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**CENTRI-VOLTAMMETRIC MOLYBDENUM
DETERMINATION AND ELECTROCHEMICAL
POLYOXOMOLYBDATE FORMATION
AND ITS CHARACTERIZATION**

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ÖZET

SANTRİ-VOLTAMMETRİK MOLİBDEN TAYİNİ VE ELEKTROKİMYASAL POLİOKSOMOLİBDAT OLUŞUMU VE KARAKTERİZASYONU

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Bu tez kapsamında santrifüj ile voltammetri yöntemlerini bir araya getirerek eser elementlerin duyarlı analizini gerçekleştirebilecek bir yöntemin eser metallere özellikle molibden iyonlarının analizine uygulaması incelenmiştir. Bu yöntemin uygulanmasında iki işlevli santrifüje uyumlu yeni bir ölçüm hücresi tasarlanmış ve bu hücrede bir çöktürücü reaktif varlığında Mo(VI) tayini gerçekleştirilerek oldukça duyar sonuçlar elde edilmiştir.

Mo(VI) analizi için oksin, pirogallol kırmızısı ve kupferon gibi reaktiflerle verdiği yapıların camımsı karbon elektrot üzerinde santrifüj yardımıyla biriktirilmesi yoluna gidilmiş, çözelti koşullarının yanı sıra voltammetrik parametreler ve santrifüjleme hızı ve süresi de optimize edilmiştir. Çalışılan reaktifler içinde en iyi sonuçlar pirogallol kırmızısı ile alınmış ve kalibrasyon grafiklerinden belirtme sınırı 1.4×10^{-8} M olarak saptanmıştır. Katalitik etkisi saptanan klorat varlığında belirtme sınırında iyileşme görülmüştür.

Bu tez çalışmasının diğer bir açılımı ise kloroasetik asit ortamında GCE üzerinde molibden iyonu varlığında gözlenen zamana bağlı pik artışlarından yola çıkılarak, potansiyel altında ve santrifüj etkisiyle varlığı belirlenen polioksomolibdat (POM) moleküllerinin incelenmesidir. Elektrot yüzeyinde Mo(VI) iyonlarının Mo(V) türüne çalışılan potansiyel aralığında indirgenmesi hücre bileşimi tarafından da katalizlenmiş ve kendiliğinden biriken dev moleküllerin oluşumuna yol açmıştır.

Büyük yüke sahip bu dev moleküllerin oluşumunu kanıtlamada değişik teknikler kullanılmıştır. Elektrokimyasal empedans spektroskopisi kullanılarak camımsı karbon elektrot yüzeyinde sentezlenen bu bileşiklerinin PZC noktasındaki kaymalar ve elektrokimyasal kuartz kristal mikrobals ile ölçülen altın elektrot yüzeyinde toplanan madde miktarındaki zamanla artışlar bu oluşumları doğrulamaktadır.

Bu nano boyutlu POM'ların yapısal karakterizasyonu Atomik Kuvvet Mikroskop (AFM), Taramalı Elektron Mikroskop (SEM) kullanılarak aydınlatılmıştır. Bu POM'lu elektrotlar yüzeyine Ru, Au ve Fe nanopartikülleri döngüsel voltammetri yardımıyla oluşturulmuş ve yüzey görüntüleri elde edilmiştir.

Anahtar kelimeler: Santri-voltammetri, Mo(VI), oksin, kupferron, PGR, POM, empedans, döngüsel voltammetri.

ABSTRACT
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DETERMINATION AND ELECTROCHEMICAL
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Centri-voltammetry is a novel method which combines the selectivity of co-precipitation technique with the sensitivity of voltammetry. This study describes an application of Centri-voltammetric method to the determination of biologically essential trace elements, molybdenum ion in particular. For this purpose, a dual function voltammetric cell adjustable to centrifuge was designed and utilized in determination of molybdenum ions with various carrier reagents namely, oxine, pyrogallol red and cupferron. Optimization studies include solution properties as well as voltammetric measurement and centrifuge parameters. Among the reagents used pyrogallol red was studied in detail and the reduction peak the complex was exploited for analytical purposes. The limit of detection from calibration curves were calculated as 1.4×10^{-8} M and better sensitivity was obtained in chlorate ions known to have a catalytic effect on the reaction.

Another challenge of the thesis was emerged during the centri-voltammetric studies based on the observations of peak growing by centrifuging the solution containing Mo(VI) ion alone. Cyclic voltammograms clearly indicated the formation of a self assembled structure on the electrode surface by time. This phenomenon differs in some aspects from the previous studies reported in the literature as no reducing agent was added to the cell. Here, a self established structure on the electrode surface via reduction of Mo(VI) to Mo(V) at the GCE surface under potential applied and catalyzed by the cell composition.

Electroanalytical techniques as well as modern surface analysis methods were used in their characterization.

Electrochemical impedance measurements have given a shift in PZC values and electrochemical quartz crystal microbalance measurements have proven an establishment on the gold electrode surface by time.

The surface analysis of these molecules was accomplished via Atomic force microscopy and scanning electron microscopy techniques. A few metallic ions were applied to the electrode surface to produce nanoparticles of Ru, Au and Fe and the potential was cycled to stabilize their presence. The surface characteristics of these modified electrodes have been examined.

Keywords: Centri-voltammetry, Mo(VI), oxine, cupferron, PGR, POM, impedance, cyclic voltammetry

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ABBREVIATIONS

AAA	: Acetic acid/acetate,
AdCtSV	: Adsorptive-catalytic stripping voltammetry
AFM	: Atomic force microscopy
BB	: Boric acid/borate buffer.
BSEs	: Backscattered electrons
CAA	: Chloroacetic acid/acetate,
CV	: Cyclic voltammetry
DL	: Detection limits
DME	: Dropping mercury electrode
DP	: Differential puls
DPASV	: Differential pulse Anodic Stripping Voltammetry
DPSV	: Differential pulse stripping voltammetry,
EDX	: Energy dispersive spectroscopy
EIS	: Electrochemical impedance spectroscopy
EQCM	: Electrochemical quartz crystal microbalance
FMN	: Flavin mononucleotide
GCE	: Glassy carbon electrode
GF-AAS	: Graphite furnace atomic absorption spectrometry
HMDE	: Hanging mercury drop electrode
ICP-MS	: Inductively coupled plasma system with mass detection
ITO	: Indium-doped tin oxide
LLS	: Laser light scattering
LSV	: Linear Sweep Voltammetry,
MTFE	: Preparation of Mercury Thin Film Electrode
PGR	: Pyrogallol red
POM	: Polyoxomolybdate
PSA	: Potentiometric stripping analysis
PZC	: Potential of zero charge
S/N	: Signal-to-noise ratio
SCE	: Standard calomel electrode
SE	: Secondary electrons
SEM	: Scanning electron microscope
SMDE	: Static mercury dropping electrode
STM	: Scanning tunneling microscopy
SWV	: Square wave voltammetry,
TEM	: Transmission electron microscope
TPN	: Total parenteral nutrition

1. INTRODUCTION

The determination of the concentration of a particular element in a sample, irrespective of its chemical form is part of the broad analytical field of elemental analysis. There has been a steady drift away from the classical methods of analysis to instrumental techniques over the past six decades. Furthermore, the ever increasing demand for more rapidly obtained analytical information from clinical, environmental and industrial samples, in which a large number of analytes at low concentrations have strongly influenced the development of modern instrumental analytical chemistry (Laitinen, 1975).

The significance of ultratrace levels of metal ions in environmental, industrial or clinical samples has led to continued efforts for enhancing the sensitivity of analytical methods. The methods to increase the signal (S) for enhancing the signal-to-noise ratio (S/N) with the aim of attaining extremely low detection limits (DL) are being searched. The analyst must often deal with a wide variety of samples, which require the application of the most appropriate technique for separation and preconcentration of the analyte (Wang, 1999).

1.1. Preconcentration Techniques

Preconcentration is of importance in terms of sensitivity and selectivity of the analysis. Several methods have been proposed to separate and preconcentrate trace metal ions according to the nature of the samples, the concentrations of the analytes and the measurement techniques.

Solvent extraction and more recently solid phase extraction techniques are often used for that purpose along with ion exchange, membrane filtration and electroanalytical techniques.

In voltammetric stripping techniques, preconcentration step usually includes an electrochemical deposition process on the electrode surface at a controlled potential under stirred conditions. Various advances during the past 50 years have forced the detectability of voltammetric techniques from the submicromolar level for pulse voltammetric techniques to the subpicomolar level in connection to adsorptive-catalytic stripping voltammetry (AdCtSV) (Wang, 1999).

There are many levels of electrochemical complexity that contribute to the enhancement of the voltammetric signal, including accumulation, adsorption, catalysis (including enzyme-related) or recycling. The recycling or repetition occurs naturally in the catalytic processes as a chain reaction or catalyst regeneration. Coupling several of these gives the overall gain G , an amplification factor in detectability, as

$$G = \kappa \alpha \beta \gamma$$

where α , accumulation factor; β , adsorption effect; γ , catalytic effect; and κ , a factor contributing to change in DL due to improvements in signal measuring (analytical instrumentation) and in signal (chemometric) processing (Wang, 1999).

Among the preconcentration/ separation methods, *co-precipitation* is one of the most effective multi-element preconcentration methods due to its simplicity, rapidity and ability to attain a high enrichment factor.

1.2. Precipitation and Co-precipitation

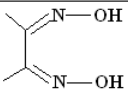
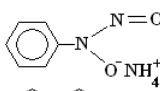
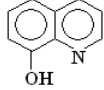
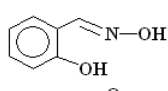
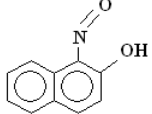
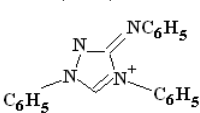
The physical properties of a precipitate are influenced by the mode of its formation as well as by the specific compound involved. Precipitates and their properties depend on the processes of nucleation, crystal growth and aging. Nucleation is the process occurring in a supersaturated solution that results in the formation of the smallest particles that are capable of growth to larger ones. Crystal growth consists of diffusion of material to the surface, followed by deposition. Aging has been defined to include all irreversible structural changes that occur in a precipitate after it has formed (Laitinen, 1975).

Nucleation and crystal growth are convenient to use the term *relative supersaturation*, defined by $\frac{Q-S}{S}$ where Q is the concentration of the mixed reagents before precipitation occurs and S is the solubility of the precipitate at equilibrium.

It is well established that to obtain large crystals and minimize the supersaturation, it is necessary to precipitate from dilute solution, to add dilute precipitating reagents slowly, with effective stirring. If the precipitate solution is hot, this will increase S as well. Finally, it is required to precipitate at as low a pH as is possible to still maintain quantitative precipitation.

Table 1.1 summarizes the common organic precipitating agents widely used in analytical chemistry to precipitate metal ions including heavy metals.

Table 1.1 Common organic precipitating agents (Mizuike, 1983)

Name	Structure	Ions precipitated
Dimethylglyoxime		$\text{Ni}^{2+}, \text{Pd}^{2+}, \text{Pt}^{2+}$
Cupferron		$\text{Fe}^{3+}, \text{VO}_2^+, \text{Ti}^{4+}, \text{Zr}^{4+}, \text{Ce}^{4+}, \text{Ga}^{3+}, \text{Sn}^{4+}$
8-Hydroxyquinoline (oxine)		$\text{Mg}^{2+}, \text{Zn}^{2+}, \text{Cu}^{2+}, \text{Cd}^{2+}, \text{Pb}^{2+}, \text{Al}^{3+}, \text{Fe}^{2+}, \text{Bi}^{3+}, \text{Ga}^{3+}, \text{Th}^{4+}, \text{Zr}^{4+}, \text{UO}_2^{2+}, \text{TiO}^{2+}$
Salicylaldoxime		$\text{Cu}^{2+}, \text{Pb}^{2+}, \text{Bi}^{3+}, \text{Zn}^{2+}, \text{Ni}^{2+}, \text{Pd}^{2+}$
1-Nitroso-2-naphthol		$\text{Co}^{2+}, \text{Fe}^{3+}, \text{Pd}^{2+}, \text{Zr}^{4+}$
Nitron		$\text{NO}_3^-, \text{ClO}_4^-, \text{BF}_4^-, \text{WO}_4^{2-}$
Sodium tetraphenylborate	$\text{Na}^+ \text{B}(\text{C}_6\text{H}_5)_4^-$	$\text{K}^+, \text{Rb}^+, \text{Cs}^+, \text{NH}_4^+, \text{Ag}^+$
Tetraphenylarsonium chloride	$(\text{C}_6\text{H}_5)_4 \text{As}^+ \text{Cl}^-$	$\text{Cr}_2\text{O}_7^{2-}, \text{MnO}_4^-, \text{ReO}_4^{2-}, \text{WO}_4^{2-}, \text{ClO}_4^-, \text{I}_3^-$

A flotation technique has been effectively employed for the preconcentration of trace elements. When an organic precipitant such as dithizone or 2-mercaptobenzothiazole was added to an aqueous sample solution as a dissolved state in a miscible solvent with water, trace analyte were quantitatively co-precipitated on organic precipitates and then floated by bubbling gas without surfactants (Mizuike, 1983).

The organic molecule has an advantage of capability to react selectively with analyte ions and form the precipitate. Kim et al, have reported that 8-hydroxyquinoline was chosen as an organic precipitant for the determination of trace Cd(II) and Pb(II) in water samples (1999).

The reagent, 8-hydroxyquinoline, can stoichiometrically and selectively react with Cd(II) and Pb(II) to form insoluble precipitates depending on the pH of solution as given in Figure 1.1.

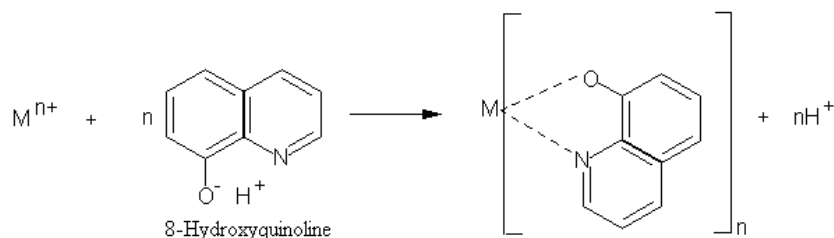


Figure 1.1 The complex formation reaction between 8-hydroxyquinolin and metal ions

Precipitates formed can be contaminated by other ions present in the solution. There are three types of contamination; co-precipitation, post-precipitation and adsorption. Co-precipitation includes only the contamination of a precipitate by normally soluble substances. Post-precipitation involves the formation of a second insoluble substance on a precipitate already formed, as a result of differences in rates of precipitation. Adsorption is the principal source of contamination of precipitates that have large surfaces, for example, flocculated colloids (metal sulfides, silver halides, hydrous oxides) (Mizuike, 1983).

Co-precipitation has been widely applied in preconcentration procedures for determining metal ions in many matrixes. The advantages of this technique are its simplicity and the fact that various analyte ions can be preconcentrated and separated simultaneously from the matrix.

The main requirement for this technique is that the collector or carrier reagent should separate the analyte easily from the matrix solution. This can be done by filtering, centrifuging and washing of the precipitate. Inorganic or organic co-precipitants have been used as efficient collectors of trace elements (Table 1.2). However, this process is slow and samples sometimes have to be kept over-night for a complete co-precipitation (Mizuike, 1983).

Inorganic co-precipitants such as manganese dioxide and aluminum, gallium, cerium (IV), erbium, iron (III), magnesium, samarium and zirconium hydroxides have been widely and successfully used for preconcentrating trace lead ions from different media. A fast procedure for separation and preconcentration using ultrasound-assisted coprecipitation was accomplished with manganese dioxide (Nakayama, 2005).

Inorganic including hydroxides, phosphates and organic co-precipitants like dithiocarbamate, diethyl-dithiophosphate, dimethyl amino benzilidene-rhodanine, etc. have been used. Rubeanic acid (dithiooxamide) is a reagent for preconcentration and determination of various metal ions from different matrixes (Soylak, 2006).

In a recent study, a co-precipitation system by dysprosium hydroxide is proposed for Pb(II), Cu(II), Ni(II), Co(II), Cd(II) and Mn(II) ions (Soylak, 2007). The proposed procedure was applied to the determinations of analytes contents of table salt and natural water samples.

Table 1.2 Inorganic and organic co-precipitants used for metal ions

Co-precipitant	Preconcentrated elements
Fe(OH) ₃	As, Cd, Co, Cr, Cu, Ni, Pt, Se, V, Zn
Fe(OH) ₃ + Ti(OH) ₄	V
Al(OH) ₃	Zr
Zr(OH) ₄	As, Cr, Pb, Sb
Mg(OH) ₂	Fe, Mn
MnO ₂	Mo, Sb
CuS	Cd
SrCO ₃	Cd
Ca(COO) ₂	Sc
2-Mercaptobenzimidazol	Au
1-Nitroso-2-naftol	U
α - benzoinoxime	Mo
Thionalide	Ag, As, Cu, Sb
α - benzyldioxime	Ni
Oxine	Cu, Fe, Mn
5,7- dibromo-8-hydroxyquinoline	Co, Cr, Cu, Fe, Mn, Zn
Oxine + tannic acid + thionalide	Al, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, Ge, Mn, Mo, Ni, Pb, Ti, V, Zn
PAN	Cr, Cu, Eu, Fe, Hg, Mn, Ni, Zn
Polyvinylpyrrolidin+ thionalide	Cd, Cu, Fe, Hg, Pb, Se, Sn, Te, Zn
6-anilino-1,3,5- triazine-2,4- dithiol	Cd, Cu, Pb
DDTC	Cd, Cr, Ca, Fe, Mn, Pb, Sb, Ti, Zn
Diethylammonium diethyldithiocarbamate	Cd, Cr, Cu, Hg, Ni, Pb

Even though co-precipitation techniques provide a simple and efficient way of preconcentration for trace elements, the laborious and time consuming sample preparation procedure limits the broader application of co precipitation methods. Alternatively, a practical and sensitive method for application of co-precipitation in trace analysis was proposed by H. Tural (Kırgöz, 2004). The method combines the co-precipitation technique with voltammetry and therefore, it is called “**Centri-voltammetry**”. Next section describes the merits of the method.

1.3. Centri-voltammetry

Centri-voltammetry is a novel method which combines the advantages of centrifugation and voltammetry. A **centrifuge** is a machine used to separate solids from liquid by using centrifugal forces. Centripetal acceleration occurs with all rotating bodies (Figure 1.2) (Whelan, 1987). Consider a point P rotating about a centre O with constant angular velocity (ω). According to Newton’s second law:

Force = Mass x Acceleration.

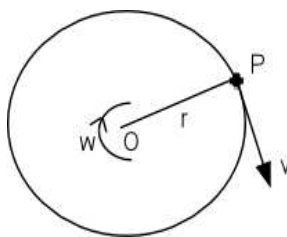


Figure 1.2 Centripetal Forces

The force required to make a body travel in a circular path is called *Centripetal Force* and it always pull towards the centre of rotation. Centripetal force (F_c) = mv^2/r and $F_c = mrw^2$.

On the other hand Newton's third law states that "*Every force has an equal and opposite reaction*". Therefore, the opposite and equal of centripetal is the ***Centrifugal Force***.

In Centri-voltammetry, centrifugation plays a vital role, as it is the core of preconcentration step. The analyte should be deposit as a thin film on the electrode surface from the solution via centrifugal force. Thickness of the film depends on centrifuge time and speed.

Specially designed cell compatible to both voltammetry and centrifuge was proved to facilitate as a flat surface for the deposit (Kırgöz, 2004). The analyte can then be pre-concentrated with an appropriate carrier by means of centrifugation and directly measured on the same electrode. Therefore, it is a practical and sensitive method for application of co-precipitation in trace analysis eliminating those time consuming and laborious decantation, filtration and dissolution steps.

Various carrier materials can be used for this purpose. Early study with Centri-voltammetry includes the enrichment process with a co precipitant $\text{Al}(\text{OH})_3$ (Kırgöz, 2004). A thin layer of alumina was produced by means of centrifugation at the electrode surface to facilitate for adsorption and then preconcentration of lead ion as a test material. Resulting DP peak current at -0.5 V was used in optimization of the experimental parameters and it was shown that lead ions can be determined at nanomolar levels.

Alternatively, the method can be applied with a non-conductive layer maintained as a thin film on the electrode surface by means of centrifugation. Amberlite XAD-7 resin was shown to be used for enrichment of the same test material on the electrode surface with a similar sensitivity (Kırgöz, 2005). The sensitivity of the method was found comparable with those of stripping techniques such as anodic stripping voltammetry (ASV).

The main difference of this technique from the conventional stripping voltammetric techniques lies on the deposition process. In anodic stripping voltammetry (ASV), only a small part of the analyte is pre-concentrated at the electrode surface under controlled conditions to represent the concentration of the bulk solution. However, in Centri-voltammetry, it is possible to accumulate most of the analyte on the electrode surface by maintaining the bulk deposition with an appropriate reagent. This constitutes an advantage for attaining lower detection limits by using higher bulk volumes. In fact, in a recent study carried out in this lab, mercuric ions were chemically reduced by a proper reagent and then collected onto a gold film electrode by means of centrifuge. This method provides a very sensitive method for mercury attaining to picomolar level (Ürkmez, 2004).

Alternatively, 8-hydroxyquinoline can be used as a precipitant since it constitutes complexes with several metal ions. NaBH_4 is a reducing agent providing deposition of heavy metal ions in metallic state on the electrode surface. Therefore, these materials can be used for determination both metal ions and organic compounds.

1.4. The Aim of the Thesis

In this thesis, application Centri-voltammetric method was exploited to determination of to selected metal ions in different matrixes. Molybdenum ion, in particular, was investigated as it is a biologically essential trace element. This element plays an important role in a wide variety of plants, animals and alloys. For this purpose, various carrier reagents namely, 8-hydroxyquinoline, pyrogallol red and cupferron were employed to maintain the selectivity and the sensitivity in molybdenum determination. The centrifugation employed forces the complex to precipitate as a thin film on the electrode surface from bulk solution.

Another challenge of the thesis was emerged during the centri-voltammetric studies based on the observations of peak growing by centrifuging the solution containing Mo(VI) ion alone. The most probable explanation for that was the glassy carbon electrode provides a flat and reductive surface appropriate for a self assembled structure by the aid of centrifuging. Electroanalytical techniques as well as modern surface analysis methods were used in their characterization.

The spherical keplerate POMs including molybdenum have unique values among the POMs discovered so far, owing to their sizes and structural complexity. Keplerate could possibly function as a discrete, nanoscale, multivalent building block for the construction of novel composite materials. As the multidisciplinary approach in science keeps increasing, it is just a matter of time before the widening of the scope of this field becomes a reality. One course should always be to weigh the cost factors against the value of the application.

1.5. Chemical Properties and Determination of Molybdenum

Molybdenum is a transition metal. The principal ore of molybdenum is molybdenite (molybdenum disulfide). Molybdenum is an essential trace mineral in animal and human nutrition. It is found in several tissues of the human body and is required for the activity of some enzymes. Organic forms of molybdenum are found in living matter, from bacteria to animals, including humans (Thomas, 1985).

In spite of its low abundance, molybdenum deficiency in humans is rare but it has been described. A patient on long-term total parenteral nutrition (TPN) developed a syndrome characterized by hypouricemia, hypermethioninemia, low urinary sulfate excretion, tachycardia, tachypnea and mental and visual disturbances.

Molybdenum has important role in enzymatic redox reaction. In biological systems, molybdenum is an essential constituent of enzymes, which catalyze redox reaction, oxidation of aldehydes, xanthine, and other purines and reduction of nitrates and molecular nitrogen (Thomas, 1985). Molybdenum deficiency in animals results in retarded weight gain, decreased food consumption, impaired reproduction and a shortened life expectancy.

High intake of molybdenum is antagonistic to copper and can produce a condition in animals known as molybdenosis. Molybdenum-containing compounds, such as tetrathiomolybdate are currently in clinical trials for the treatment of metastatic cancer and Wilson disease.

Molybdenum is found widespread in the environment and plays an important role in our ecosystem. The predominant form in which molybdenum occurs at neutral pH in water is MoO_4^{2-} . So far the estimation of Mo in a wide variety of samples including soil, sediments and geological matrices is based on thiocyanate methods of molecular absorption spectroscopy (Das, 2006). They include conventional thiocyanate methods in which Mo forms an orange red complex with the thiocyanate ions and improved thiocyanate methods where the sensitivity is increased through the formation of ternary ion association complexes.

The polarographic determination of molybdenum uses the reduction of Mo(VI) to Mo(V) and from Mo(V) to Mo(III) (Sander, 1994). Molybdenum can be determined by graphite furnace atomic absorption spectrometry (GF-AAS) and plasma emission (ICP-AES) or inductively coupled plasma system with mass detection (ICP-MS).

The low solubility of molybdenum in mercury and the unique redox properties limit the application of electrochemical methods for the direct determination of molybdenum in water samples.

Lanza et al. have studied the determination of molybdenum in steel samples by DP polarography (1980). In nitric acid medium Mo(VI) gave a reduction peak at -0.2 V and was determined in ppb ranges. Stadlober et al. have determined Mo(VI) by anodic stripping voltammetry (1997). A carbon paste electrode modified in situ with cetyltrimethyl ammonium bromide was used and the detection limit was reported as 0.04 $\mu\text{g/L}$.

Indirect methods provide more sensitive results. In recent years, several adsorptive cathodic stripping voltammetric (AdSV) procedures have been developed for molybdenum determination via its complexes with several organic molecules as give in Table 1.3.

Ensafi et al have described a highly sensitive adsorptive voltammetric method for simultaneous determination of copper and molybdenum (2004). The method is based on the reduction of complexes of these metal ions with pyrogallol red at a hanging mercury drop electrode (HMDE). The reduction peak was observed at -0.22 V versus Ag/AgCl electrode in supporting electrolyte potassium chlorate. The detection limit of molybdenum was calculated as 0.1 µg/L.

The complex of molybdenum with 8-hydroxquinoline was analyzed by cathodic stripping square-wave voltammetry based on the adsorptive collection onto HMDE. In this technique, the determination limit of molybdenum was found 0.5 µg/L (Sun, 2000).

Table 1.3 Voltammetric Methods For Mo(VI) Determination

Method	Reference	Ligand / catalyst	LOD (ng/mL)
Polarography	Lanza, 1980	-	10
DPSV	Jiao, 1992	Cupferron	0,01
DPSV	Yokoi, 1992	Mandelic acid / chlorate	0,001
Polarography	Jiang, 1994	Methyl orange / hydrazine	0.3
SWV	Net, 1994	Oxine	0.15
DP AdCSV	Adeloju, 1995	Oxine	0.5
LSV	Gao, 1996 (a)	Oxine / chlorate	0,0002
LSV	Gao, 1996 (b)	α -Benzoin oxime / chlorate	2
LSW	Gao, 1996 (c)	α -Benzoin oxime	0.014
SWV	Li, 1997	Dihydroxy naphthalene / bromate	0,006
DP ASV	Standlober, 1997	Cetyltrimethyl ammonium bromide	0.04
SWV	Li, 1997	Dihydroxy naphthalene /bromate	0,006
DP AdCSV	Sander, 1999	Chloranilic acid	0,07
SWV	Quentel, 1999	1,10- Phenanthroline	5,7
SWV	Sun, 2000	Oxine	0.1
SWV	Fraga, 2000	p-cresol derivative / chlorate	0,06
LSW	Prokhorova, 2000	Azo compounds	14
DP AdCSV	Safawi, 2001	Metil thymol blue / chlorate	0.002
DPSV	Ensafi, 2002	Cupferron	0.06
DPSV	De Andrade, 2003	α -Benzoin oxime	0.020
DPSV	Ensafi, 2004	Pyrogallol red / chlorate	0,1
DPSV	Ensafi, 2005	Tiron / chlorate	0,006

DPSV: Differential pulse stripping voltammetry, SWV : Square wave voltammetry, DP AdCSV: Differential pulse Adsorptive Cathodic Stripping Voltammetry, LSV: Linear Sweep Voltammetry, DPASV: Differential pulse Anodic Stripping Voltammetry

Another important aspect of molybdenum is its capability to form *polyoxometalates (POM)* molecules. POMs are the polyoxoanions of the early transition elements, including vanadium, molybdenum and tungsten (Krishnan, 2007). The early explorations relating to molybdenum blue solutions were made by C.W. Scheele and J.J. Berzelius over two centuries ago.

POMs are a valuable class of compounds because they span a wide range of potential applications in material science, medicine, catalysis and pigments owing to their rich electrochemical, photochemical and catalytic properties. One appealing characteristic of POMs is their capacity to accept several electrons, which makes them particularly well-suited to act as electron reservoirs (Zhu, 2003).

Numerous studies of polyoxometalates with coordinated ligands have also been related to the antitumor activity as well as activity against HIV. It has been demonstrated that the formation of 1:1 $[\text{Mo}_7\text{O}_{24}]^{6-}$ flavin mononucleotide (FMN) complex in the mitochondria on the tumor cell inhibited ATP generation. Reaction between cysteine and sodium molybdate resulted in the formation of a novel surface within the cavity of nano-structured ring-shaped cluster, $\text{Na}_3 [\text{Mo}_{154}\text{O}_{462}\text{H}_{14}(\text{H}_2\text{O})_{48} (\text{HO}_2\text{C}-(\text{NH}_3^+)\text{HC}-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\text{CH}(\text{NH}_3^+)-\text{COO}^-)_{11}] \cdot x\text{H}_2\text{O}$ ($x \approx 250$). The salt of polyoxomolybdate {Mo132} is; $(\text{NH}_4)_{42}[\text{Mo}^{\text{VI}}_{72}\text{Mo}^{\text{V}}_{60}\text{O}_{372} (\text{CH}_3\text{COO})_{30}(\text{H}_2\text{O})_{72}] \cdot \text{ca.}300\text{H}_2\text{O}$, as first published in 1998 by Müller and his collaborators. Its anion has a beautiful hollow spherical structure, with an icosahedral symmetry; composed of 12 $\{\text{Mo}^{\text{VI}}_6\}$ fragments and 30 $\{\text{Mo}^{\text{V}}_2\}$ fragments (Zhu, 2003, Zhai, 2008).

These solutions can be prepared by partially reducing Mo(VI) in acidic aqueous solution and can be separated from various molybdenum blue solutions (Müller, 1998). POMs are called as *keplerate* because of their very similar structure to that of C_{60} .

The spherical keplerate POMs including molybdenum have unique values among the POMs discovered so far, owing to their sizes, structural complexity and striking aesthetic beauty. These molecules include a mixture of ions both Mo(V) and Mo(VI) and are based on a very fundamental cluster skeleton $\{(\text{pent})_{12}(\text{link})_{30}\} = \{(\text{Mo})\text{Mo}_5\}_{12}\{(\text{link})_{30}\}$, where pent is the basic pentagonal $\{(\text{Mo})\text{Mo}_5\}$ unit and link refers to linker groups (Figure 1.3). The center of the structure is the locus of one of the C_5 axes of symmetry. The pentagonal building block occurs at the center of the C_5 axis in the keplerate (Zhu, 2003). POMs are demonstrated as $\{\text{Mo}_{132}\}$, $\{\text{Mo}_{154}\}$, $\{\text{Mo}_{368}\}$, etc (Jia, 2006), and as heteromolecule $\{\text{Mo}_{72}\text{Fe}_{30}\}$ (Müller, 2000, Liu, 2002, Cui, 2009) (Figure 1.4). Charge of $\{\text{Mo}_{132}\}$ keplerate is -42.

Keplerate could possibly function as a discrete, nanoscale, multivalent building block for the construction of novel composite materials. The high negative charge that Keplerate $\{\text{Mo}_{132}\}$ carries could be used to establish strong electrostatic interactions between the organic host and inorganic guest, leading to solid self-assembly processes (Zhu, 2003). Moreover, the 60 Mo(V) can be replaced by 30 Fe(III) ions, leading to the formation of giant molecules $(\text{Mo(VI)}_{72}\text{Fe(III)}_{30}\text{O}_{252}\text{L}_{102}\text{ca.180 H}_2\text{O with L} = \text{H}_2\text{O/CH}_3\text{COO}^-/\text{Mo}_2\text{O}_{8/9}^{n-}, \{\text{Mo}_{72}\text{Fe}_{30}\})$ with novel magnetic properties (Figure 1.5).

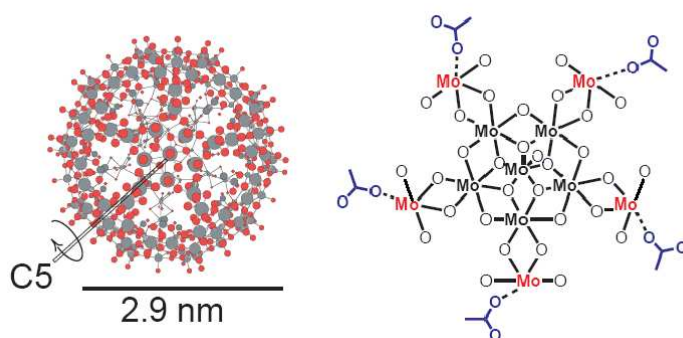


Figure 1.3 Crystal Structure of keplerate $\{\text{Mo}_{132}\}$ giant molecule and the corresponding pentagonal building blocks $\{(\text{Mo})\text{Mo}_5\}$. Mo atoms in black are oxidation state VI and the Mo atoms in red are oxidation state V (Zhu, 2003).

