MOLECULAR AND ELECTRONIC STRUCTURE
OF HYDROLIZED PLATINUM ANTICANCER DRUGS
AS REVEALED BY X-RAY ABSORPTION, IR, UV-VIS
SPECTROSCOPIES AND DFT CALCULATIONS

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The present study deals with the electronic structure of the bioactive anticancer drugs based on platinum(II) complexes: cisplatin $PtCl_2(NH_3)_2$, carboplatin $PtC_6H_{12}N_2O_4$ and oxaliplatin $PtC_8H_{14}N_2O_4$, which are being used in cancer treatment. The purpose of the work was to examine the molecular and electronic structure of platinum(II) coordination complexes when they undergo hydrolysis, which is crucial in order to better understand their antitumor properties. The density functional theory (DFT) was used to investigate the electronic structure of the platinum(II) complexes under study. The process of hydrolysis was simulated, and the structure and geometry of hydrolyzed platinum complexes were determined. The electronic structure, energy levels of occupied and unoccupied MOs and the distribution of the total and partial electron density of states (DOS) were shown and the UV-Vis and oscillation spectra of the hydrolyzed platinum(II) complexes were calculated. The theoretical calculations were verified by the experimentally obtained data by applying the method of X-ray absorption at PtL_3 edge as well as UV-Vis and IR spectroscopic techniques.

INTRODUCTION

The present work examines the platinum(II) coordination complexes as anticancer agents: cisplatin ($PtCl_2(NH_3)_2$), carboplatin ($PtC_6H_{12}N_2O_4$) and oxaliplatin ($PtC_8H_{14}N_2O_4$) which are now finding increasing application in cancer treatment. The antitumor effect of the platinum(II) coordination complexes is based on their interaction with DNA nitrogenous bases of the damaged cell, which results in forming the inner and outer spiral strands inhibiting DNA synthesis and its further replication. When compounds bind to DNA, the coordination complexes undergo the following stages: hydrolysis, degradation, binding to biological objects and transport systems of an organism. Studying the mechanisms of how Pt-based antineoplastic drugs interact with DNA of a damaged cell is essential for understanding their antitumor effect and improving their efficiency as well as for developing novel antitumor agents.

The anticancer properties of *cis*-diaminedichloro-platinum(II) were discovered in the 60s of the 20th century by Rosenberg et al., its clinical use being approved by the Food and Drug Administration, FDA, US) in 1978. Cisplatin is known to act by interacting with DNA nitrogenous bases, which causes distortions in the double-helical structure of DNA. As a consequence, cellular processes are disrupted such as transcription and replication directing a cell to cell death through apoptosis or necrosis. Getting into a water solution cisplatin is hydrolyzed forming new aqueous complexes. After that cisplatin penetrates a cell by means of passive diffusion [1, 2] as well via copper transport protein (Ctr1p).

Carboplatin (*cis*-diamine (1,1-cyclobutanedicarboxylato) platinum(II), approved by FDA in 1989 is a second-generation antineoplastic agent widely used to treat ovarian and lung cancers. Unlike cisplatin, carboplatin being less active and toxic, can be used in lower doses in combination with other therapies.

Oxaliplatin (*trans*-RR-cyclohexane-1,2-diamine) oxalatoplatinum(II)) is a third-generation antineoplastic agent containing 1,2-diaminocyclohexanein its structure, which significantly enhances its binding to DNA. Such a change in the structure of this platinum drug predetermines the range of its antitumor efficiency being different from other platinum derivatives. Formation of interstrand crosslinks in DNA occurs quicker compared to that of cisplatin and carboplatin. Experimental studies have revealed the cytostatic effect on human cancer cell lines. The antitumor effect of platinum-containing drugs falls into four successive stages: 1) hydration of molecules; 2) accumulation in tumor cells *via* passive and/or active absorption; 3) activation of platinum-based drug; 4) binding to nucleic acid and forming different Pt-DNA adducts; 5) cellular responses to DNA damage.

