late the ratios, Q_{TA}, Q_{TB}, Q_{TC}, Q_{TD}, Q_{TE}, Q_{TF}, Q_{SA}, Q_{SB}, Q_{SC}, Q_{SD}, Q_{SE}, Q_{SF}, of the peak area of methanol, acetaldehyde, acetone, 2-propanol, 1-propanol and *t*-butanol to that of the internal standard. Q_{TA}, Q_{TB}, Q_{TC}, Q_{TD}, Q_{TE} and Q_{TF} are not larger than Q_{SA}, Q_{SB}, Q_{SC}, Q_{SD}, Q_{SE} and Q_{SF}, respectively.

Internal standard solution—A solution of tetrahydrofuran in ethanol (99.5) (1 in 500)

System operating conditions—

Detector: A hydrogen flame-ionization detector.

Packing: A globular porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (packed column)

Column temperature: A constant temperature of about 100°C.

Carrier gas: Nitrogen

System suitability test—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, methanol, acetaldehyde, ethanol, acetone, 2-propanol, 1-propanol, *t*-butanol and the internal standard are eluted in this order with well separation and with peak areas big enough to be determined. The peaks of the substances appear after ethanol may be shown on the tail of ethanol peak.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of methanol to that of the internal standard is not more than 10%.

68. Water Determination (Karl Fischer Method)

Water Determination is a method to determine water content in sample materials, utilizing the fact that water reacts with iodine and sulfur dioxide quantitatively in the presence of a lower alcohol such as methanol, and an organic base such as pyridine. The reaction proceeds in the manner shown in the following equation:

$$I_2 + SO_2 + 3C_5H_5N + CH_3OH + H_2O \rightarrow 2(C_5H_5N^+H)I^- + (C_5H_5N^+H)^-OSO_2OCH_3$$

In this measurement there are two methods different in iodine-providing principle: one is the volumetric titration method and the other, the coulometric titration method. In the former, iodine is previously dissolved in a reagent for water determination, and water content is determined by measuring the amount of iodine consumed as a result of reaction with water. In the latter, iodine is produced by electrolysis of Karl Fisher reagent containing iodide ion. Based on the quantitative reaction of the generated iodine with water, the water content in a sample specimen can be determined by measuring the quantity of electricity which is required for the production of iodine during the titration.

$$2I^- \rightarrow I_2 + 2e^-$$

1. Volumetric titration

Apparatus

Generally, the apparatus consists of automatic burettes, a titration flask, a stirrer, and equipment for amperometric titration at constant voltage or potentiometric titration at constant current.

The Karl Fischer TS is extremely hygroscopic, so the apparatus should be designed to be protected from atmospheric moisture. Desiccants such as silica gel or calcium chloride for water determination are used for moisture protection.

Reagents

(1) Chloroform for water determination—To 1000 mL of chloroform add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of chloroform. Preserve the chloroform, protecting it from moisture. The water content of this chloroform should not be more than 0.1 mg per mL.

(2) Methanol for water determination—To 1000 mL of methanol add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of methanol. Preserve the methanol, protecting it from moisture. The water content of this methanol should not be more than 0.1 mg per mL.

(3) Propylene carbonate for water determination—To 1000 mL of propylene carbonate add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear propylene carbonate layer. Preserve this protecting from moisture. The water content should not be more than 0.3 mg per mL.

(4) Diethylene glycol monoethyl ether for water determination—To 1000 mL of diethylene glycol monoethyl ether add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of diethylene glycol monoethyl ether. Preserve the diethylene glycol monoethyl ether, protecting it from moisture. The water content of this diethylene glycol monoethyl ether should not be more than 0.3 mg per mL.

(5) Pyridine for water determination—Add potassium hydroxide or barium oxide to pyridine, stopper tightly, and allow to stand for several days. Distill and preserve the purified and dried pyridine, protecting it from moisture. The water content of this pyridine should not be more than 1 mg per mL.

(6) Imidazole for water determination—Use imidazole for thin-layer chromatography, of which the water content should not be more than 1 mg per g.

