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Electrochemical metallization with Sn of (E)-4-((4-nitrobenzylidene)amino)phenol in non-aqueous media: Characterization and Biological Activity of the Organotin Compound

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In this paper we report the metallization *in situ* of (E)-4-((4-nitrobenzylidene) amino) phenol (ENBA), an organotin compound of this radical with Sn(IV) ions electrogenerated from a sacrificial tin anode in an electrochemical cell. This metallization was possible because, in a previous research project, our group established the electrogeneration potential of the imine group dianion radical. The metallization was carried out at 25 ± 1º C in ACN using tetrabutylammonium tetrafluoroborate as supporting electrolyte. The organotin compound formation was confirmed by spectrophotometric and energy dispersive X-ray microanalysis (EDX) techniques. Finally, the cytotoxic activity of the ligand and its coordination compound were evaluated. According to the results of this research both compounds have a potential as cytotoxic agents. The results allowed to establish the relationship between the structure and the cytotoxic activity of the ligand and its coordination compound.

Keywords: Metallization; Sn complexes; Schiff bases; dianion radical; cytotoxic activity.

1. INTRODUCTION

Cancer is one of the most dangerous diseases today, not only for the patient pain or depression it causes, but also for the side effects of anti-cancer treatments. Chemotherapy, one of the most widespread treatments, uses drugs to reduce the ability of cancer cells to grow and reproduce; this treatment can be very effective against these cells, but it also affects other internal systems causing secondary damage to the body. Although scientific research in pharmacology has received significant attention and resources, development of anti-cancer drugs with low side effects for the patient is still insufficient.

Schiff base compounds have a double bond between carbon and nitrogen (C=N). This double bond resides in active cell sites and serves to carry out the biochemical reactions necessary for the human body. These compounds have not only anti-tumor properties, but also antimicrobial, antifungal and anti-inflammatory properties [1-7].

Our group has studied the application of the electrochemical metallization technique to obtain coordination compounds of some porphyrins and Schiff bases [8-11]. In particular, in [11] we report the electrochemical characterization by sweep potential techniques of the ENBA Schiff base and its biological activity. Figure 1 shows the ENBA Schiff base molecule. From this electrochemical characterization, we determined the formation potential of the radical dianion of ENBA. Knowing this potential it is possible to carry out the electrochemical metallization with transition metals in a controlled-potential electrolysis process. The coordination compounds from these types of ligands have been reported and a tendency has been observed for them to reduce toxicity in the metabolic processes of non-cancerous cells. In addition, these compounds increase cytotoxicity [12-14].

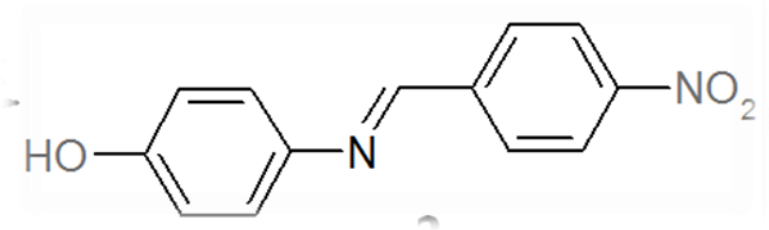

Figure 1. Molecular structure of ENBA

As a result, the application of the coordination compounds of Schiff base compounds with transition metals in the treatment of human diseases is a fast expanding area of biomedical chemistry [15-17].

Sn(IV) complexes have been reported to have antibacterial activity, which makes them attractive for biomedical applications. Given the importance of the Schiff bases and their coordination compounds with Sn(IV) in biomedicine [18-19], this paper describes the electrochemical metallization of (E)-4-((4-nitrobenzylidene)amino)phenol with Sn(IV). The process was carried out *in situ*, in an electrochemical cell with sacrificial tin anodes. The paper also reports the evaluation results of the cytotoxic and antibacterial activities of the ligand and its organotin compound.

