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Resolving alternative structure determinations of indapamide using ^{13}C solid-state NMR†‡

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The conflict between alternative crystal structures in the Cambridge Structural Database for the diuretic drug indapamide hemihydrate (IND) has been resolved with the aid of ^{13}C solid-state NMR. IND is seen to contain multiple distinct molecules in the asymmetric unit (Z′ = 4) rather than exhibiting disorder in the orientation of sulfonamide groups. The NMR crystallographic approach is a more effective tool for distinguishing between alternative structures than naïve judgements of quality based on crystallographic refinement agreement factors.

Repositories of crystal structure data, such as the Cambridge Structural Database (CSD),[1] are invaluable resources for researchers working in diverse areas of molecular solid-state chemistry and materials science. As discussed in a recent survey[2] of over 3000 "repeat structures" in the CSD, it is increasingly common to find multiple structure determinations for what appears to be the same solid form. This creates an obvious dilemma for an ordinary user of structural databases. The problem is compounded when there are questions of disorder in the crystal structure. Disorder is not uncommon, with about 6% of structures published in CSD between 2000 and 2017 containing comments (added during data curation) about disorder. While some of this disorder may be crystallographically and chemically uninteresting (*e.g.*, alternative positioning of hydrogen atoms in methyl groups), other types of disorder are structurally significant.

A particularly interesting subset of the CSD disorder comments, found in about 10% of cases, refer to "disorder by symmetry". This is used for cases when a fragment of the structure is disordered across two (or more) sets of atomic positions that do not individually reflect the space group symmetry; only the superposition of possible positions respects the symmetry. Such "disorder by symmetry" either means that the choice of space group or unit cell is incorrect (a crystallographic error), or that this fragment is indeed disordered. While additional diffraction experiments, including at different temperatures, can help identify the presence of disorder, due to dynamics or the incorrect selection of space group or unit cell, the user of an individual crystallographic result cannot resolve this ambiguity. In this context, spectroscopic techniques, which probe local chemical environments, can resolve the nature of crystallographic disorder, without requiring new diffraction-quality single crystals.[3–5]

Indapamide is a classic diuretic drug used in the treatment of hypertension,[6,7] and is marketed as a crystalline hemihydrate, hereafter IND. Previous studies have focussed on the solid-state characterisation of additional forms of IND,[8–10] while recent work has considered the amorphous[11–14] and cocrystal forms.[14,15] Reflecting the importance of solid-state characterisation for drug approval, there are multiple structures for IND in the CSD. We show here how ^{13}C solid-state NMR readily identifies the most plausible crystal structure and confirm this with a new diffraction study.

There are three deposited crystal structures for IND in the CSD (Version 5.40), each with a distinct reference code. The oldest structure, **FOCCAD**,[9] was derived from powder X-ray diffraction (PXRD) data. The **VAGKUM**[10] structure was the result of a detailed single crystal structure determination (SCXRD) of IND, whilst most recently, **WOCPEM**[14] was derived serendipitously from an unsuccessful attempt to co-crystallise IND with gliclazide. Table 1 summarises the key crystallographic data.

There are well-known challenges in determining small-molecule crystal structures from PXRD data, but with careful data collection and analysis, robust structure determinations can give reliable structural models.[16,17] Moreover, NMR is strongly complementary to PXRD, either for aiding structural determination or for validating derived structures.[3] The **FOCCAD** structure determination omitted hydrogen atoms in

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Table 1 Crystallographic information for the three alternative structures of IND reported in the CSD

	FOCCAD	VAGKUM	WOCPEM	WOCPEM NEW
Formula unit	$C_{16}H_{16}ClN_3O_3S \cdot 0.35H_2O$		$C_{16}H_{16}ClN_3O_3S \cdot \frac{1}{2}H_2O$	
M_r / g mol^{-1}	372		374.83	
Temperature / K	298	100	150	120
Wavelength / Å	1.54	1.54	0.71	0.71
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P2/a^a$	$I2/a$	$P2_1/c$	$P2_1/c$
a / Å	23.811(3)	15.059(9)	30.060(11)	30.140(10)
b / Å	9.6940(9)	9.6218(6)	9.6685(3)	9.6025(4)
c / Å	15.114(2)	23.508(14)	23.573(10)	23.461(8)
β / °	91.66(3)	92.60(16)	92.33(4)	92.59(10)
V / Å^3	3487.2(3)	3402.6(4)	6845.4(5)	6783.2(4)
Z, Z'	4, 1	8, 1	16, 4	16, 4
Data/restraints/parameters	(See text)	3036	12492	18001
		3	41	61
		261	948	979
$R_1 [I \geq 2\sigma(I)]$	0.0560	0.0369	0.0868	0.0842

