# SYNTHESIS, CHARACTERIZATION, BINDING INTERACTIONS WITH BIOMOLECULES AND CYTOTOXIC STUDIES OF MOLYBDENOCENE COMPLEXES

By

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A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

CHEMISTRY

UNIVERSITY OF PUERTO RICO

MAYAGÜEZ CAMPUS

2009

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## Abstract

Bis-(cyclopentadienyl) molybdenum dichloride or molybdenocene dihalides, like other metallocenes dihalides, have shown antitumor properties in different human tumors. In contrast to titanocene dichloride, molybdenocene dichloride has a higher water solubility and high stability under physiological pH conditions. The present work was aimed to synthesize and characterize molybdenocene complexes, to study their interactions with biologically important molecules such as calf-thymus DNA or human serum albumin (HSA) and to evaluate their cytotoxic activity. Two complexes were synthesized: $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl. Both complexes were characterized spectroscopically by NMR, IR and Mass Spectrometry. Also, an electrochemical characterization was pursued using cyclic voltammetry. The interactions between the molybdenocene complexes with calf-thymus DNA and with HSA, in buffer conditions, were studied by cyclic voltammetry. Binding studies by cyclic voltammetry experiments showed a possible interaction of the molybdocene dichloride with albumin and very weak, almost negligible interaction with DNA. The malonate and maltolato complexes did not show any interaction with DNA or HSA. In order to assess, in more details, the interaction of molybdenocene complexes with DNA or HSA we performed titrations using UV-Vis spectrometry, but no conclusive evidence of interaction can be extrapolated from these titrations. The cytotoxic activities of the molybdenocene dichloride and of the synthesized complexes were investigated on the HT-29 colon cancer cell line and on the MCF-7 human breast cancer cell line. Also the cytotoxic activities of the vanadocene dichloride and [$Cp_2V$(maltolato)]Cl complex on HT-29 were studied.

## Resumen

El dihaluro de molibdenoceno, al igual que otros dihaluros de metalocenos han demostrado propiedades antitumorales contra diferentes tumores humanos. Contrario al dicloruro de titanoceno, el dicloruro de molibdenoceno tiene alta solubilidad en agua y alta estabilidad a pH fisiológico. Este trabajo está enfocado en la síntesis y caracterización de complejos derivados de dicloruro de molibdenoceno, estudiar su interacción con moléculas biológicas como el DNA y la albúmina (HSA) y evaluar su actividad citotóxica. Dos complejos fueron sintetizados: $Cp_2Mo$(malonato) y [$Cp_2Mo$(maltolato)]Cl. Ambos, se caracterizaron espectroscópicamente por RMN, IR y espectroscopia de masa. Además, se utilizó voltametría cíclica para caracterizarlo electroquímicamente. Las interacciones entre los complejos de molibdenoceno con ADN (calf-thymus DNA) y albúmina de suero humano (HSA) fueron estudiadas por voltametría cíclica. Esta técnica mostró una posible interacción entre el molibdenoceno y la albúmina, pero una interacción menor y casi descartable con el ADN. Los complejos de malonato y maltolato no mostraron interacción con el ADN o con la albúmina. Para evaluar con más detalle estas interacciones, se utilizo la técnica UV-Vis. Estas titulaciones no mostraron la formación de bandas nuevas o puntos isosbésticos, pero si un aumento en la absorción de la banda a 260 nm lo cual sugiere una posible interacción con el ADN. Sin embargo, no se puede extrapolar ninguna evidencia contundente de interacción a partir de estas titulaciones. Los análisis de citotoxicidad del bicloruro de molibdenoceno y de los complejos sintetizados fueron investigados en las líneas celulares de cáncer de colon HT-29 y cáncer de seno MCF-7. También, se evaluó la actividad citotóxica del dicloruro de vanadoceno y de su derivado [$Cp_2V$(maltolato)]Cl en HT-29.

*To my parents: Raúl y Mita*

*And to my sisters: Ineida, Seni y Dini*

## Acknowledgements

In the course of my graduate studies in the University of Puerto Rico, Mayagüez Campus I received the assistance of many people, that without which I can not see complete my work. It is with great pleasure that I thank the effort all these people put up and their support in my research and to all these that bring me their cheer through this time.

My most sincere gratitude to my advisor, Dr. Enrique Meléndez, for giving me the opportunity of been part of his work team. Thank you, Dr. Meléndez, for allowing me to conduct this research under your continuous guidance and support. Thanks to my graduate committee, Dr. Carmen A. Vega, Dr. Mayra E. Cádiz, and Dr. Félix R. Román for their considerations and suggestions to this work.

Thanks to my lab partners: D. Acevedo, J. Lamboy, R. Hernández, Li Ming Gao and J. Vera. Thanks to Dr. Félix Román and his graduate student, Miguel Florián, for the opportunity to use his Mass Spectrophotometer facilities at the Chemistry Department, University of Puerto Rico, Mayagüez Campus. I would like to acknowledge Dr. Oscar Peráles and his graduate students, Yarilyn Cedeño and Celia Osorio, for their help and effort in the performance of the IR experiments at the Engineering Department, University of Puerto Rico, Mayagüez Campus. My gratitude to Dr. Jaime Matta and his research team, especially to Esperanza Negrón and Luisa Morales from the Ponce School of Medicine, Department of Pharmacology, Toxicology and Physiology, in Ponce Puerto Rico, for allowing me to use his facilities to carry on the cytotoxic experiments. Thanks to Dr. Virgilio Salvo and to Yainyrette Rivera from the Ponce School of Medicine, Department of Pharmacology, Toxicology and Physiology, in Ponce Puerto Rico, for

facilitating me the MCF-7 cell line in order to evaluate the cytotoxic activity of my complexes.

I want to express a genuine gratitude to all my friends, for their unconditional support and cheer. Thanks to Edward for your comprehension and love, and for all the weekends and nights that he stayed with me in the lab. Thanks to my sisters and to my parents for believing in me and for giving me strength to continue on. Finally, thanks to the most important being, who gave me all the opportunities in my life, God. Thank you Lord, because without you nothing is possible and I wouldn't be a able to complete this work.

# Table of Contents

# List of Tables

**Table** **Page**

# List of Figures

# List of Appendixes

**Appendix** **Page**

# 1. Introduction

Cancer is one of the principal causes of death in developed countries, such as the United States. Thousands of patients die each year from different types of cancer. Therefore, it is indispensable to continue studying all aspects that this disease involves.

Cancer is a disorder characterized by uncontrolled division and spread of abnormal cells. This spread can occur through invasion of adjacent tissues or by metastasis, where abnormal cells are transported through the lymphatic system toward far organs. The unregulated spread is a result of the mutation of the genes that encode for proteins that control cell division. If the spread is not controlled, malignant cells will continue to scatter to all tissues and organs, and can result in death.

Cancer can be caused by external factors like exposure to chemicals, radiation, and tobacco smoke, or to others environmental pollutants. Cancer can also be caused by internal factors such as hereditable mutations and hormonal disorders. All these factors can initiate or promote carcinogenesis [1].

There are many types of cancer and its symptoms depend on the location and the character of malignancy or on how advanced is the metastasis process. Although cancer can affect people of all ages, the probability of getting certain types of cancer depends of the sex of the person and tends to increase with age. The most common types of cancers in males are prostate, lung and bronchus, colorectal, bladder and melanoma. On the other hand, the most common types in females are breast, lung and bronchus, colorectal, uterine corpus and ovarian cancers. In children, the most common types cancers are Leukemia, brain and and neurablastoma [1].

Some of the most known cancer treatments include surgery, radiation and chemotherapy. Although, the type of treatment depends on the location and phase of the disease, the most used treatment is chemotherapy.

Chemotherapy consists in supplying anticancer agents that are able to destroy malignant cells. There are several modern pharmaceutical drugs currently used in chemotherapy containing metal complexes. The first antitumor metal complex used was cis-platin, a platinum containing complex. Platinum based drugs such as cis-platin, carboplatin and oxaliplatin, (**Figure1-1**) are commonly used in combination therapies for numerous solid tumors. These platinum based drugs work similarly, binding to DNA, preventing its transcription, replication and also avoiding cell division [2, 3]. However its effectiveness is often limited by its toxicity to other healthy tissues and organs. Also, tumor cells have demonstrated resistance to them [4].

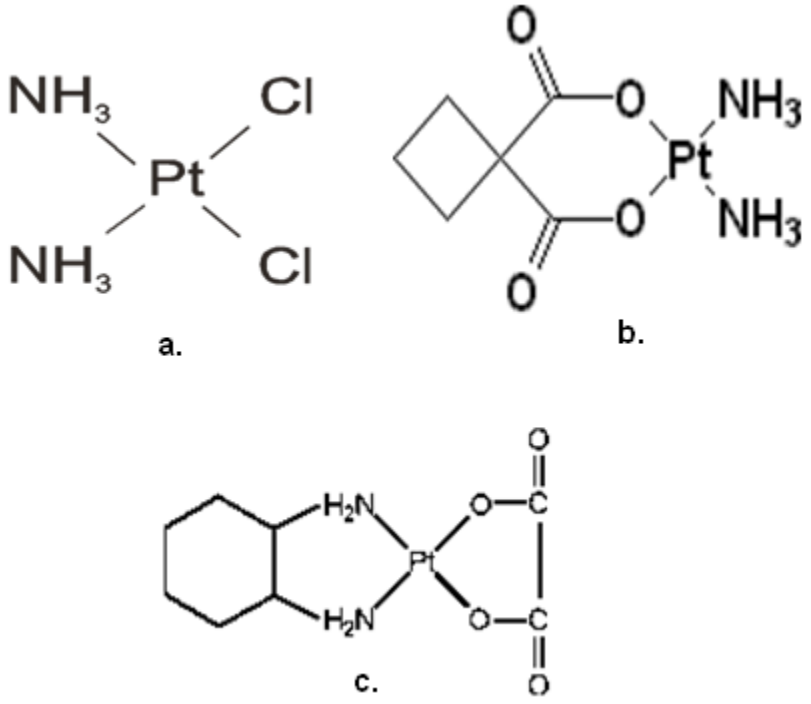



**Figure 1-1:** Structures of platinum complexes: a. cis-platin, b. carboplatin, c. oxaliplatin.

Nowadays, a range of metallocene dihalides, including titanocene, molybdocene, vanadocene and niobocene dichlorides, have been under study. These organometallic complexes exhibit strong antitumor properties. It is known that the chemical stability and coordination chemistry of metallocenes are different to that of platinum drugs, suggesting that metallocene dihalides have different mechanism of action. However, only for titanocene dichloride there is enough biological, structural and activity data. Nevertheless, one of its disadvantages is its low stability at physiological pH [10].

Of all metallocenes studied, molybdocene dichloride presents two essentials characteristic for clinical studies, these are: stability at physiological pH and water solubility. These two characteristics make easier its study because they allow us to have a better idea of the *in vivo* behavior of these molybdenum complexes under physiological environment. Also, the synthesis of water–soluble molybdenocene complex is crucial because water, the universal solvent, is abundant, inexpensive, a non toxic solvent and is an important component of a physiological media. In addition, these properties will make possible the design of new pharmaceuticals formulations or better forms of drug administration in order to facilitate patient treatment.

Based on the above arguments, the importance of continuing research to synthesize and characterize molybdocene based complexes is evident, especially with a broader range of possibilities to fight various types of cancer with high antitumor activity but with less toxicity possible. Hence, it is necessary to continue the research in this field in order to develop new drugs against cancer to provide a better quality of life to each cancer patient and to our society. For these reason, the research goal involves the synthesis, spectroscopic and electrochemical characterization of molybdenocene

complexes. It also includes the evaluation of their interactions with biologically important molecules such as DNA and Human serum albumin by using UV-Vis spectroscopy and Cyclic Voltammetry techniques. Moreover, this dissertation presents the cytotoxic activity of the synthesized complexes on two human cancer cell lines, HT-29 colon cancer and MCF-7 breast cancer.

## 1.1 Objectives

The research efforts are focused on the synthesis, the spectroscopic and electrochemical characterization of molybdenocene complexes, their interactions with biological important molecules and in the evaluation of their biological activity into tumor cells.

The specific aims can be summarized as follows:

a. To examine the coordination properties of molybdocene with different oxygen containing chelating ligands in order to improve their antitumoral properties.

b. To study molybdocene complexes solubility in water.

c. To characterize each complex by different spectroscopy techniques such as NMR, UV and IR spectroscopies and electrochemical methods such as cyclic voltammetry.

d. To study the molybdocene complexes interaction, with Calf-Thymus DNA and Human Serum Albumin as model biological compounds, using CV and UV-Vis spectroscopy.

e. To evaluate the cytotoxic activity of molybdenocene complexes on tumor cells such as HT29 human colon cancer cell line and MCF-7 human breast cancer cell line.

## 1.2 Literature Review

### 1.2.1 Platinum complexes as antitumor agents

A large amount of modern pharmaceuticals currently used to treat cancer are organic drugs. However, inorganic compounds with metallic elements have drawn the attention of researchers since the platinum antitumor properties were discovered. The use of platinum complexes in cancer treatments began in the 1960's when Barnett Rosenberg, professor at Michigan State University, observed a particular result when he was investigating the effects of electric field currents on cell growth. He noticed that cell division was restrained due to a chemical agent that was formed by the slow reaction of the platinum electrode with the ammonium chloride solution in presence of light and electrical current [2]. This chemical species was cis-diamminedichloroplatinum or *cis*-platin. These results prompted him to test cis-platin as an anticancer drug. This platinum-based drug was approved by the American Food and Drug Administration (FDA) for use in testicular and ovarian cancer treatments in 1978 [2].

Platinum based drugs destroy cancer cells by forming covalent DNA adduct. These Pt-DNA adducts generate a bending which interferes in the DNA transcription and replication mechanism, triggers the cell death [2, 3]. Nowadays, cis-platin and its analogues are used in cancer therapy. However, the effectiveness of cis-platin in clinical treatment has been restricted because tumor cells have developed resistance after continuous treatment with cis-platin and also by its high toxicity to normal cells [4]. There are different factors that can cause this resistance to platinum complexes. In ovarian cancer, for example, in agreement with Selvakumaran et al. (2003), these factors could be attributed to the following resistance mechanisms: decreased of drug

accumulation, increased the inactivation process, and improved the DNA repair capacity and tolerance of DNA to avoid damage [9]. Although, cis-platin and its analogues are currently used in therapies of some solid tumors such as ovarian and prostate cancers, researches continue their study in order to increase their effectiveness. As a result of these limitations, researchers have concentrated on the study and development of new non platinum metal complexes against cancer.

### 1.2.2 Metallocenes dihalides as antitumor agents

A wide range of metal complexes that have shown antitumoral activity are currently under study, including metallocenes. The metallocenes were first introduced in 1979 by Harmut Kopf and Petra Kopf-Maier with their study of the titanocene dichloride [$Cp_2TiCl_2$] anitumor activity [13, 14]. Metallocenes are complexes in which a transition metal is coordinated to two cyclopentadienyl ligands and two halide or acido ligands. The metallocenes dihalides $Cp_2MX_2$ (M = Ti, Mo, Nb, V; X = halide), **Figure1-2**, exhibit antitumor properties against numerous human tumors [10, 11, 12, 13, 14].

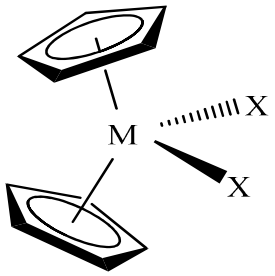



**Figure 1-2:** Structure of metallocenes dihalides

The antitumor activity of metallocenes is dependent of the central metal (M). When M = Ti, V, Nb or Mo the complexes are active; if M = Ta or W are moderate active and if M is Zr or Hf are inactive [15, 16]. In 1994 Murray and Harding studied the effect of the central metal (Ti, Mo, Zr, Hf) and halide ligands ($F^-$, $Cl^-$, $Br^-$, $I^-$) on the interaction of metallocene dihalides with nucleic acid constituents by NMR spectroscopy. They reported that "the metal center strongly influences the nature and extent of interaction between metallocene dichlorides and DNA subunits" [15, 16]. They found a formation of complexes between nucleotides and active metallocenes (Ti, Mo) but not with biological inactive metallocenes (Zr, Hf). They also found that titanocene complexes are stable for hours at pH 2-5. Therefore, they proposed that the mechanism of action of these classes of biological active metallocenes is through DNA interactions.

