Multi-purpose X-ray diffractometer equipped with $K\alpha_1$ optical system and *ab initio* powder crystal structure analysis

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1. Introduction

Crystal structure determination is an important part of understanding the physico-chemical properties of crystalline materials. Some organic compounds used in synthesizing pharmaceutical products display polymorphisms, having the same or similar chemical composition but different crystal structures. The pharmacological properties of these organic compounds may differ significantly among different polymorphs. Therefore, it is necessary to determine the crystal structures of the different materials.

The method of single crystal structure determination has generally been applied to characterize crystalline material. However, due to the poor quality of crystal, micro crystals or twin crystals, data collection and/or the single crystal structure analysis cannot be employed on some samples. Furthermore, even if single crystals with the good properties are obtained, there may be some restrictions to the measurement environment. For example, the crystals might have to be kept at low temperature or in the mother liquor. There is another method for determining single crystal structures, which uses powder diffraction data. Generally, it is easier to obtain a well-behaved powder sample than a single one.

Structure determination from powder diffraction data has been applied to powder samples of single crystals. This has been accelerated by the rapid progress in instruments and software. At the same time, however, the powder crystal structure determination has an intrinsic disadvantage. The problem is that diffraction peaks in a whole pattern profile observed from a powder crystal are sometimes difficult to be resolved because of overlapping of more than two reflections. One source of overlapping reflections is the use of $K\alpha_1$–$K\alpha_2$ radiation. Overlapping reflections can be caused by the X-ray wave length used or the optics. In other words, it is a key point to observe the whole pattern profile with less overlapping so that the structure determination from powder diffraction data can be successful.

A molecular complex containing more than two chemical compounds in a unit cell is called "cocrystal[(1)]". Presently, a cocrystal which includes an Active Pharmaceutical Ingredient (API) is often an ingredient for new pharmaceuticals. The physico-chemical properties, such as the solubility, the rate of solution and the hygroscopicity of API itself, of the cocrystal are suggested are designed to be superior to the native API. Thus, pharmaceutical companies have been trying to make a variety of cocrystals. It is necessary to determine the pharmaceutical properties for each of the chemical compounds contained in the cocrystals. Moreover, since a cocrystal may be difficult to produce in single crystal form, *ab initio* powder crystal structure determination is a necessary tool.

The separation of the characteristic $K\alpha$ radiation into the $K\alpha_1$ and $K\alpha_2$ components is often difficult because of the low angular resolution of the optics utilized in general laboratory instruments used in the powder diffraction measurement. As a result, the presence of profiles observed by $K\alpha_1$ and $K\alpha_2$ lines makes the structure determination from powder diffraction data more difficult. In the present technical note, a multi-purpose X-ray diffractometer equipped with a high-resolution multilayer mirror, eliminating the $K\alpha_2$ component, is introduced. Moreover, an application of *ab inito* power crystal structure analysis is also introduced, utilizing the above mentioned instrument. The high-resolution multilayer mirror system is "$K\alpha_1$ optical system".

2. X-ray diffractometer and optical system for powder diffraction measurement

2.1. Multi-purpose X-ray diffractometer

The powder diffraction data described in section 4

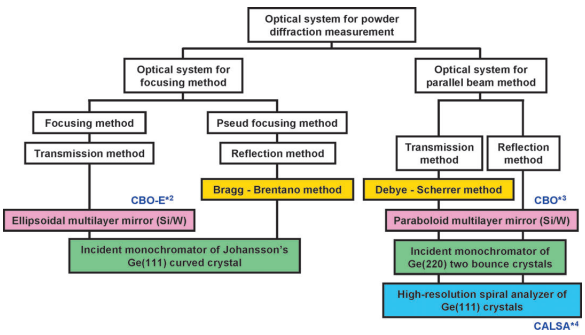

Fig. 1. Diagram of optical system used in SmartLab.

*1 Bragg-Brentano pseudo-focusing system
*2 CBO-E: optic to converge the incident X-ray beam
*3 CBO : optic to collimate the incident X-ray beam
*4 CALSA: analyzer optic to eliminate the defused X-ray on the receiving side

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Fig. 2. A unit of $K\alpha_1$ optical system using a Johansson's Ge(111) curved crystal.