^{a} Space group in the CSD. Original reference gives $I2/a$.

the structure solution step, which involved direct space Monte Carlo simulated annealing with only 9 variable parameters (3 translational, 3 orientational and 3 torsion angles). In the structural refinement step, the hydrogen atoms were placed geometrically, leading to two additional refineable torsion angles, but there was no modelling of the sulfonamide group disorder. This approach gave an excellent Rietveld fit to the observed PXRD data,[9] but DFT geometry optimisation of the determined structure resulted in significant atomic displacements (see Table S1, ESI† and associated discussion). Hence, **FOCCAD** was not considered further.

The **VAGKUM** and **WOCPEM** structures are closely related, and, as shown in Fig. S1 (ESI†), have virtually identical simulated PXRD patterns. **VAGKUM** has a unit cell volume that is a factor of two smaller, and involves disorder where the sulfonamide group is disordered over two positions with almost equal occupancies. The water molecule is also "disordered by symmetry" (see Fig. S3 and S4, ESI†). **WOCPEM** has a larger unit cell, with 4 formula units in the asymmetric unit ($Z' = 4$), as opposed to $Z' = 1$ for **VAGKUM**. This larger unit cell has no disorder, but the R factor is considerably larger (9% compared to 4%). Overlaying the four IND molecules in the **WOCPEM** asymmetric unit, Fig. 1 (b), shows that they adopt an identical conformation, differing only in the sulfonamide group orientation.

Fig. 1 (a) Molecular structure of the formula unit of indapamide hemihydrate. (b) Overlay of the four distinct indapamide molecules in the asymmetric unit of **WOCPEM** (see text).

VAGKUM and **WOCPEM** essentially describe the same structure but differ in how the sulfonamide group is modelled. This is an example of a molecular organic crystal exhibiting pseudosymmetry.[18–21] The four crystallographically independent molecules in the asymmetric unit are related by symmetry except for differences in the sulfonamide group orientation, which could quite plausibly be explained by disorder. The larger unit cell of **WOCPEM** also removes the "disorder by symmetry" of the water molecule, but this too could be the result of disorder rather than indicating a problem with the structure determination.

Without new evidence, either from repeat single-crystal studies or complementary experimental techniques, the choice of the most plausible structural model is not straightforward, especially as such two-fold disorder of the sulfonamide group is common.[22–24] Disorder of included water molecules is also chemically plausible and consistent with the large ADPs (Fig. S3–S5, ESI†), so there is no argument to be made based on chemical intuition. The R factors are also not decisive; as discussed further below, a lower R factor does not necessarily indicate a more reliable structural model.[25]

