

REPORT

MATERIALS SCIENCE

Hydrogen positions in single nanocrystals revealed by electron diffraction

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The localization of hydrogen atoms is an essential part of crystal structure analysis, but it is difficult because of their small scattering power. We report the direct localization of hydrogen atoms in nanocrystalline materials, achieved using the recently developed approach of dynamical refinement of precession electron diffraction tomography data. We used this method to locate hydrogen atoms in both an organic (paracetamol) and an inorganic (framework cobalt aluminophosphate) material. The results demonstrate that the technique can reliably reveal fine structural details, including the positions of hydrogen atoms in single crystals with micro- to nanosized dimensions.

Crystal structure analysis by diffraction techniques is the leading method for the determination of the atomic structure of crystalline solids. Despite all the advances in structure analysis, problems still remain that are very hard to solve. One challenge is the structure analysis of micro- and nanocrystalline materials, which do not form crystals large enough for single-crystal x-ray diffraction (SC-XRD), and their analysis by x-ray or neutron powder diffraction is hampered by reflection overlap and the coexistence of multiple phases. An additional challenge is the localization of hydrogen atoms. With only one electron, hydrogen atoms have the lowest x-ray and electron scattering power of all atoms, and they often have large atomic vibrations, making their detection difficult. At the same time, knowledge of the distribution of hydrogen atoms in a structure is essential for understanding the properties of many materials.

Electron diffraction is the method of choice for the determination of crystal structures from single nanosized crystals. Over the past decade, electron crystallography has been developed to a level approaching x-ray crystallography in terms of both the robustness of the structure determination and the accuracy of the inferred structure models. The milestones have been the

development of the precession electron diffraction (PED) (1) and electron diffraction tomography (EDT) methods (2, 3). These methods now allow almost routine determination of the crystal structures of many substances, including framework-type materials (4), pharmaceuticals (5), macromolecules (6), and proteins (7). However, until recently, structure models have been only roughly estimated, an accurate refinement has been almost impossible, and detection of fine structural features such as positions of hydrogen atoms has been beyond the capabilities

of this method. The reason is that the standard method for structure refinement uses the kinematical diffraction theory, which is not well suited for electron diffraction. This situation is changing now with the introduction of so-called dynamical refinement of electron diffraction data. This method exploits the dynamical diffraction theory in the calculation of the diffracted intensities and allows a more accurate and reliable structure analysis. Attempts to use this approach in the past (8–13) were limited to oriented diffraction patterns. Recently, the dynamical refinement has been applied to precession electron diffraction tomography (PEDT) data (14), which has made the method applicable to complete three-dimensional (3D) structure analysis (15, 16).

Reports on hydrogen atom localization by electron diffraction can be found in the works of Vainshtein and co-workers, but they used macroscopic powder samples (17). The potential to locate hydrogen atoms by the method of dynamical refinement was mentioned in (16), but no convincing demonstration was provided. Here we present a 3D structure analysis by electron diffraction of single micro- to nanosized crystals, including the localization of hydrogen positions, for an organic pharmaceutical material (paracetamol) with a well-known structure and an inorganic framework material (cobalt aluminophosphate) with an unknown structure.

Determining the positions of hydrogen atoms is important for understanding the functionality of active pharmaceutical ingredients. Structure solutions for pharmaceuticals are especially challenging, because often no single crystals large enough for x-ray analysis are available. A further serious complication is the sensitivity of organic crystals to an electron beam (18). EDT has been previously reported as a viable technique for