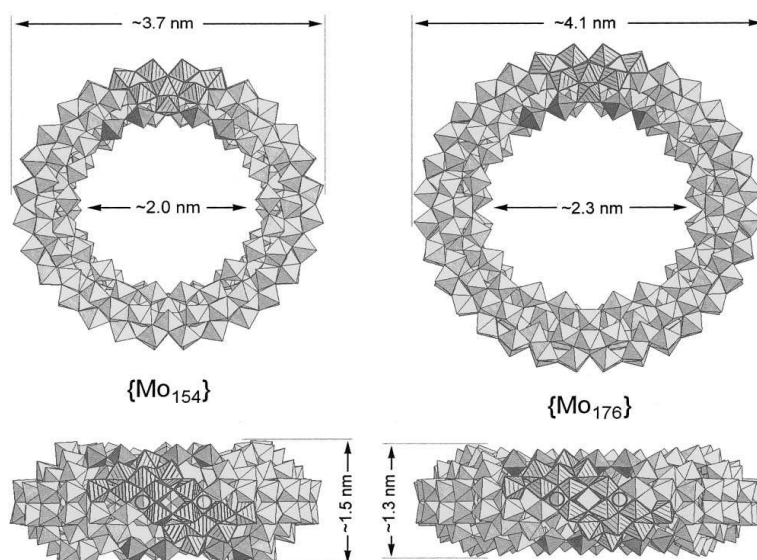


Figure 1.4 Polyhedral representations of the $\{\text{Mo}_{154}\}$ and the $\{\text{Mo}_{176}\}$ clusters showing three different building groups (the individual polyhedra represent the Mo_n coordination geometries). One $\{\text{Mo}_8\}$ group is outlined, two $\{\text{Mo}_2\}$ groups are shown in dark gray, and two equatorial $\{\text{Mo}_1\}$ units are shown encircled (Müller, 2001)

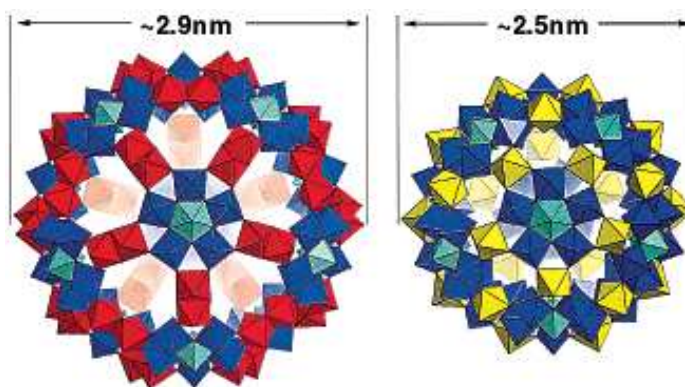


Figure 1.5 Structures of $\{\text{Mo}_{132}\}$ and $\{\text{Mo}_{72}\text{Fe}_{30}\}$ “Keplerate” giant molecules.
(Müller, 2001)

Nanometer scaled POMs are divided different structural classes such as Keggin-type anions $[\text{XM}_{12}\text{O}_{40}]^q$, (where $\text{X}=\text{P}$, Si , Ge etc., and $\text{M}=\text{Mo}$, W , V etc.) and Wells-Dawson type anions $[\text{X}_2\text{M}_{18}\text{O}_{62}]^q$, (where $\text{X}=\text{P}$, Si , Ge etc., and $\text{M}=\text{Mo}$, W , V etc. (Song, 2007). These nano-scale structures were characterized by scanning electron microscope (SEM), atomic force microscopy (AFM) (Wang, 2005), scanning tunneling microscopy (STM), laser light scattering (LLS) and transmission electron microscope (TEM) (Song, 2005).

Self-assembly processes involving Mo(V) lead to a structurally diverse family of well defined wheel-shaped nanoparticles or clusters, and it has been shown recently that such compounds can be connected in several different ways to design extended solids; they still exhibit a nanometer-sized cavity, presenting new perspectives for a novel host–guest chemistry, such as a huge surface area, which explains in part the high affinity toward adsorbents.

Polyoxomolybdate have been used in the field of magnetic, optics, electrochemistry and material sciences. Therefore, POMs are also very attractive in the electrode modifications, and attachment of POMs to electrode surface can be achieved by electrodeposition, adsorption, polymer matrixes, sol-gel techniques, carbon paste electrodes, self assembly (Müller, 2001) and layer by layer deposition (Wang, 2005).

The inherently functional polyoxometalates are complex protic acids and are used as solid acid (Kozhevnikov, 1998) and redox catalysts (Weinstock, 1998). The applications of POMs are centered primarily on their redox properties, photochemical response, ionic charge, conductivity, and ionic weights (Katsoulis, 1998).

The majority of the patent and applied literature is devoted to the applications of the Keggin type heteropolyacids (HPA) and their salts. Primarily $\text{H}_3\text{PMo}_{12}\text{O}_{40}$, $\text{H}_3\text{PW}_{12}\text{O}_{40}$, $\text{H}_4\text{SiMo}_{12}\text{O}_{40}$, and $\text{H}_4\text{SiW}_{12}\text{O}_{40}$ are used as the main examples for many applications.

Recently, electroactive and ambient temperature ionic liquids have been prepared using tetraalkyl phosphonium ions and polyoxometalates (Rickert, 2007). It may be possible to synthesize a wide range of ionic liquids by pairing the anionic polyoxometalate with an appropriate cation.

There are two generic families of POMs, the isopoly compounds, (also called isopolyanions or isopolyoxometalates) that contain only the d^0 metal cations and oxide anions and the heteropoly compounds (also called heteropolyanions) that contain one or more p-, d-, or f-block “heteroatoms” in addition the other ions (Schinazi, 1998).

The heteroatoms in the heteropoly compounds can reside in either buried (not solvent accessible) or surface (solvent accessible) positions in the POM structure. Over half of the elements in the periodic table are known to function as heteroatoms in heteropoly compounds. As heteropoly compounds are more numerous and their structural and electronic properties are easier to modify synthetically than those of the isopoly compounds, the former have dominated the medically oriented research on POMs to date.

POMs function within coatings, membranes, or thin films. These include corrosion resistant coatings, POMs in sol-gel matrixes and in conductive and nonconductive polymer membranes, and POMs as surface modifiers of substrates. POMs are also used as pigments, toners, wood pulp bleaching agents, reagents for chemical/biochemical analysis and for nuclear waste processing, and some miscellaneous applications.

A large number of application patents and publications deal with the building of membrane-based devices and sensors that exploit the high ionic conductivity of POMs. The most common uses of these membranes are in ion selective electrodes, in gas detection apparatus, in solid-state electrochromic devices, and in liquid and solid electrolytic cells.

Of great technological importance of our times is the storage of energy in a compact form that can be easily charged and discharged as lightweight batteries and/or capacitors. Polyoxometalates have been utilized in modifying activating carbon electrodes to improve their storage charge capacity and subsequently fabricate electric double-layer capacitors with optimized energy storage properties.

The polyoxometalates that are capable of reversible redox reactions provide a secondary charge storage mechanism without decreasing the charge storage ability of the activated carbon. Dilute solutions of heteropolyacids were used to activate vitreous carbon or graphite electrodes resulting in potential gains up to 1 V vs the unmodified versions for the reaction of H₂ evolution from water. The electrodes were treated by keeping them at -1.2 V vs SCE for several minutes in heteropolyacid solutions of the Keggin or Dawson type. ESCA analysis of the electrode surface after treatment showed the presence of POM elements (i.e. silicon and tungsten). The intended use of the modified electrodes was for an electrocatalytic cell for hydrogen production (Katsoulis, 1998).

Significant developments on electrode modification by POMs have taken place aiming to improve the reaction rates of fuel cells and produce economically sufficient currents for practical use. Electrocatalysts that are incorporated into the fuel cell electrodes are required to have high activity for the electrochemical reduction of oxygen, as well as being able to withstand acidic conditions. Recent studies have shown that POMs exhibit these attributes (Pang, 2009).

Electrochemistry of POM molecules synthesized was studied in different areas such as modifying POM on glassy carbon, adsorbing POMs on carbon fiber electrode with cathodic pretreatment, casting carbon paste electrode mixing with sol-gel and preparing carbon nanoparticles (Krishnan, 2007).

Practical applications of polyoxometalates in these areas depend on the successful preparation of thin polyoxometalate-containing films. Many reports were published about effective approaches for the immobilization of POM onto an electrode surface as a means to develop a cost-effective, regenerable chemical and biosensors. POM was immobilized on the electrode surface by forming Langmuir–Blodgett films and self-assembled films. However, their analytical applications were very limited, because Langmuir–Blodgett films were easily desorbed inorganic solvent, and self-assembled films were unstable in positively biased potential. Although these approaches have been shown to be promising, new materials and immobilization approaches are still needed to develop POM-based sensors with improved sensitivity, stability and reactivity.

Cyclic voltammetry and electrochemical admittance were used to characterize the self assembly process of nano scaled spheres of POM at a static mercury dropping electrode (SMDE) (Krishnan, 2007). The results have indicated that self assembly process could be detected after 8 hour and completed after one day.

Another attractive characteristic of POMs is their capacity to accept several electrons, which makes them particularly well-suited to act as electron reservoirs. POM acts as a photocatalyst for the reduction of metal ions by organic electron donors. This reaction can be used in recovery of metals or synthesis of colloidal nanoparticles (Troupis, 2006).

This property also makes them very useful in the preparation of modified electrodes to reveal its catalytic activity towards several electrochemical processes including nitrite reduction.

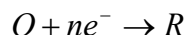
Many reports were published about effective approaches for the immobilization of POM onto the electrode surface to develop cost-effective, regenerable chemical and biosensors. However, the attempts have failed since self assembled films were unstable in positively biased potential. New approaches are required to develop POM based sensors with improved sensitivity and selectivity.

Stable multilayer films were assembled by alternate adsorption of negatively charged POM and positively charged polyelectrolytes and ability of these electrodes to facilitate multielectron transfer is from their aqueous dispersions and the regular growth of the multilayer films (Wang, 2005). The multilayer-film-modified ITO electrode was used for the detection of electrocatalytic activity towards the reduction of nitrite, bromate and hydrogen peroxide.

1.6 Fundamentals of Voltammetric Techniques

Voltammetry represents a wide range of electrochemical techniques those can be used to study the solution composition through current-potential relationships in an electrochemical cell and with the current time response of a microelectrode at a controlled potential.

The existence of polarized electrodes was recognized and utilized in a practical way. All voltammograms are recorded with a three-electrode system consisting of a reference, counter and the working electrode. The aim of the technique is accomplished by monitoring the transfer of electron during the redox process onto surface electrode of analyte:



where, O and R are the oxidized and reduced forms, respectively. Such reaction will occur in a potential region that makes the electron transfer thermodynamically or kinetically favorable. The potential of electrode can be used to establish the concentration of the electroactive species at the surface according to the Nernst equation:

$$E = E^o + \frac{0.059}{n} \log \frac{C_O}{C_R}$$

where, C_O and C_R are the concentrations of the oxidized and reduced forms of the electroactive species at the electrode surface.

Polarography is a subclass of voltammetry in which the working electrode is dropping mercury. Because of the special properties of this electrode, is wide cathodic range. Polarography has been widely used for the determination of many important reducible species.

This classical technique was invented by J. Heyrovsky in Czechoslovakia in 1922. The excitation signal used in conventional (DC) polarography is a linearly increasing potential ramp. We can obtain the Ilkovic equation for the limiting diffusion current:

$$(i_d)_l = 708nD^{1/2}m^{2/3}t^{1/6}C$$

Here, $(I_d)_l$ will have microamperes (μA), when D is in cm^2/s , m is in mg/s , t in second, C is $mmol/L$. In equal, the current can be taken 607 instead of 708 when taking mean of oscillations. When all the device parameters are kept constant, this equation becomes as given below;

$$i_d = KC$$

The relationship between current and potential is derived by the Nernst equation (Wang, 2001).

$$E = E_{1/2} + \frac{0.059}{n} \log \frac{i_d - i}{i}$$

Differential-pulse voltammetry is an extremely useful technique for measuring trace levels of organic and inorganic species. Two current samples are taken during each drop's life time. One is taken immediately before applying the potential pulse, and the second the drop is dislodged. For each cycle the first current difference value is instrumentally subtracted from the second. A plot of this current difference versus applied (dc ramp) potential produces a stepped peak-shaped incremental derivative voltammogram.

Square-wave voltammetry is a large-amplitude differential technique in which a waveform composed of a symmetrical square wave, superimposed on a base staircase potential, is applied to the working electrode. The current is sampled twice during each square-wave cycle, once at the end of the forward pulse and once at the end of reverse pulse.

The difference between the two measurements is plotted vs. the base staircase potential. With this technique concentration between 10^{-7} - 10^{-8} M can be measured.

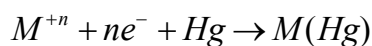
Alternating current voltammetry uses a small-amplitude sinusoidal potential modulation of a single frequency that is superimposed on a slowly changing dc potential ramp. The dc potential is effectively constant during the lifetime of each drop if a polarographic method is used. Usually, the alternating potential has a frequency of 50-100 Hz and amplitude of 10-20 mV. The height of the AC voltammetric peak is proportional to the concentration of the analyte and for a reversible reaction, to the square root of the frequency. Reversible electrode reactions yield a detection limit around 5×10^{-7} M (Tural, 2003).

Stripping analysis is an extremely sensitive electrochemical technique for measuring trace metals. With this technique concentration can be determined about 10^{-10} M. This method is applied two steps. During the first step, analyte is electrochemically deposited onto the surface of an electrode consisting of a thin film or a drop mercury or solid electrode by controlled potential electrolysis. Since the metals are preconcentrated into the electrode by factors of 100-1000, detection limit are lowered.

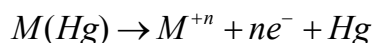
In the second step which is known as the stripping step, the deposited analyte is redissolved by scanning the potential. Different versions of stripping analysis can be employed, depending upon the deposition and measurement steps.

Anodic stripping voltammetry (ASV) is used primarily to determine the concentration of trace metals that can be preconcentrated at an electrode by reduction. Either a hanging mercury electrode or a thin-film mercury electrode can be used. Very electropositive metal ions, such as mercury (II), gold (III), silver and platinum (IV), are deposited on solid electrodes such as glassy carbon.

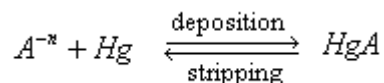
In the first step, a deposition potential is chosen that is more negative than the half-wave potential of the metal. The metal ions reach the mercury electrode by diffusion and convection, where they are reduced and concentrated as amalgams:



In the second step, the potential is scanned anodically. During the anodic scan, the amalgamated metals are reoxidized.

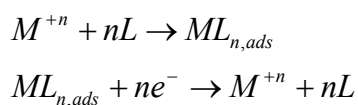


Cathodic stripping voltammetry involves anodic deposition of analyte, followed by stripping in a negative-going potential scan:

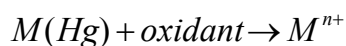


This technique is used to measure a wide range of organic and inorganic compounds capable of forming insoluble salts with mercury. Among these are various thiols and penicillin, as well as halide ions, cyanide and sulphide.

Adsorptive Cathodic Stripping Voltammetry (AdCSV) involves the reduction of the metal in adsorbed complex. The response of the surface-confined species is directly related to its surface concentration, with the adsorption isotherm providing the relationship between the surface and bulk concentrations of the adsorbed. Basic principle of method is contained the accumulation and stripping steps such as ASV. However, the current is obtained by scan negative-going potential.



Potentiometric stripping analysis (PSA) differs from ASV in the method used for stripping the amalgamated metals. The concentrated metals are reoxidized by an oxidizing agent (such as O₂ or Hg(II)) that is present in the solution:



The oxidation can be carried out by passing a constant anodic current through the electrode. During the oxidation step, the variation of the working electrode potential is recorded a stripping curve.

In voltammetric studies usually mercury based or solid electrodes are used. In the dropping mercury electrode (DME), mercury drops form at the end of the capillary tube as a result of gravity. Unlike the HMDE, the mercury drop of a DME grows continuously and has a finite lifetime of several seconds. At the end of its lifetime the mercury drop is dislodged, either manually or by gravity, and replaced by a new drop.

Among the carbon based electrodes, glassy carbon electrode (GCE) is the most widely used electrode. This electrode can be used in flow injection systems with great success after polishing with alumina powder on a piece of cloth. Electrochemical conditioning is also applied to activate the surface. The surface structure of the electrode can be seen in Figure 1.6.

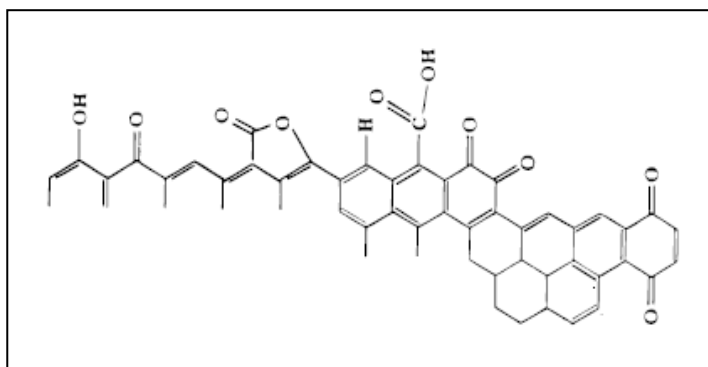


Figure 1.6 The surface structure of GCE (Chen, 1996)

Cyclic voltammetry consist of scanning linearly the potential of a stationary working electrode, using a triangular potential waveform. Single or multiple cycles can be used.

During the potential sweep, the potentiostat measures the current resulting from the applied potential. The current at the stationary working electrode is measured under diffusion-controlled, mass-transfer conditions. Behavior CV of compound is showed cathodic (reduction) and anodic (oxidation) waveform. Therefore, mechanism of reaction can be explained whether reversible or irreversible.

Admittance (Y) and impedance (Z) are interrelated as given below;

$$Y \equiv Z^{-1} \equiv Y' + jY''$$

Admittance is a powerful tool because it provides an opportunity to vary the frequency as well as the potential over a wide range of both variables, i.e., it can provide complimentary information provided by double layer capacitance and electro-capillary measurements (Krishnan, 2007). When the surface of the electrode is negatively charged the hydrogen of the water molecules will be oriented towards the electrode.

In a spherical polyoxomolybdate ion, there are 132 oxygen atoms of the type Mo outside and pointing away from the sphere, 72 oxygen atoms from water molecules bonded to the Mo inside the sphere and pointing towards the center of the sphere, and the rest of the oxygen atoms on the surface of the sphere, with an overall charge of -42 for the ion. With such a large negative charge around the polyoxomolybdate, there should be competing influences of orientation of water towards the electrode or the surface charge of the spherical ion. Admittance can give us some information on the nature of solute-solvent interactions near the electrode double layer.

The impedance is measured as a function of the frequency of the ac source. The technique where the cell or electrode impedance is plotted vs. frequency is called *electrochemical impedance spectroscopy (EIS)*. The theory is to interpret the equivalent resistance (R) and capacitance (C) values in terms of interfacial phenomena.

The mean potential of the working electrode (dc potential) is simply the equilibrium potential determined by the ratio of oxidized and reduced forms of the couple. Measurements can be made at other potentials by preparing additional solutions with different concentration ratios. The faradaic impedance method is capable of high precision and is frequently used for the evaluation of heterogeneous charge-transfer parameters and for studies of double-layer structures (Bard, 2001).

The electrochemical impedance spectroscopy (EIS) method is very useful in characterizing an electrode corrosion behavior. The electrode characterization includes the determination of the polarization resistance corrosion rate and electrochemical mechanism. The effectiveness of this method allows the analysis of the alternating current impedance data, which is based on modeling a corrosion process by an electrical circuit.

EIS allows one to quantitatively determine several coating properties without affecting the coating and its performance. It also facilitates detection of changes in a coating's behavior at a small fraction of the exposure time required for those changes to be detectable by the traditional mechanical or visual methods. The barrier properties of organic coatings create a high electrical resistance across the coating thickness.

Capacitance–potential curves have been recorded by starting from constant potential and scanning the potential either to positive or negative values up to the region where the oxidative or reductive monolayer desorption, respectively, takes place.

The technique of AC Impedance Spectroscopy is one of the most commonly used techniques in electrochemistry, both aqueous and solid. A small amplitude AC voltage of frequency (f) is applied between the working and reference electrode, superimposed to the catalyst potential U_{wr} , and both the real (Z_{Re}) and imaginary (Z_{im}) part of the impedance Z ($= U_{wr}/dI = Z_{Re} + iZ_{im}$) are obtained as a function of f . Upon crossplotting Z_{im} vs Z_{Re} , a Nyquist plot is obtained (Vayenas, 2002).

The *electrochemical quartz crystal microbalance (EQCM)* is a very useful technique for detecting small mass changes at the electrode surface that accompany electrochemical processes. In 1880, Jacques and Pierre Curie discovered that when stress was applied to some crystals, such as quartz, it resulted in an electrical potential across the crystal whose magnitude was proportional to the applied stress. This behavior is known as the *piezoelectric effect*.

The discovery was reviewed by Cady in 1946. Later the Curies discovered that the application of a potential across the crystal resulted in a corresponding mechanical strain. This effect, called the *converse piezoelectric effect*, is the operational basis of EQCM. The piezoelectric effect occurs only in materials that are acentric, that is, materials that crystallize into noncentrosymmetric space groups. This results in dipole effects that are the source of the piezoelectric effect (Bagotsky, 2006).

The core of an EQCM is a sliver (300 μ m thick and 1.3 to 2.6 cm in diameter) of quartz that is sliced from a quartz rod at an angle of approximately $35^{\circ}15'$ with respect to the crystallographic x -axis. This is referred to as the *AT cut*.

The electric field is applied using two electrodes that are vapor deposited on opposite faces of the quartz disc. The electrodes are arranged so that only the circular parts at the center are in register. The disc is mounted at the bottom of a tubular Teflon cell with O-rings. Alternatively, it can be mounted in a glass pipe joint attached to the bottom of a glass cell. In operation, one electrode is exposed to the air and the other is in contact with the electrolyte.

Typical quartz crystals used in QCM experiments have a 1-inch diameter and $f_o = 5\text{MHz}$. The frequency of oscillations is sensitive to mass changes of the crystal surface as expressed by the *Sauerbrey equation*:

$$\Delta f = - 2 f_o m n / (\rho \mu)^{1/2} = - C_f m$$

where Δf is the frequency change caused by addition of mass per unit area, m , to the crystal surface, n is the harmonic number of the oscillation ($n=1$ for 5 MHz with a 5 MHz crystal).

μ is the shear modulus of quartz ($2.947 \times 10^{11} \text{ g/cm.s}^2$), and ρ is the density of quartz (2.648 g/cm^3). The constants are usually lumped together to yield a single constant, the sensitivity factor, C_f , which has the value of $56.6 \text{ Hz cm}^2/\mu\text{g}$ for 5 MHz crystal in air (Bard, 2001).

1.7 Probe Microscopic Techniques

Atomic force microscopy (AFM) was developed about five years after STM. While STM is limited to the surfaces of conducting specimens, AFM can be used to study nonconducting materials, such as insulators and semiconductors, as well as electrical conductors. As shown in Figure 1.7, AFM again uses a sharp tip, about 2 mm long and down to a minimum of 20 nm in diameter, which is scanned closely over the specimen surface.

Scanning probe microscopes were developed in most recent years and appear to change our understanding of electrode processes. The high resolution data of surface properties are clarified by this family of microscopes (Figure 1.7). The various scanning probe microscopes have similar subcomponents but different sensing probes. These high-resolution microscopes rely on sensing the interactions between a probe tip and the target surface, while scanning the tip across the surface.

Different types of interactions can be sensed by the tip to yield different imaging signals. Among the various scanning probe microscopies, *atomic force microscopy*, *scanning tunneling microscopy*, and *scanning electrochemical microscopy* have been useful for imaging electrode surfaces directly (under potential control), and have thus dramatically improved the understanding of electrode reactions.

Scanning probe microscopes have also been useful for creating nanostructures, through patterned movement and arrangement of nanoparticles and selected molecules.

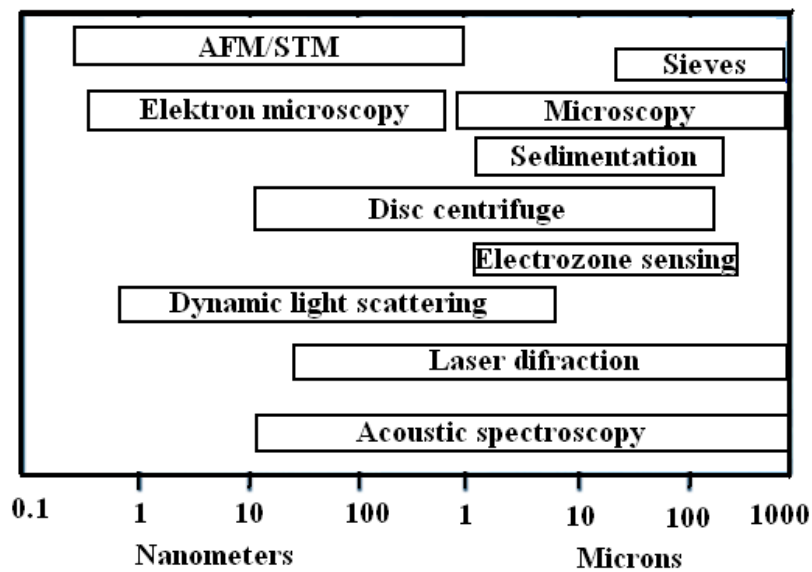


Figure 1.7 Comparison of the imaging of many types 2D and 3D profiling and imaging instruments (Rosenauer, 2003).

Atomic force microscopy (AFM) has become a standard technique to image with high resolution the topography of surfaces. AFM is a mechanical imaging instrument that measures the three dimensional topography as well as physical properties of a surface with a sharpened probe. This powerful probe microscopy operates by measuring the force between the probe and the samples.

Unlike traditional microscopes, the AFM does not rely on electromagnetic radiation, such as photon or electron beams, to create an image. The probe consists of a sharp tip (made of silicon or silicon nitride) attached to a force-sensitive cantilever (Figure 1.8).

AFM probe is very sharp; typically less than 50 nanometers in diameter and the areas scanned by the probe are less than $100\text{ }\mu\text{m}$. In practice the heights of surface features scanned with an AFM are less than $20\text{ }\mu\text{m}$. Scan times can range from a fraction of a second to many tens of minutes depending on the size of the scan and the height of the topographic features on a surface. Magnifications of the AFM may be between 100 X and 100,000,000 X in the horizontal (x-y) and vertical axis. The tip scans across the surface (by a piezoelectric scanner), and the cantilever deflects in response to force interactions between the tip and the substrate. Such deflection is monitored by bouncing a laser beam off it onto a photodetector.

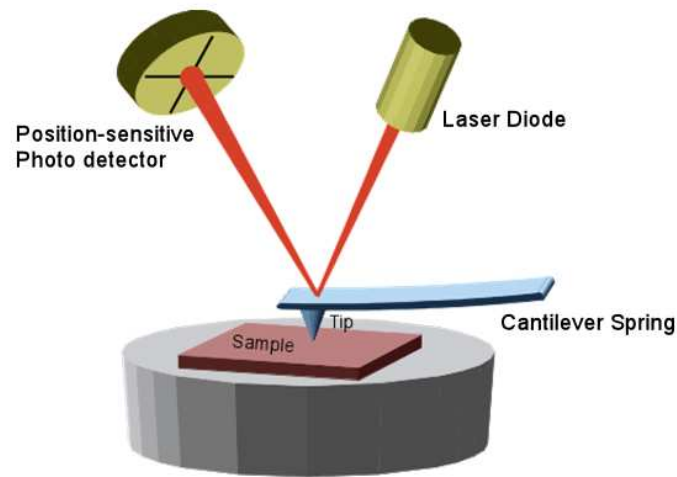


Figure 1.8 Illustration of an AFM cantilever /probe used in an AFM sensor (Nanomanyetik Catalogue).

The measured force is attributed to repulsion generated by the overlap of the electron cloud at the probe tip with the electron cloud of surface atoms. It partly depends on the nature of the electrode, the distance between the electrode and the tip, any surface contamination, and the tip geometry. An image is created as the probe is translated across the surface, while sensing the interaction of the force fields by the cantilever beam. An AFM could be used with not only conductive surface and substrate, but also it is used for insulating materials. AFM is capable of resolving features in three dimensions of a few nanometers with scan ranges up to a hundred microns. The surface structure of a wide range of materials can thus be explored. The technique is very useful for observing changes in electrode surfaces caused by adsorption, etching or underpotential deposition.

The *scanning electron microscope (SEM)* consists a source of electrons; lenses for focusing them to a fine beam; facilities for sweeping the beam in a raster; arrangements for detecting electrons (and possibly other signals) emitted by the specimen; and an image-display system (Figure 1.9). Secondary-electron (SE) images, which show topographic features of the specimen, are the most commonly used type. Backscattered-electron (BSE) images are principally used to reveal compositional variations (Reed, 2005).

The magnification of a scanning image is equal to the ratio of the size of the image as viewed by the user to that of the raster scanned by the beam on the specimen.

The minimum magnification is determined by the maximum angle through which the beam can be deflected, and depends on the working distance, being least when this is greatest. The typical minimum magnification is about 10, with a scanned area of the order of 1 cm^2 . Magnification can be increased by reducing the amplitude of the scanning waveform.

Resolution, defined as the size of the smallest detail clearly visible in the image, is limited not only by the diameter of the electron beam but also by the interaction between the electrons and the specimen. Therefore, correct focus setting is achieved by adjusting the control to obtain the sharpest possible image of fine detail in the specimen, preferably with the magnification set to a high value.

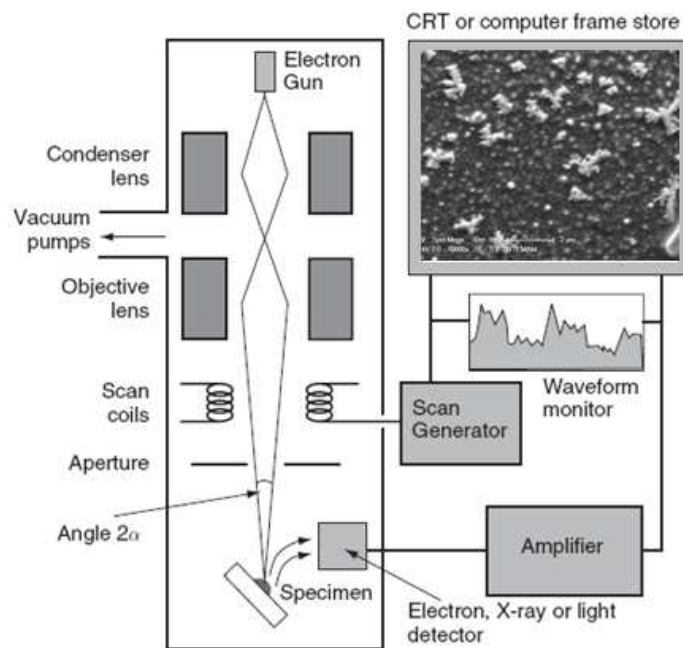


Figure 1.9 Schematic diagram of the layout of a SEM (Kelsall, 2005)

Secondary electrons (SE) are emitted from very near the surface of the sample, with energies of a few electron volts. The SE yield increases with decreasing angle between beam and specimen surface. *Backscattered electrons (BSEs)* have very high energies and low yields compared to secondary electrons. The ET detector may be used to collect only BSEs by making the grid bias slightly negative so as to exclude secondary electrons. However, only those electrons travelling in line-of-sight towards the detector will contribute to the relatively low signal and therefore images will show very strong shadowing contrast and a low signal-to-noise ratio (Kelsall, 2005).

EXPERIMENTAL

2.1. Instrumentation

Centri-voltammetric measurements were carried out using a BAS 100B voltammetric analyzer. A three-electrode system consisted of the glassy carbon electrode or gold disc electrode (i.d. 2 mm) as the working electrodes, a platinum wire as the auxiliary electrode and a Ag/AgCl electrode as the reference electrode was used. Cyclic and differential pulse (DP) mode and time base mode were used throughout the electroanalytical studies. All experiments were carried out at ambient temperature and high purity nitrogen kept flowing over the solution during electrochemical experiments.

Hettich Universal 320 R was used for centrifugation and the pH measurements were made with WTW handheld meter pH 330i ion analyzer. Bandelin Sonorex model ultrasonic bath was used for cleaning of electrodes. Ultra pure water was obtained by Millipore Milli Q (18.2 M Ω) water system. All spectrophotometric measurements was made by using VARIAN UV/VISIBLE containing double beam way. Absorbance measurements were carried out at 468 and 517 nm in 1.0 cm quartz cell.

Electrochemical impedance measurements were made with Ivium stat. QCM measurement was used by CH INSTRUMENTS 400A Series. Nanomanyetik and Ambios Q-Scope Atomic Force Microscopy were used for the characterization of Mo-POMs on the electrode surfaces. Philips XL30 SFEG and Micro Tech Polar 10 Spatter Coater were used for SEM measurements.

