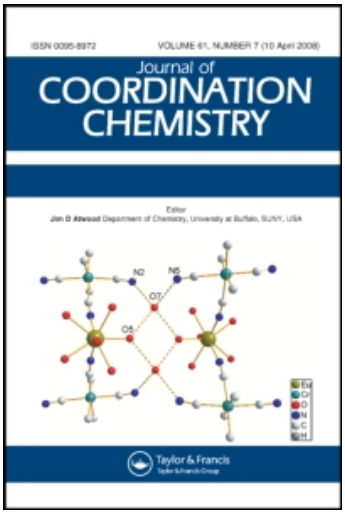


**Journal of Coordination Chemistry**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

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First published on: 13 December 2010

**To cite this Article** Castello, W. S. , Spera, M. B. M. , Gomes, A. F. , Gozzo, F. C. , Lustri, W. R. , Formiga, A. L. B. and Corbi, P. P.(2011) 'Synthesis, spectroscopic characterization, and antibacterial assays *in vitro* of a new platinum(II) complex with methionine sulfoxide', Journal of Coordination Chemistry, 64: 2, 272 — 280, First published on: 13 December 2010 (iFirst)
**To link to this Article: DOI:** 10.1080/00958972.2010.540325
**URL:** http://dx.doi.org/10.1080/00958972.2010.540325

*Journal of Coordination Chemistry*
Vol. 64, No. 2, 20 January 2011, 272–280

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# Synthesis, spectroscopic characterization, and antibacterial assays *in vitro* of a new platinum(II) complex with methionine sulfoxide

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(*Received 29 July 2010; in final form 19 October 2010*)

A new platinum(II) complex with methionine sulfoxide was synthesized and characterized by chemical and spectroscopic techniques. Elemental analyses, mass spectrometric measurements (electrospray ionization quadrupole time-of-flight mass spectrometry), and thermal analyses of the solid compound fit the composition $[(C_5H_{10}NO_3S)Pt(\mu\text{-}Cl)_2Pt(C_5H_{10}NO_3S)]\cdot 2.5H_2O$. Infrared spectroscopic data indicate coordination of the ligand to Pt(II) through the nitrogen of $NH_2$ and the sulfur of the S=O group. $^1H$-$^{15}N$ nuclear magnetic resonance spectroscopic data confirm nitrogen coordination. Antibacterial activities were evaluated by antibiogram assays using the disc diffusion method. The platinum(II) complex showed antibacterial activity against Gram-negative *Pseudomonas aeruginosa* bacterial cells.

*Keywords*: Platinum(II); Methionine sulfoxide; NMR spectroscopy; Mass spectrometry; Antibacterial activity

## 1. Introduction

Platinum complexes have been investigated as antineoplastic agents since the clinical introduction of cisplatin, *cis*-diamminedichloridoplatinum(II), in the 1970s for the treatment of ovarian, lung, testicular, and bladder carcinomas [1, 2]. The mechanism of action of cisplatin and also carboplatin, a second generation compound based on the cisplatin structure, seems to involve covalent binding of platinum(II) to nitrogens of purine deoxyribonucleic acid (DNA) bases, which primarily leads to cellular apoptosis. The successful application of cisplatin for the treatment of various types of cancers has stimulated ongoing investigations of new complexes of platinum(II), palladium(II), gold(I), gold(III), ruthenium(II), and ruthenium(III) and their anticancer activities [3–7]. Auranofin, a phosphine–gold(I) complex used as an antiarthritic drug, exhibited potent cytotoxic activity against human cancer cells *in vitro* and also increased the

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*Journal of Coordination Chemistry*
ISSN 0095-8972 print/ISSN 1029-0389 online 
DOI: 10.1080/00958972.2010.540325

survival time of mice with P388 leukemia [8]. Gold(III)–porphyrin complexes were also described in the literature as promising substances for cancer treatment in terms of their cytotoxic potency and specificity for DNA [9].