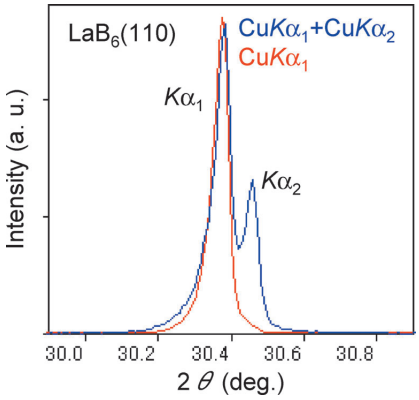

Fig. 3. Two profiles of 110 reflection of LaB_6 powder (NIST SRM 660a) observed with Bragg-Brentano's focusing system and with $K\alpha_1$ optical system, respectively.

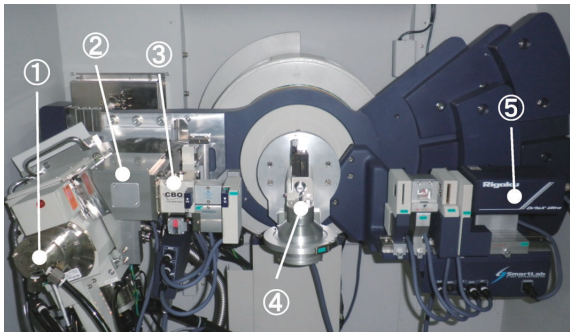

Fig. 4. Components of $K\alpha_1$ optical system.
① Rotating anode X-ray tube (45 kV−200 mA: 9 kW).
② Ge(111) curved crystal: Johansson's monochromator.
③ Multilayer ellipsoidal mirror (Si/W): CBO-E.
④ Capillary rotating sample stage.
⑤ Rapid one-dimensional detector: D/teX Ultra[3].

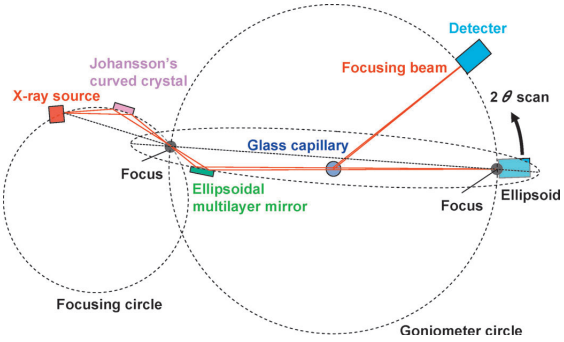

Fig. 5. A schematic view of $K\alpha_1$ optical system.

were collected with a fully-automated multi-purpose X-ray diffractometer, the SmartLab[2]. SmartLab is a reconfigurable system with the possibility of equipping with additional axes, such as an in-plane axis and a chi axis, or changing the X-ray generator type from a sealed X-ray tube to a rotating anode X-ray tube. Using the control software, SmartLab Guidance (SLG), an operator can select the optimal optical system for a sample category, such as a bulk, a thin film or a powder, and a measurement purpose according to the guidance given by SLG. Figure 1 shows a diagram of the optical system used in SmartLab.

2.2. $K\alpha_1$ optical system

In the *ab initio* powder crystal structure analysis described in the present article, the powder diffraction data was collected with an optical system designed to produce only $K\alpha_1$ radiation. This avoids the overlapping reflections due to characteristic $K\alpha_1$–$K\alpha_2$. In the $K\alpha_1$ optical system, the X-ray beam is converted into a monochromatic X-ray of Cu$K\alpha_1$ radiation by using an incident monochromator. Figure 2 shows a unit of the $K\alpha_1$ optical system using a Johansson's monochromator, a Ge(111) curved crystal. In Fig. 3, two profiles of 110 reflection of LaB_6 powder (NIST SRM 660a) observed with the standard Bragg-Brentano focusing system[*1] and with the $K\alpha_1$ optical system, respectively, are shown. The elimination of the $K\alpha_2$ line profile is clearly shown.

Figure 4 shows the $K\alpha_1$ optical system, consisting of the five optical components from ① to ⑤, which was utilized in the high-resolution measurement of this application. In Fig. 5, a schematic view of the $K\alpha_1$ optical system equipped with an ellipsoidal multilayer mirror (CBO-E[*2]) is given. In the optical configuration, the high-intensity X-ray beam is obtained with a rotating anode X-ray tube and the $K\alpha_1$ X-ray beam is extracted from the incident X-ray beam by the Johansson monochromator, which is focused on the detector plane with CBO-E[*2]. Furthermore, use of a one-dimensional detector, the D/teX Ultra, in place of a scintillation counter as shown in Fig. 5, allows for high-resolution and high-speed transmission geometry measurement for this powder diffraction experiment.

