If a viscosimeter is repaired, it must be recalibrated, since even minor repairs frequently cause significant changes in the value of its constant, k.

⟨921⟩ WATER DETERMINATION

Many Pharmacopeial articles either are hydrates or contain water in adsorbed form. As a result, the determination of the water content is important in demonstrating compliance with the Pharmacopeial standards. Generally one of the methods given below is called for in the individual monograph, depending upon the nature of the article. In rare cases, a choice is allowed between two methods. When the article contains water of hydration, the Method I (Titrimetric), the Method II (Azeotropic), or the Method III (Gravimetric) is employed, as directed in the individual monograph, and the requirement is given under the heading *Water.*

The heading *Loss on drying* (see *Loss on Drying* ⟨731⟩) is used in those cases where the loss sustained on heating may be not entirely water.

METHOD I (TITRIMETRIC)

Determine the water by *Method Ia,* unless otherwise specified in the individual monograph.

Method Ia (Direct Titration)

Principle—The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as Karl Fischer Reagent, the sulfur dioxide and iodine are dissolved in pyridine and methanol. The test specimen may be titrated with the *Reagent* directly, or the analysis may be carried out by a residual titration procedure. The stoichiometry of the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative concentrations of the *Reagent* ingredients, the nature of the inert solvent used to dissolve the test specimen, and the technique used in the particular determination. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen; however, other suitable solvents may be used for special or unusual test specimens.

Apparatus—Any apparatus may be used that provides for adequate exclusion of atmospheric moisture and determination of the endpoint. In the case of a colorless solution that is titrated directly, the endpoint may be observed visually as a change in color from canary yellow to amber. The reverse is observed in the case of a test specimen that is titrated residually. More commonly, however, the endpoint is determined electrometrically with an apparatus employing a simple electrical circuit that serves to impress about 200 mV of applied potential between a pair of platinum electrodes immersed in the solution to be titrated. At the endpoint of the titration a slight excess of the reagent increases the flow of current to between 50 and 150 microamperes for 30 seconds to 30 minutes, depending upon the solution being titrated. The time is shortest for substances that dissolve in the reagent. With some automatic titrators, the abrupt change in current or potential at the endpoint serves to close a solenoid-operated valve that controls the buret delivering the titrant. Commercially available apparatus generally comprises a closed system consisting of one or two automatic burets and a tightly covered titration vessel fitted with the necessary electrodes and a magnetic stirrer. The air in the system is kept dry with a suitable desiccant, and the titration vessel may be purged by means of a stream of dry nitrogen or current of dry air.

Reagent—Prepare the Karl Fischer Reagent as follows. Add 125 g of iodine to a solution containing 670 mL of methanol and 170 mL of pyridine, and cool. Place 100 mL of pyridine in a 250-mL graduated cylinder, and, keeping the pyridine cold in an ice bath, pass in dry sulfur dioxide until the volume reaches 200 mL. Slowly add this solution, with shaking, to the cooled iodine mixture. Shake to dissolve the iodine, transfer the solution to the apparatus, and allow the solution to stand overnight before standardizing. One mL of this solution when freshly prepared is equivalent to approximately 5 mg of water, but it deteriorates gradually; therefore, standardize it within 1 hour before use, or daily if in continuous use. Protect from light while in use. Store any bulk stock of the reagent in a suitably sealed, glass-stoppered container, fully protected from light, and under refrigeration.

A commercially available, stabilized solution of Karl Fischer type reagent may be used. Commercially available reagents containing solvents or bases other than pyridine or alcohols other than methanol may be used also. These may be single solutions or reagents formed in situ by combining the components of the reagents present in two discrete solutions. The diluted *Reagent* called for in some monographs should be diluted as directed by the manufacturer. Either methanol or other suitable solvent, such as ethylene glycol monomethyl ether, may be used as the diluent.

Test Preparation—Unless otherwise specified in the individual monograph, use an accurately weighed or measured amount of the specimen under test estimated to contain 2 to 250 mg of water. The amount of water depends on the water equivalency factor of the *Reagent* and on the method of endpoint determination. In most cases, the minimum amount of specimen, in mg, can be estimated using the formula:

$$FCV/KF$$

in which F is the water equivalency factor of the *Reagent,* in mg per mL; C is the used volume, in percent, of the capacity of the buret; V is the buret volume, in mL; and KF is the limit or reasonable expected water content in the sample, in percent. C is generally between 30% and 100% for manual titration, and between 10% and 100% for the instrumental method endpoint determination. [NOTE—It is recommended that the product of FCV be greater than or equal to 200 for the calculation to ensure that the minimum amount of water titrated is greater than or equal to 2 mg.]