The toxicity of platinum(II)-based agents is directly related to the ease with which a molecule is hydrolyzed. Platinum(II) coordination complexes having highly labile ligands such as water or nitrate are extremely toxic, whereas bis-carboxylate ligands which are hydrolyzed less slowly are known to be less toxic. Carboplatin was specifically developed to minimize the side effects after treating cancer patients with cisplatin. This was achieved by substituting the dichloride ligands with 1,1-cyclobutanedicarboxylate hydrolyzed as quickly as $10^{-8}s^{-1}$ in comparison with $10^{-5}s^{-1}$ for cisplatin. Due to its low reactivity carboplatin can be injected in much higher doses (300-450 mg/m^2) than cisplatin (20-120 mg/m^2) depending on administration protocols. After the hydrolysis stage carboplatin provides the same active component as cisplatin as well as forms the same DNA adducts.

The purpose of the present study is to examine the molecular and electronic structure of platinum(II) coordination complexes as they undergo hydrolysis, this being the first stage in how platinum-based drugs exert their effects, which is critical for understanding their antitumor properties.

EXPERIMENTAL

To investigate the molecular and electronic structure of platinum(II) coordination complexes (cisplatin, carboplatin, oxaliplatin) undergoing the hydrolysis stage, X-ray absorption spectroscopy at PtL_3 edge along with infrared and UV-Vis spectroscopies were used. For this purpose, platinum compounds were dissolved in different concentrations of distilled water.

The X-ray absorption spectra at PtL_3 edge were measured using a laboratory X-ray absorption spectrometer Rigaku R-XAS Looper (Japan). The Si(620) crystal-monochromator, Ar-300 I_0 detector and SSC detector were used in fluorescence mode to take measurements of platinum coordination complexes and their aqueous solutions. The voltage on the X-ray tube was $U = 25$ kV, the current was $I = 100$ mA. Aqueous solutions of platinum drugs were measured in a cuvette in fluorescence mode.

The UV-Vis absorption spectra of the platinum(II) complexes in aqueous solutions were measured by a UV-2600 spectrophotometer (Shimadzu Corp., Japan), in a wavelength range from 190 nm to 900 nm and a resolution of 1 nm.

Fourier Transform Infra-Red (FTIR) spectra of the powders of platinum(II) coordination complexes and their liquid samples were recorded using a laboratory FTIR spectrometer FSM 1202 (InfraSpec, Russia). Its wavenumber range was 400-5000 cm^{-1}, spectral resolution did not exceed 0.5 cm^{-1}. A tablet compression method was used for preparing samples, the base component being pure KBr powder which was previously dried and milled. The platinum drugs in aqueous solutions were measured in a cuvette using reflectance mode.

THEORY

Theoretical modelling of molecular and electronic structures of platinum(II) complexes (cisplatin, carboplatin, oxaliplatin) was carried out by the ADF modelling suite [3-5] designed for quantum chemical studies of molecular systems within the framework of Kohn–Sham DFT. The ADF software package is used to accurately model chemical and physical properties of molecular systems and provides a wide range of exchange-correlation energy functionals as well as treatment of relativistic effects, including the spin–orbit interaction. The ADF basis sets make use of Slater-type orbitals (STOs) functions and range from minimal DZ up to more precise directories TZ2P and QZ4P. The ADF modelling suite allows researchers to calculate electron density of states and electronic structure, to compute and view the energy levels of occupied and unoccupied molecular orbitals (MO), to distribute total and partial densities of states (DOS) in the energy bands of highest occupied and lowest unoccupied MOs as well as to calculate the formation energy and perform automated structure optimization without imposing restrictions on the symmetry for one-, two- and three-dimensional systems. The analytically calculated gradients and the second derivative of energy allowed determining the energy minimum of the system, transient conditions, reaction patterns and harmonic frequencies with IR intensities. The ADF software package takes into account the presence of solutions, solvation and the influence of the surrounding electric field. Also various LDA approximations, including improvements for GGA, are implemented in ADF. By the present time a number of theoretical approaches have been realized in ADF for calculating exchange-correlation potential. To optimize the molecular structure of platinum complexes and to calculate their electronic structure, optical and oscillation spectra, the TZ2P basis set and GGA approximation with OPBE exchange-correlation potential were used.

The multiple scattering formalism implemented by FEFF9.0 code [6-9] was used to calculate the total and partial densities of states (DOS). To calculate and analyze X-ray absorption spectra the finite-difference method (FDM) realized in FDMNES2011 code [10, 11] was applied.

RESULTS AND DISCUSSION

The ADF software package helps to optimize the coordination complex structure in order to minimize the energy when determining the optimal lengths and bond angles. Using ADF we performed the molecular geometry optimization of the platinum(II) coordination complexes. The spatial structure of the optimized platinum(II) coordination complexes as well as some important lengths and bond angles are shown in Fig. 1.