According to Harding et al., titanocene dichloride is the most studied of metallocenes dichlorides, but its mechanism of action has not been determined accurately. Nevertheless, titanocene dichloride is in phase II clinical trials, but its poor solubility and stability at physiological pH are limitations for a suitable formulation [10].

On other hand, Pérez et al. studied the interaction of water soluble titanocene complexes with sulfur-containing amino acids. In that study they synthesized new water-soluble complexes with titanocene and amino acids (L-cysteine; D-penicillamine; L-methionine). They characterized these complexes using those spectroscopy techniques NMR, UV and IR, and also elemental analysis and cyclic voltammetry. They found that at low pH, the ligands were lost faster than cyclopentadienyl rings. However, at physiological pH, the formation of an insoluble titanium compound was observed after complex decomposition [17].

Guo et al. (2000) established that titanocene dichloride forms complexes with human transferrin. This protein of 80 kDa is responsible for the iron delivery to proliferating cells and the possible responsible to transport Ti (IV) to target places in the cell. It is suggested that the Cp ligands protect or stabilize by hydrophobicity the metal center, and allow a hydrolytic release of the titanium ion into the iron binding site of the transferrin [18].

Cardona and Meléndez studied the interaction between transferrin, titanocene and titanocene complexes (L-cysteine; D-penicillamine; L-methionine) by different spectroscopic methodologies [19, 20]. This study was aimed to evaluate the influence of the amino acid ligands on titanium ion upon loading into the iron binding sites of transferrin. The NMR results demonstrated that Ti (IV) binds into the iron binding sites, loading the C-lobe first [20]. An analytical methodology using ICP-AES combined with dialysis was developed to determine the amount of titanium loaded into apo-transferrin. It was determined that to titanocene dichloride and $Cp_2Ti(penicillamine)_2Cl_2$ load both C- and N-lobes of transferrin while for $Cp_2Ti(cysteine)_2Cl_2$, cysteine competes with apo-transferrin for Ti(IV) ions [20]. Also transferrin binding studies were performed with $Cp_2TiCl_2$, $Cp_2VCl_2$and $Cp_2MoCl_2$ using UV-Vis spectroscopy [20]. While transferrin is uploaded by Ti(IV) and V(IV) ions, $Cp_2MoCl_2$ does not release molybdenum ions into C- and N-lobes of transferrin [20].

Different to titanocene dichloride, molybdocene dichloride has not been studied in detail and no mechanism of action is known. It is known that molybdocene dichloride is the “only metallocene in which the two cyclopentadienyl rings remain metal bound in water at pH 7.0” [21]. However, Harding and co workers studied the interaction between

molybdocene dichloride and oligonucleotides using NMR spectroscopy and found that molybdocene-DNA complexes were not formed [21]. Therefore, the idea that the mechanism of action of molybdocene involves the formation of DNA-molybdenocene adducts was ruled out.

On the other hand, Vera et al. investigated molybdocene-oligonucleotide binding at physiological pH using NMR and cyclic voltammetry and found opposite results. They have monitored the interaction of the self complementary oligonucleotide CGCATATATGCG with molybdocene dichloride at physiological pH and at pH of 6.5. They found that interactions between molybdocene and DNA can exist but these are weak [22].

Alternatively, Waern et al. studied of the interaction of molybdocene with biological ligands, such as amino acid and proteins, which contain nitrogen, oxygen and sulfur donor ligands. They were focused on establishing the affinity of molybdocene dichloride toward these ligands in water at physiological pH. They found that molybdocene dichloride forms highly water soluble and air stable complexes with cysteine and glutathione through the deprotonated thiol groups. However, no coordination complexes were formed between the amino or carboxylate groups of the cysteine, histidine, alanine or lysine. This implies that sulfur containing amino acids and proteins are attractive targets for molybdocene dichloride. Also this study demonstrated that each metallocene dihalides have different mechanisms of action [27].

### 1.2.3 Metallocenes dihalides and Human Serum Albumin interaction

Human serum albumin (HSA) is a globular protein of 66.5 kDa (**Figure 1-3**). According to Sugio et al. this protein is the most abundant protein in the blood plasma and it is found at a concentration of 5g/100mL in the blood [32]. It is capable of binding a broad range of ligands, including metal ions ($Cu^{+2}$, $Zn^{+2}$), hydrophobic ligands (fatty acids), charged aromatic ligands and many drugs [32, 33]. HSA consist of a single peptide chain of 585 amino acids which forms three homologous domains containing pairs of disulfide bridges and one cysteine free. Each domain is divided in two subdomains [32]. The subdomains IIa and IIIa are hydrophobic cavities, known as site I and site II, that act as binding sites for various ligands [32].

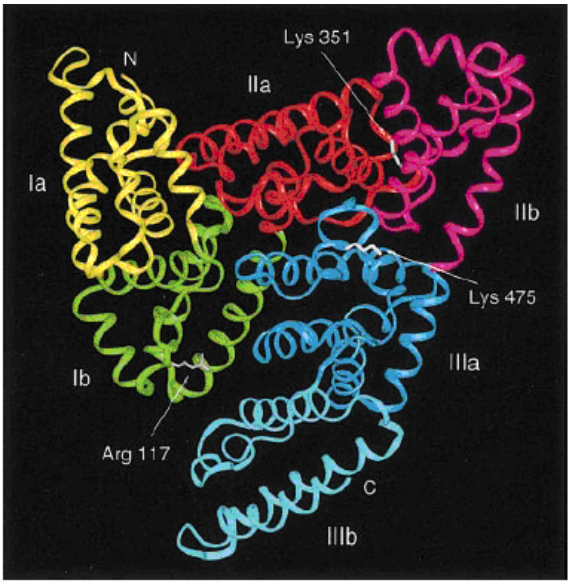



**Figure 1-3:** Structure of Human Serum Albumin.
Taken from Sugio, S. et al., *Protein Eng.* **1999**, *12*, 439-446. [32]

Ravera and coworkers evaluated the interaction of titanocene dichloride with human serum albumin electrochemically. The electrochemical experiments consisted in observing the variation of the Ti (IV)/Ti (III) reduction peak currents before and after the

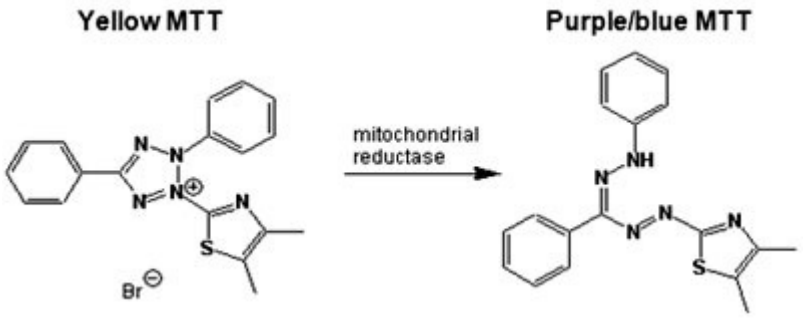
addition of HSA solution. They plotted the normalized peak currents versus the [HSA]/ [Ti] ratio at different pH and perform a parallel experiment but with DNA single and double strand. They found that the HSA has a higher affinity to titanocene dichloride than the DNA. Also, they found that at denaturing conditions (pH of 3) the interaction with albumin is strongest due to the protonation of the $-NH_2$ and carboxylates groups which remain non-coordinated, allowing the total exposure of the binding sites [34].

On the other hand, Tinoco et al. studied the interaction between Ti (IV) and human serum albumin and compared it with Ti (IV)-transferrin interaction. They demonstrated that both routes of transport for Ti (IV) are possible. They found that the Ti (IV) binds, mainly in complex form, at different sites depending on the ligands coordinating Ti (IV). They proposed that the titanocene dichloride binds to HSA by the hydrophobic sites interacting with the Cp rings or by the direct coordination of the oxygen atoms of protein residues to the metal. As a result of this interaction and since HSA has metal binding sites suitable for coordination with softer or intermediate metals, this HSA transport mechanism has been proposed for molybdocene complexes [33].

Harding and coworkers studied the interaction between molybdocene dichloride and HSA, and between molybdocene dichloride and DNA by using radiotracers. They monitored the $^{99}Mo$ by a gamma counter and measured the Mo content associated to the HSA or DNA. They found that molybdocene dichloride has higher affinity to HSA than for DNA, since molybdenum coordinates preferably with deprotonated thiols than the binding sites of the DNA [35].

### 1.2.4 Metallocenes dihalides cytotoxic studies

Since the discovery of cisplatin properties as antitumoral and its action on several solid tumors, researches are directed their efforts toward the evaluation of other metals compounds cytotoxicity potential or activity. One of the assays used for this purpose is the MTT assay. This assay is a colorimetric method in which the cell viability was determined by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan in the cell mitochondria, **Equation 1-1**. The formazan purples crystals produced by the mitochondria are dissolved in Triton and the absorbance of this solution is measured spectroscopically.




**Eq. 1-1**

Plotting the absorbances versus the metal compound concentration will give the minimal concentration of the complex required to kill the half of the tumor cells ($IC_{50}$). This dose-response curve, give the cytotoxic effectiveness of the compound under study.

In a recent publication, Gao et al., used the MTT assay to study the antitumor activity of titanocene dichloride complexes in which the Cps were functionalized with carbomethoxy and carboethoxy groups. They modified the Cp with these polar electrowithdrawing groups in order to make the Ti-Cp bonds more stable at physiological

conditions, and as a result, enhanced the metal capacity to bind the biological target, in the case of titanocene, the transferrin [37]. They wanted to evaluate if these modifications improve the antitumor activity of the of titanacene complex. According to Gao et al., the titanocene dichloride exhibits, as cisplatin does, cytotoxic activity against various tumor cells, but it has less toxic effects than the platinum compound [37]. This fact prompted them to prove the titanocene dichloride and its functionalized complexes effectiveness against HT-29 cancer colon cells line. They found that the compounds with the carbomethoxy groups did not showed cytotoxic activity against this cell line in contrast to those which have the carboethoxy substituents. However, the effectiveness of the last complexes is poor or none in comparison with the titanocene dichloride since its $IC_{50}$ is higher than the titanocene dichloride [37].

Also, in other study our research group of researchers evaluated the capacity and cytotoxic activity of $[Ti_4(maltolato)_8(\mu\text{-}O)_4]$. They found that this stable complex, at physiological pH, do not transfer to the transferring, different to those that have functionalized the Cp rings previously reported [37]. Nevertheless, the titanocene-maltolato complex has shown higher antitumor activity than other titanocene complexes studied on the colon cancer cell line HT-29 [38]. They could not find evidence or direct correlation between the ability of the titanium ion to bind the biological target or transporters and cytotoxic activity, at least in the colon cancer cell line HT-29 [37]. It is of great importance to study if this behavior is repetitive in other metallocenes dihalides derivatives, such as molybdenocene derivatives, and/or in other human tumor cells lines.

This general literature review provided great aid in our effort to interpret the experimental findings for the synthesis, characterization and the behavior of the

molybdenocene and molybdenocene complexes in presence of biologically important molecules such as DNA and HSA. Also, it has brightened on the understanding of the cytotoxicity experiments.

# 2. Molybdenocene Derivatives Synthesis and Characterization

## 2.1 Materials and Methodology

### 2.1.1 Materials for $Cp_2MoCl_2$ derivatives synthesis

Malonic acid, disodium monohydrate salt 98% ($C_3H_2O_4.2Na$), 3-hydroxy-2-methyl-4-pyrone 99% ($C_6H_6O_3$) and bis(cyclopentadienyl)molybdenum dichloride 97% ($C_{10}H_{10}Cl_2Mo$) were obtained from Aldrich and used as purchased. The purity of both the molybdenocene and the ligands was verified initially by $^1H$ NMR spectroscopy. A NaOH 1M solution was used to adjust the pH.

### 2.1.2 Methodology for $Cp_2MoL$ (L= malonate, maltolato) complexes Synthesis

#### 2.1.2.1 Synthesis of bis(cyclopentadienyl)molybdenum(IV) malonate

In a three neck round bottom flask of 50 mL, was charged with 50mg (0.168mmol) of $Cp_2MoCl_2$ and 27.9mg (0.168mmol) of sodium malonate and 10 ml of deionized water, under nitrogen was added (**Figure 2-1**). The resulting pH of the solution was about 6.0 and a dark green color was formed. The reaction mixture was stirred overnight and the final pH was between 3.5-4.5. At this point, the reaction mixture volume was reduced to about 3 mL and the product precipitated with addition of few drops of acetone. The isolated solid was finally purified by column chromatography using Lipophilic Sephadex (20-100μm, from Aldrich) as stationary phase and methanol

as solvent. A bright green solid was isolated upon removal of methanol, obtaining the product in about 90 % yield. $^{1}$H NMR ($D_2O$, ambient) δ: 5.85 (s, 5H, Cp), 3.27 (s, 2H, $CH_2$). Reflectance IR: 3101(mw), 2908(w), 1605(s), 1366(s), 1180(w), 1065(w), 1018(w), 965(w), 935(w), 833(m), 802(m), 718(m), 648(w), 579(m). ESI-MS (positive mode), *m/z* (relative intensity): $Cp_2Mo(malonate)^+$, 325 (55.4), 327 (41.8), 328 (70.3), 329 (74.1), 330 (51.6), 331 (100), 333 (43.1). Anal. Calc. for $C_{13}H_{12}MoO_4$: C, 47.28; H, 3.66. Found: C, 46.99; H, 3.63.

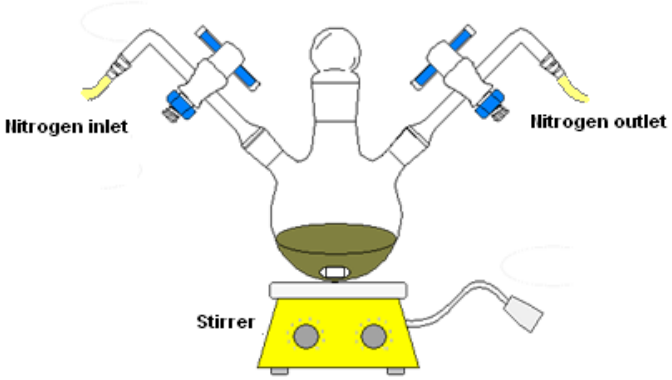



**Figure 2-1:** Schematic representation of the equipment used the preparation of $Cp_2Mo(malonate)$ complex.

### 2.1.2.2 Synthesis of bis(cyclopentadienyl(maltolate)molybdenum(IV) chloride

In a three neck round bottom flask of 50 mL, 100mg (0.337mmol) of $Cp_2MoCl_2$ and 42.5mg (0.337mmol) of 3-hydroxy-2-methyl-4-pyrone (maltol) were loaded and dissolved with 20mL of deionized and degasificated water (**Figure 2-2)**. The initial pH was 5.6 and it was adjusted to 10 with 1.0 M of NaOH. The initial color of the solution

was amber and after 24 hrs the solution turned brown. The solvent was removed in vacuum and the resulting brown complex was purified by column chromatography using Lipophilic Sephadex (20-100μm, from Aldrich) as stationary phase and methanol as solvent. The complex was obtained in 84.4% yield. The complex is highly hygroscopic. $^1$H NMR ($D_2O$, ambient) δ: 8.05 (d, 1H, J = 5 Hz, H-6), 6.72 (d, 1H, J = 5 Hz, H-5), 5.88 (s, 10H, Cp), 2.27 (s, 3H, $CH_3$). Reflectance IR: 3086(mw), 3047(w), 2970(w), 1597(m), 1543(s), 1473(s), 1435(m), 1381(m), 1265(s), 1203(s), 1018(m), 918(w), 841(s), 725(mw), 594(m). ESI-MS (positive mode), *m/z* (relative intensity): $[Cp_2Mo(maltolato)-H]^+$, 349 (8.6), 350 (32.7), 351 (63.5), 352 (76.9), 353 (46.4), 354 (100), 355 (16.3). Anal. Calc. for $C_{16}H_{17}MoO_4Cl$ ($[Cp_2Mo(maltolato)]Cl\cdot H_2O$): C, 47.49; H, 4.23. Found: C, 47.54; H, 4.27.

