EGE UNIVERSITY

MASTER THESIS

HEAD- SPACE VOLTAMMETRY: A NOVEL VOLTAMMETRIC METHOD FOR VOLATILE ORGANICS AND A CASE STUDY FOR PHENOL

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Chemistry Department

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Kemal Volkan ÖZDOKUR tarafından yüksek lisans tezi olarak sunulan "**Head- Space Voltammetry: A Novel Voltammetric Method For Volatile Organics And A Case Study For Phenol**" başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi'nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve **06.01.2012** tarihinde yapılan tez savunma sınavında aday oybirliği/oyçokluğu ile başarılı bulunmuştur.

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ÖZET

UÇUCU ORGANİK BİLEŞİKLER İÇİN
YENİ BİR VOLTAMMETRİK YÖNTEM: TEPE BOŞLUKLU
VOLTAMMETRİ VE FENOL ANALİZİNE UYGULAMASI

ÖZDOKUR, K. Volkan

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Eser analizde analitlerin önderiştirilmesi duyarlık ve kimi zaman da seçimlilik açısından büyük önem taşır. Voltammetrik sıyırma analizlerinde bu aşama genellikle kontrollü potansiyel altında karıştırılan çözeltilerden analizlerin elektrot yüzeyinde elektrokimyasal biriktirilmesini içerir. Bu tez çalışmasında ise analitin voltammetrik analiz öncesi elektrot yüzeyine tepeboşluğu koşullarında önderiştirilmesine ilişkin yeni bir yöntemin ilk bulguları yer almaktadır. Bu nedenle yöntemin adı Tepe Boşluklu Voltammetri olarak öne sürülmüştür ve bu yöntemin performasını sınamak için zehirli etkisi bilinen fenol bileşiğinin analizine uyarlanmıştır.

Tez çalışmasında gerek önderiştirme (polimerizasyonda döngü sayısı, vial pH'ı, karıştırma hızı, süresi, ortama eklenen tuz miktarı, sıcaklık ve vial hacmi) ve gerekse ölçüm aşamasına (hücre pH'ı, ve bileşimi) ilişkin denel parametrelerin optimizasyonu sonucu kalibrasyon grafiği oluşturulmuş ve fenol için belirtme sınırı 1.8×10^{-7} M olarak hesaplanmıştır. Geliştirilen bu yöntem atık su benzetme örneklerine uyarlanmış ve yüksek gerikazanımlar (%93) elde edilmiştir.

Anahtar sözcükler: voltammetri, tepe boşluğu, fenol, polipirol, elektro polimerleşme

ABSTRACT

HEAD- SPACE VOLTAMMETRY:
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ORGANICS AND A CASE STUDY FOR PHENOL

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In trace analyses, the preconcentration of the analyte plays a vital role in terms of sensitivity, and in some case the selectivity. In stripping techniques, this step usually includes an electrochemical deposition process on the electrode surface at a controlled potential under stirred conditions. Present study includes the earliest results of a novel method which combines the headspace (HS) preconcentration of the analyte on the electrode prior to the voltammetric analysis. Therefore, the method was called Headspace-Voltammetry. Phenol molecule was chosen to test the effectiveness of the method as the phenolic compounds have been proved to be toxic and therefore, monitoring of these compounds is important to evaluate the risk and the effectiveness of posterior water treatment.

In this thesis, we studied both preconcentration parameters (polymerization cycle number, vial pH, stirring rate and exposure time, salt amount, vial temperature, sample volume) and measuring cell parameters (cell pH and composition). Under optimized conditions, the calibration curves were plotted and detection limit of this method was found 1.8×10^{-7} M. This method was tested on artificial waste water and recovery percentage was calculated as 93%

Keywords: voltammetry, head space analysis, polypyrrole, phenol, electropolymerization

ACKNOWLEDGEMENT

CONTENTS

ABBREVIATIONS AND SYMBOLS

Symbol	Explanation
AE	Auxiliary electrode
AdSV	Adsorptive stripping voltammetry
4-AAP	4-aminoantipyrine
BDD	Boron doped diamond electrode
BR	Britton Robinson buffer
CE	Counter electrode
CPE	Carbon paste electrode
CV	Cyclic voltammetry
Dp	Differential pulse
GC	Gas chromatography
GE	Graphite electrode
GPE	Gum phase extraction
HPLC	High-performance liquid chromatography
HS	Headspace
LLE	Liquid–liquid extraction
PANI	Polyaniline
PPy	Polypyrrole
PTh	Poly-thiophene
OTT	Open tubular trapping
PA	Polyacrylate
PDMS	Polydimethylsiloxane
RE	Reference electrode
SCE	Saturated calomel electrode
SDE	Single drop extraction
SDS	Sodium dodecyl sulfate
SE	supporting electrolyte
SPE	Solid–phase extraction
SPME	Solid phase micro extraction
SW	Square wave (voltammetry)
VOC	Volatile organic compound
WE	Working electrode

1. INTRODUCTION

1.1. Preconcentration for Volatile Compounds

Environmental pollution has arisen as a consequence of the human activity and that man has to solve or at least to control pollution in water, soil and air (Bruzzoniti et al, 2000). Contaminants can roughly be divided into organic, inorganic and metal species pollutants. Reliable and sensitive methods are required for the determination of these contaminants in aquatic environment.

Instrumentation techniques have undergone phenomenal developments in last two decades and among them chromatography provides a unique tool for the determination of organic contaminants in aquatic environment. However, complete non invasive measurements are still not possible in most cases and one or more pretreatment steps are necessary. Sample preparation steps provide the separation of matrix components from the pollutants of interest, cleanup, and therefore signal enhancement (Mitra, 2003). On the other hand, conventional detection methods coupled with separation techniques do not always provide the sensitivity required for low amounts of pollutants. Therefore, enrichment and matrix removal procedures are needed.

Preconcentration of contaminants from water samples, and generally sample preparation steps, are often accomplished by extraction techniques, based on enrichment by liquid–liquid extraction (LLE) or solid–phase extraction (SPE) techniques. Sorption methods are widely used in the chemical processes for the isolation, separation, and preconcentration of substances. The high efficiency of sorption processes ensures the priority development of these methods.

Growing demand for faster and greener preconcentration techniques has resulted into intensive research. In order to produce a solvent-free, fast and simple sampling extraction fiber, solid phase micro extraction (SPME) was developed in the early 1990s by Pawliszyn. SPME is based on the equilibrium partitioning of target analytes between the sampled matrix and a stationary phase, coated on a fused silica fiber (Pawliszyn, 1998).

Despite the wide application potential of SPME, it suffers from some limitations namely limited sorption capacity, the fragility of the fused-silica rod, and limited lifetime of the fiber. In order to overcome these limitations, extraction techniques such as gum phase extraction (GPE), in which a trapping material is used in a packed-bed configuration, and in-tube SPME, evolved from open tubular trapping (OTT), have been developed (Demeestere, 2007).

Preconcentration technique may differ according to the physicochemical properties of the analyte, i.e. polarity, solubility in a solvent and volatility. Volatile organic compounds (VOCs) can be defined as organic compounds whose vapor pressures are greater than or equal to 0.1 mmHg at $20^{o}C$ (Mitra, 2003). An important feature of VOC analysis is that in most cases the analytes are first transferred to a vapor phase and then analyzed by an instrument. Gas chromatography (GC) is the instrumental method of choice for the separation and analysis of volatile compounds. In addition, gas sensors are very promising tools for efficient and effective detection and on-line monitoring of hazardous gases in air (Oh, 2011). They can also used for monitoring volatile components evolved from foods to determine food quality. A great variety of physical and chemical gas sensors have been developed: including artificial noses. Electrochemical gas sensors exist for a limited range of electrochemically active gases however; some of these types of gas sensors have a low sensitivity and may display relatively poor selectivity in the analysis of complex gas mixtures (Kaisheva, 1997).

For gaseous samples, pre-concentration devices are widely used for achieving improved detection sensitivity for various analytical instruments, especially for less sensitive compact instruments designed for on-site detection (Song, 2007). Most of the developed devices were constructed based on adsorbing materials packed in a tube for trapping the samples, pumps for collecting samples, and a system for thermal desorption of the trapped samples. Popular adsorbents used in these devices were charcoal, Tenax-TA, cotton and glass wool.

Analytical methods for determination of trace amounts of VOCs in water samples generally require an appropriate preconcentration step. The target species can be enriched by purging the aqueous phase with a gas stream followed by trapping or by solvent extraction methods.

Headspace (HS) sampling allows the introduction of volatile compounds from the sample matrix directly into a gas chromatographic (GC) instrument (Mitra, 2003). The technique is relatively simple when compared to other injection techniques, such as purge and trap mentioned above. In HS technique, aqueous sample is transferred to a vial placed on a thermostatic stirrer to drive the volatile components into the space above the level of the liquid sample. An aliquot of the vapor phase is introduced via a gas tight syringe into a GC equipped with capillary column.

Modern HS techniques described include static and dynamic headspace extraction, SPME and single drop extraction (SDE) which a single drop of organic solvent was contained at the end of a PTFE rod (Buczewski et al., 2002). SDE is a simple method for reducing solvent consumption. In this technique, pure solvents or mixtures can be used for the selective extraction of different organic species. However, stirring or sonification of samples in SDE experiments caused damage to the organic drop whereas stirring the liquid sample increases extraction efficiency by SPME. On the other hand SDE method represents a cheap and attractive alternative to SPME. Examples of applications in environmental, clinical, food and pharmaceutical analysis are encountered in the literature.