Conductive ITO glass and homemade glassy carbon electrodes were used for SEM and AFM images of POMs. ITO or tin-doped indium oxide is a solid solution of (In_2O_3) and (SnO_2), typically 90% In_2O_3 , 10% SnO_2 by weight. Typically 2x1 cm of glass was cut and placed in the cell.

Decomposition of milk samples was accomplished by using CEM Mars 5 microwave system and molybdenum content of milk samples is determined by Agilent 7500 CE model ICP-MS.

2.2. Reagents and Solutions

All reagents were of analytical-reagent grade. All solutions were prepared with ultra pure water from a Millipore water system. Stock solutions of metal ions were prepared by dissolving an appropriate amount of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, KClO_3 , KClO_4 , HCl , NH_4Cl , HClO_4 , NaOH , H_2SO_4 , catechol, hydrosulfate/sulfate, chloroacetic acid and sodium nitrite. Stock solution of Mo(VI) (1000 mg/L) was prepared by dissolving Na_2MoO_4 salt in water by heating and total H_2SO_4 concentration was completed to 0.37 M. 8-Hydroxyquinoline (0.1 mol/L) was used as the precipitation reagent. Pyrogallol Red (PGR) solution (5×10^{-3} M) was prepared by dissolving a mixture of (1:1) EtOH/ H_2O . Cupferron standard (1.5×10^{-3} M) was prepared by dissolving in water.

Britton-Robinson (BR) buffer was prepared by dissolving boric acid (5.0 g), orthophosphoric acid (2.7 mL) and glacial acetic acid (2.3 mL) in water and diluting to 1.0 L. The pH of this solution was adjusted with 1.0 M NaOH. The pH of chloroacetic acid buffers (0.1 M) was adjusted to pH 2-4 by using with NaOH.

2.3. Methods

Centri-voltammetric procedure: The cell was made of Delrin material and drilled to be suitable for centrifugation with a height of 8.5 cm, external diameter of 6.7 cm and bottom diameter of 4.5 cm (Fig.2.1). By this means total weight of the centri-cell was reduced to 225 g.

A conical glass was closely placed in the cell for avoiding the contact of the complex with the cell material. The cell consists of a glassy carbon electrode placed at the bottom providing a flat surface for collecting the precipitate. The GCE was activated by polishing with different grade of Al_2O_3 slurry (0.05–3 micron) on a synthetic cloth then rinsing with pure water and ultrasonicated for 3 minutes in ultrapure water. After rinsing with ultrapure water, the electrode is inserted to the bottom of the cell. Upon addition of 5.0 mL of supporting electrolyte, the cell is closed with a glass cap. Reference and auxiliary electrodes and nitrogen tubing are inserted through the holes of the cap drilled for this purpose.

Dissolved oxygen was removed by bubbling nitrogen gas for 5 min. The electrode potential is scanned from 0 to -900 mV with a rate of 50 mV/s for recording background current. Then, 100 μL of standard solution of complexing reagent is added and the voltammograms are recorded in the same scale. Small volume of standard Mo(VI) solution is spiked into this solutions. Analyte is pre-concentrated onto the working electrode placed at the bottom of the cell by means of centrifugation at certain driving force. Then the potential is scanned in negative directions to monitor the current arisen from the complex reduction

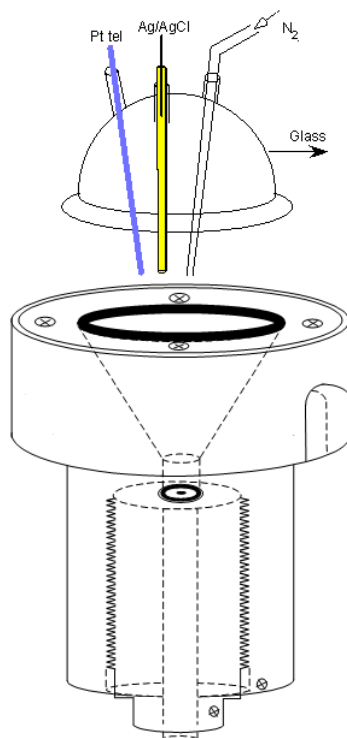


Figure 2.1. Centri-voltammetric cell system (Koçak cell)

Preparation of Mercury Thin Film Electrode (MTFE): The GCE is placed at the bottom of the cell and 10 mL of mercury plating solution (2×10^{-5} M Hg^{2+} in 0.1 M HClO_4) is placed in the cell. The MTFE is formed by applying a deposition potential of -0.5 V for 120-300 s. During this step nitrogen gas was bubbled thorough the solution for stirring. The cell was rinsed and the procedure given above is performed.

Preparation of copper amalgam film on GCE: Amalgam electrodes are more popular in recent days since they offer less toxic alternative.

The procedure similar to the one given above is applied to prepare copper-amalgam electrode. Only difference is the content of plating solution i.e. Hg(II) and Cu(II) ions in equal concentration of 2×10^{-5} M. The amalgam film was made from the electro-reduction of these ions onto the electrode surface by applying a potential of -0.5 V for 90-300 s. During this step nitrogen gas was bubbled thorough the solution for stirring. The cell was rinsed and 10 mL of supporting electrolyte containing analyte ions was placed into the cell.

Cyclic voltammetric procedure: 5.0 mL of supporting electrolyte solution contain 0.1 M chloroacetic acid is transferred into the centri-voltammetric cell. Glassy carbon or gold disc electrode is used as the working electrode. Blank voltammogram is recorded at between +1.0 V and -1.0 V. Then, Mo(VI) ions are added into the solution and voltammogram is recorded at a scan rate of 9-400 mV/s.

Spectrophotometric procedure: Blank spectrum was recorded with 0.1 M chloroacetic acid. The absorbance was recorded between 200-800 nm wavelengths in the presence of 2×10^{-4} M Pyrogallol red in chloroacetic acid solution at different pHs. Upon addition of Mo(VI) onto this solution, the spectrum was recorded.

At the fixed wavelength, Mo(VI)/PGR mole ratio was determined by using Job's method. For this purpose, 2×10^{-4} M solutions of PGR and Mo(VI) were prepared in 0.1 M chloroacetic acid medium. Appropriate ratio of this solution was diluted to 5.0 mL and the absorbance of complex was recorded in 517 nm.

Microwave digestion of milk samples: The milk sample was purchased from local market in a package. 0.5 g of milk sample was weighed and transferred into the Teflon cell of microwave digestion system. Then, 5 mL of concentrated nitric acid is added to the milk samples and total volume is completed with ultrapure water to 50 mL. Microwave digestion procedure is showed in Table 2.1.

Table 2.1 Microwave sample preparation

Max. Power	Ramp (min.)	Pressure (psi)	Temp. (°C)	Hold (min)
600 W	15	195	190	10

Digested sample was divided into two portions: one for direct ICP-MS analysis and the other portion was heated till dryness to remove nitric acid as it cause interference in voltammetric studies. The residue was dissolved in 5 mL of pH 3 chloroacetic acid containing 0.1 M chlorate and 8×10^{-5} M PGR.

The Synthesis of POM including Molybdenum: The synthesis procedure was followed as described in the literature (Müller, 1998). Hydrazine sulfate (0.8 g) was added to a solution of ammonium molybdate four hydrate (5.6 g) and ammonium acetate (12.5 g) mixture. The mixture was stirred for 10 min. During this time the colorless solution has changed to blue-green. Then, 50% acetic acid (83 mL) was added and kept open at around 20°C without stirring.

The solution has slowly changed color to dark brown and the precipitate began to appear within a few hours. After 4 days, the precipitated crystals were filtered off over a glass frit, washed with 90% ethanol, and finally dried in air.

Sample Preparation for AFM and SEM Images: Deposition of molybdenum species on the GCE electrodes was accomplished by cycling the potential in chloroacetic acid solution containing $5 \cdot 10^{-4}$ M Mo(VI) ions. The potential was cycled between 0.0 to -0.9V scanning 10 cycles after waiting for 10 min.

Deposition of molybdenum species on the ITO conductive glass is quite similar to that of GCE but, differs in Mo(VI) concentration being $1 \cdot 10^{-3}$ M. The potential was cycled in the same range but, 30 successive scans were made.

RESULTS AND DISCUSSION

Initial studies were focused on the utilization of designed and constructed new Centri-voltammetric cell for the use of oxine (8-hydroxyquinoline) as the complexing reagent for several metal ions in aqueous solutions. The complexes formed in different pH media were centrifuged on a bare glassy carbon (GCE) or mercury thin film (MTFE) to compare the performances of the electrodes. Here, the main idea was to test the effect of pH for distinguishing the metal complexes individually. Amalgamated electrodes were also tested to evaluate the performances as they offer a potential to replace the toxic mercury based electrodes. However, the attention was mostly paid to the centri-voltammetric determination of molybdenum ions in aqueous solutions by using several complexing reagents. The advantages of the method over Adsorptive cathodic stripping voltammetry were also discussed.

Second part of the study embraces the results of early attempts to use the electrochemical systems as a tool for the synthesis of self assembled structures, polyoxomolybdates, on the electrode surface and their characterizations with close images and other measurement systems.

3.1. Determination of Metal Ions with Complexing Reagents

3.1.1. *Studies with oxine*

Mercury thin film electrodes (MTFEs) along with the sensitivity and selectivity inherited from mercury towards many metal ions also provide a flat surface suitable for centrifuging. Therefore, it was utilized in Centri-voltammetric studies.

The MTFE was prepared by affecting the electrode potential at -0.5 V for 180 s in plating solution as given in Experimental Section. The electrode was cleaned with ultra pure water and 10 mL of acetate buffer (pH: 4.76) was added into the cell. After the nitrogen gas was purged, the solution was spiked with 1×10^{-3} M oxine solution and the potential was scanned in negative direction. Small volume of standard solution of Cd(II) was added to give 1×10^{-7} M concentration and then, cell content was centrifuged at 1000 rpm for 10 minutes. Figure 3.1 shows the resulting DP voltammograms and the peak current arisen from the complex reduction.

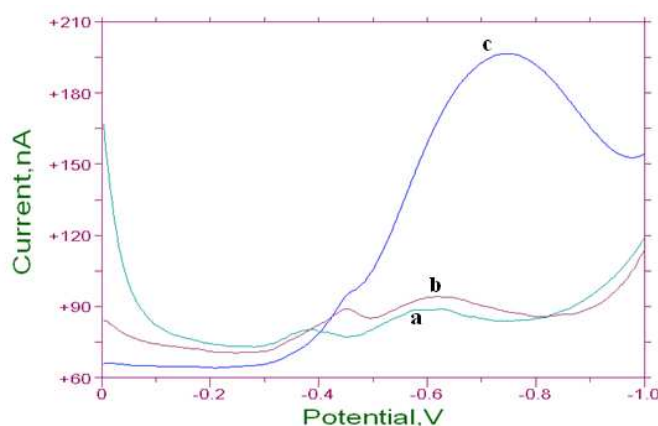


Figure 3.1 DP Voltammograms recorded with a) 0.1 M pH 4.76 acetate buffer, b) after addition of 8-Hydroxyquinoline to be 1×10^{-3} M and c) addition of Cd(II) ions to give 1×10^{-7} M concentration following the centrifugation step.

As shown in Figure 3.1, the background current is about 70 nA and upon addition of oxine and Cd(II) ions and after centrifugation, a wide reduction peak corresponding to the complex at -0.71 V is observed. The peak current clearly increased about 100 nA after centrifugation.

The enhancement in the peak height and therefore, the sensitivity gained by centrifuging can be improved by using higher speed and other solution characteristics. Here, the reduction peak corresponds to the reduction of Cd(II) in oxine complex. The peak characteristics can be improved by optimizing the conditions to obtain sharper and higher peaks. Alternatively, this method can be applied for electroinactive metal ions via reduction of the ligand in the complex deposited on the electrode surface by centrifugal forces.

Nickel, for instance, does not dissolve in mercury and therefore, anodic stripping voltammetry is not suitable for this ion. Therefore, indirect methods, mostly cathodic stripping methods are developed for its determination those utilize dimethylglyoxime as the reagent (Dugo, 2004).

Similarly, the GCE was placed in the voltammetric cell and 10 mL of pH 8.4 borate buffer was transferred to the cell. Following the nitrogen passage, background current was monitored. After addition of 5×10^{-4} M oxine, small volume of Ni^{2+} standard solution was added to the cell to give 5×10^{-6} M. Then the cell content was centrifuged at 1000 rpm for 10 min. The resulting voltammograms have given a small peak not well distinguished from the background current.

It is well known that the solution pH has a significant effect on the formation and the stability of the complex. Therefore, experiments were repeated with pH 9.0 borate buffer solution to see any difference. Indeed, a well established peak at -768 mV with a peak current of 124.8 nA was obtained in this medium (Figure 3.2).

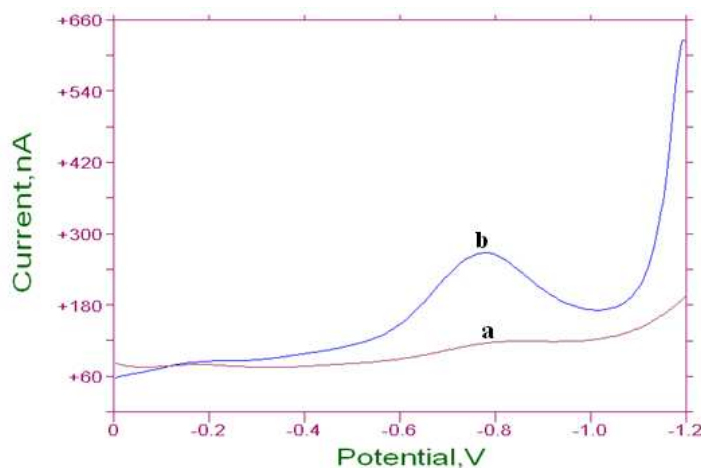


Figure 3.2 Voltammograms recorded at a GCE in a) 5×10^{-4} M oxine in 0.1 M pH 9.0 borate buffer, and b) after addition of 5×10^{-6} M Ni^{2+} and centrifugation at 1000 rpm for 10 min.

The type of the buffer ions can also affect the results as well as the pH. Therefore, Britton-Robinson (BR) buffer solutions with pH 9.0 were tested and quite similar results were obtained.

The experiment was repeated with a freshly prepared MTFE in pH 9.0 BR buffer and a small peak at around -0.4 V was obtained. Upon deposition at -0.7 V for 90 s, the potential was scanned again from 0 V to negative potential values. The complex peak was split at more negative potentials.

In conclusion, the method is found promising for nickel determination as an alternative to AdCSV method that utilize dimethylglyoxime reagent. But, the optimization of the method and other analytical approaches will be the subject to a future study.

Molybdenum ions are thought to be interesting to study as they give complexes with oxine. Therefore, their interaction was studied at a MTFE in different pH media but, no satisfactory results were obtained. Figure 3.3 shows the centri-voltammograms obtained in 0.1 M KClO_3 in pH 3.76 acetate buffer.

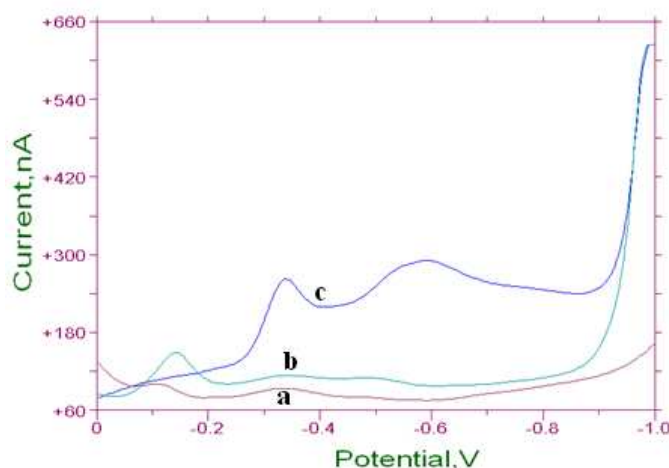


Figure 3.3 Voltammograms recorded at a MTFE in a) 0.1 M KClO_3 in pH 3.76 acetate buffer, after addition of b) 5×10^{-4} M oxine and c) 1×10^{-5} M Mo(VI) and centrifugation at 1000 rpm for 10 min

A peak at around -0.1 V can be seen in the presence of oxine alone and increases with centrifugation. This could be attributed to a copper impurity in the solution that can be detected by means of complex formation with oxine. The complex peak can be seen at -0.35 V and another wide peak has appeared at around -0.6 V.

As an alternative, direct determination method developed for Mo(VI) was investigated. Lanza et al. have studied DP polarographic determination of molybdenum in steel samples at an SMDE (1980).

The same supporting electrolyte consisting of nitric acid solution was used but the electrode was changed to copper amalgam electrode as an alternative to SMDE. However, additional peaks were observed for copper and copper oxine peaks at -0.1 V. Therefore, the copper amalgam film thickness was decreased on GC electrode by controlling the deposition time, and resulting voltammograms gave a lower background current and complex peak of copper has diminished (Figure 3.4).

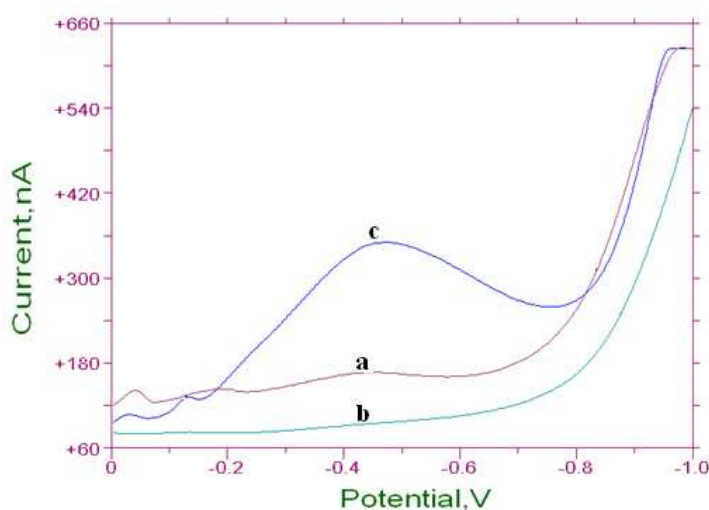


Figure 3.4 Voltammograms recorded at a copper amalgam electrode in a) 2 M NH_4NO_3 containing 0.25 M HNO_3 , b) after addition of 5×10^{-4} M oxine, and c) after addition of 1×10^{-5} M Mo(VI) and centrifugation at 1000 rpm for 10 min

On the other hand, the peak related to the Mo(VI) complex has shifted to more negative potential values. Therefore, further studies were carried out with GCE as it provides better sensitivity and is not subjected to mechanical deformation as mercury film electrodes.

On search for a proper electrolyte medium for determination of Mo(VI) with oxine, HNO_3 was omitted since it reacts with the electrode material and the studies were carried out with HCl solutions. 0.1 M HCl solution, spiked with 1×10^{-5} M oxine and then with 5×10^{-6} M Mo(VI) standard solution, was centrifuged at 1000 rpm for 10 min. The complex peak can be observed at -0.45 V as given in Figure 3.5.

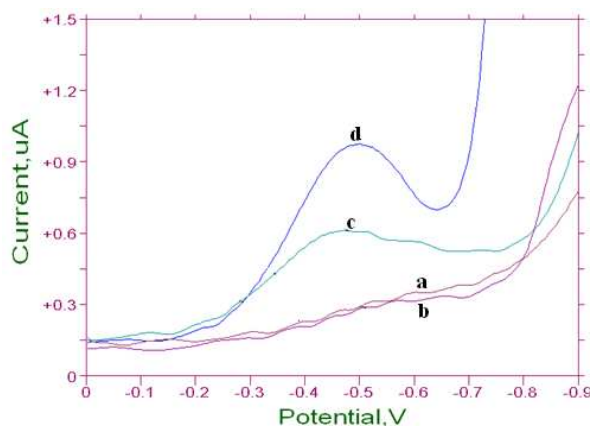


Figure 3.5 The influence of centrifuge on voltammograms recorded for 0.1 M HCl solution alone, and after addition of b) 1×10^{-5} M oxine and 5×10^{-6} M Mo(VI) and after centrifugation at c) 1000 rpm and d) 1500 rpm for 10 min

As can be seen from the figure, oxine is reduced at GCE and a reduction peak arises at potentials more negative than -0.8 V (Figure 3.5 b) giving a peak at -0.9 V.

It was also deduced from the figure that centrifugation speed has a key effect on the results as the peaks become steeper and higher at 1500 rpm. However, the centrifuge system used does not allow attaining to higher speed levels as the empty cell weight is about 395 g .

Figure 3.6 displays the voltammograms recorded for 1×10^{-3} M oxine and after addition of 5×10^{-5} M Mo(VI) ion discriminating the effectiveness of both electrochemical and centrifugal deposition of the complex species. Background currents for supporting electrolyte and in the presence of oxine have been very low and upon addition of Mo(VI) standard into the solution, a small and wide peak has appeared at -0.42 V.

After deposition of the complex onto the electrode surface by applying a potential of -0.6 V for 60 s, the potential was scanned in negative directions. The peak current was found as $6.5 \mu\text{A}$. The procedure was repeated after 10 min centrifugation at 2000 rpm and the reduction peak current was doubled ($13.0 \mu\text{A}$). This clearly illustrates the effectiveness of the centrifugation on the result.

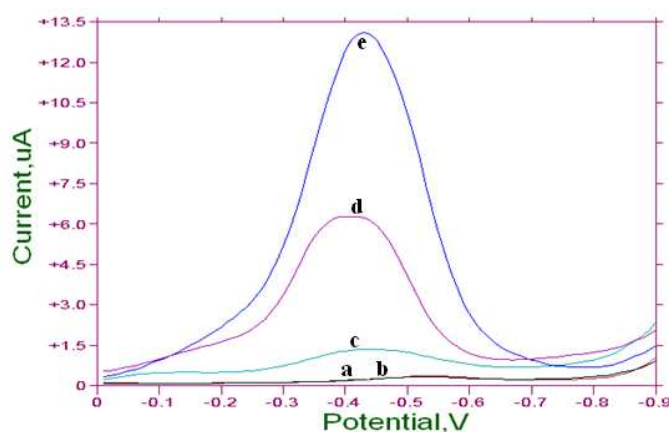


Figure 3.6 Centri-voltammograms obtained at a GCE in a) pH 2.3 HCl solution and after addition of b) 1×10^{-3} M oxine, c) 5×10^{-5} M Mo(VI), d) after applying a potential of -0.6 V for 60 s e) after centrifugation at 2000 rpm for 10 min

The parameters those have a strong effect on the analyte signal namely; pH and oxine concentration, centrifuge time and speed were optimized. The extent of complex formation and their stability are strongly dependent upon the pH of the solution. Therefore, experiments were carried out at different pHs by using several buffer solutions including hydrogen sulfate/sulfate, chloroacetic acid/acetate buffer. The type of the buffer ion can also affect the results as well as the pH. Among the buffer system searched phosphate buffer were eliminated since it may form phosphomolybdate in the solution.

Formation of Mo(VI)-oxine complex was studied in $\text{HSO}_4^-/\text{SO}_4^{2-}$ buffer system at different pHs (1.3, 2.0, 2.5 and 3.0) spiked with 1×10^{-4} M oxine. Then it was added Mo(VI) standard to give 1×10^{-5} M solution and the mixture was centrifuged at 2000 rpm for 10 and 20 min. Then, the voltammograms were recorded.

Best results were obtained in pH 2.0 medium. The complex peak was not observed at pH 1.3. Lower peak current (I_p) values in $\text{pH} < 2$ media can be attributed to a lesser amount of complex formation.

As shown in Figure 3.7, the reduction peak of complex has appeared at around -0.4 V at pH 2.0 medium. But the peak has split on centrifuging for 10 min at 2000 rpm probably due to the high of concentration molybdenum. In fact, a single peak was observed working with Mo(VI) concentration less than 1×10^{-6} M.

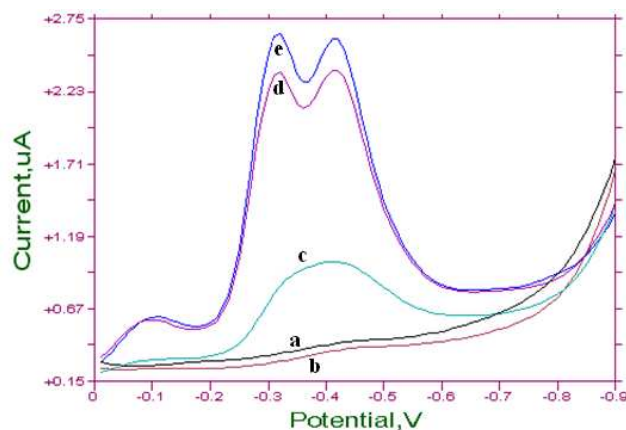


Figure 3.7 Centri-voltammograms obtained in a) pH 2.0 $\text{HSO}_4^-/\text{SO}_4^{2-}$ buffer solution and after addition of b) 1×10^{-4} M oxine, c) 1×10^{-5} M Mo(VI), after centrifugation at 2000 rpm for d) 10 min, and for e) 20 min.

Figure 3.8 shows the voltammograms obtained for 2.5×10^{-7} M Mo(VI) in pH 2.0 hydrogen sulfate/sulfate buffer. A sharp and well distinguished reduction peak at -0.5 was obtained after centrifugation at 2000 rpm for 10 and 30 min. After the recordings, the centrifugation was repeated and no difference was observed on the voltammograms recorded indicating the stability of the film deposited on the electrode surface.

In addition, chloroacetic acid/acetate buffers were tested. As Mo(VI) predominantly occurs in H_2MoO_4 form at $\text{pH} < 2$, the pH was changed between 2.0 and 3.0. Higher pHs were also tested by using acetic acid buffer solutions but no peak formation was observed for this media.

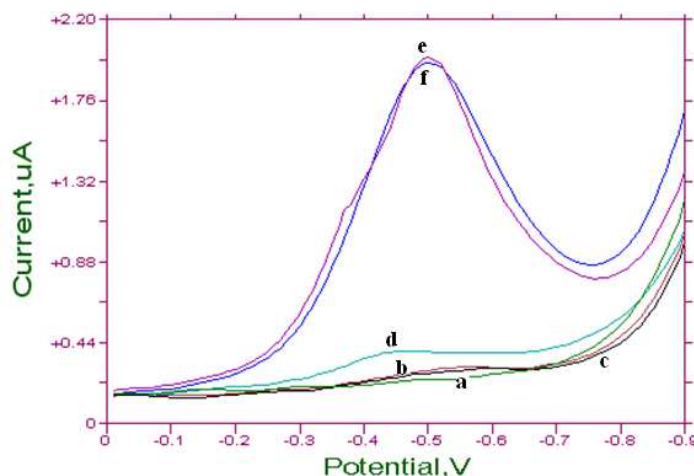


Figure 3.8 Centri-voltammograms obtained in a) 0.1 M pH 2.0 $\text{HSO}_4^-/\text{SO}_4^{2-}$ buffer, after addition of b) 1×10^{-4} M oxine and, c) 2.5×10^{-7} M Mo(VI), after centrifugation at 2000 rpm for d) 10 min and e) 30 min, and f) subsequent recording of (e)

Figure 3.9 shows the pH-Concentration change for Mo(VI) in chloroacetic acid medium drawn by Minteq program. As can be seen from the figure molybdate species dominates at $\text{pH} > 3.0$ media. Therefore, $\text{pH} < 3$ media is recommended for molybdenum determination in the literature.

As shown in Figure 3.10, the reduction peak current of complex was obtained $1.07 \mu\text{A}$ at -0.5 V after centrifugation for 2000 rpm in 10 min. The peak currents were plotted against the pH for different media as shown in Figure 3.11 and optimum pH was selected as 3.0. Higher pHs were avoided since the peak was diminished at $\text{pH} > 4.0$ and even disappeared after pH 9.0.

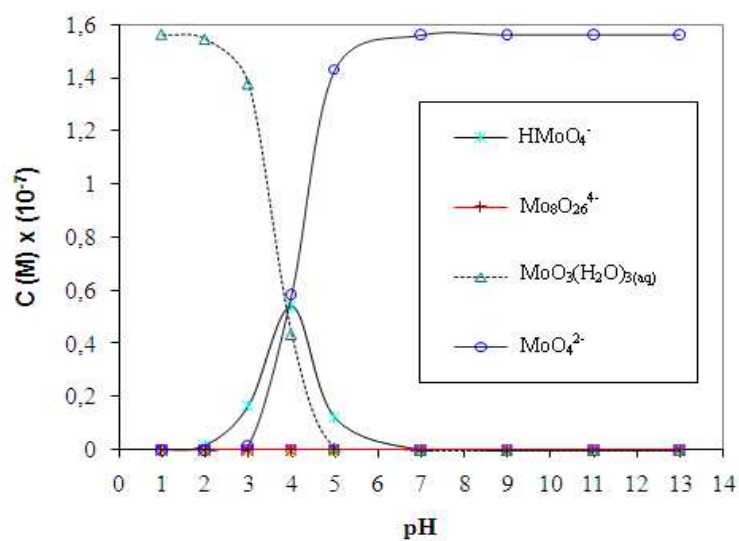


Figure 3.9 pH dependence of molybdenum species 0.1 M chloroacetic acid containing 2.5×10^{-7} M Mo(VI)

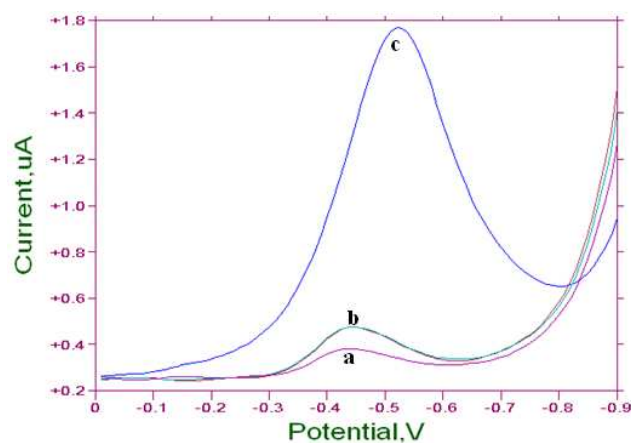


Figure 3.10 Centri-voltammograms obtained in 0.1 M pH 3.0 chloroacetic acid/acetate⁻ medium, b) after addition of 1×10^{-4} M oxine + 1×10^{-6} M Mo(VI), c) after centrifugation at 2000 rpm for 10 min

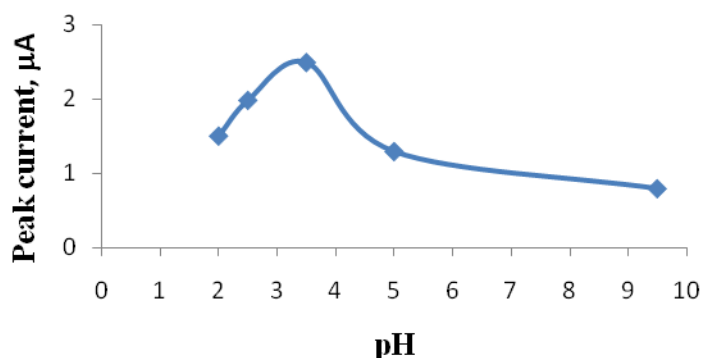


Figure 3.11 Effect of pH on Mo-oxine reduction peak currents in chloroacetic acid, acetic acid and borate buffers.

Figure 3.12 shows the dependence of peak current on centrifugation speed for a range of 500-2500 rpm applied for 10 min. The solution consists of 1×10^{-6} M Mo(VI) and 1×10^{-4} M oxine in pH 3.0 chloroacetic acid/acetate buffer. The peak current of the complex was plotted against the centrifugation speed (Figure 3.13).

As can be followed from the figures, proportionally increased amount of Mo(VI)-oxine complex deposited on GCE by centrifugal force and sharper and higher peaks were obtained at high speeds with an increased background current. However, speed rates higher than 2000 rpm were avoided as mechanical deterioration and breakage of the inner glass lining may occur.

As shown in Figure 3.14, increasing centrifugation time at constant centrifugation rate, results in an increase in reduction peak current as

expected. It was also noted that sharper peaks are obtained for longer centrifugation times.

