Drug Polymorphism

DOI: 10.1002/anie.201003185

Cisplatin: Polymorphism and Structural Insights into an Important Chemotherapeutic Drug**

*Valeska P. Ting, Marc Schmidtmann, Chick C. Wilson, and Mark T. Weller**

Cisplatin is the single most well-recognized and successful chemotherapeutic drug used today for the treatment of testicular and ovarian cancers.[1,2] Although three-dimensional structural elucidation of chemotherapeutic molecules has long been recognized in the pharmaceutical industry as vital for understanding the utility of active molecules through target receptor interactions, as well as a means of protecting the intellectual property associated with their large-scale production, cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$) has until now been perhaps the most prominent drug for which full structural information has been unavailable. The only published crystal structure of cisplatin is a low resolution determination of the "alpha" polymorph published in 1966,[3] while the solid product of patented production methods[4,5] is the "beta" polymorph. The crystal structure of the ubiquitous beta form of cisplatin has never previously been elucidated, and crucially, the relative thermal stability ranges of the two polymorphs are not known. Conversion of a prevailing solid form to a competing polymorph during transportation or storage prior to administration can drastically affect the efficacy of pharmaceuticals.[6,7] Transformation to a different polymorph can also affect practical delivery and use of pharmaceuticals, as properties such as density (which can be a factor in determining dosage) and rates of dissolution (affecting bioavailability) of distinct polymorphic forms can be dramatically different owing to variations in the strength of intermolecular bonding in the compounds. Herein we address these issues, presenting the full crystal structures of the two crystalline polymorphs of cisplatin, including precise determination of both hydrogen positions and intermolecular hydrogen bonding, which will facilitate improved modeling of the dissolution, solvation, and drug-receptor interactions of this molecule.[8–10] Furthermore, in probing the polymorphic interconversion of the two enantiotropic forms over different thermal regimes using neutron thermodiffractometry, an extraordinarily extensive thermal hysteresis range was uncovered for the reversible phase transition, indicating that the alpha and beta forms are equally stable at ambient temperatures.

Accurate determination of the 3D structure of drugs containing heavy metal centers and in which hydrogen positions and hydrogen bonding may be of importance is, however, non-routine. As the X-ray scattering contributions from hydrogen can be obscured by the scattering from heavier atoms, unequivocal location of hydrogen positions is often only obtainable using neutron scattering. Many pharmaceuticals (including cisplatin) cannot be easily obtained as large, non-twinned crystals suitable for neutron single-crystal diffraction. Thus, to obtain a complete description of the structures of the two polymorphs, including hydrogen positions, we prepared good-quality small single crystals and crystalline powders of both polymorphs. We then used the powerful combination of single-crystal X-ray diffraction (SXD) for refinement of the positions of the heavier atoms in conjunction with neutron powder diffraction (NPD) for determining the hydrogen positions to much greater accuracy. The collection of the NPD data was optimized for refinement of structural hydrogen positions[11] using the D20 high-flux powder diffractometer at the ILL in Grenoble,[12] operating in its high take-off angle configuration at a fixed wavelength of 1.87 Å.

The only previously published structure determination of cisplatin was a SXD experiment at 120 K by Milburn et al., which produced a structure in space group $P\bar{1}$, with a unit cell volume of about 256 $Å^3$, but yielded no positions for the ammonia group hydrogen atoms. Furthermore, the high reported *R* factors (ca. 9%) and an unrealistically short Pt–N bond could be attributed to the extensive twinning reported in that study. No structural study has been published on the beta form, although its synthesis has been described in a number of pharmaceutical patents.[4,5] Its powder X-ray diffraction patterns have variously been indexed to triclinic[13] and monoclinic unit cells[14] and the structure has remained elusive until now.