(7) 2-Methylaminopyridine for water determination—Distill and preserve 2-methylaminopyridine, protecting it from moisture. The water content of this 2-methylaminopyridine should not be more than 1 mg per mL.

Preparation of test solutions and standard solutions

(1) Karl Fischer TS for water determination

Prepare according to the following method (i), (ii) or (iii).

(i) Preparation 1

Dissolve 63 g of iodine in 100 mL of pyridine for water determination, cool the solution in ice bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 32 g. Then make up to 500 mL by adding chloroform for water determination or methanol for water determination, and allow to stand for more than 24 hours before use.

(ii) Preparation 2

Dissolve 102 g of imidazole for water determination in 350 mL of diethylene glycol monoethyl ether for water determination, cool the solution in ice bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 64 g, keeping the temperature between 25°C and 30°C. Then dissolve 50 g of iodine in this solution, and allow to stand for more than 24 hours before use.

(iii) Preparation 3

Pass dried sulfur dioxide gas through 220 mL of propylene carbonate until the mass increase of the solvent reaches 32 g. To this solution, cooled in ice bath, add 180 mL of propylene carbonate or diethylene glycol monoethyl ether for water determination, in which 81 g of 2-methylaminopyridine for water determination is dissolved. Then dissolve 36 g of iodine in this solution, and allow to stand for more than 24 hours before use.

The Karl Fischer TS, prepared by any one of the methods described above, must be standardized before every use, because its activity for water determination changes with the lapse of time. Further preserve the TS in a cold place, protecting it from light and moisture.

Standardization—According to the procedure described below, take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the solvent with a Karl Fischer TS to make the content of the flask anhydrous. Then, add quickly about 30 mg of water weighed accurately to the solution in the flask, and titrate the water dissolved in the solvent with a Karl Fischer TS to the end point, under vigorous stirring. Calculate the water equivalence factor, f(mg/mL), corresponding to the amount of water (H_2O) in mg per 1 mL of the test solution by using the following equation:

$$f(\text{mg/mL}) = \frac{\text{Amount of water (H}_2\text{O) taken (mg)}}{\begin{array}{c}\text{Volume of Karl Fischer TS consumed}\\ \text{for titration of water (H}_2\text{O) (mL)}\end{array}}$$

(2) Standard water-methanol solution

Preparation—Take 500 mL of methanol for water determination in a dried 1000-mL volumetric flask, add 2.0 mL of water, and adjust with the methanol to make 1000 mL.

Perform the standardization of this solution, followed by the procedure for Karl Fischer TS. Preserve it in a cool place, protecting it from light and moisture.

Standardization—According to the procedure described below, take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Then, add exactly 10 mL of Karl Fischer TS to this solution in the flask, and titrate it with the prepared standard water-methanol solution to the end point. Calculate the water concentration in the standard water-methanol solution, f'(mg/mL), by using the following equation:

$$f'(\text{mg/mL}) = \frac{f(\text{mg/mL}) \times 10\ (\text{mL})}{\begin{array}{c}\text{Volume of the standard water-methanol}\\ \text{solution consumed for titration (mL)}\end{array}}$$

Procedure

As a rule, the titration of water with a Karl Fischer TS should be performed at the same temperature as that at which the standardization was done, with protection from moisture. The apparatus is equipped with a variable resistor in the circuit, and this resistor is manipulated so as to maintain a constant voltage (mV) between two platinum electrodes immersed in the solution to be titrated. The variable current (μA) can be measured (Amperometric titration at constant voltage). During titration with Karl Fischer TS, the current in the circuit varies noticeably, but returns to the original value within several seconds. At the end of a titration, the current stops changing and persists for a certain time (usually, longer than 30 seconds). When this electric state has been attained, it is designated as the end point of titration.

Otherwise, the manipulation of the resistor serves to pass a definite current between two platinum electrodes. The variable potential (mV) can be measured (Potentiometric titration at constant current). With the progress of titration of water with a Karl Fischer TS, the value indicated by the potentiometer in the circuit decreases suddenly from a polarization state of several hundreds (mV) to the non-polarization state, but it returns to the original value within several seconds. At the end of titration, the non-polarization state persists for a certain time (usually, longer than 30 seconds). When this electric state has been attained, it is designated as the end point of titration.