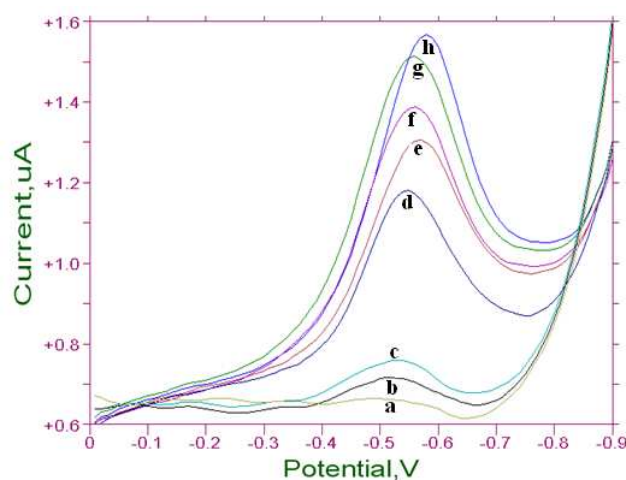


Figure 3.12 Centri-voltammograms recorded in a) 0.1 M pH 3.0 chloroacetic acid/acetate medium, b) after addition of 1×10^{-4} M oxine, c) 1×10^{-6} M Mo(VI), after centrifuging at d) 500, e) 1000, f) 1500, g) 2000, h) 2500 rpm for 10 min

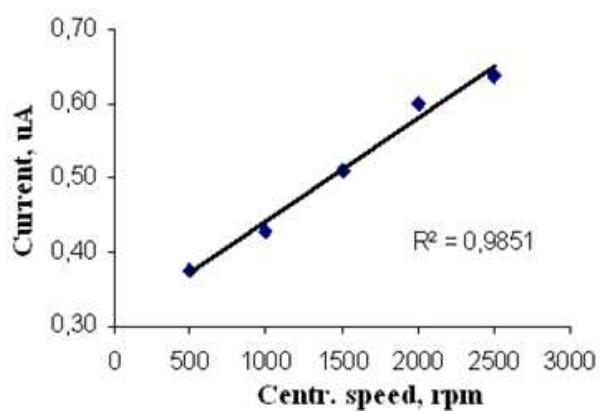


Figure 3.13 Effect of centrifugation speed on the reduction peak current of Mo(VI)-oxine complex.

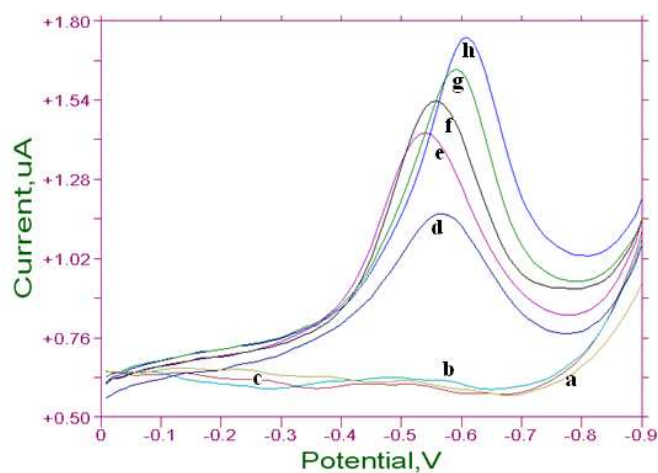


Figure 3.14 Centri-voltammograms obtained in a) pH 3.0 chloroacetic acid medium, b) after addition of 1×10^{-4} M oxine, and c) 1×10^{-6} M Mo(VI), and after centrifuging at 2000 rpm for d) 5, e) 10, f) 15, g) 20, h) 25 min.

The peak currents were plotted against the centrifuging time and a linear relationship was obtained (Figure 3.15). This indicates a proportional accumulation of the complex on the electrode surface.

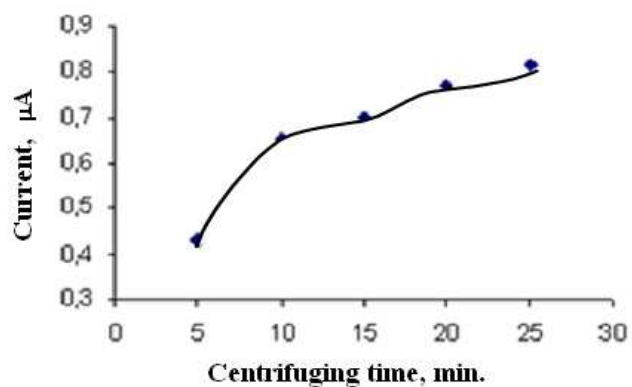


Figure 3.15 Dependence of the peak current on centrifuging time

As can be seen from the figure above, 5 min is less than satisfactory for a stable and sensitive signal formation. After centrifugation at 2000 rpm longer than 10 min, a more linear relationship can be estimated probably due to the proportional amount of complex is deposited on the electrode surface. The centrifugation speed and time were established as 2000 rpm for 10 min for further studies. Longer times with higher speed were avoided for mechanical stability.

Cyclic voltammograms (CV) were recorded to reveal the reduction mechanism of the complex formation. The supporting electrolyte consisted of pH 2.3 HCl solution was spiked with 1×10^{-3} M Mo(VI) and CVs were recorded in a potential range of -0.8 to 0 V at different scan rates between 10-500 mV/s. A reduction peak at -0.45 V and an oxidation peak at -0.3 V was observed. As shown in Figure 3.16, the peak current has increased with increasing scan rate.

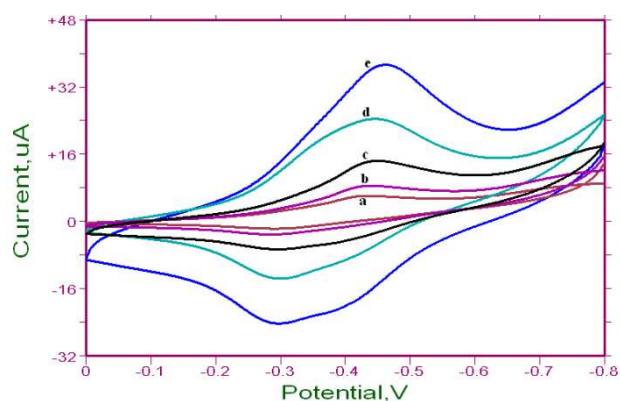


Figure 3.16 Cyclic voltammograms of 1×10^{-3} M Mo(VI) in pH 2.3 HCl medium at a scan rate of a)10, b)20, c)50, d)100, and e)200 mV/s.

The supporting electrolyte was spiked with 1×10^{-3} M oxine in another cell and no peak was observed (Figure 3.17). In the presence of Mo(VI) the peak related to the complex can be observed. The cyclic voltammograms show the effect of oxine concentration on the cyclic voltammograms of Mo(VI) in pH 2.3 HCl medium. The metal ion to ligand ratio was maintained to be in a ratio of 1:1 or 1:2.

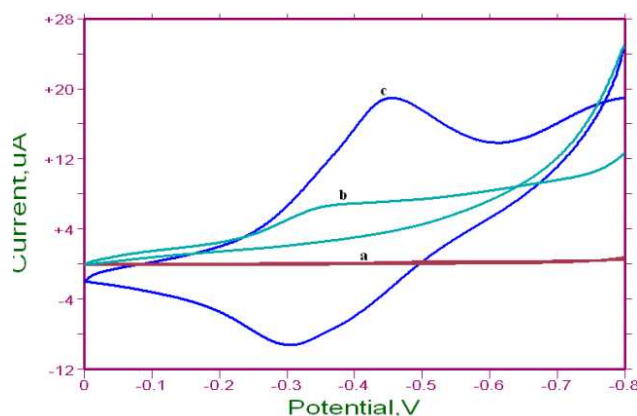


Figure 3.17 Cyclic voltammograms for a) pH 2.3 HCl medium, containing 1×10^{-3} M oxine, b) after addition of 1×10^{-3} M Mo(VI), and c) further addition of 1×10^{-3} Mo(VI) + 2×10^{-3} M oxine, scan rate was 50 mV/s.

As can be followed from the figure above, the complex peak appears at -0.45 V and an oxidation peak is observed at -0.35 V when adding oxine in twice as much of Mo(VI) ions. The potential difference between the peaks in cathodic and anodic regions indicates a slight irreversibility in the reduction mechanism. To reveal this interaction, the effect of scan rate on these peaks was investigated (Figure 3.18). As the potential scan rate increases, the reduction peaks have shifted in negative potentials indicating the tendency to irreversible process.

Furthermore, another oxidation peak around -0.45 V has become evident at high scan rates. The values were plotted against the scan rate and square root of scan rate ($v^{1/2}$) (Figure 3.19).

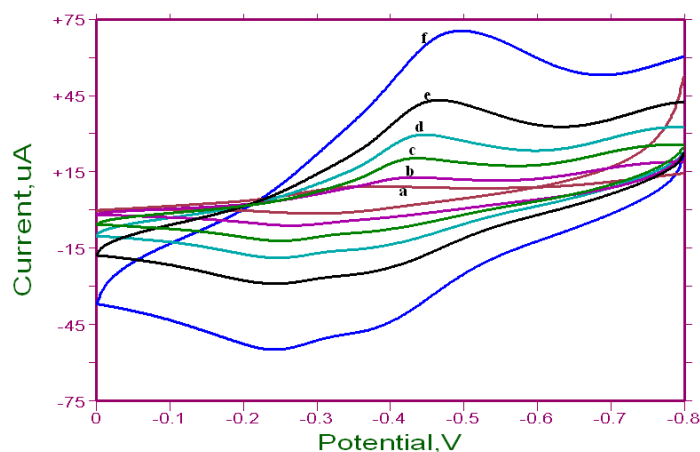


Figure 3.18 Cyclic voltammograms of 1×10^{-3} M Mo(VI) + 1×10^{-3} M oxine for scan rate of a) 10, b) 20, c) 50, d) 100, e) 200 and f) 500 mV/s in pH 2.3 HCl medium.

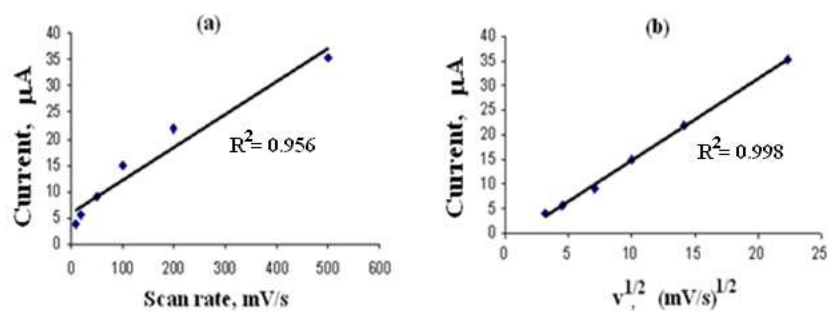


Figure 3.19 Dependence of the peak current of 1×10^{-3} M Mo(VI) + 1×10^{-3} M oxine complex on (a) scan rate b) the square root of scan rate

As can be followed from the Figure 3.19, the peak current changes linearly with $v^{1/2}$ indicating that a diffusion controlled mechanism is involved.

Further studies were carried out by using identical four cells made of white Delrin material. Delrin blocks were operated in lathe apparatus to shape in suitable centrifugal geometry and decrease the weight as well. Therefore, new cells have allowed us to experience higher speeds. Figure 3.20 shows the reduction peak of the complex at -0.5 V has built up proportionally with addition of Mo(VI) ion into the cell containing 2×10^{-4} M oxine by centrifuging at 2000 rpm.

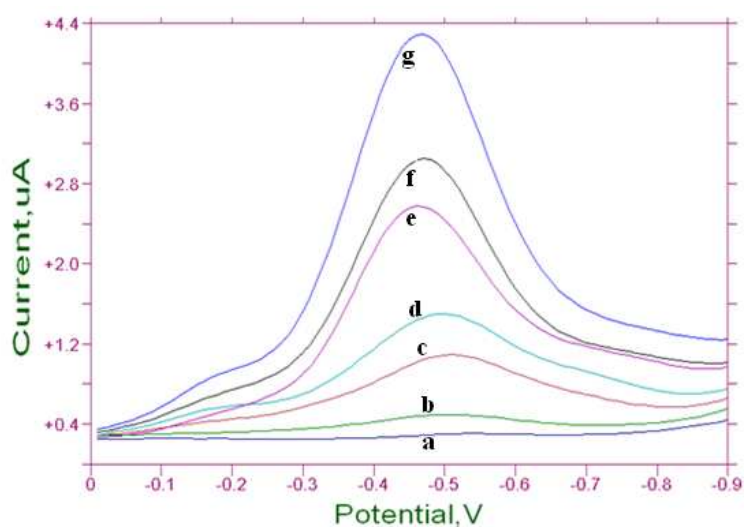


Figure 3.20 Centri-voltammograms recorded in a) pH 3.0 chloroacetic acid medium and after spiking with b) 2×10^{-4} M oxine and after addition of Mo(VI) ions to be c) 2×10^{-5} M, d) 4×10^{-5} M, e) 6×10^{-5} M, f) 8×10^{-5} M and g) 1×10^{-4} M in the cell. Centrifugation was made at 2000 rpm for 10 min.

The results so far obtained have revealed that molybdenum ions can be effectively collected on the electrode surface by the driving force of centrifugation and then voltammetric measurements can be made. Figure 3.21 shows the calibration curve constructed for Mo(VI) ion in the presence of 2×10^{-4} M oxine after centrifugation at 2000 rpm for 10 min.

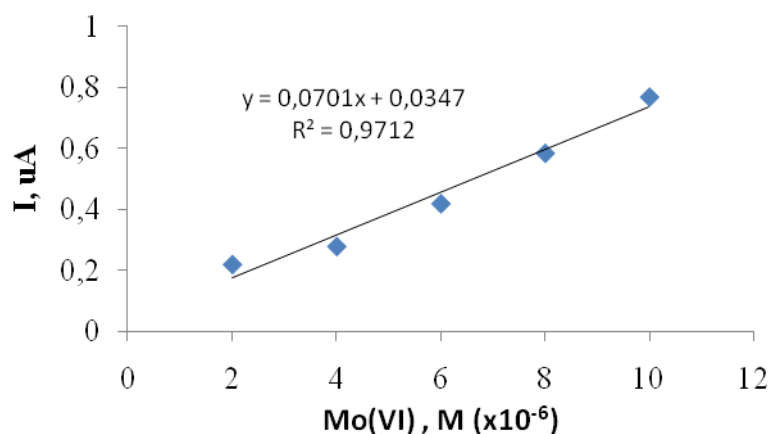


Figure 3.21 Calibration graph for Mo(VI) in the presence of 2×10^{-4} M oxine after centrifugation at 2000 rpm for 10 min.

As can be seen Mo(VI) can be determined by this means in micromolar levels. The calibration graph was found linear in the concentration range of 2×10^{-6} - 1×10^{-5} M. In fact, the signal for the lowest concentration used was in μ A level but, the background current was also high. Therefore, alternative reagents for Mo(VI) determination was searched.

3.1.2 Studies with Pyrogallol red (PGR)

PGR is a red colored reagent and, in the presence of Mo(VI), the color is changed to violet indicating a complex formation. Therefore, the complex between Mo(VI)-PGR, can be utilized in Centri-voltammetric study. The complex was collected on the surface of GCE with the aid of centrifuge. As the extent of complex formation and its stability are strongly dependent on medium pH, a 5.0 mL portion of 0.1 M chloroacetic acid solution at different pHs (2.0, 2.5, 3.0, 4.0 and 5.0) was added to the cell and spiked with 1×10^{-4} M Pyrogallol red.

Figure 3.22 shows the Centri-voltammograms recorded for 1×10^{-4} M PGR before and after addition of Mo(VI) ion and with and without centrifugation in pH 2.5 chloroacetic acid buffer. Upon scanning the potential in negative direction, the complex was reduced at -0.45 V. At this medium, the reduction peak of chloroacetic acid was also observed at about -1.3 V.

Experiments were repeated in pH 4.0 chloroacetic acid buffer and the reduction peak of the complex was observed at -0.5 V. However, the peak current of chloroacetic acid was decreased and the peak potential was shifted towards more positive direction (Figure 3.23).

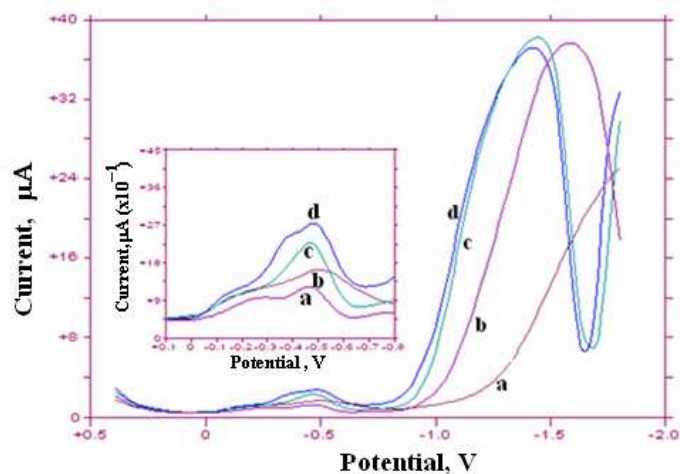


Figure 3.22 Centri-voltammograms obtained for a) 0.1 M pH 2.5 chloroacetic acid, b) after addition of 1×10^{-4} M PGR and centrifugation at 1000 rpm for 5 min, c) 2×10^{-5} M Mo(VI) and after centrifugation at 1000 rpm for 5 min, d) 10 mg/L Mo(VI) and after centrifugation at 1000 rpm for 5 min

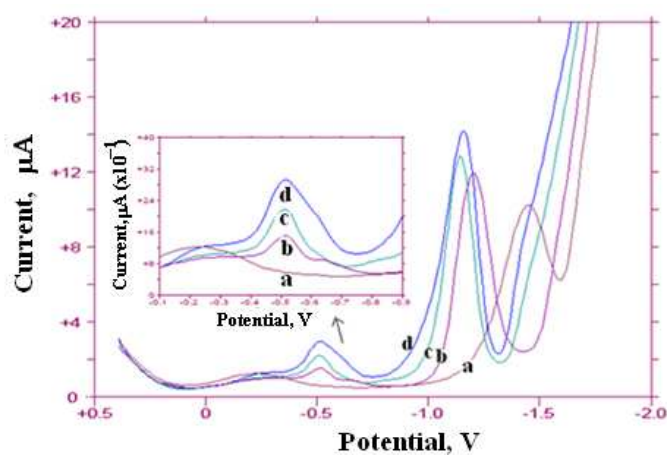


Figure 3.23 Centri-voltammograms obtained for a) 0.1 M pH 4.0 chloroacetic acid, b) after addition of 1×10^{-4} M PGR and centrifugation at 1000 rpm for 5 min, c) 2×10^{-5} M Mo(VI) and after centrifugation at 1000 rpm for 5 min, d) 1×10^{-4} M Mo(VI) and after centrifugation at 1000 rpm for 5 min.

The complex formation between Mo(VI) and PGR and its deposition on the electrode surface by means of centrifugation were also investigated in Britton-Robinson media at different pHs (2.0, 3.0, 4.0 and 5.0). Best results were obtained with pH 3.0 BR solutions where the complex peak was appeared at -0.5 V. However, considering the presence of phosphate ions in BR buffer, further experiments were performed in pH 3.0 chloroacetic acid solutions as used for oxine.

So far, no data for the metal ion to ligand ratio for this complex was observed in the literature survey. UV-VIS spectrophotometric measurement of Mo(VI) complex with PGR were carried out to investigate the complex stoichiometry.

The absorbance of Pyrogallol red solution (6×10^{-5} M) was scanned at wavelength range of 200-800 nm by changing pH in a wide range (Figure 3.24). For this purpose pH 2-4, pH 5-6 and pH 8-11 ranges were maintained by using chloroacetic acid, acetic acid/acetate and borate buffers, respectively.

PGR has displayed two absorbance peaks at 270 nm and 467 nm. Optimum pH was searched for analytical purposes. Maximum absorbance of PGR was showed at pH 2.0 and pH 8.0 (Figure 3.25). Data obtained from the figure were plotted against the pH (Figure 3.26). Optimum pH was selected as pH 3.0 since the complex of Mo(VI) with PGR is more stable in acidic medium.

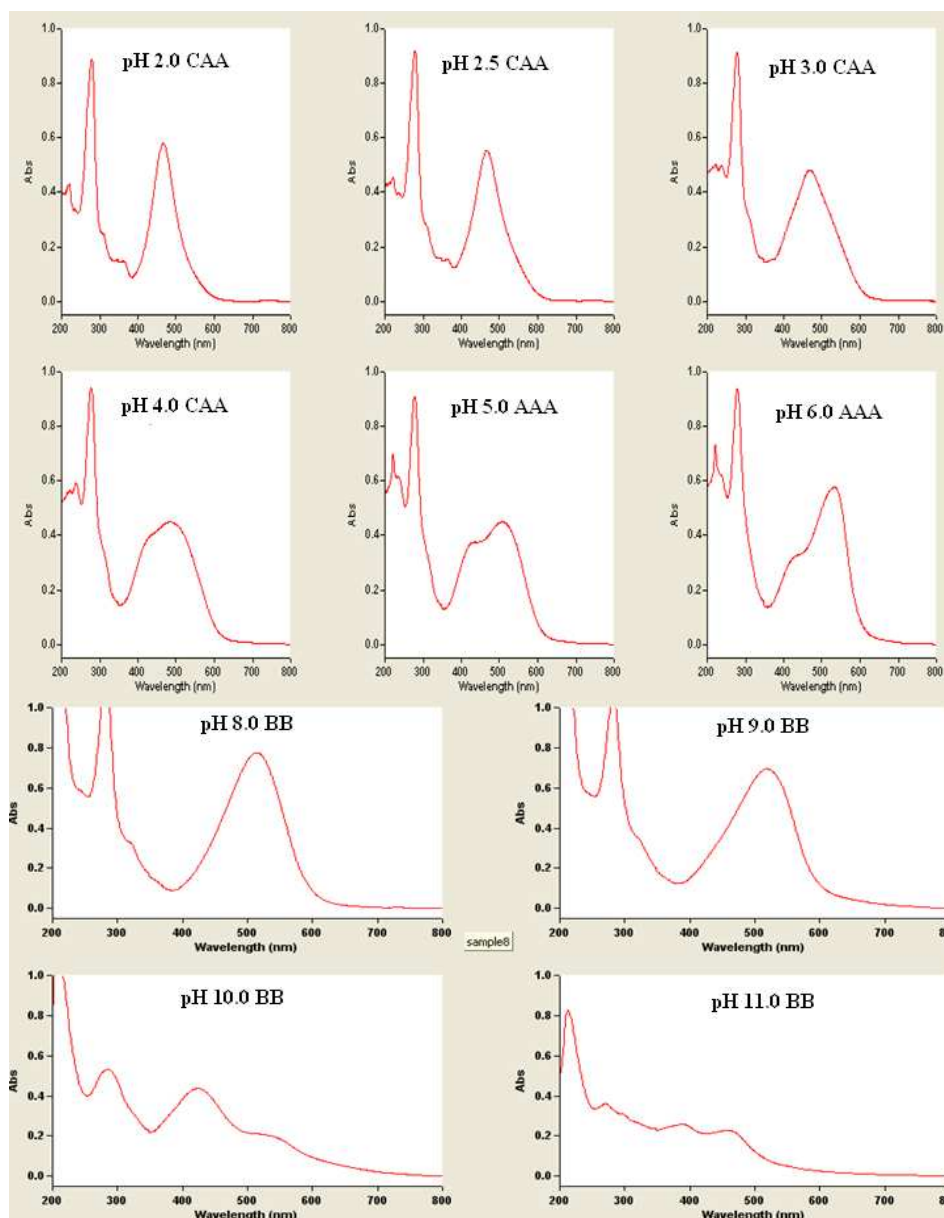


Figure 3.24 UV-Vis spectra for 6×10^{-5} M PGR alone in pH 2-11 media where CAA: chloroacetic acid/acetate, AAA: acetic acid/acetate, BB: Boric acid/borate buffer.

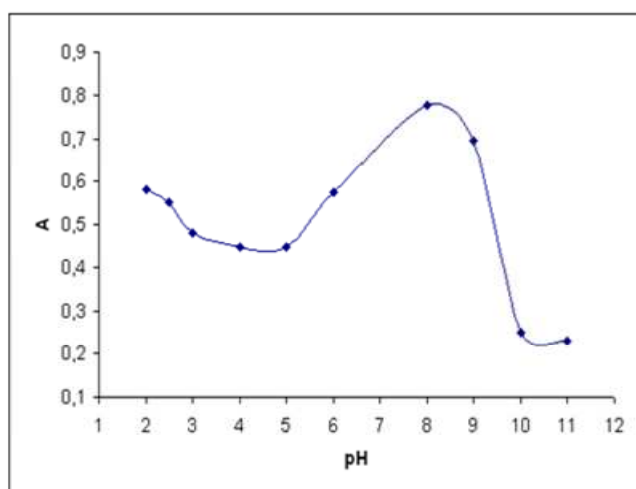


Figure 3.25 Effect of pH on the absorbance of 6×10^{-5} M PGR reagent alone.

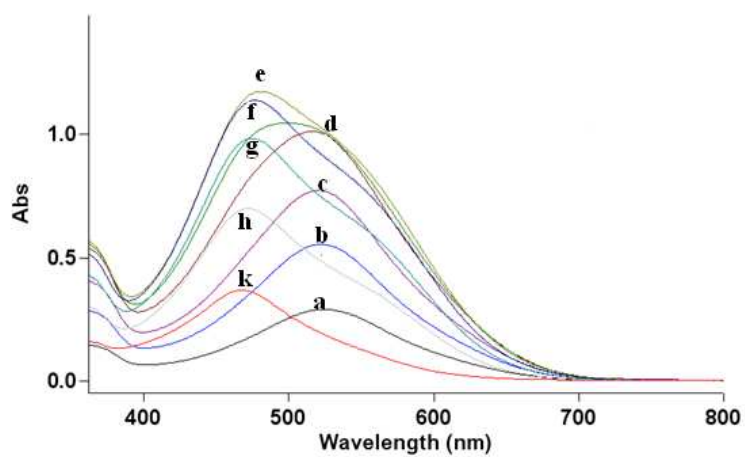


Figure 3.26 Visible absorbance spectrum of the PGR-Mo(VI) complex. Mole fractions of PGR were changed from 0.1 to 0.9 in 0.1 increments from spectrum number (a) to (k).

Furthermore, the number of PGR binding to Mo(VI) was calculated by using Job's methods (Figure 3.27). Maximum wavelength of the complex was appeared in 517 nm. Absorbance values of the complex were plotted against mole fraction and it was revealed from Job curves, the complex forms in 1:1 Mo-PGR stoichiometry.

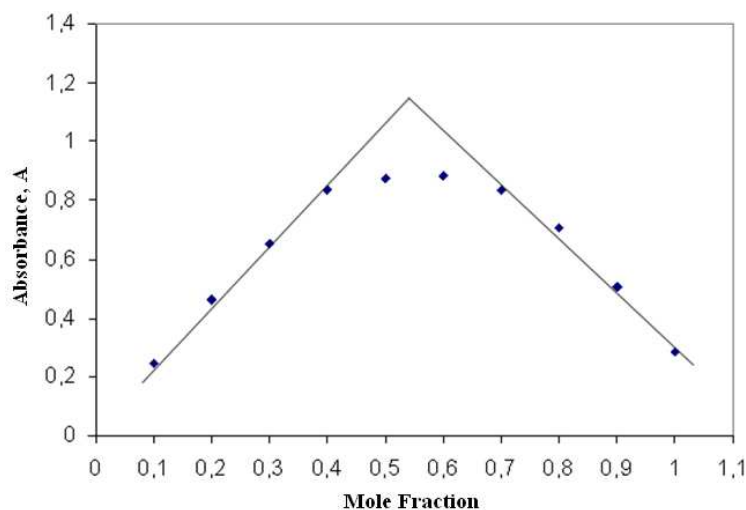


Figure 3.27 Job's curve for the complex between pyrogallol red and Mo(VI) ion

On revealing the stoichiometry of the complex, cyclic voltammetry was used to reveal the mechanism underlying the reduction process. The measurements were done with the GC electrode immersed in 0.1 M pH 3.0 chloroacetic acid buffer solution in a potential range of 0.5 to -2.0 V at a rate of 50 mV/s. The reduction of chloroacetic acid was observed at -1.58 V (Figure 3.28).

After addition of standard solution of Mo(VI) to be 1×10^{-3} M in the cell, a reduction peak at -0.5 V was obtained (Figure 3.29).

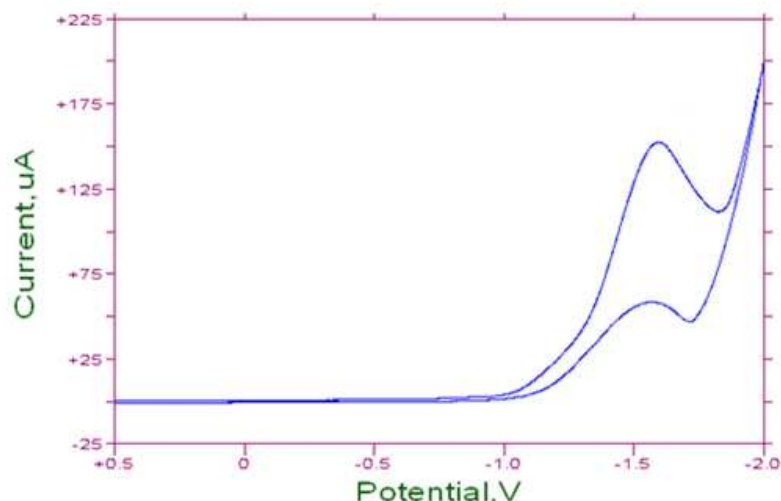


Figure 3.28 Cyclic voltammograms of pH 3.0 chloroacetic acid

Upon addition of PGR into this solution to be 5×10^{-5} M, a reduction peak at -0.55 V and an oxidation peak at -0.36 V was observed for the complex. At this point, chlorate ion was added to be 0.1 M in the cell to see any difference.

As given in Table 1.3, chlorate or bromate ions are used to improve the reduction peak current of the complex in AdCSV studies. Clearly, the combination of effective preconcentration and catalytic reaction improves the sensitivity.

As shown in Figure 3.29, chlorate ion has a profound effect on the complex peak and increased the peak height almost three times. While the potential is scanned in the negative direction, Mo(VI) in the PGR complex is reduced to Mo(V) which is immediately oxidized back to Mo(VI) by the chlorate, completing the catalytic cycle.

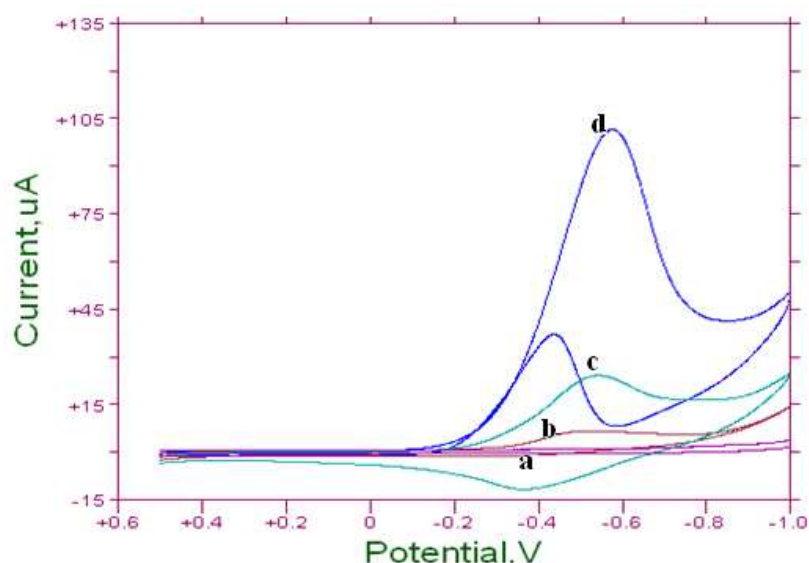


Figure 3.29 Cyclic voltammograms recorded in a) 0.1 M pH 3.0 chloroacetic acid solution and after addition of b) 1×10^{-3} M Mo(VI), c) 5×10^{-5} PGR, d) 0.1 M ClO_3^- , scan rate was 50 mV/s.

Further studies were carried out by using identical four cells made of white Delrin material. The concentration of PGR is an important parameter to be optimized. For this purpose, 5 mL of chloroacetic acid was added to the each cell and then PGR reagent was added in a concentration range of 2×10^{-5} – 1×10^{-4} M. The cell content was spiked with Mo(VI) ions and then centrifugation was performed.

The effect of PGR concentration the complex peak in the presence of 6×10^{-8} M Mo(VI) was given in Figure 3.30. For lower Mo(VI) concentrations, 8×10^{-5} M PGR was used in the cell.

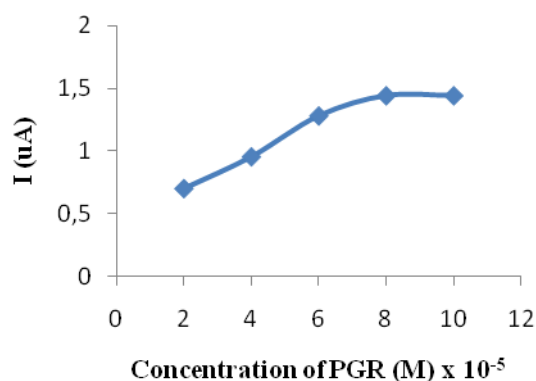


Figure 3.30 The effect of PGR concentration on the peak current of 6×10^{-8} M Mo(VI)

As shown in Figure 3.30, the peak current has increased with PGR concentration in the presence of 6×10^{-8} M Mo(VI) ions and leveled off after 8×10^{-5} M PGR. Even though it was revealed that complex forms in 1:1 ratio, it is necessary to use much higher amount of the ligand to ensure the formation of the complex in the equilibrium and obtain a stable signal in this manner.