Fig. 2 shows electronic structure and the energy levels of the occupied and unoccupied MOs of the platinum(II) coordination complexes. The gap in the energy levels between the highest occupied and lowest unoccupied MOs is approximately 2.5-2.9 eV. The reactivity of the complexes depends on the energy of molecular valence orbitals. Carboplatin (HOMO: –5.23 eV) possesses more stable ligands, in comparison with cisplatin (HOMO: –5.12 eV), while oxaliplatin (HOMO: –4.51 eV) is a less stable compound.

The calculation of electron density of states helps to determine the electron configuration of MOs. The highest occupied MOs (HOMO, HOMO-1) of cisplatin are formed mainly from of Cl $3p$ states and Pt $5d$ states, creating bonds between Pt and Cl⁻ ions. The highest occupied MOs of carboplatin are formed chiefly by means of O $2p$ states and Pt $5d$ states. The electron cloud overlap on the top of molecular valence orbitals forms the bonds between Pt–O and cyclobutane-1,1-dicarboxylate ligand. The molecular levels corresponding to Pt–N bonds are located on lower energy levels, which ensures greater stability of diamine ligand. The highest occupied MOs of oxaliplatin are mainly composed of O $2p$ states and Pt $5d$ states. The electron cloud overlap on the top of molecular valence orbitals forms the bonds between Pt–O and a group of atoms of oxalate ligand. The Pt–O bonds belong to higher energy levels compared to Pt–N, which means the Pt–O bonds are weaker than Pt–N. During hydrolysis the bonds between Pt–O and oxalate ligand break down forming mono- and two-water complexes. The contribution of atomic energy levels and the highest occupied and lowest unoccupied MOs of oxaliplatin are shown in the diagram, in Fig. 3. The calculations for total and partial electron density of states (DOS) are given in Fig. 4.
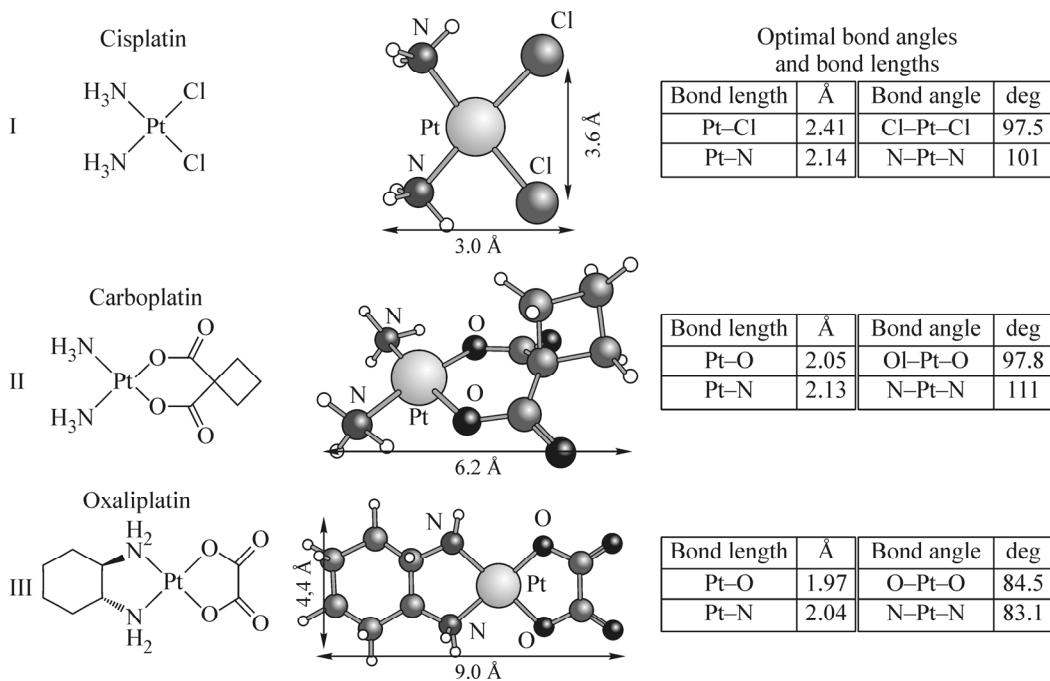
Fig. 1. The spatial structure of the optimized platinum(II) complexes.

