UNIVERSIDAD NACIONAL AUTÓNOMA

DE MÉXICO

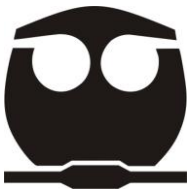
FACULTAD DE QUÍMICA

ESTUDIO BIOLÓGICO Y CARACTERIZACIÓN ELECTROQUÍMICA DEL COMPLEJO Cu (II) 1,8-BIS-(2-PIRIDIL)-3,6-DITIOCTANO (PDTO)

ACTIVIDAD DE INVESTIGACIÓN

QUE PARA OBTENER EL TÍTULO DE

QUÍMICO



PRESENTA

CARLOS DAYAN RODRÍGUEZ TORRES

MÉXICO, D.F. **2009**

JURADO ASIGNADO:

PRESIDENTE: **Profesor:** **Lena Ruiz Azuara**

VOCAL: **Profesor:** **José Alejandro Baeza Reyes**

SECRETARIO: **Profesor:** **Armando Marín Becerra**

1er. SUPLENTE: **Profesor:** **María Isabel Gracia Mora**

2° SUPLENTE: **Profesor:** **José de Jesús García Valdés**

SITIO DONDE SE DESARROLLÓ EL TEMA: LABORATORIO 210, DEPARTAMENTO DE QUÍMICA INORGÁNICA Y NUCLEAR, FACULTAD DE QUÍMICA, UNAM

ASESOR DEL TEMA: DRA. LENA RUIZ AZUARA

SUPERVISOR TÉCNICO : DR. LUIS ANTONIO ORTÍZ FRADE

SUSTENTANTE : CARLOS DAYAN RODRÍGUEZ TORRES

“….aquí va la cita de alguien que lo hace parte de un mismo…”
Dayan Rodríguez

Index

PROTOCOLO DEL PROYECTO

Nombre del alumno: Carlos Dayan Rodríguez Torres
Correo electrónico: dayan93@yahoo.com
Número de cuenta: 403100307
Carrera: Química

Nombre del asesor: Dra. Lena Ruiz
Correo electrónico del asesor: lena@servidor.unam.mx
Teléfono del asesor: 5622.3529

Supervisor técnico: Dr. Luis A. Ortíz Frade

Lugar a realizar el proyecto: Laboratorio 210 del Departamento de Química Inorgánica y nuclear. Posgrado Edificio B de la Facultad de Química

Antecedentes

Se ha probado la actividad citostática, citotóxica y antineoplásica de compuestos con cobre (II) y ligantes bidentados quelatos, una diimina y un ligante aniónico, denominados CASIOPEINAS. El diseño de estos compuestos se hizo con base en la hipótesis de utilizar un metal esencial como es el cobre, para disminuir la toxicidad del anticancerígeno. Por ejemplo, una de las causas de toxicidad del cisplatino es que el platino no es un metal esencial.

Otra ventaja de utilizar cobre, es la reducción del costo del medicamento. La razón de sintetizar una familia de compuestos es la de tener diversas moléculas con una posible selectividad hacia los diferentes tipos de neoplasias, hipótesis ya comprobada para algunos de los compuestos.

Objetivo

El presente Proyecto tiene como objetivo central colaborar en la solución de uno de los problemas de Salud Pública más importantes en México y en el mundo: el CANCER. En 1976, la Dra. Lena Ruíz de la División de Estudios de Posgrado, concibió la idea de diseñar compuestos de coordinación con posible actividad anticancerígena. A partir de 1980, se inician los trabajos de constatación biológica y desde 1992 dichos trabajos se han llevado a cabo en estrecha colaboración con el Instituto Nacional de Cancerología-México, SSA y dado el éxito obtenido, la UNAM decide patentar dichos compuestos y su proceso, así como registrarlos ante la SECOFI con el nombre de CASIOPEINAS.

Resumen

En estudios preeliminares de proliferación en la línea tumoral de HeLa usando el complejo de $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$ con sus precursores de nitrato se demostró que el complejo tiene un comportamiento similar al *cis*-platino. La reducción Cu(II) / Cu(I) electroquímica reversible del complejo fue establecida por voltamperometría cíclica, cronoamperometría y espectroscopia

de impedancia electroquímica en una solución de acetonitrilo. Por otro lado la sal de cobre $Cu(NO_3)_2 \cdot 2.5\ H_2O$ presenta un comportamiento electroquímico irreversible. Al comparar los resultados electroquímicos con los biológicos nos permite proponer la reversibilidad electroquímica como un factor importante en la actividad antitumoral de los complejos con cobre.

Plan de trabajo

Etapas	Actividad
Síntesis y caracterización del compuesto de cobre	Análisis elemental, espectrometría de masas, RMN
Caracterización espectroscópica del compuesto	UV-Vis y Método de Job
	Valoraciones espectrofotométricas de Cu(II)
	Valoración espectrofotométrica del Cu(I)
Estudio electroquímico del compuesto	Voltamperometría cíclica del complejo
	Valoración conductimétrica del complejo con Cu(I) y Cu(II)
	Mediciones de conductividad
	Valoración conductimétrica de $Cu(NO_3)_2$ con el ligante
	Estudios de VC (potencial de inversión y velocidad de barrido)
Estudio de las interacciones del compuesto con biomoléculas	Pruebas de solubilidad y estudio electroquímico con bases púricas y pirimídicas
	UV-Vis de bases púricas y pirimídicas con agua
	UV-Vis del complejo en agua
	Estudio electroquímico con una sola base
	Cronoamperometría del complejo
	Estudio electroquímico con un compuesto donador π

