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S14

A SYNCHROTRON TOOL USED TO PROCESS HOME SOURCE DATA

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Laboratory instruments for X-ray diffraction measurements are becoming more powerful with new technologies such as the liquid anode sources or HPAD detectors. It allows efficient diffraction experiments, including data for native SAD phasing, on an increasing number of protein samples in house, without the need of a synchrotron source. The crucial part of the diffraction measurement is data processing. It is a very much standardized process for synchrotron data and users are used to processing data with tools such as XDS [1]. However, in house data collection has a few specific parameters compared to synchrotron: the measurement consists of several runs with various geometry settings and the geometries are non-orthogonal. Even though XDS can process such individual runs, settings are non-trivial and behind the scope of a general user. Therefore, *Xdskappa* was introduced to simplify processing of in house data using XDS. *Xdskappa* automatically generates input for XDS and runs XDS on multiple datasets simultaneously. Moreover, a possibility of manual fine tuning remains. *Xdskappa* is distributed under the GNU General Public License 3 [2].

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2. GNU General Public License, Free Software Foundation, https://www.gnu.org/licenses/gpl-3.0.en.html.

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Session IV

S15

DIFFRACTION LIMIT IN MACROMOLECULAR CRYSTALLOGRAPHY

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Choice of the high resolution diffraction limit is an important step in macromolecular structure determination. Nowadays, there are several criteria for resolution cut-off estimation and this can lead to some confusion. To cite P. Evans: *"An appropriate choice of resolution limit is difficult and sometimes seems to be performed mainly to satisfy referees."* [1] Data from high resolution contain important information, which is needed to clarify structure details. It is also a significant part (tens of percent) of all dataset observations. Thus these data are not negligible and have a remarkable influence on the resulting structure model.

Diffraction data from a crystal of a FAD-dependent enzyme were collected on beam line P13, Petra III, Hamburg, using a PILATUS 6M detector. Diffraction spots were visible by eye up to resolution of 2.2 Å (Fig. 1). Various modern approaches of diffraction data processing were applied [2-3].

Attempts to estimate the diffraction limit using numerous criteria were performed. The main focus was on the R-factor analysis using the refinement statistics R_{work} and R_{free}. This criterion links crystallographic model and data quality [4], in contrast with conservative criteria, *e.g.*, $I/$, R_{merge}, or $CC_{1/2}$, that evaluate data quality without relation to model. Impact of various processing approaches on the overall structure quality has been analyzed. The resulting diffraction limit varied from 2.1 Å to 1.7 Å with respect to

the selected procedure. The final option 1.9 Å was estimated according to the R-factor analysis mainly, however, also values of the signal-to-noise ratio *I/* were taken into account.

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S16

CRYSTAL STRUCTURES OF HETEROSPIN COMPLEXES BASED ON Ni(II) AND TCNQ

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Complexes based on TCNQ (7,7',8,8'-tetracyanoquinodimethane) anion-radicals as carriers of unpaired electrons represent an extensively studied group of materials. Due to the ability of $TCNQ^-$ to directly coordinate to metal atoms and, in addition, to form supramolecular chains via hydrogen bonding and interactions, this species offers a great deal of possibilities for crystal engineering [1, 2].

The present study shows crystal structures of three nickel(II) complexes with 2,2'-bipyridine (*bpy*) and its derivate 4,4'-dimethyl-2,2'-bipyridine (*4,4'-dmbpy*) containing $TCNQ^-$ in various forms. All three compounds were prepared by the reactions of hot methanolic/ethanolic solutions of Ni(II) salt with *bpy*/*4,4'-dmbpy* and LiTCNQ, followed by crystallization.