1.2. Phenol and Phenolic compounds

Phenol is a monosubstituted aromatic hydrocarbon and it exists as a colorless or white solid. Phenol gives off a sweet smell detectable to most people at 40 ppb in air and at about 1–8 mg/L in water. It evaporates more slowly than water and is moderately soluble in water (EPA, 2002).

Phenols are also a class of analytes of major environmental importance. They are produced through both natural and anthropogenic processes and can be found in the aquatic environment as biodegradation products of humic substances, lignins and tannins or as derivatives of plastics, dye industries and pulp processing (Bruzzoniti et al., 2000). It is naturally occurring in some foods, in human and animal wastes, and in decomposing organic material, and it is produced from the metabolism of aromatic amino acids.

Phenol has been isolated from coal tar, but it is now synthetically manufactured. Currently, the largest use of phenol is as an intermediate in the production of phenolic resins, which are used in the plywood, adhesive, construction, automotive, and appliance industries. Phenol is also used in the production of synthetic fibers such as nylon and for epoxy resin precursors such as bisphenol-A.

Phenol is toxic to bacteria and fungi, and it is used as a slimicide and disinfectant. Because of its anesthetic effects, phenol is used in medicines such as lotion, ear and nose drops, throat lozenges and sprays and antiseptic lotions. The greatest potential source of exposure to phenol is in the occupational setting, where phenol is used in manufacturing processes. Most of the phenolic compounds are known to be toxic and might denature the proteins at low concentrations. Thus, monitoring of phenolic compounds is important to evaluate the risk and the effectiveness of posterior water treatment.

1.3. Determination Methods for Phenolic Compounds

The most commonly used analytical methods are spectrophotometric methods based on the oxidative coupling of phenols with 4-aminoantipyrine (4-AAP) (American Public Health Association). However, the use of large amounts of toxic solvents such as chloroform is major drawback.

Phenolic pollutants in water are usually in similar structure. In addition to their low concentration levels, the similarity in reactivity and properties for all the phenolic compounds makes their separation a difficult task. Therefore, gas chromatography (GC) has been widely used for the analysis of phenols, usually with a derivatization and preconcentration step. However, derivatization increases the sample preparation time and introduces a possible source of errors. Alternatively phenols can be determined by high-performance liquid chromatography (HPLC) with different detection systems such as ultraviolet (UV) detection, photodiode, mass spectrometry, and fluorescence detection (Zhao et al, 2001). On the other hand, electrochemical techniques appear to be very promising because they ensure practically good analytical performance characteristics without expensive and sophisticated instrumentation.

It is common knowledge that most phenols can be easily oxidized at various types of anodes leading, in the first stage, to the formation of phenoxy radicals. Further polymerization of these primary oxidation products results in the formation of polymeric films which cause electrode deactivation. This behavior is in line with the fact that, during phenol oxidation, the deactivation of the electrodes occurs more slowly in acidic solutions than in neutral or alkaline ones (Spataru et al 2010)**.** In order to cope with this problem gas-enzyme sensors have been developed. Amperometric gas sensors find applications in various fields and enzyme-based gas sensors can broaden the applications due to the superior selectivity based on the substrate specificity of the enzyme. Since the enzyme requires an aqueous environment, the interface between the gaseous analyte and the aqueous sensor electrolyte is of special importance.

Another drawback of the electrochemical method for phenol determination is that the electrode surface can be impaired by the presence of surface active materials and electroactive impurities in the matrices. As an alternative, headspace technique can be used prior to the voltammetric measurement since the interference from non-volatile components of the sample will be eliminated. Despite the vast number of publication on voltammetric determination of pollutants in water matrices are encountered in the literature, only a few studies those require special design to utilize as a biosensor placed above the sample solution (Kaisheva, 1997, Yu, 2003).

In this thesis, it was presented a much simpler method that utilizes a voltammetric electrode modified with a conducting polymer as a head space preconcentration tool for volatile compounds and transducer as well. The GCE surface was modified by electropolymerization, and it was slotted in a sealed vial and placed over the solution in heated gently on a hotplate with a stirrer for phenol preconcentration. After a predetermined time, the electrode is carefully removed and then, it is immersed in an electrolyte solution for subsequent voltammetric determination.

Electrochemical and chemical plating of conducting polymer is an attractive alternative for surface modification of electrode in recent years. The advantages of electrochemical method are that it can be controlled easily, and a uniform and stable coating is obtained.

Among the conducting polymers, polypyrrole (PPy) has good environmental stability, facile synthesis and higher conductivity than many other conductive polymers. The surface is more flexible, because polymer can be formed conveniently by changing dopant ions or using substituted pyrrole monomers under controlled electrochemical conditions.

Polypyrrole was used as an HS sorbent for phenolic compound determination by GC (Alizadeh et al., 2007). In electrochemically synthesis of PPy, the porous and uniform polymer film is directly electrodeposited on the surface of electrode from an aqueous solution containing pyrrole and electrolyte, using one of the electrochemical techniques.

1.4. Formation of Polypyrrole on the GCE Surface

Historically, the story of most conducting polymers is tightly connected to electrochemistry, since most of the classical conducting polymers, like the ones based on heterocycles (polypyrrole, PPy, poly-thiophene, PTh, polyaniline, PANI), were initially synthesized by electrochemical oxidation. The mechanism for electrochemical polymerization, is shown in the scheme (Fig 1.1). It consists of an oxidation step generating a cation radical followed by coupling and deprotonation steps generating the dimer; this being more easily oxidized than the monomer, the same reaction suite occurs to produce longer species until precipitation takes place at the electrode/electrolyte interface.

1.5. Fundamentals of Voltammetry

The term voltammetry is applied to that group of electroanalytical techniques in which the current that flows through an electrochemical cell is measured as the potential applied to the electrodes in the cell is varied. The essential difference between voltammetric and other potentiodyamic techniques is that in voltammetry an electrode with a small surface area ($< 10^{-5}$ mm^2) is used to monitor the current produced by the species in solution reacting at this electrode in response to the potential applied to it. Because the electrode used in voltammetry is so small, the amount of material reacting at the electrode can be ignored. This is in contrast to the case in coulometry where large area electrodes are used so that all of a species in the cell may be oxidised or reduced (Thomas, 2001).
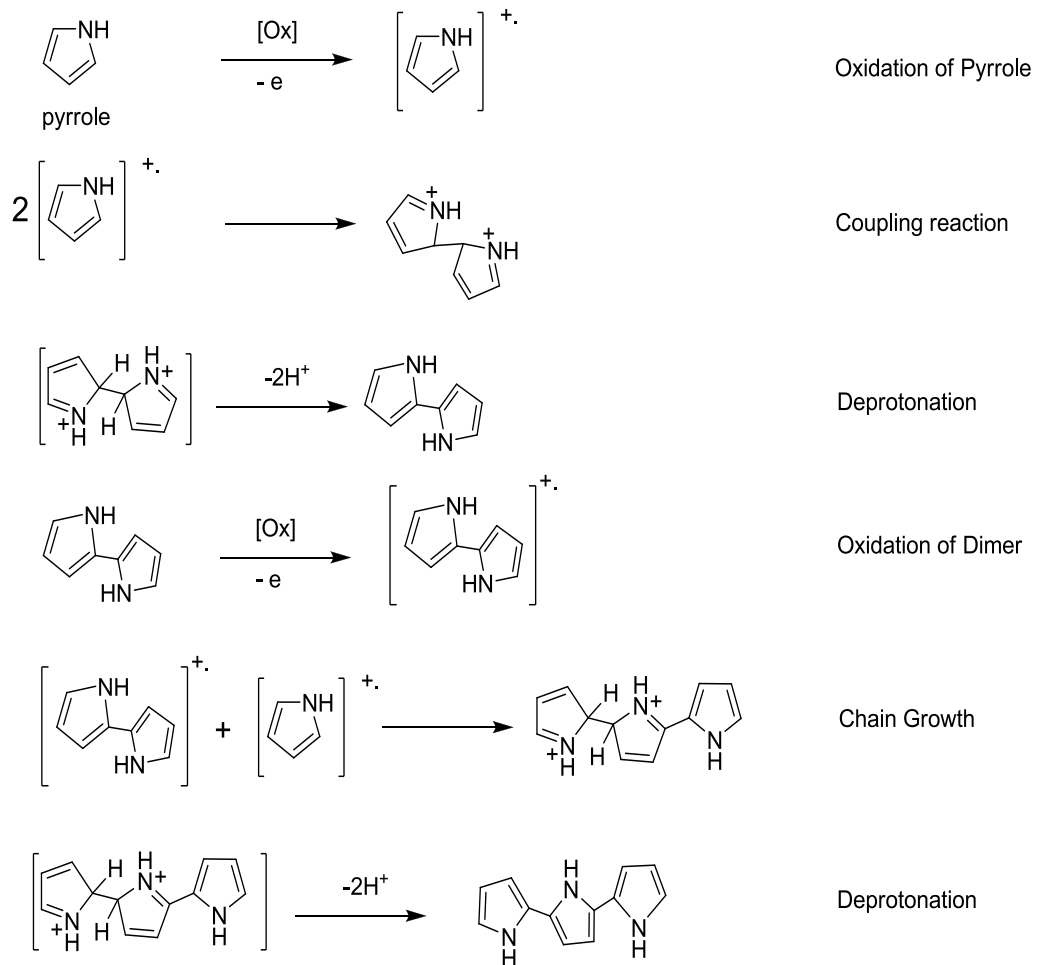
Figure 1.1 The sheme for electrochemical polymerization of pyrrole
(Skotheim, 2007)

Polarography is the special technique where the mercury, flowing through a fine capillary and these droplets are used as the small electrodes in a voltammetric cell. This name is derived from the fact that the electrode can be polarized i.e. no direct current flows across its interface with the solution even though there is a potential difference across this interface.