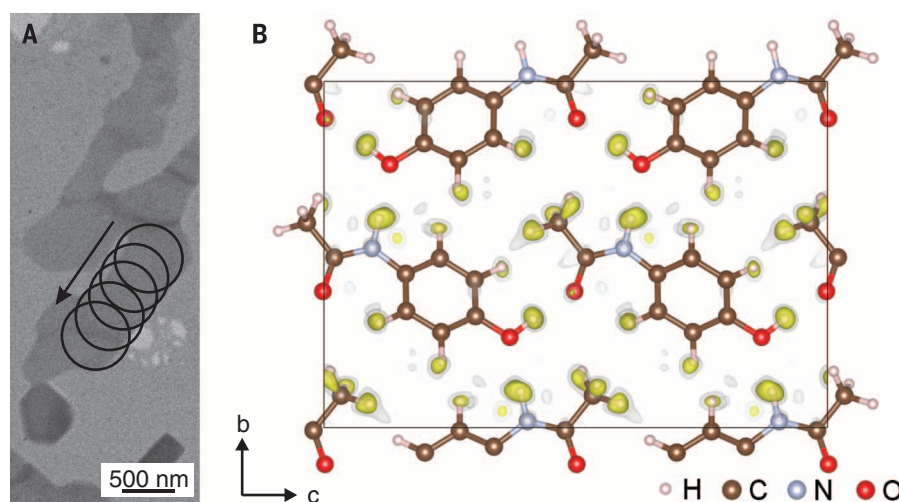


Fig. 1. Paracetamol PEDT structure analysis. (A) Transmission electron microscopy (TEM) image of a crystal of paracetamol form II. Circles schematically show the movement of the electron beam on the sample during data collection to minimize the effect of radiation damage. (B) One layer of the crystal structure viewed along axis *a*, with a superimposed difference potential map showing maxima at the positions of the hydrogen atoms. Isosurface levels are $2\sigma[\Delta V(\mathbf{r})]$ (light gray) and $3\sigma[\Delta V(\mathbf{r})]$ (yellow).

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structure determination of organics from single micro- to nanosized crystals (19); however, direct localization of hydrogen atoms remained an unattained goal. We selected paracetamol form II to demonstrate that PEDT combined with the dynamical structure refinement is sufficiently accurate and powerful to locate the positions of hydrogen atoms on electron beam-sensitive crystals of a pharmaceutically important molecule.

PEDT data collection and analysis were performed on the crystal shown in Fig. 1A [table S1, (20)]. The material is beam-sensitive, and a deterioration of the crystal quality was apparent after just 30 s of irradiation by about 0.4 electrons $\text{\AA}^{-1} \text{s}^{-1}$. To minimize the beam damage and record a sufficiently complete data set, a small beam was slowly scanned over the crystal (Fig. 1A). The structure of paracetamol without hydrogen atoms was solved *ab initio* by using the program Superflip (21), and it is in good agreement with the published data (22, 23). The dynamical refinement of the structure yielded a difference potential map $[\Delta V(\mathbf{r})]$, where V is electrostatic potential and \mathbf{r} is the positional vector in the unit cell; see the supplementary text for details on the calculation, which allowed localization of all hydrogen atoms (Fig. 1B and movie S1). The standard deviation (σ) of $\Delta V(\mathbf{r})$ is 0.146 $e \text{\AA}^{-1}$; the heights of the maxima corresponding to the hydrogen atoms range between 3.73σ and 5.82σ . The height of the highest noise maximum is 3.14σ . The addition of the hydrogen atoms to the structure model and the application of standard geometrical restraints on their positions and constraints on temperature factors (table S4) led to a decrease of the refinement figures of merit (R factors) by about 3% (data S1). A model with freely varying hydrogen positions reached R factors only about 0.1% lower than those of the restrained model (data S2). The average C-H distance in the free model is 1.15 \AA , with the largest deviation from the expected 1.09 \AA being 0.13 \AA , whereas the largest angular deviation from the expected 120° and 109.5° is about 10° . A comparison of the molecules between the two structure models is shown in fig. S1. Both the free and restrained refinements are stable and yielded results comparable to those obtained from SC-XRD (23). For comparison, the refinement using the kinematical approximation yielded a much more noisy difference potential map with only five maxima at hydrogen positions above the noise level (fig. S2 and supplementary text).

Once validated with paracetamol form II, the technique was applied to localize hydrogen atoms in an inorganic framework material with positional disorder involving hydrogen atoms. The preparation of zeolites and other framework materials is important because of their widespread use as catalysts, sorbents, ion exchangers, and food additives, among other applications (24–26). The reduction of crystal size in these materials leads to substantial challenges in terms of structure determination (27–30). In metal-containing aluminophosphates, synthesized through isomorphous substitution of P^{5+} and/or Al^{3+} (31), hy-