2. EXPERIMENTAL

2.1 Materials and measurements

All starting materials were purchased from Aldrich Chemical Company. Solvents were used without any purification. Infrared spectra were recorded using a Perkin Elmer Universal FT-IR spectrophotometer equipped with an ATR accessory with a single reflection ZnSe ATR crystal. UV spectra were obtained with a 100Conc VARIAN Cary UV/VIS spectrophotometer. A 932AA GBC

atomic absorption spectrophotometer and a JSM-6701F JEOL were used to identify the Sn(IV) complex.

2.2. Electrochemical studies

The electrochemical characterization of the ENBA was carried out in a PGSTAT 30 AUTOLAB potentiostat/galvanostat. The experimental procedure and the obtained results were reported in [11], where we established the electrogeneration potential of the imine group dianion radical in this Schiff base. Knowing this potential value is necessary to achieve the Schiff base metallization by the imine group and not by the OH group as it would happen if using the classical chemical method. For this reason, this metallization takes place in an electrochemical cell using sacrificial anodes made of the metal of interest.

2.3. Electrochemical synthesis

Electrosynthesis was carried out in a non-divided cell using a reticulated vitreous carbon working electrode, a tin foil (electrolytic degree) as sacrificial anode, and a silver wire as pseudo-reference electrode. This procedure consisted of an electrolysis at a controlled potential of –1.69 V vs. Fc/Fc+; this potential is a few millivolts lowest than the dianion radical formation potential.

2.4. In vitro cytotoxicic assay

Human lymphoblastic leukemia MOLT-4 cells were employed to test cytotoxic effects of the ENBA and its complex with Sn(IV). MOLT-4 cells were maintained in RPMI media supplemented with 10% Foetal Bovine Serum (Gibco), 100 IUmL-1 penicillin and 100 μgmL-1 streptomycin. Cell line culture was carried out at 37o C in an Incubator (Shel Lab, TC-2323) with a 95% air and 5% CO2 atmosphere. Cytotoxicity of both compounds was tested at 30, 10, 5, and 1 ppm. Leukemia cells were seeded in 24-well tissue culture plates with $5x10^4$ $cellswell^{-1}$. Immediately after platting, each compound was added to the cells. The experiments were carried out three times. All dilutions were prepared with fresh culture media and plates were incubated for 24 h. Microscopic analysis was carried out to search for morphological changes in cells. Surviving cells were measured by the MTS method [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] and related to the mock cell population by measuring absorbance at 590 nm to establish cell viability.

2.5. Antibacterial assays

A bacterial strain of *Salmonella typhimurium* (ATCC 14028) was tested in a Minimal Inhibitory Concentration (MIC) assay. This assay allows determining by visual inspection of the cells the lowest concentration for which cell growth is inhibited after incubation for each compound. Cultures were prepared in TSB media in 96 well plates and, immediately after preparation, they were

incubated at 37 ^{o}C during 24 h. Samples were tested against bacteria in dilutions from 1:200 to 1:0.15. Once incubation time elapsed, the absorbance of the wells was read at 540 nm. The minimal concentration for growth inhibition was estimated by reference to a mock culture as positive control. The same MIC assay was performed with ampicillin to contrast assay results.

3. RESULTS AND DISCUSSION

3.1 Electrochemical synthesis: ENBA metallization

ENBA metallization was conducted as described in the experimental subsections 2.2 and 2.3. This procedure consisted of a controlled-potential electrolysis at a potential of –1.69 V vs. Fc/Fc^{+}, a few milivolts lower than the electrogeneration potential of the imine group dianion radical in this Schiff base. This electrogeneration potential was established by pulse differential voltammetry as described in [11]. The solution color changed from orange to violet because of the ENBA reduction. In addition, a beige solid was formed as a precipitate. The electrochemical process was carried out for 45 min and yielded an electrolytic efficiency of 47% and a chemical yield of 42%. Because of these low electrolytic efficiency and chemical yield values, we are currently working on designing a better electrochemical cell configuration.