The electrochemical cell used in voltammetry consists of two electronic conductors called electrodes immersed in an ionic solution as a conducting phase containing the substance of analytical interest called the analyte. At the solution-electrode interface, the analyte and the interfacial processes are exploited in electro-analytical chemistry. The application of a voltage or a current from an external source to the electrodes produces an electrical response from the analyte in the cell solution. The nature and magnitude of this response may be used to both identify and quantify the analyte.

The small electrode used to monitor the response of the analyte is known as the working electrode (WE). Even though only a negligible amount of material is involved at the working electrode, a high current density develops at its surface as the surface area is too small. The WE may be constructed from a wide variety of conducting materials including various forms of carbon and metals such as mercury, gold or platinum. These electrodes may be stationary, rotating or, in the case of mercury, flowing with respect to the cell solution.

The second electrode in the simple cell, called a counter electrode (CE),- serves two purposes. It is used to control the potential applied to the working electrode and to complete the circuit for carrying the current generated by the processes occurring at the WE. In the former role it must act as a reference electrode (RE). The ideal reference electrode must be able to maintain a constant potential at its interface with the cell solution irrespective of any current that may flow across this interface. This can only be achieved if the reaction that controls the potential of this electrode is very fast and if there are no significant changes in the ion concentration profile in the locality of the interface-conditions. Commonly used reference electrodes are the Ag/AgCl electrode or SCE (saturated calomel electrode). These electrodes are robust, easily constructed and maintain a constant potential over long periods of time.

The solution in the cell will have a resistance to the flow of current and as a consequence there will be a potential drop across the cell. Although this may be minimized by adding a high concentration of a supporting electrolyte (SE) to the cell solution it can never be eliminated. The auxiliary electrode, AE, is immersed to the cell to complete the current carrying circuit. The area of this AE must be large compared to that of the WE. The addition of the auxiliary electrode means that the counter electrode now is used only to control the potential of the working electrode and so becomes a true reference electrode. Since no current flows through the potential controlling circuit, the size of the reference electrode can be reduced and a conducting salt bridge can be placed between the variable composition cell solution and the reference electrode so that the composition of its solution will remain constant. The increase in resistance resulting from these changes does not affect the potential control of the working electrode.

1.6. The Aim of the Thesis

Electroanalytical applications have an increasing importance since the modification of the electrode surfaces provide a more selective and sensitive surfaces as well as their increased electrocatalytic activation. However, the electrode surface can be impaired by the presence of surface active materials and electroactive impurities in the matrices.

Headspace technique provides a practical tool for preconcentration of volatile organic compounds prior to their measurement with mostly chromatographic methods. In this thesis, an effective method was developed in which a voltammetric electrode modified with a conducting polymer as a head space preconcentration tool for volatile compounds and transducer as well. Any interference from non-volatile components of the sample will be eliminated by this means. For this purpose, the GCE surface was modified by electropolymerization, and it was slotted in a sealed vial in which the solution in heated gently on a hotplate with a stirrer. Phenol was chosen to test the performance of the method. After a predetermined time, the electrode is carefully removed from standard phenol solution and then, it is immersed in an electrolyte solution for subsequent voltammetric determination.

One of the important tasks is the choice of the monomer for the electropolymerization. Most of the commercial polymeric sorbents such as polyacrylate (PA) and polydimethylsiloxane (PDMS) are known to be insulator and therefore, electroactive volatile organic compounds cannot be detected by voltammetric methods after HS sampling. Polypyrrole (PPy) have good environmental stability, facile synthesis and higher conductivity than many other conductive polymers. In electrochemically synthesis of PPy, the porous and uniform polymer film is directly electrodeposited on the surface of electrode from an aqueous solution containing pyrrole and electrolyte, using one of the electrochemical techniques. Literature survey has revealed that no study was carried out for electrochemical detection of phenol after HS preconcentration. This is the pioneering work for differential pulse voltammetry combined with HS preconcentration by using dodecyl-sulphate doped PPy-modified GC electrode was used for determination of phenol in water samples.

2. EXPERIMENTAL

2.1 Materials

Pyrrole (Py) from Alfa-Aeser, sodium dodecyl sulfate (SDS), conc. sulfuric acid, sodium hydroxide, glacial acetic acid, o-phosphoric acid, boric acid, sodium chloride and phenol were obtained from Merck used with no further purification in experiments. The water was supplied from Ultrapure water (Millipore Q). Britton Robinson buffer systems were prepared by using 0.04 M glacial acetic acid, o-phosphoric acid and boric acid mixture.

2.2 Equipment

Voltammetric studies were carried out with Palmsens Compact Stat model potentiostat and Autolab PGS 101 potentiostat. A special voltammetric cell was designed for voltammetric measurement and electropolymerization (Figure 2.1). The cell consists of three electrodes namely; platinum wire was used as the auxiliary electrode, Ag/AgCl electrode as the reference electrode and glassy carbon electrode (GCE) as the working electrode. pH adjustments were made with Orion 4 star pH-meter.

2.3. Electrode Modification

Polypyrrole coating was formed on the GCE surface by electropolymerization. For this purpose, the electrode potential was cycled at a rate of 20 mV s^{-1} between 0.5 – 1.2 V for several times in the presence of 0.1 M pyrrole in 7.10^{-3} M SDS as described elsewhere (Alizadeh, 2007).
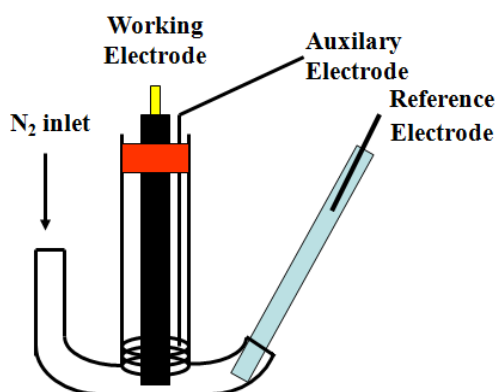

Figure 2.1 Voltammetric cell used for electropolymerization.

As shown in the Figure 2.2, the peak around 1.0 V in first cycle has shifted to more negative potentials upon cycling and then, disappeared. This peak belongs to pyrrole oxidation and oxidation product of this reaction can oxidize more easily than pyrrole to form polypyrrole (Skotheim, 2007).

At the end of the polymerization step, a thin black film was observed on the electrode surface. This film may include dimeric pyrrole compounds which must be removed in order to record a stable background current. For this purpose, the electrode was immersed into 0.01 M H_2SO_4 solutions and then, the potential was cycled between 0 to 1.2 V for 25 times at 100 mV s^{-1}. At the end of the scan, the electrode was washed with ultrapure water. Then, the electrode was immersed in 1.0 mL of pH 7.0 BR buffer and stable background voltammograms were recorded by dp voltammetry with a 50 mV s^{-1} scan rate. The pulse amplitude was selected as 25 mV. The electrode was washed with ultrapure water and dried with N_2 gas prior to the preconcentration step.
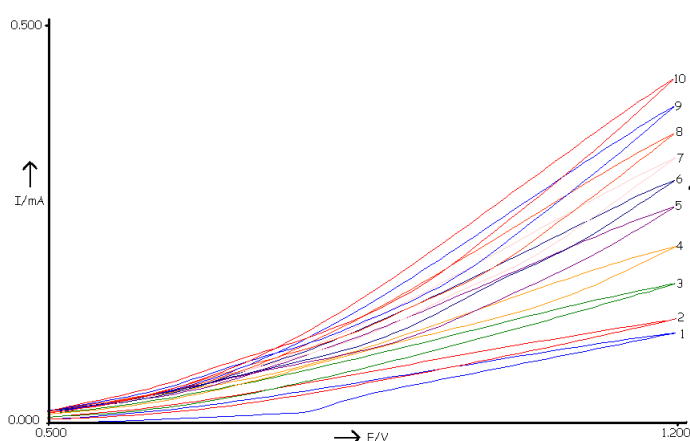
Figure 2.2 Cyclic voltammograms recorded for a GCE in 0.1 M pyrrole solution containing $7x10^{-3}$ M SDS

2.4. Preconcentration and Voltammetric Analysis

The electrode coated with the polymeric film was carefully slotted top of the vial containing certain volume of sample solution prepared in sulfuric acid. All the optimization experiments were carried out in the presence of $2x10^{-4}$ M phenol unless otherwise stated. After the proper amount of salt was added, the solution was heated on a hotplate and the magnetic stirrer was switched on. Schematic representation of the preconcentration device can be seen in Figure 2.3.