Ruthenium complexes have also been considered as anticancer drugs. Biological activities of *trans*-tetrachlorido(dimethyl sulfoxide)imidazoleruthenate(III), or NAMI-A, have been described. The NAMI-A complex reduces the formation of metastases and appears to inhibit the growth of tumors [1]. More recently, ruthenium(II) complexes $[RuCl_2(NO)(dppp)(L)]PF_6$ [dppp = 1,3-*bis*(diphenylphosphino)propane; L = pyridine, 4-methylpyridine, 4-phenylpyridine, and dimethyl sulfoxide] were synthesized and studied as potential anticancer drugs. The *in vitro* evaluation of these nitrosyl complexes revealed their cytotoxic activity from 7.1 to 19.0 $\mu mol\,L^{-1}$ against MDA-MB-231 breast tumor cells, being more active than cisplatin which was used as a reference compound [10].

The cytotoxic and antitumor activities of palladium(II) complexes have also been investigated [11, 12]. According to the literature, new mononuclear and multinuclear palladium(II) compounds with reduced cisplatin resistance and higher specificity than cisplatin have been studied. Similar to platinum(II) agents, DNA seems to be the main target of the palladium(II) complexes in the cell [11].



Metal complexes have also been considered as antimicrobial agents. Synthesis and characterization of new complexes with ampicillin and amoxicillin were recently described [13]. The antimicrobial activities of such compounds were tested on four different microorganisms and compared to the free drugs. The Cu(II) complexes were shown to be the most active against *Bacillus subtilis* and *Escherichia coli*. Also, a new gold(I) complex with *N*-acetyl-L-cysteine exhibiting effective antibacterial activity against *Staphylococcus aureus* (Gram-positive) and *E. coli* (Gram-negative) bacteria was synthesized in our laboratories [14]. Platinum(II) and palladium(II) complexes have also been synthesized and their antibacterial properties evaluated. A new platinum(II) complex obtained by reaction between *cis*-diamminediaquaplatinum(II) and 5-nitrosouracil exhibited antimicrobial activity against *E. coli* and *Aspergillus niger* with minimum inhibitory concentration (MIC) ranging from 75 to 100 $\mu mol\,mL^{-1}$ [15]. More recently, silver(I) and platinum(II) complexes with acesulfame were studied against different microorganisms. The silver(I) complex exhibited antimicrobacterial activity *in vitro* against *Mycobacterium tuberculosis*, responsible for tuberculosis, with a MIC value of 11.6 $\mu mol\,L^{-1}$. The silver(I) complex also showed a promising activity against Gram-negative (*E. coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Enterococcus faecalis*) microorganisms. The platinum(II) complex with acesulfame, $K_2[PtCl_2(ace)_2]$, was evaluated for antiviral properties against dengue virus type 2 (New Guinea C strain) in Vero cells and showed good inhibition of dengue virus replication at 200 $\mu mol\,L^{-1}$ when compared to vehicle-treated cells [16]. In addition, palladium(II) and platinum(II) complexes with bioactive Schiff bases were synthesized and tested against two pathogenic bacteria (*E. coli* and *Pseudomonas cepacicola*) using the disc diffusion method [17]. According to the published data, the palladium(II) and platinum(II) complexes inhibited the growth of the bacterial strains to a greater extent than the free Schiff bases and comparable to streptomycin.

Methionine sulfoxide ($C_5H_{11}NO_3S$, MetSO) is a sulfur-containing amino acid present in vegetables like carrots and onions. Crystallographic data of two platinum(II) complexes with MetSO have been described [18, 19]. The composition of the complexes was defined as $[PtCl_2(C_5H_{11}NO_3S)]$ and $[Pt_2(C_5H_9NSO_3)_2]$. In the first case, ligand

coordination to platinum(II) occurs through N and S, while in the second coordination was shown to be through N, O, and S forming a square-planar geometry. No biological studies were reported for such complexes. More recently, nickel(II), cobalt(II), iron(II), copper(II), and also palladium(II) complexes with MetSO were synthesized in our laboratories [20–22]. In the case of the palladium(II) complex, coordination was through nitrogen of $NH_2$ and oxygen of $COO^-$, forming a square-planar geometry. Biological results showed modest antitumor activity of the Pd(II)–MetSO complex, with inhibition of 20% of HeLa tumorigenic cell viability at a concentration of $200\,\mu mol\,L^{-1}$ [21]. This article describes the synthesis, characterization, and initial biological assays of a new platinum(II) complex with MetSO.

