Razi University

Faculty of Chemistry
Department of Analytical Chemistry

Ph.D. Thesis

Title of the Thesis:

Fabrication of electrochemical sensors and biosensors based on new composites and modifiers
&
Improvement of selectivity and sensitivity of electrochemical detection of some drugs and pollutants in real samples with the aid of molecularly imprinted polymers

Supervisor:

Prof. Mohammad Bagher Gholivand

By:

Mehdi Khodadadian

June 2014

To:

My Parents

Acknowledgments

First, I thank my God for his benefice and mercy all throughout my life.

I would like to thank my supervisor, Prof. Mohammad B. Gholivand, for the support, guidance and time he devoted to my studies.

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Most of all, I express my deep appreciations to my parents for tireless encouragement, support, inspiration, friendship, and love.

Mehdi Khodadadian

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Abstract

Part One:

In part one we mainly focused on the design and fabrication of electrochemical sensors and biosensors based on new composites and modifiers. In chapter two of part one, a bi-layer modified glassy carbon electrode (GCE) was prepared by depositing appropriate amounts of multilayered graphene (GR) on the surface of GCE, followed by electrodepositing copper hexacyanoferrate (CuHCF) nano-particles on the graphene layer. The combination of graphene and CuHCF considerably improved the current response of the GCE towards the oxidation of captopril. Studies showed that the best response of the modified electrode could be achieved within neutral pHs. Amperometric signals were linear over the concentration ranges of 0.2 to 5.8 µM and 5.8 to 480 µM. The detection limit of the method was 0.09 µM. The modified electrode was used for electrocatalytic determination of captopril in some real samples.

In chapter three of part one, a voltammetric method has been developed for the simultaneous determination of captopril (CPT) and hydrochlorothiazide (HCT) in pharmaceutical combinations and clinical samples using a graphene/ferrocene composite carbon paste (GR/Fc/CP) electrode. The electrochemical behaviors of CPT and HCT were individually and simultaneously investigated at the surface of GR/Fc/CP electrode. In differential pulse voltammetric (DPV) mode and under optimized experimental conditions, CPT and HCT gave linear responses over the concentration ranges 1.0‑430 µM and 0.5‑390 µM ($r^2 > 0.99$), respectively.

In chapter four of part one, cholesterol oxidase (ChOx) and catalase (CAT) were co-immobilized on a graphene/ionic liquid-modified glassy carbon electrode (GR-IL/GCE) to develop a highly sensitive amperometric cholesterol biosensor. The H_2O_2 generated during the enzymatic reaction of ChOx with cholesterol could be reduced electrocatalytically by immobilized CAT to obtain a sensitive amperometric response to cholesterol. An excellent sensitivity of 4.163 mA mM^{-1} cm^{-2}, a response time less than 6 s, and a linear range of 0.25-215 µM (R^2>0.99) have been observed for cholesterol determination using the proposed biosensor. The apparent Michaelis–Menten constant (K_M^{app}) was calculated to be 2.32 mM.

In chapter five of part one, a glassy carbon electrode (GCE) modified with multiwalled carbon nanotubes (MWCNTs) and a hydrophobic ionic liquid (IL), i.e. 1-Butyl-3-methylimidazolium hexafluorophosphate ([bmim] [PF_6]), was used for the simultaneous

voltammetric determination of theophylline (TP) and guaifenesin (GF) in pharmaceutical formulations and biological samples. The results showed that not only the oxidations of TP and GF were facilitated at modified electrode, but also the peak-to-peak separation at MWCNT–IL/GCE (252 mV) was larger than that observed at unmodified GCE (165 mV). Under the optimized conditions, TP and GF exhibited linear ranges of 0.5 to 98.0 μM (R^2>0.99) and 1.5 to 480.0 μM (R^2>0.99), respectively.

