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Keywords: powder diffraction, synchrotron radiation, protein crystallography

MS.10.4

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Pseudo-polymorphic transition of pharmaceutical crystals revealed by SDPD method

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Many pharmaceutical crystals show pseudo-polymorphic transition via hydration / dehydration processes depending on their storage environment or mechanical treatment. Sometimes, even after dehydration, the XRD pattern does not change significantly showing "isomorphic desolvation" which attracted much interest. Also, as their physicochemical properties such as color, stability, and solubility largely differ depending on the crystal structures, the structural investigation of the transition is important especially to utilize the pharmaceutical polymorphic crystals as API. However, after the transition, single crystal integrity tends to degrade and powdery crystals are formed. In such case, *ab initio* Structure Determination from Powder X-ray Diffraction data (SDPD) is efficient technique. We have succeeded to reveal several solid-state structure rearrangement phenomena so far by using the technique [1-7].

Herein, some examples of the pseudo-polymorphic transitions relating "isomorphic desolvation" are presented, which have been investigated by SDPD technique.

Cephalexin (cephem antibiotic) has five hydrated forms and their reversible transformations are induced by the change of relative humidity. Three pseudo-polymorphs (anhydrate, monohydrate, and dihydrate) were successfully analyzed by SDPD technique. As the structure has three independent API molecules, the number of parameters was adjusted during the direct space calculations. Water molecules of hydration were located from the residual map, cavity volume, and geometrical considerations. The hydrate phases show water tunnel structure between L-shaped building blocks that were formed by three independent cephalexin molecules connected by hydrogen bonds. In the hydration process, the blocks slide each other to increase the tunnel volume from 0 to 280 Å^3 (see figure), which is accompanied by elongation of the *a*-axis length by 17%. Such "sliding block" mechanism enables the large structural change with retention of crystallinity.

Erythromycin is used as a macrolide antibiotic drug in the dihydrate form. It released the water molecules at 355K or at dry condition to form anhydrate phase that has analyzed by SDPD technique. The structure (isomorphic desolvate) has void tunnel regions that were occupied by water molecules in the dihydrate phase. Thus, it should be transformed to more stable anhydrate structure.

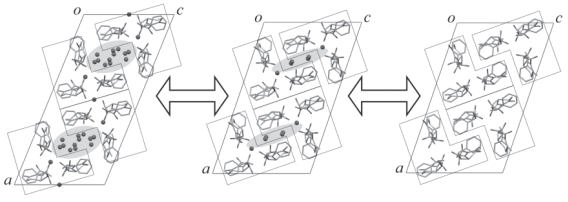

Fig. Cephalexin dihydrate, monohydrate, and anhydrate

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Keywords: polymorphism, pharmaceutical, hydration

MS.10.5

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Structure Determination of Barbiturate Derivative Using X-Ray Powder Diffraction

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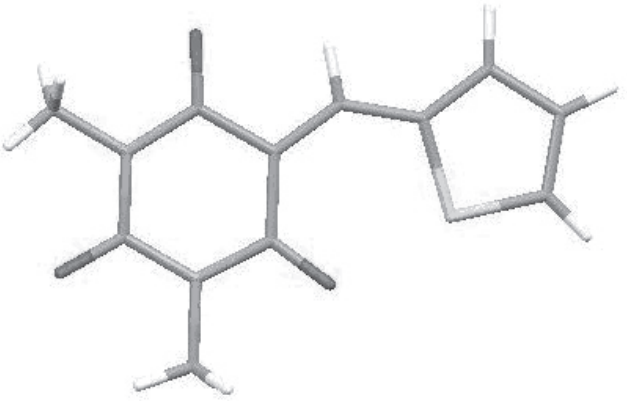
The barbiturate derivative namely 1,3-dimethyl-5[(2-thienyl)methylene]-2,4,6-pyrimidinetrione ($C_{11}H_{10}N_2O_3S$) has numerous biological activities as anti-inflammatory, anticonvulsant and antibacterial. The molecular structure of this compound has been determined by the method of simulated annealing as implemented in Dash program from high resolution laboratory X-ray powder diffraction data collected at ambient conditions. It has been found that the compound crystallize in the monoclinic space group $P2_1/c$ with lattice parameters a=7.2384Å, b=13.2319 Å, c=13.8221 Å, β =123.74° and unit cell volume=110.89 Å^3. The crystal structure was refined using Rietveld refinement method on a data collected at 1.5 Å resolution yielded R-Bragg values of 7.91% and R_{wp} value of 6.4%. The molecules are stacked in parallel layers and are stabilized by hydrogen bonds.