The small yellow crystals of the beta form we recovered by recrystallization were found to transform to the alpha form at low temperatures, so whilst the SXD measurements of the alpha form were conducted at 100 K (in keeping with Milburn's experiments), data on the beta form were collected at 220 K. The SXD data obtained from the two polymorphs were indexed to two distinct triclinic unit cells. The unit cell dimensions of the plate-like yellow crystals of the alpha form correspond well with those reported by Milburn et al., as do the fractional coordinates of the Pt and Cl atoms, although the

[*] Dr. V. P. Ting, Prof. M. T. Weller
School of Chemistry, University of Southampton
Southampton SO17 1BJ (UK)
Fax: (+44) 238-0593-3781
E-mail: mtw@soton.ac.uk

Dr. M. Schmidtmann, Prof. C. C. Wilson
WestCHEM, Department of Chemistry
University of Glasgow, Glasgow G12 8QQ (UK)

[**] We thank Dr. P.Henry for technical advice, Dr. J. Armstrong for collection of SXD data, the EPSRC for funding, and the Institut Laue-Langevin for beam time.

Supporting information for this article, including crystallization methods, details on SXD and NPD data collection and refinement, QM calculations, solubility measurements, and associated references, is available on the WWW under http://dx.doi.org/10.1002/anie.201003185.

extracted Pt–N bond lengths are more physically meaningful here (2.048(3) Å, compared to 2.05(4) and 1.95(3) Å from Milburn's structure). The SXD data from the needle-like crystals of the beta form were indexed to a larger $P\bar{1}$ triclinic cell with the *b* axis approximately double that of the alpha form (volume ca. 526 $Å^3$, compared to 256 $Å^3$). These unit cells differ from those previously reported in the literature,[13,14] though ours is related to that reported by Kirik et al.[13] The SXD was used to accurately determine the atomic coordinates of the non-hydrogen atoms, and subsequent simultaneous refinement against the SXD data and 4 h NPD data sets at 100 K and 220 K for the alpha and beta phases, respectively, enabled the identification of the H atom positions by Fourier difference analysis (see Supporting Information for Fourier maps). This method permitted the orientations of the ammonia groups, and thus the hydrogen bonding interactions between the square planar moieties, to be determined for the first time. The full refined structures for the alpha and the beta forms are shown in Figure 1; the

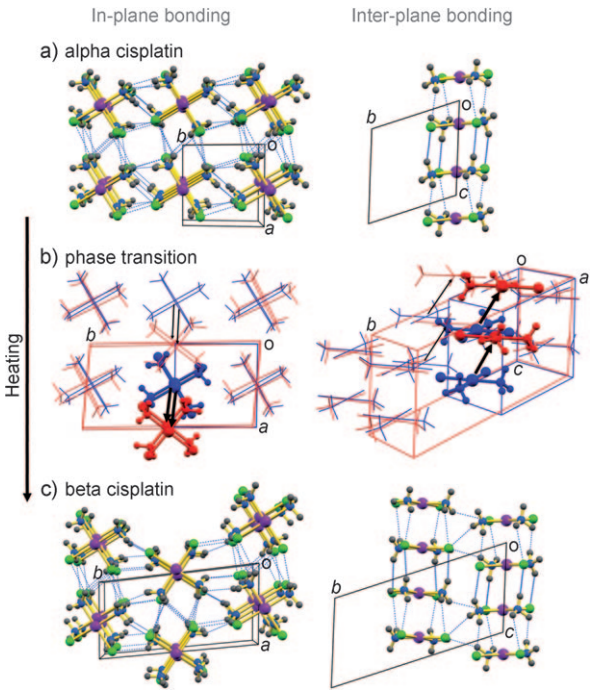

Figure 1. a) The structure of alpha cisplatin. b) The overlaid unit cells of the alpha and beta forms, showing the transformation from the low-temperature alpha form (blue) to the high-temperature beta form (red). c) The structure of the beta form. For each compound, the interlayer bonding in the *ab* plane is shown on the left and the interlayer H-bonding along the stacks along *c* is shown on the right. Hydrogen bonding is shown as dashed blue lines and the unit cell is shown in black.

refined atomic coordinates and selected bond lengths and angles (as well as the refined profile fits to the NPD data) are given in the Supporting Information. Refinement of both forms resulted in physically meaningful N–H bonds and angles (average N–H bond distance 1.00(5) Å, average H-N-H angle 107(7)°). Figure 1 shows the alternating arrangement of the NH_3 groups and the Cl atoms in each stack of square planar molecules along the *c* axis. In both forms, the central Pt atoms appear staggered along *c*, with Pt-Pt-Pt angles ranging from about 160 to about 170°. It is likely the structure adopts this conformation (rather than a perfectly stacked arrangement of Pt atoms) as a result of hydrogen bonding, which directs the packing of the cisplatin molecules in the solid state.