Part Two:

Part two of this work deals with the application of molecularly imprinted solid phase extraction (MISPE) as a powerful sample cleanup and preconcentration technique before quantitative analysis by electrochemical methods such as voltammetry and amperometry. In chapter two of part two, molecularly imprinted polymers (MIPs) with high selectivity toward methocarbamol have been computationally designed and synthesized based on the general non-covalent molecular imprinting approach. A virtual library consisting of 18 functional monomers was built and possible interactions between the template and functional monomers were investigated using a semiempirical and quantum mechanics approach. On the basis of computational results, acrylic acid (AA) and tetrahydrofuran (THF) were found to be the best choices of functional monomer and polymerization solvent, respectively. After MISPE the drug could be determined either by differential pulse voltammetry (DPV), on a glassy carbon electrode modified with multiwalled-carbon nanotubes (GC/MWNT), or high performance chromatography (HPLC) with UV detection. A comparative study between MISPE-DPV and MISPE-HPLC-UV was performed.

In chapter three of part two, molecularly imprinted polymers (MIPs) were used as selective solid-phase extraction sorbents before flow injection (FI) amperometry for the efficient preconcentration and determination of 4-nitrophenol (4-NP) in water samples. A pencil graphite electrode modified with multiwalled carbon nanotubes (MWCNTs/PGE) with good sensitivity and excellent anti-fouling ability was used to detect 4-NP by FI amperometry. SPE-cartridges containing 100 mg of MIP and a sample volume of 50 mL resulted in a preconcentration factor of 97.7, which was sufficient to analyze 4-NP at the maximum level permitted by the Environmental Protection Agency (EPA) (60 μg L^{-1}). Standard calibration graph for 4-NP yielded a linear range of 5-300 μg L^{-1} (3.5-210) × 10^{-8} M by MISPE-FI method. The detection limit was 1.5 μg L^{-1}. The proposed method was applied to the determination of 4-NP in lake and river water samples.

Publications

1. M.B. Gholivand, M. Khodadadian, *"Simultaneous voltammetric determination of theophylline and guaifenesin using a multiwalled carbon nanotube-ionic liquid modified glassy carbon electrode",* **Electroanalysis,** Accepted.

2. M.B. Gholivand, M. Khodadadian, "*Amperometric cholesterol biosensor based on the direct electrochemistry of cholesterol oxidase and catalase on a graphene/ionic liquid-modified glassy carbon electrode*", **Biosensors and Bioelectronics** 53 (2014) 472–478.

3. M.B. Gholivand, M. Khodadadian, M. Omidi, "*Amperometric sensor based on a graphene/copper hexacyanoferrate nano-composite for highly sensitive electrocatalytic determination of captopril*", **Materials Science and Engineering C** 33 (2013) 774–781.

4. M.B. Gholivand, M. Khodadadian, "*Simultaneous Voltammetric Determination of Captopril and Hydrochlorothiazide on a Graphene/Ferrocene Composite Carbon Paste Electrode*", **Electroanalysis** 25 (2013) 1263 – 1270.

5. M.B. Gholivand, M. Khodadadian, "*Rationally Designed Molecularly Imprinted Polymers for Selective Extraction of Methocarbamol from Human Plasma*", **Talanta** 85 (2011) 1680–1688.

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CHAPTER FOUR:
SIMULTANEOUS VOLTAMMETRIC DETERMINATION OF CAPTOPRIL AND HYDROCHLOROTHIAZIDE ON A GRAPHENE/FERROCENE COMPOSITE CARBON PASTE ELECTRODE

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Part One

Fabrication of electrochemical sensors and biosensors based on new composites and modifiers

Chapter One

Theoretical Review

1-1-1 Electroanalytical Techniques

Electroanalytical techniques are concerned with the interplay between electricity and chemistry, namely the measurements of electrical quantities, such as current, potential, or charge, and their relationship to chemical parameters. Such use of electrical measurements for analytical purposes has found a vast range of applications, including environmental monitoring, industrial quality control, and biomedical analysis. Advances in the 1980s and 1990s—including the development of ultramicroelectrodes, the design of tailored interfaces and molecular monolayers, the coupling of biological components and electrochemical transducers, the synthesis of ionophores and receptors containing cavities of molecular size, the development of ultratrace voltammetric techniques or of high-resolution scanning probe microscopies, and the microfabrication of molecular devices or efficient flow detectors–have led to a substantial increase in the popularity of electroanalysis, and to its expansion into new phases and environments. Indeed, electrochemical probes are receiving a major share of the attention in the development of chemical sensors. In contrast to many chemical measurements that involve homogeneous bulk solutions, electrochemical processes take place at the electrode-solution interface. The distinction between various electroanalytical techniques reflects the type of to the target analyte(s) and is thus termed the indicator (or working) electrode. The second one, termed the reference electrode, is of constant potential (that is, independent of the properties of the solution). Electrochemical cells can be classified as electrolytic (when they consume electricity from an external source) or galvanic (if they are used to produce electrical energy) [1].