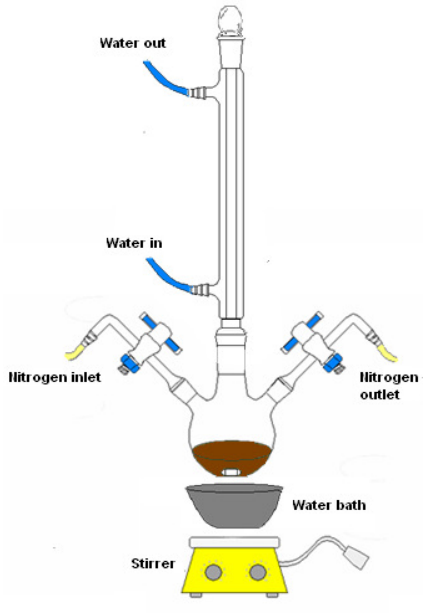



**Figure 2-2:** Schematic representation of the equipment used for the preparation of $[Cp_2Mo(maltolato)]Cl$ complex.

### 2.1.3 Mass Spectroscopy Characterization

The complexes were dissolved in a formic acid 0.2% solution prior to the mass spectra analysis. Solutions of 1ppm, for both complexes were used. A Bruker Daltonics Esquire 6000 instrument was used to record the Mass spectral data. The electrospray positive ion was used as ionization mode during the MS experiment.

The experimental intensities were reported relative to the parent peak on each mass spectrum. In order to determine the theoretical isotopic distribution patter of the $[M]^+$ *m/z* peaks, the Molecular Weight Calculator Software available on http://jjorg.chem.unc.edu/personal/monroe was used.

### 2.1.4 $^1$H NMR Characterization

Once purified, as described in section 2.1.2, the synthesized molybdenum complexes were dissolved in $D_2O$ 99.9% D (Aldrich) inside the NMR tube to obtain a concentration of $1x10^{-2}$ M. The pH of the solutions was ~3. $^1$H spectra were recorded on a 500 MHz (11.74 Tesla magnetic field), Bruker Advance spectrometer, equipped with a Linux computer and with a VT unit controller for temperature control. Chemical shifts are referenced to water peak.

### 2.1.5 IR Characterization

Since the infra red characterizations were performed using a reflectance IR, the complexes do not require sample preparation before the analysis. The IR spectra were obtained using a reflectance IR-FT spectrophotometer Scimitar Serie Digilab FIS 1000 instrument, equipped with a Digilab software resolution 4.

### 2.1.6 UV Characterization

Solutions of 4.0 $x10^{-4}$ M molybdenocene dichloride and molybdenocene complexes were prepared respectively, using Tris buffer as solvent. The Tris buffer used for each study through UV-Vis, was prepared by dissolving (in deionized water) the exact amount of weighted grams of Tris-HCl, NaCl and $NaHCO_3$ needed to obtain the following concentrations: 0.1 M Tris-HCl / 10 mM NaCl / 25 mM $NaHCO_3$. To adjust the buffer solution pH to physiological pH a NaOH 1.0 M was used. The experiment was performed at room temperature.

UV-Vis spectra were recorded with a double beam Lambda Bio 20 Perkin Elmer spectrophotometer, equipped with a temperature controller, and quartz cells were used. The system is interfaced with a 586 Nokia Computer System and the spectral handling was carried out using WinLab Software (PerkinElmer).

### 2.1.7 CV Characterization

A 100mM Tris/ 10mM NaCl buffer solution was used to dissolve the molybdenocene dichloride and molybdenocene complexes, in order to obtain $5.0x10^{-4}$ M solutions and as a supporting electrolyte. The pH was adjusted to 7.4 with a solution of NaOH 1.0 M. Electrochemical characterizations were performed at ambient temperature using a Voltammetric analyzer model BAS CV-50W provided with the software version 2.0, 1995. The three-electrode system was used; the working electrode was glassy carbon electrode (BAS model MF-2014) embedded in a CTFE housing, the measurements were taken against an Ag/AgCl, 3M NaCl reference electrode and Pt wire was used as a counter electrode (BAS CV-50W). The analyte solutions contained in the

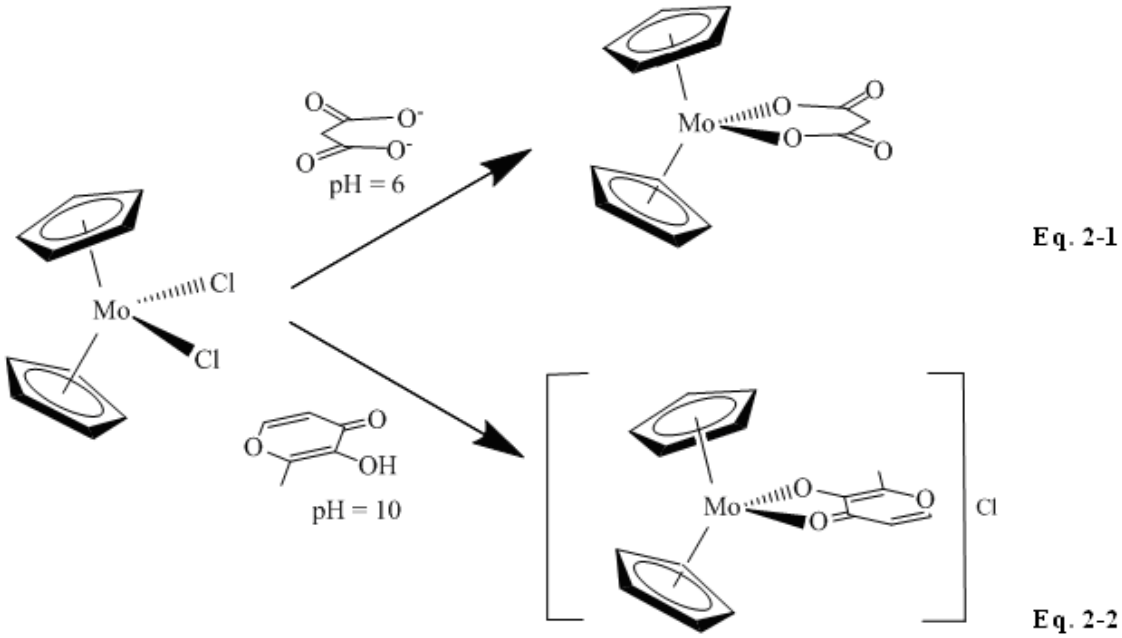
electrochemical cell was purged with nitrogen (UHP 99.99% pure) in order to remove any dissolved oxygen.

## 2.2 Results and Discussions

### 2.2.1 Synthesis of $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl complexes

The synthesis of $Cp_2Mo$(malonate) was performed by reacting $Cp_2MoCl_2$ and sodium malonate in a 1:1 ratio in water, **Equation 2-1**. The pH is kept at 6.0 to assure the malonate to be deprotonated. The solution was stirred overnight under anaerobic conditions and at room temperature. After 24 hours, the solution changed from green to dark green color and had a pH of 3.5 - 4.5. The complex was obtained in 98.9 % yield.

Likewise, the reaction of $Cp_2MoCl_2$ with maltol in a 1:1 ratio, in water at pH of 10 and at 60 °C afforded [$Cp_2Mo$(maltolato)]Cl, **Equation 2-2**. The solution was stirred overnight under a nitrogen atmosphere and was heated at 60 °C. After 24 hours, a color change was noticeable and its pH was about 3. The complex was obtained in 84.4% yield. This complex is highly hygroscopic.




A solubility test was performed with the purpose of selecting a suitable solvent to purify each product. Then the complexes were finally purified by column chromatography Lipophilic Sephadex (20-100μm, from Aldrich) as stationary phase and methanol as solvent. Elemental analyses were performed by Atlantic Microlab in order to corroborate the composition of the compounds.

### 2.2.2 Solubility Properties

Knowing the solubility of the synthesized compounds is a crucial step to perform other experiments and because our objective is to obtain compounds able to dissolve at physiological conditions, which consist of about 65% of water. In order to fulfill these requirements different solvents were used to evaluate the solubility properties of the synthesized molybdenum complexes, at 25°C. Solubility tests results for $Cp_2Mo$(malonate) and $Cp_2Mo$(malonate) complexes are summarized in **Table 2-1**.

| Solvent | Soluble | Partially Soluble | Insoluble |
|---|---|---|---|
| **Water** | **X** | | |
| **Ethanol** | **X** | | |
| **Methanol** | **X** | | |
| **Dimethyl sulfoxide** | **X** | | |
| **Dichloromethane** | | **X** | |
| **Acetone** | | | **X** |
| **Acetonitrile** | | | **X** |
| **Tetrahydrofuran** | | | **X** |
| **Chloroform** | | | **X** |
| **Benzene** | | | **X** |
| **Toluene** | | | **X** |

**Table 2-1:** Solubility test for $Cp_2MoL$ (L= malonate, maltol) complexes

As is shown in the table above, both complexes were soluble in polar solvents, but insoluble in non polar solvents. The complexes dissolve in polar protic solvents, such as water, because these type of solvents contain acidic protons able to potentially interact with the oxygen (C=O) of the solutes via hydrogen bonding. In the case of dimethyl sulfoxide (DMSO), which is an aprotic polar solvent, the solubility is due to its great dipolar moment. Since the starting materials, molybdocene and ligands, of both complexes are water soluble therefore the aqueous solubility of the products was predictable.

### 2.2.3 Elemental analysis.

Pure samples were sent to Atlantic Microlab to determine the composition of the new molybdenocene complexes (**Table 2-2**). The experimental results indicate that the malonate complex contains two Cp rings per malonate ligand. This corroborates the proposed formula for this complex, $Cp_2Mo$(malonate).

Likewise, $Cp_2Mo(maltolato)Cl.H_2O$ was submitted for elemental analysis. The experimental data supports the proposed formula. In addition, the elemental analysis data suggests that the complex is a hydrate containing one molecule of water per molybdenocene. In order to corroborate the identity of the new molybdenocene complexes, we also characterized these species by Mass Spectrometry (MS).

| Compound | % C | | % H | |
|---|---|---|---|---|
| | Theory | Found | Theory | Found |
| $Cp_2Mo(malonate)$ | 47.28 | 46.99 | 3.66 | 3.63 |
| $[Cp_2Mo(maltolato)]Cl.H_2O$ | 47.49 | 47.54 | 4.23 | 4.27 |

**Table 2-2:** Percent of composition of $Cp_2MoL$ (L= malonate, maltol) complexes

### 2.2.4 MS Characterization Results

Mass spectroscopic characterization by electrospray positive ion mode in presence of formic acid was performed on the new complexes. The theoretical *m/z* peaks corresponding to $Cp_2Mo(malonate)$ and $[Cp_2Mo(maltolato)]Cl$ complexes were 331 and 389 respectively. However, the experimental data indicates that the *m/z* peak were 331 for $Cp_2Mo(malonate)$ complex and 353 for $[Cp_2Mo(maltolato)]Cl$. These results suggest that a mono-positively charged species $[M]^+$ is formed for $Cp_2Mo(malonate)$ complex, where M is the molecular ion $[Cp_2Mo(malonate)]^+$. In contrast, in $[Cp_2Mo(maltolato)]Cl$ complex, the parent peak observed correspond to $[Cp_2Mo(maltolato)\text{-}H]^+$ under electrospray ionization conditions. This is expected in this complex since the chloride is not covalently bound to Mo(IV), it is ionic in nature. The theoretical mass spectrometric isotopic distribution for $Cp_2Mo(malonate)$ and $[Cp_2Mo(maltolato)]Cl$ complexes were

calculated using Molecular Weight Calculator Program available on http://jjorg.chem.unc.edu/personal/monroe and compared with the experimetal ones, see **Table 2-3a; 2-3b**. It is evident that the experimental values have the same pattern as the predicted theoretical values, suporting the proposed formula for these new species.

| Mass | Fraction | Intensity | |
|---|---|---|---|
| | | Calculated | Experimental |
| 324.9876 | 0.127553 | 57.46 | 55.42 |
| 325.9876 | 0.018359 | 8.27 | - |
| 326.9876 | 0.081778 | 36.84 | 41.81 |
| 327.9876 | 0.148479 | 66.88 | 70.32 |
| 328.9876 | 0.164495 | 74.1 | 74.08 |
| 329.9876 | 0.105283 | 47.43 | 51.64 |
| 330.9876 | 0.221996 | 100 | 100 |
| 331.9876 | 0.031557 | 14.22 | - |
| 332.9876 | 0.086616 | 39.02 | 43.05 |
| 333.9876 | 0.012251 | 5.52 | - |
| 334.9876 | 0.001501 | 0.68 | - |
| 335.9876 | 0.000132 | 0.06 | - |
| 336.9876 | 1.04E-05 | 0 | - |

**Table 2-3a:** Isotopic Abundances for $Cp_2Mo(malonate)^+$ ion.

| Mass | Fraction | Intensity | |
|---|---|---|---|
| | | Calculated | Experimental |
| 347.00838 | 0.123747 | 56.66 | - |
| 348.00838 | 0.021835 | 10 | 8.58 |
| 349.00838 | 0.079707 | 36.49 | 32.66 |
| 350.00838 | 0.146591 | 67.12 | 63.54 |
| 351.00838 | 0.164134 | 75.15 | 76.90 |
| 352.00838 | 0.107081 | 49.03 | 46.37 |
| 353.00838 | 0.218413 | 100 | 100 |
| 354.00838 | 0.037434 | 17.14 | 16.28 |
| 355.00838 | 0.084653 | 38.76 | - |
| 356.00838 | 0.014551 | 6.66 | - |
| 357.00838 | 0.001697 | 0.78 | - |
| 358.00838 | 0.00015 | 0.07 | - |
| 359.00838 | 1.05E-05 | 0 | - |

**Table 2-3b:** Isotopic Abundances for $[Cp_2Mo(maltolato)-H]^+$ ion.

### 2.2.5 $^{1}$H NMR Characterization Results

**Figure 2-3** shows the $^{1}$H NMR spectrum ($D_2O$) of the $Cp_2Mo$(malonate) complex. This complex is characterized by only two singlets peaks. The signal at 3.3 ppm was attributed to the $CH_2$ of the malonic ligand, which compared with the free ligand spectrum, moves downfield. This is because the electron density around the $CH_2$ is reduced as a result of the coordination of its oxygen atoms (C-O) with the metal center. The peak at 5.9 ppm corresponds to the cyclopentadienyl protons of the molybdenocene. As a reference, the $^{1}$H NMR spectrun of $Cp_2MoCl_2$ in $D_2O$ showed two peaks. These signals are attributed to the hydrolysis of $Cp_2MoCl_2$ in $D_2O$ forming $Cp_2Mo(D_2O)_2^{2+}$ (at 6.0ppm) and $Cp_2Mo(OD)(D_2O)^{+}$ (at 5.8ppm) [22]. When the $Cp_2MoCl_2$ is coordinated with the ligand these signals disappear and only one peak is observed, which is indicative of the complex formation and its high purity. Table 2.2 summarized the $^{1}$H NMR data.

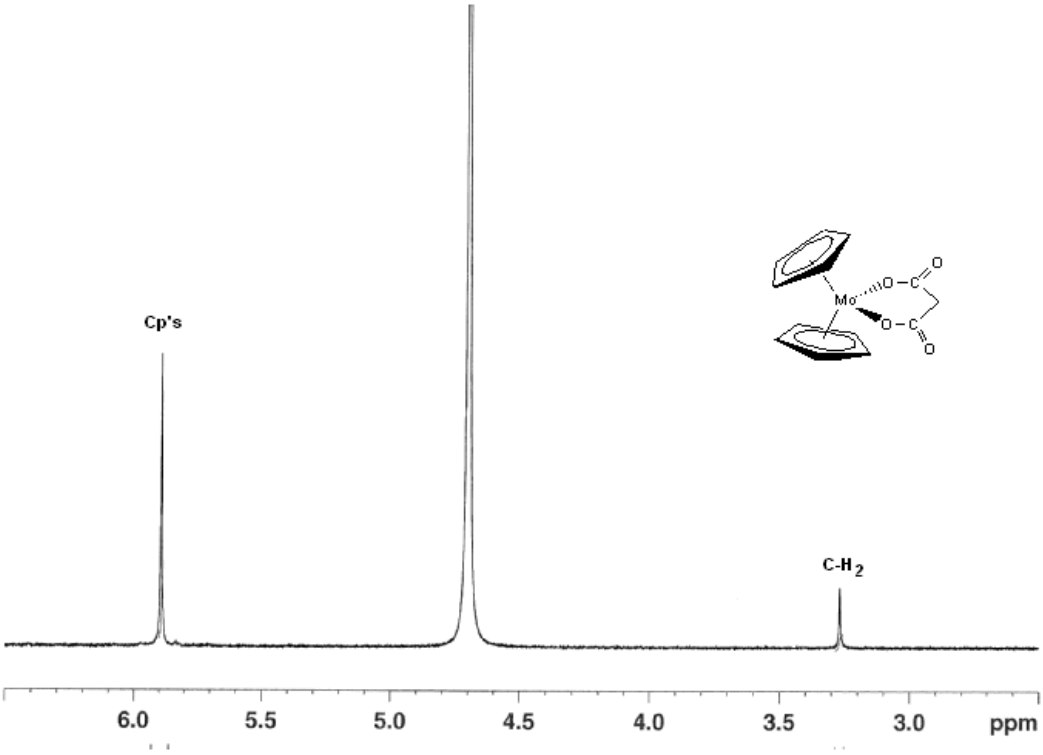



**Figure 2-3:** The 500-MHz $^{1}$H NMR spectrum of $Cp_2Mo$(malonate) spectrum in $D_2O$ at room temperature.

Formation of [$Cp_2Mo$(maltolato)]Cl complex, by the interaction between molybdenocene dichloride and maltol, was also studied by $^1H$ NMR spectroscopy (**Figure 2-4**). The $^1H$ NMR spectra reveals one resonance at 5.9 ppm, which was due to Cp protons. Also, it shows a singlet at 2.3 ppm and two doblets at 6.7 ppm and 8.0 ppm which belongs to the maltol, methyl and diene protons respectively. In comparison with the free ligand spectrum, down field shifts are observed for H(5) and H(6) protons. These are the protons adjacent to thee ketonic and alkoxy oxygens of maltol ligand.