At the end of the preconcentration time, the electrode was carefully driven back out from the vial and immersed into the voltammetric cell containing BR buffer for subsequent measurement. Stripping voltammograms are recorded at a scan rate of 50 mV s^{-1} and dp mode is applied with a 25 mV pulse amplitude.
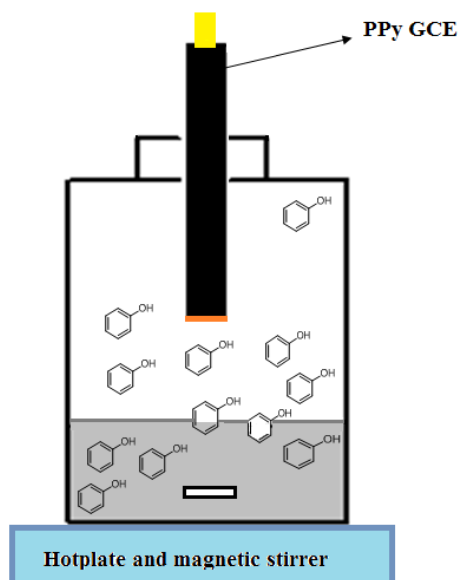
Figure 2.3 Experimental set-up for preconcentration step of the method
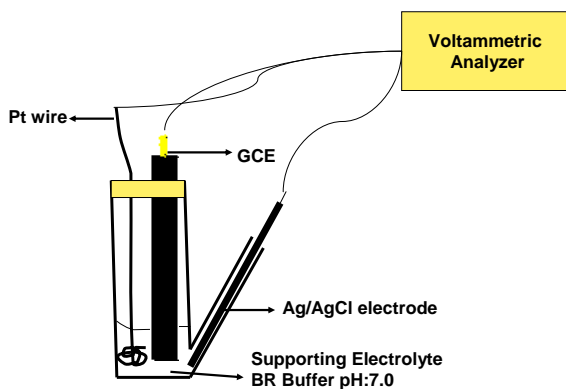
Figure 2.4 The cell used for voltammetric measurements

2.5 Preparation of waste water simulation

Synthetically concocted waste water was prepared according to a procedure given elsewhere (Timur, 2003). The composition was composed of $(NH_4)_2SO_4$ (0.5g), $MgSO_4$ (1.0000 g), $MnSO_4$ (0.100 g), $FeSO_4$ (0.0050g) and known amounts of phenol in 1 L of tap water. The pH of this solution was made 2.0 by simply adding H_2SO_4 solution.

3. RESULTS AND DISCUSSION

3.1. Optimization Studies

In this thesis, the method developed has two stages; first the preconcentration of the analyte into the polymeric coating of the GCE placed on top of the vial and subsequent voltammetric measurements in the cell. Therefore, the parameters those affect the both stages were investigated in the vial and in the cell as well.

The cell pH: Initial studies were conducted to see the effect of pH on the electrochemical signal of phenol at a GCE surface. Figure 2.1 shows the effect of pH on the dp voltammetric response of $2x10^{-4}$ M phenol at a Ppy modified GCE. The polimerization was accomplished as given in Experimental Section.

Britton Robinson (BR) buffer systems was used throughout the study in pH range of 3.0-11.0 to maintain the similar ionic species in the solution. The oxidation peak of phenol has shown a dependence on the medium pH and the peak current has increased first and then, decreased as the pH increases. The results were found in agreement with a previous study (Hu, 2001).
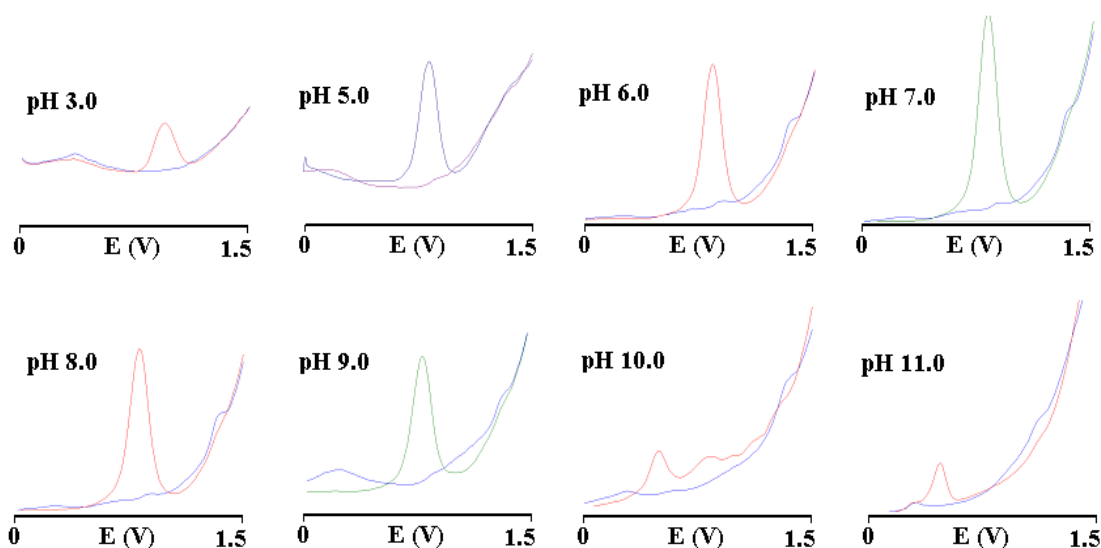

Figure 3.1 Dependence of the dp voltammetric response of $2x10^{-4}$ M phenol prepared in Britton Robinson buffer systems on the medium pH at a Ppy modified GCE.

The peak charactesitics were given in Table 3.1. As can be followed from the table, maximum peak current was observed in pH 7.0. This is also consisted with the graph given in Figure 3.2. Further experiments were performed at this pH. The calibration graph constructed at this pH can be seen in Figure 3.3.

Table 3.1 The effect of pH on the peak characteristics of $2x10^{-4}$ M phenol prepared in Britton Robinson buffer systems at a Ppy modified GCE

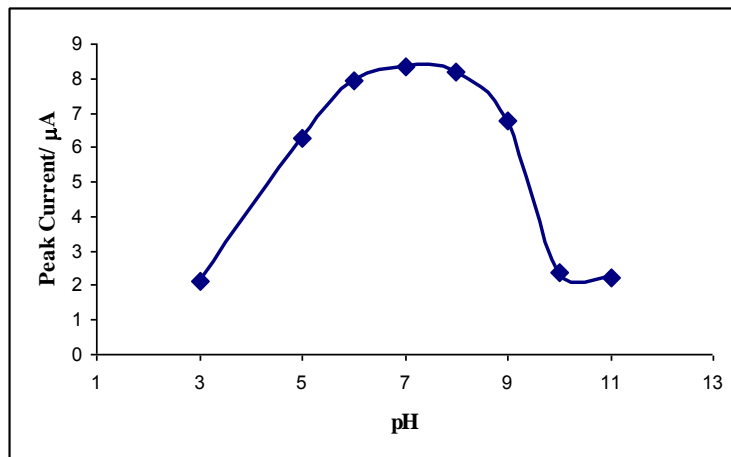| pH	3.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0
Ep_1(V)	-	-	-	-	-	-	0.49	0.45
Ip_1(μA)	-	-	-	-	-	-	1.907	2.227
Ep_2(V)	0.93	0.83	0.83	0.82	0.80	0.80	0.80	-
Ip_2(μA)	2.108	6.267	7.916	8.245	8.207	6.766	0.466	-

Figure 3.2 The effect of cell pH on direct voltammetric measurement of $2x10^{-4}$ M phenol
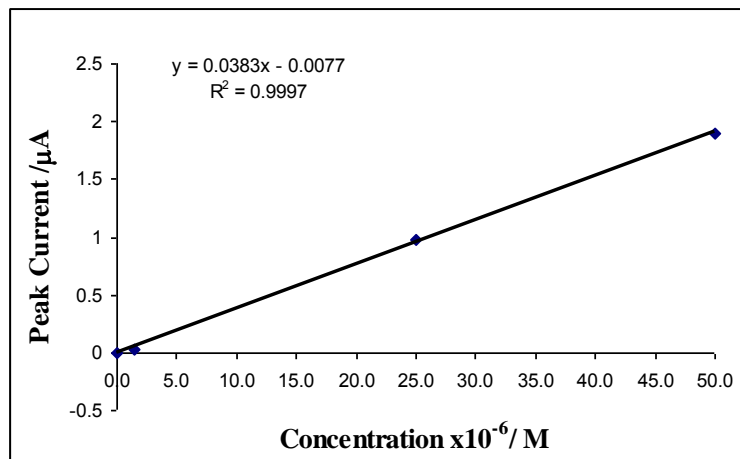

Figure 3.3 Calibration graph for direct voltammetric measurement of phenol at a PPy modified GCE in pH 7.0 BR buffer