In the case of back titration, when the amperometric titration method is used at constant voltage, the needle of microammeter is out of scale during excessive presence of Karl Fischer TS, and it returns rapidly to the original position when the titration system has reached the end point. In the case of the potentiometric titration method at constant current in the back titration mode, the needle of the millivoltmeter is at the original position during excessive presence of Karl Fischer TS. Finally a definite voltage is indicated when the titration system has reached the end point.

Unless otherwise specified, the titration of water with Karl Fischer TS can be performed by either one of the following methods. Usually, the end point of the titration can be observed more clearly in the back titration method, compared with the direct titration method.

(1) Direct titration

Unless otherwise specified, proceed by the following method. Take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Take a quantity of sample specimen containing 5 to 30 mg of water, transfer it quickly into the titration flask, dissolve by stirring, and titrate the solution to be examined with Karl Fischer TS to the end point under vigorous stirring.

In the case of an insoluble sample specimen, powder the sample quickly, weigh a suitable amount of the sample containing 5 to 30 mg of water, and transfer it quickly into the titration vessel, stir the mixture for 5 - 30 minutes, protecting it from moisture, and perform a titration under vigorous stirring. Alternatively, in the case of a sample specimen which is insoluble in the solvent for water determination or which interfere with the Karl Fisher reaction, water in the sample can be removed by heating under a stream of nitrogen gas, and introduced into the titration vessel by using a water evaporation technique.

Though the titration procedure should be performed under atmospheric conditions at low humidity, if the effect of atmospheric moisture cannot be avoided, for instance, if a long time is required for extraction and titration of water, a blank test must be done under the same conditions as used for the sample test, and the data must be corrected, accordingly.

$$\text{Water } (H_2O)\ \% = \frac{\begin{array}{l}\text{Volume of Karl Fischer}\\ \text{TS consumed for}\\ \text{titration (mL)}\end{array} \times f(\text{mg/mL})}{\text{Amount of sample (mg)}} \times 100$$

(2) Back titration

Unless otherwise specified, proceed by the following method. Take a suitable quantity of methanol for water determination in the dried titration vessel, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Take a suitable quantity of sample specimen having 5 - 30 mg of water, transfer the sample quickly into the titration vessel, dissolve it in the solution by stirring, add an excessive and definite volume of Karl Fischer TS, and then titrate the solution with the standard water-methanol solution to the end point under vigorous stirring.

In the case of an insoluble sample specimen, powder the sample quickly, weigh a suitable amount accurately, transfer it quickly into the titration vessel, and add an excessive and definite volume of Karl Fischer TS. After stirring for 5 - 30 minutes, with protection from moisture, perform the titration under vigorous stirring.

$$\text{Water } (H_2O)\ \% = \frac{\left[\begin{array}{l}\text{Volume of Karl}\\ \text{Fischer TS}\\ \text{added (mL)}\end{array}\right] \times f(\text{mg/mL}) - \left[\begin{array}{l}\text{Volume of the standard water-}\\ \text{methanol solution consumed}\\ \text{for titration (mL)}\end{array}\right] \times f'(\text{mg/mL})}{\text{Amount of sample (mg)}} \times 100$$

2. Coulometric titration

Apparatus

Usually, the apparatus is comprised of a titration flask equipped with an electrolytic cell for iodine production, a stirrer, and a potentiometric titration system at constant current. The iodine production system is composed of an anode and a cathode, separated by a diaphragm. The anode is immersed in the anolyte solution for water determination and the cathode is immersed in the catholyte solution for water determination. Both electrodes are usually made of platinum-mesh.

Because both the anolyte and the catholyte solutions for water determination are strongly hygroscopic, the titration system should be protected from atmospheric moisture. For this purpose, silica gel or calcium chloride for water determination can be used.

Preparation of anolyte and catholyte solutions for water determination

Electrolytic solutions shall consist of an anolyte solution and a catholyte solution, the preparations of which are described below.

