Int. J. Electrochem. Sci., 12 **(2017)** 11316 – 11329, doi: 10.20964/2017.12.66

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Cisplatin-chemotherapeutic Drug Interactions with the Surface of Some Metal Bioimplants in Physiological Serum

Adriana Samide[*], *Roxana Grecu, Bogdan Tutunaru, Cristian Tigae*[*], *Cezar Spînu*

University of Craiova, Faculty of Sciences, Department of Chemistry, Calea Bucuresti 107i, Craiova, Dolj, Romania
[*]E-mail: samide_adriana@yahoo.com; ctigae@yahoo.com

Received: 13 August 2017 / *Accepted:* 6 October 2017 / *Published:* 12 November 2017

The effect of cisplatin-chemotherapeutic drug (CisP), IUPAC name: azanide; dichloroplatinum(2+), on materials used as bioimplants, namely 304L stainless steel (SS) and titanium was investigated in physiological serum (PS) using the cyclic voltammetry, the potentiodynamic polarization and the electrochemical impedance spectroscopy (EIS) associated with UV-Vis spectrophotometry. The experimental data were reported to those obtained for platinum electrode, as the comparison and discussion standard. It has been established that the cisplatin-bioimplants interactions are dependent on the substrate, highlighting weakly CisP-SS links, moderate CisP-Ti bonds, the strongest being CisP-Pt interaction. The electrochemical measurements showed that, the presence of CisP in the physiological serum leads to degradation of the bioimplant surface, affecting the material/electrolyte interface layer, but the platinum surface does not have susceptibility to the drug. UV-Vis spectrophotometry showed that, after successive electrochemical measurements CisP was degraded, the highest electrochemical instability being recorded on 304L stainless steel. Corroborating the results of electrochemical and spectrophotometric measurements revealed the following types of interactions: hydrogen/chlorine bridges are established between CisP and SS; the CisP-Ti_xO_y links are most likely to occur in the titanium case; metal-metal bonds in the case of platinum electrode.

Keywords: cisplatin; bioimplant; interaction; electrochemical measurements; UV-Vis spectrophotometry.

1. INTRODUCTION

Cisplatin, with the molecular formula: *cis*-$[PtCl_2(NH_3)_2]$, was first synthetized in 1845 by Michael Peyrone. Many platinum compounds have been obtained and evaluated as potential chemotherapeutic agents. Also, a variety of Ru, Ti, Nb, Mo and Re complexes have been found to display anticancer activity. Among the platinum (II) drugs (transplatin, ormaplatin, enloplatin,

carboplatin, oxaliplatin, lobaplatin), cisplatin (CisP) is used for the cure of testicular, ovarian, head, neck, bladder, cervical, melanoma, lymphomas and other type of cancer [1].

The action molecular mechanism of cisplatin shows that in the cytoplasm, the chloride atoms are displaced by water molecules yielding to a hydrolyzed compound that can react with the sulfur and nitrogen from nucleic acids, these being originated from cancer cells, causing apoptotic death [2].

Some studies [3] show that nitric oxide synthesis and intracellular Ca^{2+} may influence the cisplatin resistance. The tests on cisplatin and transplatin adsorption on mesoporous silica nanoparticles show a rapid adsorption and a slowly release of platinum species [4]. V. Levet et al. [5] obtained a formulation for controlled-release of cisplatin dry powder for inhalation against lung cancer.

The hydrolysis of cisplatin yields the monohydrate derivative which is most important in anticancer activities, the dose of cisplatin being limited because of its nephrotoxicity and severe nausea [6]. The cisplatin hydrolysis products are more toxic than the parent drug itself. Sodium thiosulfate was successfully used to reduce toxic side effects of cisplatin hydrolysis products [7]. Because of the severe side effects, cisplatin is administrated in small doses at low concentrations. For these reasons, the determination of cisplatin, from biological samples, has been performed by different analytical techniques (UV-Vis spectrophotometry, phosphorescence, atomic absorption spectrometry, gas chromatography, capillary electrophoresis, high-performance liquid chromatography) [8-13]. SH-containing molecules (e.g. glutathione) are responsive to drug resistance [14]. However, cisplatin and cisplatin analogues confer protection against cyanide poisoning [15]. The electro-permeability of cisplatin and bleomycin delivery is used on cutaneous and subcutaneous tumors after IV injection of drug [16].

The differential pulse voltammetry (DPV) method has been applied for electrochemical determination of an antitumor platinum (IV) complex [17]. The cytostatics based on platinum have been detected in contaminated wastewater, by flow injection analysis with electrochemical detection [18]. The graphene oxide dispersed multi-walled carbon nanotubes composite modified glassy carbon electrode was used for the electrochemical determination of cisplatin drug in 0.05 M KCl in the absence and in the presence of 0.5 % serum [19]. The experimental results shows a good linearity in the concentration range from 1.3 μM to 26.0 μM. CisP was also determined by adsorptive transfer stripping technique using a metallothionein modified hanging mercury drop electrode with a detection limit of 0.5 μM [20].

In the case of cancer treated patients, the studies present opposite views on the effect of the primary bone-integration of metallic implants [21].

The current study investigated the degenerative effect of cisplatin-bioimplat interactions in the physiological serum (PS) using cyclic voltammetry, potentiodynamic polarization and electrochemical impedance spectroscopy (EIS). The cisplatin (CisP) analysis before and after electrochemical measurements was performed by UV-Vis spectrophotometry. The interpretation of the experimental data obtained for CisP on implant electrodes, such as 304L stainless steel (SS) and titanium (Ti), was reported to platinum electrode (Pt) due to the behavioral predictability of the drug on this substrate. The physiological serum was chosen as study solution, because in the hospital, prior to administration,

CisP solution having the concentration of 1.0 g L^{-1} is diluted with physiological serum, or possibly PS containing glucose.