## 2. Experimental

### 2.1. *Reagents and equipments*



DL-MetSO (99%), potassium hydroxide, and potassium tetrachloroplatinate(II), 98% were purchased from Sigma–Aldrich Laboratories. Elemental analyses for carbon, hydrogen, and nitrogen were performed using a CHNS Perkin Elmer 2400 Analyzer. Infrared (IR) spectra from 4000 to $450\,cm^{-1}$ of the potassium salt of MetSO (KMetSO) and the Pt(II)–MetSO complex were measured using a ABB Bomen Fourier transform infrared (FT-IR) MB Series spectrophotometer; samples were prepared as KBr pellets. The $^1H$-$^{15}N$-NMR data were acquired on a Bruker AVANCE II 300 MHz spectrometer using a 5-mm probe at 303 K. The Pt(II)–MetSO complex was analyzed in deuterated dimethyl sulfoxide solution, while KMetSO was analyzed in deuterium oxide solution. Samples were referenced to dimethylformamide. Thermal analysis was performed on a SEIKO EXSTAR 6000 simultaneous TGA/differential thermal analyzer (DTA) under the following conditions: synthetic air, flux rate of $50\,cm^3\,min^{-1}$ with a heating rate of $10^\circ C\,min^{-1}$, from 25°C to 900°C. The residue of thermal treatment at 900°C was analyzed on a Shimadzu XRD-7000 diffractometer using Cu-K$\alpha$ radiation ($\lambda = 1.5406$ Å) with graphite monochromator at room temperature. The sample was scanned from 1.4° to 50° ($2\theta$) with a scanning rate of $2.0^\circ\,min^{-1}$. Electrospray ionization quadrupole time-of-flight mass spectrometry (ESI–QTOF-MS) measurements were carried out in a Waters Xevo QTOF instrument. Samples were solubilized in $H_2O$/MeCN 50 : 50 containing 0.1% formic acid to a concentration between 10 and $20\,\mu mol\,L^{-1}$. Such solutions were then directly infused into the instrument's nanoESI source at a flow rate of $1\,\mu L\,min^{-1}$. Typical acquisition conditions were capillary voltage 3 kV, cone voltage 30 V, and source temperature 100°C. The spectra were acquired in the positive ion mode (ESI+) at a scan rate of 1 Hz. The instrument was previously calibrated with phosphoric acid oligomers ($H_3PO_4$ 0.005% in $H_2O$/MeCN 50 : 50) ranging from $m/z$ 100 to 2000.

### 2.2. *Synthesis*

The platinum(II) complex with MetSO was synthesized by the reaction of 3.0 mL of an aqueous solution of potassium tetrachloroplatinate(II)–$K_2[PtCl_4]$ ($5.0 \times 10^{-4}$ mol) with 3.0 mL of an aqueous solution of MetSO ($5.0 \times 10^{-4}$ mol). The pH of the solution was

adjusted to 10 with a dilute potassium hydroxide aqueous solution. Synthesis of the complex was carried out with stirring at room temperature for 12 h. The pale yellowish solid obtained was vacuum-filtered, washed with cold water, and dried in a desiccator over $P_4O_{10}$. Elemental analysis led to the following composition for the complex: $Pt_2Cl_2(C_5H_{10}NO_3S)_2 \cdot 2.5H_2O$. Anal. Calcd for $Pt_2Cl_2(C_5H_{10}NO_3S)_2 \cdot 2.5H_2O$ (%): C, 14.4; H, 3.02; and N, 3.36. Found (%): C, 14.4; H, 3.04; and N, 3.32. No crystals were obtained in order to perform a full X-ray structure characterization. The Pt(II)–MetSO complex is soluble in dimethyl sulfoxide, but insoluble in water, ethanol, methanol, acetonitrile, chloroform, acetone, and hexane. Since the complex was obtained in alkaline medium, the KMetSO was also prepared by reacting equimolar quantities of MetSO and KOH in aqueous solution. The salt is the ionic form of the free ligand.