The crystal structure of $[Ni(bpy)_3]_2(TCNQ\text{-}TCNQ)(TCNQ)_2 \bullet 6H_2O$ (**1**) was found to be composed of bulky complex cations $[Ni(bpy)_3]^{2+}$, uncoordinated $TCNQ^-$ anion-radicals, uncoordinated -dimerized $(TCNQ\text{-}TCNQ)^{2-}$ dianions and three crystallographically independent, uncoordinated water molecules which are strongly disordered (Fig. 1 and Fig. 2). The nickel(II) atom within the $[Ni(bpy)_3]^{2+}$ complex cation is hexacoordinated by three bidentate aromatic ligands (Fig. 1). The Ni-N bond lengths range from 2.078(2) to 2.109(2) Å which are in the usual range of values. The -dimerized dianion $(TCNQ\text{-}TCNQ)^{2-}$ with a central C-C bond length of 1.653(11) Å is disordered with a pair of anion-radicals overlapped by their exo groups. The occupation ratio for disordered anion-radicals was refined to 0.753(9): 0.247(9). Both forms of TCNQ, the anion-radicals and the -dimerized dianions, form supramolecular chains via stacking of their external $C(C?N)_2$ groups. These chains alternate in the structure with layers of $[Ni(bpy)_3]^{2+}$ complex cations. The water solvate molecules along with the $TCNQ^-$ anion-radicals form a three-dimensional supramolecular structure via O-H···O and O-H···N hydrogen bonds. The ionic crystal structures of two other compounds containing the *4,4'-dmbpy* ligand will be discussed during the oral presentation.

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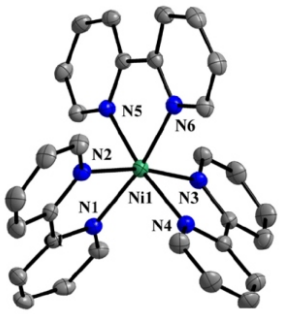

Figure 1. Coordination environment of nickel(II) atom in $[Ni(bpy)_3]^{2+}$

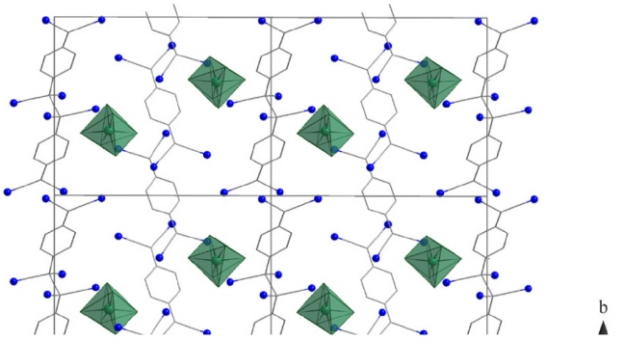

Figure 2. Supramolecular chains of TCNQ with $[Ni(bpy)_3]^{2+}$ cations

S17

STRUCTURAL AND SPECTROSCOPIC STUDY OF COPPER(II) DIPICOLINATE COMPOUNDS

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Despite multiple reports and investigations performed on dipicolinate compounds, great attention to their further study is still being paid, because of their structural variability, interesting properties and applications in many fields such as material chemistry, medicine and bioinorganic chemistry [1, 2]. Compounds of copper(II) containing nicotinamide or its derivatives as N-donor ligands have been chosen considering that they could be involved into the hydrogen-bond networks through the carboxamide group [3] and thus are suitable candidates for construction of supramolecular architectures and together with dipicolinate ligand, which can bind to a metal centre in a variety of coordination modes [4, 5], are of interest from a supramolecular chemistry point of view. Therefore using the above-mentioned ligands four copper(II) dipicolinate complexes have been synthesized, namely {[Cu(pca)(dipic)(H_2O)]·H_2O} (**1**), [Cu(nia)(dipic)(H_2O)] (**2**), {[Cu(mnia)(dipic)(H_2O)]·$2H_2O$} (**3**) and {[Cu(inia)(dipic)]$_n$·$2CH_3OH$} (**4**) (dipic = anion derived from pyridine-2,6-dicarboxylic acid also well-known as dipicolinic acid, pca = pyrazinecarboxamide, nia = nicotinamide, mnia = *N*-methylnicotinamide and inia = isonicotinamide). Crystal structures of all the compounds have been determined, supramolecular contacts such as hydrogen bonding described and all prepared compounds have been also studied by EPR spectroscopy. The X-ray diffraction analysis revealed that all of the copper(II) complexes are of distorted square-pyramidal geometry with five-coordinated central copper(II) atoms through one nitrogen donor atom of nicotinamide or its derivatives, one nitrogen donor atom and two oxygen donor atoms of a tridentate chelating dipicolinate anion and one oxygen donor atom from coordinated water molecule (besides complex (**4**)). In the complex **4** anion of dipicolinic acid has one carbonyl oxygen atom of carboxylate group acting as bridging to the neighbouring copper(II) atom, what resulted into the formation of polymeric compound. The complex molecules to each other and solvent molecules in the cases, where uncoordinated solvent molecules (water or methanol) are located in the crystal structure, are linked together through complicated hydrogen-bond networks in all cases and form either 2D or 3D supramolecular frameworks. The X-band EPR spectra of all prepared copper(II) complexes were recorded at room, as well as at low temperature 98 K. The g-factor relation $g_{//} > g_{+} > 2.0023$ confirmed the d_x^2-$_y^2$ ground electronic state.