The pH of vial: The sample pH is one of the most important parameter as it determines the head space concentration of the phenol in the headspace. In extraction studies of organic compounds those display weak acidic character, the pH $<$ pK_a -2 media is recommended to maintain the molecular form in the solution (Mitra, 2003). Considering the weak acidic character of phenol (pKa 9.90), the effect of sample pH on the extraction from water samples was studied. The electrode was exposed for 15 min to 10 mL of solution at different pHs (2.0-10.0) by using different ratio of sulfuric acid and sodium hydroxide and each spiked with phenol standard to be 2×10^{-4} M in the vial. The solution was stirred at 400 rpm throughout the preconcentration step. Figure 3.4 shows the voltammograms obtained and the peak characteristic are given in Table 3.2.
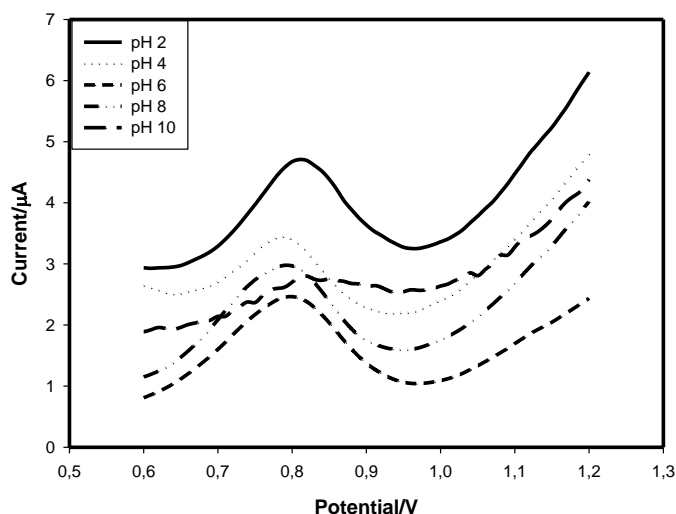
Figure 3.4 The effect of pH on the head space preconcentration of 2×10^{-4} M phenol in the vial for 15 min at 70°C prior to the voltammetric determination.

Table 3.2 The effect of vial pH on the characteristics of the voltammetric signal recorded at pH 7.0 BR buffer after exposure to 2×10^{-4} M phenol for 15 min at 70°C.

pH	2.0	4.0	6.0	8.0	10.0
Ep(V)	0.79	0.80	0.80	0.80	0.80
Ip(μA)	1.902	1.597	1.587	1.603	0.439

It follows from the table above that maximum recovery is obtained at pH 2.0. At lower pHs, the acid–base equilibrium of phenol shifts significantly toward

the neutral form, which have high vapor pressure and greater affinities toward the film, and the extraction efficiencies are, therefore, increased.

The next step was designed to see the effect of sulfuric acid concentration on the voltammetric signal. Figure 3.5 shows the effect of sulfuric acid concentration used in head space conditions. The electrode was exposed for 15 min to 10 mL of sulfuric acid solution at different concentrations spiked with phenol standard to be $2x10^{-4}$ M in the vial.
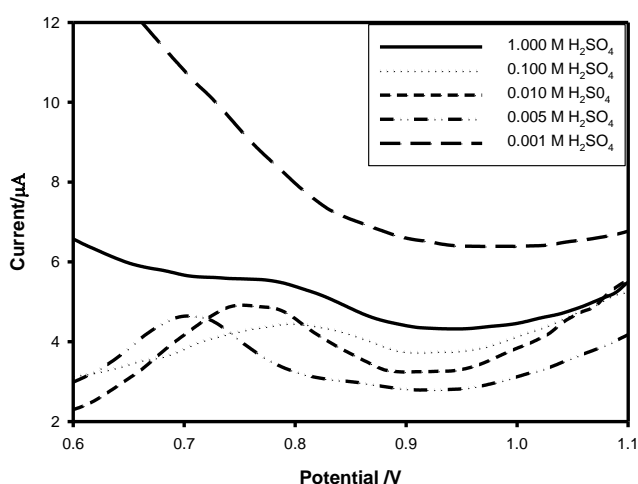

Figure 3.5 The effect of sulfuric acid concentration on the voltammetric signal for 15 min exposure to $2x10^{-4}$ M phenol at 70°C.

The voltammetric signal characteristics were given in Table 3.3. It is clear that acid concentration has a major effect on the voltammetric signal. The best results were obtained with 0.01 M sulphuric acid solution in the vial and therefore, this concentration was used for further studies.

Table 3.3. The effect of sulfuric acid concentration on the voltammetric peak characteristics of $2x10^{-4}$ M phenol preconcentration for 15 min at 70°C.

H_2SO_4 conc (M)	1.000	0.100	0.010	0.005	0.001
Ep(V)	0.79	0.80	0.75	0.70	-
Ip(μA)	0.425	0.946	2.267	2.107	-

PPy Thickness: Cyclic voltammetry is a suitable tool for preparation of a homogenous and reproducible polymeric film on the GCE surface. The film thickness can easily be controlled by changing the potential range scanned and the

repetitive number of the cycles in the monomer solution. The film thickness along with the porosity and active surface area of the electrode has a major influence on the amount of analyte preconcentrated.

In addition, the polymeric surface should maintain the conductive character necessary to function as a transducer. This feature is the main difference from the polymeric fibers used in chromatographic studies those inserted to injection port shortly after the preconcentration for thermal desorption. On the other hand, in head space voltammetry, the electrode has dual purpose for preconcentration and subsequent voltammetric measurement. The number of cycle used in electropolymerization is one of the most important parameter to be optimized for tuning the thickness and therefore, the performance of the electrode in both steps.

In this study GCE surfaces were coated with a number of cycles in 0.1 M pyrrole solution containing 7×10^{-3} M SDS as the dopant as given earlier. As shown in Figure 3.6, the anodic peak current of 2×10^{-4} M phenol has given an increase with the cycle number from 1 to 5. Table 3.4 summarizes the results obtained for repetitive cycling number on the peak characteristics of 2×10^{-4}M phenol. The results were given as the average of three replicates. After 5 cycles, high background current was observed and subsequent 5 cycles for coating was chosen for further experiments.
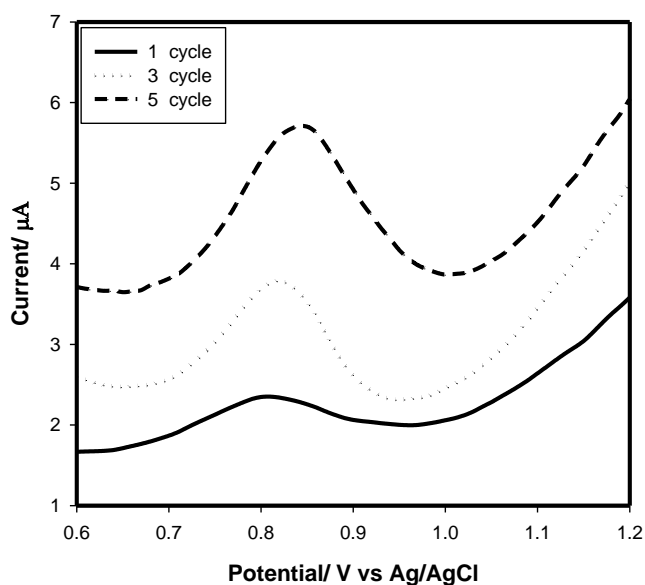
Figure 3.6 The effect of the cycle number on the voltammetric signal of phenol in pH 7.0 BR buffer after preconcentration from 0.01 M H_2SO_4 solution for 15 min at 70°C.

Table 3.4 The effect of the cycle number on the peak characteristics of 2×10^{-4}M phenol in pH 7.0 after preconcentration from 0.01 M H_2SO_4 solution for 15 min at 70°C.

Cycle numbers	1	3	5
Ep(V)	0.80	0.82	0.84
Ip(μA)	0.513	1.630	1.942

Amount of salt added to the vial: Salting out is a term used for addition of an inert salt to an aqueous solution to enhance the evaporation of volatile components based on the principle that their solubility decreases at high salt concentrations. In high ionic strength, the water molecules are attracted by the ions dissolved in the solution in favor of increasing the volatility of the organic compounds as the number of water molecule available are decreased. In this study, the amount of salt added to the sample solution was studied in a concentration range of 0 - 200 g L^{-1} and resulting voltammograms can be seen in Figure 3.7.
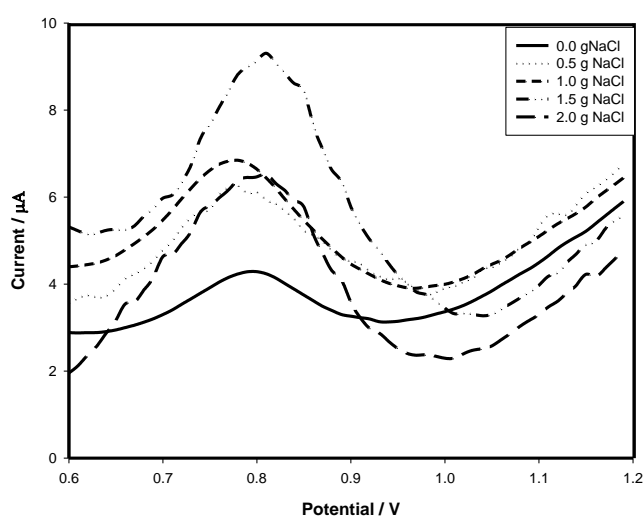

Figure 3.7 The effect of salt amount in the sample solution. Voltammetric signals were recorded after preconcentration of 2×10^{-4}mol L^{-1} phenol in 0.01 M H_2SO_4 solution for 15 min at 70°C.

The peak characteristics are given in Table 3.5 and the peak currents were plotted against the salt amount added to the aqueous sample solution in Figure 3.8. All results were plotted as the average of three replicates. Best results were obtained with 150 g L^{-1} salt amount.