Where the specimen under test is an aerosol with propellant, store it in a freezer for not less than 2 hours, open the container, and test 10.0 mL of the well-mixed specimen. In titrating the specimen, determine the endpoint at a temperature of 10° or higher.

Where the specimen under test is capsules, use a portion of the mixed contents of not fewer than 4 capsules.

Where the specimen under test is tablets, use powder from not fewer than 4 tablets ground to a fine powder in an atmosphere of temperature and relative humidity known not to influence the results.

Where the monograph specifies that the specimen under test is hygroscopic, use a dry syringe to inject an appropriate volume of methanol, or other suitable solvent, accurately measured, into a tared container, and shake to dissolve the specimen. Using the same syringe, remove the solution from the container and transfer it to a titration vessel prepared as directed for *Procedure.* Repeat the procedure

with a second portion of methanol, or other suitable solvent, accurately measured, add this washing to the titration vessel, and immediately titrate. Determine the water content, in mg, of a portion of solvent of the same total volume as that used to dissolve the specimen and to wash the container and syringe, as directed for *Standardization of Water Solution for Residual Titrations,* and subtract this value from the water content, in mg, obtained in the titration of the specimen under test. Dry the container and its closure at 100° for 3 hours, allow to cool in a desiccator, and weigh. Determine the weight of specimen tested from the difference in weight from the initial weight of the container.

Standardization of the Reagent—Place enough methanol or other suitable solvent in the titration vessel to cover the electrodes, and add sufficient *Reagent* to give the characteristic endpoint color, or 100 ± 50 microamperes of direct current at about 200 mV of applied potential.

For determination of trace amounts of water (less than 1%), it is preferable to use a *Reagent* with a water equivalency factor of not more than 2.0. *Purified Water,* sodium tartrate dihydrate, a USP Reference Standard, or commercial standards with a certificate of analysis traceable to a national standard may be used to standardize the *Reagent.* The reagent equivalency factor, the recommended titration volume, buret size, and amount of standard to measure are factors to consider when deciding which standard and how much to use.[1] For *Purified Water* or water standards, quickly add the equivalent of between 2 and 250 mg of water. Calculate the water equivalency factor, F, in mg of water per mL of reagent, by the formula:

$$W/V$$

in which W is the weight, in mg, of the water contained in the aliquot of standard used; and V is the volume, in mL, of the *Reagent* used in the titration. For sodium tartrate dihydrate, quickly add 20 to 125 mg of sodium tartrate dihydrate ($C_4H_4Na_2O_6 \cdot 2H_2O$), accurately weighed by difference, and titrate to the endpoint. The water equivalence factor F, in mg of water per mL of reagent, is given by the formula:

$$W/V\ (36.04/230.08)$$

in which 36.04 is two times the molecular weight of water and 230.08 is the molecular weight of sodium tartrate dihydrate; W is the weight, in mg, of sodium tartrate dihydrate; and V is the volume, in mL, of the *Reagent* consumed in the second titration. Note that the solubility of sodium tartrate dihydrate in methanol is such that fresh methanol may be needed for additional titrations of the sodium tartrate dihydrate standard.

Procedure—Unless otherwise specified, transfer enough methanol or other suitable solvent to the titration vessel, ensuring that the volume is sufficient to cover the electrodes (approximately 30 to 40 mL), and titrate with the *Reagent* to the electrometric or visual endpoint to consume any moisture that may be present. (Disregard the volume consumed, because it does not enter into the calculations.) Quickly add the *Test Preparation,* mix, and again titrate with the *Reagent* to the electrometric or visual endpoint. Calculate the water content of the specimen, in mg, taken by the formula:

$$SF$$

in which S is the volume, in mL, of the *Reagent* consumed in the second titration; and F is the water equivalence factor of the *Reagent.*

[1] Consider a setup in which the reagent equivalency factor is 5 mg/mL, and the buret volume is 5 mL and an instrumental endpoint. Standard amounts equivalent to between 2.5 mg and 22.5 mg of water (10% to 90% of buret capacity) could be used based on the buret and the reagent equivalency factor. The upper end of this range would involve an excessive amount of sodium tartrate dihydrate. If *Purified Water* or a standard is weighed, an analytical balance appropriate to the amount weighed is required.