Potentiometry, which is of great practical importance, is a static (zero current) technique in which the quantitative information about the sample is obtained from measurement of the potential established across a membrane. Different types of membrane materials, possessing different ion-recognition processes, have been developed to impart high selectivity. The resulting potentiometric probes have thus been widely used for several decades for direct monitoring of ionic species such as protons or calcium, fluoride, and potassium ions in complex samples.

Controlled-potential (potentiostatic) techniques deal with the study of charge transfer processes at the electrode-solution interface, and are based on dynamic (no zero current) situations. Here, the electrode potential is being used to derive an electron-transfer reaction and the resultant current is measured. The role of the potential is analogous to that of the wavelength in optical measurements. Such a controllable parameter can be viewed as "electron pressure," which forces the chemical species to gain or lose an electron (reduction or oxidation, respectively). Figure 1-1-1 presents elrctrodes configuration of controlled-potential techniques [2].

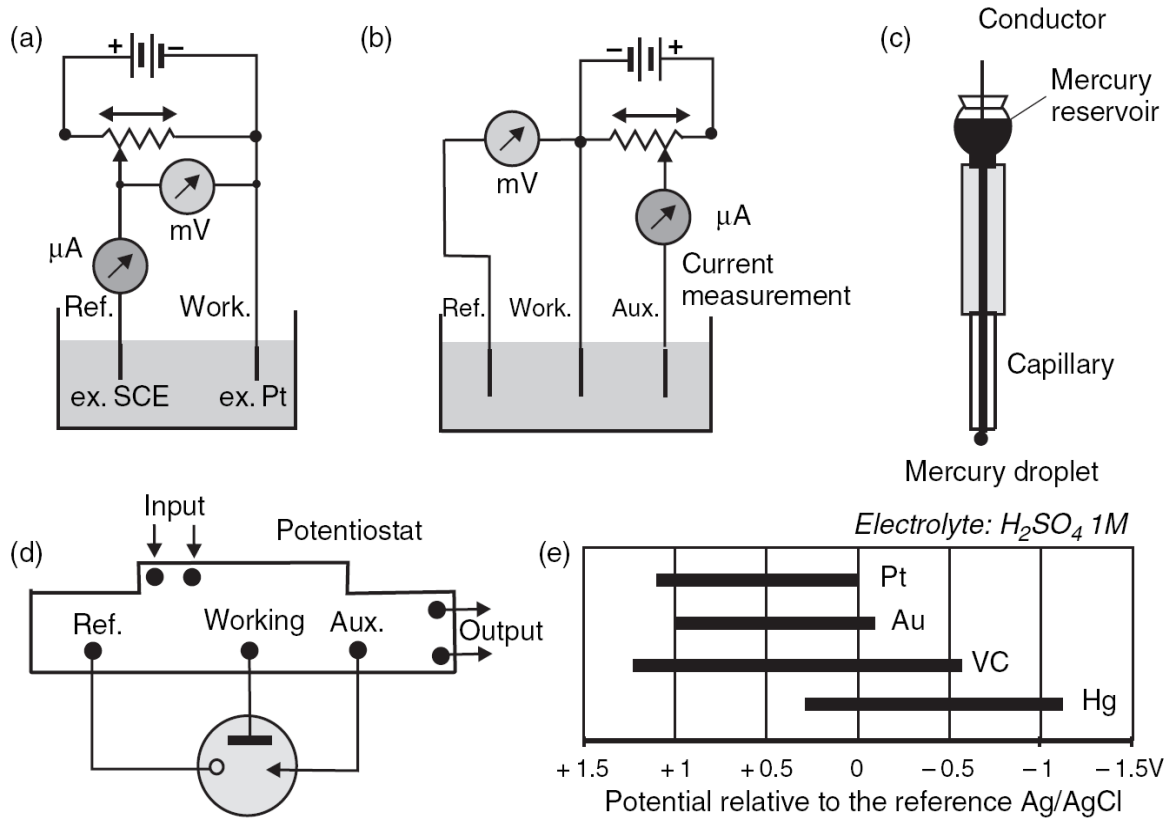

Fig. 1-1-1 Schematics showing 2- and 3-electrode cells in potentiostatic experiments. (a) Circuit diagram with two electrodes that is not used in practice as this assembly has the major inconvenience that the current passes through the reference electrode which should be avoided because its potential will be modified; (b) DC set-up in which no current passes through the reference electrode (a consequence of its high impedance); (c) A model indicator electrode (hanging-drop mercury electrode). (d) Representation of a measuring cell controlled by a potentiostat; (e) Ranges of use for the four principal working electrodes in sulfuric acid 1M as support electrolyte (VC stands for vitreous carbon). The mercury electrode can be used over a wide cathodic range with electrolytes such as KCl or NaOH (−2 V). Although overlaps are observed with different electrodes, their sensitivities can be quite different for a given species [2].

Accordingly, the resulting current reflects the rate at which electrons move across the electrode-solution interface. Potentiostatic techniques can thus measure any chemical species that is electroactive, in other words, that can be made to reduce or oxidize. Knowledge of the reactivity of functional group in a given compound can be used to predict its electroactivity. Nonelectroactive compounds may also be detected in connection with indirect or derivatization procedures.

