Carboplatin and oxaliplatin decomposition in chloride medium, monitored by XAS.

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The stability of carboplatin and oxaliplatin aqueous solutions has been studied under different chloride ions concentration and pH conditions. For both compounds, we demonstrate the chloration of the platinum first coordination shell.

Keywords: platinum antitumoral drugs, carboplatin, oxaliplatin, cisplatin, solution stability.

1. Introduction

Platinum(II) complexes are largely used for antitumoral therapy. Among them, carboplatin and oxaliplatin are second generation drugs belonging to the cisplatin structural family. Each of them have specific therapeutic activities and toxicity. Peculiarly, carboplatin and oxaliplatin present less toxicity (Wong and Giandomenico, 1999).

The stability of carboplatin in the presence of chloride ions, provided under perfusion conditions, has been largely studied (Lederer, M. and Leipzig-Pagani, 1998, Valière *et al*, 1996, Benaji *et al*, 1994, Prat *et al*, 1994, Hadfield *et al*, 1993, Van der Vigh, 1991). A degradation has been shown, with a probable production of cisplatin, that would increase the drug toxicity. Nevertheless, the degradation products have not been clearly identified in these studies. Such a degradation may also be feared in the case of oxaliplatin.

In a preliminary study, we have shown a progressive transformation of carboplatin into cisplatin under well-defined chloride concentration and pH conditions (Curis *et al.*, 2000). In this study, we consider the behaviour of oxaliplatin in comparison with the carboplatin one.

2. Experiments

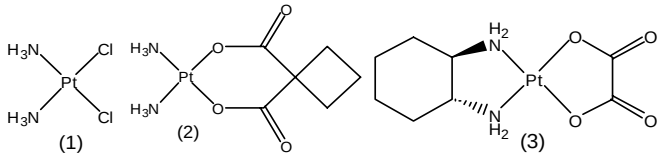
The solid samples were prepared as pellets. The cisplatin was purchased from Sigma chemicals; the carboplatin used was obtained from a pharmaceutical preparation from Bristol-Myers Squibb ("paraplatine", ref. 331 720.0), and the oxaliplatin from the pharmaceutical preparation from Sanofi ("eloxatine", ref. 559 649 2). In the case of the kinetic studies, we used freshly prepared solutions. Some solutions were prepared a long time before in order to check the stability in a larger scale.

All the spectra were recorded at LURE (Orsay, France), in transmission mode, on the EXAFS 13 experimental station (beamline D41, DCI storage ring, with a current of about 250 mA), at the L_{III} platinum edge (11,564 eV). A Si (1 1 1) double-crystal or channel-cut monochromator was used; the energy was calibrated using the L_{III} edge of a gold foil. Measurements were made from 11,400 to 12,600 eV with a step of 3 eV and a counting time of 1 s/point. The resulting recording time is about 20 min for each spectrum.

The spectra were extracted (with E_0 taken at half height of the edge: 11,564 eV) and fitted using LASE (Curis), EXAFS98 (Michalowicz, 1991), Crystalff (Provost and Michalowicz) and FEFF (Rehr *et al*, 1991) softwares. The Fourier transforms were calculated in the range 2-12 $Å^{-1}$ for all solid samples and 2-10 $Å^{-1}$ for all liquid samples; in both cases, the Fourier transforms are not corrected for the phase-shift. The fits were completed using the crystallographic data in the case of cisplatin, carboplatin and oxaliplatin compounds (respective references: Milburn and Truter, 1966, Beagley *et al*, 1985, Bruck *et al*, 1984).

3. Results and discussion

3.1 Carboplatin and oxaliplatin EXAFS spectra



The cisplatin (1), carboplatin (2), and oxaliplatin (3) solid state EXAFS spectra show clear differences (fig. 1).

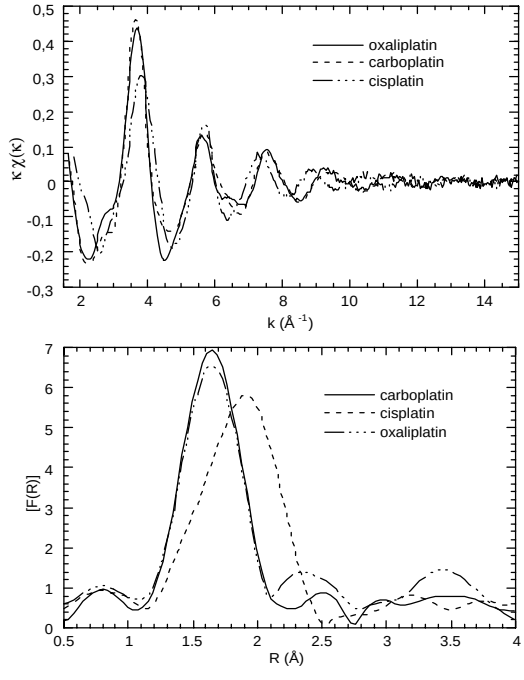


Figure 1
Experimental EXAFS spectra and their FT modulus of carboplatin, oxaliplatin and cisplatin.