3.2 Spectroscopic characterization of Sn-ENBA

UV-Vis spectrophotometry spectrum of ENBA (Figure 2) shows the band of the π-π* imine bond at 373 nm. The 373 nm band does not appear in the UV-Vis spectrum of Sn-ENBA (Figure 3), which indicates the coordination with the nitrogen of the imine group.

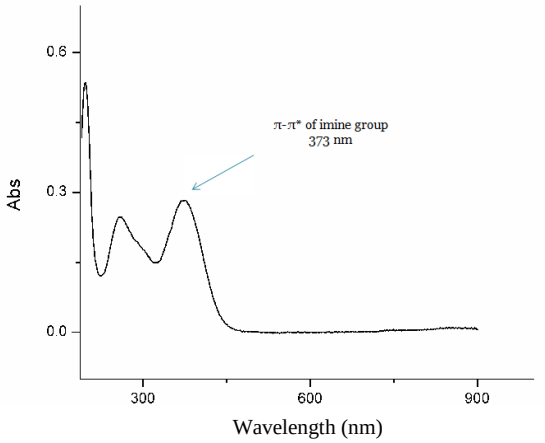

Figure 2. Absorption spectrum of ENBA in ACN.

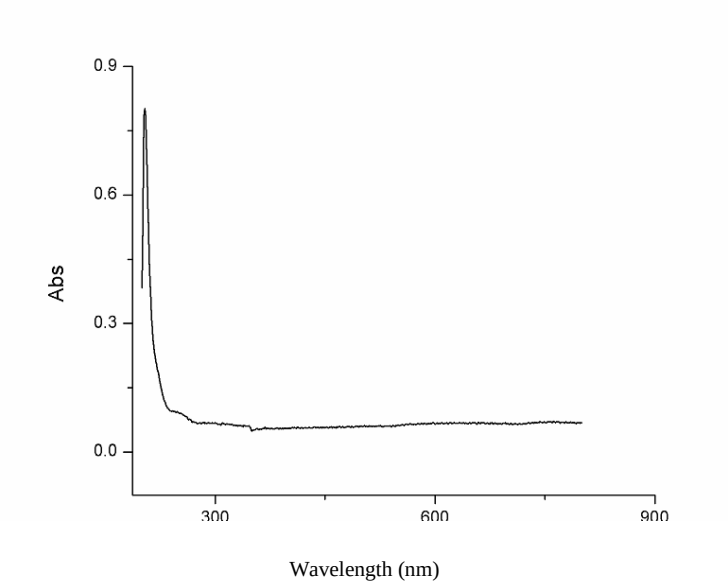

Figure 3. Absorption spectrum of Sn-ENBA in ACN.

The infrared spectra of ENBA and Sn-ENBA (Figure 4) show the disappearance of the C=N bond in the Sn-ENBA spectrum, which indicates a coordination bonding between ENBA and Sn.

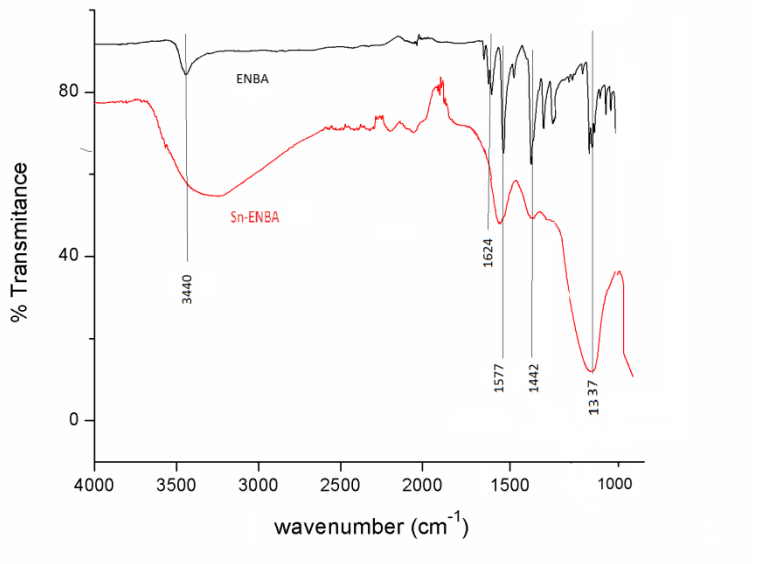

Figure 4. IR spectra of Sn-ENBA and ENBA.