The advantages of controlled-potential techniques include high sensitivity, selectivity towards electroactive species, a wide linear range, portable and lowcost instrumentation, speciation capability, and a wide range of electrodes that allow assays of unusual environments. Several properties of these techniques are summarized in Table 1-1-1. Extremely low (nanomolar) detection limits can be achieved with very small sample volumes (5-20 ul), thus allowing the determination of analyte amounts of 10~13 to 10~15mol on a routine basis. Improved selectivity may be achieved via the coupling of controlled-potential schemes with chromatographic or optical procedures.

Table 1-1-1

Properties of Controlled-Potential Techniques [1].

Technique[a]	Working Electrode[b]	Detection Limit (M)	Speed (time per cycle) (min)	Response Shape
DC polarography	DME	10^{-5}	3	Wave
NP polarography	DME	5×10^{-7}	3	Wave
DP polarography	DME	10^{-8}	3	Peak
DP voltammetry	Solid	5×10^{-7}	3	Peak
SW polarography	DME	10^{-8}	0.1	Peak
AC polarography	DME	5×10^{-7}	1	Peak
Chronoamperometry	Stationary	10^{-5}	0.1	Transient
Cyclic voltammetry	Stationary	10^{-5}	0.1–2	Peak
Stripping voltammetry	HMDE, MFE	10^{-10}	3–6	Peak
Adsorptive stripping voltammetry	HMDE	10^{-10}	2–5	Peak
Adsorptive stripping voltammetry	Solid	10^{-9}	4–5	Peak
Adsorptive-catalytic stripping voltammetry	HMDE	10^{-12}	2–5	Peak

[a]DC = direct current; NP = normal pulse; DP = differential pulse; SW = square wave; AC = alternating current.

[b]DME = dropping mercury electrode; HMDE = hanging mercury drop electrode; MFE = mercury film

1-1-2 Electrochemical Sensors

A chemical sensor is a small device that can be used for direct measurement of the analyte in the sample matrix. Ideally, such a device is capable of responding continuously and reversibly and does not perturb the sample. By combining the sample handling and measurement steps, sensors eliminate the need for sample collection and preparation. Chemical sensors consist of a *transduction element* covered with a chemical or biological *recognition layer* (Fig 1-1-2). This layer interacts with the target analyte and the chemical changes resulting from this interaction are translated by the transduction element into electrical signals. The development of chemical sensors is currently one of the most active areas of analytical research. Electrochemical sensors represent an important subclass of chemical sensors in which an electrode is used as the transduction element [3]. Such devices hold a leading position among sensors presently available, have reached the commercial stage, and have found a vast range of important applications in the fields of clinical, industrial, environmental, and agricultural analyses. The field of sensors is interdisciplinary and future advances are likely to derive from progress in several disciplines.