Figure 3.31 shows the effect of Mo(VI) ions on the centri-voltammograms of PGR in pH 3.0 chloroacetic acid solution. The peak of 1×10^{-4} M PGR alone was observed at -0.57 V. Upon addition of Mo(VI) to the cell in controlled amount and centrifuging at 2000 rpm for 10 min, this peak has decrease while another peak at -0.48 V related to the complex has appeared. The height of this peak has built up and reached to 1.6 μ A levels at 1×10^{-5} M Mo(VI) concentration while the free ligand peak was disappeared.

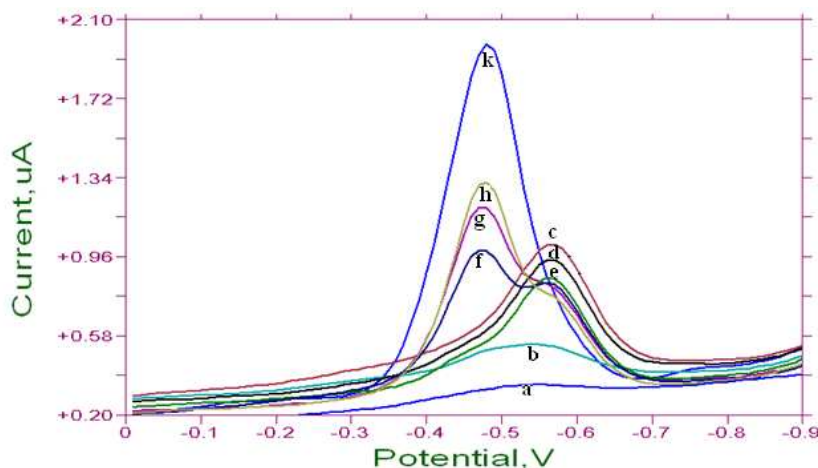


Figure 3.31 Centri-voltammograms of a) pH 3.0 chloroacetic acid, and after addition of PGR to be b) 1×10^{-5} M and c) 1×10^{-4} M and after addition of Mo(VI) ions to be d) 2×10^{-7} M, e) 4×10^{-7} M, f) 6×10^{-7} M, g) 8×10^{-7} M, h) 1×10^{-6} M and k) 1×10^{-5} M in the cell. Centrifugation was made at 2000 rpm for 10 min.

So far the influences of solution characteristics and centrifugation parameters on the complex peak current were investigated. Besides, parameters related to the measurement mode might have a strong effect on the signal as well.

In ASV measurements usually low scan rates, such as 10 mV/s, were employed. However, in AdCSV measurements as the analyte accumulated on the electrode surface as a thin film of a reducible complex, rapid scan of the potential results in the reduction of all assessable species giving higher peak formation (van den Berg, 1986).

Similar to AdCSV, centri-voltammetry provides the accumulation of the complex as a thin film on the electrode surface only with the aid of centrifugation not the electrochemical deposition.

Therefore, the effect of scan rate was studied in a range of 5-100 mV/s for a cell containing 3×10^{-6} M Mo(VI) and 8×10^{-5} M PGR. The results are given in Table 3.1.

Table 3.1 The effect of potential scan rate on the peak current of 3×10^{-6} M Mo(VI) and 8×10^{-5} M PGR

Scan rate (mV/s)	I(μ A)
5	0,18
10	0,26
25	0,29
50	0,38
100	0,52

As can be followed from the table, scan rate of potential has a significant effect on the peak height. On the other hand, high scan rates might alter the peak shape giving a wider peak formation. Therefore, 50 mV/s was chosen for further studies.

The reproducibility of the peak current was tested upon repetitive measurements simultaneously in identical cells for two different PGR concentrations. As can be seen from Table 3.2, method developed a reproducible peak for both concentrations. To maintain the sensitivity, high PGR concentration was preferred in calibration studies.

Under the conditions determined above, centri-voltammetric measurements were performed to obtain a calibration curve for Mo(VI) ion. Figure 3.32 shows the voltammograms obtained for 2×10^{-8} - 1×10^{-7} M Mo(VI) in the presence of 8×10^{-5} M PGR recorded in pH 3.0 chloroacetic acid medium after centrifugation at 2000 rpm for 10 min. The peak currents were plotted against the concentration (Figure 3.33).

Table 3.2 The reproducibility of the peak currents of 2×10^{-6} M Mo(VI)-PGR complex in pH 3.0 medium. Centrifugation was made at 2000 rpm for 10 min.

Cell No	1×10^{-5} M PGR		1×10^{-4} M PGR	
	E (mV)	I (μ A)	E (mV)	I (μ A)
1	-540	0,138	-570	0,575
2	-510	0,126	-570	0,549
3	-560	0,134	-570	0,587
Mean		0,133		0,570
ss		0,006		0,019

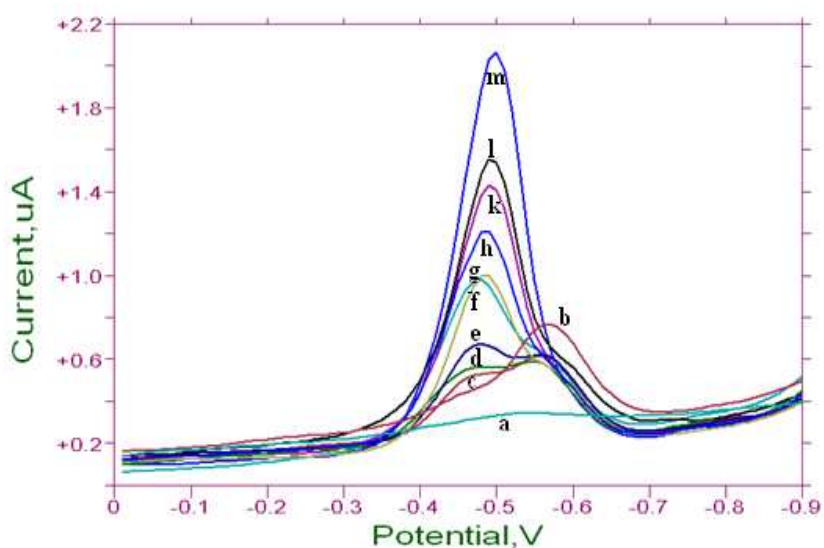


Figure 3.32 Centri voltammograms obtained for a) pH 3.0 chloroacetic acid, b) 2×10^{-8} , c) 4×10^{-8} , d) 6×10^{-8} , e) 8×10^{-8} , f) 1.0×10^{-7} , g) 1.2×10^{-7} , h) 1.4×10^{-7} , k) 1.6×10^{-7} , l) 1.8×10^{-7} and m) 2.0×10^{-7} Mo(VI) in the presence of 8×10^{-5} M PGR in medium after centrifugation at 2000 rpm for 10 min.

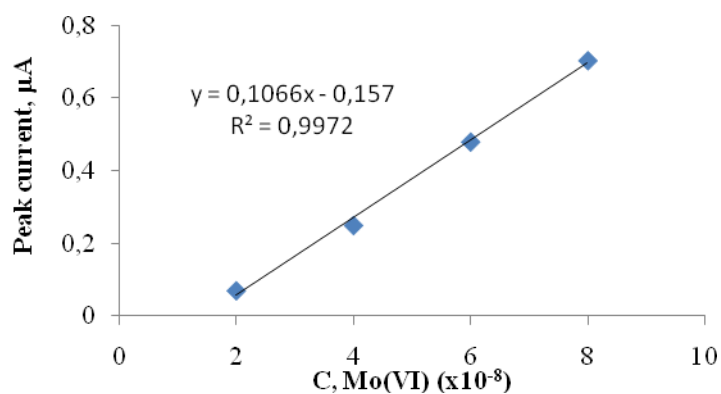


Figure 3.33 Calibration curve for Mo(VI) ion in the presence of 8×10^{-5} M PGR and after centrifuging at 2000 rpm for 10 min.

As clarified earlier, chlorate ion catalysis the reduction of the complex and therefore, another calibration graph in the presence of chlorate ion was constructed and given in Figure 3.34.

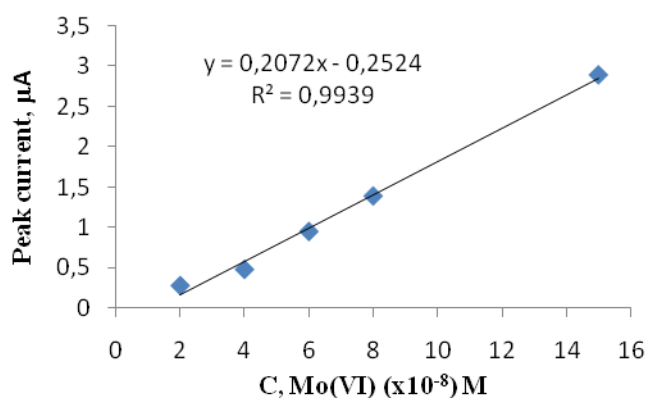


Figure 3.34 Calibration graph for Mo(VI) ion in the presence of 8×10^{-5} M PGR and 0.1 M chlorate ion after centrifuging at 2000 rpm for 10 min.

As can be followed from the figures above, Mo(VI) can be determined at almost nanomolar levels giving a detection limit of 1×10^{-8} M (approximately 1 ng/mL). This sensitivity attained can be satisfactory to determine the molybdenum levels in different matrixes such as milk and tomato food samples given in the literature.

The interference of other ions was also studied by spiking the solution with divalent cations to see their effect. No interference was observed from Pb(II) ion even for a concentration ratio of 1:50 (Figure 3.35 A). As can be seen from the Figure 3.35 B, in pH 3.0 chloroacetic acid medium containing 8×10^{-5} M PGR has given well separated reduction peaks for Cu(II), Pb(II) and Cd(II) ions added in 10 or 50 times of the Mo(VI) ion.

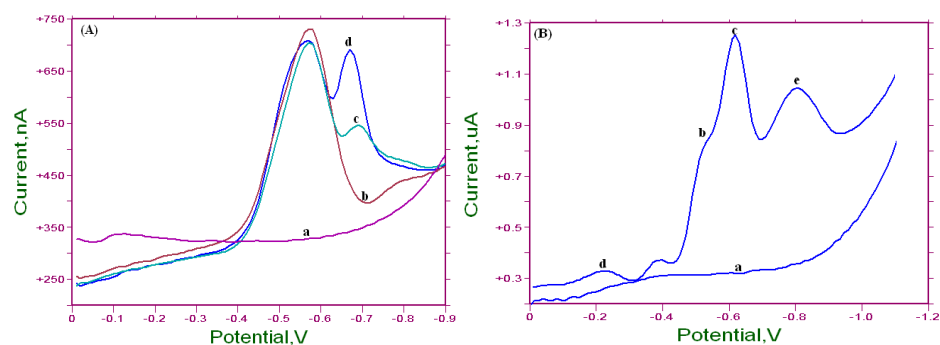


Figure 3.35 Centri-voltammograms of (A) a) pH 3.0 chloroacetic acid, b) 2×10^{-6} M Mo(VI) in the presence of 8×10^{-5} M PGR, c) 7 mg/L d) 15 mg/L Pb(II). (B) a) pH 3.0 chloroacetic acid, b) 2×10^{-6} M Mo(VI) in the presence of 8×10^{-5} M PGR, c) 10 mg/L Pb(II), d) 2 mg/L Cu(II), e) 2 mg/L Cd(II) in the cell.

Centrifugation was made at 2000 rpm for 10 min.

3.1.3 Studies with cupferron

Cupferron is a well known complexing agent for molybdenum as well as several other metal ions. Initial studies were conducted to evaluate the cyclic voltammetric behavior of the complex at a GCE.

The cyclic voltammograms were recorded at pH 3.0 chloroacetic acid medium which was optimized earlier for the Mo(VI)-oxine complex. Figure 3.36 shows the cyclic voltammograms of 1×10^{-3} M Mo(VI) and in the presence of cupferron in a concentration range of $1.25 - 5.0 \times 10^{-4}$ M.

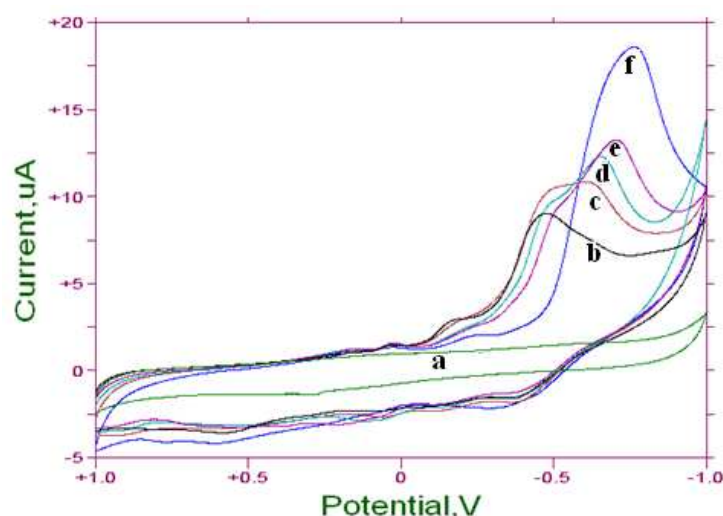


Figure 3.36 Cyclic voltammograms for a) pH 3.0 0.1 M chloroacetic acid, b) after addition of Mo(VI) standard to be 1×10^{-3} M, then addition of cupferron to be c) 1.25×10^{-4} , d) 2.5×10^{-4} , e) 3.75×10^{-4} , f) 5.0×10^{-4} M. Scan rate: 50 mV/s.

As can be followed from the Figure 3.36, Mo(VI) ion has given a peak at -0.45 V. Upon addition of cupferron, this peak has increased in size and has shifted to negative potentials as expected.

Here, it should be noted that cupferron reagent alone has given a reduction peak at -0.65 V and this peak has also increased on addition of Mo(VI) to the solution.

Figure 3.37 shows the Centri-voltammograms recorded for 5×10^{-5} M cupferron and spiking with Mo(VI) ion and after centrifugation in pH 3.0 chloroacetic acid buffer. Upon scanning the potential in negative direction, the reagent alone is reduced at -0.55 V (b) and this peak has increased after 10 min centrifugation at 2000 rpm (c).

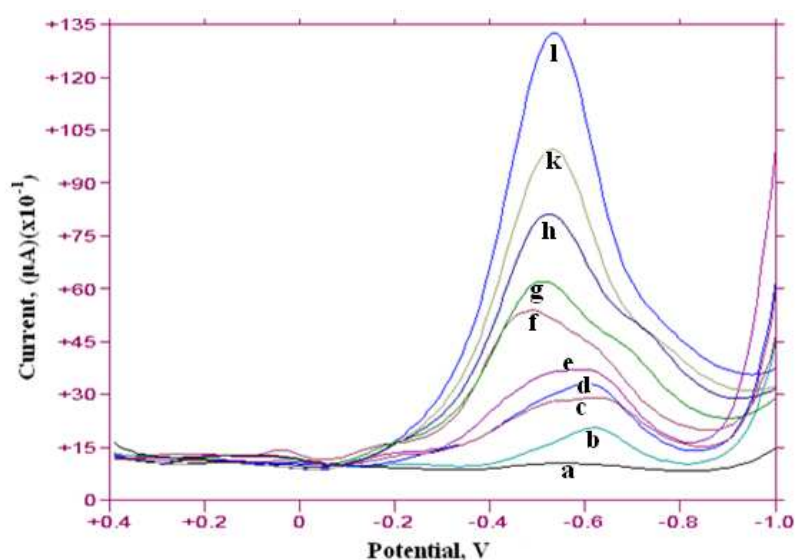


Figure 3.37 Centri-voltammograms obtained for Mo(VI)

Equal amount of Mo(VI) was added to the solution and the peak has given a shoulder at more positive potentials where the Mo(VI) alone reduces (d) and 10 min centrifugation has resulted in a suppressed peak formation (e).

After addition of Mo(VI) and cupferron standards, a decrease on this shoulder was observed (f) and this shoulder has built up upon centrifugation at increased rate or time (g-l). Data obtained from Figure 3.37 were listed in Table 3.3.

Table 3.3 The peak characteristics of the Centri-voltammograms in Figure 3.37

No	The cell content and procedure	E_p (mV)	I_p (μ A)
a	Blank (0.1 M pH 3.0 chloroacetic acid)	-	-
b	Blank + 5×10^{-5} M cupferron	-620	1,05
c	centrifugation at 2000 rpm for 10 min	-610	1,85
d	+ 5×10^{-5} M Mo(VI)	-510	1,47
e	centrifugation at 2000 rpm for 10 min	-500	2,50
f	+ 1×10^{-4} M cupferron + 1×10^{-4} M Mo(VI)	-480	3,57
g	centrifugation at 2000 rpm for 10 min,	-510	4,29
h	centrifugation at 2500 rpm for further 10 min,	-520	6,10
k	centrifugation at 2500 rpm for further 15 min,	-530	7,59
l	centrifugation at 2500 rpm for further 20 min,	-530	10,62

As pointed out in a previous study carried out with HMDE, first electron transfer reaction of Mo(VI) to Mo(V) is shifted slightly to negative potentials in the presence of cupferron due to the complexation (Sander, 1999). The second reduction step of Mo(V) to Mo(III) in the complex occurs at more negative potentials (about -0.75 V).

The reason for the splitting of this signal was deduced as a separation of the two-electron process into two one-electron processes, or, a reduction peak of the excess cupferron present. Similarly, the peak at -0.5 V has a shoulder at more negative potentials indicating the reduction of the ligand itself.

Calibration graph for Mo(VI) in the presence of 5×10^{-5} M cupferron was constructed and given in Figure 3.38.

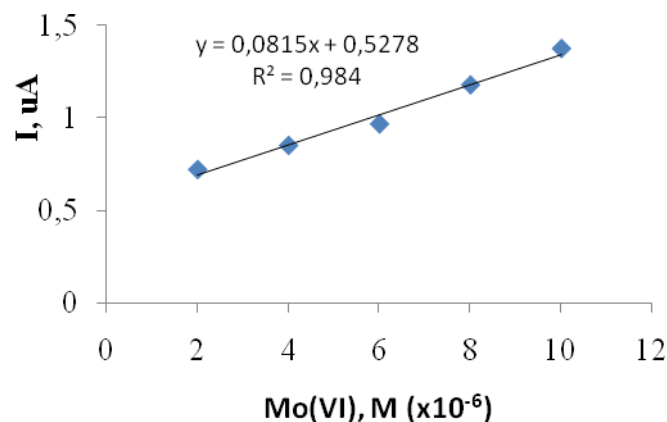


Figure 3.38 Calibration graph for Mo(VI) in the presence of 5×10^{-5} M cupferron after centrifuging at 2000 rpm for 10 min

According to the results above, the increase in height of the shoulder on the peak at -0.53 V indicated that Mo(VI) alone can give a reduction peak and therefore, an experiment was designed to see the effect of centrifugation on the reduction peak of molybdenum ion alone. The results of this study were given in Section 3.2.

3.1.4 The comparison and Application of the Methods

The methods developed for Mo(VI) were compared each other and characteristics of the calibration graphs were given in Table 3.4. According to their limit of detection levels, the method that utilize PGR reagent were found more sensitive.

Table 3.4 Analytical characteristics of the centri-voltammetric methods developed for molybdenum ion determination

Reagent	Linear range (M)	Equation	R ²	LOD (M)
Oxine	2×10^{-6} - 1×10^{-5}	$y = 0,070x + 0,035$	0,971	1×10^{-6}
Cupferon	2×10^{-6} - 1×10^{-5}	$y = 0,081x + 0,528$	0,984	1×10^{-6}
PGR	2×10^{-8} - 1×10^{-7}	$y = 0,106x - 0,157$	0,9972	1.4×10^{-8}
PGR + chlorate	2×10^{-8} - 1.5×10^{-7}	$y = 0,207x - 0,252$	0,9939	1×10^{-8}

Although, the AdCSV methods depending on electrochemical deposition on a mercury drop have given better detection limits, the use of such toxic electrode materials are not preferred lately. Besides, these methods suffer from interference of some metal ions (Ensafi, 2005).

The selectivity of the method was tested upon addition of several metal ions in the cell those can form complexes with PGR as given above and it was stated that potentially interfering ions namely copper, lead and cadmium have given a well separated peak and no suppression of the molybdenum peak was observed for 10 to 50 fold concentration ratio.

Glassy carbon electrode offers a non-toxic and flat surface available for centrifuging. The cell volume can be increased and therefore, the sensitivity can be improved as the analyte driven to the electrode surface from the bulk of the solution.

The method was applied to the milk samples which are known to contain a certain amount of molybdenum ions probably due to the enzyme structure.

Figure 3.39 shows the standard addition centri voltammograms obtained for milk samples. The sample was treated as given in experimental section and then, centri-voltammetric procedure was applied.

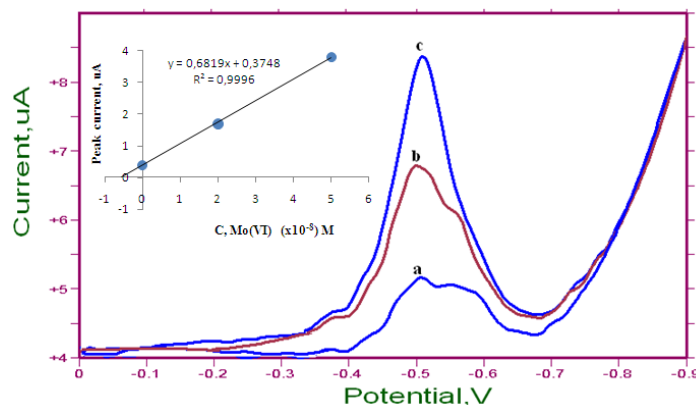


Figure 3.39 Centri-voltammogram of a) milk sample, after addition of Mo(VI) standard to be b) 2×10^{-7} M and c) 5×10^{-7} M in the cell and resulting standard addition calibration curve.

As can be seen from the figure, standard addition graph was constructed and baseline correction was made. The results were found in agreement being 0.132 ng/mL for centri-voltammetry and 0.136 ng/mL for ICP-MS.

The calibration curve for ICP-MS was found linear in a range of 5-100 ng/mL ($R^2 = 0.9988$) and detection limit was calculated as 2.15 ng/mL. Germanium ion was used as internal standard. The recovery ratio for ICP-MS was also calculated as 98% for addition of 25 ng/mL Mo(VI) into milk sample.

3.2 Polyoxomolybdate Formation in Aqueous Solution

In the progress of centri-voltammetric studies with molybdate ions, a rather unusual peak formation was noted in the absence of any complexing reagent. Centrifugation has unexpectedly resulted a peak grow in chloroacetic acid medium and this occurrence was found worth to study in detail as given below.

3.2.1 Centri-voltammograms of Mo(VI) in Chloroacetic acid

The peak characteristics of Mo(VI) alone depending on its concentration and centrifugation process were investigated at a GCE in centri-voltammetric cell. After recoding the voltammograms of 0.1 M chloroacetic acid solution (pH 3.0), the solution was spiked with Mo(VI) standard solution and then, the potential was readily scanned and after the centrifugation in negative directions (Figure 3.40). Chloroacetic acid itself reduces at the GCE but, no interference was observed as the peak takes place at very negative potentials; around -1.2 V.

Rather high concentrations of Mo(VI) were added to the cell in order to clarify the effect of centrifugation undoubtedly. Two reduction peaks were observed for Mo(VI) at -0.15 and -0.45 V. Interestingly, the peak current increases after 10 min centrifugation at 2000 rpm. This finding shows us Mo(VI) ions somewhat deposited on the electrode surface without any complexing reagent. This phenomenon was investigated in detail by changing the main parameters and observing the corresponding changes.

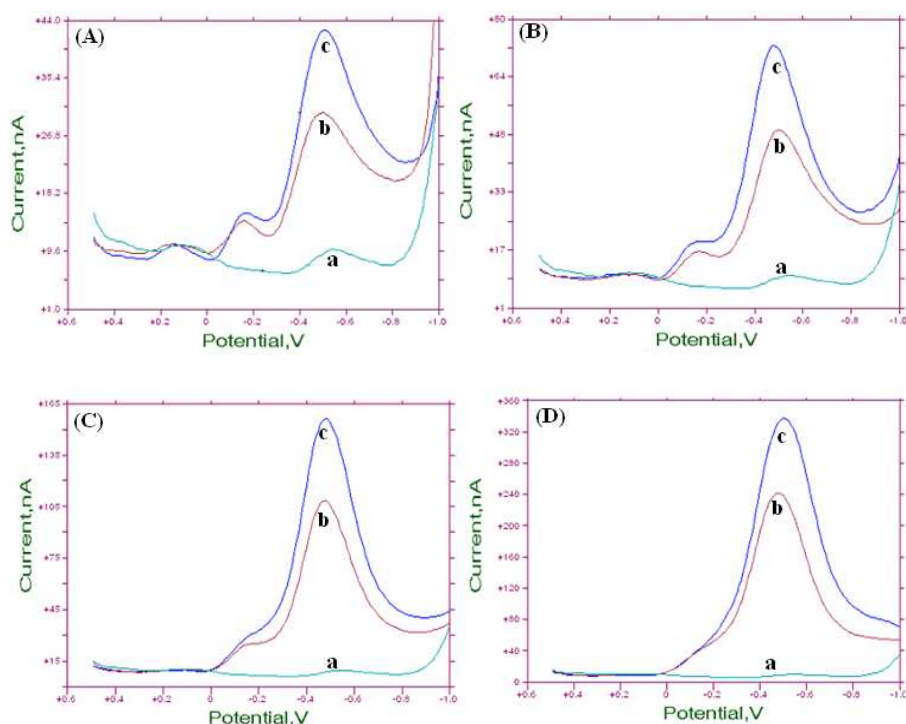


Figure 3.40 Centri-voltammograms obtained for Mo(VI) alone recorded in pH 3.0 chloroacetic acid (a) and direct potential scan after addition of Mo(VI) to be A) 40, B) 60, C) 80 D) 100 mg/L Mo(VI) in the cell (b), and after centrifugation at 2000 rpm for 5 min (c) at GCE.

On the other hand, as given in Table 1.3, ClO_3^- ions were used in several studies since they lead a catalytic effect on the Mo(VI) complex with organic reagents. Here, the effect of chlorate ions on the reduction peak of Mo(VI) ion alone was investigated by centri-voltammetry. The solution containing 100 mg/L Mo(VI) was spiked with 0.01 M chlorate ion and it was centrifuged and then voltammograms were recorded (Figure 3.41).

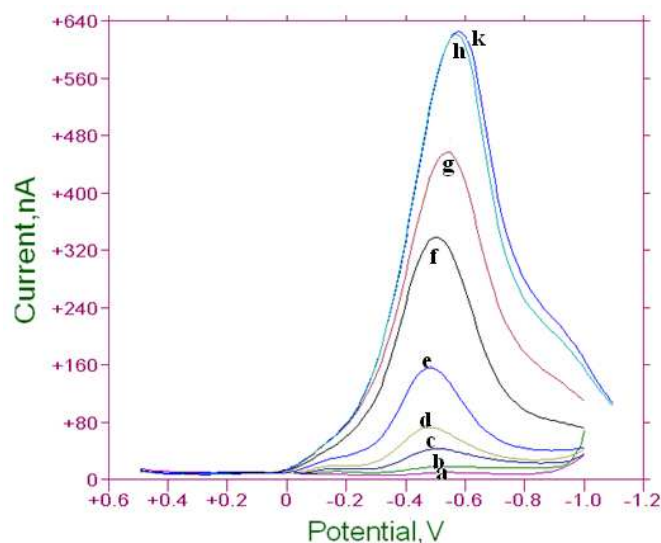


Figure 3.41 Centri-voltammograms obtained for a) 0.1 M pH 3.0 chloroacetic acid, after addition of Mo(VI) to be b) 20, c) 40, d) 60, e) 80 and f) 100 mg/L and after centrifugation at 2000 rpm for 5 min and on addition of ClO_3^- to be g) 0.01 M, h) 0.05 M, and k) further centrifugation at 2000 rpm for 5 min.

As can be seen from Figure 3.41, the peak currents have noticeably enhanced indicating a catalytic effect on reduction of molybdenum. Overall data were summarized in Table 3.5.

Figure 3.42 shows the effect of ClO_3^- concentration in a wider range on the cyclic voltammograms of Mo(VI) in pH 3.0 chloroacetic acid medium (CAA). As can be seen from the Figure 3.41, the peaks were improved in the presence of increasing amount of chlorate ion while the peak potential shift in negative direction. The data from the figure was listed in Table 3.6.

Table 3.5 Peak characteristics of Mo(VI) from Figure 3.41

Cell content	Cent.*	E (mV)	I (nA)	ΔI (nA)
pH 3.0 Chloroacetic acid	-	-540	2,94	
20 mg/L Mo(VI)	-	-520	3,12	
20 mg/L Mo(VI)	+	-520	5,38	2,26
40 mg/L Mo(VI)	-	-480	14,89	
40 mg/L Mo(VI)	+	-500	24,74	9,85
60 mg/L Mo(VI)	-	-500	30,91	
60 mg/L Mo(VI)	+	-480	49,49	18,58
80 mg/L Mo(VI)	-	-480	80,83	
80 mg/L Mo(VI)	+	-480	121,9	41,07
100 mg/L Mo(VI)	-	-480	192,6	
100 mg/L Mo(VI)	+	-500	271,5	78,9
100 mg/L Mo(VI) + 0.01 M ClO_3^-	-	-540	349,3	77,8
100 mg/L Mo(VI) + 0.05 M ClO_3^-	-	-570	479,3	207,8
100 mg/L Mo(VI) + 0.05 M ClO_3^-	+	-570	472,5	-6,8

*Centrifugation at 2000 rpm for 5 min

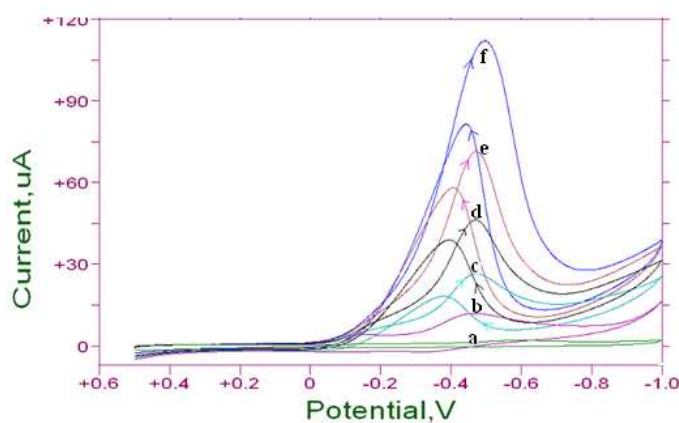


Figure 3.42 Cyclic voltammograms for a) 0.1 M pH 3.0 chloroacetic acid, b) after addition of 1×10^{-3} M Mo(VI), and then after spiking the solution with ClO_3^- ions to be c) 0.01M d) 0.05 M, e) 0.1 M and f) 0.15 M in the cell.

Table 3.6 Effect of ClO_3^- concentration on the peak reduction of Mo(VI)

No	Cell Content	E_{peak} (mV)	I (μA)
a	pH 3.0 CAA	-	-
b	0.001 M Mo(VI)	-468	4,73
c	0.01 M ClO_3^-	-465	12,4
d	0.05 M ClO_3^-	-463	21,5
e	0.10 M ClO_3^-	-465	35,2
f	0.15 M ClO_3^-	-487	66,8

Chlorate concentration has displayed a profound effect on the peak current. As the results have shown, the peak currents increase proportionally with increasing chlorate concentration up to 0.15 M.

On the other hand, a reverse peak formation was observed on anodic scan in the presence of the catalyst. This peak is probably related to the reduction of a product during the potential scan which is still in cathodic region. Indeed, this is a typical case for most of the catalytic reactions but, no satisfactory explanation was observed in the literature.

Furthermore, chloroacetic acid itself might play a role in the catalysis. In order to reveal this effect, an experiment was designed to compare the performances of the GCE with gold disc electrode.

Glassy carbon and gold electrodes were polished and cyclic voltammograms of chloroacetic acid were recorded at both electrodes by scanning the potential from 0.0 V to - 1.2 V or to -1.8 V. Scan rates were changed in a range of 9 - 400 mV/s (Figure 3.43 and Figure 3.44).