### 2.3. *Biological assays*

Three referenced bacteria (*E. coli* – ATCC 25922, *P. aeruginosa* – ATCC 27853, and *S. aureus* – ATCC 25923) were used in this study. Antibiogram assay was performed using the disc diffusion method [23, 24]. The sensitivity of Pt(II)–MetSO complex was tested in Mueller–Hinton (MH) agar plates. The microorganisms (*E. coli*, *P. aeruginosa*, and *S. aureus*) were transferred to separate test tubes containing 5.0 mL of sterile brain heart infusion (BHI) medium and incubated for 18 h at 35–37°C. Sufficient inocula were added in new tubes until the turbidity equaled 0.5 McFarland ($\sim 1.5 \times 10^8$ CFU mL$^{-1}$). The bacterial inocula diluted with BHI (McFarland standard) were uniformly spread using sterile cotton swabs on sterile Petri dishes MH agar.

Sterile filter paper discs of 10 mm in diameter were aseptically impregnated with 1.6 mg of Pt(II)–MetSO according to the following procedure: 20.0 mg of the platinum(II) complex was dissolved in 500 μL of dimethyl sulfoxide, homogenized in a vortex, and 40 μL of the solution was collected with a micropipette and transferred to the paper discs. Sterile discs impregnated with 1.6 mg of pure MetSO, used as a negative control, were prepared as follows: 20.0 mg of MetSO was dissolved in 500 μL of distilled water and 40 μL of the homogenized solution was transferred to the paper discs.

Discs impregnated with Pt(II)–MetSO and pure MetSO were dried and sterilized in a vertical laminar flow under UV radiation for 45 min before the experiment. All impregnated discs were placed on the surface of the solid agar. The plates were incubated for 18 h at 35–37°C and examined thereafter. Clear zones of inhibition formed around the discs were measured and the complex sensitivity was assayed from the diameter of the clear inhibition zones (in millimeters). Experiments were performed in triplicate and the results were compared to other platinum(II) complexes described in the literature against the same bacterial strains.

## 3. Results and discussion

### 3.1. *$^1$H-$^{15}$N-NMR spectroscopic measurements*

The structural formula of MetSO with hydrogen and carbon numbering is shown in figure 1. The $^{15}$N chemical shifts for KMetSO and for Pt(II)–MetSO were indirectly obtained from the 2-D spectra *via* the heteronuclear [$^1$H-$^{15}$N] multiple bond coherence

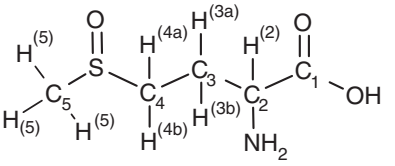


Figure 1. Schematic structural formula of MetSO showing hydrogen and carbon numberings.

technique (HMBC), as described for the palladium(II) complex with MetSO [21]. The assignment of the nitrogen resonance was performed by its correlation with protons $H^{(3a)}$ and $H^{(3b)}$. Analysis of the HMBC spectrum of KMetSO permitted identification of the $^{15}N$ isomer shift of the $NH_2$ group at 37.8 ppm while in the complex, the $^{15}N$ isomer shift appears upfield at −3.10 ppm. The observed $\Delta\delta = -40.9$ ppm confirms coordination of MetSO to platinum(II) through $NH_2$.

