Experimental report

27/02/2017

Proposal:	**5-12-319**	**Council:**	4/2015

Title: Mapping complex hydrogen-bonded networks in quinoline-based antimalarial drugs

Research area: Chemistry

This proposal is a new proposal

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Samples: Chloroquine diphosphate dihydrate

Instrument	Requested days	Allocated days	From	To
D19	7	6	08/09/2015	15/09/2015
			27/11/2015	02/12/2015

Abstract:

This proposal is devoted to the structural and crystallographic investigation of the hydrogen bond (HB) network in high-quality single crystals of the antimalarial drug chloroquine by single-crystal neutron diffraction at low T (down to 100 K). This experiment will complement high-resolution X-ray diffraction results, allowing to clarify the role of hydrogen bonds in setting up the molecular recognition process.

Scientific council: 4/2015

Proposal 5-12-319

Experiment dates: From 08/09/2015 to 15/09/2015 and from 27/11/2015 to 02/12/2015

Mapping complex hydrogen-bonded networks in quinoline-based antimalarial drugs

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Experiment Report

Summary. This experiment was carried out at the 4-circle single-crystal diffractometer at the D19 thermal neutron beamstation. It was devoted to the structural and crystallographic investigation of the hydrogen bond (HB) network in high-quality single crystals of the antimalarial drug chloroquine (CQ) by single-crystal neutron diffraction at low T (< 120 K).

Aims of the experiment. We growed large and well-formed monoclinic ($P2_1/c$) samples of the salt chloroquine diphosphate dihydrate by sol-gel methods. Preliminary X-ray diffraction experiments at T = 100(2) K up to $\sin\vartheta/\lambda$ = 1.0 Å^{-1} revealed that complex patterns of hydrogen bonds are formed, which involve both the phosphate groups and the co-crystallized water molecules. Phosphate ions form infinite chains parallel to the monoclinic *b* axis, while CQ molecules keep their main fused ring system orthogonal to the chains, setting in the free space among them through strong N–H···O HBs.[1] The role of the two water molecules is less clear, even though they should help to coordinate phosphate ions. Thus, this work aims at: (i) accurately determine the experimental CQ diphosphate dihydrate structure with the highest precision up to date; (iii) Provide an accurate estimate of the anisotropic thermal motion for both hydrogen and heavy atoms, to eventually achieve a better deconvolution of the static electron charge density from X-ray data.

Description of the experiments performed:

Samples numbered as **#1** and **#2** (batch 2, obtained through solvent diffusion crystallization methods) were tested and deemed too small to produce suitable diffraction patterns.

A preliminary data set was then collected at T = 100 K (hot neutrons, λ = 1.1695 Å). The sample mounted (**#3**, batch 1 from gel crystallization, approximately 5x3x2 mm) resulted to be twinned. There was some amount of superposition among reflections, especially at low Bragg angles, resulting in the worsening of the overall statistics (bad equivalents, some exception to the $P2_1/c$ extinction rules, difficulties in indexing the pattern). We believe that (i) the twin individual is a minority non-merohedral mate, on the basis of the lower average intensities of the off-lattice spots; (ii) just a certain amount of reflections suffer from diffraction contributions due to the parasite. Unfortunately, it was not possible to correctly indicize the minority lattice even by employing low-filtering recipes on the harvested intensities. The data collection at 100 K was nevertheless completed, as the sample was large enough to provide accurate enough statistics for the reflections of the main lattice without parasitic contributions.

Sample **#4** was also discarded due to twinning. Sample **#5** was regular, well-shaped, with dimensions approx. 4 x 2 x 2 mm. It was mounted with a 0.5 wide beam guide and, after a preliminary data collection at RT, cooled down to 20 K for a complete data collection. Unfortunately, we were not able to determine a reliable unit cell. Therefore, we resorted to a complete analysis of the 100 K dataset.

Data reduction.

Can correction, which takes into account absorption from the vanadium cylinder mounted around the crystal as a heat shield, was applied throughout. Indeed, can-corrected data perform better in terms of internal agreement (R_{int}, equivalents), model-to-data agreement and agreement statistics. In general, can correction improves the both the quality of the dataset and the structural model, providing a better description of the anisotropic thermal motion. We also checked performances of a different spot shape library, based in turn on a very good quality crystal (croconic acid), on the integration performances of the 100 K dataset collected on the sample #3. Both can-corrected and can-uncorrected data were taken into account, in conjunction with International Tables SFAC values [2] and a maximum resolution of 0.55 $Å^{-1}$ in $\sin\theta/\lambda$. However, we found that the quality of the data significantly worsened, whereas the attenuation correction had just a very minor influence on the overall statistics.

Data refinements.

All data refinements were carried out by means of the shelxl-14 [3] program. Available X-ray structural models were employed as suitable starting points. As for the 100 K dataset, the X-ray cell was used throughout and never refined. The following strategy was applied to develop a sensible structural model:

1) Preliminary refinements against all the dataset (up to 120 deg in 2θ);
2) Localization of the H atoms from the Fourier residuals;
3) Refinement of the anisotropic thermal parameters for all the atoms, H included.

