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The presence of polymorphism in oxytetracycline hydrochloride shown by X-ray powder diffraction techniques

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Abstract. The characterization of several polymorphs of oxytetracycline hydrochloride, a commonly used antibiotic of the tetracyclines family, was carried out after its crystallization under different conditions: slow evaporation, rapid crystallization, and vapour diffusion with different solvents. The solvents used included water, ethanol, methanol, ether, ethyl acetate, toluene, dichloromethane and dioxane. The different products obtained were analyzed by X-Ray Powder Diffraction, NMR, FT-IR, and Thermal Analysis (TGA and DSC).

Introduction

Polymorphism is an aspect of special interest in the characterization of pharmaceuticals because this phenomenon usually modifies the physicochemical and pharmaceutical properties of an Active Pharmaceutical Ingredient (API). It can affect intrinsic properties of the solid state such as melting point, solubility, chemical stability, density, bioavailability, among others. The pharmaceutical solids are mostly small organic molecules that form molecular crystals which are held together by weak inter- and intramolecular forces (mainly, hydrogen bonds and Van der Waals attractions). The different structural forms of these materials are due to the diverse dispositions that their constituent molecules can adopt in the solid state. The formation of different crystalline modifications can also be induced by the incorporation of water (hydrates) and organic solvents (solvates) in their structures. It has been estimated that between 56% [1] and 87% [2] of all the drug substances, show some kind of polymorphism or *pseudopolymorphism*. Some active ingredients with long, "flexible" molecules may be difficult to crystallize and tend to produce amorphous materials during precipitation. Also, they can exhibit partial order between that of a crystalline phase and an amorphous state.

Oxytetracycline hydrochloride, a common antibiotic of the tetracyclines family, is usually prescribed as a bacteriostatic agent and used for cutaneous and minor infections. In the present work, the characterization of several polymorphs of this compound was carried out after its crystallization under different conditions. The different products obtained were analyzed by X-ray Powder Diffraction, FT-IR, and NMR spectroscopy. Thermal Analysis (TGA and DSC) were also employed. These results are part of the work carried out by Toro in his *Licenciatura* Thesis [3].

Experimental section

Sample treatment

Oxytetracycline hydrochloride ($C_{22}H_{23}N_2O_9 \cdot HCl$) as provided by *PROULA-Medicamentos* was recrystallized under different conditions, using a variety of solvents: water, ethanol, methanol, ether, ethyl acetate, toluene, dichloromethane and dioxane. The recrystallization process was carried out by slow evaporation, rapid crystallization, and vapour diffusion, using 0.30 g of sample in 10-15 ml of each solvent.

Diffraction data collection

The powder diffraction patterns were recorded at room temperature in a Phillips PW-1250 Goniometer, automated by Crystal Logic Inc., using CuKα radiation (λ = 1.5418 Å). This instrument is equipped with a secondary graphite monochromator and a scintillation detector, and it was operated at 40 kV and 25 mA. A fixed scatter slit of 0.2° and a 1 mm receiving slit were used. The data were registered in the interval of 5-70° (2θ), in steps of 0.02° with a counting time of 10 s/step. The profile fit of each pattern was carried out with the MDI JADE 5 software [4]. The positions of the diffraction maxima were established after profile fitting the pattern using split Pearson functions. The indexing of each pattern was performed with the program DICVOL04 [5].

Nuclear magnetic resonance

^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance DRX-400 spectrometer, using DMSO as the solvent. TMS was used as an internal standard. Chemical shifts (δ) and coupling constants (J) values are reported in ppm and Hz, respectively. The bidimensional study was performed using the Heteronuclear Multiple Bond Correlation (HMBC) technique.

Results and discussion

Commercial material

Oxytetracycline hydrochloride (OxyHCl) as provided by *PROULA-Medicamentos* is a brilliant yellow solid which decomposes at 215°C. The NMR spectra clearly showed the chemical nature of the material under study (figure 1). Table 1 contains the peak assignment of the ^{1}H-NMR spectrum. The diffraction pattern registered for this phase, shown in figure 2, is different from the only report for this material contained in the PDF-2 (32-1812) [6] and from the pattern calculated using PLATON [7] with the data reported in the CSD [8] (REFCODE TERMYC [9])