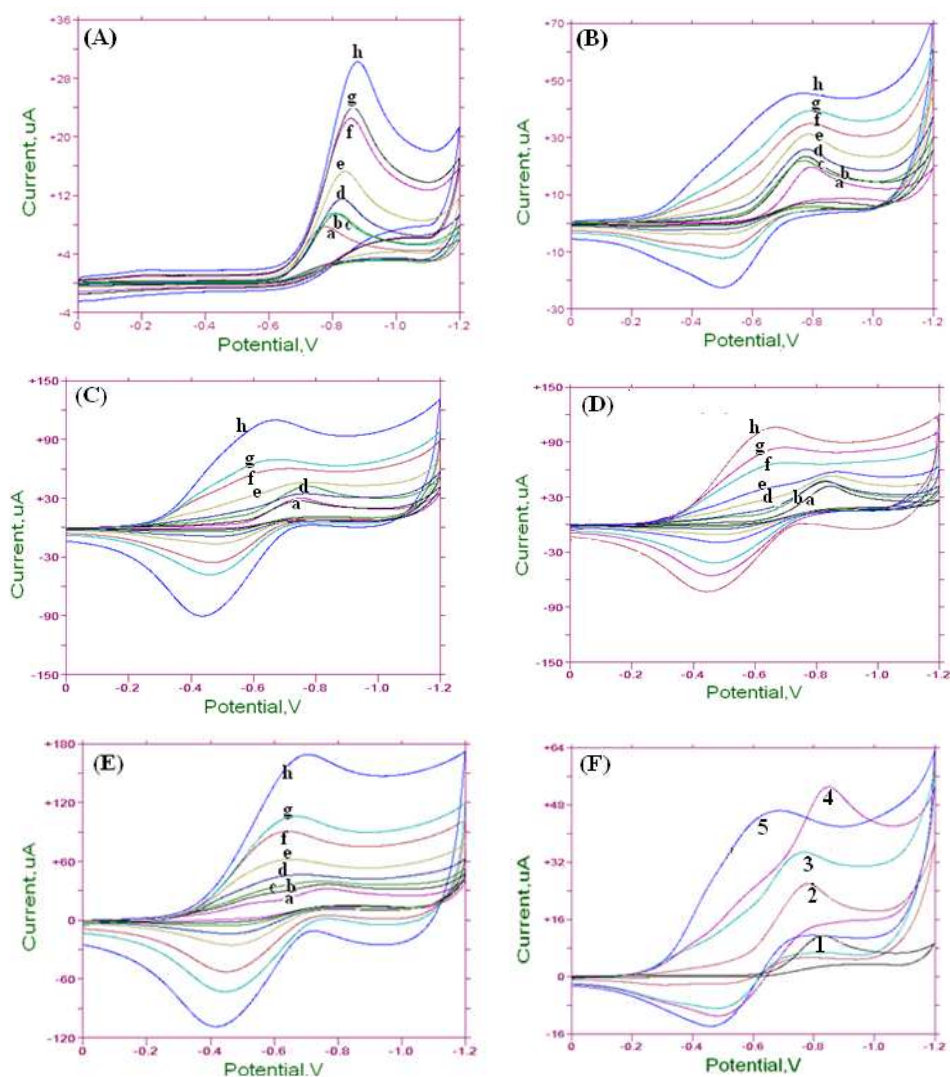


Figure 3.43 Cyclic voltammograms of A) pH 4.0 chloroacetic acid, after addition Mo(VI) to be B) 2×10^{-4} M, C) 4×10^{-4} M, D) 6×10^{-4} M, E) 8×10^{-4} M and then addition of chlorate to be 0.01 M at scan rates of (a-h) 9, 16, 25, 49, 81, 169, 225 and 400 mV/s and F) overlaid voltammograms recorded at, 1) pH 4.0 CAA, 2) 2×10^{-4} , 3) 4×10^{-4} , 4) 6×10^{-4} M Mo(VI) and 5) 0.01 M ClO_3^- at a gold disc electrode with a scan rate of 49 mV/s.

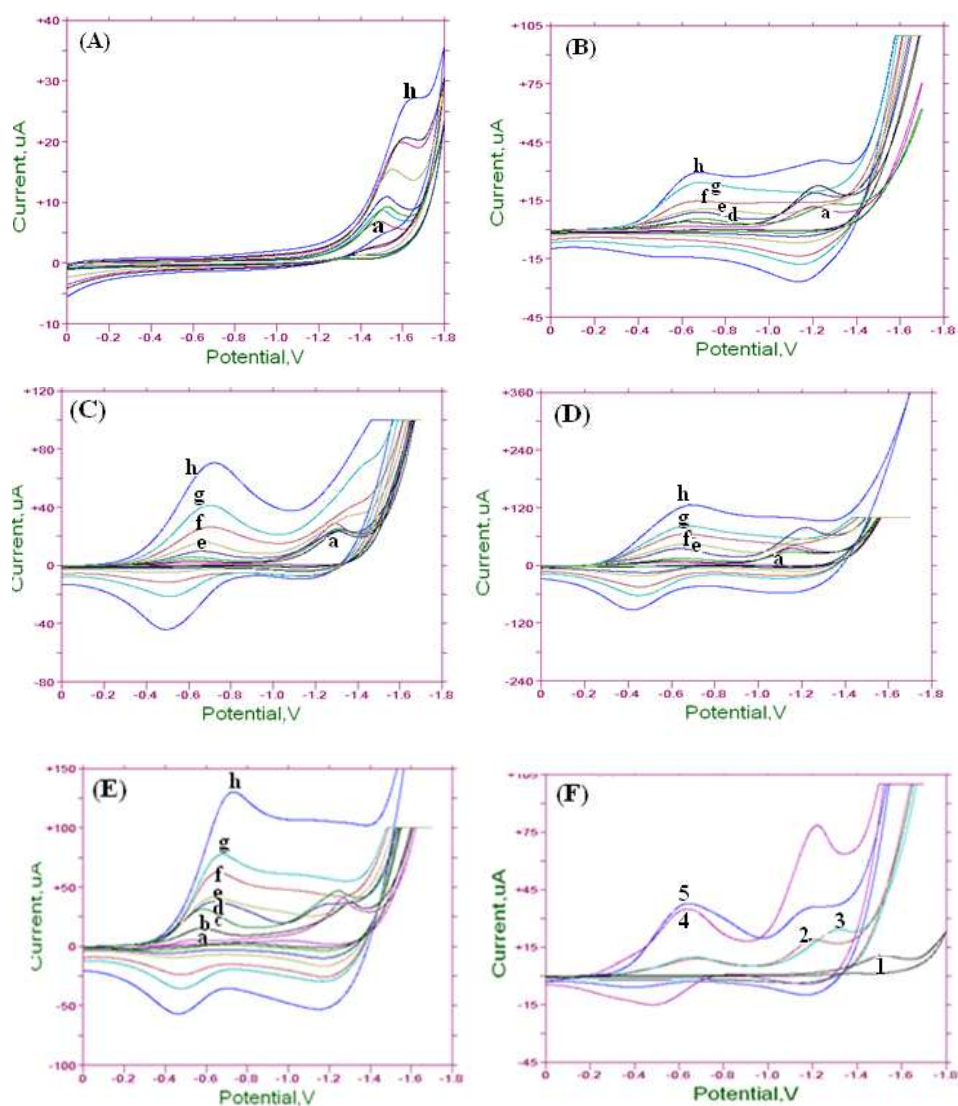


Figure 3.44 Cyclic voltammograms of A) pH 4.0 chloroacetic acid, after addition Mo(VI) to be B) 2×10^{-4} M, C) 4×10^{-4} M, D) 6×10^{-4} M, E) 8×10^{-4} M and then addition of chlorate to be 0.01 M at scan rates of (a-h) 9, 16, 25, 49, 81, 169, 225 and 400 mV/s and F) overlaid voltammograms recorded at, 1) pH

4.0 CAA, 2) 2×10^{-4} , 3) 4×10^{-4} , 4) 6×10^{-4} M Mo(VI) and 5) 0.01 M ClO_3^- at a GCE with a scan rate of 49 mV/s.

As shown in Figure 3.43 and 3.44, peak currents tend to grow with increasing scan rate. The reduction peak of chloroacetic acid on gold electrode was observed at more positive potentials than GC electrode. Upon addition of Mo(VI) ions into the cell, the peak was noticeably increased. To prove any catalytic effect, the dependence of peak currents and the potentials on the scan rate was investigated to perform the diagnostic tests. Peak current/square root of scan rate ratios ($I_p/v^{1/2}$) were plotted against the scan rate (v) and as shown in Figure 3.45 and 3.46 a decrease with scan rate was obtained. This change proves the catalytic effect of chloroacetic acid on the reduction of Mo(VI) ion.

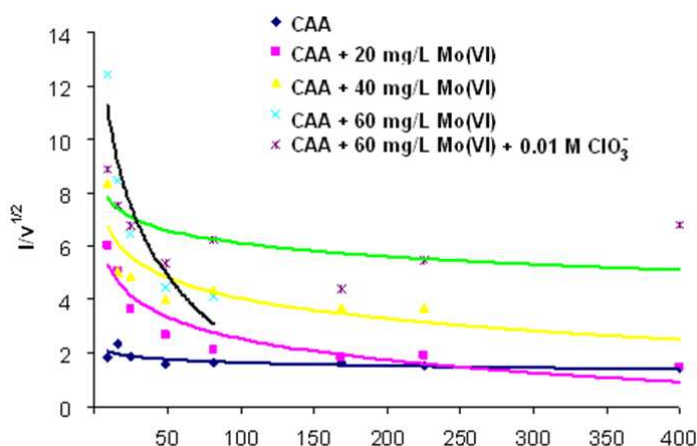


Figure 3.45 Dependence of peak current of Mo(VI) in the presence of chloroacetic acid on the scan rate at gold disc electrode

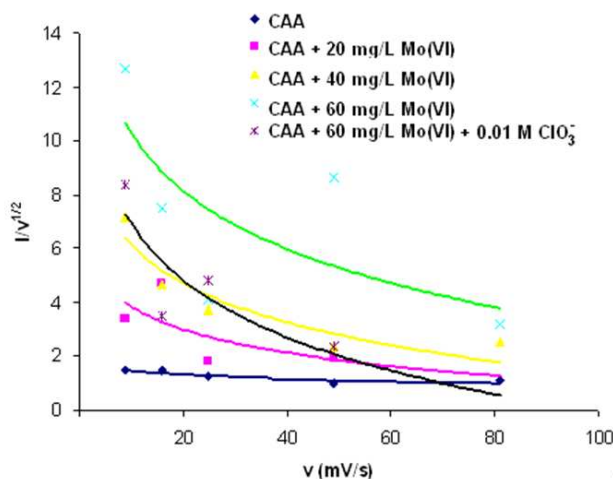


Figure 3.46 Dependence of peak current of Mo(VI) in the presence of chloroacetic acid on the scan rate at glassy carbon electrode

Figure 3.47 shows the cyclic voltammograms recorded for 5×10^{-5} M Mo(VI) in pH 3.0 chloroacetic acid medium and shows the effect of time passed during centrifuge step. The data listed in Table 3.7 clearly indicates formation of a self assembled structure on the electrode surface by time.

This phenomenon differs in some aspect from the previous studies as no complexing reagent was added to the cell. Here, a self established structure on the electrode surface via reduction of Mo(VI) to Mo(V) at the GCE surface catalyzed by the cell composition.

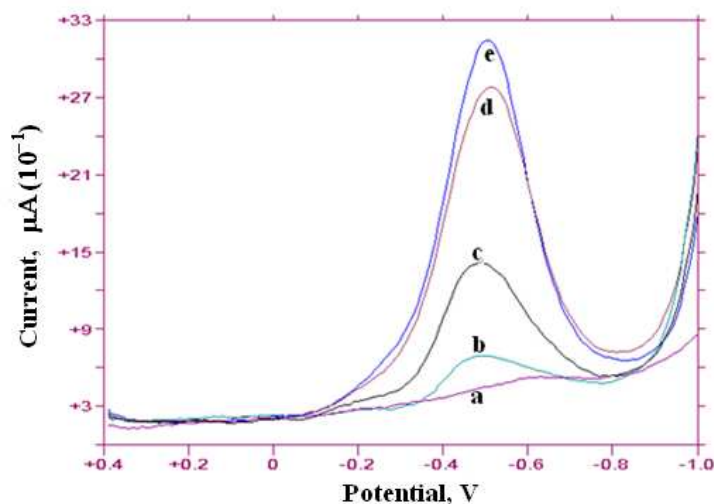


Figure 3.47 Centri-voltammograms obtained at GCE in a) pH 3.0 chloroacetic acid, b) after addition Mo(VI) to be 5×10^{-5} M and after centrifuging at 2000 rpm for c) 10 min, d) 15 and e) 20 min

Table 3.7 The effect of centrifuge time on the peak current of molybdenum species in chloroacetic acid medium at a GCE.

No	Reagent	E_p (mV)	I_p (nA)
a	0.1 M pH 3.0 chloroacetic acid	-610	0,70
b	5×10^{-5} M Mo(VI)	-480	3,12
c	centrifugation at 2000 rpm for 10 min,	-490	10,18
d	centrifugation at 2000 rpm for 15 min,	-510	22,57
e	centrifugation at 2000 rpm for 20 min,	-500	26,60

This unusual increase on the reduction peak indicates that Mo(VI) ions somewhat deposited on the electrode surface without any complexing reagent or precipitation reactions. Besides, the persistent of

the residue at the electrode surface even polishing with alumina slurry has proved that the product strongly attached to the electrode surface.

This phenomenon has led us to examine the possibility of a self assembled structure via reduction of Mo(VI) to Mo(V) at the GCE surface. A reasonable explanation for this establishment is the polyoxomolybdate (POM) formation at GCE surface enhanced by the driving force of centrifugation.

The experiment was repeated at pH 2.0 HCl medium to see the effect of acidity onto the residue formation. Much broader peaks (> 400 mV) were observed at around -0.45 V with higher peak currents. It was observed that HCl medium was not satisfactory to clear off the GCE surface and then, nitric acid was utilized for surface cleaning.

Figure 3.48 shows the decline of the current by time in the presence of 0.1 M HNO_3 . The electrode potential was affected at 1.0 V and the current was high at the beginning and then diminished by stripping the deposited structures from the surface to background current in 50 s.

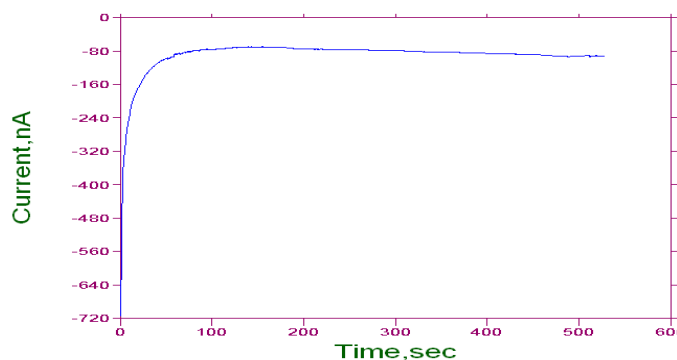


Figure 3.48 Chronoamperograms of GCE with self assembled structure recorded in 0.1 M HNO_3 at 1.0 V

In the light of the literature, POM is formed in solutions at room temperature by simply adding the appropriate reagents and allowing the self assembled structure. Therefore, a study was intended to see the effect of time on the formation of POM species on the cyclic voltammograms without centrifugation (Figure 3.49). Data were summarized in Table 3.8.

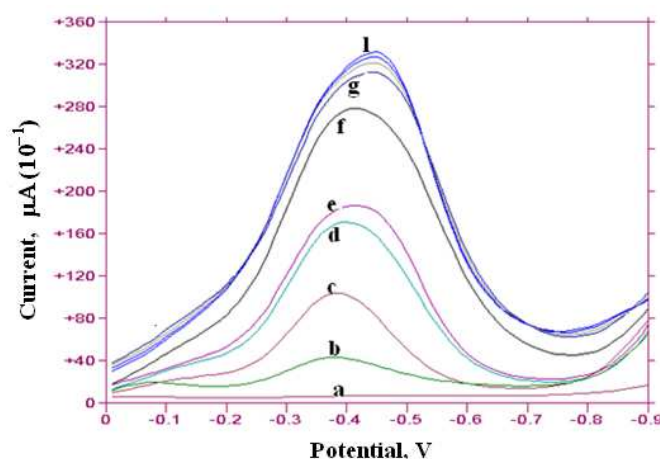


Figure 3.49 Centri-voltammograms obtained for a) pH 2.0 HCl medium, b) after addition of Mo(VI) to be 2×10^{-4} M and then waiting for c) 10, d) further 10 min, e) successive scan and f) waiting for further 10 min, g-l) successive scans

Table 3.8 Data from Figure 3.49

No	Reagent and procedure	E_p (mV)	I_p (nA)
b	2×10^{-4} M Mo(VI)	-380	27
c	waiting for 10 min,	-390	87,4
d	waiting for 10 min,	-400	150,7
e	2.scan	-420	163
f	waiting for 10 min,	-410	239,1
g	2.scan	-440	250,8
h	3.scan	-440	259,9
k	4.scan	-440	265,8
l	5.scan	-440	265,8

Elucidation of the peak values reveals that extent of self assembled structure is strongly time dependent. According to Table 3.8, 2×10^{-4} M Mo(VI) has given 27 μ A peak current and this current has increased three times simply waiting for 10 min and then doubled for further 10 min. The peak has grown almost ten times by waiting half an hour. This can be a sign of the formation of self assembled structure on the electrode surface, in the presence or absence of centrifugation this indicates a kinetic process which proves formation of a self-assembled supramolecular structure as is the case for POMs.

This procedure might serve as a novel way for POM formation on the electrode surface. Indeed, as far as we have searched, many techniques were tested to immobilize the POMs synthesized chemically on the electrode surface. Here, electrochemical synthesis has been accomplished in chloroacetic acid medium by simply cycling the potential from 0 V to -0.9 V with a rate of 50 mV/s.

On the other hand, to see any difference of this procedure including cycling the potential, from other deposition processes such as adsorptive stripping, a -0.5 V potential was applied between the electrodes for 10 min prior to the scan from 0 V to -0.9 V with a rate of 50 mV/s.

Figure 3.50 shows the effect of deposition step on resulting DP stripping voltammograms. It can be concluded that self assembled POM formation results in similar size peak formation with adsorptive stripping technique. However, the peak potentials differ about 40 mV indicating a different reduction mechanism is involved.

Here, it should be noted that the solution content is also important for self assembled structure formation. Slower developments of the peaks by time along with their smaller sizes in HCl solution have indicated a somewhat different surface reaction is undergone. Clearly, chloroacetic acid has a substantial effect on the peak characteristics.

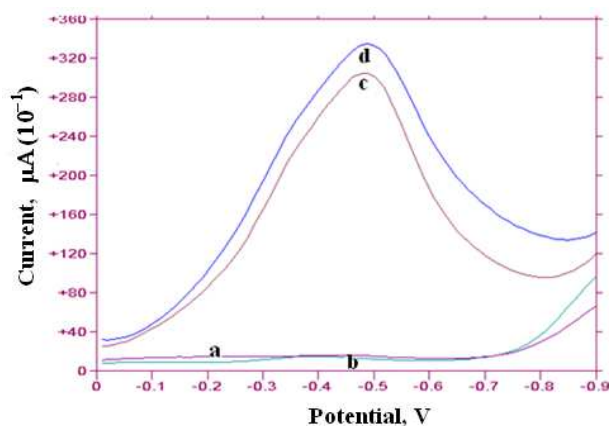


Figure 3.50 DP voltammograms obtained in a) pH 2.0 HCl, b) 2×10^{-4} M Mo(VI), c) deposition at -0.5 V for 10 min, d) further deposition for 10 min.

The effect of centrifugation speed was also tested. Figure 3.51 shows the DP voltammograms obtained after centrifugation of 5×10^{-5} M Mo(VI) solution at different speed values for 10 min. As can be seen from the figure, the peak heights and areas have increased upon increasing the speed as expected. Higher speeds have helped to develop self assembled structure on the electrode surface. The results were summarized in Table 3.9.

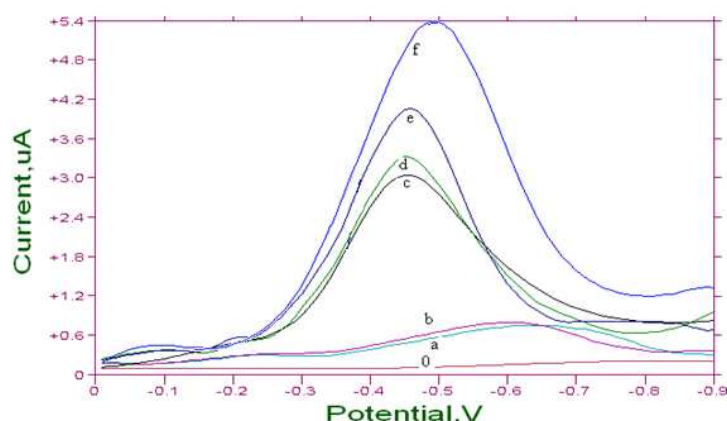


Figure 3.51 Centri-voltammograms of 5×10^{-5} M Mo(VI) in pH 3.0 chloroacetic acid medium after centrifuging at a) 1000, b) 1500, c) 2000, d) 2500, e) 3000 and f) 3500 rpm for 10 min.

Table-3.9 The dependence of peak currents on centrifugation speed

No	Speed (rpm)	E_p (mV)	I_p (μ A)
a	1000	-640	0,46
b	1500	-610	0,46
c	2000	-450	2,47
d	2500	-450	2,74
e	3000	-460	3,36
f	3500	-490	4,60

As can be seen from the table given above, the peak has grown by increasing centrifugation speed. However, considering the time elapsed during the centrifugation, another experiment was designed to see clarify this dual effect and four cells have been used to obtain time dependent voltammograms in parallel with and without centrifugation. Figure 3.52 shows the related voltammograms.

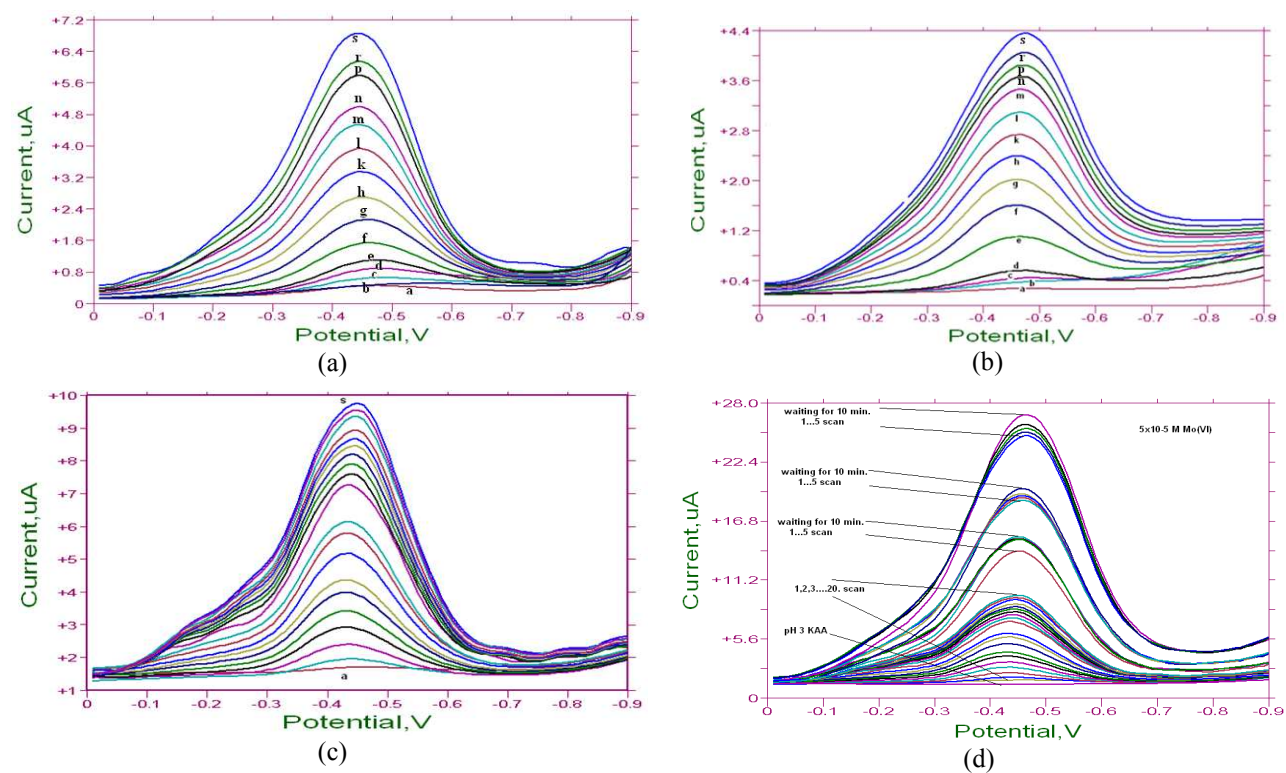


Figure 3.52 Time dependent DP voltammograms of 2×10^{-5} M Mo(VI) a) every ten min, b) in the presence of centrifugation at 2000 rpm for 10 min. c) successive 20 scan, d) successive scan with waiting periods for 5×10^{-5} Mo(VI).

As can be deduced from the time-dependent voltammograms, centrifugation might hinder the POM formation and the peak current was found lesser in comparison to that of without centrifugation. Furthermore, successive scan of 5×10^{-5} M of Mo(VI) have resulted even larger peak currents. Considering that each scan takes 25 s, the increase in the peak current is much larger than the voltammograms recorded every ten min. This reflects the effect of potential cycling on POM formation. Then, the cell was disconnected for 10 min and successive voltammograms were recorded afterwards (Figure 3.52 d).

The increase in the peak current has continued and after one and a half hours, the peak current has started to diminish and the current-time profile was given in Figure 3.53. This could be probably due to the excess coverage of the surface by self assembled POM structure and therefore, electron transfer is hindered.

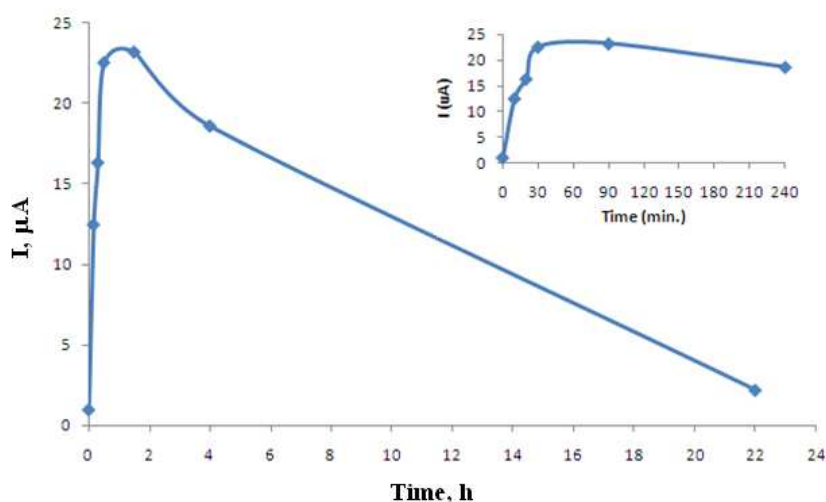


Figure 3.53 Time dependent change in peak current of 5×10^{-5} M Mo(VI) in pH 3.0 chloroacetic acid medium at a GCE

Here, it might be questioned any effect of POM formation on the centri-voltammetric determination of Mo(VI) in the presence of a complexing reagent. To clarify the effect of this formation, time dependent voltammograms were recorded in the presence of equal concentrations of PGR and Mo(VI) being 1×10^{-4} M in pH 3.0 chloroacetic acid medium. Figure 3.54 shows the peak current at -0.48 V corresponding to the reduction peak of the complex.

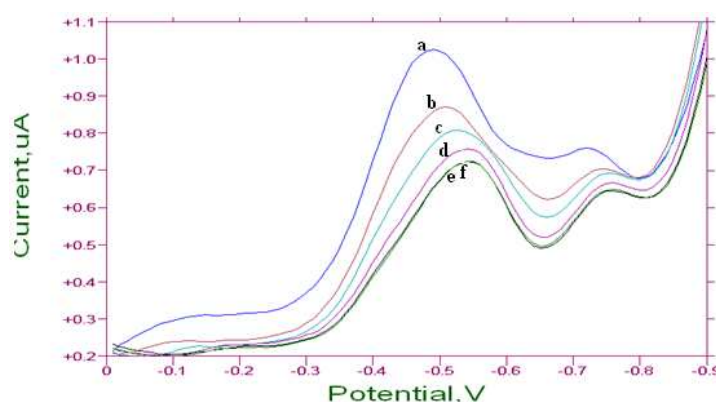


Figure 3.54 Successive DP voltammograms of 1×10^{-4} M Mo(VI) in the presence of equal concentration of PGR in pH 3.0 chloroacetic acid medium.

Successive scans given above have revealed that the peak actually decreases in the presence of a complexing reagent and therefore, it was concluded that Mo(VI) cannot form POM on the electrode surface as the free molybdenum ion has undergone complexation.

On the other hand, former studies carried out with HMDE have not published any data reporting a self assembled structure. This is the first time electrochemical synthesis of POM structure was reported for a GCE.

We believe that surface of GCE serve as a pattern for establishment of such a supramolecular structure (Figure 1.6) in the presence of chloroacetic acid and cycling the potential between 0 V to negative potentials. It can be proposed that OH groups on the GCE facilitates as interaction sites where Mo(VI) ions build up POM structure.

Thus, the structures assembled on the electrode surface should be characterized with several techniques. Prior to these characterization studies, the chemical synthesis was also accomplished to compare the cyclic voltammetric behavior of chemical and electrochemical synthesized POM structures.

3.2.2 Chemical Synthesis of POMs in Aqueous Solutions

Further experiments were focused on the POMs chemically obtained in solution phase. The synthesis procedure was followed as described in the literature (Müller, 1998). Mo(VI) standard solution was added to 1:1 diluted acetic acid solution containing ammonium acetate and hydrazine sulfate. The mixture was stirred for 10 min and it was noted that solution has changed from colorless to bluish green. Then, the solution was left open for the reaction without stirring at around 20°C. The color of the solution changed slowly to dark brown and the precipitate began to appear within few hours.

The POM was filtered, air dried and then weighted. A small portion was dissolved in 50 mL of ultrapure water and an aliquot of this solution (0.1 mL) was injected into pH 3.0 chloroacetic acid as supporting electrolyte and cycled between 0.5 and -0.9 V potential range.

The scan rate was changed in a range of 9 - 625 mV/s to clarify the reduction mechanism. Figure 3.55 shows the effect of scan rates on the reduction peaks of chemically obtained POMs. The data were listed in Table 3.10.

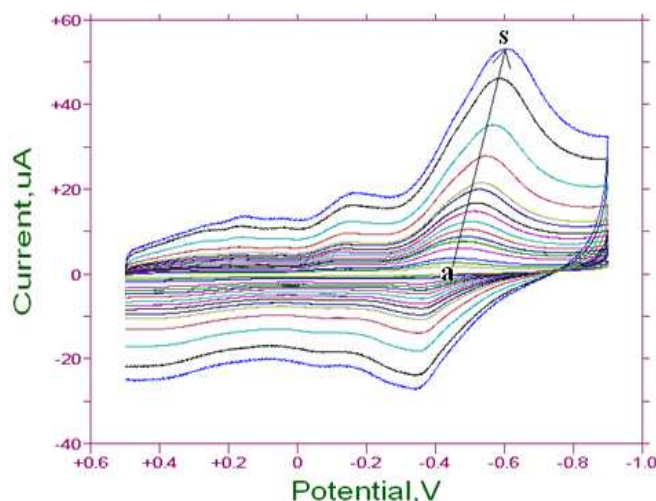


Figure 3.55 Cyclic voltammograms of 40 mg/L POM in pH 3.0 chloroacetic acid medium at various scan rates (a to s: 9 to 625 mV/s)

The peak potential has shifted from -424 to 600 mV as the scan rate increases, indicating irreversibility of the reduction in some degree. Besides, additional peak can be observable at -0.2 V at high scan rates. This peak was also reported in former studies carried out in sulphate solutions (Huang, 2008).

The peak currents were plotted against scan rate as given in Figure 3.56. Linear relationship with scan rate indicates that reduction is adsorption controlled.

Table 3.10 Effect of scan rates on the POM peak current.

No	mV/s	$v^{1/2}$	$E_p(\text{mV})$	$I(\mu\text{A})$	$I/v^{1/2}$
a	9	3	-424	0,815	0,272
b	16	4	-429	1,340	0,335
c	25	5	-445	2,027	0,405
d	49	7	-476	3,736	0,534
e	64	8	-472	4,441	0,555
f	81	9	-483	5,554	0,617
g	100	10	-497	6,782	0,678
h	125	11	-506	7,955	0,712
i	144	12	-512	9,919	0,827
k	169	13	-520	11,000	0,846
l	196	14	-526	13,260	0,947
m	225	15	-534	14,280	0,952
n	289	17	-546	18,580	1,093
p	400	20	-572	23,320	1,166
r	529	23	-587	30,730	1,336
s	625	25	-600	35,110	1,404

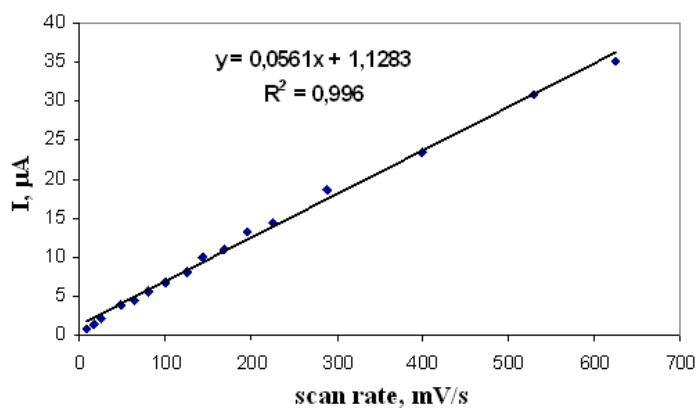


Figure 3.56 Dependence of peak current on scan rates

The peak current to square root of scan rate were plotted against the scan rate and given in Figure 3.57. The trend in the plot shows that it may follow EC mechanism. Therefore, it can be concluded that POM structure in aqueous solution adsorbs at the electrode surface and then electron transfer around -0.5 V occurs and reduced part of the Keplerate may rearrange at the electrode surface. After a series of measurement, the electrode was cleaned chronoamperometrically and change in the current by time was obtained similar to Figure 3.48.