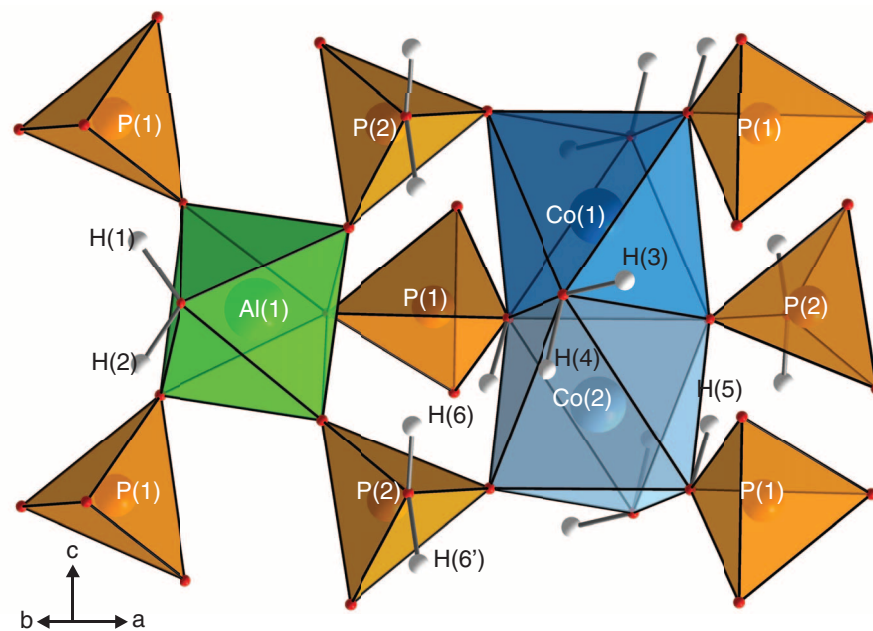


Fig. 2. A view of the cobalt aluminophosphate (CAP) structure along $[-2 -1 0]$, showing the environment of the $\text{Al}(1)\text{O}_6$, $\text{Co}(1)\text{O}_6$, and $\text{Co}(2)\text{O}_6$ octahedra. The $\text{Co}(2)\text{O}_6$ octahedron is only partially occupied by Co. Each polyhedron is labeled with the name of the central cation. Hydrogen atom positions with their labels are also shown. Red spheres, oxygen.

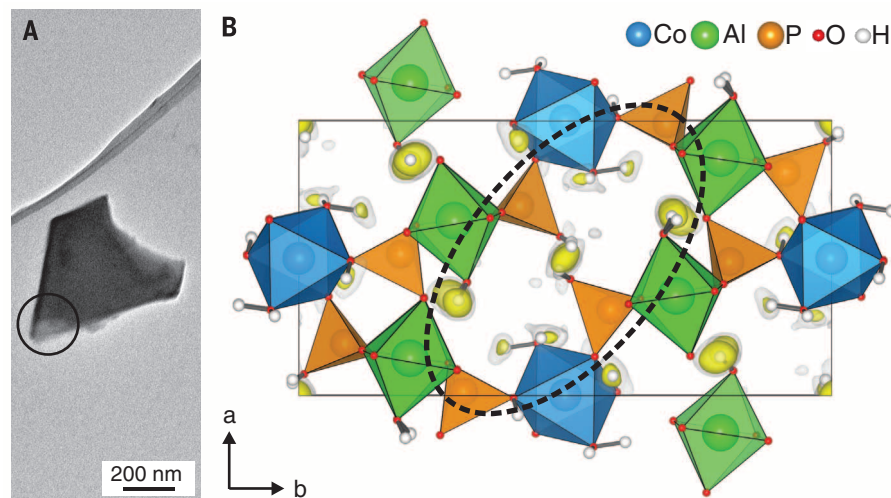


Fig. 3. PEDT structure analysis of CAP. (A) TEM image of a typical crystal of CAP. The black circle shows the part of the crystal illuminated by the electron beam during data collection. (B) Projection of the CAP structure along axis c , with a superimposed difference potential map showing maxima at the positions of the hydrogen atoms. Isosurface levels are $2\sigma[\Delta V(\mathbf{r})]$ (light gray) and $3\sigma[\Delta V(\mathbf{r})]$ (yellow). The dashed ellipse marks a channel running along c .

drogen atoms and/or water molecules are often linked to the metal center and affect the reactivity and catalytic properties. Cobalt aluminophosphate with the chemical formula $\text{Co}_{1.13}\text{Al}_2\text{P}_4\text{O}_{20}\text{H}_{11.74}$ (CAP) was prepared by using a new synthetic approach in which the structure of layered co-

balt methylphosphonate (32) is opened under hydrothermal treatment in the presence of aluminum, phosphorus, and water (20).