Method Ib (Residual Titration)

Principle—See the information given in the section *Principle* under *Method Ia.* In the residual titration, excess *Reagent* is added to the test specimen, sufficient time is allowed for the reaction to reach completion, and the unconsumed *Reagent* is titrated with a standard solution of water in a solvent such as methanol. The residual titration procedure is applicable generally and avoids the difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly.

Apparatus, Reagent, and **Test Preparation**—Use *Method Ia.*

Standardization of Water Solution for Residual Titration—Prepare a *Water Solution* by diluting 2 mL of water with methanol or other suitable solvent to 1000 mL. Standardize this solution by titrating 25.0 mL with the *Reagent,* previously standardized as directed under *Standardization of the Reagent.* Calculate the water content, in mg per mL, of the *Water Solution* taken by the formula:

$$V'F/25$$

in which V′ is the volume of the *Reagent* consumed, and F is the water equivalence factor of the *Reagent.* Determine the water content of the *Water Solution* weekly, and standardize the *Reagent* against it periodically as needed.

Procedure—Where the individual monograph specifies that the water content is to be determined by *Method Ib,* transfer enough methanol or other suitable solvent to the titration vessel, ensuring that the volume is sufficient to cover the electrodes (approximately 30 to 40 mL), and titrate with the *Reagent* to the electrometric or visual endpoint. Quickly add the *Test Preparation,* mix, and add an accurately measured excess of the *Reagent.* Allow sufficient time for the reaction to reach completion, and titrate the unconsumed *Reagent* with standardized *Water Solution* to the electrometric or visual endpoint. Calculate the water content of the specimen, in mg, taken by the formula:

$$F(X' - XR)$$

in which F is the water equivalence factor of the *Reagent*; X′ is the volume, in mL, of the *Reagent* added after introduction of the specimen; X is the volume, in mL, of standardized *Water Solution* required to neutralize the unconsumed *Reagent;* and R is the ratio, V′/25 (mL *Reagent*/mL *Water Solution*), determined from the *Standardization of Water Solution for Residual Titration.*

Method Ic (Coulometric Titration)

Principle—The Karl Fischer reaction is used in the coulometric determination of water. Iodine, however, is not added in the form of a volumetric solution but is produced in an iodide-containing solution by anodic oxidation. The reaction cell usually consists of a large anode compartment and a small cathode compartment that are separated by a diaphragm. Other suitable types of reaction cells (e.g., without diaphragms) may also be used. Each compartment has a platinum electrode that conducts current through the cell. Iodine, which is produced at the anode electrode, immediately reacts with water present in the compartment. When all the water has been consumed, an excess of iodine occurs, which usually is detected electrometrically, thus indicating the endpoint. Moisture is eliminated from the system by pre-electrolysis. Changing the Karl Fischer solution after each determination is not necessary because individual determinations can be carried out in succession in the same reagent solution. A requirement for this method is that each component of the test specimen is compatible with the other components, and no side reactions take place. Samples are usually transferred into the vessel as solutions by

means of injection through a septum. Gases can be introduced into the cell by means of a suitable gas inlet tube. Precision in the method is predominantly governed by the extent to which atmospheric moisture is excluded from the system; thus, the introduction of solids into the cell may require precautions, such as working in a glove-box in an atmosphere of dry inert gas. Control of the system may be monitored by measuring the amount of baseline drift. This method is particularly suited to chemically inert substances like hydrocarbons, alcohols, and ethers. In comparison with the volumetric Karl Fischer titration, coulometry is a micromethod.

Apparatus—Any commercially available apparatus consisting of an absolutely tight system fitted with the necessary electrodes and a magnetic stirrer is appropriate. The instrument's microprocessor controls the analytical procedure and displays the results. Calibration of the instrument is not necessary, as the current consumed can be measured absolutely.

Reagent—See the manufacturer's recommendations.

Test Preparation—Where the specimen is a soluble solid, an appropriate quantity, accurately weighed, may be dissolved in anhydrous methanol or other suitable solvents.

Where the specimen is an insoluble solid, an appropriate quantity, accurately weighed, may be extracted using a suitable anhydrous solvent, and may be injected into the anolyte solution. Alternatively, an evaporation technique may be used in which water is released and evaporated by heating the specimen in a tube in a stream of dry inert gas. The gas is then passed into the cell.

Where the specimen is to be used directly without dissolving in a suitable anhydrous solvent, an appropriate quantity, accurately weighed, may be introduced into the chamber directly.