2. MATERIALS AND METHODS

2.1. Materials

The materials were purchased from Sigma Aldrich, these having the following characteristics: titanium purity ≥ 99.9%; 304L stainless steel (alloy: FeNi18Cr10) contains (wt %): C-0.03%; Ni-18%; Cr-10%; Mn-2.0%; Fe up to 100%. Cisplatin was purchased from a local deposit of expired drugs as bottled solution in brown flask containing 50 mL aqueous solution with 50 mg CisP (CisP concentration of 1.0 g L^{-1}). For testing, the solution was diluted by ten-fold with physiological serum (solution of 0.9% NaCl in water), obtaining CisP concentation of 0.33 mmol L^{-1}.

2.2. Electrochemical measurements

The electrochemical measurements were carried out with an electrochemical system VoltaLab 40, with VoltaMaster 4 software. The glass electrochemical cell with three electrodes was used. The working electrodes were successively platinum and the two tested implants, 304L-SS and titanium, under plate form having an active area of 1.0 cm^2. In all cases, platinum plate (area of 1.0 cm^2) provided the auxiliary electrode, and Ag/AgCl electrode was used as a reference.

The cyclic voltammetry was performed in potential window of -1.5 V and 1.5 V, with the potential scan rate of 100 mV s^{-1} at room temperature (23 ± 1 ^oC), after prepolarization of electrodes at open circuit a time of 4.0 minutes.

The potentiodynamic polarization measurements were accomplished at room temperature, with a potential scan rate of 1.0 mV s^{-1}, in the potential range from -1.5 V to 1.5 V, the potential restrictions of the tested material being controlled by the software. The potentiodynamic curves were processed as semi-logarithmic curves and, in the allowed zone, as linear diagram in the potential range of ±20 mV with respect to the potential at zero current value, E(i=0). The electrochemical impedance spectroscopy was performed soon after the potentiodynamic polarization, at metal specific-open circuit, in the frequency range of 10^5 Hz and 10^{-1} Hz, with an AC perturbation signal of 10 mV.

2.3. UV-Vis spectrophotometry

The UV-Vis spectrophotometry was used for analysis of 0.33 mmol L^{-1} CisP in physiological serum, before and after cyclic voltammetry and after EIS. The UV-Vis spectra were recorded in wavelength range between 800 and 200 nm being set to the interest area of 400 and 200 nm. The UV-Vis Varian-Cary 50 spectrophotometer, with CaryWin software, was used. The analysis report was accurately obtained for each case.

3. RESULTS AND DISCUSSION

3.1. UV-Vis spectrophotometry of Cisplatin

The UV-vis spectrum of 0.33 mmol L^{-1} CisP in physiological serum (PS) is displayed in Figure 1. This shows that, CisP presents a maximum adsorption peak at 208 nm (charge-transfer band) [4] and a large shoulder around 280 nm attributed to *d-d* transitions of the square planar Pt^{2+} ion [4, 22]. A detail in wavelength range between 240-300 nm is inserted in Fig. 1, which clearly displays a split adsorption maximum above 280 nm, most likely due to the distortions characteristic of the *d-d* transitions within a plane square structure [4].

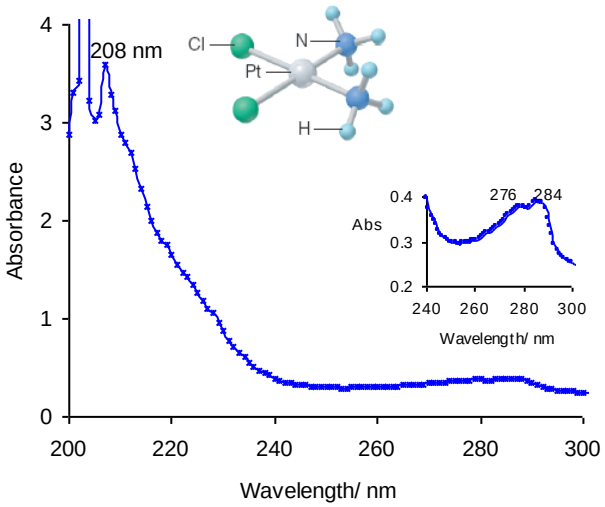


Figure 1. Chemical structure and UV-Vis spectrum of 0.33 mmol L^{-1} CisP in physiological serum

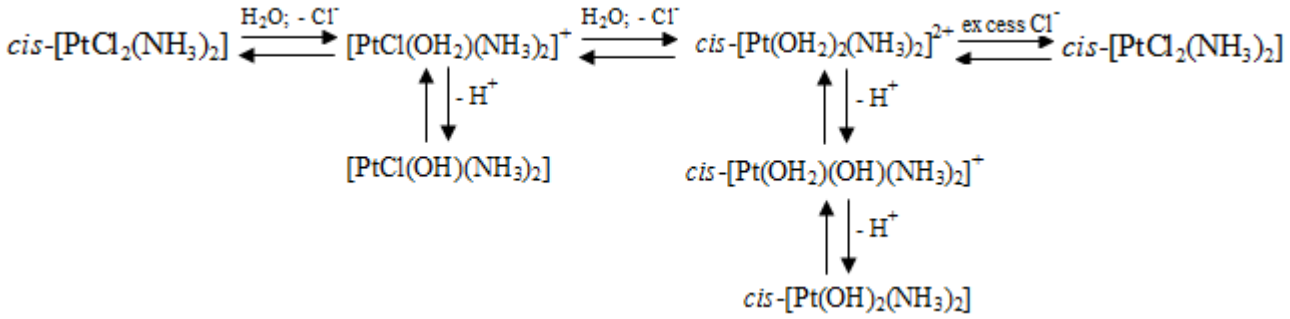
3.2. Cyclic voltammetry (CV)

The cyclic voltammograms recorded on the platinum, 304L stainless steel and titanium electrodes in PS without and with CisP are illustrated in Figs. 2a-c.