CAP crystallizes in the space group $P2_1/n$. The lattice parameters were determined by x-ray powder diffraction as $a = 8.4927(3) \text{\AA}$, $b =$

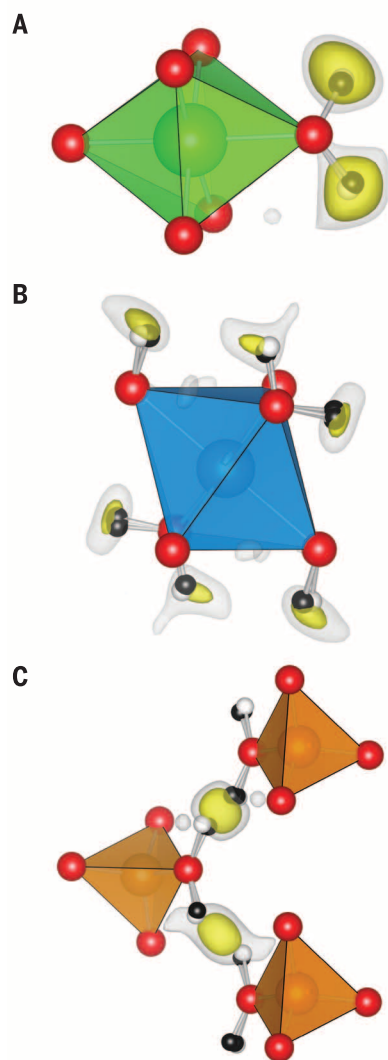


Fig. 4. Isosurface representation of the difference potential map of CAP. Isosurfaces of the potential map are shown in the vicinity of (A) $\text{AlO}_5 \cdot \text{H}_2\text{O}$, (B) $\text{CoO}_2(\text{OH})_2 \cdot 2\text{H}_2\text{O}$, and (C) PO_3OH groups (coloring is as in Fig. 3). Isosurface levels are $2\sigma[\Delta V(\mathbf{r})]$ (light gray) and $3\sigma[\Delta V(\mathbf{r})]$ (yellow). Hydrogen positions from the DFT calculations and from the PEDT refinement are shown in black and white, respectively.

$16.5278(7) \text{ \AA}$, $c = 5.0844(2) \text{ \AA}$, and $\beta = 90.638(3)^\circ$ at room temperature. The structure consists of a regular stacking of $\text{CoP}_4\text{O}_{12}(\text{OH})_2 \cdot 2\text{H}_2\text{O}$ and $\text{AlO}_5 \cdot \text{H}_2\text{O}$ layers along crystal axis b (Figs. 2 and 3). The principal cobalt site Co(1) exhibits a reduced site occupancy, and an additional octahedral site Co(2) is partially occupied by cobalt (Fig. 2). Whenever the Co^{2+} cation occurs in the position Co(2), charge balance must be maintained by replacing two $(\text{OH})^-$ groups with O^{2-} .

Initially, data sets from six CAP crystals were used for PEDT dynamical refinements [table S3, (20)]. The refinements showed that the partial occupancies at the two cobalt sites varied in a

broad interval, indicating a variable degree of redistribution of Co atoms between the Co(1) and Co(2) positions (fig. S4A). This redistribution also affected positions of other atoms (fig. S4B) and resulted in a slight rearrangement of the structure (fig. S5). The observed differences could be explained by the structural changes that the material underwent upon irradiation by the beam of high-energy electrons. Therefore, another batch of data sets was collected under very low dose conditions [table S3, (20)]. The resulting six data sets had less variable Co distribution and Co occupancies that were much closer to the values from SC-XRD [fig. S4B; see the supplementary text for details]. These six data sets were used for the final structure analysis.

It was also possible to synthesize CAP crystals that were sufficiently large for SC-XRD. Two crystals were used for structure analysis. The derived structure model was used as an independent validation of the PEDT results, but not all hydrogen positions could be identified [fig. S3 and data S3; see the supplementary text for details].