The positions of all the H atoms in CQ and H_2PO4^- ions were secured. However, those belonging to water molecules were much more difficult to be located. Thus, we worked with Cu sphere data (99 deg in 2θ), but just the residual corresponding to the water molecule O1w could be located. Due to low scattering power at high angles, we then restricted our refinement to a further subset corresponding to 0.55 $Å^{-1}$ in $\sin\theta/\lambda$, i.e. up to 80 deg in 2θ. We first constrained the positions of water hydrogen atoms to those found by difmap, and then we relaxed them after applying a SADI constrain for both the water molecules. The latter were found to be disordered. Just the position of H13w, bonded to O1w, is perfectly ordered and we accordingly refined it anisotropically. The other two hydrogens, H11w and H12w, are forced by symmetry to occupy a couple of non-equivalent positions with sof = 0.5. Actually, O1w is inversion-related to another O1w molecule, resulting in a very short apparent contact (1.297 Å) between H11w atoms (Figure 1). The disorder is here due to the setting up of a frustrated zig-zag HB motif between adjacent H_2PO4^- pillars (Figure 1). The orientations of one half of the H atoms are free due to the lacking of an acceptor in their close neighborhood, while it is not possible to unequivocally define to which oxygen most of the other H atoms are covalently bonded. Likely, all the possible motifs are present in the crystal and this translates into disorder, which should be (at least in part) static as it persists down to ≈ 100 K. Besides, it is easy to see that perfect $P2_1/c$ symmetry is incompatible with the regular H-bond network (Figure 1).

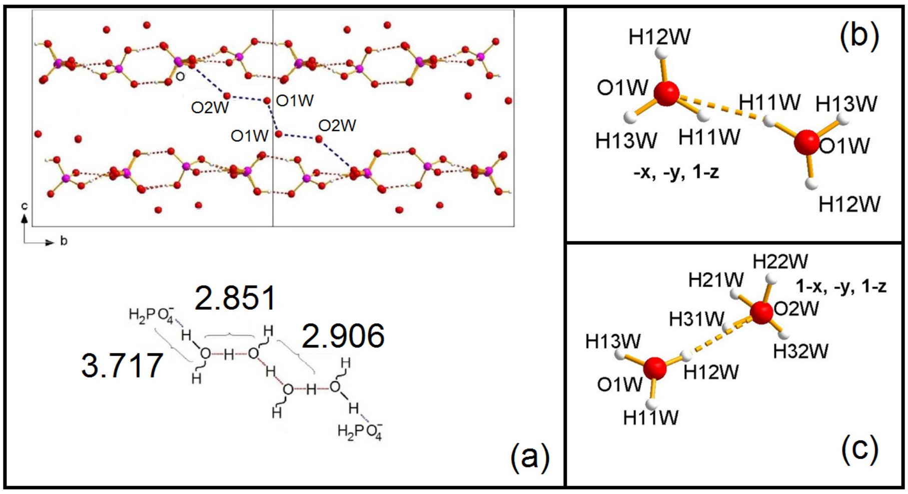

Figure 1. (a) Frustrated HB motif between adjacent phosphate pillars. Red spheres are O atoms, purple spheres P atoms. Atom labels and relevant X-ray O···O distances are also highlighted. (b) Disorder model for inversion-related O1w water oxygen artoms. (c) Same as (b), for the O1w-O2w HB systems.

Indeed, any H atom bonded to inversion-related O9 atoms along the O9···O9 direction would produce a O1w–H···H–O1w steric clash. This means that the $P2_1/c$ symmetry of the crystal emerges thanks to the statistical average of allegedly equivalent configurations in the H-bonded frustrated water motif.

The other water molecule (O2w, Figure 1) is present in two possible non-equivalent orientations, with relative weight as high as ≈ 70 % and 30 %. It should be noted that the main conformation is involved in a strong HB with the O3 oxygen atom of the P1 phosphate group.

Conclusions.

The currently employed dataset at 100 K consisted of 8921 reflections up to 2θ = 72 deg, corresponding to 2564 unique data with R(int) = 0.921 and R(sigma) = 0.0596. The final least-squared model refined to R1=0.0905 for intense ($I>2\sigma(I)$) reflections, R1= 0.0983 for all the data, wR2 = 0.2356 and goodness-of-fit 1.098, in conjunction with maximum residuals as high as +1.99/–1.24. Actually, this model can hardly be considered as final, as 497 inconsistent equivalents on strong reflections are still present together with 20 exceptions to $P2_1/c$ systematic extinction rules. We attribute such discrepancies to twinning issues. Moreover, comparison with periodic DFT optimization of the water orientation [1] by means of the CRYSTAL09 program, showed that the computed conformations are dissimilar with those found experimentally (O3-H21w-O2w-H32w was -87.57° in CRYSTAL and -125.65(9)° from the experiment, O8-H13w-O1w-H12w was 125.91° in CRYSTAL model 1 and 16.0(1)° from the experiment). We must conclude that a more accurate data treatment of twinning, or even a new data collection on a higher quality sample, is mandatory to obtain a more reliable model.

References

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[3] G. M. Sheldrick, Acta Cryst. A 2008, 64, 112-122