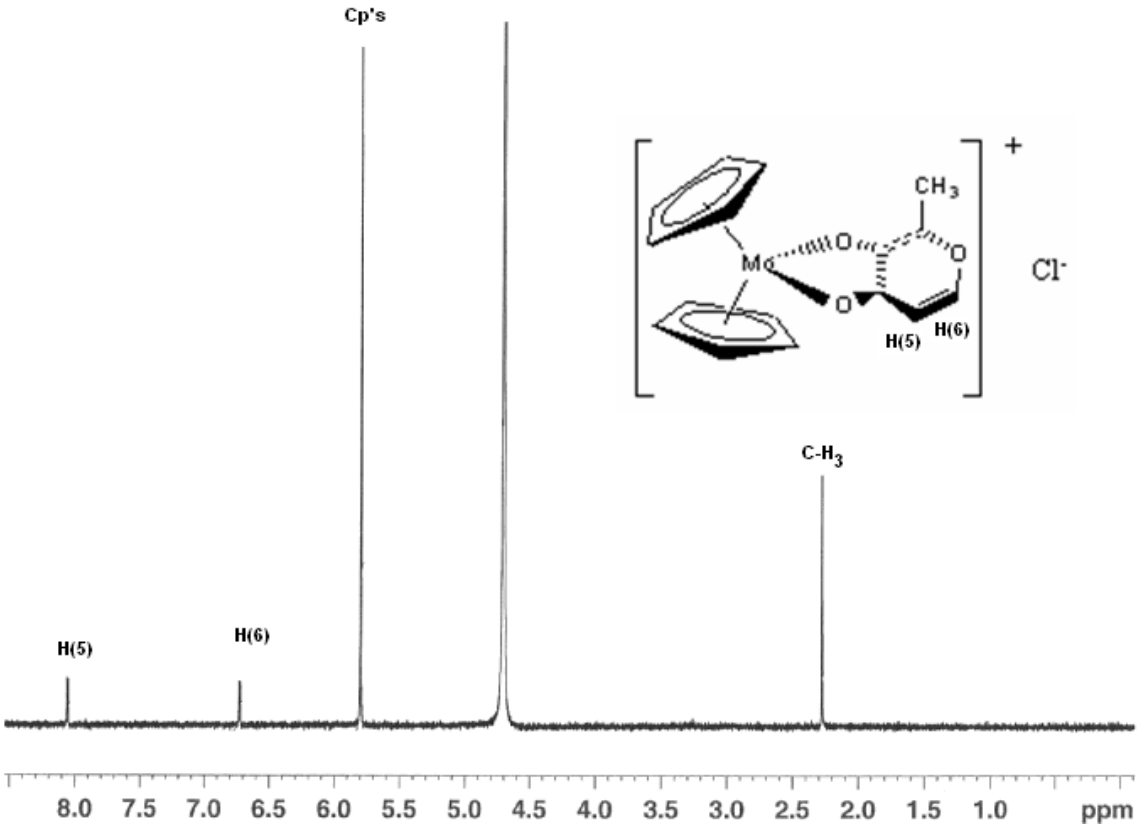



**Figure 2-4:** The 500-MHz $^1H$ NMR spectrum of [$Cp_2Mo$ (maltolato)]Cl in $D_2O$ at room temperature.

**NMR SPECTRA AND CORRELATION CHART**

| Compound | Proton | Shift (ppm) | Integral | Multiplicity |
|---|---|---|---|---|
| $Cp_2MoCl_2$ | Cp(I) | 5.92 | 5 | Singlet |
| | Cp(II) | 6.10 | 5 | Singlet |
| $L_1$= malonate | C**H**$_2$ | 3.01 | - | Singlet |
| $L_2$ = maltol | $C_5$**H**$_2O_2$ | 7.91<br>7.90 | 1 | Doublets |
| ($C_6H_6O_3$) | $C_5$**H**$_2O_2$ | 6.42<br>6.41 | 1 | Doublets |
| | C**H**$_3$ | 2.29 | 3 | Singlet |
| | O**H** | - | 1 | Singlet |
| $Cp_2MoL_1$ | Cp | 5.89 | 5 | Singlet |
| | C**H**$_2$ | 3.27 | 1 | Singlet |
| $[Cp_2MoL_2]Cl$ | Cp | 5.88 | 10 | Singlet |
| | $C_5$**H**$_2O_2$ | 8.05<br>8.04 | 1 | Doublets |
| | $C_5$**H**$_2O_2$ | 6.72<br>6.71 | 1 | Doublets |
| | C**H**$_3$ | 2.27 | 3 | Singlet |

**Table 2-4:** $^1$H NMR signals (ppm) for ligands and their complexes in $D_2O$, at 25ºC.

### 2.2.6 IR Characterization Results

Reflectance infrared analysis was performed for molybdenocene, molybdenocene complexes and free ligands in order to determine their functional groups. The infrared spectrum of free molybdenocene showed a weak band at 1427 $cm^{-1}$ and a medium at 3094 $cm^{-1}$ corresponding to C=C stretching and to C-H stretch of cyclopentadienyl rings. After binding to malonate, $Cp_2Mo$(malonate) shows ν(Cp) stretching band at 3102 $cm^{-1}$. The free malonate showed strong bands at 1574 $cm^{-1}$ and 1350 $cm^{-1}$ which belong to C=O asymmetric and symmetric stretching. These low frequencies were due to the carboxylic salt, in which the CO bonds are delocalized. The $Cp_2Mo$(malonate) spectrum showed that these bands were shifted to 1605 $cm^{-1}$ and 1366 $cm^{-1}$. The increases in frequencies are the result of malonate chelation and as a result more localized double bond character on the carbonyls. For the free ligand and the complex the C-H bending of $CH_2$ was overlapping with the carbonyl symmetric band at 1350-1470$cm^{-1}$. The presence of the functional groups bands, of the molybdenocene and the free ligand, but shifted, as can be seen in **Figure2-5**, suggests the formation of the $Cp_2Mo$(malonate) complex.

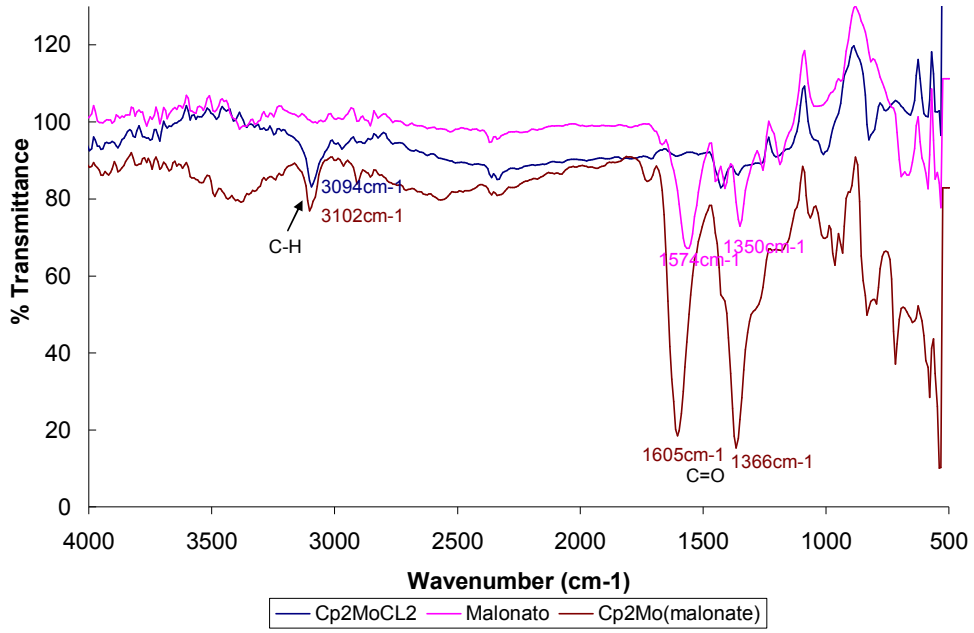



**Figure 2-5:** Infrared spectrum of $Cp_2MoCl_2$; malonic acid and $Cp_2Mo$(malonate) complex.

Likewise IR spectral data for [$Cp_2Mo$(maltolato)]Cl and its free ligand were recorded. The maltol spectrum showed strong bands at 1612 $cm^{-1}$ and 1254 $cm^{-1}$, 1203 $cm^{-1}$ and a broad band at 3248 $cm^{-1}$ corresponding to C=O, C-O and O-H stretching. Also, the maltol spectrum showed a weak bands at 3071 $cm^{-1}$ which can be attributed to the C-H stretching of olefinic group. In addition, it showed two strong bands at 686 $cm^{-1}$ and 849 $cm^{-1}$ resulting for the bending of the C-H group. In the $Cp_2Mo$(malonate) complex spectrum, these bands are present but with different shift due to the coordination of the maltolato group to the molybdenocene. Moreover, the band at 3248 $cm^{-1}$ corresponding to O-H stretching in free maltol spectrum, is not observed in the [$Cp_2Mo$(maltolato)]Cl spectrum suggesting that the interaction between molybdenocene and ligand is via the oxygen of the hydroxy group of the maltol. **Figure 2-6** shows an

infrared spectra obtained for free molybdenocene, free maltol and for [$Cp_2$Mo(maltolato)]Cl.

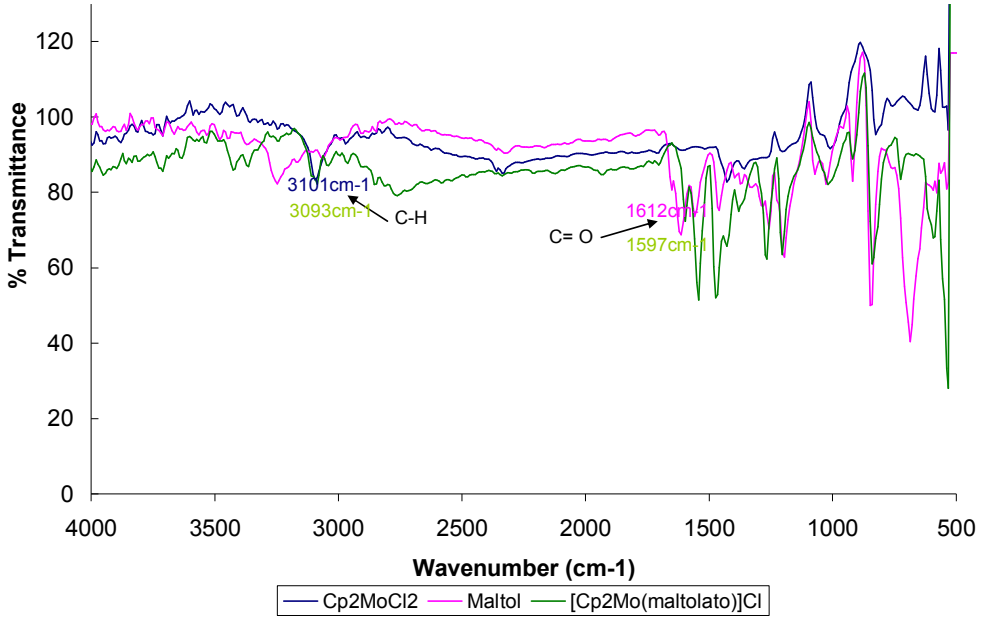



**Figure 2-6:** Infrared spectrum of $Cp_2MoCl_2$; maltolato and [Cp2Mo(maltolato)]Cl complex.

### 2.2.7 UV-Vis Characterization Results

Electronic spectra were recorded for the new synthesized species and to the starting materials (**Figure 2-7**), using 100mM Tris-HCl / 10 mM NaCl / 25 mM $NaHCO_3$as buffer solution, and at room temperature. Under these conditions the molybdenoce exhibits an absorption band at 385nm, which is attributed to d-d transitions (since its $\varepsilon = 96\ M^{-1}cm^{-1}$). When the electronic spectrum was taken for the $Cp_2Mo$(malonate) complex this band is shifted to the red, to a higher wavelength, at 402nm ($\varepsilon = 102\ M^{-1}cm^{-1}$). This shift suggest that the electronic environment around Mo(IV) has changed as a result of malonate coordination. The free malonate does not exhibit absorption bands at any wavelengths of the ultraviolet-visible region. Likewise, for [$Cp_2$Mo(maltolato)]Cl a similar shift is observed in the d-d transition, appearing as a shoulder at 411 nm ($\varepsilon = 195\ M^{-1}cm^{-1}$).

In the case of the free maltol it can be seen that it shows two absorption bands at 215 nm and 272 nm ($\varepsilon = 1919\ M^{-1}cm^{-1}$ and $1600\ M^{-1}cm^{-1}$ respectively). However, when the complex between Mo and maltol was formed, these two bands moved toward to the red. These bands appeared at 262 nm and at 330 nm respectively ($\varepsilon = 4845\ M^{-1}cm^{-1}$ and $3003\ M^{-1}cm^{-1}$ respectively). All complexes exhibit $\pi$-$\pi^*$(Cp) transitions about 225 nm.

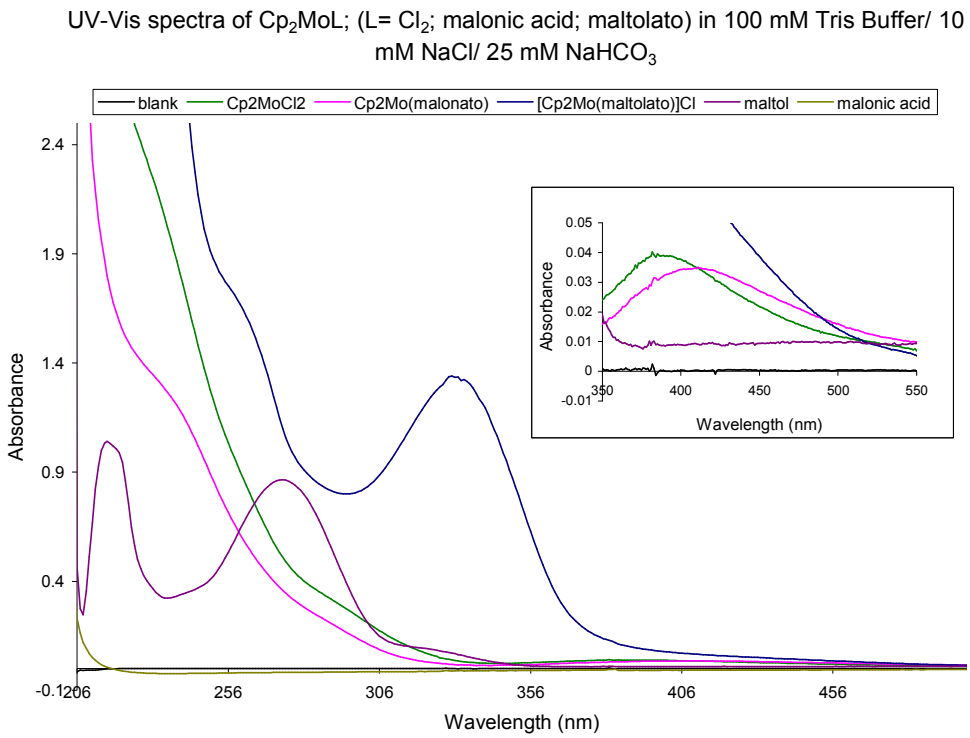



**Figure 2-7:** UV-Vis spectrum of $Cp_2MoCl_2$; $Cp_2Mo$(malonate); [$Cp_2Mo$(maltolato)]Cl; malonic acid and maltol a) UV-Vis scan from 600 to 200 nm; b) Amplified spectra from 600 to 350 nm.