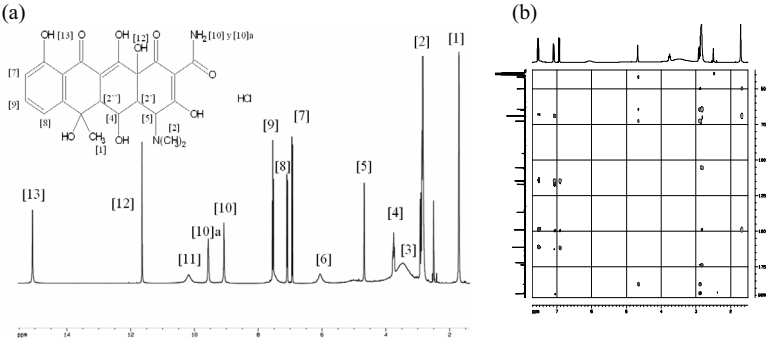


Figure 1. (a) ^{1}H-NMR spectrum registered for commercial OxyHCl. (b) HMBC spectrum for OxyHCl.

Table 1. Peak assignment of the 1H-NMR spectrum of a commercial OxyHCl.

Signal	δ (ppm)	Multiplicity
H[1]	1.71	(t) 3H
H[2]	2.87	(s) 6H
H[2']	2.89	Overlapping with H[2]
H[2'']	2.92	Overlapping with H[2]
H[4]	3.76	(t) 1H
H[5]	4.67	(s) 1H
H[7]	6.92 (J=8 Hz)	(d) 1H
H[8]	7.08 (J=8 Hz)	(d) 1H
H[9]	7.54 (J=8 Hz)	(t) 1H
H[10]	9.07	(s) 1H
H[10]a	9.56	(s) 1H
H[12]	11.54	(s) 1H
H[13]	15.08	(s) 1H

Crystallization by slow evaporation in water

The crystallization of OxyHCl in water, by slow evaporation at room temperature, produced a brown-opaque solid which decomposes at 210ºC. Its diffraction pattern is different from the one obtained for the commercial sample (figure 3) and from the pattern calculated using the data for TERMYC extracted from the CSD. However, it is very similar to the pattern reported in PDF-2 (32-1812). The indexing of this pattern, carried out using DICVOL04, suggests that this phase crystallizes in an orthorhombic unit cell with parameters: ***a***=12.009(3) Å, ***b***=11.450(3) Å, ***c***=15.808(6) Å, and *V*=2183.1(8) Å^3. These parameters are similar to those reported more than fifty years ago by Robertson, *et al*. [10]. It is noteworthy that when the quantity of the API is doubled, using the same crystallization conditions, the product is predominantly amorphous.

(a) (b)

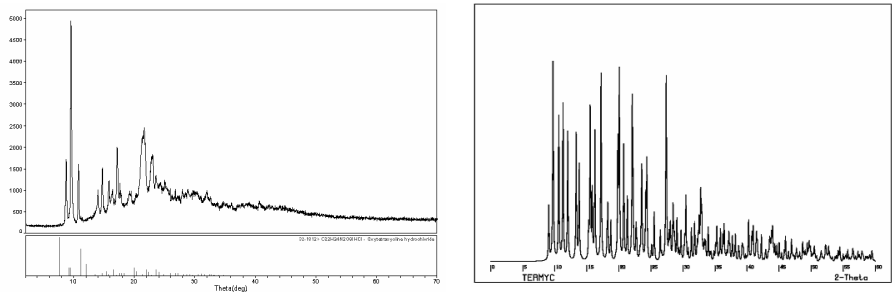
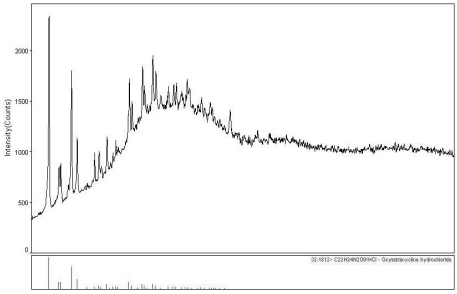


Figure 2. Powder Diffraction pattern (a) recorded for commercial OxyHCl and (b) calculated using the data for OxyHCl (REFCODE TERMYC) extracted from the CSD.



Crystal System	Orthorhombic
Unit cell parameters	***a***=12.009(3)Å, ***b***=11.450(3)Å, ***c***=15.808(6)Å, *V*=2183.1(8)Å^3
Figure of merit	M_{20}=14,1 F_{25}=22,2 (0,0180, 75)

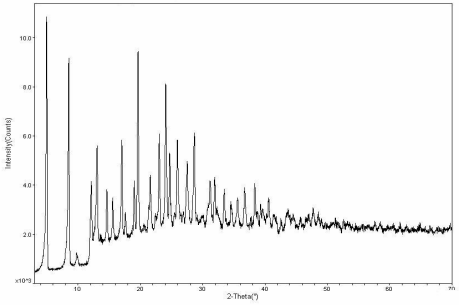
Figure 3. Powder Diffraction pattern recorded for OxyHCl recrystallized in water.