The remarkable differences were observed for CisP behaviour, as follows: (i) on platinum electrode (Fig. 2a) the decrease of current density is observed, but the large loop of hystheresis was formed compared to that recorded in the absence of CisP; (ii) overlapped hysteresis were recorded on 304L stainless steel (Fig. 2b), apparently there was no significant change on the voltammogram in the presence of CisP compared to that obtained in its absence; unlike platinum, potentials are much smaller, meaning that steel is relatively passive only up to 0.5 V, after which the current density sharply increases, reaching a value of 2.0 times higher than on platinum; CisP influences the titanium voltammogram recorded in the physiological serum (Fig. 2c), thus favoring the formation of a reduced hysteresis and the decrease of the current density from 1.7 mA cm^{-2} to 0.7 mA cm^{-2}, at potential value of 1.5 V.

According to literature data [1, 23] CisP may undergo some reversible transformations, as shown in Scheme 1. In physiological serum, the formation probability of both cations,

$[PtCl(OH_2)(NH_3)_2]^+$ and *cis*-$[Pt(OH_2)_2(NH_3)_2]^{2+}$ is relatively low, but not totally excluded and certainly dependent on the substrate type that interacts with CisP during the measurements.



Scheme 1. Cisplatin reversible reactions

Based on above mentioned and on the UV-Vis spectrophotometry results (Figs. 2a'- c') specific comments regarding cyclic voltammograms recorded on platinum, 304L stainless steel and titanium are necessary. Examining the voltammogram corresponding to the platinum electrode, the formation of a large oxidation peak, like a shoulder, around 1.1 V potential is nuanced, that could be attributed to some overlapping processes, such as: platinum oxidation and/or other specific oxidation processes of CisP. There is a net difference between the oxidation peak recorded in the absence of CisP, the latter being noted at potential value of 0.93 V, closer to the oxidation potential of Pt to PtO (0.98 V) and having a correspondent peak on the cathode scan at the same potential. The gap between the two peaks is due to CisP transformation processes, these being partially reversible (see the peak shown on the cathode scan), but the formation of a plateau, in its presence between 1.0 V and 0.1 V is observed, suggesting platinum surface changes due to its interaction with CisP. These can due by some metal-metal interactions such as Pt-Pt bridges [24] which can generate a stable surface. The UV-Vis scans displayed in Fig. 2a' show that, after the cyclic voltammetry the characteristics of CisP adsorption maximum are changed. The absorbance increases without returning to the baseline, suggesting that the CisP transformation reactions are reversible and/or its interaction with the platinum electrode could occur. Also, it could be generated the electrochemical instability of a small part of CisP.

Analyzing the UV-Vis spectra recorded on 304L stainless steel in PS (Fig. 2b'), apparently the same spectrophotogram changes are noted, but there is a higher probability of adsorption of CisP on the substrate by means of chlorine and hydrogen bridges and certainly not an interaction of metal-metal type. As can be seen in the detail inserted in Fig. 2b, CisP modifies the appearance of the plateau, in the potential range between -0.3 and 0.3 V, but it is difficult to specify, only from the CV, if these are temporary or the CisP-SS have stability over time.