Consequently, hydrolysis of cisplatin will be accompanied by the loss of Cl ions, a cyclobutane-1,1-dicarboxylate ligand for carboplatin and by a loss of oxalate ligand for oxaliplatin with further bonding to mono- and two water complexes. Taking cisplatin and carboplatin complexes as an example we compared molecular energy levels of cisplatin and hydrolyzed complexes. Fig. 5 presents a diagram illustrating the MOs energy levels in the hydrolyzed complexes compared to those of cisplatin. For the hydrolyzed platinum(II) complexes a more stable geometry is sure to be the one in having OH_2 (diaquacomplex), whereas the platinum(II) complexes containing OH ligands appear to be less favorable than with Cl ions.
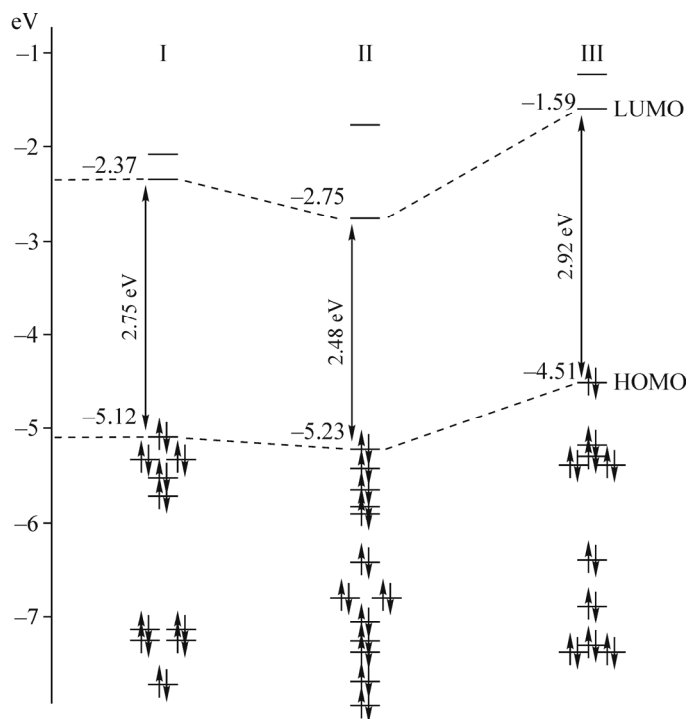
**Fig. 2.** The energy levels of MOs of platinum(II) complexes.

MOs energy levels for oxaliplatin
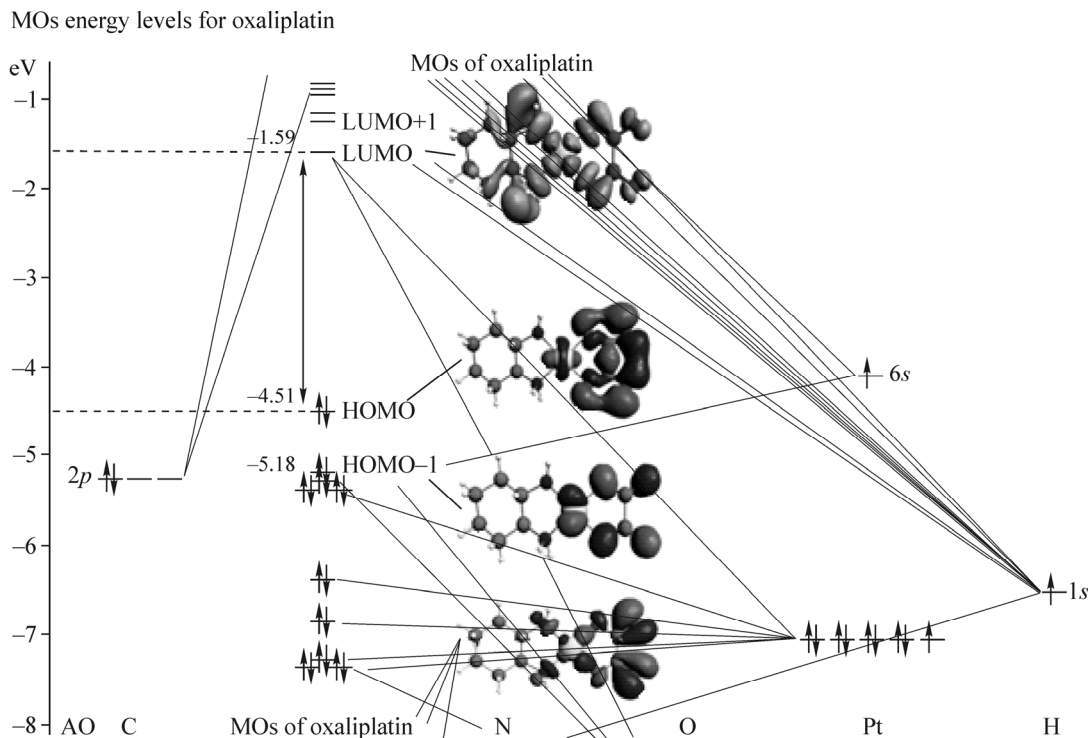
Fig. 3. MOs energy levels for oxaliplatin.