Preparation—Any of methods (1), (2), and (3) can be used for the preparation of the electrolytes for coulometric titration.

(1) Preparation 1

Anolyte for water determination—Dissolve 102 g of imidazole for water determination in 900 mL of methanol for water determination, cool the solution in ice bath, and pass dried sulfur dioxide gas through the solution, which is kept below 30°C. When the mass increase of the solution has reached 64 g, the gas flow is stopped and 12 g of iodine is dissolved by stirring. Then drop a suitable amount of water into the solution until the color of liquid is changed from brown to yellow, and add methanol for water determination to make up 1000 mL.

Catholyte for water determination—Dissolve 24 g of diethanolamine hydrochloride in 100 mL of methanol for water determination.

(2) Preparation 2

Anolyte for water determination—Dissolve 40 g of 1,3-di(4-pyridyl)propane and 30 g of diethanolamine in about 200 mL of methanol for water determination, and pass dried sulfur dioxide gas through the solution. When the mass increase of the solution has reached 25 g, the gas flow is stopped. Add 50 mL of propylene carbonate, and dissolve 6 g of iodine in the solution. Then make up the solution to 500 mL by addition of methanol for water determination and drop in a suitable amount of water until the color of liquid is changed from brown to yellow.

Catholyte for water determination—Dissolve 30 g of choline hydrochloride into methanol for water determination and adjust the volume to 100 mL by adding the methanol.

(3) Preparation 3

Anolyte for water determination—Dissolve 100 g of diethanolamine in 900 mL of methanol for water determination or a mixture of methanol and chloroform for water determination(3 : 1), and pass dried sulfur dioxide gas through the solution. When the mass increase of the solution has reached 64 g, the gas flow is stopped. Dissolve 20 g of iodine in the solution, and drop in a suitable amount of water until the color of liquid is changed from brown to yellow.

Catholyte for water determination—Dissolve 25 g of lithium chloride in 1000 mL of a mixture of methanol for water determination and nitromethane (4 : 1).

Procedure

Take a suitable volume of an anolyte for water determination in the titration vessel, immerse in this solution a pair of platinum electrodes for potentiometric titration at constant current. Then immerse the iodine production system filled with a catholyte for water determination in the anolyte solution. Switch on the electrolytic system and make the content of the titration vessel anhydrous. Next take an accurately weighed amount of a sample specimen containing 0.2 - 5 mg of water, add it quickly to the vessel, dissolve by stirring, and perform the titration to the end point under vigorous stirring.

When a sample specimen cannot be dissolved in the anolyte, powder it quickly, and add an accurately weighed amount of the sample estimated to contain 0.2 - 5 mg of water to the vessel. After stirring the mixture for 5 - 30 minutes, with protection from atmospheric moisture, perform the titration under vigorous stirring. Alternatively, in the case of an insoluble solid or a sample containing a component which interferes with the Karl Fisher reaction, water in the sample can be removed by heating, and carried by a nitrogen gas flow into the titration vessel, by using a water evaporation technique.

Determine the quantity of electricity (C) [= electric current (A) × time (s)] required for the production of iodine during the titration, and calculate the water content (%) in the sample specimen by use of the following equation.

Though the titration procedure should be performed under atmospheric conditions at low humidity, if the effect of atmospheric moisture cannot be avoided, for instance, if a long time is required for extraction and titration of water, a blank test must be done under the same conditions as used for the sample test, and the data must be corrected, accordingly.

$$\text{Water } (H_2O)\ \% = \frac{\text{Quantity of electricity required for iodine production (C)}}{10.72\ (\text{C/mg}) \times \text{Amount of sample (mg)}} \times 100$$

10.72: quantity of electricity corresponding to 1 mg of water (C/mg)

69. X-Ray Powder Diffraction Method

X-Ray powder diffraction measurement is a method for measuring characteristic X-ray diffraction angles and intensities from randomly oriented powder crystallites irradiated by a monochromated X-ray beam. In this diffraction process, electrons in the component atoms of crystallites are forced to vibrate by the incident X-ray beam, and make sharp coherent scatters of the X-ray. The diffraction pattern from a crystalline specimen is specific for the crystal form of the specimen. Therefore the X-ray powder diffraction method can be used for qualitative and quantitative evaluation of crystal forms, formation of polymorphism, and crystalline solvates. The random arrangement of molecules in non-crystalline substances makes them poor interference scatters of X-rays, resulting in broad, diffuse maxima in the diffraction patterns. Thus, for non-crystalline substances and/or materials with poor crystallinity, the utility of this method is limited. However, the X-ray diffraction method can be used for estimating the crystallinity of materials like plastics.