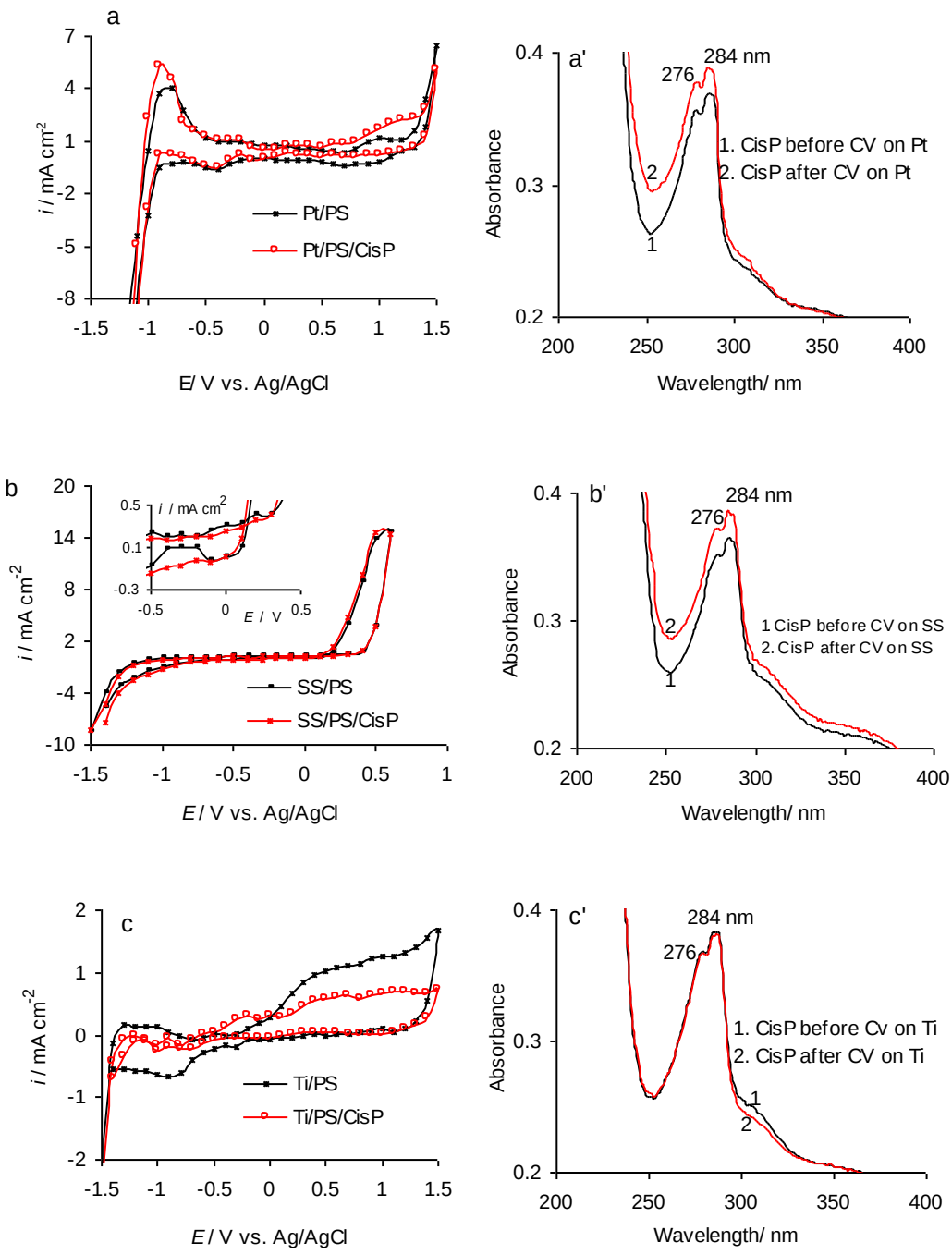


Figure 2. Cyclic voltammograms (a, b, c) and UV-Vis spectra of Cisplatin in physiological serum, before and after CV (a', b' c') recorded on: a – platinum electrode; b – 304L stainless steel; c – titanium electrode

On the titanium electrode, CisP behaves very differently compared to platinum and 304L stainless steel, marking some changes (Fig. 2c) as follows: (i) compressed hysteresis and exposed to a much lower current density; (ii) cathodic low intensity processes suggesting a certain substrate stability due to the formation of a CisP-Ti_xO_y type bond, which consumes a very small amount of drug from the reaction environment; (iii) weakly oxidation processes with the formation of short-lived species (Scheme 1) which, in the presence of Cl^- excess, are quickly transformed to CisP. These are confirmed by the UV-Vis spectrophotometry (Fig. 2c'), which shows overlapped scans of CisP, with a slightly

alteration of baseline after CV, in the wavelength range of 300-340 nm, indicating the consumption of a small amount of CisP from environment.

3.3. Potentiodynamic polarization

To support the above-mentioned, the potentiodynamic polarization was performed in PS on platinum, 304L stainless steel and titanium electrodes. The semi-logarithmic curves were recorded in the potential range of -1.5 and 1.5 V, these being shown in Figure 3.

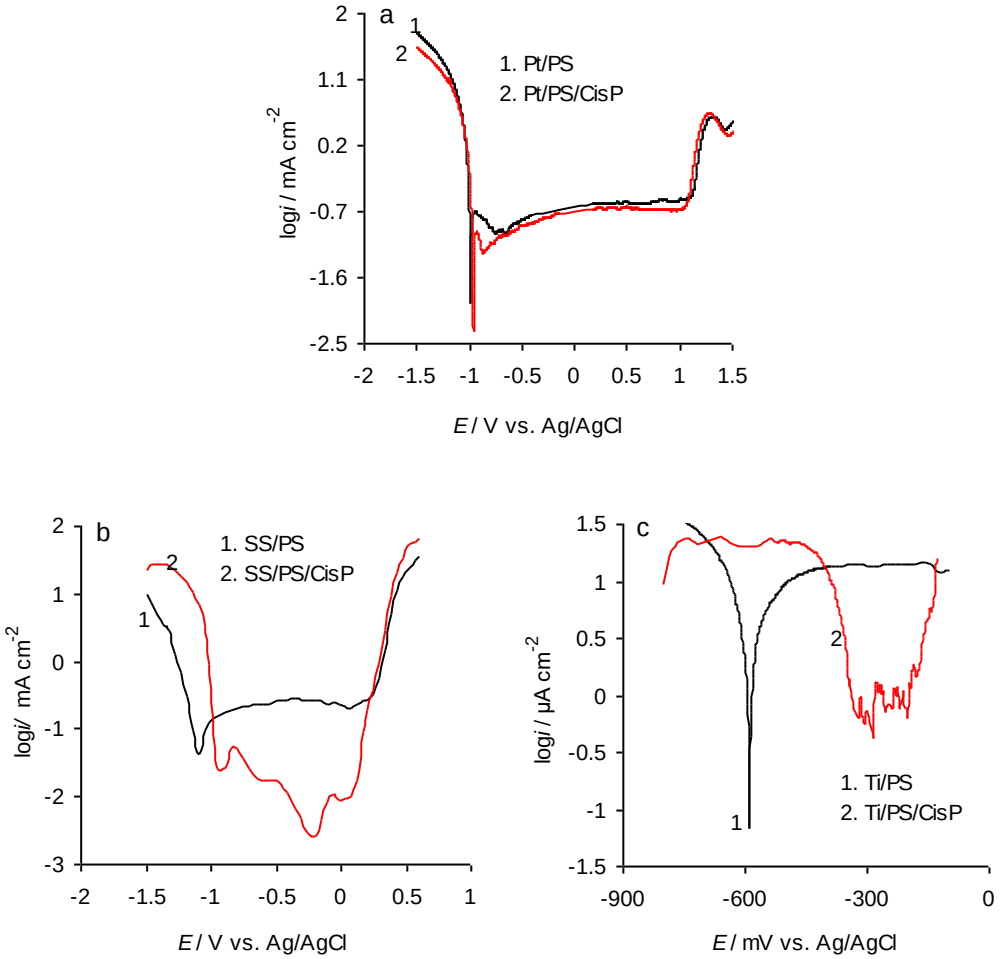


Figure 3. Potentiodynamic polarization curves recorded in physiological serum without and with cisplatin on: a – platinum electrode; b – 304L stainless steel electrode; c – titanium electrode

As shown in Fig. 3a, in the presence of CisP, the characteristic plateau, in the potential range of -1.0 and 1.0 V, is located at lower current density, which gives the substrate a high stability, especially due to strong metal-metal bonds which are cleaved over 1.0 V, when the current density sharply increases and overlapped curves are highlighted indicating a platinum trans-passivation process.