### 2.2.8 CV Characterization Results

An electrochemical characterization was performed, using cyclic voltammetry, in order to observe the electrochemical behavior of the molybdenocene complexes in aqueous system. This is very important because there are many oxidation processes that could result in damages to biological molecules and because not only Mo(IV) but Mo(VI) also possesses anti-cancer properties

As it can be seen in the voltammogram **(Figure 2-8)**, the three molybdenum (IV) species exhibits an irreversible behavior under aqueous conditions. In accordance with Rodríguez et al., [25] the $Cp_2MoCl_2$ shows an irreversible oxidation peak at 700mV, which is attributed to the formation of a Mo (V) electrodeficient and reactive species, or the formation of a stable metal-oxo complex, that prevents the reverse reduction. The same irreversible behaviors were observed for the two Mo-complexes. The $Cp_2Mo$(malonate) complex exhibits an oxidation peak at 790mV and the [$Cp_2Mo$(maltolato)]Cl complex exhibits an oxidation peak at 800mV. Both complexes showed oxidation potentials higher than the parent compound, $Cp_2MoCl_2$, which suggest higher stability in aqueous solution under physiological conditions.

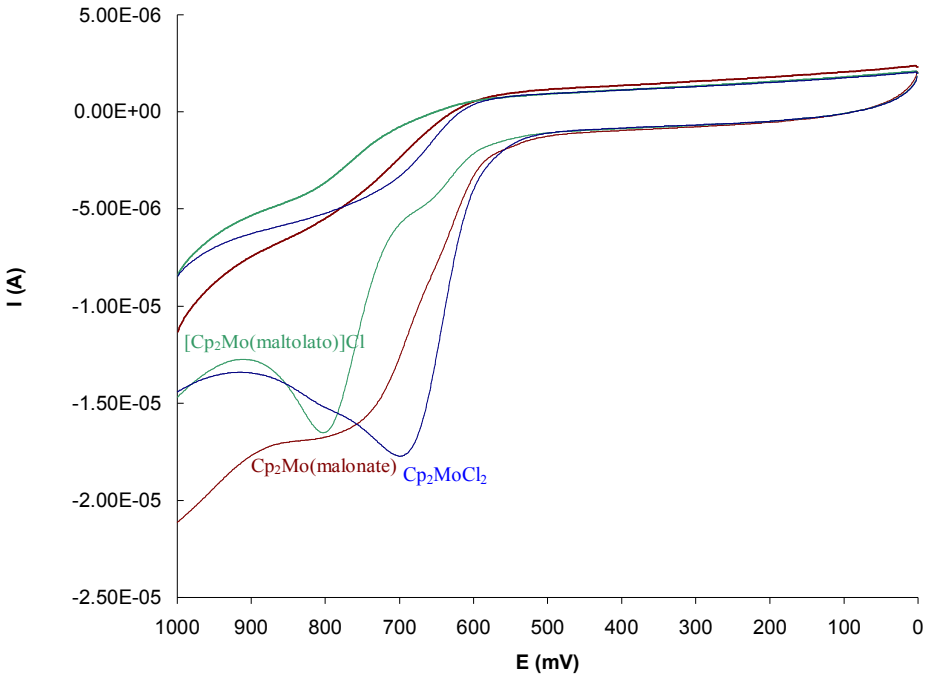



**Figure 2-8:** CVs of $Cp_2MoCl_2$; $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl complexes.

# 3. Biological interactions of molybdenocene dichloride and its derivatives with calf thymus DNA and human serum albumin (HSA)

## 3.1 Material and Methods

### 3.1.1 Cyclic voltammetry electrochemical studies

#### 3.1.1.1 Preparation of ferrous sulphate solution for voltammetric analyzer calibration

A solution of ferrous sulphate 3 mM was prepared in 1M HCl in order to calibrate the voltammetric analyzer. It was used a three electrode arrangement, where glassy carbon was the working electrode, the auxiliary electrode was a platinum wire and the reference electrode was Ag/AgCl in 3 mM NaCl. The Ag/AgCl potential in 3 mM NaCl is at 209 mV with respect to Standar Hydrogen Electrode (SHE) and the literature value for $Fe^{+2}/Fe^{+3}$ in 1 M HCl is 700 mV [25]. The Eº determined was 494 mV with respect to the Ag/AgCl reference electrode therefore, the $FeSO_4$ in 1 M HCl was 703 mV vs SHE. Consequently, the potential measurements uncertainty was ±3.0 mV.

#### 3.1.1.2 Preparation of 100 mM Tris/ 10 mM NaCl buffer solution

A Tris buffer solution was used as supporting electrolyte in each cyclic voltammetry (cv) analysis in order to facilitate the electrochemical process of the electroactive species in solution. A buffer solution of 100 mM Tris / 10 mM NaCl was prepared dissolving 15.7600 g (100 mmoles) of Tris –HCl and 0.5844 g (10 mmoles) of NaCl quantitatively transferred to a 1000 mL volumetric flask, with deionized water. A NaOH 1.0 M solution was used to adjust the Tris buffer solution to physiological pH. These conditions were required in order to resemble physiological environment of cytosol and blood.

### 3.1.1.3 Preparation of calf thymus DNA solution

Deoxyribonucleic acid sodium salt, from calf thymus was obtained from Sigma Aldrich. It was used as purchased. It was dissolved (1 mg) in 10 ml of 100 mM Tris buffer solution and its concentration was determined using an UV-Vis spectrophotometer. Concentration in the order of $10^{-5}$ M was obtained. The Beer-Lambert equation, (**Equation 3-1**) where A is the absorbance at 260 nm, ε is the absorptivity coefficient in $M^{-1}cm^{-1}$(ε =6600) and b is the width in centimeter of the quartz cell, allows to determine the DNA concentration spectrophotometrically.

$$A = \varepsilon b C \qquad \textbf{Eq. 3-1}$$

### 3.1.1.4 Preparation of human serum albumin (HSA) solution

The human serum albumin (99%) was obtained from Sigma-Aldrich (agarose gel electrophoresis, lyophilized powder) and used as purchased. A quantity of 0.0068 g of HSA was transferred quantitatively to a 10 mL volumetric flask and dissolved with the 100 mM Tris/ 10 mM NaCl buffer solution in order to obtain a concentration in the order of $10^{-5}$ M. Its concentration was verified by UV-Vis using the Beer-Lambert equation, (**equation 3-1**). However, for HSA the A is the absorbance at 280 nm, ε is the absorptivity coefficient in $M^{-1}cm^{-1}$(ε =35495) and b is the width in centimeters of the quartz cell.

### 3.1.1.5 Preparation of molybdenocene and molybdenocene complexes solutions

The proper amounts of each compound, molybdenocene (Sigma Aldrich) or molybdenocene complexes were weighted and dissolved with 100 mM Tris/ 10 mM NaCl buffer solution in order to obtain a concentration of 5.00 x $10^{-4}$M for each complex. The molybdocene dichloride solution was sonicated during 20 minutes. The pH of molybdocene and molybdenocene complexes solutions were adjusted to 7.4 with NaOH 1.0 M.

### 3.1.1.6 Preparation of DNA/HSA and complex for interaction studies

In order to perform the biological interactions, 3.0 mL of approximately 5.0 x $10^{-4}$ M of the molybdenocene dichloride or molybdenocene complexes was titrated with 2.5 x $10^{-5}$ M solution of DNA or HSA. Four aliquots of 250 μL of the DNA (or HSA) solution were added to the complex solution. After each addition, a voltammogram was obtained. Before and after titration, the pH was measured in order to ensure that it remained constant at physiological pH during the titration. Since the potential ($E_{pa}$) is more sensitive to the coordination environment than the current, it was used to determined the percentage of interaction between the metal and the DNA and between the metal and the HSA, using **Equation 3-2** [24, 25].

$$Interaction(\%) = \frac{E_{pa}(Cp_2MoCl_2(aq)) - E_{pa}(Cp_2MoCl_2(aq) - DNA\,(or\ HSA))}{E_{pa}(Cp_2MoCl_2(aq))} x100 \quad \textbf{Eq. 3-2}$$

### 3.1.1.7 Solutions and electrode surface pretreatment

Solutions were purged with nitrogen gas (UHP 99.99% pure) for 120 seconds before recording each cyclic voltammogram, in order to remove the dissolved oxygen.

The working electrode, glassy carbon, was polished with 0.05 μm alumina slurry and rinsed with deionized water. This working electrode cleaning process was made before each electrochemical measurement. A sweep between 0 to 1000 mV was performed on the buffer solution in order to detect any metal or biological molecule deposition.

### 3.1.1.8 CV instrument and instrumental parameters

The instrument used was a voltammetric analyzer model BAS 50W (from Bioanalytical Systems, Inc.) with a three-stand electrode cell. The instrumental parameters used to perform the electrochemical experiment were the followings: the potential window had a maximal potential of 1000 mV and a low potential of 0 mV, the sweep was performed in the negative direction. The scan rate was 100 mV/s and the sensitivity was 10 μA/V. Two segments are set in order to obtain a cyclic voltammogram.

Electrochemical measurements were performed on the buffer solutions and DNA/ HSA in buffer solutions, with the same parameter mentioned above, in order to verify their electrochemical inactivity in this sweep window. All measurements were performed in triplicate.

### 3.1.2 Ultraviolet Visible spectroscopy studies

#### 3.1.2.1 Preparation of 100 mM Tris/ 10 mM NaCl buffer solution

A solution of 100 mM Tris / 10 mM NaCl was used as buffer in all the experiment. It was prepared as described in section 3.1.1.2. A NaOH 1.0 M solution was used to adjust the Tris buffer solution to physiological pH.

#### 3.1.2.2 Preparation of Calf Thymus DNA and Human serum albumin solutions

The DNA and HSA were prepared as described in sections 3.1.1.3 and 3.1.1.4 respectively. Concentrations of both, DNA and HSA, in order of $10^{-5}$ M were obtained. Their pH was adjusted to 7.4.

#### 3.1.2.3 Preparation of molybdenocene and molybdenocene complexes solutions

The proper amounts of each complex, molybdenocene (Sigma Aldrich) or molybdenocene complexes were weighted and dissolved with 10 mL of sodium chloride 10 mM solution (0.0745 g of NaCl in 100.0 mL deionized water) in order to obtain a concentration of $1.00 \times 10^{-3}$ M for each complex. Molybdocene dichloride solution was sonicated (during 20 minutes). Molybdocene and molybdenum complexes solutions have a pH of approximately 3.

#### 3.1.2.4 Preparation of DNA/HSA and complex for interaction studies

In order to perform the DNA (or HSA) interaction, 2.0 mL of approximately $2.5 \times 10^{-5}$ M DNA/HSA was titrated with $1.0 \times 10^{-3}$ M solution of the molybdenocene complex. Each aliquot was added at 30 minutes intervals. After each 30 minutes a scan

from 600 nm to 200 nm was performed. Before and after titration, the pH was measured in order to ensure it remained constant at physiological pH during the titration.

## 3.2 Results and Discussions

### 3.2.1 Cyclic Voltammetry Studies

Since cyclic voltammetry is a powerful electrochemical technique, it was used to monitor the interaction between each molybdenocene complex and calf thymus DNA or human serum albumin.

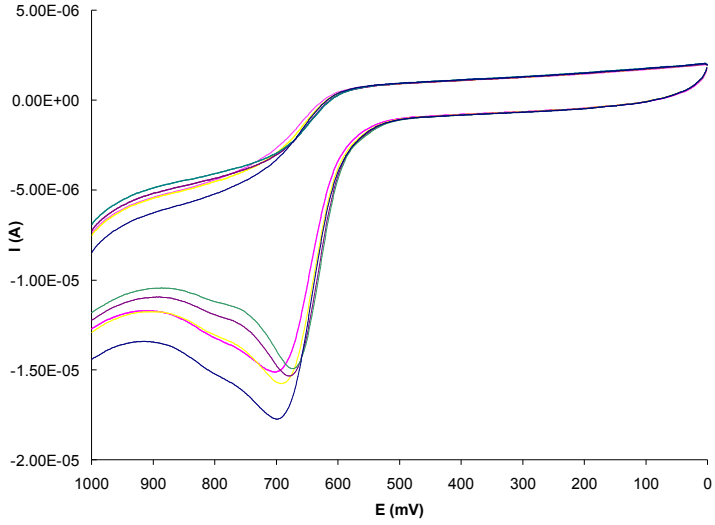
#### 3.2.1.1 DNA/HSA: molybdenocene dichloride interaction

In order to monitor the electrochemical behavior (coordination environment) of $Cp_2MoCl_2$ in presence of a biological molecule, a titrimetric analysis was performed. Four aliquots of 250 μL of 100 mM Tris/ 10 mM NaCl buffer (as a blank), DNA ($2.5x10^{-5}$M) or HSA ($2.5x10^{-5}$M) were added to the metal solution consecutively. A voltammogram was recorded after each addition, and the oxidation current ($I_{pa}$) and the oxidation potential ($E_{pa}$) were obtained. The $E_{pa}$ was used to evaluate the percentage of interaction between the metal and DNA or HSA, because it is more sensitive to the coordination environment than the current [25].

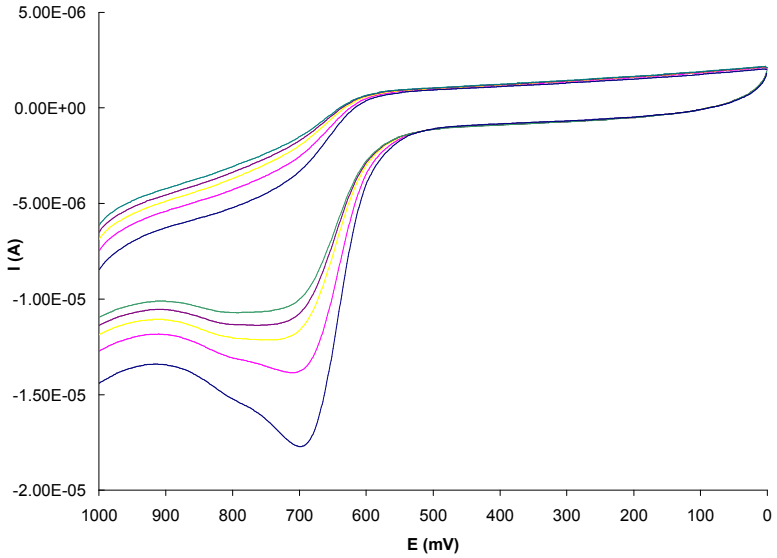
**Figure 3-1a** shows the $Cp_2MoCl_2$ voltammogram after the addition of buffer at pH of 7.4. As it can be seen, the $E_{pa}$ do not have any change after each addition. However, when the aliquots of DNA were added (**Figure 3-1b**), the $E_{pa}$ moves toward more positive values (anodic shift) suggesting the formation of "$Cp_2$Mo-Tris" species [22]. From the $E_{pa}$ data, the interaction percent obtained was 2.4% (±0.9), which was within the value reported by Rodriguez et al. [25]. On other hand, **Figure 3-1c** shows the

$Cp_2MoCl_2$ behavior after additions of HSA. It is notable that after each HSA addition, the $E_{pa}$ moves toward higher potentials and the oxidation peak eventually disappears. This gives a maximum interaction percent of 15% (±2) between Mo(IV)-HSA. Although, the interactions between $Cp_2MoCl_2$ and DNA or HSA were weak, these results are indicative of a strongest interaction between the molybdenocene-HSA than between the molybdenbocene-DNA. These findings suggest that the albumin protein should be a preference target for the $Cp_2MoCl_2$ than the direct linkage with the DNA.