3. *Ab inito* powder crystal structure determination from powder diffraction data

3.1. Difference between diffraction data from a single crystal and a powder crystal

In Fig. 6, diffraction images observed from a single crystal and a powder crystal with a two-dimensional detector are shown. The diffraction image (Fig. 6(a)) from the single crystal is spotty, indicative of a single

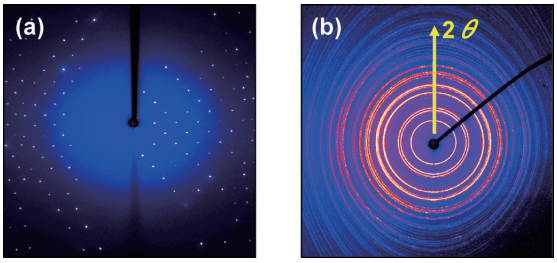

Fig. 6. Two dimensional diffraction images from (a) single crystal and (b) powder crystal.

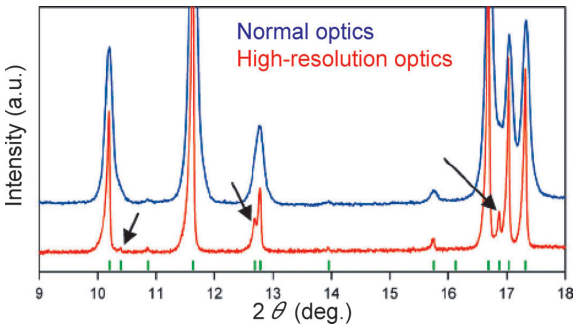

Fig. 7. Two powder diffraction patterns of indomethacin observed with a normal optic and the $K\alpha_1$ optical system, respectively.

crystal. However, the diffraction image (Fig. 6(b)) from the powder crystal is continuous and circular, which forms a so-called Debye-Scherrer ring, due to the random orientation of the numerous constituent crystalline grains. Therefore, in a powder diffraction profile, a 2θ–I diagram is the data and is given as a section along the radial direction from the center of the ring. Figure 7 shows two powder diffraction profiles observed for indomethacin: the blue profile is observed with a normal optical system and the red profile observed with a high resolution optical system. For the blue profile, couples of neighboring peaks are overlapped each other, so that the smaller of the overlapped peaks are hidden by the larger to be undetectable. However, for the red profile, those smaller peaks are clearly separated from the larger.

3.2. Rietveld analysis and *ab initio* powder crystal structure determination

Crystal structures of a large number of powder crystals have so far been determined by the Rietveld method[4] which was developed by H. M. Rietveld in 1969. The Rietveld method can be applied to a powder crystal for which the crystallographic information, such as the space group, the molecular formula, the lattice constants, etc., are known. In other words, it is necessary for the Rietveld analysis to have an initial crystal structure model.

On the other hand, the *ab initio* powder crystal structure determination is not necessary to have an initial crystal structure model.

3.3. *Ab initio* powder crystal structure determination

The software package PDXL[5], which is developed by Rigaku for unifying powder diffraction data processing, was used for determining the crystal structure of a mestanolone and salicylic acid cocrystal described in section 4. PDXL provides all the necessary tools for solving powder crystal structures.

Three indexing software programs, of ITO13[6], DICVOL06[7] and N-TREOR[8] are incorporated into PDXL. For determining powder crystal structures, any of the three methods, Direct Methods[9], Direct Space Method[10] and CF Mehtod (Charge Flipping Method)[11], can be applied. For Direct Methods EXPO2009[12], which was developed by C. Giacovazzo, *et al.*, is used in PDXL. In Fig. 8, basic procedures used for *ab initio* powder crystal structure determination are shown.

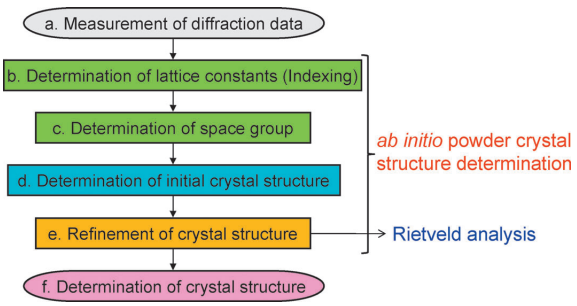

Fig. 8. Basic procedures used for *ab initio* powder crystal structure determination.

4. Successful example of *ab initio* powder crystal structure determination

4.1. Sample

In this section, *ab initio* powder crystal structure determination is demonstrated on mestanolone and salicylic acid cocrystal consisting of a protein assimilation hormone drug, mestanolone, and an antipyretic analgesic drug, salicylic acid. In Fig. 9, the molecular structures of mestanolone and salicylic acid are shown.