### 3.2. *Mass spectrometric measurements*

The Pt(II)–MetSO complex, $[Pt_2Cl_2(C_5H_{10}NO_3S)_2]$, was observed in the ESI–QTOF mass spectrum as singly charged $[M+H]^+$ and $[M–Cl]^+$ ions of $m/z$ 786.9 and 750.9, respectively (figure 2a), which confirmed the presence of two chlorides and indicated the formation of a dimer. The experimental isotope patterns for these ions match theoretical predictions (figure 2b and c), confirming their identities. Mass errors were −16.8 ppm for $[M–Cl]^+$ (Calcd $m/z$ 750.9705, exp. $m/z$ 750.9579) and +4.4 ppm for $[M+H]^+$ (Calcd $m/z$ 786.9471, exp. $m/z$ 786.9506).

### 3.3. *IR spectroscopic data*

The Pt(II)–MetSO IR spectrum was analyzed in comparison to that of KMetSO. The IR spectra of KMetSO and Pt(II)–MetSO are provided as supplementary material.

The amino group involvement in ligand coordination to platinum(II), as proposed for the $^1H$-$^{15}N$-NMR spectroscopic measurements was confirmed by analyzing the IR data. According to Nakamoto [25], the presence of two resolved absorption bands in the range 3300–3000 $cm^{-1}$ are indicative of coordination of the amino group to metal centers. In the case of Pt(II)–MetSO, the presence of one resolved band at 3250 $cm^{-1}$ and a shoulder at 3140 $cm^{-1}$ attest to coordination of the nitrogen of the amino group to Pt(II). These bands can be assigned to the asymmetric and symmetric stretching modes of coordinated $NH_2$, respectively. Broadening and poor resolution of these bands is due to the presence of hydration water which forms hydrogen bonds with the Pt(II)–MetSO complex. Moreover, the absence of the $\delta_{as}(NH_2)$ vibration in the spectrum of the Pt(II)–MetSO complex constitutes further proof of the involvement of $NH_2$ coordination. The $\delta_{as}(NH_2)$ vibration frequency of KMetSO appears at 1531 $cm^{-1}$.

Sulfur coordination of MetSO to Pt(II) is proposed by comparing the S=O absorption in IR spectra of KMetSO and the Pt(II)–MetSO complex. According to the literature, if coordination occurs through sulfur, the S=O stretching in the IR spectrum shifts to higher frequency. On the other hand, if coordination occurs through oxygen, the characteristic $\nu$(S=O) stretching frequency is shifted to lower energy [25]. The S=O vibration in KMetSO was observed at 1015 $cm^{-1}$ while in the spectrum of the complex