**a.**




**b.**




**c.**

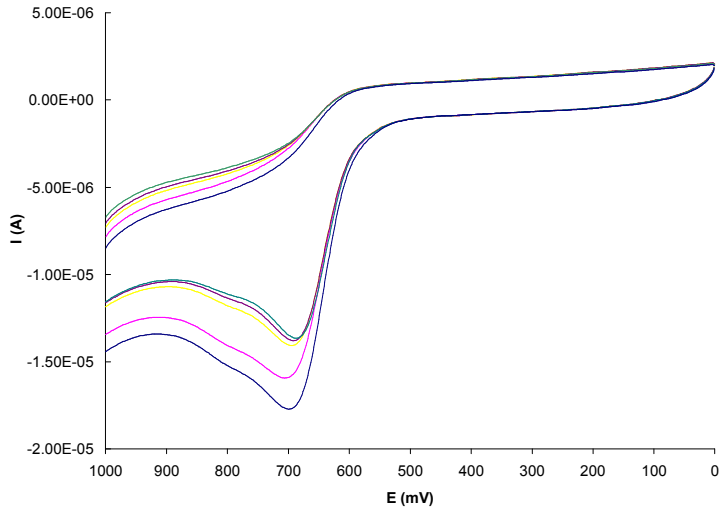



**Figure 3-1:** Cyclic Voltammograms for the tritation of $Cp_2MoCl_2$ with (a) 100 mM Tris/ 10 mM NaCl buffer (b) DNA and (c) HAS

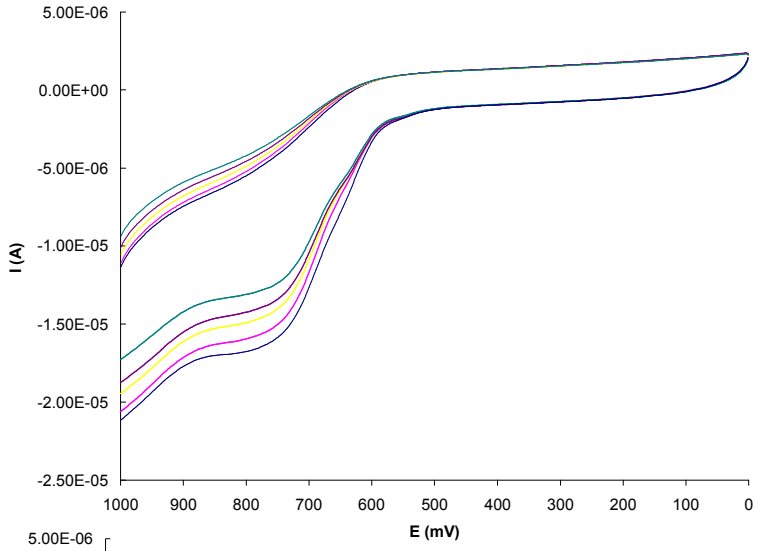
### 3.2.1.2 DNA/HSA: molybdenocene complexes interaction

Likewise, similar experiments were performed on the new molybdenocene complexes. Aliquots of 250μL of DNA or HSA were added to the metal solution until complete 1000μL and its electrochemical behavior was monitored after each addition. For the first complex $Cp_2Mo$(malonate), **Figure 3-2a** and **Figure 3-2b** show voltamograms after addition of buffer and DNA. The maximum percent of interaction was calculated, using the $E_{pa}$; it was of only 1.3% (±0.4), demonstrating negligible interaction between these species. However, when the HSA (**Figure 3-2c**) was added, the maximum interaction was of 3.2% (±0.4). The substitution of the Cl ligands in $Cp_2MoCl_2$ with the bidentate ligand, malonate, decreases the complex interaction with both, DNA and HSA.

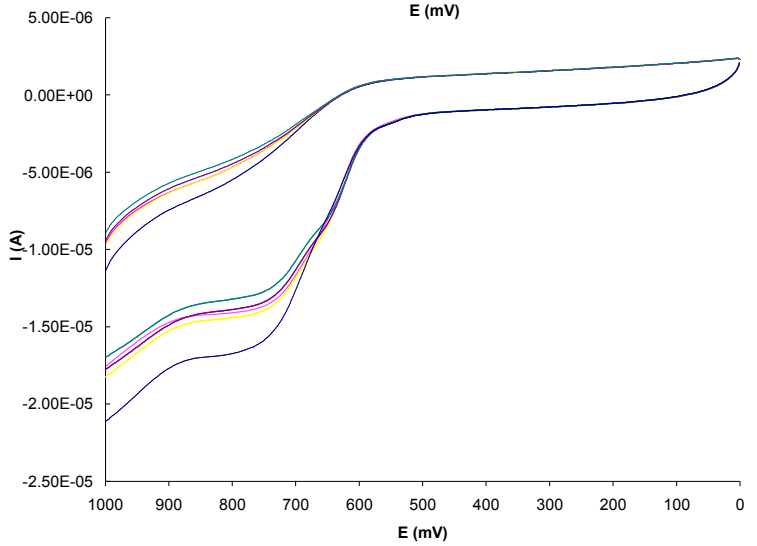
For the second complex, [$Cp_2Mo$(maltolato)]Cl, the maximum percent of interaction obtained for DNA and HSA were of 0.3% (±0.3) and 0.9% (±0.4) respectively. These negligible interactions can be explained based on steric interactions and the lack of available coordination sites on Mo(IV) center. Since the maximum percent of interaction, for both DNA and HSA titration experiments, was less than 1%, they were considered negligible, because values are within the experimental error (**Figures 3-3a, 3-3b, 3-3c**).

Although the percent values obtained from molybdenocene derivatives complexes are smaller than those obtained for $Cp_2MoCl_2$ interaction with DNA or HSA, the same pattern was observed. In both molybdenocene derivatives, the affinity for albumin is higher than for the DNA.

**a.**




**b.**




**c.**

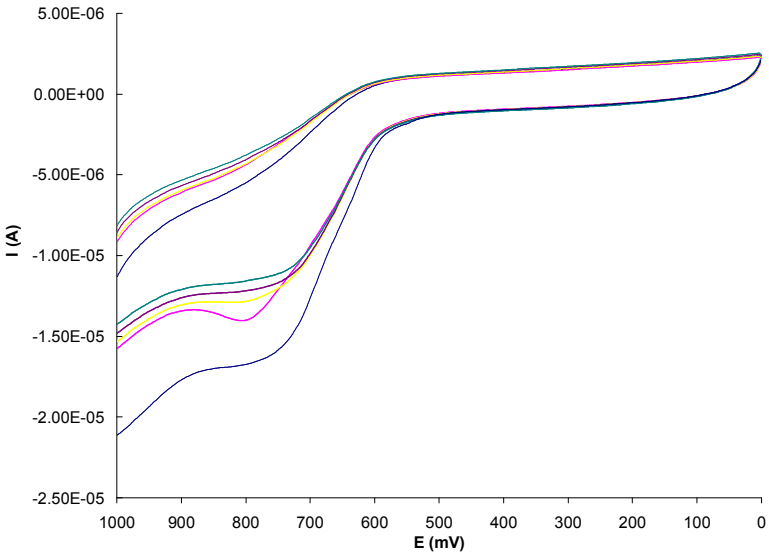
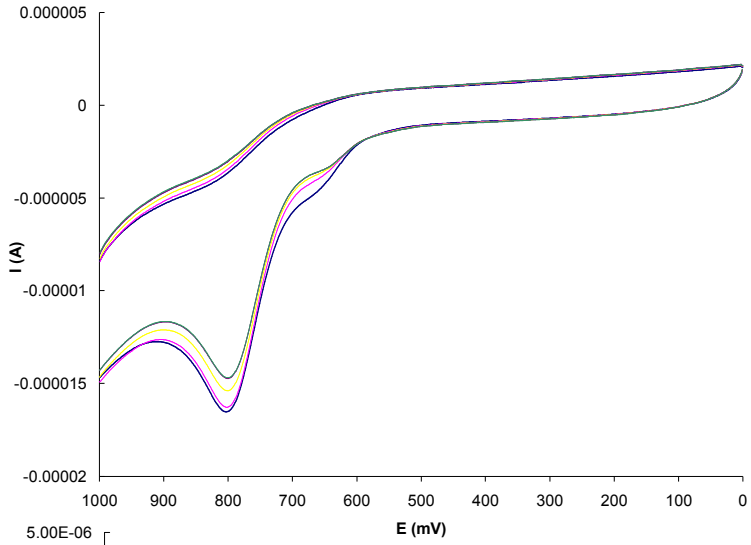
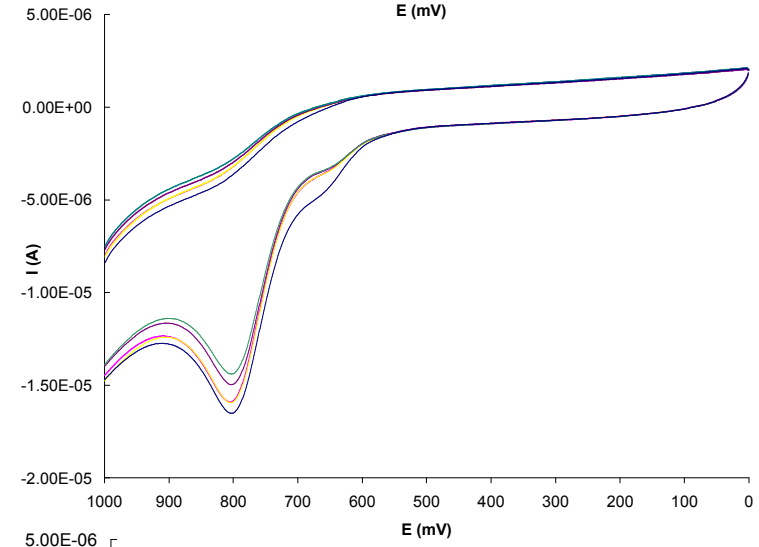



**Figure 3-2:** Cyclic Voltammograms for the tritation of $Cp_2Mo$(malonate) with (a) 100 mM Tris/ 10 mM NaCl buffer (b) DNA and (c) HSA

**a.**




**b.**




**c.**

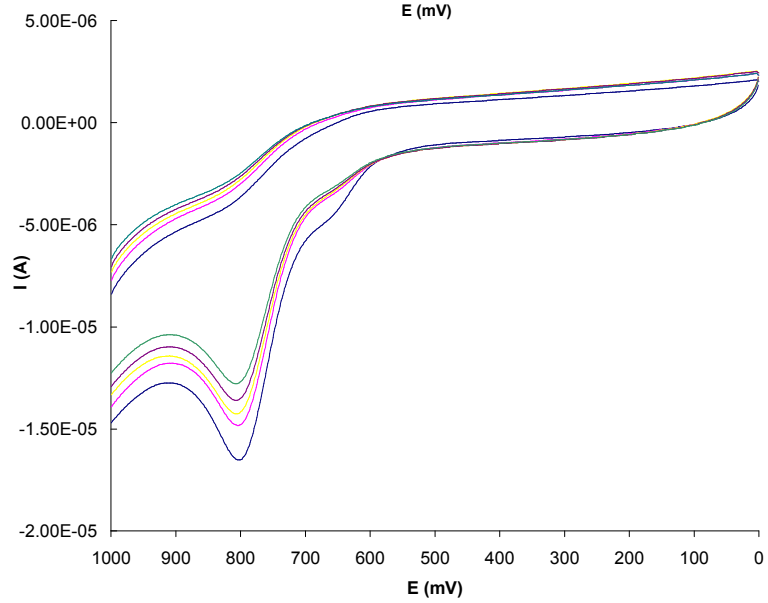



**Figure 3-3:** Cyclic Voltammograms for the tritation of $[Cp_2Mo(maltolato)]Cl$ with (a) 100 mM Tris/ 10 mM NaCl buffer (b) DNA and (c) HSA

Upon examination of these results, we reconsidered these titrations based on the fact that the possible interactions between molybdenocenes and HSA are mainly hydrophobic [33, 34] and since there is no change in coordination environment, the potential is not as sensitive as the current to estimate binding interactions. A change in current could be associated to a change in the diffusion as a result of molybdenocene incorporating in the hydrophobic pocket of HSA. In this regard, using the change current, molybdenocene dichloride showed a maximum of 32% (±7) of interaction with HSA, while $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl showed only 11% (±3) and 15% (±4) respectively. This suggests that molybdenocene dichloride has more affinity to HSA than $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl, following the same pattern observed using the oxidation potentials but we believe that these numbers are more realistic since they show that molybdenocene-HSA interaction could be significant.

**Table 3-1** summarizes the percents of interaction for the DNA-metal complex binding, using the anodic potentials and the HSA-metal complex binding, using the anodic potentials and currents.

| Compound | DNA | HSA | |
|---|---|---|---|
| | % ($\Delta E_{pa}$) | % ($\Delta E_{pa}$) | % ($\Delta I_{pa}$) |
| $Cp_2MoCl_2$ | 2.4 (±.9) | 15 (±2) | 32 (±7) |
| $Cp_2Mo$(malonate) | 1.3 (±.4) | 3.2 (±.4) | 11 (±3) |
| [$Cp_2Mo$(maltolato)]Cl | 0.3 (±.3) | 0.9 (±.4) | 15 (±4) |

**Table 3-1:** Percent of interaction of DNA/HSA -metal complexes bindings

### 3.2.2 UV-Vis Spectroscopy Studies

In order to confirm the results obtained by cyclic voltammetry, UV-Vis spectroscopy studies were performed. This technique was used because most of the proteins show an absorption band at 280nm, due to the presence of aromatic residues of tyrosine (Tyr) and tryptophan (Trp). Also, DNA has an absorption band at 260nm. These characteristics allow us to use of this technique as an effective method to determine protein and DNA concentrations and their Mo(IV)-interactions.

#### 3.2.2.1 DNA/HSA: molybdenocene dichloride interaction study

Titrimetic analysis of calf thymus DNA and molybdenocene dichloride was performed, in which aliquots of the metal solution were added to a DNA solution until obtaining a ratio of 1: 10 (DNA: molybdenocene). A UV-Vis spectrum (**Figure 3-4**) was recorded from 600 to 200 nm to DNA in Tris buffer solution (100mM Tris /10mM NaCl) and after each addition of the metal complex. It is observed that the only band present is the band at 260nm corresponding to the DNA, no other bands were formed. Also, an isosbestic point is not developed during the course of this titration. Although there is an increase in the absorbance of the band at 260 nm which may imply possible interaction of $Cp_2MoCl_2$ with DNA, using this data alone cannot sort out the type of interaction present.

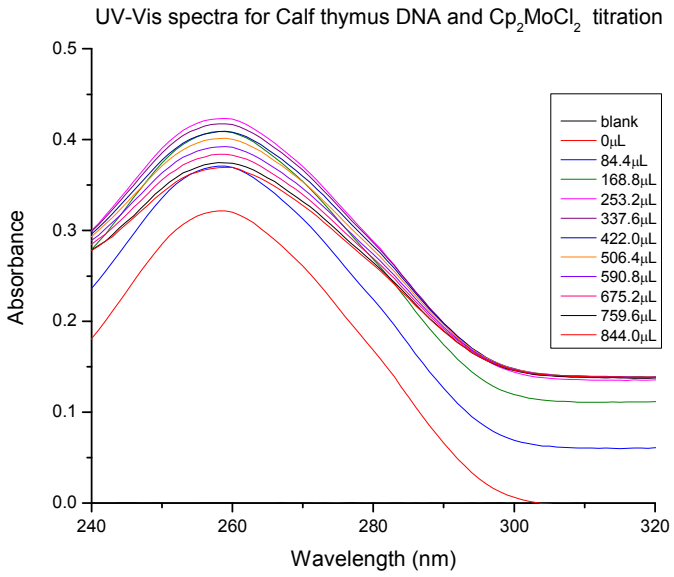



**Figure 3-4:** UV-Vis spectra of Calf thymus DNA and $Cp_2MoCl_2$ titration in 100mMTris/10mM NaCl buffer solution.

The same experiment was conducted with the human serum albumin (HSA) in Tris buffer solution (100mM Tris /10mM NaCl) and molybdenocene dichloride until completing a ratio of 1: 24 (HSA: $Cp_2MoCl_2$). In this experiment, aliquots of the metal solution were added to HSA solution in order to perform a titration. A UV-Vis spectrum was recorded after each addition. As it can be seen in **Figure 3-5**, no bands different to the HSA band at 280 nm are formed. Moreover, no isosbestic peaks are present in the spectra, which mean that none of these species, the free HSA and molybdenocene and complex HAS-$Cp_2MoCl_2$, coexist in equilibrium. Based on these results, no interaction is observed between the HSA and the molybdenocene dichloride, or at least it cannot be detected by UV-Vis spectroscopy.