Polyhedron xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

Biological study and electrochemical characterization of Cu(II) and 1,8-bis-(2-pyridyl)-3,6-dithiaoctane (***pdto***) complex

Dayan Rodríguez-Torres [b], Juan Carlos García-Ramos [b], Juan Manríquez [a], Rafael Moreno-Esparza [b], Mario Altamirano Lozano [c], Ignacio González [d], Isabel Gracia-Mora [b], Lena Ruiz-Azuara [b,1], Rene Antaño Lopez [a], Luis Ortiz-Frade [a,*]

[a] *Electrochemistry Department, Centro de Investigación y Desarrollo Tecnológico en Electroquímica S.C. Parque Tecnológico Querétaro, Sanfandila, Pedro de Escobedo, CP 76703 Querétaro, Mexico*
[b] *Facultad de Química, UNAM, Cd. Universitaria, Circuito Interior, CP 04510 México, D.F., Mexico*
[c] *Facultad de Estudios Superiores Zaragoza, UNAM, Batalla 5 de Mayo s/n Esq. Fuerte de Loreto. Col. Ejército de Oriente Iztapalapa, CP 09230 México, D.F., Mexico*
[d] *Universidad Autónoma Metropolitana-Iztapalapa, Departamento de Química, México, D.F., P.O. Box 55-534, 09340 México, Mexico*

ARTICLE INFO

Article history:
Received 13 December 2008
Accepted 30 January 2009
Available online xxxx

Keywords:
Cell growth inhibition
Chronoamperometry
Cyclic voltammetry
Electrochemical impedance spectroscopy
pdto
Copper complexes

ABSTRACT

Preliminary proliferation assays in human tumor cervix line HeLa, using the coordination compound $[Cu(\textbf{pdto})H_2O]^{2+}$ (***pdto*** = 1,8-bis-(2-pyridyl)-3,6-dithiaoctane) and its precursors $Cu(NO_3)_2 \cdot 2.5H_2O$ and ***pdto***, were carried out. The results showed that the copper complex has a behavior similar to that of the reference drug *cis*-platin. No biological activity for the non-coordinated ligand and the copper salt was found. It was established by cyclic voltammetry, chronoamperometry, and electrochemical impedance spectroscopy, that the complex $[Cu(\textbf{pdto})H_2O]^{2+}$ presents an electrochemical reversible Cu(II)/Cu(I) reduction, in acetonitrile solution, meanwhile, the copper salt $Cu(NO_3)_2 \cdot 2.5H_2O$ exhibited an electrochemical irreversible behavior. A comparison between biological and electrochemical results corresponding to $[Cu(\textbf{pdto})H_2O]^{2+}$ and $Cu(NO_3)_2 \cdot 2.5H_2O$ let us to proposed, the electrochemical reversibility, as one important factor in the antitumoral activity of the copper complex. Due to the nature of the studies presented in this work, other factors like intercalation properties with DNA cannot be neglected in the antitumoral activity of the complex.

1. Introduction

Natural defense mechanisms against powerful carcinogenic species, such as the superoxide radical ($O_2^{\cdot-}$), have been extensively studied in mammalian cells. For instance, it has been reported that the bovine copper superoxide dismutase (Cu-SOD) is an enzyme that catalyzes the two-step disproportionation reaction of the superoxide radical to generate oxygen (O_2) and hydrogen peroxide (H_2O_2), Eqs. (1) and (2) [1,2]:

$$\text{Cu(II)-SOD} + O_2^{\cdot-} \rightarrow \text{Cu(I)-SOD} + O_2 \quad (1)$$

$$\text{Cu(I)-SOD} + O_2^{\cdot-} + 2H^+ \rightarrow \text{Cu(II)-SOD} + H_2O_2 \quad (2)$$

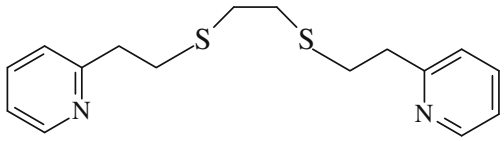
According to these chemical reactions, mimetic Cu-SOD complexes present a good catalytic activity when the redox potential of the Cu(II)L/Cu(I)L couple is in the range from −330 mV (versus NHE at pH 7; $O_2/O_2^{\cdot-}$) to +890 mV (versus NHE at pH 7; $O_2^{\cdot-}/H_2O_2$) [1,2].

The redox potentials of the Cu(II)L/Cu(I)L couple, for $[Cu(\textbf{pdto})H_2O]^{2+}$ complex (***pdto*** = 1,8-bis-(2-pyridyl)-3,6-dithiaoctane, see Scheme 1) 620 mV versus NHE (acetonitrile solution), and 580 mV versus NHE (water solution) suggest the use of this complex as a promising antitumoral drug, with a probably SOD-type behavior [3–5]. However, to the best of our knowledge, no biological study has been reported.