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S18

CRYSTAL STRUCTURE OF EDARAVONE COCRYSTALS

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We have focused on characterization of cocrystallization products of edaravone, a neuroprotective agent, and phenolic acids in order to prove the formation of cocrystals. New derivates of edaravone were prepared because it could improve physicochemical properties of this drug.

The goal of qualitative phase analysis is to determine what phases are present in sample, such as reactants, cocrystals with different stoichiometric ratios of starting materials and their polymorphs and pseudopolymorphs.

The preparation of cocrystals was performed, with tree experimental procedures, namely solvent drop grinding, solvent cocrystallization and slurring method at 1:1, 2:1, and 1:2 molar ratios with different solvents. Mainly, powder samples were prepared.

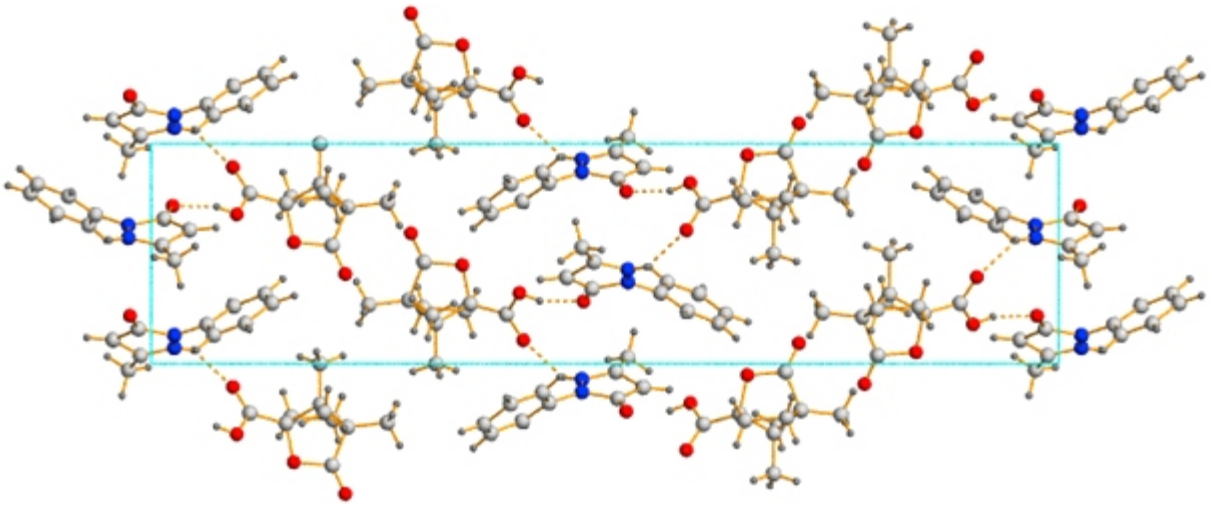

Figure 1. Crystal structure of cocrystal Edaravon: Camphanic acid 2:1 (view along axis c)