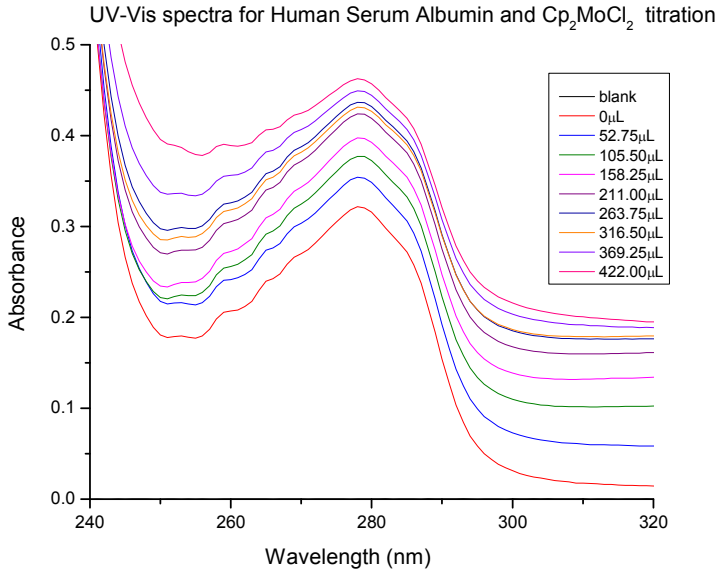



**Figure 3-5:** UV-Vis spectra of Human Serum Albumin $Cp_2MoCl_2$ titration in 100mMTris/10mM NaCl buffer solution.

### 3.2.2.2 DNA/HSA: molybdenocene derivatives interaction studies

Titrimetric analysis of calf thymus DNA and molybdenocene complexes "$Cp_2MoL$", where L is malonaic acid or maltolato, were performed. In both analyses, aliquots of the "$Cp_2MoL$" complex were added to the DNA solution reaching a final ratio of 1:10 (DNA: complex). The UV-Vis spectra were recorded from 600 to 200 nm after each addition.

Like the results obtained for the interaction between DNA and $Cp_2MoCl_2$ in section 4.2.2.1, no isosbestic point are observed but the band at 260 nm increases. **Figure 3-6** shows the UV-Vis spectra of DNA-$Cp_2$Mo(malonate) titration, which may suggest a possible interaction with DNA but we cannot conclude if this exist with the electronic spectra.

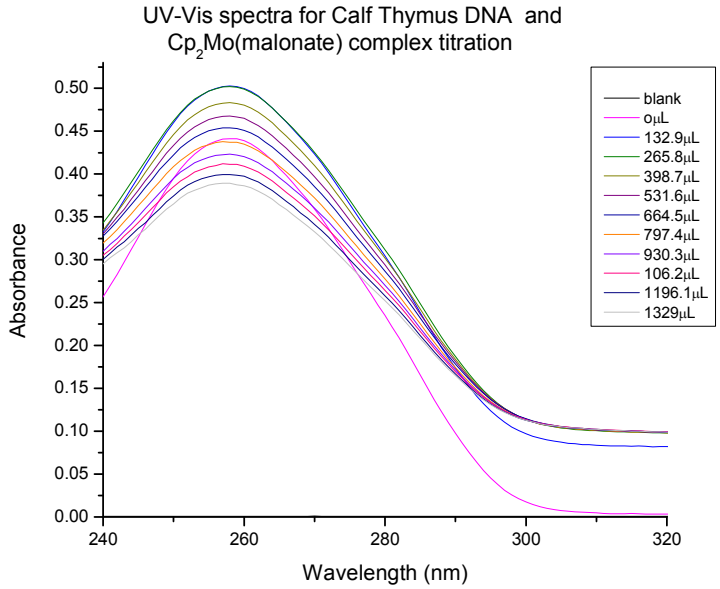



**Figure 3-6:** UV-Vis spectra of Calf thymus DNA and $Cp_2Mo$(malonate) in 100mMTris/10mM NaCl buffer solution.

Also, a titrations between human serum albumin and $Cp_2Mo$(malonate) or [$Cp_2Mo$(malotolato)]Cl, were performed. Aliquots of the desired complex were added to the HSA in order to monitor its behavior. A final ratio of 1:24 (HSA: metal complex) was obtained. As for the molybdenocene dichloride, $Cp_2Mo$(malonate) complex did not show evidence of any interaction with DNA or with HSA (**Figure 3-7**), since no bands and isosbestic points were formed. Neither, [$Cp_2Mo$(maltolato)]Cl complex, shows any interaction with HSA.

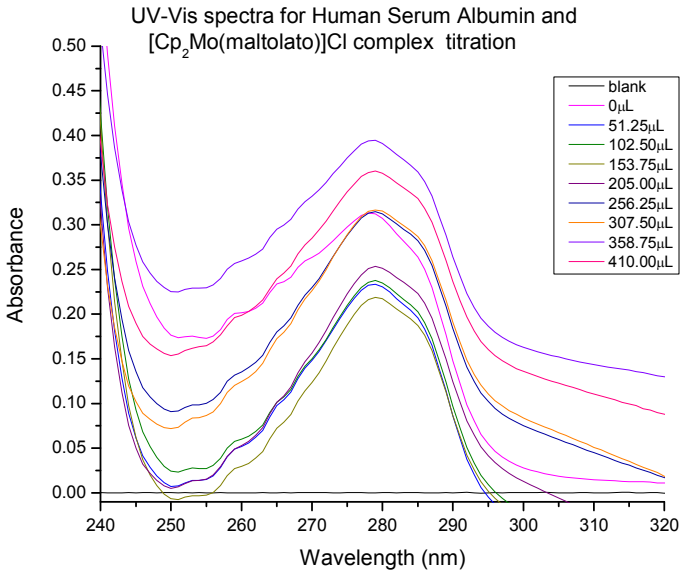



**Figure 3-7:** UV-Vis spectra of HSA-$Cp_2Mo$(malonate) interaction in 100mMTris/10mM NaCl buffer solution.

# 4. Cytotoxic Studies

## 4.1 Materials and Methods

### 4.1.1 Complexes

Bis(cyclopentadienyl)molybdenum dichloride 97% and Bis(cyclopentadienyl)vanadium dichloride 97% were obtained from Aldrich and used as purchased. $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl were synthesized as described section 2.2.1 and 2.2.2. The [$Cp_2V$(maltolato)]Cl complexes was provided from J. Lamboy, current member of the research laboratory of Dr. Enrique Meléndez at University of Puerto Rico at Mayagüez.

### 4.1.2 Cell lines culture

The analyses were performed into to different cell lines, HT-29 and MCF-7, both obtained from American Type Culture Collection (ATCC HTB-38 and ATCC HTB-22). The colon cancer cell line, HT-29, was grown under 95% Air / 5% $CO_2$ (USP grade) atmosphere at 37 °C. The growth medium used was McCoy's 5A (ATCC) complete medium adjusted by supplier to contain 1.5 mM L-glutamine and 2.2g/L sodium bicarbonate. In addition, this medium was supplemented with 10% (v/v) fetal bovine serum (ATCC) and with 1% (v/v) antibiotic-antimycotic (Sigma).

The breast cancer cell line, MCF-7, was grown and maintained, as well as HT-29, at 37°C and 95% Air/5% $CO_2$ (USP grade). This cell line was grown in Dubelcco's Modification of Eagle's Media (DMEM) from Cellgro, which is supplemented by supplier with L-glutamine, 4.5g/L glucose and sodium pyruvate. This complete media

was supplemented with 10% (v/v) fetal bovine serum (ATCC), and with 1% (v/v) antibiotic-antimycotic (Sigma).

### 4.1.3 Cell viability assay

The 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (yellow tetrazolium MTT) was obtained from Sigma and used as purchased, in order to determine the cytotoxicity of molybdenocene and its derivatives and vanadocene.

A 100 μL suspension with an initial population of 10,000 to 15,000 (for HT-29 cell line) cells per well were seeded in a 96 well plates (VWR) and after 24hrs of incubation, a dose of the metal complex was added. Complexes concentrations were between 8-8470 μM (ten data points evenly distributed, one concentration per column of eight wells) dissolved in 5%DMSO (Sigma) and 95% medium. Experiments were performed in quadruplicate plates. The plates were leave at 37°C and 95% Air/5% $CO_2$ for 72 hours. Two to four hours before the completion of the 72 hours of incubation, a solution of MTT (1.0 mg/mL) was added and incubated. When time was completed and the purple formazan insoluble product was observed, the cell media was removed and plates were washed with cold Phosphate Buffer Solution (PBS). The PBS was prepared with sodium chloride, potassium chloride, sodium phosphate and potassium phosphate (all from Sigma-Aldrich) dissolved in double distilled, deionized and autoclaved water. The PBS solution was autoclaved and filtered through cellulose-acetate 0.2μm filters.

For the MCF-7 cell line, an initial population of cells per well greater than that for the HT-29 was required because MCF-7 cells have a doubling time (ATCC) greater than HT-29.

At this point 200 μL per well of a detergent solution, 10% (v/v) Triton X-100 (Sigma) in 2-propanol (Fisher), was added and left at 37 °C in order to dissolve the formazan product.

The absorbencies of the resulted colored solutions were measured at 570nm in the Micro Plate Reader with background subtraction at 630 nm. The instrument used was the 340ATTC Microplate Reader from SLT Lab Instruments equipped with a temperature control unit and interfaced with a computer with winSeLecT software. The $IC_{50}$, a metal complex concentration necessary to inhibit cell proliferation by 50%, was calculated by fitting the data to a four-parameter logistic plot using the SigmaPlot software from SPSS Company.

All MTT protocol was performed in a dark room. Experiments were designed in order to contain blanks well, which contained only cell with the medium; control wells, which were untreated cells but the medium contains 5% DMSO, and test wells, which were cells treated with the metal compound at different concentrations.

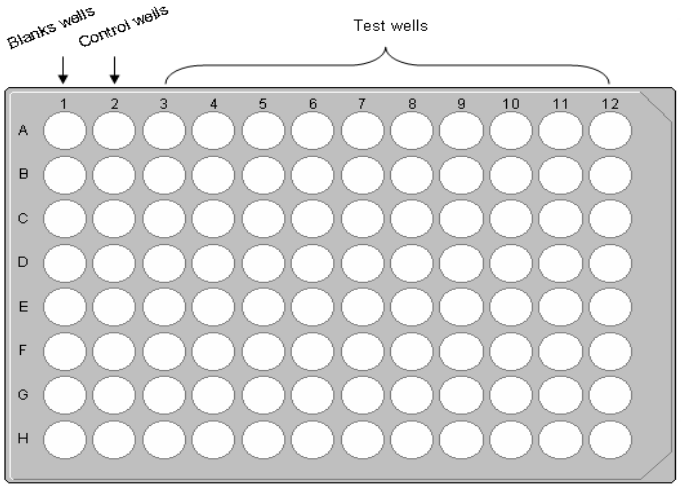



**Figure 4-1:** A 96-well plate seeding protocol.

## 4.2 Results and Discussions

### 4.2.1 $Cp_2MoCl_2$ and $Cp2MoCl_2$ derivatives cytotoxicity results on the colon cancer cell line, HT-29

A MTT assay was performed to the molybdenocene dichloride and molybdenocene derivatives, $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl, in order to determine their biological activity. The colorimetric assay was done on the human colon cancer cell line HT-29 for a period of time of 72 hours. The complexes concentration ranges were from $2.09x10^{-4}$ to $8.47x10^{-3}$ M. **Figure 4-2** shows a dose response curve for the $Cp_2MoCl_2$ and molybdenocene complexes on HT29 cell line.

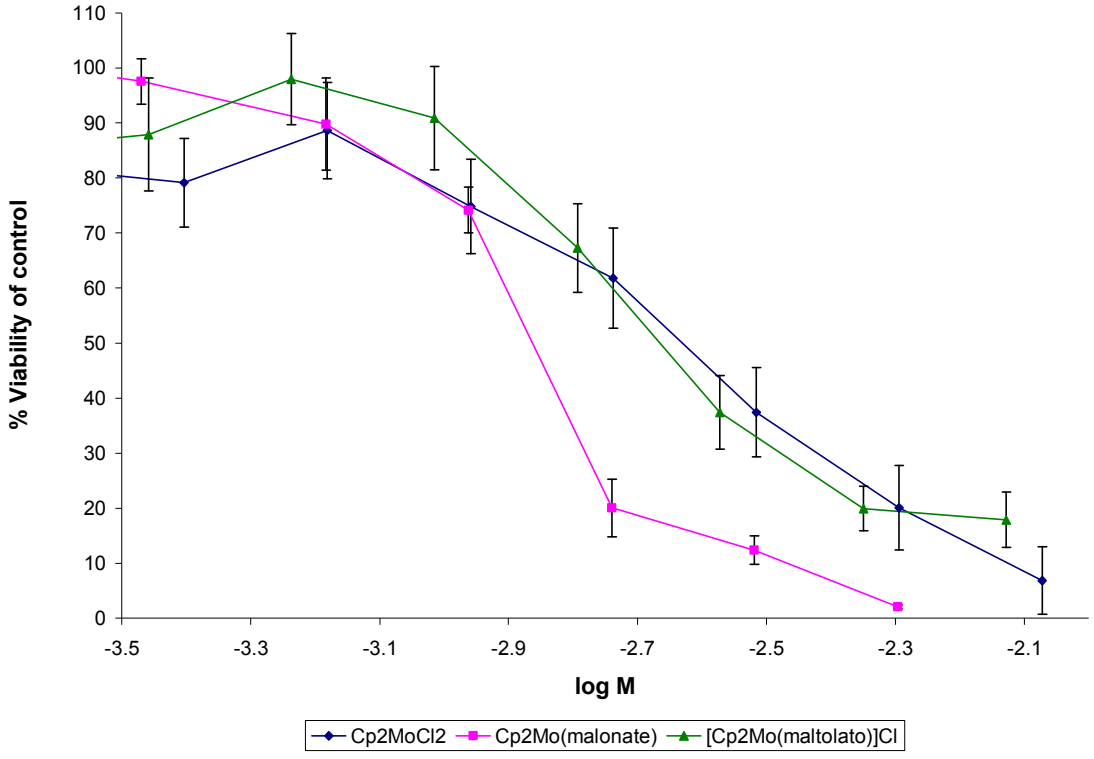



**Figure 4-2:** Dose-response curve f or $Cp_2MoCl_2$, $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl against HT-29 at 72 hours of drug exposure.

The cytotoxic values ($IC_{50}$) against HT-29 obtained for the $Cp_2MoCl_2$, $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl complexes were 2.7(±0.3)mM, 1.31(±0.02)mM and 1.9(±0.3)mM respectively. Although there are in the same order of magnitude, it can be seen that the concentration the $Cp_2MoCl_2$ requires to inhibit 50% of cells proliferation is more than those of molybdenocene derivatives complexes. The addition of these bidentate ligands, malonate and maltol, does not improve greatly the cytotoxic activity of the $Cp_2MoCl_2$. However, if the two complexes are analyzed separately, it can be seen that the $Cp_2Mo$(malonate) complex was more effective than the [$Cp_2Mo$(maltolato)]Cl complex. The main difference between the structures of both complexes is the aromatic ring in the ([$Cp_2Mo$(maltolato)]Cl) complex. This implies that the aromatic ring of the [$Cp_2Mo$(maltolato)]Cl complex causes an impediment that avoids or limits its activity on the cell. These results can be compared with those obtained from the biological interactions, in which the $Cp_2Mo$(malonate) interacts more with the DNA or HSA, than the [$Cp_2Mo$(maltolato)]Cl complex. By contrast, the $Cp_2MoCl_2$ turn out to be less cytotoxic than the molybdenocene derivatives, but it has more interaction with the HSA or DNA.

### 4.2.2 $Cp_2VCl_2$ and [$Cp_2V$(maltolato)]Cl cytotoxic results on the colon cancer cell line, HT-29

Vanadocene and vanadocene-maltolato cytotoxic activities were studied on HT-29 human colon cancer cell line (**Figure4-3**). The ranges of concentration studied were from $7.92x10^{-6}$ to $6.40x10^{-4}$ M in a period of time of 72 hours.