Table 3.5 Dependence of voltammetric signal onto the salt concentration in the vial.

NaCl (g L^{-1})	0	50	100	150	200
Ep(V)	0.80	0.80	0.78	0.81	0.81
Ip(μA)	2.148	2.608	2.717	4.984	4.702
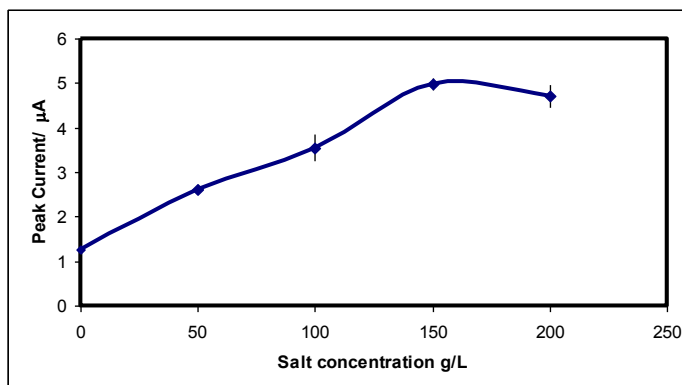
Figure 3.8 Dependence of voltammetric signal onto salt concentration in the vial.

Vial temperature: Preconcentration of a volatile species strongly depends on the temperature. The volatile analytes can effectively dissociate from the matrix at elevated temperatures and move into the headspace for rapid extraction by the polimeric coating on the electrode surface. However, the coating/headspace distribution coefficient also decreases with an increase of temperature resulting in a decline in the amount of the analyte extracted. Therefore, headspace temperature should compromise these two opposite effects. A temperature range of 30–75 C was used to study the extraction temperature on the extraction efficiency of phenol. As shown in Table 3.6, the extraction effiency increases at elevated temperature. However, a significant decrease in adsorption capacity was observed when temperature increased up to 75°C. It should be noted that adsorption is generally an exothermic process and therefore, the partition coefficient of analytes between headspace and fiber decreases at high temperatures. Best results were obtained at 60°C and this finding was found in agreement with former studies (Alizadeh, 2007). Therefore, the vial temperature was set to 60°C for further studies.
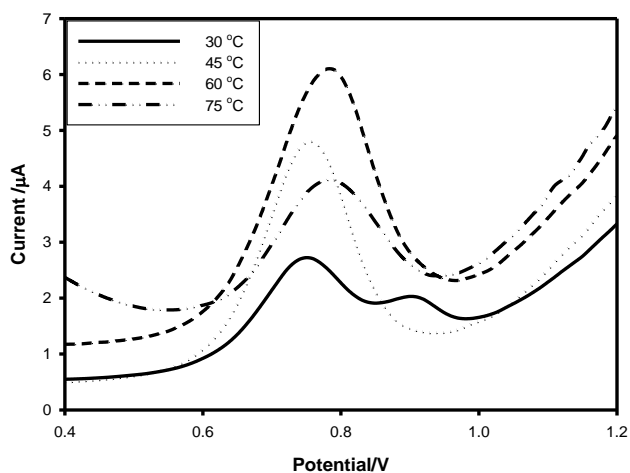
Figure 3.9 Voltammograms obtained after preconcentration in 2×10^{-4} mol L^{-1} phenol in 0.01 M H_2SO_4 solution containing 150 g L^{-1} NaCl for 15 min at different temperatures.
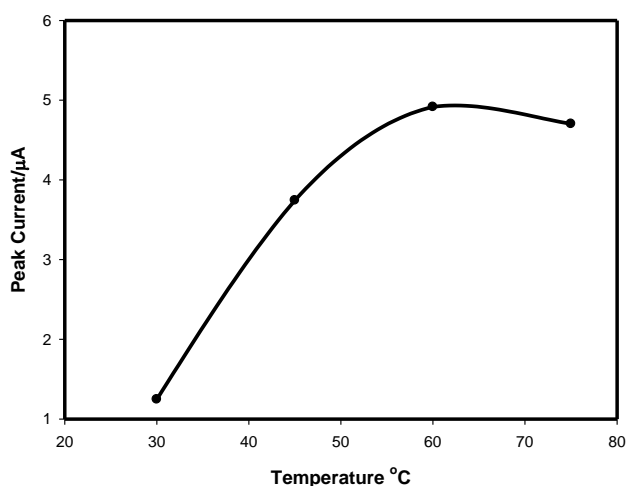

Figure 3.10 Dependence of voltammetric signal onto preconcentration temperature

Exposure time: The time elapsed on top of the vial in the solution head space strongly effects the amount of analyte that can be preconcentrated in the polymeric surface of the electrode. Since the maximum amount of analyte preconcentrated at the surface verify the sensitivity of the method, this parameter should be optimized. This effect was studied in the range of 5–60 min at 60°C and the stirring rate constant at 400 rpm. A series of 2×10^{-4} M standard phenol solutions were prepared and the variation of the analytical signal for each analyte was studied as a function of exposure time. Figure 3.9 shows the voltammograms obtained for these exposure time studies. Results were obtained in three replicates to ensure reproducibility.
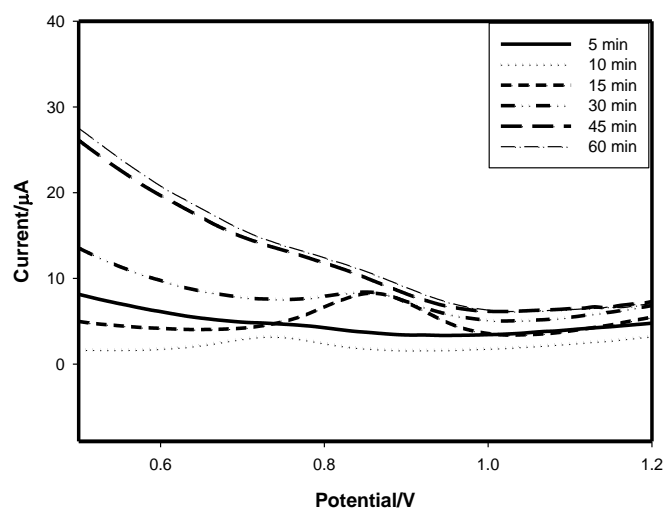
Figure 3.11 dp voltammograms obtained after preconcentration in 2×10^{-4} mol L^{-1} phenol in 0.01 M H_2SO_4 solution containing 150 g L^{-1} NaCl at $60^{\circ}C$.

The peak current data obtained from Figure 3.11 were plotted against the exposure time in Figure 3.12. All results were obtaining in an average of three replicates. Exposure times longer than 30 min have resulted signal loss probably due to desorption of adsorbed phenol molecules and therefore, 30 min was selected as a reasonable compromise between preconcentration and analysis time.
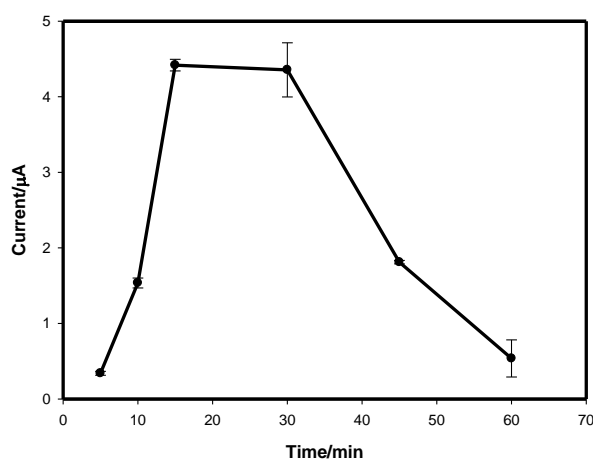
Figure 3.12 Dependence of voltammetric signal onto head space exposure time.

Agitation: Stirring the sample increases the mass transfer in the aqueous phase and induces the convection in the headspace. Therefore, equilibrium between the aqueous and headspace can be achieved more rapidly. Figure 3.13 shows the voltammograms obtained after 30 min exposure time applied for 10 mL of the sample solutions containing 150 g L^{-1} NaCl at various stirring speeds.

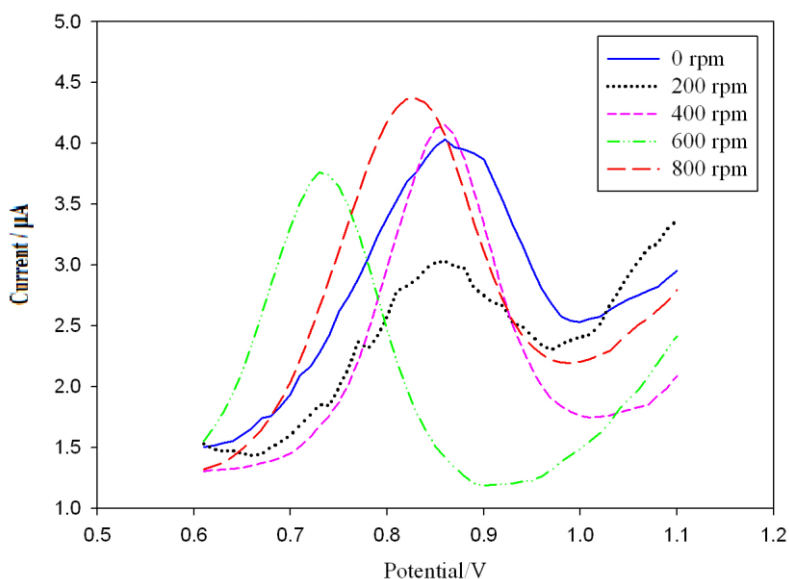Figure 3.13 dp voltammograms obtained after preconcentration in 2×10^{-4} mol L^{-1} phenol in 0.01 M H$_2$SO$_4$ solution containing 150 g L^{-1} NaCl at 60°C with different stirring rate.