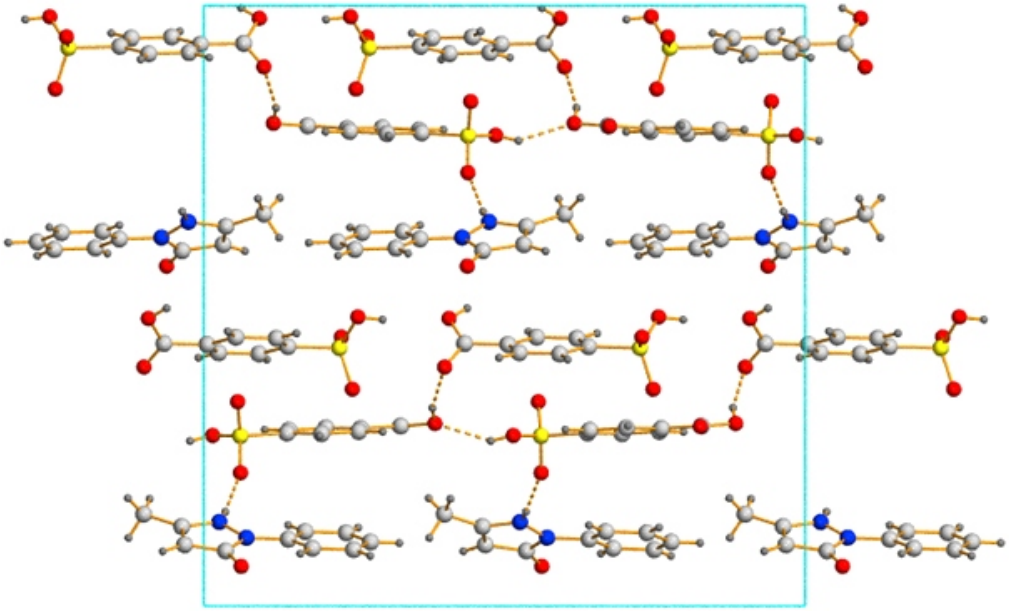

Figure 2. Crystal structure of cocrystal Edaravon: 4- Sulfobenzoic acid 1:2 (view along axis c)