Crystallization by vapour diffusion of ether into a methanol solution.

When the crystallization took place under vapour diffusion of ether into a methanol or an ethanol solution in a refrigerator, very thin light-brown needles were obtained in both cases. Their melting points were very similar, 210ºC and 213ºC. The diffraction patterns of these materials were very similar and also similar to the patterns of the products obtained by other

essays: vapour diffusion of dichloromethane in ethanol:dicloromethane 2:3 solution at room temperature; vapour diffusion of toluene in ethanol:toluene 1:1 at 65ºC; vapour diffusion of toluene in methanol:toluene 1:1 at 65ºC and vapour diffusion of ether in ethanol:ether 2:3 under refrigeration.

Figure 4 shows the diffraction pattern recorded for the OxyHCl obtained by vapour diffusion of ether into an ethanol:ether solution in a refrigerator. The indexing of the patterns carried out using the program DICVOL04 produced consistently a monoclinic unit cell with parameters: ***a***=20.978(6) Å, ***b***=10.524(8) Å, ***c***=10.316(9) Å, β=119.59(3)°, and V=1980(1) Å^3.



Crystal System	Monoclinic
Unit cell parameters	***a***=20.978(6)Å, ***b***=10.524(8)Å, β=119.59(3)° ***c***=10.316(9)Å, V=1980(1)Å^3
Figure of merit	M_{20}=15,1 F_{25}=11,5 (0,0082, 264)

Figure 4. Powder Diffraction pattern recorded for OxyHCl obtained by vapour diffusion of ether in an ethanol:ether solution.

Additional crystallization experiments of the API under study in methanol and ethanol produced a yellow-opaque powder which decomposes at 211ºC. An amorphous character dominates the patterns. However, a few diffraction maxima are observed. The positions of these maxima do not coincide with the positions of the only report found in the Powder Diffraction File or with the positions registered for the previous samples.

Concluding remarks

These results indicate that oxytetracycline hydrochloride (OxyHCl) exhibits several polymorphic modifications that, to our knowledge, have not been previously reported. These modifications have been induced by changing the crystallization solvent and the crystallization method. The different polymorphs need to be characterized properly in order to fully understand the behaviour of this important antibiotic. In spite of our efforts, attempts to prepare single crystals of these polymorphs were unsuccessful.

References

1. Henck, J.-O., Griesser, U. J. & Burger, A., 1997, *Pharm. Ind.*, **59**, 165.
2. Stahly, G. P. 2003, at the American Chemical Society ProSpectives *Polymorphism in Crystals: Fundamentals, Predictions and Industrial Practice*, Tampa, FL, Feb 23–26. Cited by Hilfiker, R., Blatter, F. & von Raumer, M., 2006, in *Polymorphism in the Pharmaceutical Industry*, edited by R. Hilfiker (Weinheim: Wiley-VCH), pp. 1-19.
3. Toro, R., 2005, "Caracterización por Técnicas de Difracción de Rayos-X, RMN, FT-IR y TGA-DSC de diversos compuestos farmacológicamente activos, sometidos a distintas condiciones de cristalización". Tesis de Licenciatura, Universidad de Los Andes, Mérida, Venezuela.
4. JADE: *XRD Pattern Processing and Identification Program*. Materials Data, Inc., Release 5.0.
5. Boultif, A. & Loüer, D., 2004, *J. Appl. Cryst*., **37**, 274.
6. ICDD, Powder Diffraction File. 2002, International Centre for Diffraction Data, 12 Campus Boulevard, Newtown Square, PA 19073-3272, USA.
7. Spek, A.L., 2003, *J. Appl. Cryst.*, **36**, 7. *PLATON: A Multipurpose Crystallographic Tool.* Utrech University, Utrech, The Netherlands.
8. Allen, F.H., 2002, *Acta Cryst.*, B**58**, 380.
9. Cid-Dresdner, H., 1965, *Z. Kristallogr., Kristallgeom., Kristallphys., Kristallchem.*, **121,**170.
10. Robertson, J., Robertson, I., Eiland, P.F. & Pepinsky, R., 1952, *J. Amer. Chem. Soc.*, **74**, 841.

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