4.2. Analysis results

4.2.1. Determination of the lattice constants (Indexing)

The lattice constants of a powder sample are determined by indexing peaks which are found in a whole diffraction profile. Indexing produces a unit cell for the powder sample through analysis of reciprocal space unit vectors from the above peaks. Since the peaks in the high angle range in the powder diffraction data are subject to superposing with another peaks having the same diffraction angles, peaks, about 20 to 30, in the low angle range are usually utilized for the indexing. However, the edges of the peaks in the low angle range are subject to overlapping with those of the neighboring peaks due to the low resolution of the optic. Therefore, with the high resolution $K\alpha_1$ optical system, these peaks in the low angle range can be resolved. In this report,

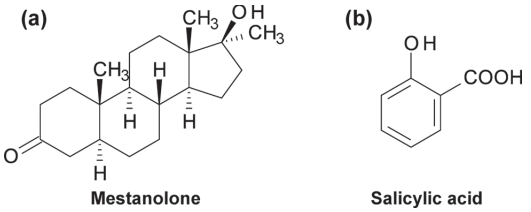

Fig. 9. Molecular structures of (a) mestanolone and (b) salicylic acid.

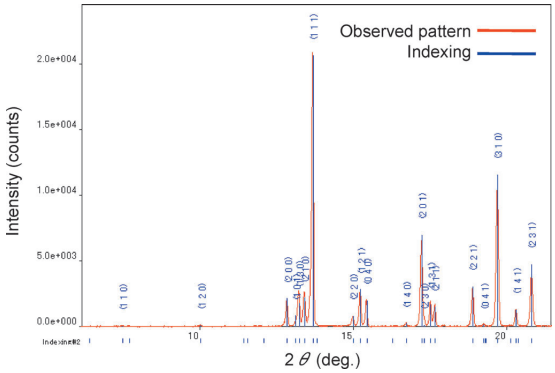

Fig. 10. An observed whole pattern profile and indexed peaks.

the indexing program N-TREOR embodied in PDXL was utilized as the first step in the *ab initio* powder crystal structure analysis of the cocrystal. The lattice constants, $a=7.724$ Å, $b=13.764$ Å and $c=22.920$ Å, are thus obtained. Figure 10 shows the observed whole diffraction profile and the indexing, indicating that all reflections in the cocrystal are reasonably indexed with the above lattice constants.

4.2.2. Determination of the space group

By examining the difference between the observed peaks and those calculated from the lattice constants obtained in indexing, the space group of the cocrystal was determined to be $P2_12_12_1$ based on extinction rules.

4.2.3. Determination of the initial crystal structure

The direct space method, particularly applicable to organic compounds, was used for initial structure determination. The direct space method is a technique in which parameters, such as the position of the gravity center, the orientation, the torsion angles at the rotatable bonds, etc., for an organic molecule are given from a calculated model in a unit cell, are refined. In other words, the whole diffraction profile is calculated so as to be consistent with the observed whole diffraction profile.

Next, the above refinement parameters for a molecular model of the cocrystal of the mestanolone and salicylic acid are calculated with the SA (Simulated Annealing method[13]) which is a global optimization method. As referred to the following sections, an initial crystal structure obtained is determined by taking account of the *R* factor (reliability factor) and the reasonability of the crystal structure.

Table 1. Lattice constants and reliability factors obtained from structure refinement.

Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Lattice constant	a = 7.723(5) Å	α = 90°
	b = 13.764(5) Å	β = 90°
	c = 22.928(5) Å	γ = 90°
Lattice volume	2437.09 Å^3	
Reliability factor	R_{wp} = 7.06 %, S = 1.18	

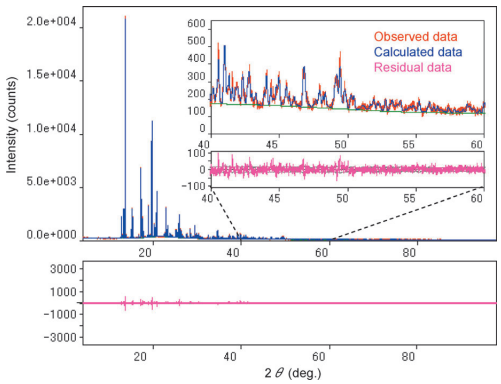

Fig. 11. Whole diffraction pattern obtained by the least squares refinement.

