Bromine K-Edge X-Ray Absorption Near-Edge Structure Analysis on Hydrobromide-Salt Crystals and the Solid Dispersion of Active Pharmaceutical Ingredients

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Introduction
Evaluating the crystal forms of active pharmaceutical ingredients (APIs) is critically important in the quality control of drug formulations. This is because the crystal polymorphs usually have different physicochemical properties such as solubility and stability, and the contamination of polymorphs or polymorphic phase transition may result in reduced efficacy of the formulations. API crystal polymorphs have been evaluated by powder X-ray diffraction (PXRD), thermal analysis and IR absorption spectroscopy, and also by Raman spectroscopy and solid-state NMR spectroscopy. Recently, X-ray absorption fine structure spectroscopy was demonstrated to discriminate between the crystal forms of APIs.\(^1\)\(^,\)\(^2\) X-ray absorption spectra show sharp rises, called edges, at the electron orbital energies of X-ray absorbing elements. The spectra shapes at the edges and up to around 50 eV above the edges are called X-ray absorption near edge structures (XANES), and their shapes are unique depending on the electronic states of the X-ray absorbing atoms.\(^2\)\(^,\)\(^3\) Weak interatomic interactions in API crystals, such as hydrogen bonds and halogen bonds, were shown to perturb the XANES shape by affecting the electronic states of the X-ray absorbing atoms. This means that XANES spectroscopy can be used to identify the crystal forms of APIs. XANES spectroscopy is highly specific to X-ray absorbing elements, and this characteristic is advantageous when analyzing the APIs in pharmaceutical formulations, especially specific elements included only in the API but not in excipients. XANES spectroscopy has been applied to APIs containing chloride ions\(^4\) and covalently-bound chloride atoms.\(^1\)\(^,\)\(^4\)\(^,\)\(^5\) The energy of the chloride K-edge (absorption by K-shell electrons) is as low as 2472 eV. When XANES spectroscopy was applied to APIs containing the elements of higher atomic numbers, the absorption-edge energies are high and the perturbations on the XANES shapes by the weak interatomic interactions in API crystals might be much smaller. In this study, we performed the bromine K-edge XANES spectroscopy analysis to evaluate its applicability at the higher energy range. Bromine elements are included in APIs as counter ions and their K-edge energy is as high as approximately 13470 eV. The model APIs are crystals of eletriptan hydrobromide, dextromethorphan hydrobromide and the crystal of scopolamine hydrobromide (Fig. 1a).

Experimental
Materials
ELT α-form crystals were purchased from Indo-Swift Laboratory Ltd. (Chandigrah, India), Eudragit E-100 from Evonik Japan Co., Ltd. (Tokyo, Japan), dextromethorphan hydrobromide monohydrate (DXM) from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), and scopolamine hydrobromide trihydrate (SCP-T) from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All other reagents used were of the highest grade available from commercial sources.

Preparation of ELT Monohydrate Crystals and Solid Dispersions
ELT monohydrate crystal powders were prepared by the slow evaporation of the dichloromethane solution of ELT α-form. SD of ELT in Eudragit E-100 (ELT/E-100 SD) was prepared by the solvent evaporation method. One hundred milligrams of ELT α-form were dissolved in dichloromethane 20 mL, and 1625 mg of E-100 were dissolved in the mixed solution of 50 mL dichloromethane and 15 mL of methanol. The ELT solution and the E-100 solution were mixed at a volume ratio of 1/1.15 (ELT/E-100 weight ratio of 1/5), and the mixture was evaporated by blowing dry nitrogen gas to obtain SD. The SD formation was confirmed by PXRD.

PXRD Analysis and Single Crystal Structure Analysis
PXRD measurements were performed using a MiniFlex 600 (Rigaku Corporation, Tokyo, Japan) with Cu-Kα radiation at room temperature. The PXRD profiles of the crystal pow-
ders were compared with those calculated from the crystal structures deposited in the Cambridge Crystallographic Data Center (CCDC) to confirm their crystal forms: the CCDC numbers of ELT α-form crystal and ELT monohydrate crystal, and dextromethorphan hydrobromide monohydrate were 806079, 718149 and 2047035, respectively.

The crystal structure of SCP-T was determined in this study by single crystal X-ray diffraction analysis. An SCP-T single crystal was picked out from the purchased powders. Single crystals of scopolamine hydrobromide sesquihydrate (SCP-S) were obtained by drying the aqueous solution of SCP-T. Their diffraction data were collected at 93 K using XtaLAB P200 system diffractometer (Rigaku Corporation) and Mo-Kα X-ray. Structure determinations and crystallographic refinements were performed using SHELX, SHEXL and shelXle. Statistics of the crystallographic data were summarized in supplementary Table S1. The single crystal structures of SCP-T and SCP-S were deposited in the CCDC with numbers of 2113078 and 2113079, respectively.