The bond length of Pt–OH$_2$ diaquacomplex is 2.25 Å, in contrast to that of Pt–N is 2.17 Å. In the diaquacomplex, the Pt–OH bond length is 2.11 Å and the Pt–N bond length is 2.25 Å.

The simulation data for hydrolyzed complexes was compared with the experimental results for hydrolyzed platinum(II) complexes obtained by means of X-ray absorption at PtL_3 edge as well as UV-Vis and IR spectroscopies.

In Fig. 6 the normalized experimental X-ray spectrum at PtL_3 edge of the hydrolyzed carboplatin and the theoretical spectra of carboplatin molecule, one- and two water carboplatin complexes are compared. By analyzing the obtained curves, it can be seen that absorption spectra at PtL_3 edge appears sensitive to the local environment of the Pt atom surrounded by different ligands. The most distinctive features in the shape of the spectra and their energy peak positions can be observed in the range of C and D peaks.

The calculated spectrum of the diaquacomplex provides the most accurate description of the experimental spectrum. However, to describe the experimental spectrum of the hydrolyzed platinum(II) complex it is necessary to take into account other hydrolyzed complexes as well.
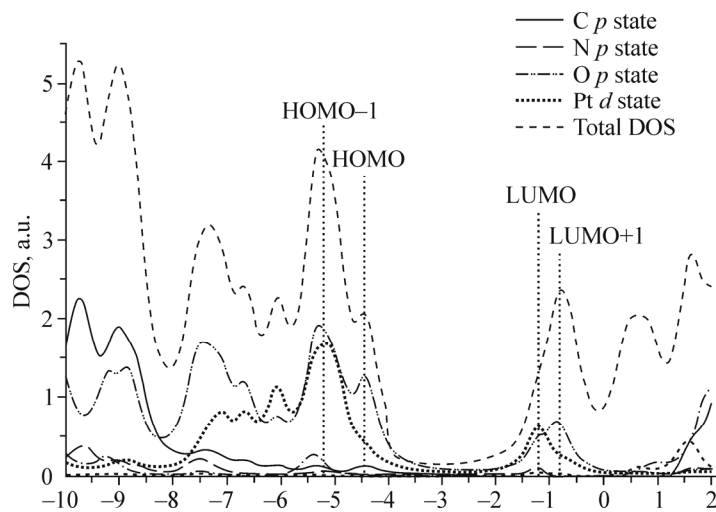

**Fig. 4.** The total and partial electron density of oxaliplatin.
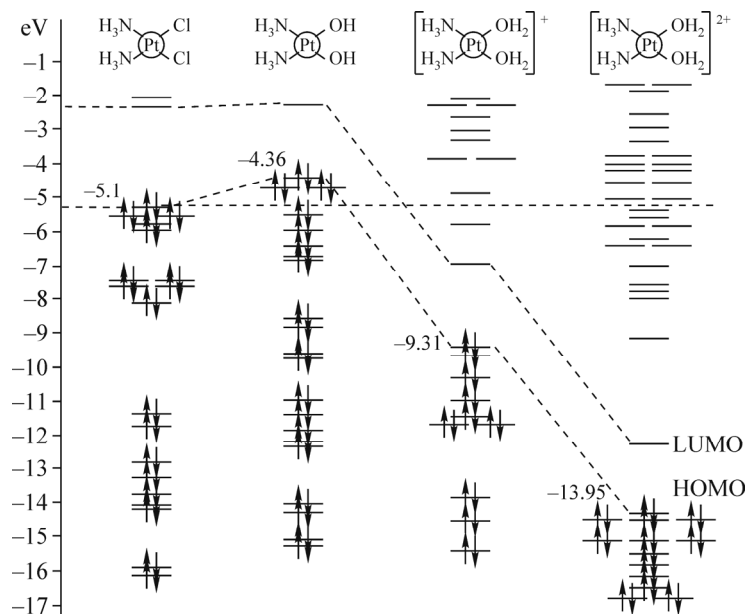
Fig. 5. MO energy levels for hydrolyzed complexes of cisplatin(I).