Subsequent recordings have revealed that preconcentration efficiency reaches a maximum and remains constant above 600 rpm (Figure 3.14). Thus, further experiments were carried out at a stirring rate of 600 rpm.
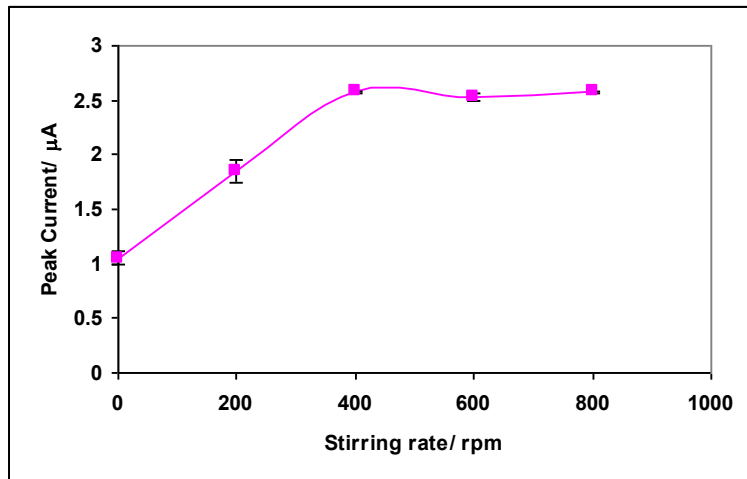

Figure 3.14 Dependence of voltammetric signal onto the stirring rate during head space sampling procedure

Sample volume: The volume of the vials was chosen as 20 mL since 10 mL of sample solutions was used. The head space concentration of the volatile species are expected to be dependent on the volume above the solution and therefore, the effect of sample volume should be studied.

The sample volume was changed in between 5-18 mL and therefore, the heas space volumes were changed from 15-2 mL, accordingly. Figure 3.15 shows the voltammograms recorded in pH 7.0 BR buffer after preconcentration above 0.01 M H_2SO_4 solution containing 150 g L^{-1} NaCl and spiked with $2x10^{-4}$M phenol for various sample volumes for 30 min exposure time under stirring at 600 rpm. The peak currents were plotted against the sample volume and given in Figure 3.16. As can be followed from the figure, 5 mL sample and therefore, 15 mL of headspace volume was found optimum and used for calibration studies. This can be concluded as the more analyte evaporates to reach the equilibrium as the headspace volume increases.

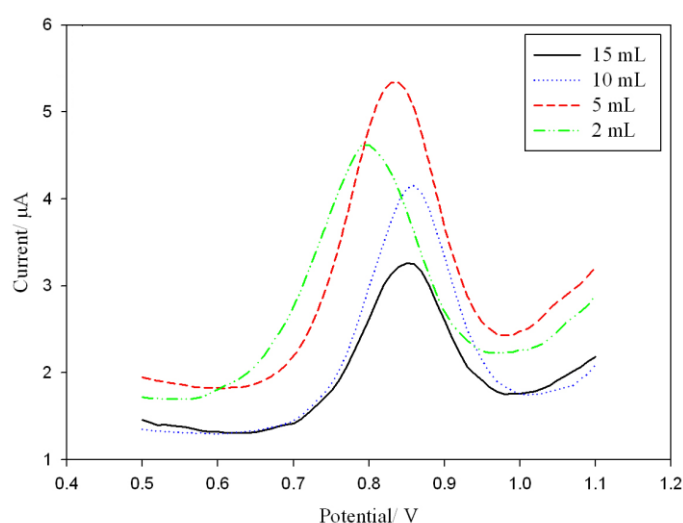Figure 3.15 dp voltammograms obtained after preconcentration in $2x10^{-4}$mol L^{-1} phenol in different volumes of 0.01 M H_2SO_4 solution containing 150 g L^{-1} NaCl at 60°C.
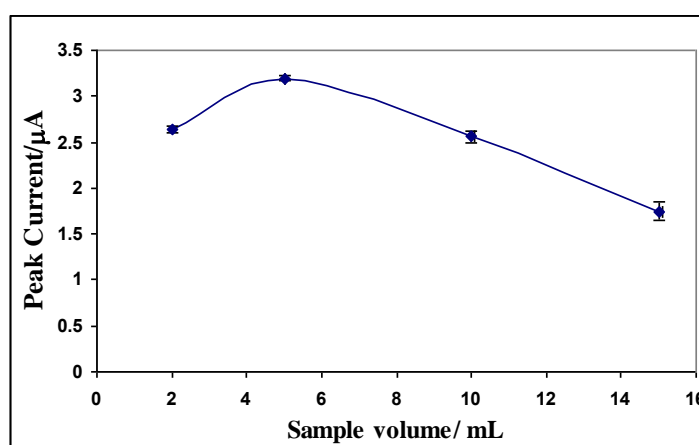

Figure 3.16 Dependence of voltammetric signal onto headspace volume

As a whole, Table 3.6 lists the experimental conditions found optimum for headspace voltammetric determination of 2×10^{-4}M phenol.

Table 3.6 Optimized conditions for HS-voltammetric determination of phenol

Parameter	Optimum Value
Cell pH	7.0
Sulphuric Acid Concentration (M)	0.01
Salt Concentration (g L^{-1})	150
Exposure Time (min)	30
ExposureTemperature (^{o}C)	60
Stirring Rate (rpm)	600
Headspace Volume (mL)	15

3.2. Quantitative analysis

Under optimized conditions given above, analytical characteristics of the method were studied. Figure 3.17 shows the voltammograms obtained for different phenol concentration and calibration graph constructed for phenol determination was given in Figure 3.18.
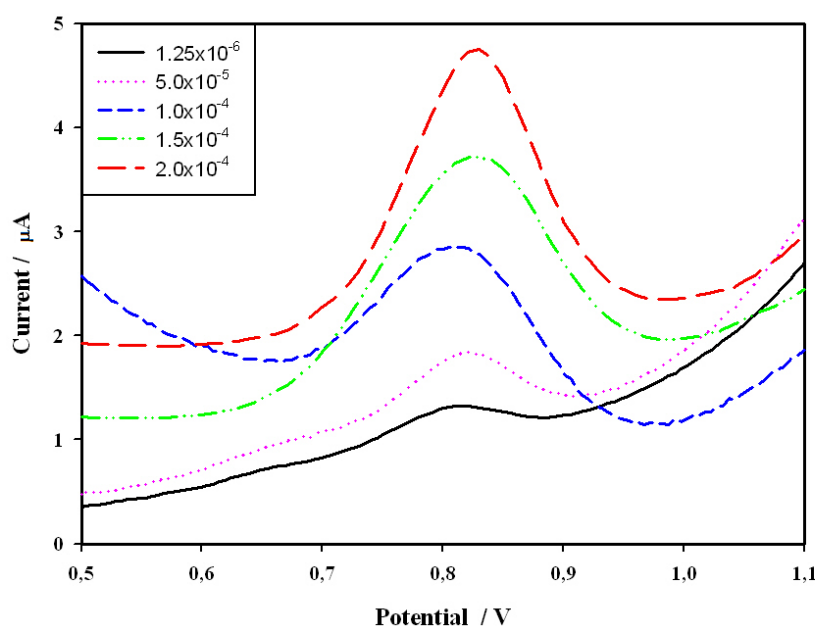
Figure 3.17 The HS voltammograms obtained for 1.25×10^{-6}, 5.0×10^{-5}, 1.0×10^{-4}, 1.5×10^{-4} and 2.0×10^{-4} M phenol

Figure 3.18 The HS-voltammetric calibration curve for phenol in high
concentration range