The stoichiometry of the structure and the bond-valence considerations (33) show that six hydrogen atoms per formula unit should be expected, if the occupancy of the Co(2) position is assumed to be zero. One water molecule is attached to the Al(1)O_6 octahedron, another to the Co(1)O_6 octahedron, and two more hydrogen atoms to two different PO_4 tetrahedra (Fig. 2). Out of these six positions, only the hydrogen atoms bonded to the AlO_6 octahedron are not affected by the disorder. These atoms can be observed in the difference potential maps from almost all PEDT data sets (fig. S6).

Although the identification of these fully occupied hydrogen positions was straightforward, localization of the remaining positions was more challenging. Maxima were visible in the individual difference potential maps at expected hydrogen positions, but they were close to the noise level. To improve the data completeness, enhance the signal from the hydrogen atoms, and suppress the noise in the difference potential map, the structure model was refined against a combination of data from all six crystals. In this refinement, the hydrogen atoms bonded to the AlO_6 octahedron appeared with even better clarity (Fig. 4A and movie S2), and the remaining positions of the hydrogen atoms were also revealed (Fig. 4, B and C, and movie S2). The hydrogen atom H(5) is present only when the Co(2) position is vacant, hence its occupancy factor is about 74%. Similarly, the orientation of the water molecule attached to the Co(1)O_6 octahedron is expected to vary with changes in the Co(2) occupancy, making the position of atom H(4) also occupied to only about 74%.

The ultimate challenge was the localization of the hydrogen atoms associated with the $\text{P(2)O}_3\text{OH}$ groups. These groups form a chain along crystal axis c , and each pair of neighboring $\text{P(2)O}_3\text{OH}$ groups is linked by an inversion center. The difference potential map exhibits a maximum around each of the inversion centers

(Fig. 4C and movie S2). The only structure model compatible with this observation is a model with two hydrogen positions related by an inversion center, each occupied with 50% probability. The maxima in the difference potential map due to these partially occupied hydrogens are close to each other and diffuse, resulting in one broad maximum. The density functional theory (DFT) optimizations (supplementary text) confirm this model (Fig. 4C). Hence, the PEDT data allow the detection of even these positions with 50% occupancy and poor localization.

The standard deviation σ of the difference potential map obtained from the combined data set is 0.144 e \AA^{-1} . The heights of the maxima at the hydrogen atom positions range from 3.34σ to 6.86σ . When maxima directly at the position of cations are neglected [3.45σ at Co(1) and 2.91σ at Al(1)], the highest noise maximum has a height of 2.34σ . For comparison, the refinement using the kinematical approximation yielded a very noisy difference potential map, which could not be used for identification of hydrogen positions (fig. S7).

On the basis of the difference potential map, the hydrogen atoms were placed in the positions of the maxima and refined with distance restraints (O-H distance = 1.0 \AA). The addition of the hydrogen atoms to the structure model decreased the refinement R value R_{obs} by 0.88%, and the refined positions remained close to the positions optimized by DFT, further confirming the correctness of the inferred model and the stability of the refinement (Fig. 4).

The examples presented here demonstrate that it is possible to locate hydrogen atoms in single submicrometric crystals of organic and inorganic materials by using dynamical refinement with electron diffraction data. The detection of hydrogen atom positions is one of the vital challenges in crystal structure analysis, with implications for understanding the properties and functions of the materials. The sensitivity of the presented approach allows even partially occupied and disordered hydrogen positions to be located and other structural details to be revealed. With the development of electron nanocrystallography and the recent progress toward lower data collection time and electron dose (5), the present work opens the route to detailed structure analysis of electron beam-sensitive substances such as porous materials, hydrates, and complex organics.

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Supplementary Text

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Editor's Summary

Pin the tail on the hydrogens

X-ray diffraction has been the method of choice for determining the positions of atoms within a crystal. However, the technique works better for atoms with higher atomic numbers and requires single crystals of a minimum size. Palatinus *et al.* used electron diffraction, a technique of increasing importance for analyzing very small crystals, to identify the positions of hydrogen atoms in organic and inorganic materials (see the Perspective by McCusker).

Science, this issue p. 166; see also p. 136

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