Based on the exposed above, we decided to explore antitumoral properties of the $[Cu(\textbf{pdto})H_2O]^{2+}$ complex over human cervix tumor cell line HeLa, in order to understand in a easy way whether this biological activity can be related to simple redox properties or not. The electrochemical characterization of the complex $[Cu(\textbf{pdto})H_2O]^{2+}$, and its precursor $Cu(NO_3)_2 \cdot 2.5H_2O$, was performed using cyclic voltammetry, chronoamperometry, and electrochemical impedance spectroscopy techniques. In all electrochemical measurements acetonitrile was employed as solvent due to its high potential range. Water solution was not used since $[Cu(\textbf{pdto})H_2O]^{2+}$ presented adsorption processes on the electrode.

* Corresponding author.
E-mail address: lortiz@cideteq.mx (L. Ortiz-Frade).
[1] Previously Lena Ruiz-Ramírez.

0277-5387/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.poly.2009.01.033



Scheme 1. Structure of the ligand = 1,8-bis-(2-pyridyl)-3,6-dithiaoctane (***pdto***).

2. Experimental

2.1. Chemicals

All chemicals and solvents for the synthesis and electrochemical studies were purchased from Aldrich Chemical Co. and were used without further purification.

2.2. Synthesis

Ligand 1,8-bis(2-pyridyl)-3,6-dithioctane (***pdto***) was prepared by the method described by Goodwin and Lions. Yield 70% [6]. Elemental *Anal.* Calc. for $C_{16}H_{20}N_2S_2$: C, 63.1; H, 6.6; N, 9.2; S, 20.1. Found: C, 63.1; H, 6.2; N, 9.7; S, 20.5%.

Aquo 1,8-bis(2-pyridyl)-3,6-dithiaoctane copper(II) hexafluorophosphate, [Cu(***pdto***)(H_2O)](PF_6)$_2$ 0.304 g (1.0 mmol) of ***pdto*** was added to 0.294 g of $Cu(NO_3)_2 \cdot 2.5H_2O$ (1.0 mmol) previously dissolved in 50 mL of methanol. The solution was stirred strongly and a deep blue color was observed. This solution was treated with two equivalents of ammonium hexafluorophosphate (0.0088 g). A blue precipitate was isolated. Elemental *Anal.* Calc. for $CuC_{16}H_{21}N_2S_2OP_2F_{12}$: C, 28.4; H, 3.1; N, 4.2; S, 9.5. Found: C, 28.1; H, 3.0; N, 4.7; S, 9.3%.

2.3. Measurement of cell growth inhibition

The antiproliferative effect was analyzed on cervix human tumor cell line HeLa obtained from American Type Culture Collection (ATCC, Rockville (MD) USA). Once the cells reached a constant rate of proliferation, approximately 2×10^4 cells/well were plated in a 96-well microplate with Dulbecco's modified eagle medium (DMEM) supplemented with 10% of fetal bovine serum (FBS), and allowed to attach, incubating at 37 °C and 5% CO_2 for 24 h. At the end of incubation timing the medium was removed and cells were exposed to drugs in five different concentrations (0, 1, 10, 100 and 300 μg/mL) with fresh supplemented media for 24 h. *cis*-platin was employed as drug control at same mentioned concentrations. Cell growth was determined according to the sulforhodamine B assay, described by Skehan et al. [7,8]. Absorbance was measured at 564 nm (*Microplate reader BIO-RAD 550*) and percentage cell growth by each concentration of drug was calculated as: percentage growth = $100 * [T/C]$; where T is the absorbance of treated wells and C is the absorbance of untreated wells. The software PROBIT (*Log Probit Analysis by Maximum Likelihood/Novartis)* was used to calculate the concentration to inhibit the proliferation of the cells in a 50% (IC_{50}).

2.4. Elemental analysis

Fissons Instruments Analyzer model EA 1108 was used for elemental analysis determination, using a sulfanilamide standard.

2.5. Electrochemistry

All electrochemical measurements were performed in acetonitrile, MeCN, (HPLC grade) solution containing 0.1 M tetra-N-butylammonium tetrafluoroborate ($TBABF_4$) as supporting electrolyte. All electrochemical experiments were obtained at sample concentrations of 1×10^{-3} M in the presence of supporting electrolyte. A potentiostat/galvanostat EG&G PAR model 263 controlled by a PC software was used. A typical three-electrode array was employed for all electrochemical measurements: platinum disk as working electrode $\phi = 2$ mm, platinum wire as counter-electrode, and a silver wire as pseudo reference electrode. The silver electrode was immersed in a MeCN solution with 0.1 M tetra-N-butylammonium chloride (TBACl) in a separate compartment that was connected to the working cell through a BAS vycor™ tip. All potentials were reported versus the couple Fc/Fc^+ according to IUPAC.

Cyclic voltammetry was initiated from open circuit potential (E_{ocp}), the scan rate employed was 0.1 V/s. One step chronoamperometry experiments were performed, initiating at $E_1 = E_{ocp}$ to different potential values, E_2, using a pulse width of 3 s. Electrochemical impedance spectra were measured using a BAS-ZAHNER IM6 potentiostat–galvanostat which was controlled by the software THALES SYSTEM™ version 3.0 previously installed in a PC Pentium III. All the spectra were obtained at open circuit potential and applying to the electrochemical cell an alternant perturbation having an amplitude of 10 mV and frequencies, f, between 100 kHz and 1 Hz. Fitting data to the Randles equivalent circuit were carried out by means of the spectra simulator SIM, which is a routine also supplied by the THALES SYSTEM software.