In crystalline materials, the molecular and atomic species are ordered in a three-dimensional array, called a lattice. The smallest lattice is designated the unit cell in crystallography. A plane in the crystal can be defined by three arbitrary lattice points and there is a family of planes separated by a certain interplanar spacing from each other. Thus a family of planes is called a crystalline plane and is usually indexed by using Miller indices (*hkl*). These indices are the reciprocals, reduced to smallest integers, of the intercepts that a plane makes along the three axes composing the unit cell. Interplanar spacing for a set of parallel planes (*hkl*) is denoted by d_{hkl}.

The occurrence of X-ray diffraction and the direction of the diffracted beam by a crystallographic plane obey the Bragg's law. According to this law, X-ray diffraction occurs only when the scattered beams in a specific direction travel distances that differ by integral multiples (n), the order of the reflection, of the incident X-ray wavelength (λ). This condition is described by the equation,

$$2\, d_{hkl} \sin \theta = n\lambda$$

in which d_{hkl} denotes the interplanar spacing and θ is the angle of diffraction. The diffracted angle $2\ \theta$ due to a plane (*hkl*) is specified by the intrinsic interplanar spacing, defined by the lattice constants, and the wavelength of the incident X-ray beam.

The intensity of a coherent X-ray diffraction is dependent upon structure factors, temperature ractor, degree of crystallinity, volume and density of the powdered specimen, absorption characteristics, intensity and wavelength of the incident X-ray beam, polarization factor, multiplicity, Lorentz factor, etc. Among these factors, the polarization factor is dependent upon the monochromatizing method of the incident X-ray beam, the Lorentz factor upon geometrical eactors of the apparatus, multiplicity factor upon the crystalline systems, absorption factor upon the component atoms of the sample, crystallinity upon experimental temperature end physical properties of the specimen, and structural factor upon the position of each atom in the unit cell and atomic species.

Apparatus

Usually an X-ray diffractometer equipped with a radiation detector is used for X-ray powder diffraction measurement. The diffractometer is composed of an X-ray source, goniometer, radiation detector, and the combined controller and data processor.

X-Ray radiation sources are usually vacuum tubes, in which heated electrons are emitted from a cathode, and impinge violently against an anode under a high electric field. Since the wavelength of the generated X-rays depend upon the type of metal utilized as the anode, an appropriate anode has to be selected for the specimen to be analyzed. In general, since the generated X-rays have a wide spectrum of wavelength, an appropriate filter element or crystal monochromator must be chosen so that a monochromated beam is practically used for the diffraction analysis.

The goniometer is an angle scanning apparatus for adjusting two angles, that between the X-ray beam and the specimen surface, and that between the specimen surface and the detector. It is usually scanned with the above two angles being held equal, which is called the symmetrical reflection technique. The goniometer is equipped with a filter or a monochromator for selecting a specific X-ray beam. Further equipment may be included for heating or cooling sample specimens.

A counting apparatus is composed of a detector and a computing apparatus, of which the former converts the intensity of scattered X-rays to electrical signals, while the latter converts the obtained signals to diffraction intensity values. A proportional counter or a scintillation counter is usually used as the detector.

A combination of controller and data processor is used for controlling the goniometer angle, for recording diffraction intensity, and for data processing.

Operation Procedure

The following variables have to be selected and/or determined before performing a diffraction measurement for a given specimen: anode type, electric current and voltage for the X-ray vacuum tube, scanning speed and range of the goniometer, time constant and so on. A copper anode is most commonly employed for organic substances and polymers.

A powdered specimen is usually packed and prepared in a