The orientations of the NH_3 groups in each polymorph revealed a much more elaborate intermolecular hydrogen bonding network than was originally suggested by Milburn et al., who speculated that hydrogen bonding only existed in the direction of the *a* axis, based on their refined intermolecular N–Cl bond lengths and Pt-N-Cl angles. Significantly, the current study shows that both forms of cisplatin contain extended three-dimensional hydrogen-bonded networks, where the main interactions are found within the cisplatin stacks in the direction of the *c* axis. This finding is consistent with the observed retention of the stacked formation throughout the alpha–beta phase transition, and on the persistent orientation of the NH_3 groups (as determined experimentally and computationally), which act to minimize the H-N-Pt-Pt torsion angles, and consequently the four H···Cl distances between neighboring cisplatin molecules (see Table 1 for a list of selected H-bonding distances). These

Table 1: H···Cl bond lengths in alpha and beta cisplatin.[a]

	Alpha cisplatin		Beta cisplatin			
	H-bond	H···Cl [Å]	H-bond	H···Cl [Å]	H-bond	H···Cl [Å]
c	H1···Cl2	2.428(17)	H1···Cl2	2.43(2)	H7···Cl4	2.55(2)
c	H4···Cl1	2.516(18)	H4···Cl1	2.63(3)	H10···Cl3	2.42(3)
c	H6···Cl1	2.637(17)	H6···Cl1	2.51(3)	H12···Cl3	2.75(2)
c	H3···Cl2	2.974(15)	H3···Cl2	2.91(2)	H9···Cl4	2.65(3)
b	H2···Cl1	2.436(13)	H2···Cl3	2.53(2)	H8···Cl1	2.67(2)
b	H5···Cl1	2.533(17)	H5···Cl3	2.62(3)	H11···Cl2	2.79(2)
a	H3···Cl2	2.640(15)	H3···Cl2	2.72(2)	H9···Cl4	2.93(2)
a	H6···Cl2	2.723(15)	H6···Cl2	2.84(2)	H12···Cl4	2.42(2)
a	H3···Cl1	2.932(15)	H3···Cl1	2.84(2)	H9···Cl3	3.03(3)

[a] Alpha cisplatin data acquired at 100 K, beta at 220 K. Principal bonding directions given along unit cell vectors. Data taken from the NPD refinements.

three-dimensional networks consisting of cisplatin stacks are stabilized by further H···Cl interactions in both the *a* and *b* axial directions. To quantify the important thermal stability properties of the two forms of cisplatin, the relative stability of the two polymorphs with respect to temperature was examined by in situ variable-temperature NPD experiments. As the hydrogen bonding was of such critical importance, sample deuteration was avoided so as to remove the possibility of isotopic effects, which can be significant when investigating kinetics[15] (for example, of phase transitions). A sample of polycrystalline beta cisplatin at room temperature (ca. 2 g) was initially cooled to 100 K, then heated to 360 K, and finally returned to room temperature at a fixed rate of about 1.5° min^{-1}. Sequential refinements of the relative phase fractions show a transformation starting below 200 K from the initial beta form at room temperature to the pure alpha form (Figure 2). The alpha conformer remained stable at the lower temperatures (from 100 K), with signs of reversion to the beta