3. Results and discussion

3.1. Cell growth inhibition studies of [Cu(*pdto*)H_2O]$^{2+}$ complex

The antiproliferative effect of the compound [Cu(***pdto***)H_2O]$^{2+}$ was determined as IC_{50} in human cervix tumor cell line HeLa. From Table 1 it can be seen the similarity between the IC_{50} value of [Cu(***pdto***)H_2O]$^{2+}$ (41.8 ± 1.2 μM) and *cis*-platin (40.1 ± 0.9 μM). An inspection of values, show that the biological activity is due to [Cu(***pdto***)H_2O]$^{2+}$ complex, and not to its precursors. This fact can be related to redox properties, which may correspond to possible Cu-SOD dismutase reactions. In order to explain how the redox properties affect the biological activity, in the next sections we present a complete electrochemical characterization of the [Cu(***pdto***)H_2O]$^{2+}$ complex, and $Cu(NO_3)_2 \cdot 2.5H_2O$.

3.2. Electrochemical behavior of $Cu(NO_3)_2 \cdot 2.5H_2O$

Cyclic voltammogram of a 1×10^{-3} M $Cu(NO_3)_2 \cdot 2.5H_2O$ acetonitrile solution is shown in Fig. 1. When the scan was initiated in negative direction two reduction signals **Ic** and **IIc**, and two oxidation signals **Ia** and **IIa** are observed. The dependence of the signals **Ic** with **Ia** and **IIc** with **IIa** was established by modifying the switching potentials $E_{-\lambda}$. The cathodic and anodic peak potential values for signals **Ic**, **IIc**, **Ia** and **IIa** are: E_{pc}(**Ic**) = 0.401 V, E_{pc}(**IIc**) = −0.914 V, E_{pa}(**Ia**) = 0.615 V and E_{pa}(**IIa**) = −0.667 V, respectively.

The electrochemical processes **I** and **II** can be attributed to the consecutive reductions Cu(II) + 1e → Cu(I) and Cu(I) + 1e → Cu(0).

Table 1
IC_{50} mean values (μM) and standard deviation (SD) obtained for [Cu(***pdto***)H_2O]$^{2+}$, $Cu(NO_3)_2 \cdot 2.5H_2O$, and *cis*-platin against tumor cell line HeLa.

	IC_{50} (μM) ± SD
cis-Platin	40.1 ± 0.9
[Cu(***pdto***)H_2O]$^{2+}$	41.8 ± 1.2
$Cu(NO_3)_2 \cdot 2.5H_2O$	No activity
pdto	No activity

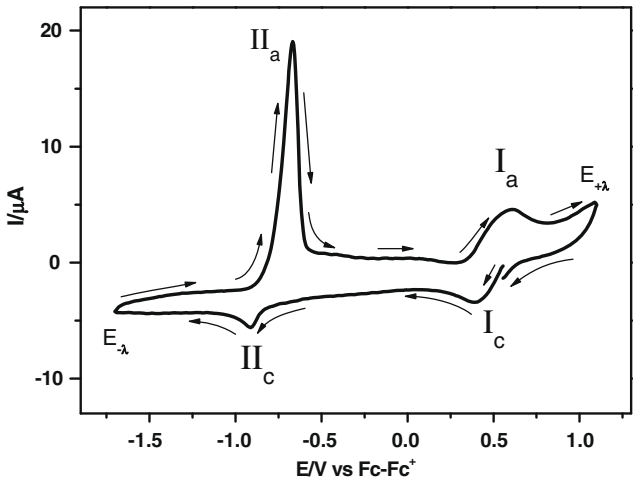


Fig. 1. Cyclic voltammogram of 1×10^{-3} M $Cu(NO_3)_2 \cdot 2.5H_2O$ in MeCN in presence of 0.1 M $TBABF_4$. The scan potential was initiated from E_{ocp} to negative direction. Scan rate 0.1 V s^{-1}. Platinum electrode was used.

However, to confirm this idea one step chronoamperometry experiments were also carried out.

From current-sampled versus potential plot $I(t) - E$, at current sampling time of 1.5 s, two reduction and one oxidation diffusion controlled limit zones are detected: $Id_{Ic}(t)$, $Id_{IIc}(t)$ and $Id_{Ia}(t)$ (see Fig. 2). This is a typical current–potential curve when soluble species of a redox couple are initially presented. Using the ratio $Id_{Ic}(t)/Id_{Ia}(t)$, it was established 90% of Cu(II) and 10% of Cu(I) in solution. The presence of the last species is attributed to its stability in acetonitrile solution [9,10].

The equation that describes the voltammogram in sampled-current voltammetry in the semi-infinite linear diffusion region with two soluble species of a redox couple is:

$$E = E_{1/2} + \frac{RT}{nF}\ln\frac{[Id_{OX}(t) - I(t)]}{[I(t) - Id_{RED}(t)]} \quad (3)$$