Keywords: structure_determination, x-ray_diffraction, antibacterial

MS.11.1

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Accurate mass, models and resolution for high-throughput structural analyses

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Efficiently characterizing biologically relevant conformations of macromolecules and their complexes is a critical challenge for

structural biology. Furthermore, unstructured proteins, RNA or DNA components provide functionally important flexibility that is key to many macromolecular assemblies throughout cell biology. As objective, quantitative experimental measures of flexibility and disorder in solution are limited, small angle scattering (SAS), and in particular small angle X-ray scattering (SAXS), provides a critical technology to assess macromolecular flexibility as well as shape and assembly at the proteomic scale [1], [2], [3]. The accurate modeling of such SAS data requires not only well-characterized homogeneous samples but also analytical tools for objective, high-throughput assessments of mass, models and resolution derived from the SAS experiment scale [4], [5]. We apply the Porod-Debye law as a powerful tool for detecting biopolymer flexibility in SAS experiments. We find that the Porod-Debye region fundamentally describes the nature of the scattering intensity decay, which captures information needed for distinguishing between folded and flexible particles. Particularly for comparative SAS experiments, proper analyses can distinguish between discrete conformational changes and localized flexibility relevant to molecular recognition and interaction networks. This approach aids insightful analyses of fully and partly flexible macromolecules that is more robust and conclusive than traditional Kratky analyses. Furthermore, we demonstrate for prototypic SAXS data that the ability to calculate particle density by the Porod-Debye criteria provides an objective quality assurance parameter suitable for SAXS modeling and validation. We have moreover defined a novel SAS invariant called the volume-of-correlation, Vc, that reflects the information contained within the entire scattering curve. Vc is specific to the structural state of the particle, yet independent of concentration and the requirements of a compact folded particle. Together with radius-of-gyration, Vc provides the means to accurately determine the molecular mass of proteins or RNA and to define a statistical measure, R_{SAS}, for improved evaluation of structural models.

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Keywords: SAXS, crystallography, macromolecular

MS.11.2

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A system for automated data analysis and interpretation for biological solution SAXS

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Small angle X-ray scattering (SAXS) is a structural method providing information about the overall structure of dissolved macromolecules and functional complexes at low resolution. The technique is increasingly demanded by the biological community thanks to the development of both cutting edge instrumentation and novel data analysis approaches enhancing the reliability of the reconstructed models. We have designed a system for automated high throughput solution SAXS encompassing both data acquisition and analysis. The system includes a Beamline Meta Server allowing for queuing of multiple samples for subsequent measurement by a robotic sample changer and remote experiment control. The primary data reduction and primary processing are done on-the-fly by a pipeline style performing radial averaging, check for radiation damages, buffer subtraction, calculation of the invariants, fast shape evaluation and reporting in convenient tabular form using XML format. The pipeline is already running on several synchrotron SAXS beamlines at the storage rings in Hamburg and Grenoble.

An integrated system DANESSA reads in the processed data from the pipeline to perform automated data analysis and model building. DANESSA includes modules for *ab initio* shape determination, high resolution structure validation, rigid body modeling, mixture analysis. The system requires a rather simple input containing *(i)* primary sequences of the objects, *(ii)* scattering profiles of the measured construct(s), and *(iii)* a table with molarities of the objects for each curve to describe the sample stoichiometry. DANESSA makes an initial assessment of the data quality and queries external databases and servers to gather available bioinformatic knowledge on the object(s). Depending on the scenario identified by the system (e.g. complex formation / deletion mutants study / dissociation / model validation), it makes a decision what are the appropriate modules to launch. Given that the full modeling cycle consists of multiple runs of multiple programs, the system employs 140-processor cluster at EMBL Hamburg. The system may run in a standalone mode from a Web interface. The details on the modules and the data flow in DANESSA as well as practical examples of its usage will be presented.

Keywords: SAXS, automation, biomolecule

MS.11.3

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Quality Control of Protein Standards for Molecular Mass Determinations by SAXS

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Forward scattering intensity, $I(0)$, is one of the important model-independent parameters that can be determined with small-angle scattering experiments. $I(0)$ is proportional to the number of particles and also to the squared particle volume, thus $I(0)$ normalized relative to the particle concentration ($I(0)/c$) is often used as a measure of molecular mass of biological macromolecules.

There are two major procedures upon estimating the particle mass from $I(0)$. One is to use known scattering length of water as a standard [1], and the other is to refer $I(0)$ of a biological standard with known molecular mass [2]. In the former case, molecular mass of protein samples can be determined mostly within ~ 10 % deviation by using 'effective' particle specific volume [3]. On the other hand, the latter procedure requires reproducible and monodispersed preparations of the biological standard and its exact concentration.

In this presentation, I will report my effort to establish a reproducible protocol of preparing a series of protein standards suited for solution scattering experiments. The size distribution of the prepared standards was checked with dynamic light scattering to be as narrow as monodispersed system. Furthermore, hydrodynamic properties of the prepared standards were carefully examined with analytical ultracentrifuge. Several factors limiting the accuracy of molecular mass determination will be discussed through a direct comparison of molecular mass from solution scattering with that from hydrodynamic measurements.