rent (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 ($\lambda$). This condition is described by the equation,

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

in which $d_{hkl}$ denotes the interplanar spacing and $\theta$ 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 specimen holder made of aluminum or glass. As a rule, the orientation of sample crystallites have to be randomized before packaging. The specimen may be ground in an agate mortar to a fine powder in order to randomize the orientation of crystallites. However, this grinding method is sometimes inappropriate due to the physical characteristics of a specimen or the measurement object.

In setting up the specimen and apparatus, coplanarity of the specimen surface with the specimen holder surface and the setting of the specimen holder at the position of symmetric reflection geometry have to be assured. Further it should be noted that the grinding procedure may affect the crystallinity of the specimen and the packaging pressure on the specimen holder may induce orientation of the crystallites.

**Identification and/or Judgement**

Identification of the specimen with the standard material can be accomplished by comparing the X-ray powder diffraction patterns with each other. Judgement of polymorphism and crystalline solvates can be done by comparison of the diffraction pattern obtained for the specimen with that of the reference material or the same material measured previously.

Comparison of two X-ray diffraction patterns should be based on the intensity ratio of diffracted peaks, and the interplanar spacings $d$. The intensity ratio is defined by the ratio of the peak intensity of a particular diffraction angle to the intensity of the standard peak, for which the strongest maxima in the diffraction pattern is usually selected. However, the diffraction angle $2\theta$ can be used as a basis for the identification, where the same wavelength of the radiation beam is utilized for the diffraction measurement of the sample and reference material. The scanning angle range for diffraction measurement is usually between 5° and 40° for ordinary organic substances, except where it is specified in particular *Monographs*. Based on the obtained X-ray diffraction patterns, the identification of a specimen with a standard material can be confirmed, if the diffraction pattern for the specimen gives diffraction peaks of the same intensity at the same diffraction angle $2\theta$, as those of the standard. If two powder crystallites ascribed to the same substance have the same crystal form, the X-ray diffraction angles should agree within $\pm 0.2°$.

**Assay**

A quantitative analysis by X-ray powder diffraction does not give a sufficiently precise result. Thus, the quantitative application of this method is limited to a few analytical problems: numerical estimation of degree of polymorphism, solvation number for crystalline solvates, and degree of crystallinity.

For a quantitative analysis of polymorphism and/or solvate, an appropriate diffraction peak has to be selected. Usually, the calibration curve method can be applied to the quantitative estimation by the X-ray analysis. Before measurement of the diffraction intensity for a sample specimen at a selected diffraction peak, a calibration curve must be prepared under the same conditions, using a series of standard samples containing known amounts of the objective substance.

Alternatively the internal standard method can also be effective in place of the above standard method. A known amount of internal standard is usually added to weighed amounts of a sample to be analyzed. Diffraction intensity ratios of the specimen to the internal standard are measured. Separately, a calibration curve for the intensity ratio against the mixing ratio of the reference material to the internal standard are prepared under the same conditions. By using the calibration curve, a quantitative analysis is possible in X-ray powder diffraction measurement. If more than two diffraction peaks ascribed to different lattice planes ($hkl$) are used, the influence of orientation of crystallites can be detected. The internal standard should have approximately the same density as the specimen and similar absorption characteristics with regard to the X-ray beam. Further the diffraction peak given by the standard should not overlap with that of the specimen to be analyzed.

Caution: Handle the apparatus with great care since X-ray may affect the human health.

# 70. Reference Standards; Reagents, Test Solutions; Standard Solutions for Volumetric Analysis; Standard Solutions; Matching Fluids for Color; Optical Filters for Wavelength and Transmission Rate Calibration; and Measuring Instruments, Appliances

Reference standards are substances which are prepared to have definite purity or definite biological action, and are used when drugs are tested physically, chemically or biologically.

Reagents are chemicals which are used in the tests of the Japanese Pharmacopoeia. Those reagents described as standard substances for volumetric analysis, special class, first class or for water determination in the Pharmacopoeia conform to the specifications of standard reagents for volumetric analysis, special class ones, first class ones, or for water determination ones of the Japanese Industrial Standards, respectively, and the testing procedures refer to the Japanese Industrial Standards. When the name of a reagent used in the Pharmacopoeia differs from that used in the Japanese Industrial Standards, the latter is shown together. When there appears the indication, "Same as the namesake monograph," the reagent conforms to the specification of the individual monograph. As to reagents for which the testing procedures are given, proceed as directed for the testing procedures of the Pharmacopoeia.

Test solutions are solutions which are prepared to be used in the tests of the Pharmacopoeia.

Standard solutions for volumetric analysis are solutions of reagents of precisely known concentrations intended primarily for use in quantitative determinations.

Standard solutions are solutions to be used as the bases for comparison in the tests of the Pharmacopoeia.

Matching fluids for color are used as references for comparison of colors in the tests of the Pharmacopoeia.

Measuring instruments are instruments or machines used for the measurements in the tests of the Pharmacopoeia.

Appliances are instruments designed to render the condi-