Characteristic semi-logarithmic curves to 304L stainless steel are shown in Fig. 3b. It is observed that, in the cathodic field, the polarization curve recorded in the presence of CisP moves in the positive direction, the drug having an inhibitory effect due to its adsorption on the steel surface through weak hydrogen/chlorine bonds. The passive field is shifted to lower current densities, but it is altered by breakdown potentials to: -0.88 V, -0.198 V, -0.603 V and 0.072 V, being caused by desorption and/or transformations/electrodegradation of CisP.

As in cyclic voltammetry, it can be observed that titanium has a completely different behavior. As shown in Fig. 3c, the addition of CisP in PS leads to a significant change of the polarization curves by the appearance a pre-passive zone in negative field between -0.9 V and -0.4 V, in comparison with the classic aspect of the polarization curve recorded in the absence of CisP. This can confirm the possibility of the formation of some interfacial bonds [25] between platinum from CisP and titanium oxides (CisP-Ti_xO_y), knowing that the oxidation/reduction processes of the titanium/its oxides occur at potential values between -1.63 V and -0.49 V, the over-layer formation being inevitable and its adhesion to the metal surface takes place [25]. At the potential value of -0.4 V, the current density sharply decreases to zero and it remains constant around this value until the potential of -0.19 V, thus displaying a passive area altered by numerous harmful interferences which could be due to the instability of specific interactions.

As known, it is very difficult to estimate the charge transfer resistance (R_{ct}) in pre-passive/passive layers that restrict the transfer of metal cations to the electrolyte. The errors can be significant, especially in the case of noble metals and unstable films with desorption/adsorption frequency, that leads to the formation of active areas on the substrate surface where the corrosion processes can be intensified. Therefore, R_{ct} was determined for platinum (Pt), 304 L stainless steel (SS) and titanium (Ti) in physiological serum (PS) in the absence and presence of CisP, from the linear shapes of the polarization curves plotted in the potential range ± 20 mV close to potential at zero current value, E(i=0). The VoltaMaster 4 software was used for experimental data fitting of the smooth polarization curves recorded in the potential range of ± 20 mV close to E(i=0), where the minimal interferences were notified. The results are presented in Figure 4.

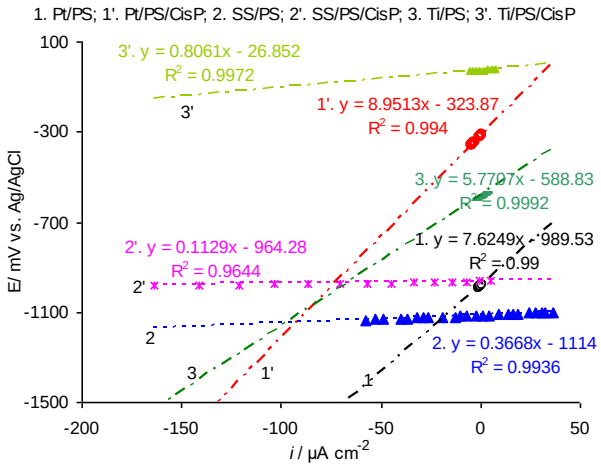


Figure 4. Linear diagram of potentiodynamic polarization curves for Pt, SS and Ti in PS without and with CisP recorded in the potential range ± 20 mV close to E(i=0)

The R_{ct} values were calculated as $(dE/di)_{E\rightarrow E\ (i=0)}$ by deriving the equations inserted in the graph from Figure 4 [26]. These are listed in Table 1.

Table 1. The R_{ct} values computed from the linear diagram drawn in the potential range close to E (i=0) for Pt, SS and Ti in PS blank solution and in PS containing CisP

Electrode/Electrolyte	E (i=0)/ mV vs. Ag/AgCl	Slope	R_{ct}/Ω cm^2
Pt/PS	-990.0	7.6249	7624.9
Pt/PS-CisP	-322.5	8.9513	8951.3
SS/PS	-1114.0	0.3668	366.8
SS/PS-CisP	-963.0	0.1129	112.9
Ti/PS	-589.0	5.7707	5770.7
Ti/PS-CisP	-27.0	0.8061	806.1

It can be observed that: (i) the R_{ct} values both in the absence and presence of CisP range as follows: Pt>Ti>SS; (ii) the presence of CisP in PS leads to the increase of R_{ct}, for the platinum electrode suggesting the layer stability; (iii) for SS and Ti electrodes, the CisP presence in PS leads to the decrease of $R_{ct,}$ confirming the surface film weak adherence, that follows more adsorption-desorption cycles [27].

3.4. Electrochemical impedance spectroscopy (EIS)

The EIS measurements were carried out after potentiodynamic polarization, in order to study the metal surface changes due to the CisP presence in PS, as well as the strength of the CisP interaction with platinum, 304L stainless steel and titanium which provide the electrochemical stability of the over-layer adhered to the metal surface.

The Nyquist diagrams are presented in Figure 5. Both in the absence and presence of CisP, the Nyquit plots are characterized by two distinct regions; (1) in high frequency range a semicircle can be observed indicating the charge transfer process where the reactions are kinetically controlled [28, 29], and consequently one phase angle maximum well defined in the Bode diagram displayed in Fig. 6a; (2) in low frequency region, a line placed about 45°, in the complex impedance plane where the processes are controlled by the mass transfer (diffusion) [28, 29]. The charge transfer resistance (R_{ct}) can be estimated by the extrapolation of high-frequency semicircle to the impedance real axis, while the solution resistance (R_s) corresponds to the highest frequency value [30]. As shown in Fig. 5a, the presence of CisP in PS leads to the occurrence of an extensive semicircle compared to that was recorded in its absence. Thus, it can be argued that the charge transfer resistance is significantly higher in the presence of CisP than that in its absence, indicating both the stability and the resistance of the surface layer.