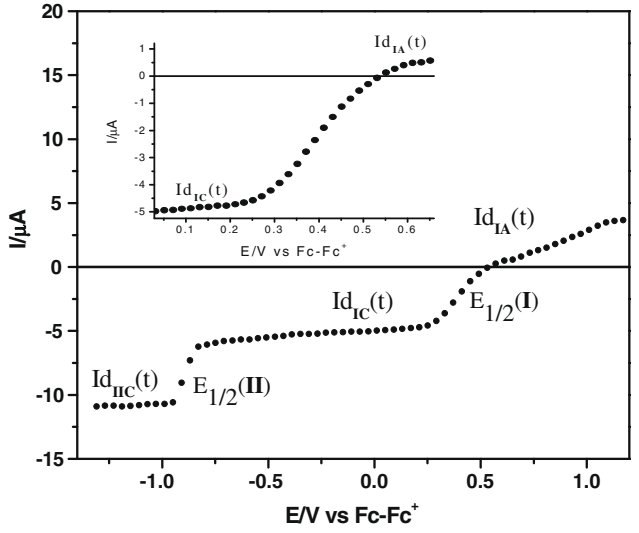


Fig. 2. Current-potential curves $I(t)$–E, constructed from chronoamperograms at different potentials E_2 from −1.560 to 1.571 V/Fc − Fc^+, corresponding to the electrochemical behavior of the copper salt $Cu(NO_3)_2 \cdot 2.5H_2O$. The $I(t)$–E plot was constructed for current sampling times 1.5 s. Inset corresponds to an expanded $I(t)$–E scale.

For a reversible system the slope value should be 0.059/n V at 25 °C and the intercept corresponds to the half wave potential $E_{1/2}$. The half wave potential is usually a very good approximation of E^0 for a reversible couple, whenever the diffusion coefficients ratio is close to the unity Eq. (4):

$$E_{1/2} = E^0 + \frac{RT}{nF}\ln\frac{D_R^{1/2}}{D_O^{1/2}} \quad (4)$$

The slope and the intercept of E versus $\log[Id_{OX}(t) - I(t)]/[I(t) - Id_{RED}(t)]$ plot were found to be 0.122 and 0.387 ($r = 0.999$) for process **I**, and 0.047 and −0.892 ($r = 0.999$) for process **II**. The slope values suggest that non-reversible behavior for processes **I** and **II** is observed.

Due to the non-reversible behavior of both processes, no formal potential E^0, can be evaluated. Instead of E^0, the values of $E_{1/2}$ for processes **I** and **II** were obtained, $E_{1/2}(I) = 0.387$ V and $E_{1/2}(II) = -0.892$ V. Considering the preferential geometry of Cu(II) and Cu(I) complexes and with all arguments presented above, the following electrochemical reactions in MeCN solution are proposed:

I $[Cu^{II}(MeCN)_n]^{2+} + 1e \rightarrow [Cu^{I}(MeCN)_{n-1}]^{+} + MeCN$

$E_{1/2}(I) = 0.387$ V

II $[Cu^{I}(MeCN)_{n-1}]^{+} + 1e \rightarrow Cu(0) + (n-1)MeCN$

$E_{1/2}(II) = -0.892$ V

4. Electrochemical behavior of the complex $[Cu(\textit{pdto})H_2O]^{2+}$

Fig. 3 shows a typical cyclic voltammogram of $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$ in acetonitrile solution, started from open circuit potential to negative direction. Before the potential switch, $E_{-\lambda}$, two reduction signals **Ic′** and **IIc′** can be observed. When the scan was completed four oxidation signals (**IIa′**, **IIa′*** , **Ia′** and **IIIa′**) and one additional reduction signal (**IIIc′**) were detected. The cathodic peak potential values E_{pc}(**Ic′**), E_{pc}(**IIc′**) and E_{pc}(**IIIc′**) for signals **Ic′**, **IIc′** and **IIIc′** are: 0.180, −1.106 and 0.340 V, respectively. On the other hand, the values of anodic peaks potentials E_{pa}(**Ia′**), E_{pa}(**IIa′**) and E_{pa}(**IIIa′**) for signals **Ia′**, **IIa′** and **IIIa′** were 0.268, −0.650 and 0.543 V.

A comparison of these peak potential values, and those obtained from Fig. 1, suggests that the signals **IIIa′** and **IIIc′** are attributed to

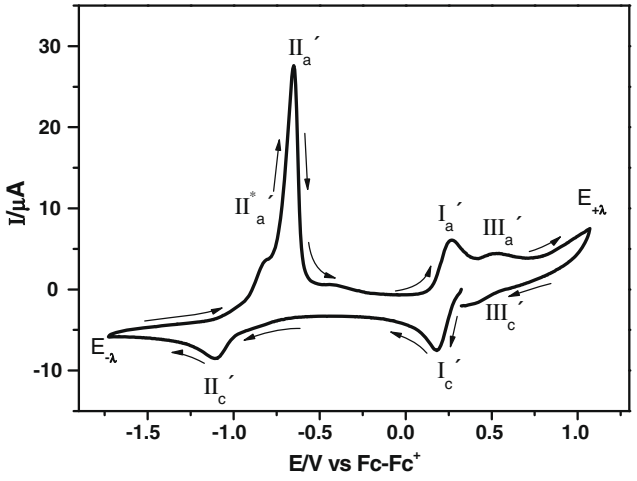


Fig. 3. Cyclic voltammogram of 1×10^{-3} M $[Cu(\textbf{\textit{pdto}})H_2O](PF_6)_2$ in MeCN in presence of 0.1 M $TBABF_4$. The scan potential was initiated from E_{ocp} to negative direction. Scan rate 0.1 V s^{-1}. Platinum electrode was used.

non-coordinated Cu(II) that comes form the dissociation of the complex. The signal **IIa′*** can be explained as the dissolution of deposited elemental copper in the presence of the ligand [11].