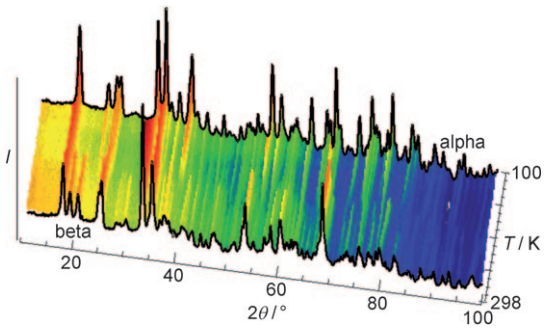

Figure 2. Sequential NPD data sets showing the transformation between the diffraction patterns of the alpha and beta forms upon cooling, from the beta form at room temperature to the alpha form at 100 K, showing the evolution and disappearance of characteristic peaks for each phase. Colors indicate peak intensity.

form starting at temperatures above about 320 K (see Figure 3); the almost pure beta form re-emerges at about 350 K (though about 10% phase fraction of the alpha form remained at the end of the heating cycle).

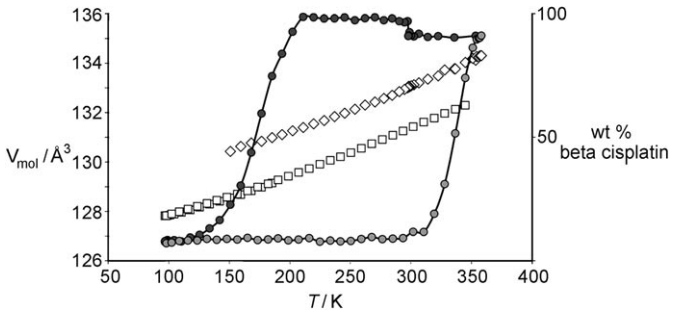

Figure 3. Relative thermal stability of the alpha and beta polymorphs. Plot of the phase fraction of the beta polymorph with respect to temperature, as refined from the variable-temperature NPD data. Light gray circles: heating cycle; dark gray: cooling. Overlaid are the refined volumes V_{mol} that a cisplatin molecule occupies in the alpha (□) and beta (◇) forms.

As the sample was returned to room temperature, the beta form remained the dominant phase. The unit cell parameters refined from NPD data sets show that, over the accessible temperature range, the density of the alpha form is consistently about 2% higher than that of the beta form (Figure 3). The unexpectedly large hysteresis apparent in the phase transition temperatures (ca. 160 K) between the temperatures of formation of the slightly denser alpha form upon cooling and the reversion to the beta form above 320 K implies that both forms are thermodynamically stable over a large temperature range. Quantum-mechanical ground-state energy calculations (see Supporting Information) indicate that the two forms are thermodynamically comparable to within 1 kJ mol^{-1}. This very low energy difference is typical of polymorphic materials and is also compatible with the critical effect of the experimental conditions on the crystallization-based synthesis of each form. The large hysteresis can be resolved by examining the structural mechanism of the phase transition. On transformation from the low-temperature alpha form to the high-temperature beta form, every second layer of cisplatin stacks is translated by half a unit cell vector in the directions of the *a* and *c* axes (shown by the black arrows in Figure 1b), accompanied by a slight rotation of approximately 25° about the *c* axis. As a consequence, all hydrogen bonds along the *b* and half the hydrogen bonds along the *a* axis are broken and reformed, leaving the stronger interplanar hydrogen bonds along the *c* direction intact. Even though the driving force behind this massive, reversible rearrangement of cisplatin molecules remains unknown, it is consistent with the large thermal hysteresis observed. Once the alpha phase has formed, the activation barrier necessary to convert into the beta phase (associated with the energy needed to overcome the in-plane hydrogen bonding) requires significantly elevated temperatures.

