigorously, titrate with Karl Fischer reagent.

The reagent is standardized by the same procedure and by either adding about 250 mg of sodium tartrate ($Na_2C_4H_4O_6 \cdot 2H_2O$) accurately weighed or by using 25 ml of a 0.2% solution of water in methanol. If the latter secondary standardization procedure is employed, the volume of Karl Fischer reagent used must be corrected by a blank titration on a 25-ml sample of the same methanol used to make the water-methanol solution.

End-point detection can be on the basis of a color change from pale yellow to dark brown or when polarized platinum electrodes are used on the basis of a "dead-stop" increase in current which lasts for at least 30 sec. An increase in potential for at least 30 sec can be used when the platinum electrodes are employed in a potentiometric titration at constant current.

Sulfonamide Diazotization Assay

REFERENCE: *USP*, XVII, p. 882; J. P. La Rocca and K. L. Water, *J. Am. Pharm. Assoc.*, 39, 521 (1950)

To an accurately weighed sample of about 500 mg of sulfonamide in a beaker is added 20 ml of hydrochloric acid and 50 ml of water. After the

sulfonamide is dissolved, the mixture is cooled to 15° and then about 25 g of crushed ice is added. The sulfonamide is titrated slowly with 0.1 *M* sodium nitrite accompanied by continuous and vigorous stirring.

The end point of the titration can be determined by streaking a glass rod which has been dipped into the titration solution across starch-iodide paste. A blue color indicates an excess of sodium nitrite and, if the color is reproducible after the mixture has stood for 1 min, this is the indication of the end point of the titration. The end point can also be determined potentiometrically using a platinum-calomel electrode system.

The Determination of Primary Aromatic Amines by an Amine Coupling Reagent

REFERENCES: A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, 128, 537 (1939); F. J. Bandelin and C. R. Kemp, *Ind. Eng. Chem., Anal. Ed.*, 18, 470 (1946)

Pipette a 5-ml sample of 0.001 to 0.005% aqueous solution of an amine salt such as benzocaine hydrochloride into a 50-ml volumetric flask containing 5 ml of 4 *N* sulfuric acid. Add 1 ml of a 0.1% solution of sodium nitrite and allow to stand 3 min before decomposing the excess nitrous acid by reaction with 5 ml of alcohol. After the mixture has stood for another 2 min, add 1 ml of a 0.1% N-(1-naphthyl)ethylenediamine solution, mix, and add water to volume. After 5 min, determine absorbance at the absorbance maximum for the compound under investigation. Concentration is determined by comparison to the absorbance produced by a series of known concentrations of reference material.

Mercuric Acetate Titration of Methylthiouracil

REFERENCE: *BP*, 1963, p. 502

An accurately weighed quantity of about 0.35 g of methylthiouracil is dissolved by warming in a mixture of 50 ml of 0.1 *N* sodium hydroxide and 200 ml of water. After cooling the solution, 10 g of sodium acetate is added and the solution acidified to a litmus paper end point with acetic acid. One milliliter of freshly prepared 0.5% w/v alcoholic solution of diphenylcarbazone is added and the mixture titrated with 0.1 *N* mercuric acetate solution until a rose-violet color lasts for 2 to 3 min.

Kjeldahl Determination

REFERENCE: *NF*, XII, p. 468

To a 500-ml Kjeldahl flask containing 1 g of substance accurately weighed is added 10 g of powdered potassium sulfate or anhydrous sodium sulfate, 500 mg of powdered cupric sulfate, and 20 ml of sulfuric acid. While the flask is inclined at a 45° angle, the mixture is gently heated until frothing has

ceased. Then heating is increased until the acid boils vigorously. Heating is continued at this rate until the solution has been a clear green color for 30 min. The flask is cooled and 150 ml of water is slowly added with continuous mixing. After the flask has been recooled, 100 ml of a 2 in 5 sodium hydroxide solution is added cautiously down the side of the inclined flask so as to form a layer of sodium hydroxide solution below the acidic digestion mixture. Several pieces of granulated zinc are added to the mixture and the flask is connected immediately to the connecting bulb of a previously assembled distilling apparatus. The distillation apparatus consists of a Kjeldahl connecting bulb and condenser with the delivery tube of the condenser dipping beneath the surface of 50 ml of a 1 in 25 boric acid solution in a 500-ml Erlenmeyer flask.

The contents of the Kjeldahl flask are gently but completely mixed before heating to distill about two-thirds of the contents of the flask into the boric acid solution. The tip of the delivery tube is washed into the boric acid solution and the ammonia is titrated with 0.5 *N* sulfuric acid using methyl red-methylene blue solution as the indicator. If a low nitrogen percentage is to be expected, 0.1 *N* sulfuric acid is used in place of the 0.5 *N* solution. The results are corrected for any contribution of a blank determination.

When nitrates or nitrites are present, a preliminary reduction by a mixture of 1 g of salicylic acid in 25 ml of sulfuric acid for a sample corresponding to 150 mg of nitrogen is required. After the mixture has stood in the Kjeldahl flask for 30 min with frequent shaking, 5 g of powdered sodium thiosulfate is added and the system mixed. Then 500 mg of cupric sulfate is added and the digestion continued as above.

Sodium Tetraphenylborate; Quaternary Amine Titration

REFERENCE: D. M. Patel and R. A. Anderson, *Drug Standards*, 26, 189 (1958)

About 300 mg of quaternary amine accurately weighed is dissolved in about 75 ml of water in a 250-ml Erlenmeyer flask. Add 0.4 ml of a bromophenol blue solution which has previously been made by dissolving 100 mg of bromophenol blue in 3.0 ml of 0.05 *N* NaOH and diluting to 200 ml with water. Add 1 ml of 1 *N* sodium hydroxide and 10 ml of chloroform; titrate with a 0.02-*M* sodium tetraphenylborate solution to the disappearance of the blue color from the chloroform layer. As the end point is approached, the titrant is added dropwise and frequent shaking is required.

QUESTIONS

Q9.1. Name specific pharmaceutical problems in which the quantitative determination of water may be of importance.

Q9.2. A 290.0-mg sample of sodium tartrate ($C_4H_4Na_2O_6 \cdot 2H_2O$) requires 10.80 ml of Karl Fischer reagent to titrate to the end point. If 15.40 ml of this same

reagent is required to titrate the water in a 250-mg sample of a medicinal compound, what is the percentage of water in the compound?

Q9.3. The reaction of the dimethyl acetal of acetone with water has been used for the determination of water. A literature method uses the carbonyl absorption at 5.7 μ as the basis for such a determination. Suggest other procedures for using this reaction for the quantitative determination of water.

Q9.4. A primary aromatic amine can be determined by several different procedures based upon the reaction of the amine with nitrous acid. Briefly outline these procedures.

Q9.5. A 0.9536-g sample in the Kjeldahl procedure requires 15.45 ml of 0.5045 *N* sulfuric acid to titrate the ammonia produced in the procedure. What is the percentage of nitrogen in the sample?

Q9.6. In the Kjeldahl determination contrast the use of boric acid for trapping the ammonia liberated to the use of hydrochloric or sulfuric acid. Why is it possible to titrate the ammonia directly with standard acid only in the boric acid procedure?

Q9.7. Amphetamine will form a water-insoluble tetraphenylborate salt. Suggest analytical procedures based upon this fact.

Q9.8. Both catalytic hydrogenation and mercuric acetate titration have been described for the determination of unsaturation. Compare the scope of applications and general advantages and disadvantages of the two procedures.

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