Where the specimen is a liquid, and is miscible with anhydrous methanol or other suitable solvents, an appropriate quantity, accurately weighed, may be added to anhydrous methanol or other suitable solvents.

Procedure—Using a dry device, inject or add directly an accurately measured amount of the sample or sample preparation estimated to contain between 0.5 and 5 mg of water, or an amount recommended by the instrument manufacturer into the anolyte, mix, and perform the coulometric titration to the electrometric endpoint. Read the water content of the liquid *Test Preparation* directly from the instrument's display, and calculate the percentage that is present in the substance. Perform a blank determination, as needed, and make any necessary corrections.

METHOD II (AZEOTROPIC—TOLUENE DISTILLATION)

Apparatus—Use a 500-mL glass flask A connected by means of a trap B to a reflux condenser C by ground glass joints (see *Figure 1*).

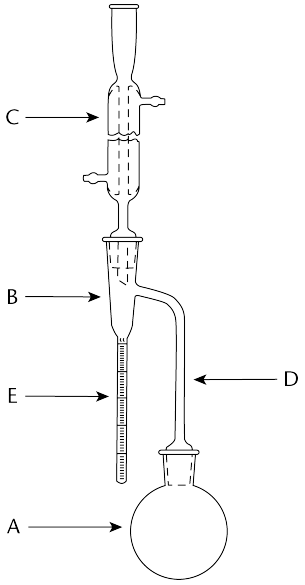

Figure 1. Toluene Moisture Apparatus

The critical dimensions of the parts of the apparatus are as follows. The connecting tube D is 9 to 11 mm in internal diameter. The trap is 235 to 240 mm in length. The condenser, if of the straight-tube type, is approximately 400 mm in length and not less than 8 mm in bore diameter. The receiving tube E has a 5-mL capacity, and its cylindrical portion, 146 to 156 mm in length, is graduated in 0.1-mL subdivisions, so that the error of reading is not greater than 0.05 mL for any indicated volume. The source of heat is preferably an electric heater with rheostat control or an oil bath. The upper portion of the flask and the connecting tube may be insulated.

Clean the receiving tube and the condenser with chromic acid cleansing mixture, thoroughly rinse with water, and dry in an oven. Prepare the toluene to be used by first shaking with a small quantity of water, separating the excess water, and distilling the toluene.

Procedure—Place in the dry flask a quantity of the substance, weighed accurately to the nearest centigram, which is expected to yield 2 to 4 mL of water. If the substance is of a pasty character, weigh it in a boat of metal foil of a size that will just pass through the neck of the flask. If the substance is likely to cause bumping, add enough dry, washed sand to cover the bottom of the flask, or a number of capillary melting-point tubes, about 100 mm in length, sealed at the upper end. Place about 200 mL of toluene in the flask, connect the apparatus, and fill the receiving tube *E* with toluene poured through the top of the condenser. Heat the flask gently for 15 minutes and, when the toluene begins to boil, distill at the rate of about 2 drops per second until most of the water has passed over, then increase the rate of distillation to about 4 drops per second. When the water has apparently all distilled over, rinse the inside of the condenser tube with toluene while brushing down the tube with a tube brush attached to a copper wire and saturated with toluene. Continue the distillation for 5 minutes, then remove the heat, and allow the receiving tube to cool to room temperature. If any droplets of water adhere to the walls of the receiving tube, scrub them down with a brush consisting of a rubber band wrapped around a copper wire and wetted with toluene. When the water and toluene have separated completely, read the volume of water, and calculate the percentage that was present in the substance.

METHOD III (GRAVIMETRIC)

Procedure for Chemicals—Proceed as directed in the individual monograph preparing the chemical as directed under *Loss on Drying* ⟨731⟩.

Procedure for Biologics—Proceed as directed in the individual monograph.

Procedure for Articles of Botanical Origin—Place about 10 g of the drug, prepared as directed (see *Methods of Analysis* under *Articles of Botanical Origin* ⟨561⟩) and accurately weighed, in a tared evaporating dish. Dry at 105° for 5 hours, and weigh. Continue the drying and weighing at 1-hour intervals until the difference between two successive weighings corresponds to not more than 0.25%.