The results of this study may have important implications for the reliable production, storage, and administration of cisplatin. The thermally induced transformations between these two polymorphs, investigated by in situ variable-temperature NPD, revealed the ease with which the polymorphic conversion to the alpha polymorphic form can occur in cisplatin. The temperatures identified for transition to the alpha polymorph are accessible during the routine lyophilization of the solid drug prior to administration, and full reversion to the commercially relevant beta form will not occur until the product has been heated to above 75 °C. However, it is unlikely that any pharmacological differences would persist once the solid drug has been solvated. The full structures of the two polymorphs reported herein suggest that small differences in the physical properties of the two solid polymorphs could feasibly exist, which would for example affect the solubility (which is dependent upon the free-energy difference between the solvated and crystalline states, and thus the strength of the hydrogen bonds within the crystal). This observation could be of relevance, as commercial beta cisplatin has a very low solubility of about 1 mg mL^{-1} in aqueous 0.9% NaCl.[16] Differences in the dissolution rates were also predicted for the same reasons based on energetics, but also due to significant differences in the crystal morphologies and resulting surface areas of the two forms. Qualitative measurements on the dissolution of each of the two polymorphs in saline solution showed that dissolution rates under these conditions were indeed affected by particle shape, with the plate-like crystals of the alpha form dissolving less readily than the needle-like crystallites of the beta form (see Supporting Information). No clear differences in the absolute solubility levels of the two polymorphs could be detected within the accuracy of our measurements, which is consistent with the small calculated energy difference between the two polymorphs.

In summary, this study has highlighted the huge potential for retrieval of accurate and in-depth information on hydrogen positions and hydrogen bonding in polycrystalline pharmaceutical products in the solid state by the powerful combination of single-crystal X-ray diffraction and neutron powder diffraction. The accurate experimental determination of the hydrogen positions in active molecules will facilitate more exact computational modeling of their dissolution and

ligand exchange processes, as well as the interactions with their biological targets, thus providing a better understanding of the mechanisms by which they function.

Received: May 26, 2010
Revised: September 10, 2010
Published online: October 28, 2010

Keywords: cisplatin · neutron diffraction · phase transitions · polymorphism

[1] A. Flechon, S. Culine, J. P. Droz, *Crit. Rev. Oncol. Hematol.* **2001**, *37*, 35 – 46.
[2] A. W. Prestayko, S. T. Crooke, S. K. Carter, *Cisplatin, current status and new developments*, New York: Academic Press, **1980**.
[3] G. H. W. Milburn, M. R. Truter, *J. Chem. Soc (A)* **1966**, 1609 – 1616.
[4] M. A. Kaplan, A. P. Granatek, US 4322391, **1982**.
[5] T. Murata, K. Ishikawa, O. Kogawa, K. Iwata, WO98/29344, **1999**.
[6] J. Bauer, S. Spanton, R. Henry, J. Quick, W. Dziki, W. Porter, J. Morris, *Pharm. Res.* **2001**, *18*, 859 – 866.
[7] D. S. Johnson, J. J. Li, *The art of drug synthesis*, Wiley, Hoboken, NJ, **2007**.
[8] A. M. Amado, S. M. Fiuza, M. P. M. Marques, L. de Carvalho, *J. Chem. Phys.* **2007**, *127*, 185 104 – 185 114.
[9] P. M. Takahara, A. C. Rosenzweig, C. A. Frederick, S. J. Lippard, *Nature* **1995**, *377*, 649 – 652.
[10] S. E. Sherman, D. Gibson, A. H. J. Wang, S. J. Lippard, *Science* **1985**, *230*, 412 – 417.
[11] M. T. Weller, P. F. Henry, V. P. Ting, C. C. Wilson, *Chem. Commun.* **2009**, 2953 – 2989.
[12] T. C. Hansen, P. F. Henry, H. E. Fischer, J. Torregrossa, P. Convert, in 4th European Conference on Neutron Scattering, IOP Publishing Ltd, Lund, SWEDEN, **2007**.
[13] S. Kirik, L. Solovyev, Blokhin, A. Mulagaleev, R. Mulagaleev, *PCPDFWIN* ref. 50-0643: β-$[Pt(NH_3)_2Cl_2]$, **1998**.
[14] J. Visser, *J. Appl. Crystallogr.* **1969**, *2*, 89 – 95.
[15] J. J. Thomas, H. M. Jennings, *Chem. Mater.* **1999**, *11*, 1907 – 1914.
[16] L. Feng, A. De Dille, V. J. Jameson, L. Smith, W. S. Dernell, M. C. Manning, *Cancer Chem. Pharma.* **2004**, *54*, 441 – 448.