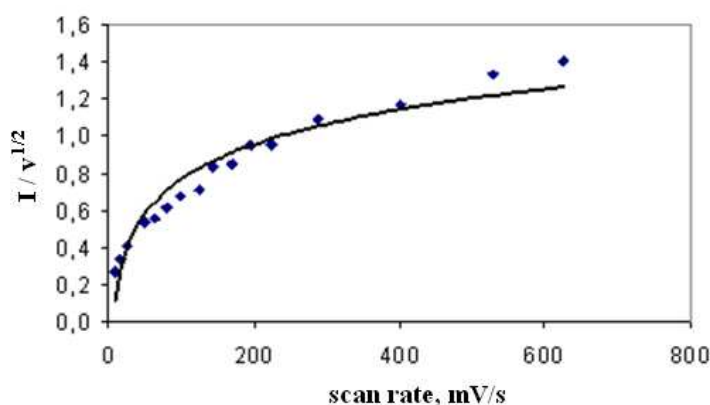


Figure 3.57 Effect of scan rates on the peak current of chemically obtained POMs at the GCE surface divided by square root of scan rate.

The reduction peak current was recorded against POM concentration and cyclic voltammograms at Figure 3.58 were obtained. The peak current has proportionally increased with addition of POM solutions to the cell and this dependence was also investigated by using linear DP scan (Figure 3.59).

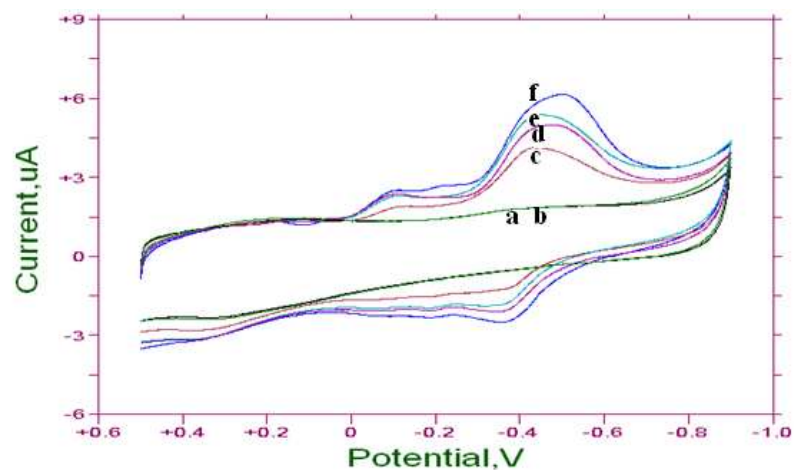


Figure 3.58 Cyclic voltammograms of a) pH 3.0 CAA, on addition of b) 2×10^{-3} M hydrazine sulfate, and after addition of POM to be c) 10, d) 20, e) 30 and f) 40 mg/L in the cell. Scan rate was 50 mV/s.

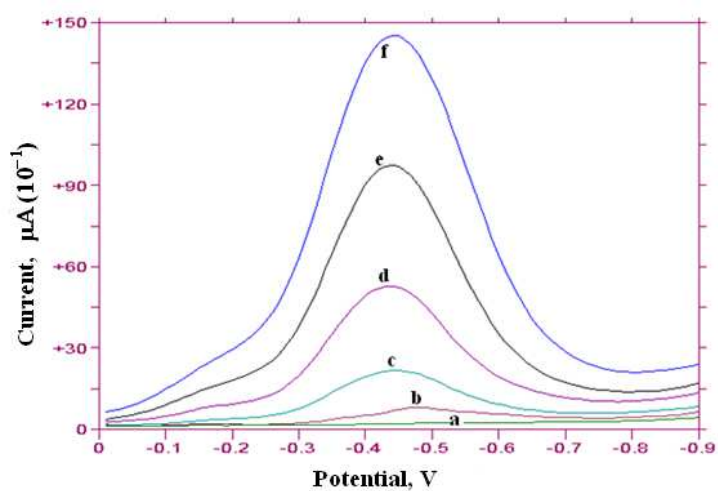


Figure 3.59 Linear DP voltammograms obtained for b) 10, c) 20, d) 30, e) 40 and f) 50 mg/L POMs added to the cell.

The effect of centrifugation was also studied by simply adding the POM solution to pH 3.0 chloroacetic acid solution. Figure 3.60 shows the difference between the peak currents in the presence and absence of centrifugation for a lower concentration range. As can be seen from the figure, peak currents have been increase by subsequent centrifugation at 2000 rpm for 10 min allowing the POM structure to cover the electrode surface.

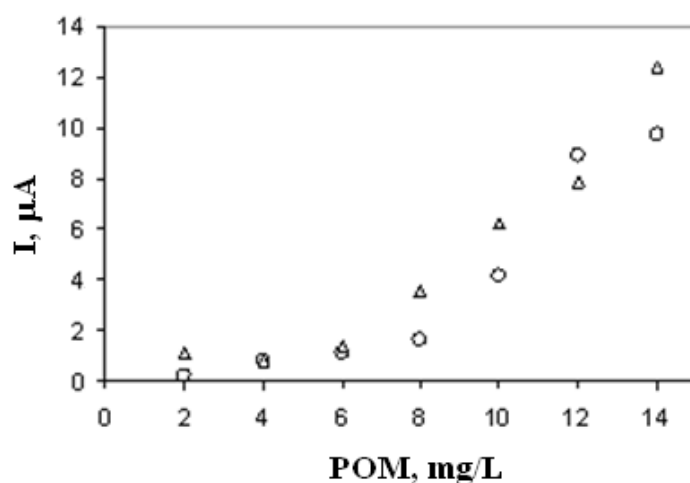


Figure 3.60 The change in peak current of POMs with (Δ) and without (o) centrifugation at 2000 rpm.

The effect of centrifugation speed was also tested. Table 3.11 summarizes the characteristics of DP voltammograms obtained after centrifugation of 2 mg/L POM solution for 10 min at different speeds.

Table 3.11 The peak characteristics of Centri-voltammograms obtained for 2 mg/L POM in pH 3.0 chloroacetic acid containing 2×10^{-3} M Hydrazine sulphate at various speed for 10 min.

Speed (rpm)	E_p (mV)	Peak current (μA)	Peak Area ($A \cdot V$) $\times 10^{-10}$
-	-480	2,89	5,89
1000	-480	3,76	8,18
1500	-480	4,21	10,37
1750	-480	6,00	10,5
2000	-480	7,64	17,5
2500	-480	9,23	24,3
3000	-480	13,4	31,8

As can be seen from the table, the peak heights have increased upon increasing the speed. The results were plotted against centrifugation speed and given in Figure 3.61.

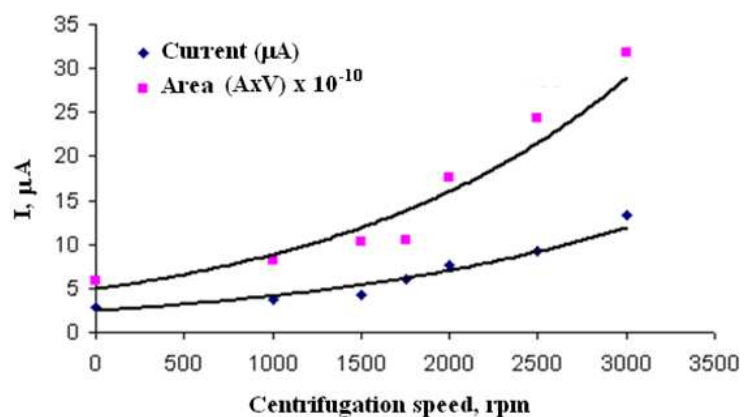


Figure 3.61 The dependence of peak currents and areas of 2 mg/L POM on centrifugation speed.

The overall results obtained with chemically produced POMs reveal that the reduction potentials of electrochemical and chemical synthesized POMs are very close indicating a quite similar structure is obtained by both methods. The peak potentials of both structure has shifted to negative values as the scan rate increases, indicating irreversibility of the reduction in certain degree. The peak current change with scan rate has revealed that reduction of chemically formed POM is adsorption controlled. It is necessary to clean the surface afterwards.

For electrochemical synthesized POMs, the persistent of the residue at the electrode surface even polishing with alumina slurry has also proved that reaction product has a strong attachment with the electrode surface. The only difference in electrochemical behavior of both POMs is the dependence of $I_p/v^{1/2}$ ratio to scan rate. In electrochemical self assembled structure, this plot has given a decrease indicating a catalytic effect while in chemically produced POMs this change has given an increase with scan rate indicating an EC mechanism might be involved.

Next studies were focused on the electrochemical techniques and related results for surface characterization. Afterwards, this POM structures on GCE will be characterized by Atomic force microscopy (AFM) and Scanning electron microscopy (SEM).

3.2.3 Surface Characterization by Electrochemical Techniques

The behaviors of polyoxomolybdate at or in the vicinity of double layer possess an interesting task. These are large Keplerate structures with very high negative charges. In addition to the free water molecules, they have several water molecules bonded inside the structures as well as on the outside surface. Besides, the metal centers have redox properties.

The huge negative charge on the POM molecule (-42) can be proven by electrochemical methods. With such a large negative charge around the polyoxomolybdate, there should be competing influences of orientation of water towards the electrode or the surface charge of the spherical ion. Admittance can give some information on the nature of solute-solvent interactions near the electrode double layer. Admittance (Y) and impedance (Z) are interrelated. Therefore, impedance measurements of POMs electrochemically synthesized on the electrode surface were carried out to see any difference at the capacitance of the electrode double layer in comparison to bare GCE. Figure 3.62 shows the change in capacitance upon centrifugation.

In order to understand the contributions of POMs attached to the surface to the admittance level, first the admittance of chloroacetic acid was measured and given in Figure 3.62a. The impedance of pH 3.0 chloroacetic acid buffer solution has displayed no peaks. The capacitance of the bare GCE surface was in nF levels and displayed a slow decrease as the potential is scanned in negative direction. The PZC point was observed as -0.2 V.

Upon addition of Mo(VI) into the cell to be 5×10^{-5} M and then, scanning the potential in negative direction for several times, POMs have been established on the electrode surface. The capacitance values were changes in μF levels (Figure 3.62b) and a PZC point was observed at -0.55 V. This big shift in PZC values clearly demonstrated the increase in electrode surface charge as highly negatively charged species is assembled on the electrode surface.

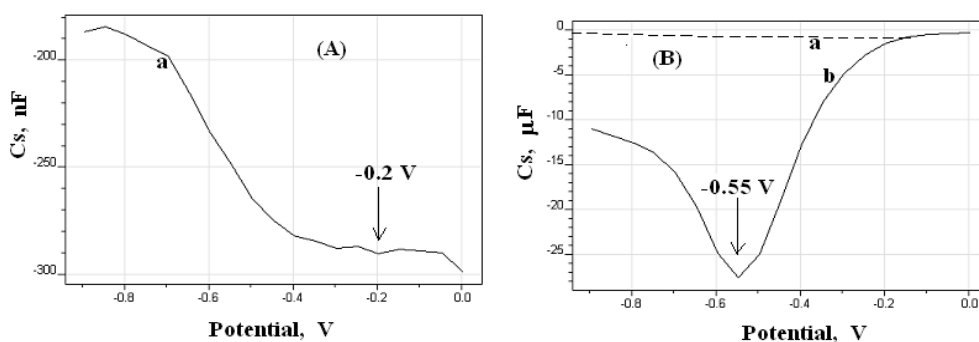


Figure 3.62 Impedance measurement of a) bare GCE in pH 3.0 chloroacetic acid buffer and b) after addition of Mo(VI) to be 5×10^{-5} M in the cell and cycling the potential in negative direction at a GCE.

The effect of a complexing reagent on the admittance measurements was also tested. Although the same PZC point was observed in the presence of 5×10^{-5} M PGR, the capacitance values were diminished in about 1 μF levels. This finding agrees well with above conclusion as the complexing reagent prevents the formation of POMs. By this means, the huge increase in the surface charge by POM formation was proven. Furthermore, the formation of self assembled POM structure can be cleared by other techniques.

Quartz crystal microbalance (QCM) is a highly sensitive in situ technique for monitoring mass changes on the electrode surface during electrochemical processes, and it has been proved to be very powerful in elucidating electrochemical reaction mechanisms.

The frequency of oscillations is sensitive to mass changes of the crystal surface as expressed by the *Sauerbrey equation* as given in Introduction Section. The frequency change Δf is related to the addition of mass per unit area of the crystal surface with a sensitivity factor which has the value of $56.6 \text{ Hz cm}^2/\mu\text{g}$ for 5 MHz crystal in air (Bard, 2001).

Figure 3.63 shows the recordings obtained for bare GCE in pH 3.0 chloroacetic acid, and after addition of Mo(VI) ion to be $5 \times 10^{-5} \text{ M}$ in the cell and then successive two and four scans to compare the frequency change afterwards.

As can be deduced from these figures, the frequency change was zero for blank, 20.17 Hz for after two potential scans. This change was the most obvious after successive four scans (129.33 Hz). This indicates that further scans can help to produce POMs and therefore, the mass accumulated at the electrode surface increases. The calculations for 3 mL of solution have revealed that the mass of the self assembled structure was 28.24 ng for B, and 181.13 ng for C at the surface of gold electrode. Clearly, the mass was increased more than 6 fold in two more potential cycles and this specifies the key role of the potential scan on POM formation on the electrode surface.

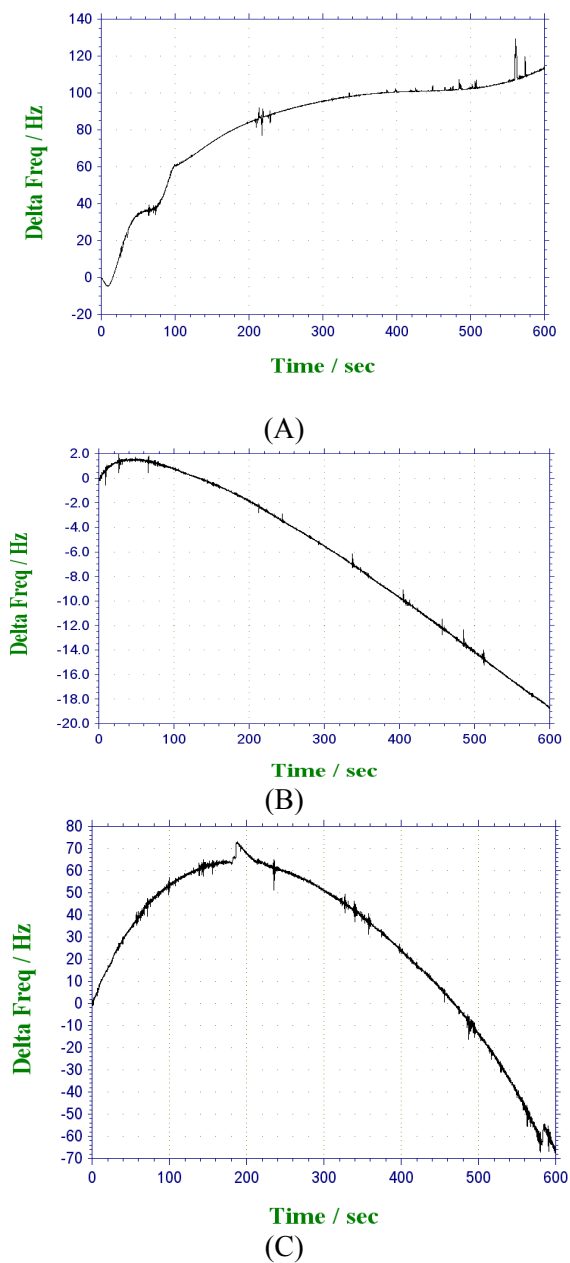


Figure 3.63 QCM measurements of A) in pH 3.0 chloroacetic acid buffer, and after addition of Mo(VI) ion to be 5×10^{-5} M in the cell and then successive cycling the potential B) twice and C) four times at a scan rate of 50 mV/s.

It should be remembered that the measurements were carried out with gold electrodes as the method requires. However, as given in Figure 3.43 and 3.44, GCE provides a better surface for formation of POM structure. Therefore, the mass found in this experiment would be much more at a GCE.

So far, electrochemical POM formation on the electrode surface was investigated by several electrochemical techniques to characterize the self assembled structure. It should be emphasized that this is the novel approach to synthesize Keplerate molecules on the GCE surface.

The role played by these giant molecules at the electrode surface-solution interphase is difficult to establish in the absence of structural information on the molecular size scale. Next section describes the probe microscopic techniques those were used to illuminate the formation of POMs on the electrode surface.

3.2.4 Probe Microscopic Techniques for Elucidating POMs

The self assembled POM structures established by affecting the potential of GCE were characterized by Atomic force microscopy (AFM) and Scanning Electron Microscopy (SEM).

Here, the major drawback is the geometry of the electrode which is 4.5 cm in height and therefore, not suitable for such measurements. The probe techniques require less altitude for sample holder being less than 3 for AFM and 1.5 cm for SEM for optimal resolution. Therefore, glassy carbon electrodes less than 1 cm in height were developed in this lab using glassy carbon rod embedded in Teflon body.

The performance of the new electrodes was tested by using the of 1×10^{-3} M $\text{Fe}(\text{CN})_6^{-3}$ in 1.0 M KNO_3 solution and cyclic voltammograms have agreed well with those of commercial electrodes. In this new electrode surface, the formation of self assembled structure was proven by recording the cycling voltammograms. Afterwards, AFM images were recorded to elucidate these surface assembled structures.

Figure 3.64 shows the AFM images of bare GCE in pH 3.0 chloroacetic acid medium containing 5×10^{-4} M Mo(VI) ion. The surface morphology has shown that mean roughness of bare electrode was about 5 nm. Upon centrifuging at 2000 rpm 10 min, the height of the molecule has increased to 66 nm and after deposition step at -0.6 V the height has increased four times reaching 217 nm. The images in phase mode which helps to discriminate the phases of the surface clearly indicates self assembled structure formation on the glassy carbon electrode surface.

The main cause of this self assembled structure is the potential applied to the electrode surface as revealed former studies. Therefore, the potential was scanned between 0 V to -0.9 V for 10 times and rather a film formation was observed with a maximum height of 578 nm. This growing evidently demonstrates the self assembled formation of molybdenum species on the electrode surface.

To interpret the data unambiguously, the electrode was brought into contact with chemically synthesized POM and the potential was scanned between 0 V to -0.9 V for 10 times. Figure 3.64 e displays the AFM images of this surface attached species.

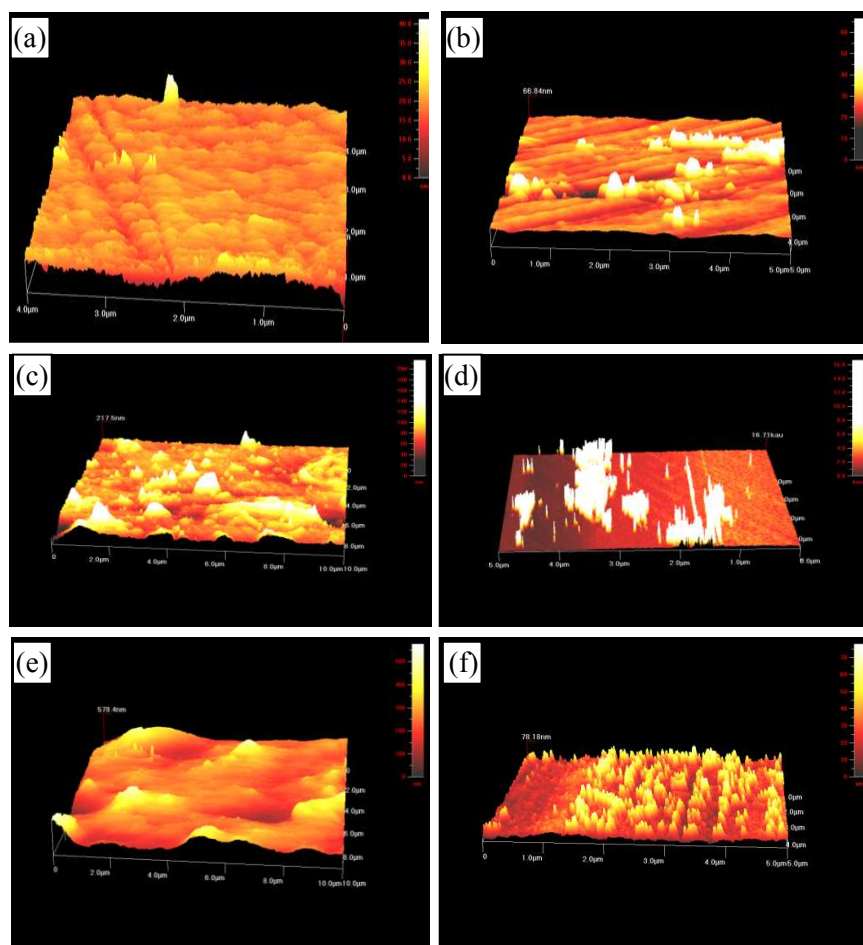


Figure 3.64 AFM images of a) bare GCE surface in pH 3.0 chloroacetic acid, b) upon centrifuging at 2000 rpm for 10 min in the presence of 5×10^{-4} M Mo(VI), c) after applying -0.6 V for 2 min, (d) phase mode of c, e) after cycling the potential between 0- -0.9 V for 10 times, f) after cycling the potential between 0- -0.9 V for 10 times in the presence of 2 mg/L chemically synthesized POM.

According to Figure 3.64 f images, the well organized structures have a mean size of 78 nm can be perceived. The difference in the sizes of electrochemical and chemical synthesized POMs can be concluded as the increased amount of molybdenum per unit area with the aid of potential applied and hence the formation of building blocks in different sizes. Thus, surface formed species constitutes another challenge for understanding at the same level of satisfaction already achieved for solution formed solid species with probe techniques.

Another challenge is the search for support materials appropriate for POM formation other than GCE. For this purpose, conductive ITO glass electrode was immersed into the pH 3.0 chloroacetic acid solution with reference and counter electrodes. After addition of Mo(VI) ions to the cell solution and cycling the potential of the electrode, the surface became green at first glance and then, gone to brown due to the particle formation while the solution color changing blue.

Figure 3.65 displays the two and three dimensional AFM images of ITO glass in the presence and absence of Mo(VI) ions in pH 3.0 chloroacetic acid solution. In Figure 3.65 a and b homogeneously distributed indiumoxide and tin oxide can be spotted as dots in bulk silicon. The mean roughness of the surface is about 60 nm.

Upon scanning the potential in the presence of 1×10^{-4} M Mo(VI) and after drying in an oven at 50°C, AFM images were taken. As can be seen from Figure 3.65 c and d, round shaped POMs with a height of 104 nm can be noticed easily.

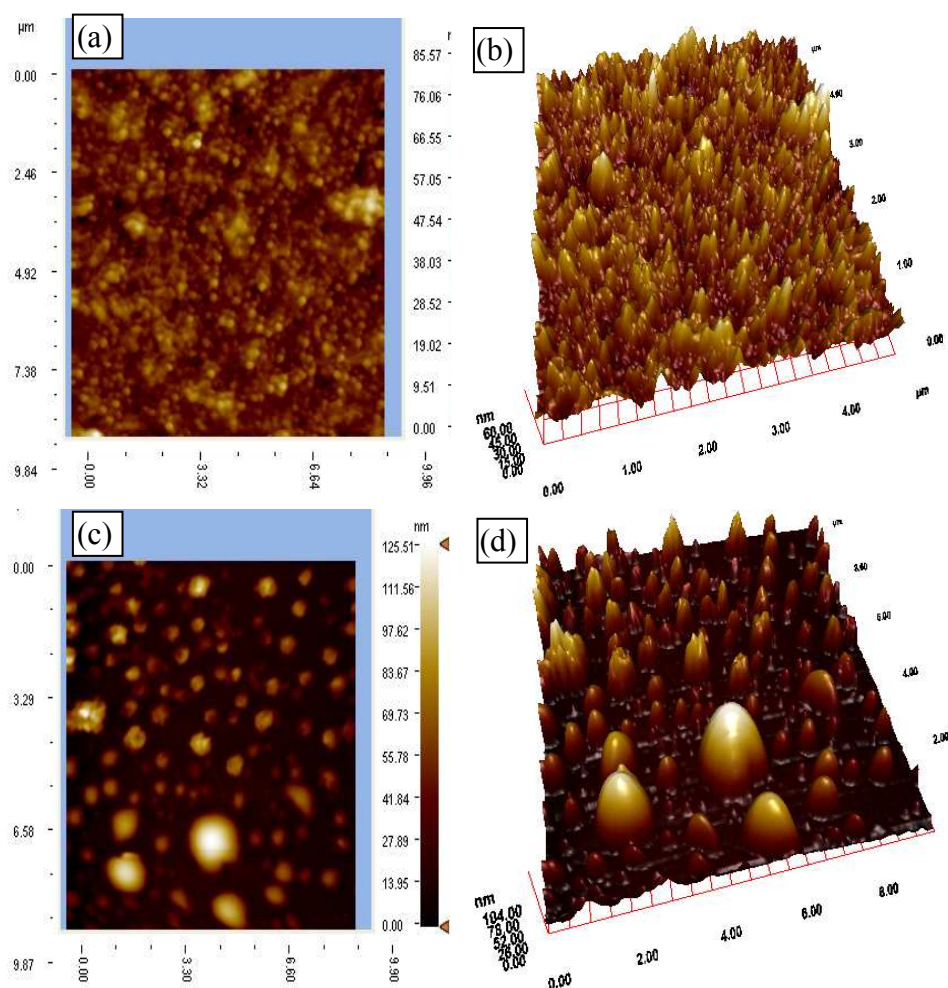


Figure 3.65 a) 2D and b) 3D AFM images of ITO glass in pH 3.0 chloroacetic acid, c) 2D and d) 3D images in the presence of POMs formed from the solution containing 1×10^{-4} M Mo(VI) and cycling the potential.

In Figure 3.66 SEM images of ITO glass can be seen. Indium and tin oxide compounds can be spotted in images magnified by 20 000 and 50 000 times (Figure 3.66 a ve b).

Later on the glass was treated with Mo(VI) solution as given in Experimental Section to form self assembled structures on the surface. SEM images of this glass can be seen in Figure 3.66 c – f for closer observation. Self assembled species have displayed a puzzle like homogeneous and transparent film formation on the electrode surface.

The crackeds on the film helps to discriminate the glass surface and the structure attached on it. Here, the edges of the film might appear due to the high concentrations of Mo(VI) ions. As the magnification ratio increases, a round shaped pattern with a mean diameter of 0.3 μm was became observable which is bigger than the pattern of ITO surface alone (e-g). These round shaped figures can be attributed to the formation of big POM molecules placed in a big puzzle like film..

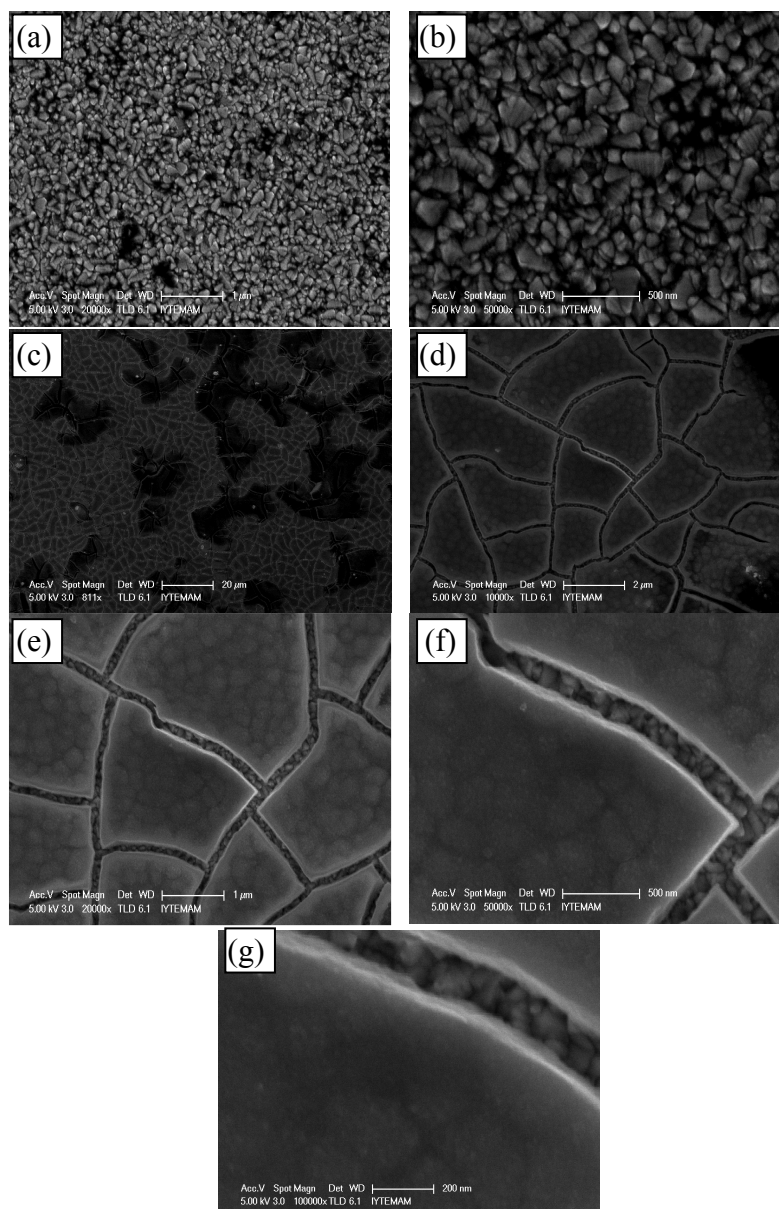


Figure 3.66 SEM images of ITO glass magnified by a) 20 000 and b) 50 000 times, and after modification with Mo(VI) ions c-g 811, 10 000, 20 000, 50 000 and 100 000 times.

The samples used in SEM images, were exposed to EDX spectra and several elements found in ITO glass structure namely Si, Sn, Na and Ca have given peaks as expected. The molybdenum was found in 25% weight percent as it builds up on the electrode surface as a self assembled structure (Figure 3.67).

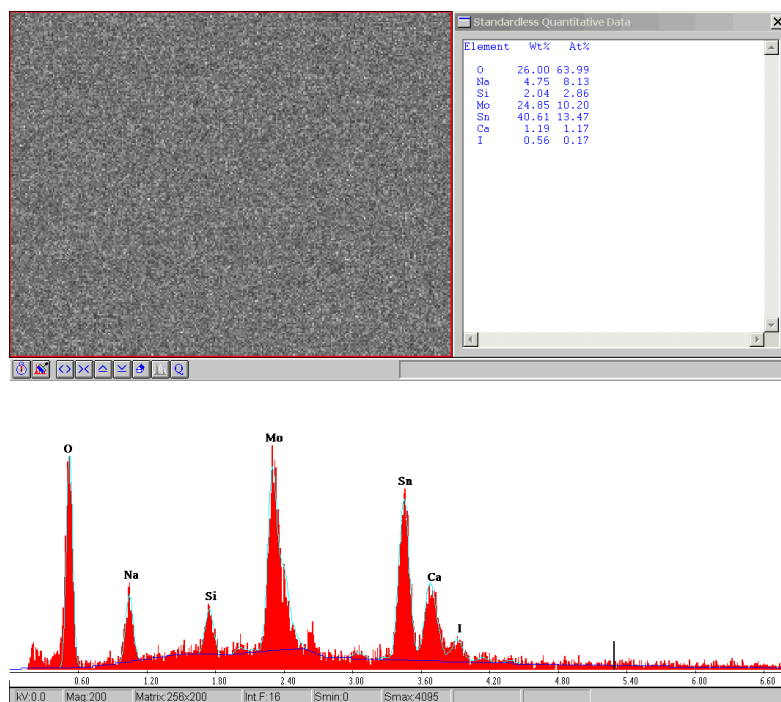


Figure 3.67 EDX analysis of ITO glass modified with molybdenum

Another attractive characteristic of POMs is their capacity to accept several electrons, which makes them particularly well-suited to act as electron reservoirs. POM acts as a photocatalyst for the reduction of metal ions by organic electron donors. This reaction can be used in recovery of metals or synthesis of colloidal nanoparticles.

Here, it was tested to use of metal ion solutions dropped onto the self assembled structure. Figure 3.68 shows the images of ITO surface having a POM layer spiked with 10 μL of 1×10^{-3} M Ru(III) solution and rinsed with pure water and then, dried in 50°C for 10 min.