The Nyquist diagram obtained for stainless steel in PS, without and with CisP, shown in Figs. 5b and 5b' is completely different from platinum. In the absence of CisP (Fig. 5b) the appearance of a capacitive loop placed at the kohm (kΩ) impedance values can be seen, suggesting that the surface layer remains stable and adherent on the 304L stainless steel. Also, an extended relatively Gaussian shape with one well defined maximum is highlighted in the corresponding Bode diagram (Fig. 6b). Significant changes of the 304L stainless steel surface morphology can be noticed in the presence of CisP that stimulates surface electrodegradation suggesting the upper-layer formation (confirmed by two phase angle maxima slightly differentiated in Bode (Fig. 6b) with low adhesion and resistance [31] due to weak interaction of CisP with the substrate.

A classical behavior of titanium can be seen in Fig. 5c. The Nyquist diagram reveals a capacitive loop appearance in the presence of CisP that causes a slightly tendency to form one phase angle maximum in Bode (Fig. 6c). In the absence of CisP (Fig. 5c), the Nyquist diagram shows an open loop with large diameter (extended range of the maximum phase angle maximum in the Bode diagram, Fig. 6c) indicating a layer resistance much higher than that evidenced in its absence.

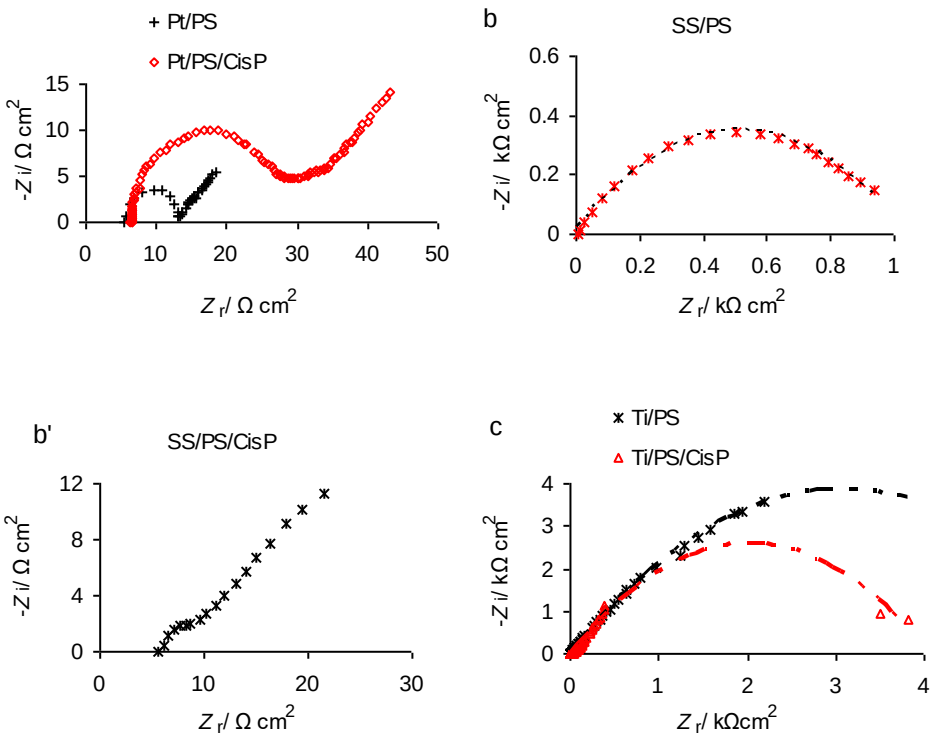


Figure 5. Nyquist diagrams recorded on platinum, 304L stainless steel and titanium electrodes in physiological serum in the absence and presence of cisplatin

To support those discussed above, the impedance response to frequency was processed as Bode diagram displayed in Figs. 6a-c.

In the case of platinum, a standard Bode diagram was obtained showing that, the presence of CisP in PS leads to an increase in the impedance response (log$Z$ = 1.65) compared to that obtained for PS blank 1.28. Also, the phase angle maximum shifted to the lower frequency area and it decreased

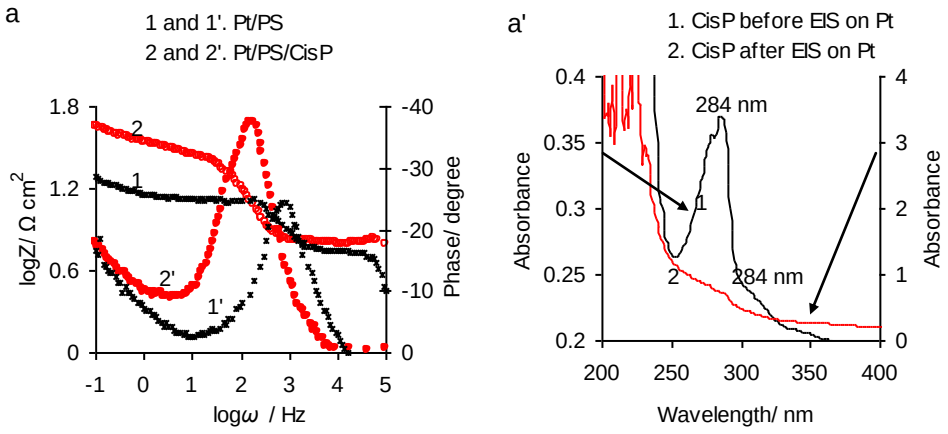
from -23.53 degrees to -36.84 degrees, indicating the stability of the layer formed on the platinum surface and, in conclusion, confirmed the strength of the Pt-Pt bonds.