NMR crystallography refers to the use of solid-state NMR, often in conjunction with first principles calculations, to resolve crystallographic questions.[3,5] IND provides an example of the complementary role of diffraction and spectroscopic experiments. The presence of multiple resonances per carbon site in the ^{13}C NMR spectrum, Fig. 2, clearly indicates that Z' has to be greater than 1. The highlighted line shapes are most obviously explained by $Z' = 4$. Although not strictly necessary to establish that $Z' > 1$, calculated chemical shifts[26] based on the **WOCPEM** structure show good agreement with the experimental data (as shown in Fig. S7, ESI†); the RMSD between experimental and calculated ^{13}C chemical shifts of 1.70 ppm is in line with literature precedents.[27,28] The calculations also helpfully confirm that the structure at ambient temperature (NMR data) is essentially indistinguishable from the structure at the measurement temperature for the single-crystal diffraction data (120–150 K).
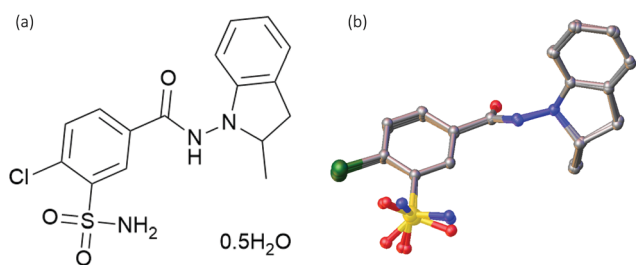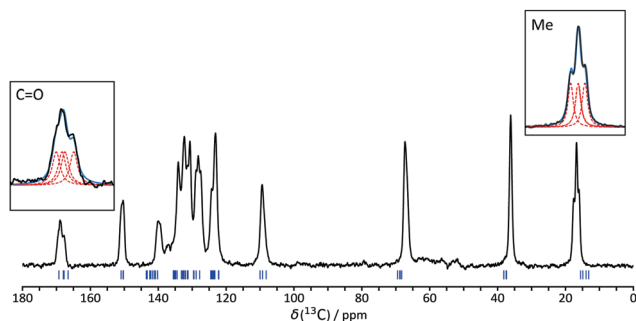
Fig. 2 ^{13}C CP/MAS NMR spectrum of IND. Highlighted peaks clearly indicate $Z' > 1$. Blue tick marks indicate DFT-calculated chemical shifts (using plane-wave basis sets) for the WOCPEM structure, whilst insets show deconvolution of the Me and CO signals into four components (see ESI† for further details).

As would be expected from Fig. 1(b), the differences in chemical shift between corresponding carbon sites within the asymmetric unit are small, and so not all peaks show resolved "crystallographic splittings". As previously observed, however, the magnitudes of the crystallographic splittings are predicted extremely well by the DFT calculation.[29] It is worth noting the sizes of the systems that can now be handled using first principles calculation; **WOCPEM** contains 280 atoms in its unit cell. Further details of the computation, including modifications required to run calculations of this size can be found in the ESI.†

The NMR studies only require powered samples and so can be applied directly on the commercial material, without needing to obtain diffraction-quality single crystals. However, one of the crystallisation routes[14] attempted in this work did provide diffraction-quality single crystals (see ESI†). The resulting structure, labelled here as **WOCPEM NEW** (CCDC deposition number: 2115849), was in excellent agreement with **WOCPEM** (Table 1).

The clear conclusion from the NMR data is that there is no disorder of the sulfonamide group, either static (which would lead to structure-less broadening of the ^{13}C resonances) or dynamic (which would lead to sharpened single resonances).[3] In terms of diffraction, the ordering of the sulfonamide orientation leads to a doubling of the unit cell and weak additional reflections. However, the typical superstructure reflection intensities, such as (300), (310) and (111), were 3 to 4 orders of magnitude weaker than other intensities for **WOCPEM NEW**, and these reflections must have been overlooked when determining the **VAGKUM** structure. Deposited reflection intensity data for the smaller unit cell would not allow this problem to be corrected, since the key reflections would normally be missing from the data set.

The fact that the **WOCPEM** structures, which are independently verified by repeat single-crystal X-ray diffraction and solid-state NMR experiments, have higher R factors (see Table 1) than the alternative **VAGKUM** structure determination may seem counter-intuitive; a non-expert user of crystallographic databases might assume that a lower R implies a

"better" structure. There are multiple factors, however, that complicate comparison of the R factors. For example, the shorter wavelength used for the **WOCPEM** studies means these involve a much larger number of reflections (including many weak reflections associated with the pseudo-symmetry). Combined with the very different unit cell sizes, this means that the *hkl* data sets are not easily compared. Overall, the R factor for the **WOCPEM** structures is reasonable for standard single-crystal diffraction structure of an average, middle-size organic molecule. Relaxing the number of restraints (from 61 for **WOCPEM NEW**) could reduce the R value but produce a less chemically sensible solution. This highlights the risks of using R naïvely to assess the relative correctness of different structural solutions.

In summary, we have described how solid-state NMR can be straightforwardly used to distinguish between conflicting structures in structural databases. NMR crystallographic methods are particularly complementary to diffraction when resolving potential issues of "disorder by symmetry" and pseudo-symmetry, since these are often associated with differences in the number of molecules in the asymmetric unit.

Conflicts of interest

The authors declare no conflicts of interest.

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