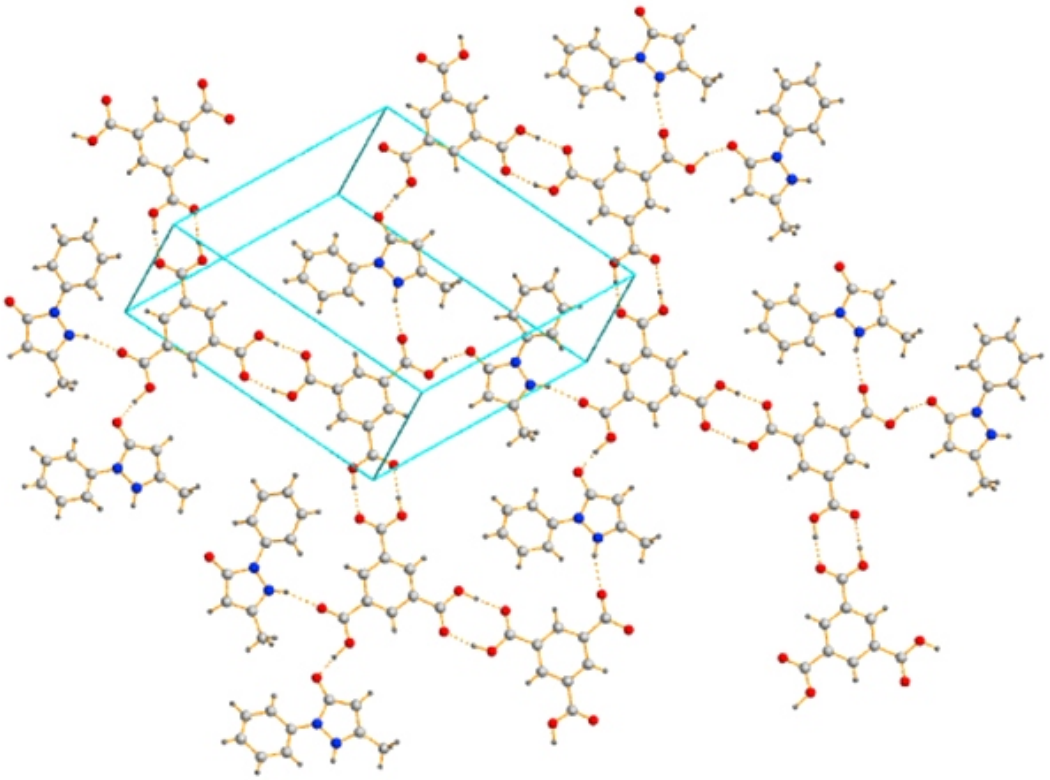

Figure 3. Supramolecular 2D framework of continuum salt- cocrystal Edaravon: Trimesic acid 1:2.

Table 1. Crystallographic data.

	Edaravon: Camphanic acid 2:1	**Edaravon: 4-Sulfobenzo-ic acid 1:2**	**Edaravon: Trimesic acid 1:2**
Chemical formula	$C_{20}H_{24}N_2O_5$	$C_{24}H_{22}N_2O_{11}S_2$	$C_{76}H_{64}N_8O_{28}$
M_r	372.41	578.56	1537.35
Cell setting Space group	Orthorhombic $P2_12_12_1$	Orthorhombic $Pn2_1a$	Triclinic P-1
T (K)	293(1)	293(1)	293(1)
a (Å)	32.9448(10)	19.911(3)	7.5440(3)
b (Å)	7.94775(16)	19.893(3)	14.9690(12)
c (Å)	7.31362(18)	6.5273(9)	16.1120(7)
(°)	90	90	81.442(5)
(°)	90	90	78.327(4)
(°)	90	90	87.653(5)
V (Å^3)	1914.98(8)	2585.4(7)	1761.89(18)

As cocrystals differ from salts or continuum salt-cocrystal only by the position of proton between the acidic and the basic functionality of the co-crystallization components, we can decide about the form of the products only after determination of their crystal structure. The position of the proton was determined indirectly by deducting it from the bond lengths C-O and C=O groups of carboxyl groups.

More then forty powder samples have been characterized, out of which ten pure crystalline products have been confirmed. Preliminary crystal structures of three products have been solved (**Table**). Crystal structure of cocrystal Edaravon: Camphanic acid 2:1 (**Fig. 1**) and cocrystal Edaravon: 4-Sulfobenzoic acid 1:2 (**Fig. 2**) have been solved by powder diffraction analysis. Continuum salt - cocrystal Edaravon: Trimesic acid 1:2 (**Fig. 3**) has been solved by a single crystal diffraction analysis.

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SL19

RANDOM MICROSEEDING APPLICATION ON HALOALKANE DEHALOGENASE DBEA CL VARIANT FROM *BRADYRHIZOBIUM ELKANII* USDA94

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A novel enzyme, DbeA, belonging to the family of haloalkane dehalogenases (EC 3.8.1.5) was isolated from *Bradyrhizobium elkanii* USDA94. This haloalkane dehalogenase is closely related to DbjA enzyme from *Bradyrhizobium japonicum* USDA110 (71% sequence identity), but has different biochemical properties. DbeA is generally less active and has a higher specificity towards brominated and iodinated compounds than DbjA. The DbeA protein was crystallised using the sitting-drop vapour-diffusion method and the crystal structure of a DbeA enzyme has been solved and deposited at Worldwide Protein Data Bank under PDB ID 4k2a. The DbeA wt structure revealed the presence of two halide-binding sites. The first chloride-binding site is located in the active site in between two halide-stabilizing residues. The second chloride-binding site is unique to DbeA and has not been previously reported in any other structure of this enzyme family. To elucidate the role of the second halide-binding site, a two-point variant DbeA Cl (I44L+Q102H) lacking this site was constructed and biochemically characterized [1]. Elimination of the second halide-binding site decreased the stability and catalytic activity, and dramatically altered the substrate specificity. The two-point substitution resulted in a shift of the substrate-specificity class, which is the first time this has been demonstrated for this enzyme family. Rational design of buried halide-binding sites represents a novel strategy for engineering of enzymes with modified catalytic properties.

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