Table 1 summarizes the infrared spectra data for ENBA and Sn-ENBA. The ENBA characteristic bands can be explained as follows: -OH at 3440 cm^{-1} is the hydroxyl group in a ring position. The band at 1624 cm^{-1} corresponds to the imine group. The bands at 1577 cm^{-1} and 1442 cm^{-1} correspond to the conjugated C=C double bonds of benzene. The band at 1337 cm^{-1} corresponds to the nitro group. The coordination bond in Sn-ENBA makes a shift on the wave number because the

insertion of Sn changes the electrostatic interaction in the molecule and causes the disappearance of the imine signal corresponding to the coordination of Sn with this organic group.

Table 1. Summary of infrared spectra results for ENBA and Sn-ENBA

Characteristic Band	ENBA Wave Number (cm^{-1})	Sn-ENBA Wave Number (cm^{-1})
Hydroxyl group in ring position	3440	3335
Imine group	1624	---
Conjugated double bonds of benzene	1577 and 1442	1593 and1448
Nitro group	1337	1342

The solid obtained was analyzed by atomic absorption spectrophotometry. The measurements show a concentration of 338 ppm of Sn, giving a yield of 33.8% weight of Sn, which is in agreement with the theoretical value of 32.9% for a coordination compound of ML 1:1, where M is Sn and L is the ligand.

3.3 Energy dispersive X-ray microanalysis of Sn-ENBA

In addition, EDX (Figure 5) was performed to confirm the presence and determine the amount of metal in the compound. The average yield was 32.01% weight of Sn. Table 2 shows the EDX data. This result agrees with the results obtained from the atomic absorption spectroscopic technique.

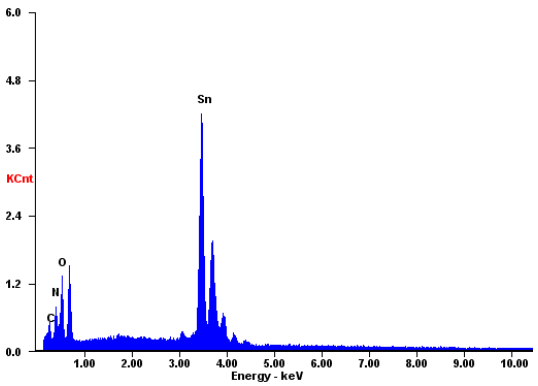

Figure 5. EDX of Sn-ENBA.

Table 2. EDX data

Sn-ENBA	Sn-L (%)
1	31.03
2	35.95
3	29.05
Yield	32.01

3.4 Summary of characterization results

The characterization of the product resulting from the metallization process described in Subsection 3.1 confirms the formation of the Sn-ENBA complex. The results of application of the UV-vis, IR, and atomic absorption spectroscopy techniques, and the EDX microanalysis allow concluding that the ligand was metalled with Sn *in situ* in the electrochemical cell.

The applied procedure was the result of our previous research work reported in [11], where we applied sweep potential techniques to determine the electrogeneration potential of the imine group dianion radical. In [11], we proposed to metall ENBA by applying electrolysis to a slightly more negative potential than the electrogeneration potential of the imine group dianion radical. This proposal was based on the idea that this method would favor the coordination of the ligand imine group with the Sn(IV) ions. These Sn(IV) ions could be electrogenerated in the oxidation process of a tin sacrificial anode. The results reported in this paper confirm the validity of the method proposed in [11]. Had we applied a chemical metallization procedure, the coordination would have taken place with the oxygen of the OH group instead of with the imine group nitrogen.