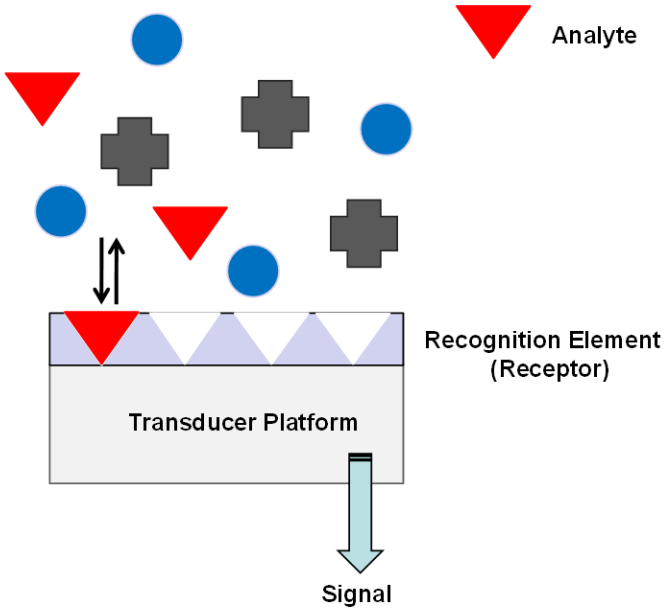

Fig 1-1-2 Schematic representation of a chemical (bio) sensor operation.

1-1-2.1 Electrochemical Biosensors

Electrochemical biosensors combine the analytical power of electrochemical techniques with the specificity of biological recognition processes [3]. The aim is to

biologically produce an electrical signal that relates to the concentration of an analyte. For this purpose, a biospecific reagent is either immobilized or retained at a suitable electrode, which converts the biological recognition event into a quantitative amperometric or potentiometric response. Such biocomponent-electrode combinations offer new and powerful analytical tools that are applicable to many challenging problems. A level of sophistication and state-of-the art technology are commonly employed to produce easy-to-use, compact, and inexpensive devices. Advances in electrochemical biosensors are progressing in different directions. Two general categories of electrochemical biosensors may be distinguished, depending on the nature of the biological recognition process: biocatalytic devices (utilizing enzymes, cells, or tissues as immobilized biocomponents) and affinity sensors (based on antibodies, membrane receptors, or nucleic acids).

1-1-2.2 Enzyme-Based Electrodes

Enzymes are proteins that catalyze chemical reactions in living systems. Such catalysts are not only efficient but are also extremely selective. Hence, enzymes combine the recognition and amplification steps, as needed for many sensing applications [4].

Enzyme electrodes are based on the coupling of a layer of an enzyme with an appropriate electrode. Such electrodes combine the specificity of the enzyme for its substrate with the analytical power of electrochemical devices. As a result of this coupling, enzyme electrodes have been shown to be extremely useful for monitoring a wide variety of substrates of analytical importance in clinical, environmental, and food samples.

The operation of an enzyme electrode is illustrated in Figure 1-1-3. The immobilized enzyme layer is chosen to catalyze a reaction, which generates or consumes a detectable species:

$$S + C \xrightarrow{\text{Enzyme}} P + C' \qquad (1\text{-}1\text{-}1)$$

where S and C are the substrate and co-reactant (cofactor), and P and C' are the corresponding products. The choice of the sensing electrode depends primarily upon the enzymatic system employed. For example, amperometric probes are highly suitable when oxidase or dehydrogenase enzymes (generating electrooxidizable peroxide or NADH species) are employed, pH-glass electrodes are used for enzymatic pathways that result in a change in pH, while gas (carbon dioxide) potentiometric devices will be the choice when decarboxylase enzymes are used.