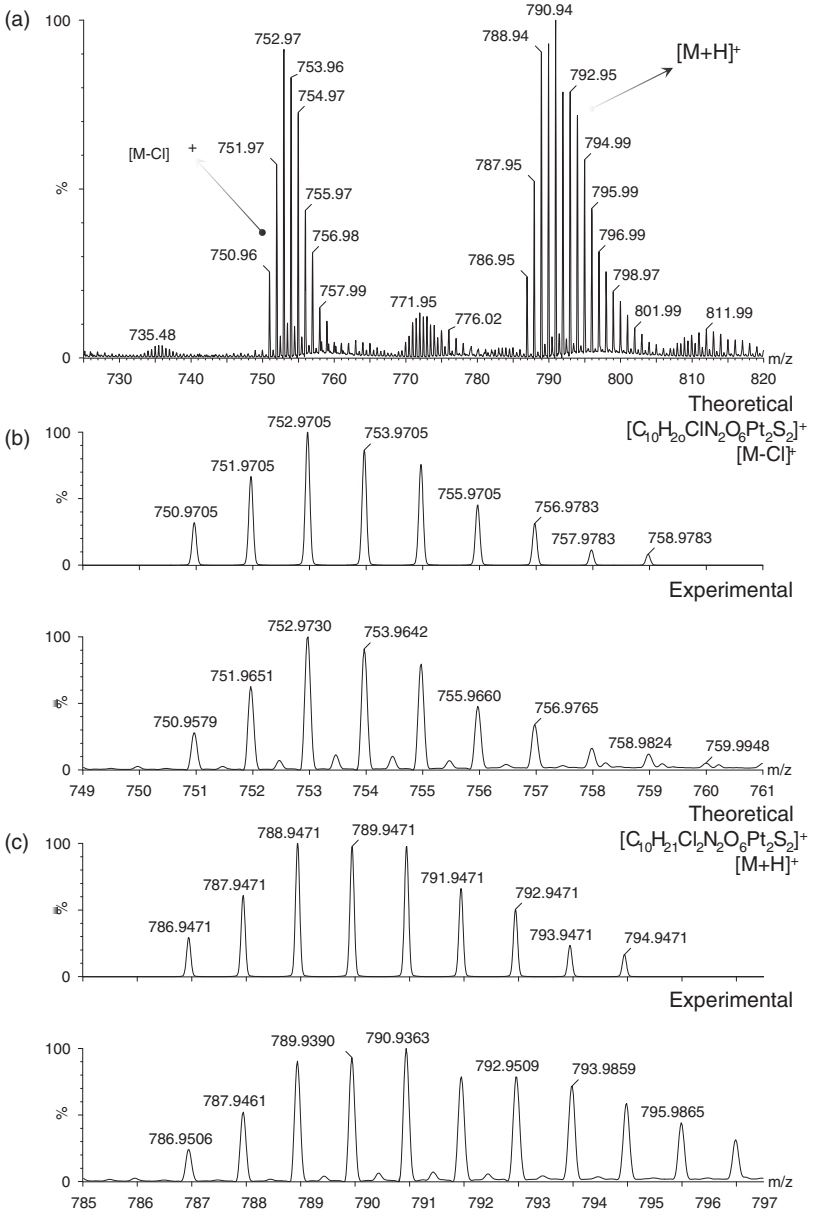


Figure 2. ESI–QTOF mass spectrum of $[Pt_2Cl_2(C_5H_{10}NO_3S)_2]$, demonstrating its presence as $[M–Cl]^+$ and $[M+H]^+$ singly charged ions of $m/z$ 750.9 and 786.9, respectively (a) and isotope pattern comparisons for (b) $[M–Cl]^+$ of $m/z$ 750.9 and (c) $[M+H]^+$ of $m/z$ 786.9.

it was shifted to higher frequencies, at 1118 $cm^{-1}$. The observed results led us to consider sulfur coordination to Pt(II).

Possible coordination of MetSO to Pt(II) through oxygen of carboxylate was also considered based on the IR data. The IR spectrum of KMetSO exhibits a strong absorption with maximum at 1583 $cm^{-1}$, which is assigned to the asymmetric stretching

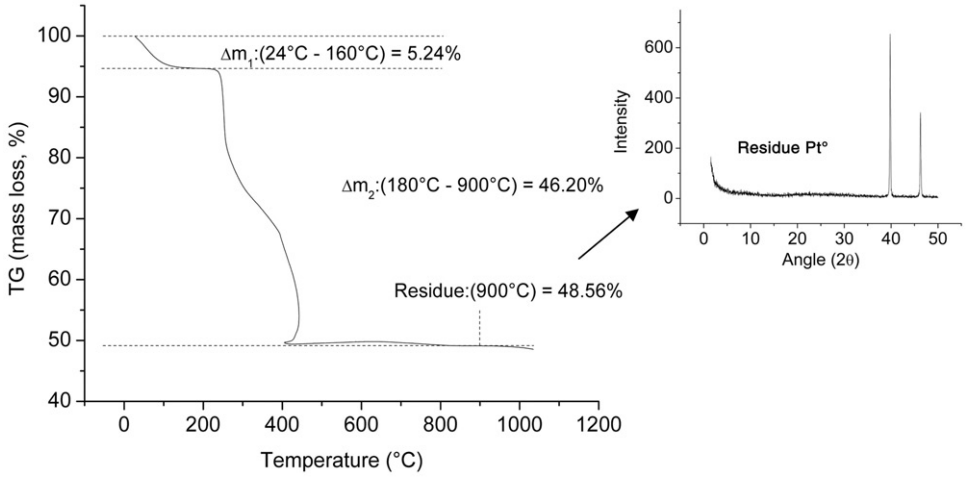


Figure 3. Thermogravimetric data for the Pt(II)–MetSO complex.