3.5 In vitro cytotoxic assay

The in vitro cytotoxic assay showed that ENBA and its complex Sn-ENBA have a cytotoxic effect against human lymphoblastic leukemia MOLT-4 cells. As Figure 6 shows, the cytotoxic activity of these compounds generally increases with the concentration, and the activity difference between them also increases. At 1 ppm, ENBA leaves 77% of surviving cells and Sn-ENBA leaves 83%. In spite of these weak effects, some morphological changes, such as nuclei swelling and cytoplasm disturbances were observed in most or the surviving cells. At 5 ppm, ENBA leaves 31.6% of surviving cells and Sn-ENBA leaves 70%. No living cells were detected in wells corresponding to 30 ppm for both compounds.

However, the Sn-ENBA solubility is smaller than the ligand solubility for concentration equal to or greater than 30 ppm, which favors the use of the Sn-ENBA compound. This compound forms microscopic aggregates in association with components of the culture medium (specifically, with complex peptides and with globulines of the added Foetal Bovine Serum). These aggregates can be endocyted by the cells and dissolved in their lysosomes, which slows down the complex incorporation to the cell membrane and, as a result, reduces the side effects.

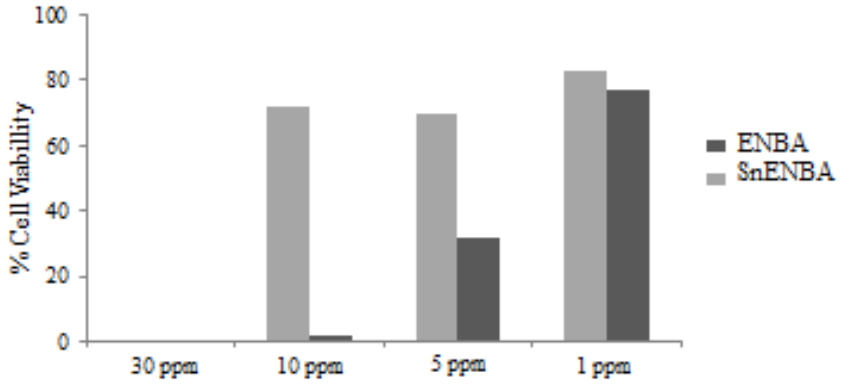

Figure 6. Cytotoxic effect of ENBA and its complex Sn-ENBA on lymphoblastic leukemia MOLT-4 cells. MCK is the mock culture.

3.3 Antibacterial assays

ENBA and its Sn-ENBA complex exhibited a significant antibacterial activity against the gram-negative bacteria *Salmonella typhimurium* (ATCC 14028). Table 3 shows that the MIC values for ENBA and Sn-ENBA (0.35 ppm and 0.46 ppm, respectively) are not too far away from the MIC value for ampicillin. Therefore, these synthesized compounds have a very promising potential as antibacterial agents.

Table 3. Effect of compounds on gram-negative bacteria *Salmonella typhimurium* (ATCC 14028).

Compound	MIC (ppm)
Ampicillin	0.25
ENBA	0.35
Sn-ENBA	0.46

4. CONCLUSIONS

Knowing the electrogeneration potential value of the imine group dianion radical established in a previous research project by our group, made it possible the metallization *in situ* of the (E)-4-((4-nitrobenzylidene) amino) phenol) Schiff base. This process led to obtaining an organotin compound of this Schiff base with the Sn(IV) ions electrogenerated from a sacrificial tin anode in an electrochemical cell. Such metallization was carried out by the imine group and not by the OH group as it would happen if using the classical chemical synthesis. The Sn-ENBA compound was formed successfully, as shown by spectrophotometric and EDX techniques. Both ENBA and Sn-ENBA have cytotoxic and antibacterial activities, but Sn-ENBA has lower level of incorporation to the cell membrane than ENBA, which reduces the side effects of Sn-ENBA.

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