For lower concentration range, the voltammograms were recorded and given
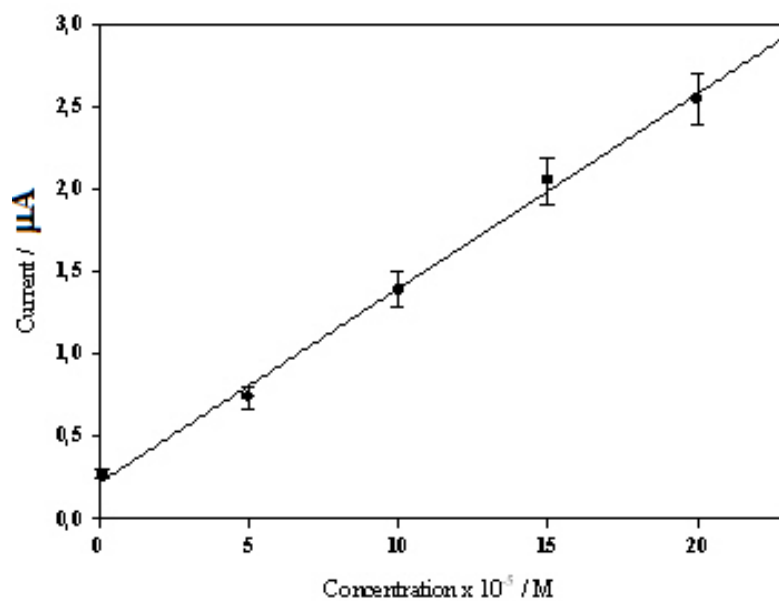in Figure 3.19. The calibration graph for submicromolar level was given in Figure
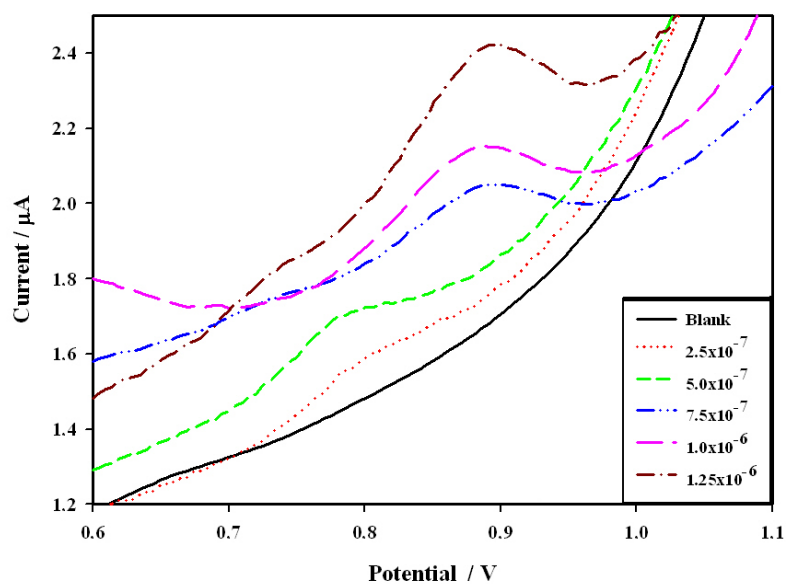3.20.

Figure 3.19 The HS-voltammograms obtained for $2.5x10^{-7}$, $5.0x10^{-7}$, $7.5x10^{-7}$,
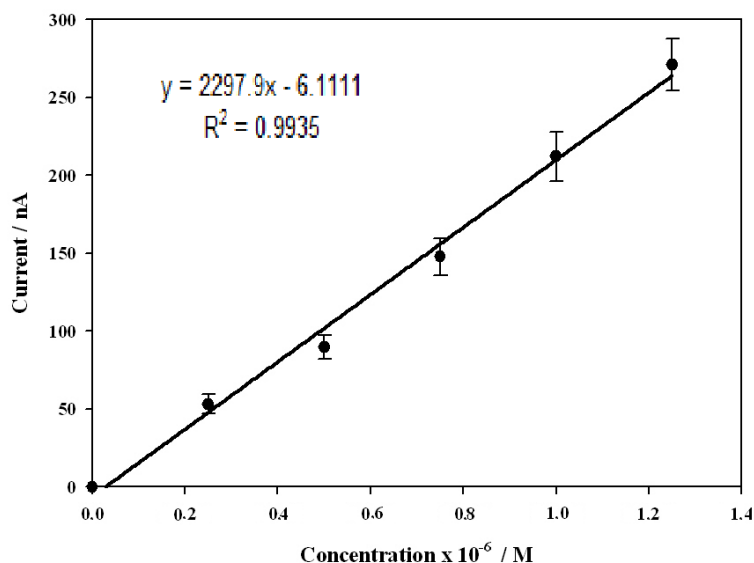$1.0x10^{-6}$ and $1.25x10^{-6}$ M phenol concentration.

Figure 3.20 The calibration curve for HS-voltammetric determination of phenol in lower concentration range

The analytical characteristics of the proposed method are given in Table 3.7with other electroanalytical methods for comparison. As can be followed from the table, the sensitivity of the method is comparable with other developed methods in addition to the selectivity gained by the volatilization of the analyte from the matrix. Direct measurement of phenol with PPy modified GCE has a detection limit of 1.14×10^{-6} M. Head space preconcentration has maintained nearly ten times more sensitive results in addition to the selectivity gained by head space preconcentration.

Table 3.7 Analytical characteristics of recently developed electroanalytical methods

Methods	Linear range	LOD	Reference
Anodic (Pt-Pty-GE)	$3 \times 10^{-4} - 1 \times 10^{-2}$ M	Not given	Spataru, 2010
CV (DAB modified GCE)	$1 \times 10^{-3} - 1 \times 10^{-12}$ M	1.0×10^{-10} M	Mülazımoğlu, 2011
AdSV (Nafion-GCE)	$8 \times 10^{-9} - 1 \times 10^{-5}$	1×10^{-9} M	Yi, 2001
SW (BDDE)	$1 \times 10^{-5} - 3 \times 10^{-5}$ M	Not given	Azevedo, 2011
SW (Pt/PPy-FeCN)	$5 \times 10^{-6} - 1 \times 10^{-4}$ M	5×10^{-6}	Lupu, 2008
Dp (CPE/polyamide)	$1 \times 10^{-5} - 1 \times 10^{-6}$	8.5×10^{-9} M	Zou, 1997
Amperometric enzyme sensor	$1.2 \times 10^{-7} - 2,6 \times 10^{-4}$ M	1×10^{-7} M	Yu, 2003
Amperometric enzyme gas sensor	$1 \times 10^{-5} - 1 \times 10^{-3}$ M	0.89×10^{-6} M	Hammerle, 2010
Ppy-GCE direct	$1.5 \times 10^{-6} - 1 \times 10^{-4}$ M	1.14×10^{-6} M*	Proposed method
HS-Voltammetry	$2.5 \times 10^{-7} - 1.25 \times 10^{-6}$ M	1.8×10^{-7} M *	Proposed method

* Calculated using $m/3S_N$ criterion.

3.3. Application of the Method

The method developed was applied to the determination of phenol in artificial waste water samples prepared in the lab. The procedure was given in Experimental Section. This sample was spiked with $4x10^{-7}M$ phenol and standard addition method was applied to calculate the recovery values. Under optimized conditions, the voltammograms were recorded and resulting standard addition curve was given in Figure 3.21. All results were obtained in three replicates to ensure reproducibility. The recovery values were calculated as 93%. This figure shows the accuracy of the method.
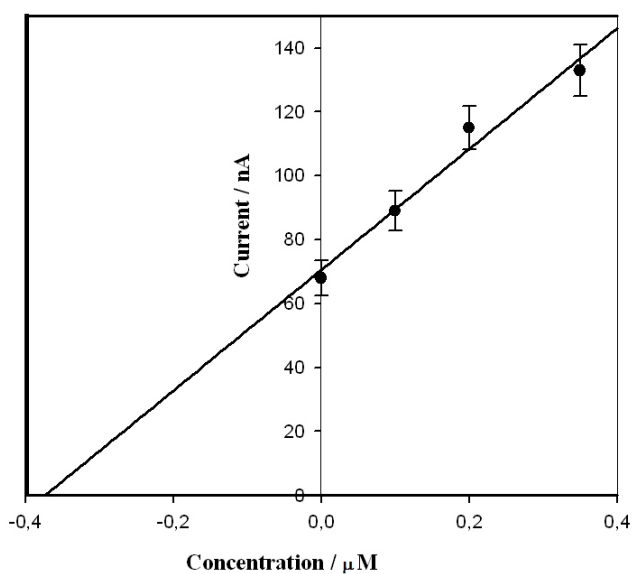

Figure 3.21 Standard addition curves obtained from phenol recovery in artificial waste water.

4. CONCLUSION

In trace analyses, the preconcentration of the analyte plays a vital role in terms of sensitivity, and in some case the selectivity. In stripping techniques, this step usually includes an electrochemical deposition process on the electrode surface at a controlled potential under stirred conditions. Present thesis represents the very first results of a novel method which combines the head space (HS) preconcentration of the analyte on the electrode with the voltammetric measurements. Headspace techniques provide a practical tool for preconcentration of volatile organic compounds prior to their measurement with mostly chromatographic methods. Here, this technique was combined with voltammetric measurement right after the preconcentration step. The method performance was tested upon using a volatile analyte; phenol molecule.

Phenolic compounds have been proved to be toxic and therefore, monitoring of these compounds is important to evaluate the risk and the effectiveness of posterior water treatment. However, the selectivity of voltammetric methods is mostly impaired due to the matrix effect. The HS-Voltammetry proposed was proven to be an efficient method for phenol determination as the analyte is evaporated from the solution and then, preconcentrated in a polymeric coating on the GCE slotted in the sample vial.

Experimental parameters such as the thickness of polymeric coating which provides an adsorbent with a high surface area, the pH of the vial and measuring cell, head space temperature and exposure time was investigated. Salting out effect was also taken into account and the salt amount was optimized. Under optimized conditions, the LOD of the method was calculated as 1.8×10^{-7} M. This level was found comparable to other electroanalytical techniques developed for phenolic compounds.

In conclusion, the method proposed in this thesis provides a very selective and sensitive alternative for phenol determination in several matrixes. Future studies will focus on possible interference by other phenolic compounds and several other polymeric coatings will be tested for attaining more sensitive and selective results.

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