Considering the first shell of coordination, the modulus of the Fourier transforms are quite similar for carboplatin and oxaliplatin. In the case of cisplatin, the replacement of two

oxygen atoms into two chloride atoms produces, as expected, a larger distance contribution to the main peak. The contribution of the two nitrogen atoms only appears as a shoulder on the left of this peak. These observations are confirmed by the fit of the spectra, using either a four nitrogen/oxygen atom shell for carboplatin and oxaliplatin or a two nitrogen atoms and two chloride atoms shell for cisplatin, using phases and amplitudes calculated by FEFF (table 1). For all compounds, large values of $\Delta E=E_{0exp}-E_{0th}$ were obtained because of the transition involved in the edge white line, as explained elsewhere (Curis *et al*, 2000, Pavankumar *et al*, 1999).

Table 1
Fitting results for the first coordination shell of carboplatin, oxaliplatin and cisplatin. Only distances, Debye-Waller factors and ΔE were fitted.

	Carboplatin	Oxaliplatin	Cisplatin	
Shell	4N/O	4N/O	2N	2Cl
R (Å)	2.028(20)	2.006(6)	2.032(7)	2.314(4)
$\sigma \times 10^2$(Å)	2.00(14)	5.23(63)	3.31(100)	3.40(40)
ΔE (eV)	-13.26(1)	-12.74(28)	-12.37(1)	

Secondary peaks appear at larger distances for carboplatin and oxaliplatin, due to higher shells of coordination. In this study, we only deal with the first coordination shell and its evolutions. The complete analysis of the spectra will be presented later.

The carboplatin and oxaliplatin spectra are unchanged in pure aqueous solution.

3.2 Oxaliplatin in the presence of a large excess of hydrochloric acid

As no assumption of oxaliplatin degradation in the presence of chloride ions has been previously shown, we have first considered the case of a large excess of hydrochloric acid. In such a case, a yellow precipitate appears within a few minutes. The absence of L_{III} platinum edge in the floating part shows that there is no more platinum in solution. The precipitate EXAFS spectrum is strongly different from the oxaliplatin one (fig. 2).

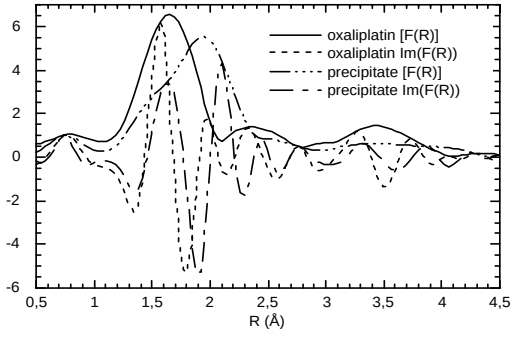


Figure 2
Exafs Fourier transform modulus and imaginary part (calculated in the range 2-12 $Å^{-1}$) of oxaliplatin and the precipitate.

The modulus of the Fourier transform shows the probable presence of chloride atoms in the first shell of coordination. This hypothesis is confirmed by the fit of the precipitate spectrum using two nitrogen and two chloride atoms in the first coordination shell (table 2, fig. 3).

Table 2
Fitting results for the first shell in the precipitate.

	R (Å)	$\sigma \times 10^2$ (Å)	ΔE (eV)
2 N	2.000(8)	6.81(71)	-8.42(18)
2 Cl	2.305(3)	5.96(43)	

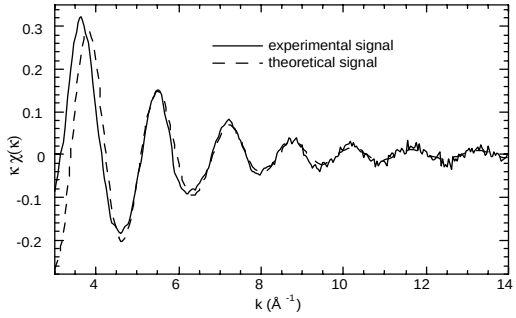


Figure 3
Experimental and theoretical EXAFS spectra for the precipitate.

3.3 Carboplatin and oxaliplatin hydrochloric solutions

For both compounds, we observed a progressive evolution of freshly prepared solutions, in the presence of hydrochloric acid (fig. 4, fig. 5). In both cases, there is an enlargement of the main peak in the Fourier transform modulus, with the appearance of a larger distance characteristic of chloride atoms in the platinum first coordination sphere, whose relative importance increases with the time. After a few hours, the carboplatin solutions spectra tend to the cisplatin one, and the oxaliplatin solutions one tend to the precipitate previously studied one.