The experimental absorption spectra of the hydrolyzed carboplatin in UV-Vis spectroscopy measurements were compared with the theoretically obtained spectra for carboplatin and the diaquacomplex as shown in Fig. 7. The changes in spectra are caused by shifting the bands of carboplatin absorption from the visual region into the IR one, corresponding to the registered absorption bands of the diaquacomplex.
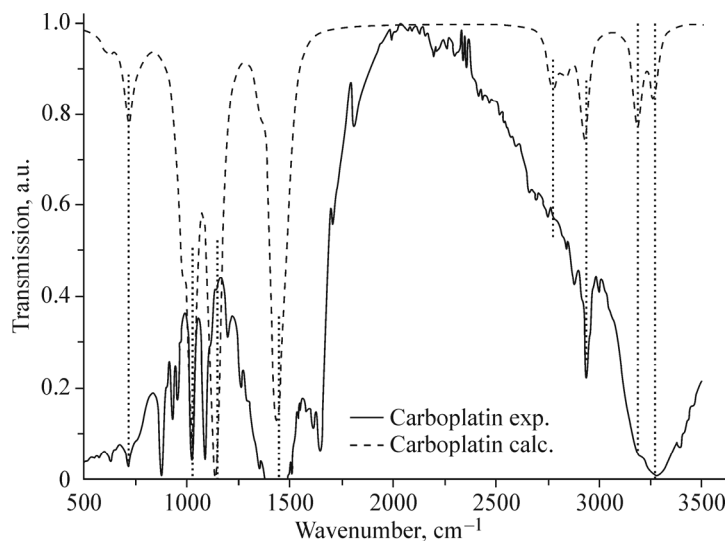
Fig. 8 compares the experimental IR spectrum of carboplatin with the calculated oscillation spectrum of its molecule, which is found to be in a good agreement with the experimental IR spectrum. Hence, the theoretical simulation of the hydrolyzed platinum(II) complexes have been experimentally proved by means of X-ray absorption at PtL_3 edge, UV-Vis and IR spectroscopy techniques. The spectroscopic techniques exploited in this study turned out to be appropriate for examining the hydrolysis of platinum(II) coordination complexes, the results of which could be used in further research into the antitumor effect of platinum-based anticancer agents.
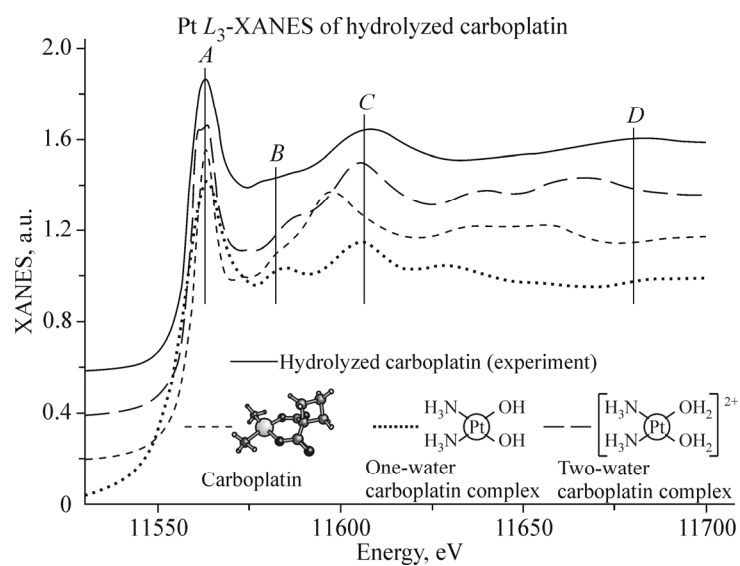
Fig. 6. The experimental and calculated spectra of X-ray absorption at PtL_3 edge for hydrolyzed carboplatin(II).
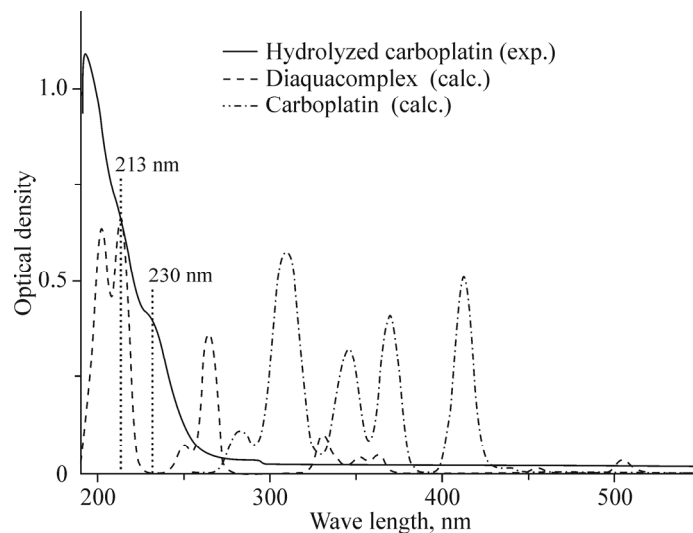
Fig. 7. IR and UV-Vis spectra of carboplatin and carboplatin aqueous solution.