In order to obtain the number of electrons exchanged in the principal processes **I′** and **II′**, current-sampled versus potential plot $I(t) - E$ was constructed from chronoamperometric experiments at current sampling time of 1.5 s. Two diffusion controlled limit zones $Id_I(t)$ and $Id_{II}(t)$ with their corresponding half wave potential $E_{1/2}(I)$ and $E_{1/2}(II)$ are observed.

For a reversible system when only one oxidized species is present in solution, a plot E versus $\log[I_d(t) - I(t)/I(t)]$ should be linear with a slope value of 0.059/n V and with an intercept value that corresponds to the formal potential E^0. Using linear fit with correlation coefficient of 0.999 for both processes, the calculated slopes were 0.065 for process **I′**, and 0.069 for process **II′**. These values clearly suggest that both systems are practically reversible with a value of one electron exchanged in both cases. From the intercept, the obtained values of formal potentials $E^0(I')$ and $E^0(II')$ for processes **I′** and **II′** were 0.165 and −1.110 V, respectively.

To confirm the number of exchanged electrons in these processes, Cottrell's analysis is presented. It is well known that for a two consecutive electron transfer, the diffusion limit currents are established with the Cottrell Equations: for the first step, Eq. (5), the slope is proportional to n_1, Eq. (7), and for the second step, Eq. (6), the slope is proportional to $(n_1 + n_2)$, Eq. (8) [12]:

$$Ox_1 + n_1 \rightarrow Red_1 \quad (5)$$

$$Red_1 + n_2 \rightarrow Red_2 \quad (6)$$

$$Id(t) = n_1 FAD_o^{1/2} \pi^{1/2} Co^* t^{-1/2} \quad (7)$$

$$Id(t) = (n_1 + n_2) FAD_o^{1/2} \pi^{-1/2} Co^* t^{-1/2} \quad (8)$$

Two linear relationships of current $I(t)$ and $t^{-1/2}$ were obtained within the two diffusion controlled zones, with the equations $I(t) = -4.9t^{-1/2} + 0.36$ ($r = 0.999$) and $I(t) = -10.0t^{-1/2} + 0.9$ ($r = 0.999$) for processes **I′** and **II′**, respectively. Considering the same value of diffusion coefficient in Eqs. (7) and (8), the ratio $Id_{II}(t)/Id_I(t) = 2$ confirms the values $n_1 = 1$ and $n_2 = 1$. With all the evidences the electrochemical processes **I′** and **II′** can be attributed to:

I′ $[Cu^{II}(\boldsymbol{pdto})H_2O]^{2+} + 1e \rightarrow [Cu^{I}(\boldsymbol{pdto})]^{+} + H_2O$

$E^0(I') = 0.165$ V/Fc − Fc$^+$

II′ $[Cu^{I}(\boldsymbol{pdto})]^{+} + 1e \rightarrow Cu(0) + \boldsymbol{pdto}$ $E^0(II') = -1.110$ V/Fc − Fc$^+$

The coordination spheres for Cu(I) and Cu(II) complexes are proposed based on previously reported X-ray structures. [13]. The reversibility presented in the electrochemical processes Cu(II)/Cu(I) is in agreement with the high flexibility of the ligand ***pdto*** towards the preferential geometry of the central atom [4–6,13–24].

In order to complete the electrochemical characterization of the complex $[Cu(\boldsymbol{pdto})H_2O]^{2+}$, and its precursor $Cu(NO_3)_2 \cdot 2.5H_2O$, electrochemical impedance spectroscopy experiments were performed. These results are discussed in the next section.

4.1. Electrochemical impedance spectroscopy studies of $[Cu(\boldsymbol{pdto})H_2O]^{2+}$ $Cu(NO_3)_2 \cdot 2.5H_2O$

Fig. 4 shows typical Nyquist plots for $Cu(NO_3)_2 \cdot 2.5H_2O$ and $[Cu(\boldsymbol{pdto})H_2O]^{2+}$. The presence of a semi-loop at high frequencies in these plots indicates that an electrochemical reaction exists at the open circuit potential (E_{ocp}). This reaction is attributed to the Cu(II)/Cu(I) equilibrium. But, according to the shape of the spectra at the low frequency range, the main contribution to the impedance is due to a semi-infinite diffusion process, which controls the overall reaction rate. It seems that the species involved in this equilibrium diffuse at the interface metal–solution. However these species remains unidentified.

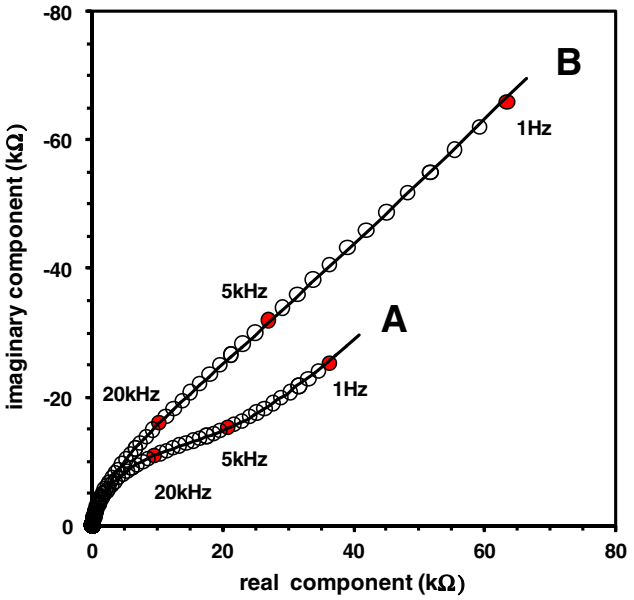


Fig. 4. Nyquist diagram for (A) $Cu(NO_3)_2 \cdot 2.5H_2O$ and (B) $[Cu(\boldsymbol{pdto})H_2O](PF_6)_2$. The concentration of samples was 1×10^{-3} M, in supporting electrolyte (0.1 M $TBABF_4$ in MeCN). EIS spectra were obtained at open circuit potential $E_{i=0}$ vs. Fc − Fc$^+$ couple with alternant perturbation 10 mV.