XANES Spectroscopy

Powders of ELT crystals or ELT/E-100 SD were mixed with the filler boron nitride using mortar and pestle, and were tableted using an eccentric tablet press and flat stick and dice with a 7mm diameter (Table 1). The tablets were sealed in polyethylene bags immediately after tableting. XANES measurements were carried out at the BLSSI beamline of the Aichi Synchrotron Radiation Center (Aichi, Japan). The X-ray energy was calibrated using peak-top energies of the Cu K-edge and the ELT α-form crystals. XANES spectra were recorded in the transmission mode at 25°C. Processing of the XANES spectra, subtracting the background and normalizing the edge jump between pre-edge and post-edges to be 1, was performed using ATHENA software. The energies of the Br-K edges, $E_0$, in each spectrum were defined as the energies where second derivatives of the edge spectra were zero. Linear-combination fitting (LCF) of the XANES spectra, implemented also in ATHENA software, was used for the quantitative analysis.  

The XANES spectra of mixtures of ELT α-form and monohydrate crystals were fitted by the least-square method with the spectrum of the linear-combination of the XANES spectra of each crystal at the energy range of 13490–13515 eV. The contents of each crystal were determined as the coefficients of the LCF.

Fig. 1. API Hydrobromide Salts and an Excipient Used in This Study
(a) Chemical structures. (b) PXRD profiles.

Table 1. Formulations of the Tablets for XAFS Analyses

| Sample # | 1     | 2     | 3     | 4     | 5     | 6     |
|----------|-------|-------|-------|-------|-------|-------|
| Crystals/SD (mg) | ELT α-form | ELT monohydrate | ELT/E-100 SD | ELTα-form E-100 Physical mixture | DXM | SCP-T |
| Boron nitride (mg) | 42.5 | 42.5 | 42.5 | 42.5 | 42.5 | 42.5 |
| E-100 (mg) | 68.6 | 68.6 | 68.6 | 68.6 | 68.6 | 68.6 |

| Sample # | 7 | 8 | 9 | 10 | 11 |
|----------|---|---|---|----|----|
| ELT α-form ratio (%) | 20 | 40 | 50 | 60 | 80 |
| ELT α-form (mg) | 3.4 | 6.9 | 8.6 | 10.3 | 13.7 |
| ELT monohydrate (mg) | 13.7 | 10.3 | 8.6 | 6.9 | 3.4 |
| Boron nitride (mg) | 42.5 | 42.5 | 42.5 | 42.5 | 42.5 |

a) According to the calculation, ELT/E-100 SD 82.3 mg contained 13.7 mg of ELT monohydrate and 68.6 mg of E-100.
Results and Discussion

PXRD profiles of purchased ELT crystals and of ELT re-crystallized from dichloromethane solution confirmed that those calculated from the single crystal structures of ELT α-form and monohydrate, respectively (Fig. 1b). ELT/E-100 SD showed a halo pattern whereas diffraction peaks were observed in the profile of their physical mixtures, confirming the formation of the SD. The PXRD profile of the purchased DXM crystals were the same as that calculated from its single crystal structure. The PXRD profile of purchased SCP-T powders indicated that the sample was a mixture of SCP-T and SCP-S (SCP-TS). SCP-T was reported to easily dehydrate at the ambient condition and some of SCP-T probably transited to SCP-S through dehydration during the sample setup and the measurement. The ratio of SCP-T:SCP-S of the mixture was estimated to be approximately 0.77:0.23 (w/w) based on the calculated and observed X-ray diffraction intensities in the PXRD profiles.

XANES spectra of the API hydrobromide salts samples (sample #1–6 in Table 1) showed almost the same \(E_0\) values of 13470–13471 eV and the highest peak-top energies of 13473–13475 eV (Fig. 2a). Unique spectral features of the samples were more prominent at 13478–13502 eV (Fig. 2b). The XANES spectra of the ELT α-form crystal and the ELT α-form/E-100 physical mixture were identical (Fig. 2c). This demonstrated that the excipient consisted of light elements such as C, H, O and N did not interfere with the XANES spectra at this energy range and that the reproducibility of the energies was estimated to be much less than 1 eV. The spectrum of ELT α-form showed a negative peak at 13480 eV and two positive peaks at 13487 and 13499 eV. The spectrum of ELT monohydrate showed a negative peak at 13480 eV and a positive peak at 13487 eV. The spectrum of ELT/E-100 SD showed a negative peak at 13481 eV and only one broad positive peak at 13491 eV. The energy of the positive peak of the SD was 4 eV higher than those of ELT crystals. Dependingly, the energy of the negative peak was also 1 eV higher than those of ELT crystals. In the XANES spectrum of DXM, the normalized absorbance at the negative peak at 13481 eV showed the highest value among those spectra and two positive peaks were observed at 13491 and 13500 eV. In the SCP-TS XANES spectra, the negative peak at 13481 eV was largest and one positive peak was observed at 13491 eV. The perturbation amplitudes from the normalized absorbance value of 1.0 at the XANES regions were −0.11 to +0.09, and these values are approximately one-third as small as those of Cl K-edge XANES.1,4 However, the identification and discrimination of the API crystals and SD were enough possible based on the energies and the absorbance values of the peaks at the XANES spectra.