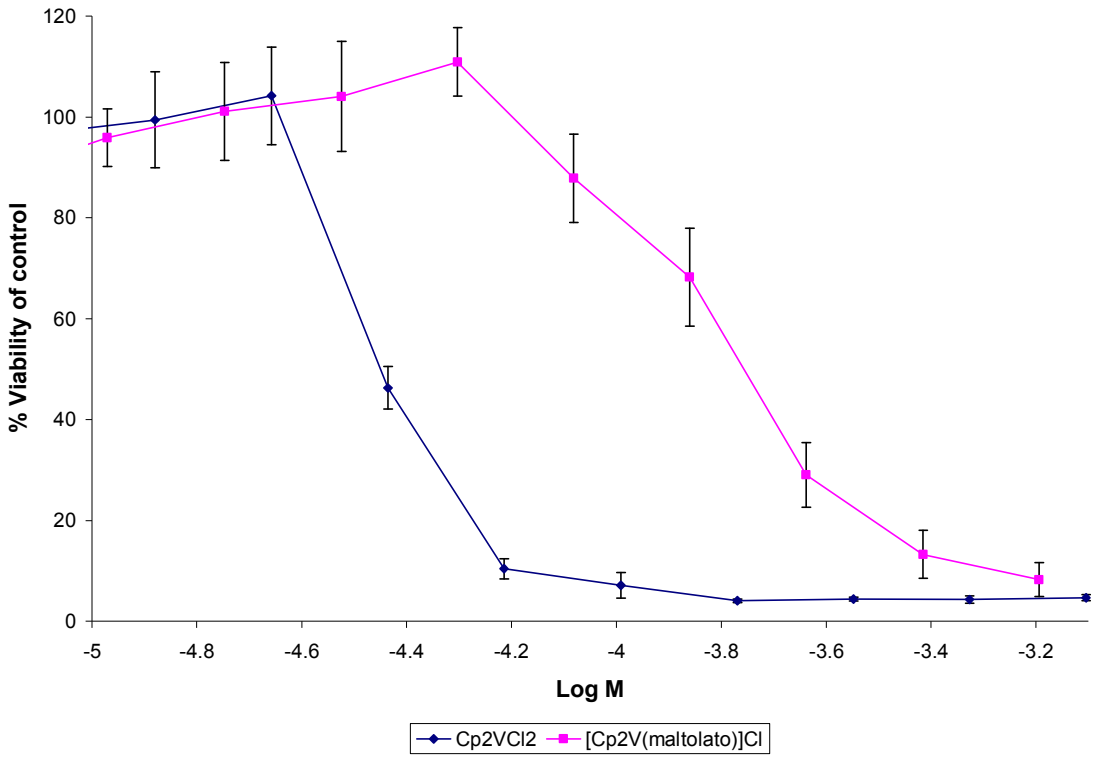



**Figure 4-3:** Dose-response curve f or $Cp_{2V}Cl_2$ and [$Cp_2$V(maltolato)]Cl against HT-29 at 72 hours of drug exposure.

As it can be observed in **Figure 4-3**, the $Cp_2VCl_2$ resulted more cytotoxic active than the [$Cp_2$V(maltol)]Cl complex. The $IC_{50}$ obtained for $Cp_2VCl_2$ was 0.0365(±0.0005)mM and 0.17(±0.02)mM for [$Cp_2$V(maltol)]Cl complex, which suggests that the first is a more powerful drug against colon cancer cells. The substitution of the cloro ligands of $Cp_2VCl_2$ by the bidentate ligand maltol decreases greatly the cytotoxic activity of the original compound.

In comparison with the $Cp_2MoCl_2$, the $Cp_2VCl_2$ is biologically more active, which is expected since the $Cp_2VCl_2$ is the most active metallocenes *in vitro* [10]. However, when the Cl ligands of the $Cp_2MoCl_2$ were substituted by the bidentate ligand, forming a chelate, it slightly improves the cytotoxic activity although the change is not significant. In contrast, in $Cp_2VCl_2$ when the chlorides are replaced by a chelating ligand

such as maltol the result is a reduction in its cytotoxic activity. This behavior can be attributed to the influence of the chelating ligand and the lack of available coordination sites on the vanadium center. The later has been proposed to be necessary to bind biologically important molecules. And express its antitumor activity.

### 4.2.3 $Cp_2MoCl_2$ and $Cp2MoCl_2$ derivatives cytotoxicity results on the breast cancer cell line, MCF-7

In order to evaluate and compare the cytotoxic activity of the $Cp_2MoCl_2$ and $Cp2MoCl_2$ derivatives in a cell line other than HT-29, a MTT assay was performed to each complex on the breast cancer cell line, MCF-7. The complexes concentration ranges were from $7.60x10^{-5}$ to $8.48x10^{-3}$ M, and similar to the colorimetric assay on HT-29 cell line, the experiments were done for a period of time of 72 hours. Contrary to the results obtained for the dose-response of the HT-29 cell line, in the MCF-7 cell line none of the molybdenum complexes exhibit antiproliferative effects (**Figure 4-4**).

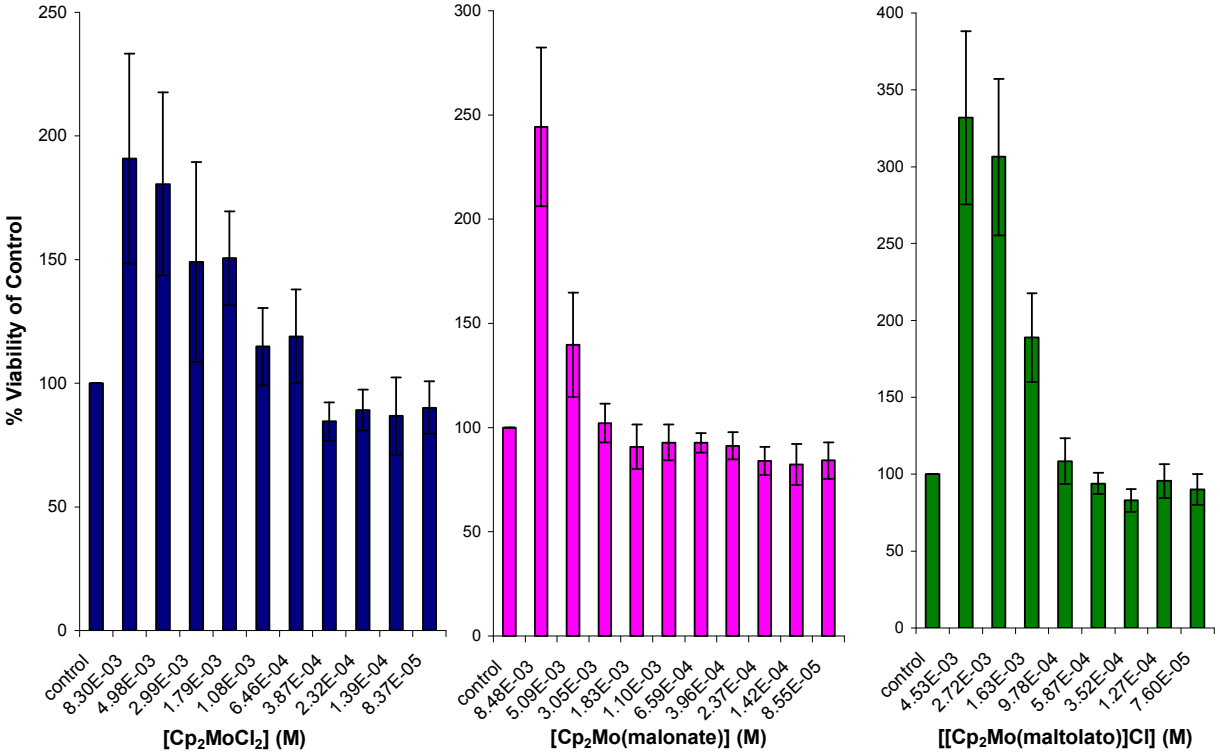



**Figure 4 -4:** Effects of $Cp_2MoCl_2$, $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl on the MCF-7 after 72 hours of drug exposure.

The previous figure (**Figure 4-4**) shows the increment in the percentage of alive cells, compared with the control (cells without drug), according to the increase of the concentration of the molybdenocene complexes. Although the three complexes showed the same behavior, the [$Cp_2$Mo(maltolato)]Cl appears to cause a mayor increase of the MCF-7 cell proliferation than both $Cp_2MoCl_2$ and $Cp_2$Mo(malonate).

Previous works [39, 40] reported a similar behavior of this cell line but with $Cp_2TiCl_2$ and some of its derivatives as titanocene tamoxifen. They found a strong proliferation of the cells in the presence of small concentrations of these drugs. They attributed this behavior to the dependence of the MCF-7 cells to hormones, containing the estrogen receptor ER(+) [39, 40]. According to Jaouen and co-workers, the estrogen receptor alpha is the main estrogen receptor present in the MCF-7. In their work they explain that this receptor contains zinc atoms which mediate the association of the DNA to an estrogen response element. Changing the zinc by other metals such as Ni(II) and Cu(II) can cause an inhibition, meanwhile none effects are observed with Co(I) and Cd(I), in the DNA-estrogen response element association. At low concentrations, the Cd(II) and Ti(IV) mimic the estrogen activity, which results in the proliferation of MCF-7 cells [40]. These reports may provide an explanation to our experimental findings; however more detailed studies need to be performed.

## 5. Conclusion and future works

The coordination properties of molybdenocene with different oxygen-containing chelating ligands have been studied in order to improve their antitumoral properties. Two oxygen-containing chelating ligands were used, malonic acid and maltol, which form the water soluble complexes $Cp_2Mo$(malonate) and [$Cp_2Mo$(maltolato)]Cl respectively. Both synthesized complexes were characterized using spectroscopic techniques such as Mass Spectrometry, $^1H$ NMR, FT-IR and UV-Vis spectroscopies. Also they were characterized by cyclic voltammetry, and elemental analyses were performed at Atlantic Microlab in order to corroborate its composition.

The molybdocene complexes' interactions with calf thymus DNA and human serum albumin were monitored by cyclic voltammetry, because it is a simple and reliable technique [25]. Voltammograms for the titration of DNA to the $Cp_2MoCl_2$ showed a 2.1% of interaction, which is in agreement with the theoretical value corresponding to a weak interaction between them at physiological pH, as reported in the literature [25]. However, when the Cl- ligands were substituted by the oxygen-containing chelating ligands, the interaction between the Mo(IV) and the DNA decreased. It is observed that the degree of interaction between Mo(IV) and DNA is smaller for the [$Cp_2Mo$(maltolato)]Cl than for $Cp_2Mo$(malonate). This behavior can be attributed to the steric hinderance that the ring of the maltolato ligand produces. On the other hand, $Cp_2MoCl_2$ shows a greater interaction with human serum albumin than with DNA, which suggests that the albumin protein should be a preference target for the $Cp_2MoCl_2$ than the direct linkage with the DNA. Nevertheless, Mo(IV) exhibits more affinity to the HSA than for the DNA, and this interaction is also weak in nature. Likewise in the Mo(IV)-DNA interaction for each synthesized complex, it is observed that the $Cp_2Mo$(IV)-HSA

interaction decreases when the oxygen-containing ligands substitutes the $Cl^-$ ligands. Also it is observable that the [$Cp_2$Mo(maltolato)]Cl complex has a weaker interaction than the $Cp_2$Mo(malonato). These negligible interactions can explained based on steric hinderance and the lack of available coordination sites on Mo(IV) center. In order to corroborate these findings, UV-Vis spectroscopy studies were performed. Titrations curves for the $Cp_2MoCl_2$ and the two new complexes were performed at physiological pH, but no conclusive evidence of interactions can be extrapolated from these titrations.

Also in this study, the cytotoxic activity of molybdenocene complexes were evaluate on the tumor cells lines HT-29 (human colon cancer cell line) and MCF-7 (human breast cancer cell line). While the $Cp_2MoCl_2$ engages in interaction with DNA or HSA, although weakly, it exhibits better cytotoxic activity on the HT-29 cell line in comparison with the synthesized complexes. Our findings demonstrated that the substitution of the cloro ligands by oxygen-containing chelating ligands does not improve greatly the cytotoxic activity on the HT-29 cells. When the cytotoxic activity of both complexes, $Cp_2$Mo(malonato) and [$Cp_2$Mo(maltolato)]Cl, were compared it is observed that $Cp_2$Mo(malonato) is slightly more cytotoxic than the second one, in analogous manner as the results of the Mo(IV)-DNA/HSA interactions.

In addition, the cytotoxic activity of the $Cp_2VCl_2$ and [$Cp_2$V(maltolato)]Cl were studied on HT-29 and MCF-7 cell lines in order to determine how the metal center changes the cytotoxic activity of isostructural complexes. The $Cp_2VCl_2$ showed a high level of cytotoxic activity in comparison with the $Cp_2MoCl_2$, which is in agreement with the literature [10]. However, in contrast to [$Cp_2$Mo(maltolato)]Cl, when the cloro ligands of the vanadocene dichloride were substituted by the oxygen-containing chelating ligand, maltolato, its cytotoxic activity was reduced by one order of magnitude. This reduction

in the V(IV) cytotoxic effectiveness can be explained based on the lack of vacant coordination sites as the result of strong chelating capabilities of the maltolato ligand.

Finally, recommendations for future studies on this project are presented. Further works must be done to describe in detail the nature of the interaction between the molybdenocene complexes with the DNA and in particular with the HSA. Other further works recommended is the evaluation of the cytotoxic activity of each compound in others cancer cells lines such as ovaries and prostate cancers, in order to obtain a complete sketch that allows and improves the development of molybdenocene complexes and other metallocenes as an effective alternative in the cancer treatment.

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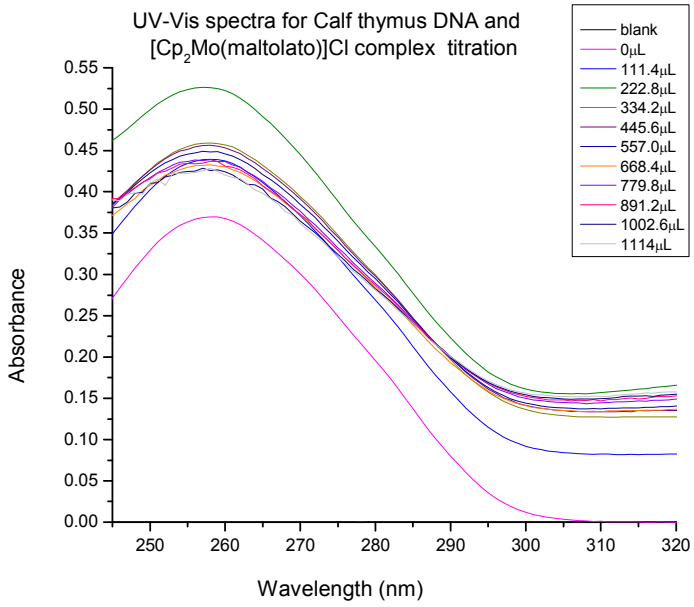
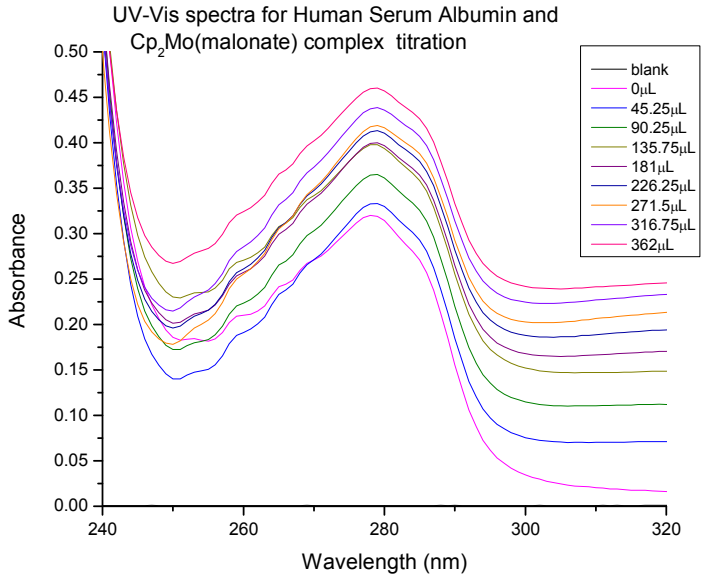
# Appendix A: Additional UV titration Spectra




**Appendix A-1:** UV-Vis spectra of Calf thymus DNA-[$Cp_2$Mo(maltolato)]Cl interaction in 100mMTris/10mM NaCl buffer solution.




**Appendix A-1:** UV-Vis spectra of HSA-[$Cp_2$Mo(maltolato)]Cl interaction in 100mMTris/10mM NaCl buffer solution.