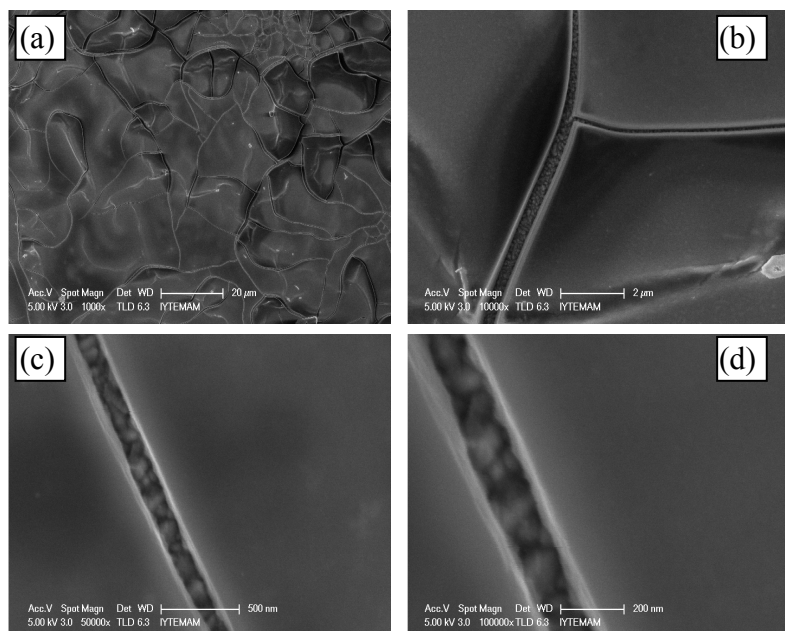


Figure 3.68 SEM images of a) POM structure on ITO glass after dropping Ru(III) solution magnified by a)1000, b)10 000, c)50 000 and d)100 000 times.

The images have shown more homogeneous film formation in the presence of Ruthenium particles on the POM structure. Bright spots on the images might be metallic particles on the POM structure. Large round shadows were not seen in closer images

The elemental contents of the structure was revealed by means of EDX measurements and given in Figure 3.69.

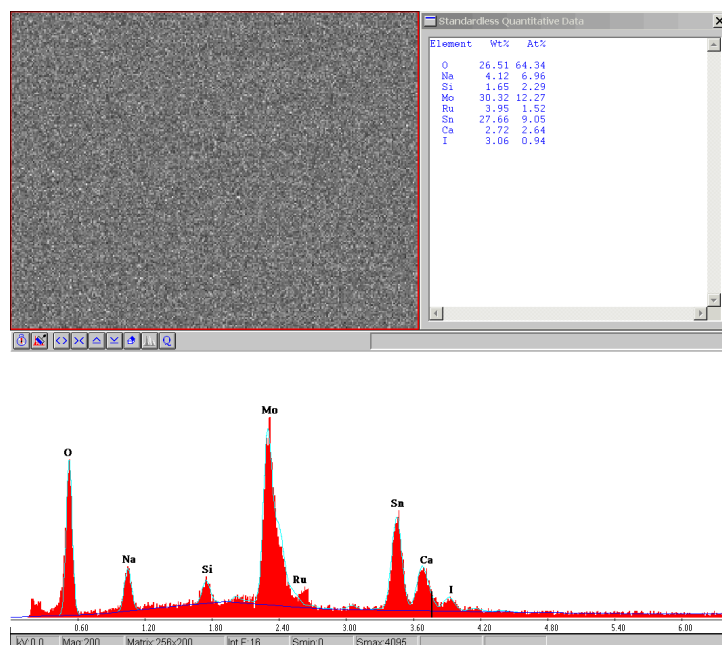


Figure 3.69 EDX analysis of Ru(III) treated ITO glass containing POM structure

As can be seen from the weight analysis, ruthenium can be detected almost 4%. The atomic ratio of Mo and O are found about 1:6 for both EDX results.

The POMs electrochemically produced on the GCE surface were investigated with SEM measurements. Figure 3.70 displays homogeneous and transparent film formation on the electrode surface similar to ITO glass. The main difference is the presence of bright spots on the film. The image in Figure 3.70 c was taken from a rather dense part of the surface film. As can be clearly seen the spots are little humps on the film. The scratches at the film edges were found thicker for GCE.

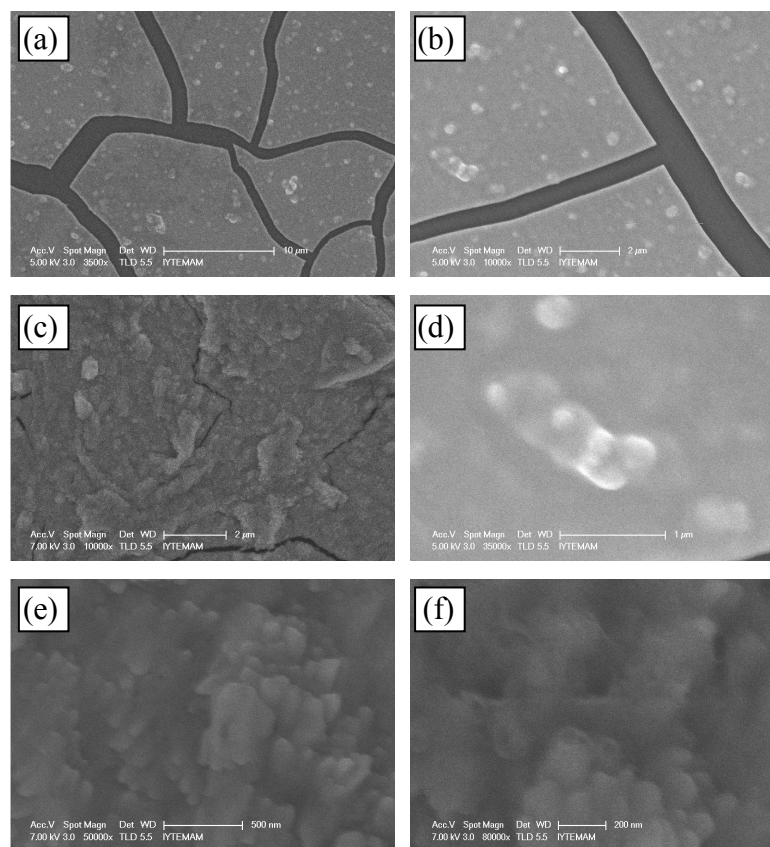


Figure 3.70 SEM images of POMs electrochemically produced at the GCE magnified by a)3 500, b)10 000, c)10 000, d)35 000, f)50 000, g)80 000 times

The EDX spectra of POMs produced on the electrode surface by electrochemical procedure can be seen in Figure 3.71. As can be deduced from the spectra carbon has the highest weight ratio as expected. Other elements are oxygen, molybdenum and sodium in order. Atomic percentages of O: Mo gives a ratio of 6.2 which agrees well with the ratio calculated for $\text{Na}_{15}[\text{Mo}^{\text{VI}}_{126}\text{Mo}^{\text{V}}_{28}\text{O}_{462}\text{H}_{14}(\text{H}_2\text{O})_{70}]_{0.5}$. $[\text{Mo}^{\text{VI}}_{124}\text{Mo}^{\text{V}}_{28}\text{O}_{457}\text{H}_{14}(\text{H}_2\text{O})_{68}]_{0.5}$. 400 H_2O molecule having a ratio of 928: 153 that is 6.06 (Müller, 1999).

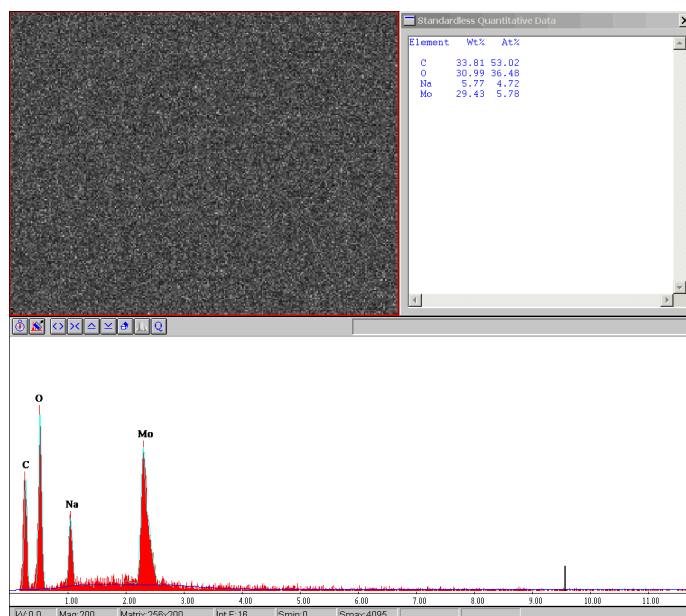


Figure 3.71 EDX spectra of POMs produced on GCE

Close images of chemically synthesized POMs according to Müller's method have been also investigated for comparison (1998). The POMs were observed as round shaped structures at first in the solution. After 4 days, the crystals were filtered, rinsed and dried.

The POMs were weighted and dissolved in chloroacetic acid solution to be 2 mg/L. The potential of the GCE was cycled in a range of 0.0 - -0.9V for 10 min to attach these giant molecules on the electrode surface. The SEM images of surface bonded POMs have displayed a triangular shape as given in Figure 3.72. The results are found in agreement with the literature (Zhu, 2003). The EDX spectra of this structure are given in Figure 3.73.

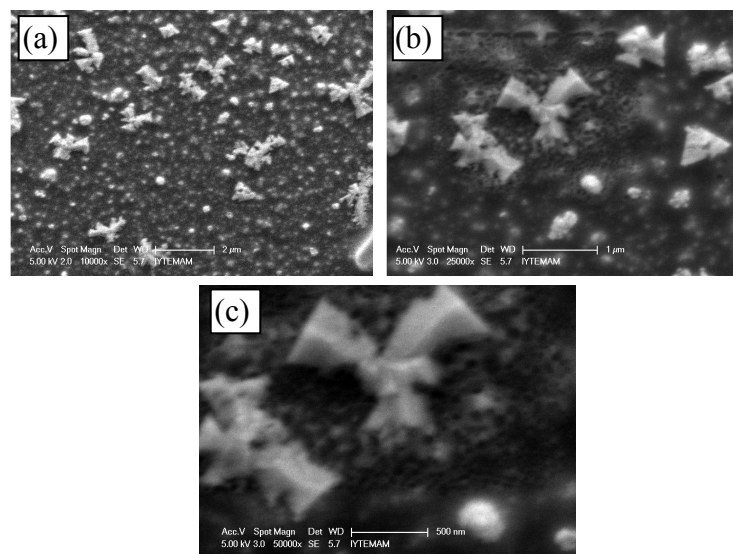


Figure 3.72 SEM images of $\{Mo_{132}\}$ POMs produced by Müller's method on GCE and magnified by a) 10 000, b) 25 000 and c) 50 000 times

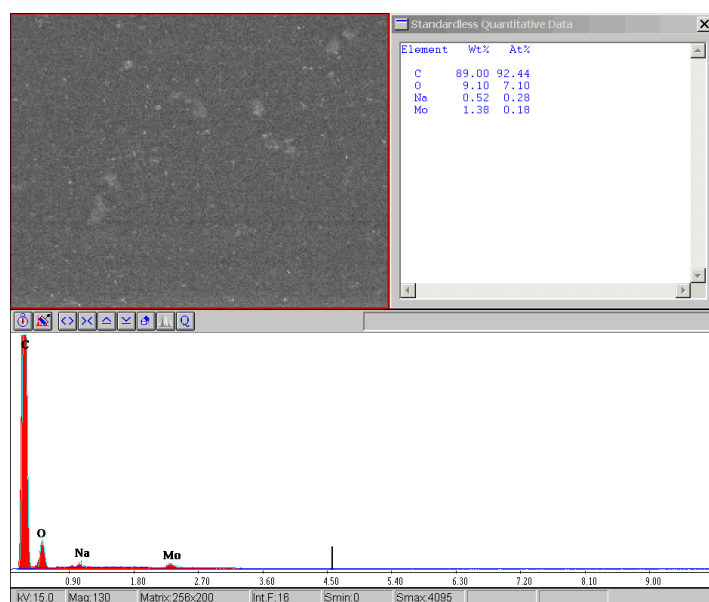


Figure 3.73 EDX of chemically produced POMs on GCE

POM acts as catalyst for the reduction of metal ions as tested before. Figure 3.74 shows the images of POM modified GCE surface spiked with 10 μL of 1×10^{-3} M Ru(III) solution, rinsed with pure water and then, dried in 50°C for 10 min. The close images of the surface have exhibited more homogeneous film formation with fewer scratches. Ru content of the film was detected in 1.64% in EDX spectra (Figure 3.75).

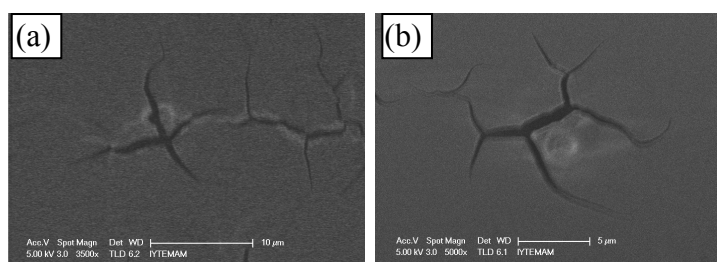


Figure 3.74 SEM images of Ru(III) modified GCE containing electrochemically produced POMs and magnified by a) 3 500 and b) 5 000 times

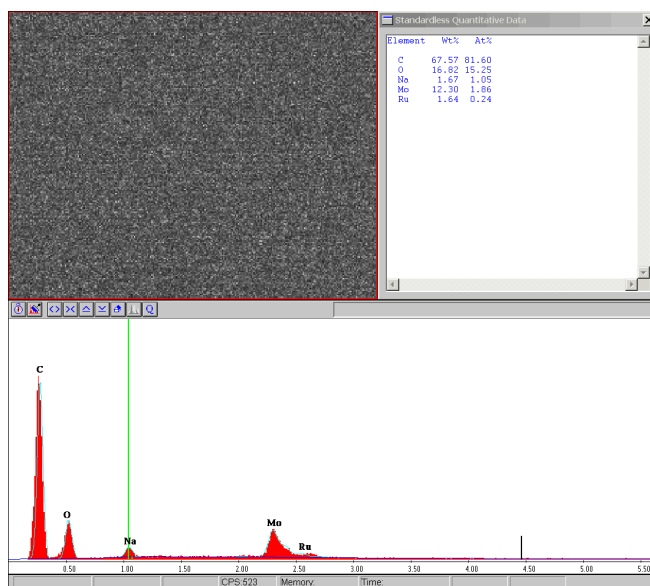


Figure 3.75 EDX spectra of Ruthenium modified GCE-POM surface

As stated before, Keplerates can be obtained with mixed metallic ions like iron with novel magnetic properties. In Mo_{132} structure, 60 of Mo(V) can be replaced by 30 Fe(III) ions, leading to the formation of giant molecules $(\text{Mo(VI)}_{72} \text{Fe(III)}_{30} \text{O}_{252} \text{L}_{102} \text{ca.180 H}_2\text{O})$ with $\text{L} = \text{H}_2\text{O}/\text{CH}_3\text{COO}^-/\text{Mo}_2\text{O}_{8/9}^{n-}, \{\text{Mo}_{72}\text{Fe}_{30}\}$.

An experiment was designed to see the possibility of iron containing POM formation on the GCE surface. Equal concentrations (1×10^{-4} M) of Mo(VI) and Fe(III) ions were added to pH 3.0 chloroacetic acid medium and the procedure was repeated. The electrode surface was rinsed with pure water and then, dried at 50°C for 10 min. SEM images of electrode surface have revealed again a homogeneous film formation except no scratches were seen (Figure 3.76).

The EDX spectra show nearly 0.2% of Fe content as well as Mo, O and massive C due to the GCE surface (Figure 3.77). The atomic ratio of Mo to O cannot be explicated unambiguously as the atomic percentages of these elements are less than 5%. More decisive methods like XRD are required to identify the structure.

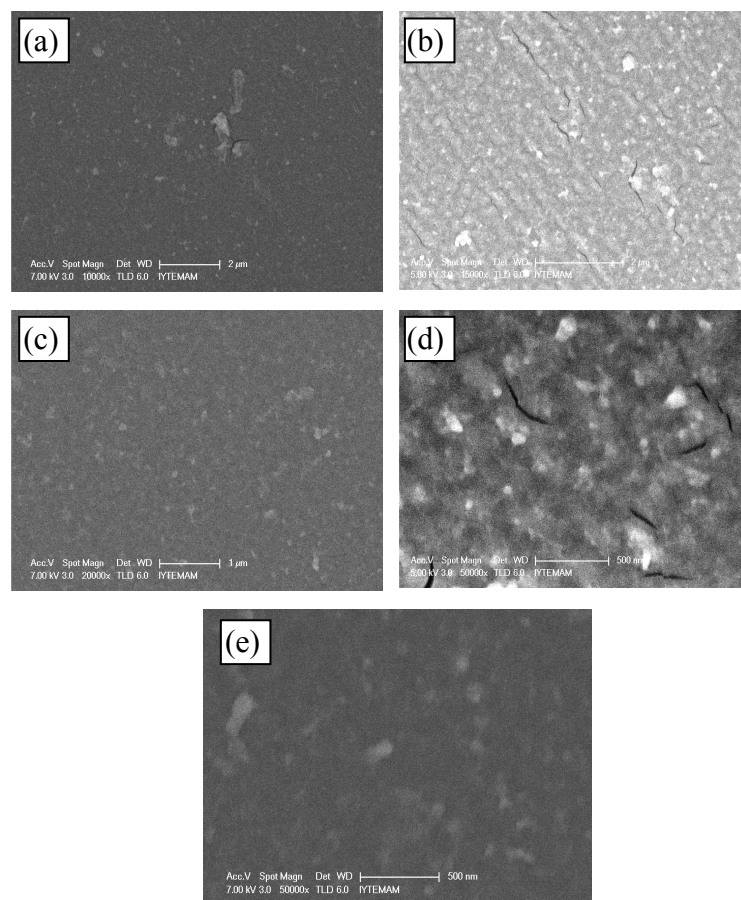


Figure 3.76 SEM images of GCE surface modified with electrochemically in the presence of equal concentrations of Mo(VI) and Fe(III) ions magnified by a)10 000, b) 15 000, c) 20 000, d) 50 000 and e) 50 000 times

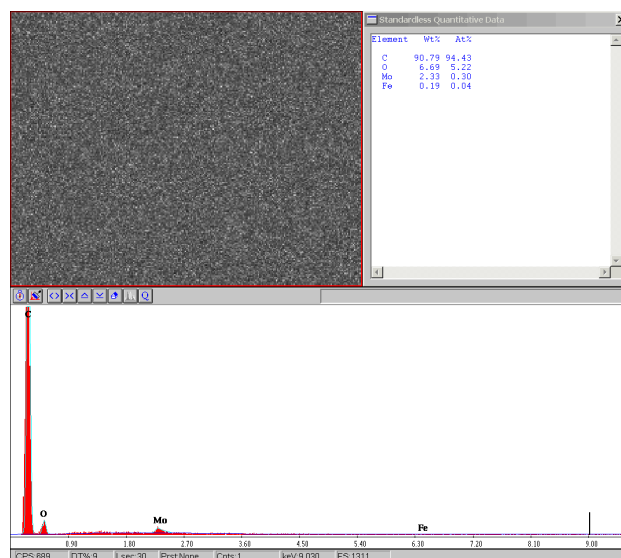


Figure 3.77 EDX of iron containing POMs at GCE

3.2.5 Concluding Remarks and Future Studies with POMs

The catalytic redox activity of polyoxometalates has attracted much attention in recent years. Heteropolyanions and especially their metal-substituted derivatives have some very useful and interesting properties including high stability of most of their redox states, the possibility to tune their redox potentials by changing the heteroions and/or the addenda ions without affecting their structure, the variability of the transition metal cations which can be incorporated into the heteropolymetalate structure, and the possibility of multiple electron transfer. These properties make heteropolyanions attractive as redox catalysts (mediators) for indirect electrochemical processes.

So far, the polyoxometalates are able to be attached onto the electrode by using an interaction between the polyoxometalates and the electrodes. There are three methods commonly used to immobilize polyoxometalates onto the electrode surface. The first method is the adsorption of polyoxometalates on the electrode surface by dip coating. The second method is to entrap polyoxometalates into polymers on the electrode surface. The third method is the electrodeposition of polyoxometalates onto the electrode surface within the already formed polyoxometalate solution under constant potential at -1.2 V.

The molecular structure that can fit into our atomic percentages in EDX measurements is the one blue mixed-crystal compound $\text{Na}_{15}[\text{Mo}^{\text{VI}}_{126}\text{Mo}^{\text{V}}_{28}\text{O}_{462} \text{H}_{14} (\text{H}_2\text{O})_{70}]_{0.5} \cdot [\text{Mo}^{\text{VI}}_{124}\text{Mo}^{\text{V}}_{28}\text{O}_{457} \text{H}_{14} (\text{H}_2\text{O})_{68}]_{0.5}$. 400 H_2O molecule having a ratio of 928: 153 that is 6.06 (Müller, 1999). This compound was reported to be precipitated within one day when an acidic (pH~1) aqueous solution sodium molybdate is reduced chemically by using sodium ditionite.

In the past, generations of chemists tried to crystallize compounds from solutions containing different types of molybdenum blue species, ending up with amorphous materials. First break-through in this field was the isolation of some crystals (Müller, 1998). Later, the same group has discovered that the extensive water shell due to the large number of water ligands is crucial for the high solubility of molybdenum blue species which prevented the isolation. The electrolytes help to destroy the extensive hydration shell of the giant molecule and unable to isolate the crystals in high yield.

However, no data were obtained in the literature as describing POM formation at the electrode surface. This is the first time, we believe, the giant POM molecule was formed on the electrode surface diffusing from the molybdenum ion and reducing at the electrode surface in chloroacetic acid medium. After characterization of the surface bonded species by modern probe techniques, the new horizons in this field have been searched.

One of the most important properties of these metal oxide clusters is the capability for reversible multivalence reduction, forming mixed-valence species. The property makes them very useful in the preparation of modified electrodes and brings about favorable catalytic properties with regard to several challenging electrochemical processes such as the reduction of nitrite, bromate and hydrogen peroxide.

An experiment was designed to compare the performance of metal doped GCE-POM electrodes with bare GCE and gold disc electrode. Although it was verified by EDX measurements the reduction of Au(III) ions on the active sites of electrodes, better results were obtained after stabilization of the surface by cycling the electrode potential 15 times between -0.4 to 0.8 at a rate of 50 mV/s.

Figure 3.78 shows DP voltammograms related with reduction of nitrite for various electrodes to compare the catalytic effects. The peak lay outs clearly demonstrates that the broad reduction peak of nitrite at GCE has shifted in positive potentials about 0.12 V in the presence of gold particles on bare and POM modified GCE being at -0.78 V but latter electrode has displayed a much higher peak current almost 3 fold.

Therefore, a calibration curve was obtained for nitrite determination and given in Figure 3.79. The voltammograms have displayed a very promising method for nitrite determination as a future study.

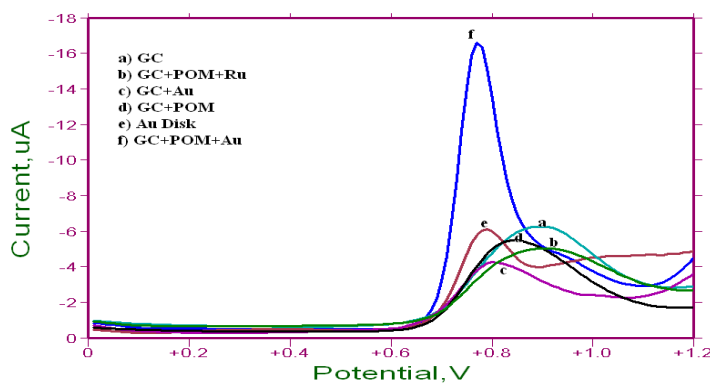


Figure 3.78 DP Voltammograms of 1×10^{-3} M nitrite on different electrodes in pH 7 phosphate buffer, a) GCE, b) Au nanoparticle doped GCE+POM, c) Au modified GCE, d) GC+POM, e) Au disc, f) Au nanoparticle doped GC+POM electrodes

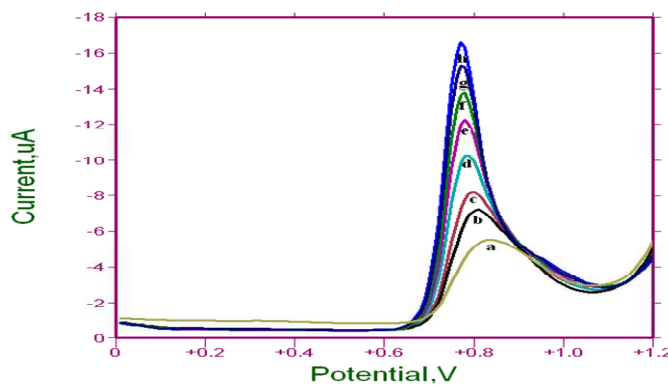


Figure 3.79 Voltammogram of a) pH 7.0 phosphate buffer, b) 5×10^{-5} , c) 1×10^{-4} , d) 2×10^{-4} , e) 4×10^{-4} , f) 6×10^{-4} , g) 8×10^{-4} , h) 1×10^{-3} M nitrite.

Another significant challenge is the possibility of the use of POMs in fuel cells. During the past ten years, significant developments on electrode modification by POMs have taken place aiming to improve the reaction rates of fuel cells and produce economically sufficient currents for practical uses.

Electrocatalysts that are incorporated into the fuel cell electrodes are required to have high activity for the electrochemical reduction of oxygen, as well as being able to withstand a working environment of relatively high temperatures and strong acid levels.

Hydrogen presents itself as a potential alternative to carboniferous fossil fuels but only with consideration of an appropriate source. While the catalytic production of hydrogen and oxygen from water splitting has yet to be effectively achieved in homogeneous solution, some of the basic building blocks in the foundation of the science needed for this reaction have emerged over the past three decades.

One of these important building blocks comes from knowledge from studies concerning hydrogen production by photochemical means (Esswein & Nocera, 1998). In anaerobic solution, photoexcitation of POMs in the presence of oxidizable substrates such as alcohols gives the blue solution accompanied by H₂ production. The rate of H₂ production is faster in the presence of a colloidal Pt catalyst, but H₂ production is inhibited in the presence of oxygen, favoring the reduction of oxygen to water instead.

Here, the effect of POMs on hydrogen evolution and methanol oxidation was tested. Figure 3.80 shows the DP voltammograms recorded in 0.1 M HCl solution at various electrodes. Notation follows the appearance order of the peak.

The large reduction current can be seen after -0.9 V for bare GCE (Figure 3.80 d). Upon modification of the electrode surface with POM and with several metal ions, the current rise has shifted in either way indicating a diverse catalytic effect depending on the type of nano particle. Gold nano particles have given the best shift (app. 0.5 V) which coincides with gold polycrystalline electrode.

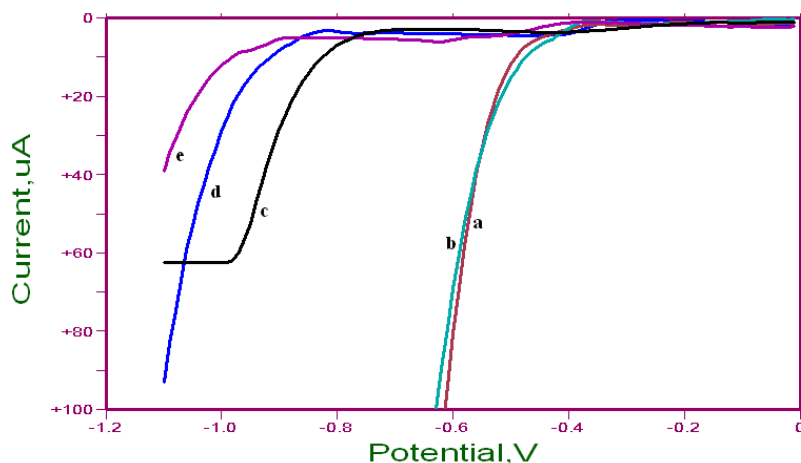


Figure 3.80 DP voltammograms for hydrogen evolution at different electrodes in 0.1 M HCl medium, a) bare gold disc, b) Au modified GCE-POM, c) Ru modified GCE-POM, d) bare GCE, e) Fe modified GCE-POM

The methanol oxidation was investigated in 1.0 NaOH medium containing 1.0 M methanol as established before in a project based on fuel cells (Dursun, 2009). Figure 3.81 shows the methanol oxidation peak at bare gold electrode and GC-POM electrodes doped with Ru and Au nanoparticles as described earlier.

As can be seen from the figure, the gold nanoparticles have displayed a well catalytic effect for methanol oxidation and a very promising material for future studies. Gold nanoparticles can substitute expensive gold disc electrodes with improved catalytic activity.

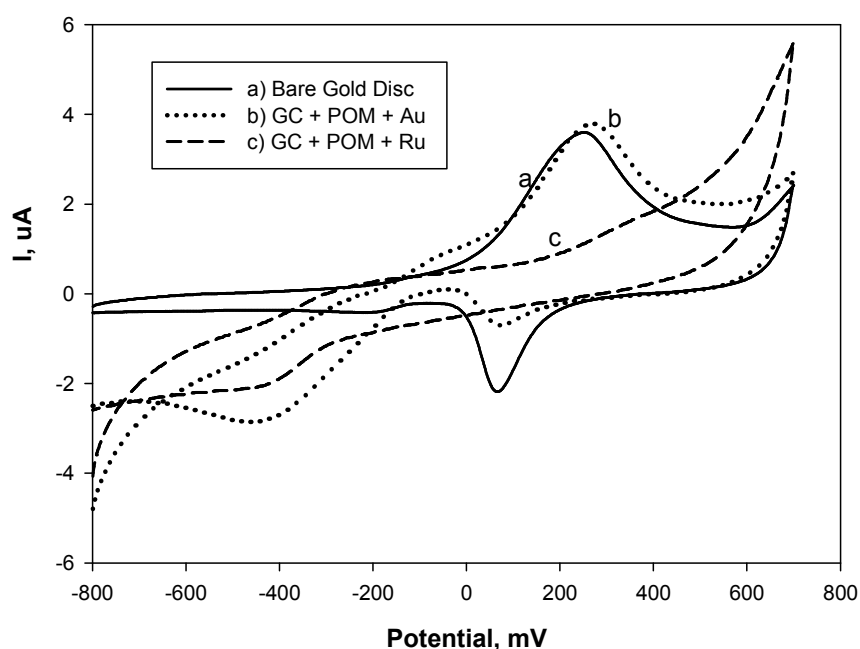


Figure 3.81 Cyclic voltammograms of 1.0 M methanol in 1.0 M NaOH solution, at a) bare gold disc electrode, and GC-POM electrodes doped with b) gold and c) Ru nanoparticles

As the multidisciplinary approach in science keeps increasing, it is just a matter of time before the widening of the scope of this field becomes a reality. One course should always be to weigh the cost factors against the value of the application. In this respect, POM modified electrodes offer wide range of possibilities of applications as excellent catalysts.

4. CONCLUSION

Analytical chemistry requires the application of the most appropriate technique for separation and preconcentration of the analyte. A practical and sensitive method for application of co-precipitation in trace analysis, called ***Centri-voltammetry***, combines the selectivity of co-precipitation technique with a sensitive method; voltammetry. The method shows similarity with Adsorptive cathodic stripping voltammetry (AdCSV), only the pre-concentration step differs as the centrifuge is employed. The centrifugation employed forces the complex to precipitate as a thin film on the electrode surface from bulk solution.

This study describes another application of Centri-voltammetric method to the determination of biologically essential trace element; molybdenum ion with various carrier reagents namely, 8-hydroxyquinoline, pyrogallol red and cupferron. Among the reagents used pyrogallol red was studied in detail and the reduction peak the complex was exploited for analytical purposes. The method was employed to milk samples to determine molybdenum contents.

Another challenge of the thesis was emerged during the centri-voltammetric studies based on the observations of peak growing by centrifuging the solution containing Mo(VI) ion alone. Cyclic voltammograms clearly indicated the formation of a self assembled structure on the electrode surface by time. This phenomenon differs in some aspects from the previous studies reported in the literature as no reducing agent was added to the cell.

Here, a self established structure on the electrode surface via reduction of Mo(VI) to Mo(V) at the GCE surface under potential applied and catalyzed by the cell composition. The most probable explanation for that is the GCE provides a flat and reductive surface appropriate for a self assembled structure by the aid of centrifuging. Electroanalytical techniques as well as modern surface analysis methods were used in their characterization.

These spherical Keplerates including molybdenum have unique values among the POMs discovered so far, owing to their sizes and structural complexity. The catalytic redox activity of polyoxometalates has attracted much attention in recent years. Practical applications of polyoxometalates largely depend on the successful preparation of thin polyoxometalate-containing films. Many reports were published about effective approaches for the immobilization of POM onto an electrode surface as a means to develop a cost-effective, regenerable chemical and biosensors. Although these approaches have been shown to be promising, no data were obtained in the literature as describing POM formation at the electrode surface. We believe, this is the first time, the giant POM molecule was formed on the electrode surface diffusing from the molybdenum ion and reducing at the electrode surface in chloroacetic acid medium. After characterization of the surface bonded species by modern probe techniques, the new horizons in this field have been searched. The GCE-POM doped with gold nanoparticles has shown very promising results for hydrogen and nitrite reduction and methanol oxidation for the future. These are new challenges for widening of the scope of this field.

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