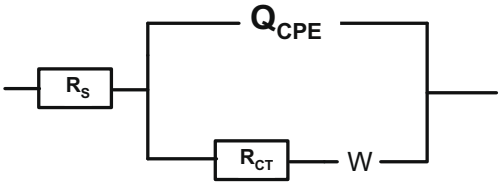
Both impedance spectra have been analyzed using a classical Randles equivalent circuit, displayed on Scheme 2. In this circuit, R_s stands for the resistance of the electrolyte, R_{tc} is the charge transfer resistance of the electrochemical reaction, *CPE* (constant phase element) and n are related to the double layer capacitance C_{dl} (see Eq. (9)) and, W is the Warburg parameter, related to diffusion processes (see Eq. (10)) [25,26]:

$$Z_{CPE} = \frac{1}{Q_{CPE}} (j\omega)^{-n} \quad (9)$$

where $\omega = 2\pi f$, $j = \sqrt{-1}$, and $0 \leqslant n \leqslant 1$ If $n = 1$ $Z_{CPE} = Z_{C_{dl}}$

$$Z_W = W(j\omega)^{-1/2} \quad (10)$$

Fig. 4 shows a good fitting (continuous line) of the Randless circuit to the experimental spectra in both cases. Fitted parameters are presented in Table 2. It is seen that R_{tc} is similar for both $Cu(NO_3)_2 \cdot 2.5H_2O$ and $[Cu(\boldsymbol{pdto})H_2O]^{2+}$ spectra, showing no significant difference in the kinetics of charge transfer of both species. In contrast, there is a significant difference in the Warburg parameter, being more important the diffusion process for the $[Cu(\boldsymbol{pdto})H_2O]^{2+}$ complex. This means that a deeper concentration gradient is built in this case, as a result of a faster electrochemical reaction. Such behavior is related to a more reversible Cu(II)/Cu(I) equilibrium, thus confirming the voltammetric and chronoamperometric results.



Scheme 2. Randles-type equivalent circuit used for fitting EIS spectra.

Table 2
Parameters of the Randles circuit fitted to the impedance spectra.

Compound	Proposed parameters
$Cu(NO_3)_2 \cdot 2.5H_2O$	$R_s = 212.4 \pm 5.5\ \Omega$, $R_{ct} = 17.9 \pm 0.2\ k\Omega$, $C_{dl} = 0.336 \pm 0.005\ \mu F$ $n = 0.933 \pm 0.002$, $W = 86.57 \pm 1.59\ k\Omega/s^{1/2}$
$[Cu(\textbf{\textit{pdto}})H_2O](PF_6)_2$	$R_s = 213.8 \pm 16.3\ \Omega$, $R_{ct} = 18.7 \pm 0.6\ k\Omega$, $C_{dl} = 0.258 \pm 0.007\ \mu F$ $n = 0.924 \pm 0.004$, $W = 246.30 \pm 2.88\ k\Omega/s^{1/2}$

It is totally clear that the complex $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$ presents more reversible electrochemical behavior than its precursor $Cu(NO_3)_2 \cdot 2.5H_2O$, due to the high flexibility of the ***pdto*** ligand towards the preferential geometry of the central atom. This is in good agreement with the fact that the value of the self-exchange electron transfer rate constant for the couple Cu(II)L/Cu(I)L (L = tetradentate ligands N_2S_2) is larger than the corresponding value of the non-coordinated couple Cu^{2+}/Cu^{+} [27]. In a simple way, we proposed that the electrochemical reversibility presented in the complex $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$ increases the reaction of electron exchange with superoxide molecule (Eqs. (1) and (2)). According to the Marcus theory, the π system in the ligand ***pdto*** and the smaller molecular reorganization in the couple $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}/[Cu(\textbf{\textit{pdto}})H_2O]^{+}$ support the idea of an easy transfer of electrons from $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$ to other chemical species in solution [28]. A correlation between the antiporoliferative effect and electrochemical reversibility of the Fe(II)/Fe(III) couple in Fe(II) complexes with thiohydrazones has been recently proposed by Kalinowski et al. [29].

Due to the nature of the studies presented in this work, it cannot be neglected that the biological activity of the complex $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$ is also related with other properties, like intercalation properties with DNA [3]. But, by simple electrochemical techniques, it is possible to proposed that electrochemical reversibility is an important factor in the antitumoral activity of the $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$ complex.