⟨941⟩ CHARACTERIZATION OF CRYSTALLINE AND PARTIALLY CRYSTALLINE SOLIDS BY X-RAY POWDER DIFFRACTION (XRPD)

INTRODUCTION

Every crystalline phase of a given substance produces a characteristic X-ray diffraction pattern. Diffraction patterns can be obtained from a randomly oriented crystalline powder composed of crystallites or crystal fragments of finite size. Essentially three types of information can be derived from a powder diffraction pattern: the angular position of diffraction lines (depending on geometry and size of the unit cell), the intensities of diffraction lines (depending mainly on atom type and arrangement, and particle orientation within the sample), and diffraction line profiles (depending on instrumental resolution, crystallite size, strain, and specimen thickness).

Experiments giving angular positions and intensities of lines can be used for applications such as qualitative phase analysis (e.g., identification of crystalline phases) and quantitative phase analysis of crystalline materials. An estimate of the amorphous and crystalline fractions[1] can also be made.

The X-ray powder diffraction (XRPD) method provides an advantage over other means of analysis in that it is usually nondestructive in nature (to ensure a randomly oriented sample, specimen preparation is usually limited to grinding). XRPD investigations can also be carried out under *in situ* conditions on specimens exposed to nonambient conditions such as low or high temperature and humidity.

PRINCIPLES

X-ray diffraction results from the interaction between X-rays and electron clouds of atoms. Depending on atomic arrangement, interferences arise from the scattered X-rays. These interferences are constructive when the path difference between two diffracted X-ray waves differs by an integral number of wavelengths. This selective condition is described by the Bragg equation, also called Bragg's law (see *Figure 1*).

$$2d_{hkl} \sin\theta_{hkl} = n\lambda$$

The wavelength, λ, of the X-rays is of the same order of magnitude as the distance between successive crystal lattice planes, or d_{hkl} (also called d-spacings). θ_{hkl} is the angle between the incident ray and the family of lattice planes, and $\sin\theta_{hkl}$ is inversely proportional to the distance between successive crystal planes or d-spacings.

The direction and spacing of the planes with reference to the unit cell axes are defined by the Miller indices {hkl}. These indices are the reciprocals, reduced to the next-lower integer, of the intercepts that a plane makes with the unit cell axes. The unit cell dimensions are given by the spacings a, b, and c, and the angles between them α, β, and γ.

The interplanar spacing for a specified set of parallel hkl planes is denoted by d_{hkl}. Each such family of planes may show higher orders of diffraction where the d values for the related families of planes nh, nk, nl are diminished by the factor 1/n (n being an integer: 2, 3, 4, etc.).

Every set of planes throughout a crystal has a corresponding Bragg diffraction angle, θ_{hkl}, associated with it (for a specific λ).

A powder specimen is assumed to be polycrystalline so that at any angle θ_{hkl} there are always crystallites in an orientation allowing diffraction according to Bragg's law.[2] For a given X-ray wavelength, the positions of the diffraction peaks (also referred to as "lines", "reflections", or "Bragg reflections") are characteristic of the crystal lattice (d-spacings), their theoretical intensities depend on the crystallographic unit cell content (nature and positions of atoms), and the line profiles depend on the perfection and extent of the crystal lattice. Under these conditions, the diffraction peak has a finite intensity arising from atomic arrangement, type of atoms, thermal motion, and structural imperfections, as well as from instrument characteristics.

The intensity is dependent upon many factors such as structure factor, temperature factor, crystallinity, polarization factor, multiplicity, and Lorentz factor.

The main characteristics of diffraction line profiles are 2θ position, peak height, peak area, and shape (characterized by, e.g., peak width, or asymmetry, analytical function, and empirical representation). An example of the type of powder patterns obtained for five different solid phases of a substance are shown in *Figure 2.*

In addition to the diffraction peaks, an X-ray diffraction experiment also generates a more or less uniform background, upon which the peaks are superimposed. Besides specimen preparation, other factors contribute to the background—for example, sample holder, diffuse scattering from air and equipment, and other instrumental parameters such as detector noise and general radiation from the X-ray tube. The peak-to-background ratio can be increased by minimizing background and by choosing prolonged exposure times.

[1] There are many other applications of the X-ray powder diffraction technique that can be applied to crystalline pharmaceutical substances, such as determination of crystal structures, refinement of crystal structures, determination of the crystallographic purity of crystalline phases, and characterization of crystallographic texture. These applications are not described in this chapter.

[2] An ideal powder for diffraction experiments consists of a large number of small, randomly oriented spherical crystallites (coherently diffracting crystalline domains). If this number is sufficiently large, there are always enough crystallites in any diffracting orientation to give reproducible diffraction patterns.