4.2.4. Refinement of the crystal structure

On the basis of the initial crystal structure determined with the direct space method, the parameters of the crystal structure such as the lattice constants are refined with the Rietveld method, and the crystal structure is finally determined by examining the reasonability. In Table 1, the lattice constants and the *R* factor are listed. In Fig. 11, a whole diffraction profile obtained by the least squares refinement is shown with the observed one. Figure 11 indicates that all peaks in the whole diffraction profile observed have a good agreement with those of the calculated whole diffraction profile. The R_{wp}*5 factor indicates the agreement of the observed and calculated values and the S*6 factor indicates the ratio of the reasonable R_{wp} factor to the obtained R_{wp} factor. We refined our structure to $R_{wp}=7.06\%$ and $S=1.18$.

4.2.5. Evaluation of the reasonability for the crystal structure

Generally, the *ab initio* powder crystal structure determination will not produce better results than the single crystal structure determination. Thus, evaluation of the reasonability for the crystal structure obtained is extremely important.

In an organic compound, when the individual atoms

*5 R_{wp}: index to show the residual of the observed and calculated data
*6 S: ratio of the R_{wp} obtained with analysis to the minimum value of R_{wp} obtained with statistics

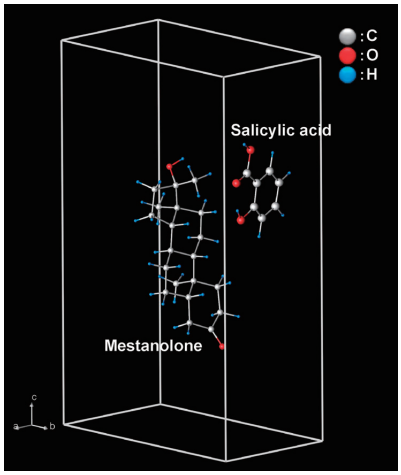

Fig. 12. Crystal structure of a mestanolone and salicylic acid cocrystal.

in the molecule are refined independently, number of the refinement parameters is increased, so that the bonding lengths and angles of the constituent atoms sometimes have been refined to unreasonable values. In PDXL, constraints (binding conditions) fixing the refinement parameters for atomic coordinates, temperature factors, bonding lengths, bonding angles, etc. are utilized. Rigid bodies may also be used. For example, the atomic positions of a benzene ring included in a molecule can be refined by treating the benzene ring as a rigid body with the binding condition.

The main points discussed in the analysis are shown below. In Fig. 12, the crystal structure of the mestanolone and salicylic acid cocrystal determined in the analysis is shown.

① Evaluation of the molecular structure

The bonding lengths, the bonding angles and the torsion angles in the crystal structure refined using PDXL were verified.

② Evaluation of the intermolecular force

When polarized groups such as −COOH, −OH, ＞C=O, etc. are included in a molecule, the bonding lengths and the bonding angles of the hydrogen bonds (O−H), formed between the molecules, must be examined. In the current analysis, it was verified that hydrogen bonds were formed between oxygen and hydrogen atoms in the cocrystal so as to stabilize the crystal structure.

③ Evaluation of the intermolecular distances and voids

By evaluating the atomic size in the analyzed molecules from the Van de Waals radiuses, the reasonable intermolecular distances and intermolecular voids were verified.

④ Finding the missing atoms

Some atoms in the initial crystal structure are not found in the analysis, however, electron density analysis of MEM (Maximum Entropy Method)[14),(15)] or Difference Fourier Synthesis enabled us to find them. In the current analysis, no missing atoms have been examined with the above method.

⑤ Positions of the hydrogen atoms

To determine the positions of hydrogen atoms, having only one electron, in the molecules with X-ray diffraction method is difficult. In the current analysis, the positions of the hydrogen atoms were assumed to be the chemically reasonable positions.

⑥ Evaluation of the mass density

It was verified that the mass density estimated from numbers of the atoms and of the atomic kinds in a unit cell was consistent with the mass density given from observation of the real cocrystal.

5. Conclusions

It has been a general technique that a powder crystal structure is determined from the powder diffraction data observed with a high resolution instrument in facilities such as SPring-8. However, in recent years, good quality data from a powder compound has come to be observed even using a laboratory instrument. Therefore, from the point of view of the working efficiency and the convenience, we have shown these experiments can be done at the home laboratory.

On the other hand, the single crystal structure determination has still been regarded as a general method for determining a crystal structure. Furthermore, data collection for an extremely small particle (～10μm) of single crystal is feasible. Sometimes, the measurement time of the data collection may take more than a few days depending on the quality of the crystal or the size. On the other hand, powder crystal diffraction data, collected with a high resolution optic, enables us to take diffraction data of sufficient high quality and enough intensity in several hours or one day. Since the peak overlap which appears in powder samples is reduced with this high resolution optic, *ab initio* powder crystal structure determination can be considered to be an important technique for examining an unknown crystal structure.

Acknowledgments

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