The Fig. 6b shows that the presence of CisP in PS greatly affects the stainless steel surface, leading to degenerative changes involving the instability of interactions between this drug and substrate. The impedance low response compared to that obtained for PS blank solution as well as an uncertainty phase angle maximum show that at the interface there was a multitude of species which exhibit inability to bind on substrate and to form a stable upper-layer. As noted in the previous paragraphs, weak bonds between CisP and the steel surface have been formed, which are easily detachable and consequently, the alloy surface degrades.

The relative passivation of the titanium illustrated in Fig. 6c by an extended range of the phase angle maximum, is no longer present in the PS containing CisP when a phase angle maximum is outlined, but a lower impedance response compared to that of titanium in PS blank solution. Thus, CisP destabilizes the oxidic layer from titanium surface, which is replaced by an upper-layer that involves CisP-Ti_xO_y bonds, but that has a temporary adherence; certain interferences due to successive adsorption-desorption processes are marked by the instability of CisP-Ti_xO_y links, as we have shown in the previous paragraphs. However, as a whole, CisP interactions with titanium are stronger than those with the 304L stainless steel, the destabilization of the surface being temporary, this could be renewable.

After EIS measurements the UV-Vis spectra of CisP were recorded and these are presented in Figs. 6a'-c'. In all cases the electrochemical instability of drug is highlighted and its electrodegradation to other compounds is nuanced. This is a concurrent process that could destabilize the metallic surface and it can obstruct the formation of an adherent layer.

The lowest electrochemical stability of CisP in the physiological serum is highlighted on 304L stainless steel electrode, the Fig. 6b' displaying the occurrence of numerous compounds in the wavelength range of 200-300 nm, compared to platinum (Fig. 6a') and titanium (Fig. 6c') when the degradation products appear on a smaller domain between 200 nm and 250 nm. Consequently the CisP electrochemical degradation is faster on SS-electrode. Moreover, the CisP UV-Vis scan recorded on titanium after EIS show that, the characteristics of the adsorption peak are still noticeable, which induces the idea that an adduct between CisP and titanium oxides could be formed, thus controlling the CisP release and consequently its electrodegradation.



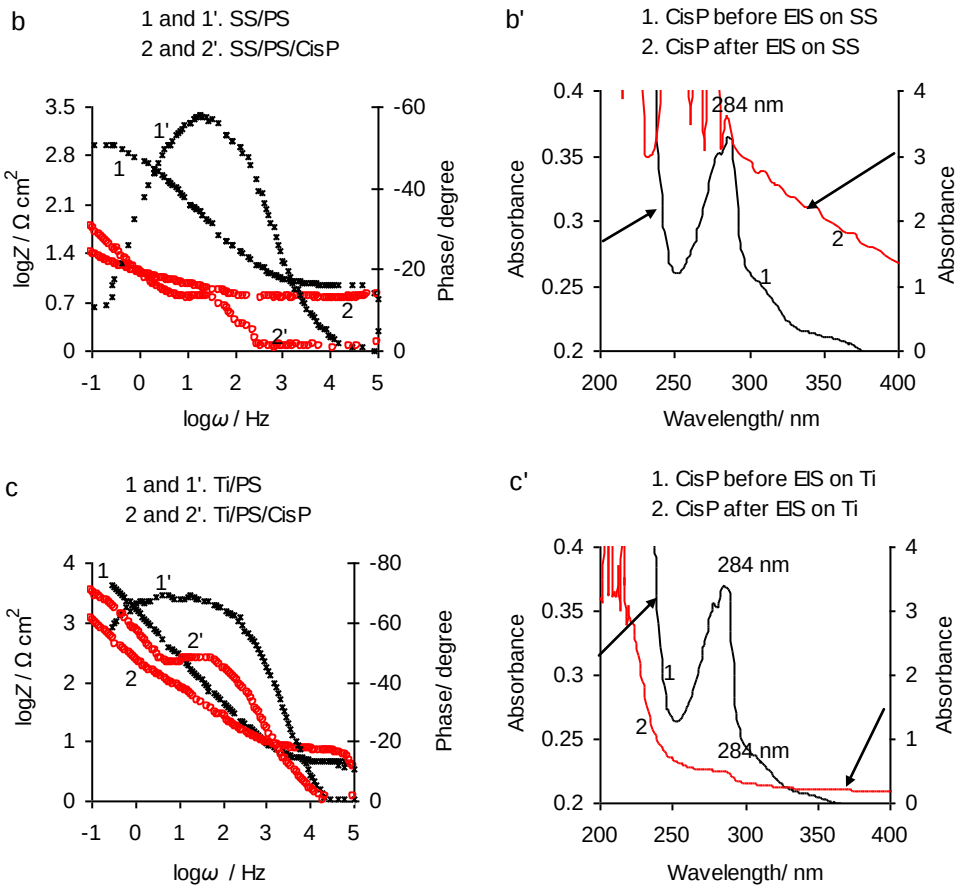


Figure 6. Bode diagrams (a, b, c) and UV-Vis spectra of Cisplatin in physiological serum, before and after EIS (a', b' c') recorded on: a – platinum electrode; b – 304L stainless steel electrode; c – titanium electrode

CisP electrodegradation has no impact on initial interactions of this drug with the metallic surfaces, but its electrochemical instability, after successive measurements, induces some changes at electrode/electrolyte interface which may affect the behaviour of substrates reflected by the Bode diagrams.

4. CONCLUSIONS

The effect of the CisP cystostatic on some bioimplants such as, 304L stainless steel and titanium was studied considering as estimation standard the platinum electrode.

The electrochemical tests performed in physiological serum infusion solution have shown that cisplatin affects the implants with degenerative effects on the upper-layer adhered on surface, at the metal/electrolyte interface or extrapolating to the human body, at the implant/tissue interface.