5. Conclusion

The similar antiproliferative effects of the complexes $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$ and *cis*-platin in human cervix tumor cell line HeLa, suggest that the copper compound could be used against other tumor cell lines. We have established, by using cyclic voltammetry chronoamperometry and electrochemical impedance spectroscopy, an electrochemical reversibility for complex $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$, and irreversibility for the starting material $Cu(NO_3)_2 \cdot 2.5H_2O$ in acetonitrile solution. This fact confirms the flexibility of the ***pdto*** ligand, towards the preferential geometry of Cu (II) and Cu(I) in their corresponding complexes. It can be proposed that antitumoral activity of the complex $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$ is related with electrochemical reversibility. Due to the studies performed in this work, additional effects like the interaction of $[Cu(\textbf{\textit{pdto}})H_2O]^{2+}$ with DNA reported before, cannot be neglected in the full understanding of the biological activity of this copper complex.

Acknowledgements

The authors thank DGAPA-UNAM (IN212500) (IN209907) and CONACyT proyecto bilateral (CNR Italaia México) (3700PE) for financial support. The authors also thank Guadalupe Osorio-Monreal for all the kind comments done to this work.

References

[1] L.W. Oberley, G.R. Buettner, Cancer Res. 39 (1979) 114.
[2] C. Urquiola, D. Gambino, M. Cabrera, M.L. Lavaggi, H. Cerecetto, M. González, A.L. Cerain, A. Monge, A.J. Costa-Filho, M.H. Torre, J. Inorg. Biochem. 102 (2008) 119.
[3] S. Mahadevan, M. Palaniandavar, Inorg. Chim. Acta 254 (1997) 291.
[4] M. Thompson, J. Whelan, D.J. Zemon, B. Bosnich, E.I. Solomon, H.B. Gray, J. Am. Chem. Soc. 101 (1979) 2482.
[5] U. Sakaguchi, A.W. Addison, J. Chem. Soc., Dalton Trans. (1979) 600.
[6] H.A. Goodwin, F. Lions, J. Am. Chem. Soc. 82 (1960) 5013.
[7] P. Skehan, R. Storeng, D. Scudeiro, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Nat. Cancer Inst. 82 (1990) 1107.
[8] L.V. Rubistein, K.D. Shoemaker, K.D. Paul, R.M. Simon, S. Tosini, P. Skehan, D.A. Scudeiro, A. Monks, M.R. Boyd, J. Nat. Cancer Inst. 82 (1990) 1113.
[9] A.J. Parker, Search 4 (1973) 426.
[10] F.A. Cotton, G. Wilkinson, Advanced Inorganic Chemistry, 5th Ed., John Wiley and Sons, New York, 1988. p. 967.
[11] C. Nila, I. González, J. Electroanal. Chem. 401 (1996) 171.
[12] A.J. Bard, L.R. Faulkner, Electrochemical Methods, Fundamentals and Applications, 2nd Ed., John Wiley and Sons, New York, 2001 (Chapter 5).
[13] G.R. Brubaker, J.N. Brown, M.K. Yoo, R.A. Kinsey, T.M. Kutchan, E.A. Mottel, Inorg. Chem. 18 (1979) 299.
[14] A.R. Amundsen, J. Whelan, B. Bosnich, J. Am. Chem. Soc. 99 (1977) 6730.
[15] J.H. Worrell, J.J. Genova, T.D. Dubois, J. Inorg. Nucl. Chem. 40 (1978) 441.
[16] A. Castineiras, M.V. Paredes, W. Hiller, Acta Crystallogr., Sect.C 40 (1984) 2078.
[17] A. Castineiras, W. Hiller, M.V. Paredes, J. Sordo, J. Strähle, Acta Crystallogr., Sect. A 40 (1984) C302.
[18] A. Castineiras, W. Hiller, M.V. Paredes, J. Sordo, J. Strähle, Acta Crystallogr., Sect. C 41 (1985) 41.
[19] D.G. Humphery, G.D. Fallon, K.S. Murray, J. Chem. Soc., Chem. Commun. (1988) 1356.
[20] A. Castineiras, G. Diaz, F. Florencio, S. García-Blanco, S.J. Martínez-Carrera, Cristallogr. Spectrosc. Res. 18 (1988) 395.
[21] A. Castineiras, G. Diaz, F. Florencio, S. García-Blanco, S.Z. Martínez-Carrera, Anorg. Allg. Chem. 101 (1988) 567.
[22] E. Bermejo, A. Castineiras, A.R. Domínguez, J. Strähle, W. Hiller, Acta Crystallogr., Sect. C C49 (1993) 324.
[23] V.V. Pavlischchuk, S.V. Koltilov, E.S. Michael, J. Prushan, A.W. Addison, Inorg. Chim. Acta 278 (1998) 217.
[24] E. Bermejo, A. Castineiras, A.R. Domínguez, J. Strähle, W. Hiller, Acta Crystallogr., Sect. C 49 (1993) 1918.
[25] J.R. Macdonald, W.B. Johnson, in: Impedance Spectroscopy: Emphazing Solid Materials and Systems, John Wiley and Sons, New York, 1987 (Chapter 1).
[26] A. Lasia, in: B.E. Conway et al. (Eds.), Modern Aspects of Electrochemistry, no. 32, Kluwer Academic/Plenum Publishers, New York, 1999 (Chapter 2).
[27] K.M. Davies, B. Guilani, Inorg. Chim. Acta 127 (1987) 223.
[28] J.E. Huheey, E.A. Keiter, R.L. Keiter, Inorganic Chemistry, 4th Ed., Harper Collins College Editions, New York, 1999. p. 557 (Chapter 2).
[29] D.S. Kalinowski, P.C. Sharpe, P.V. Bernhardt, D.S. Richardson, J. Med. Chem. Soc. 50 (2007) 6212.