The differences in the shape of XANES spectra would be ascribed to the differences in the interatomic interactions formed at the bromine ions in these samples. The asymmetric unit of the ELT α-form crystal contain three crystallographically-independent bromine ions. The bromine ion at a general position form two charge-assisted hydrogen bonds with protonated amino moieties of the methyl pyrrolidine rings (Supplementary Fig. S1a). One of the two bromine ions at special positions (occupancy 0.5) forms one hydrogen bond with a water molecule (Supplementary Fig. S1b), and the other bromine ion was surrounded by hydrophobic aromatic and aliphatic groups and formed no hydrogen bond (Supplementary Fig. S1c). In the ELT monohydrate crystal, the bromine ion forms two hydrogen bonds with the amino moiety of the indole ring and a water molecule (Supplementary Fig. S1d). In the case of ELT/E-100 SD, the interactions of the bromine ions may not be as specific as observed in crystal structures but have various interatomic geometries. Possible interactions were the hydrogen bonds with the amino moiety of the ELT indole ring and the charge-assisted hydrogen bonds with the protonated tertiary amino moieties of ELT and E-100. The XANES spectrum of the ELT/E-100 SD was the superposition of the XANES spectra of the bromine ions with such various interatomic interactions, and this may explain the differences
between XANES shapes of the SD and crystals and possibly also the broad feature of the peak at 13491 eV. The bromine ions in DXM formed two hydrogen bonds not with the amino moieties but with water molecules (Supplementary Fig. S1e). The asymmetric unit of the SCP-T crystal contained two scopolamine hydrobromide and three water molecules. Both of the bromine ions formed three hydrogen bonds with two water molecules and one disordered water molecule modeled in two positions (Supplementary Figs. S1f, g). The bromine ion in the SCP-S crystal was disordered and modeled in two positions, and formed three hydrogen bonds with two water molecules and one disordered hydroxyl moiety (Supplementary Fig. S1h). The observed XANES spectrum of SCP-TS was a superposition of those of SCP-T and SCP-S crystals in which interactions at the bromine ions varied because of the disorders of hydrogen bond donors and bromine ions. In summary, bromine ions of both ELT α-form and monohydrate crystals have hydrogen bonds with amino moieties of ELT molecules and with water molecules, and their XANES spectra had a peak at 13491 eV. XANES spectrum of ELT/E-100 SD also had a peak at 13491 eV, and was similar to that of DXM and SCP-TS. This suggests that the SD contains a small amount of water molecules, which may be hydrogen-bonded to bromine ions. In addition, the bromine ions may not be likely to form hydrogen bond with the amino moiety of ELT. Structural differences in the interatomic interactions of bromine ions were observed in terms of the numbers and species of the hydrogen bond donors such as charged and uncharged amino moieties, water molecules, and hydroxyl moieties. Based on these structural features, it may be possible to infer the interaction of bromine ions in SD from the XANES shape. However, it is difficult to make a definite conclusion from the current limited number of samples, and further investigation is necessary.

XANES spectra of the crystal powder mixtures of the ELT α-form and monohydrate crystals (sample #1, 2, 7–11) were measured to evaluate the ability of the XANES spectroscopy for the quantification of the API crystals (Fig. 3a). We performed the LCF analysis using the XANES regions from 13490 to 13515 eV, where the aspects of each spectrum were prominent. The R-factor and χ² values, which were used to assess the deviation between the measured and the fitted spectra, of all of the mixtures were 1.43–9.78 × 10⁻³ and 0.20–1.30 × 10⁻⁶, respectively, indicating that the LCF analysis was performed properly (Supplementary Fig. S2). Estimated contents of the ELT monohydrate crystals by LCF showed a high correlation coefficient of 0.9989 with the actual contents (Fig. 3b). This demonstrated the XANES spectroscopy at this high energy range can be applied for the quantitative evaluations of APIs.

**Conclusion**

Bromine K-edge XANES spectroscopy discriminated between crystals and solid dispersion of the API hydrobromide salts based on the shape of XANES. Differences in the XANES shape were ascribable to the differences in the interatomic interactions at the bromine ions. The ratio of ELT α-form crystals in the mixtures of ELT α-form and monohydrate crystals could be quantified by LCF using XANES spectra. The discriminability and quantitativeness of XANES spectroscopy toward the APIs, together with its high element specificity, indicates that the XANES spectroscopy can be a potent method for evaluating the APIs of pharmaceutical formulations.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Supplementary Materials**

This article contains supplementary materials.
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