Weaker interactions with 304L stainless steel have been highlighted compared to titanium, but both implants became susceptible in the presence of cisplatin in physiological serum, unlike the

platinum electrode that is not affected by cytostatic, most likely due to the formation of stronger interactions of metal-metal type.

It should be noted that, in the human body the potential will not reach such high levels, thus the effect of the cytostatic on the bioimplants is not in short-time, but in a long-term it may have repercussions or CisP may potentiate the negative effect of other pharmaceutical compounds.

References

1. R. A. Alderden, M. D. Hall and T. W. Hambley, *J. Chem. Educ.,* 83 (2006) 728.
2. S. Dasari and P. B. Tchounwou, *Eur. J. Pharmacol.,* 740 (2014) 364.
3. Y. Yu, Q. Xie, W. Liu, Y. Guo, N. Xu, L. Xu, S. Liu, S. Li, Y. Xu and L. Sun, *Biomed. Pharmacother.,* 86 (2017) 8.
4. Z. Tao, Y. Xie, J. Goodisman and T. Asefa, *Langmuir,* 26 (2010) 8914.
5. V. Levet, R. Rosière, R. Merlos, L. Fusaro, G. Berger, K. Amighi, and N. Wauthoz, *Int. J. Pharm.,* 515 (2016) 209.
6. R. Bandu, H. S. Ahn, J. W. Lee, Y. W. Kim, S. H. Choi, H. J. Kim and K. P Kim, *Plos One,* 10 (2015) e0134027.
7. M. Sooriyaarachchi, J. Gailer, N. V. Dolgova, I. J. Pickering and G. N. George, *J. Inorg. Biochem.,* 162 (2016) 96.
8. M. Espinosa Bosch, A. J. Ruiz Sanchez, F. Sanchez Rojas and C. Bosch Ojeda, *J. Pharmaceut. Biomed.,* 47 (2008) 451.
9. Z. Huang, A. R. Timerbaev, B. K. Keppler and T. Hirokawa, *J. Chromatogr. A,* 1106 (2006) 75.
10. P. J. Parsons, P. F. Morrison and A. F. LeRoy, *J. Chromatogr. A,* 385 (1987) 323.
11. A. Andersson and H. Ehrsson, *J. Chromatogr. B Biomed. Sci. Appl.,* 652 (1994) 203.
12. A. Andersson, H. Hedenmalm, B. Elfsson and H. Ehrsson, *J. Pharmaceut. Sci.,* 83 (1994) 859.
13. M. Verschraagen, K. der Born, T. H. U. Zwiers and W. J. F. van der Vijgh, *J. Chromatogr. B,* 772 (2002) 273.
14. E. Cadoni, E. Valletta, G. Caddeo, F. Isaia, M. G. Cabiddu, S. Vascellari and T. Pivetta, *J. Inorg. Biochem.,* 173 (2017) 126.
15. A. K. Nath, X. Shi, D. L. Harrison, J. E. Morningstar and R. T. Peterson, *Cell Chem. Biol.,* 24 (2017) 565-575.e4.
16. J. Teissié, J. M. Escoffre, A. Paganin, S. Chabot, E. Bellard, L. Wasungu, M. P. Rols and M. Golzio, *Int. J. Pharm.,* 423 (2012) 3.
17. C. S. Hernandez Dominguez and P. Hernandez, *J. Electrochem. Sci. Eng.,* 3 (2013) 81.
18. M. Kominkova, Z. Heger, O. Zitka, J. Kynicky, M. Pohanka, M. Beklova, V. Adam and R. Kizek, *Int. J. Environ. Res. Public Health,* 11 (2014) 1715.
19. L. Ye, M. Xiang, Y. Lu, Y. Gao and P. Pang, *Int. J. Electrochem. Sci.,* 9 (2014) 1537.
20. J. Petrlova, D. Potesil, J. Zehnalek, B. Sures, V. Adam, L. Trnkova and R. Kizek, *Electrochim., Acta,* 51 (2006) 5169.
21. A. el-Mahalawy, H. F. Marei, H. Abuohashish, H. Alhawaj, M. Alrefaee and B. Al-Jandan, *J. Cranio Maxill. Surg.,* 44 (2016) 337e346.
22. A. J. Di Pasqua, J. Goodisman, D. J. Kerwood, B. B. Toms, R. L. Dubowy and J. C. Dabrowiak, *Chem. Res. Toxicol.,* 20 (2007) 896.
23. J. C. Dabrowiak, J. Goodisman and A. K. Souid, *Drug Metab. Dispos.,* 30 (2002) 1378.
24. B. Lippert, C. J. L. Lock, B. Rosenberg and M. Zvagulis, *Inorg. Chem.,* 17 (1978) 2971.
25. S. J. Tauster, *Acc. Chem. Res.,* 17(1987) 389.

26. A. Samide, B. Tutunaru, A. Dobrițescu, P. Ilea, A. C. Vladu and C. Tigae, *Int. J. Electrochem. Sci.*, 11 (2016) 5520.
27. A. Samide, B. Tutunaru, C. Ionescu, P. Rotaru and L. Simoiu, J. Therm. Anal. Calorim., 118 (2014) 631.
28. S. Campuzano, M. Pedrero, C. Montemayor, E. Fatás and J. M. Pingarrón, *J. Electroanal. Chem.*, 586 (2006) 112.
29. J. R. de Sousa, M. M. V. Parente, I. C. N. DiÓgenes, L. G. F. Lopes, L. P. Neto, M. L. A. Temperini, A. A. Batista and I. Moreira, *J. Electroanal. Chem.*, 566 (2004) 443.
30. K. Bandyopadhyay, V. Patil, M. Sastry and K. Vijayamohanan, *Langmuir*, 14 (1998) 3808.
31. A. Samide, C. Negrila and A. Ciuciu, *Dig. J. Nanomater. Bios.*, 5(2010) 1001.