Fig. 8. The experimental IR spectrum and theoretical oscillation spectrum of carboplatin(II).

CONCLUSIONS

The molecular and electronic structure of the platinum(II) coordination complexes, such as cisplatin $PtCl_2(NH_3)_2$, carboplatin $PtC_6H_{12}N_2O_4$ and oxaliplatin $PtC_8H_{14}N_2O_4$, undergoing hydrolysis was examined. Their electronic structure was studied by applying the density functional theory (DFT). The study also yielded information on the reactivity and stability of chemical bonds of the platinum(II) coordination complexes under study. The process of hydrolysis was simulated and the structure and geometry of hydrolyzed platinum complexes were determined. The theoretical calculations were shown to be valid by means of X-ray absorption at PtL_3 edge and UV-Vis and IR spectroscopic techniques. The chosen spectroscopic techniques were found to be most accurate and thus appropriate for investigating the hydrolysis of platinum(II) complexes, which will definitely be of great value for further study of these complexes and their therapeutic effect in anticancer treatment.

REFERENCES

1. D. Esteban-Fernandez, E. Moreno-Gordaliza, B. Canas, M. A. Palacios, and M. M. Gomez-Gomez, *Metallomics*, **2**, 19-38 (2010).

2. N. J. Wheate, S. Walker, G. E. Craig, and R. Oun, *Dalton Trans*., **39**, No. 35, 8097-8340 (2010).

3. G. te Velde, F. M. Bickelhaupt, S. J. A. van Gisbergen, C. Fonseca Guerra, E. J. Baerends, J. G. Snijders, and T. Ziegler, *J. Comput. Chem*., **22**, 931-967 (2001).

4. *ADF2009.01*, SCM, Theoretical Chemistry, Vrije Universiteit, Amsterdam, Netherlands; http://www.scm.com.

5. S. H. Vosko, L. Wilk, and M. Nusair, *Can. J. Phys*., **58**, 1200-1211 (1980).

6. J. J. Rehr, J. J. Kas, M. P. Prange, A. P. Sorini, Y. Takimoto, and F. Vila, *C. R. Phys*., **10**, 548-559 (2009).

7. J. J. Rehr, *Radiat. Phys. Chem*., **75**, 1547-1558 (2006).

8. A. L. Ankudinov, B. Ravel, J. J. Rehr, and S. Conradson, *Phys. Rev. B*, **58**, No. 12, 7565-7576 (1998).

9. J. J. Rehr and A. L. Ankudinov, *Radiat. Phys. Chem*., **70**, 453-463 (2004).

10. Y. Joly, *Phys. Rev. B*, **63**, 125120-125129 (2001).

11. Y. Joly, *Phys. Rev. Lett*., **68**, 950-953 (1992).

12. P. Blaha, K. Schwarz, et al., *Wien2k* (2008).