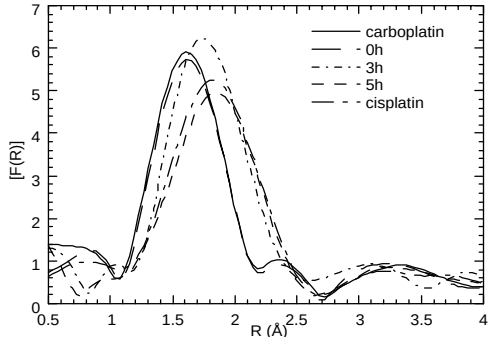


Figure 4
Modulus of Fourier transform (calculated in the range 2-10 $Å^{-1}$) for a solution of carboplatin 10 mg/ml in HCl 0.4 M at 0h, 3h and 5h after the beginning of the experiment.

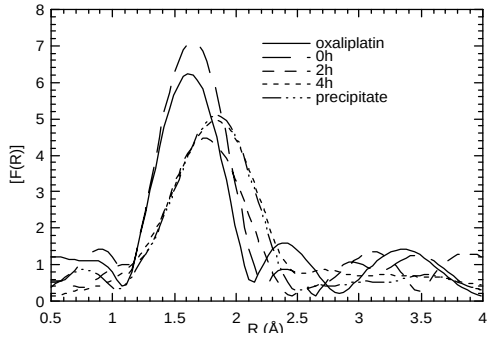


Figure 5
Modulus of Fourier transform (calculated in the range 2-10 $Å^{-1}$) for a solution of oxaliplatin 10 mg/ml in HCl 0.4 M at 0h, 2h and 4h after the beginning of the experiment.

For both compounds, the evolution is faster when the pH decreases and thus the chloride concentration increases.

3.4 Carboplatin and oxaliplatin NaCl solutions

We then studied the stability of these two complexes in presence of chloride neutral medium and we observed different behaviours.

Concerning the carboplatin solutions the EXAFS spectra show no evolution within a few hours whatever the NaCl concentration included between 0.08 M and 3 M. A fortnight later a precipitate appears for the more concentrated NaCl solution which proved to be cisplatin. For the 0.3 M NaCl concentration solution we observe no evolution (fig. 6). Nevertheless, a precipitate appeared a few weeks later.

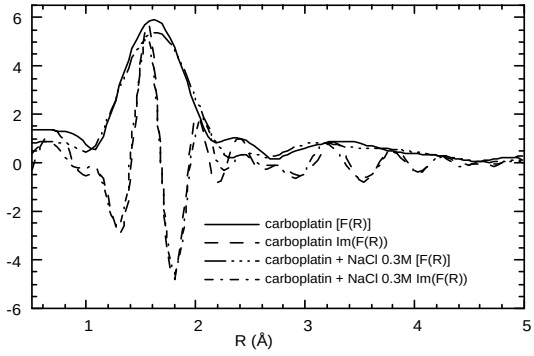


Figure 6
Modulus of Fourier transform for a solution of carboplatin 10 mg/ml in NaCl 0.3M kept 15 days in the dark.

In the case of oxaliplatin complex, a slight enlargement appears after a few hours (fig. 7), indicating that the chloration may have begun. The oxaliplatin appears thus to be less stable than the carboplatin in chloride neutral solution.

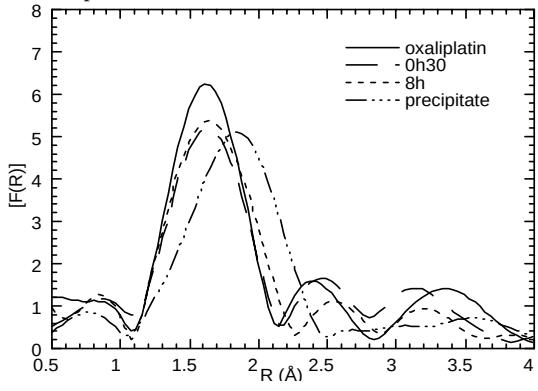


Figure 7
Modulus of Fourier transform calculated in the range 2-10 $Å^{-1}$ for a solution of oxaliplatin 10 mg/ml in NaCl 0.65 M at 0h30 and 8h after the beginning of the experiment.

4. Conclusion

We have focused our study on the evolution of the first coordination sphere of platinum in antitumoral drugs. We have demonstrated that XAFS technique is well suited to follow this evolution in presence of chloride ions. Under such conditions the oxaliplatin and carboplatin complexes show similar chlorations. In both cases we observe the displacement of two light atoms (N or O) by two chloride atoms. This reaction is favoured when the acidity of the medium or when the chloride concentration is increased. Nevertheless we noticed that the oxaliplatin is less stable than the carboplatin in neutral solutions.

For practical pharmaceutical implications, these preliminary results will be extended to physiological and perfusion conditions.

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 Received 31 July 2000 • Accepted 16 November 2000