frequency of ionized and uncoordinated carboxylate ($COO^-$) [25, 26]. In Pt(II)–MetSO, this band shifts to higher frequencies, at 1658 $cm^{-1}$. Based on the work of Freeman *et al*. [19], the results might be considered as an indication of non-coordination of the ligand through oxygen, as observed for [Pt(methionine)$Cl_2$] and Pt(methionine S-oxide)$Cl_2$] reported earlier. The presence of an intense broad band at 3500 $cm^{-1}$, assigned to $\nu$(O–H), in the Pt(II)–MetSO IR spectrum confirms the presence of hydration water [25].

### 3.4. *Thermal analysis*

The TGA curve for Pt(II)–MetSO is shown in figure 3. According to the thermogravimetric data, the hydration waters are lost from 30 to 160°C. Anal. Calcd for loss of 2.5 $H_2O$ molecules (%) 5.40; Found (%) 5.24. Ligand decomposition starts at 180°C leading to formation of the final residue at 500°C. Anal. Calcd for loss of 2 MetSO + 2 Cl (%) 48.2; Found (%) 46.2. The residue of the thermogravimetric analysis was identified by X-ray powder diffraction measurements as metallic platinum [27]. Anal. Calcd for $Pt^o$ (%) 46.8; Found (%) 48.6.

The DTA curve for Pt(II)–MetSO exhibits two exothermic peaks with maxima at 252°C and 440°C, assigned to ligand oxidation in two steps leading to formation of $Pt^o$ as the final residue of the thermal treatment.

### 3.5. *Biological activity*

Antibiotic sensitivity profiles demonstrate the antibacterial activity of the Pt(II)–MetSO complex against *P. aeruginosa* bacterial strain, as observed by the disc diffusion method. It was found that impregnated paper discs with Pt(II)–MetSO exhibit an inhibition zone for *P. aeruginosa* of $15.0 \pm 0.1$ mm, which indicates that this bacterial strain is sensitive to Pt(II)–MetSO. The results confirm potential application of

platinum(II) complexes as antibacterial agents, as described for platinum(II) and palladium(II) complexes with tetracyclines and with Schiff-base ligands against pathogenic bacteria [17, 28]. The Pt(II)–MetSO complex did not show antibacterial activity against *S. aureus* and *E. coli* under the same conditions. Also, pure MetSO, used as a negative control, did not exhibit antibacterial activity against *E. coli*, *P. aeruginosa*, or *S. aureus* in the same experimental conditions.

## 4. Conclusions

The molar composition of Pt(II)–MetSO was 1 : 1 (metal : ligand). Water content was confirmed by elemental, thermogravimetric, and IR analyses. $^{1}H$-$^{15}N$-NMR measurements confirmed nitrogen coordination of the ligand to Pt(II), while IR data also suggest sulfur coordination. The ESI–QTOF mass spectrometry supported the dimeric structure with coordination formula $[(C_5H_{10}N_2O_3S)Pt(\mu\text{-}Cl)_2Pt(C_5H_{10}N_2O_3S)] \cdot 2.5H_2O$.

Biological studies revealed the antibacterial activity of the complex against the Gram-negative (*P. aeruginosa*) bacterial strain. The observed results warrant further *in vitro* investigations against different microorganisms and the optimization of the dosage of the Pt(II)–MetSO complex.



## Acknowledgments

This study was supported by grants from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo – Brazil, proc. 2010/00534-7 and 2006/55367-2), FAEPEX, and CNPq.

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