

Review

Electrochemical Chiral Sensors and Biosensors

Marek Trojanowicz,^{a,b,*} Marzena Kaniewska^a

^a Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland

^b Institute of Nuclear Chemistry and Technology, Dorodna 16, 03-195 Warsaw, Poland

*e-mail: trojan@chem.uw.edu.pl

Received: July 22, 2008

Accepted: September 9, 2008

Abstract

Chiral molecular recognition depends on difference in stability constants of diastereomeric complexes with applied selectors, different migration of enantiomers based on the formation and dissociation of diastereomeric complexes, and on a different interaction rate of enantiomers with selector. Selected examples of electrochemical enantioselective sensors and biosensors based on different mechanisms of molecular recognition are reviewed. Chemical chiral sensors can be based on plasticized membranes incorporating a chiral ionophore, on differences of Gibbs free energy for interphase electron transfer, or on the use of molecularly imprinted polymers and electrodeposited conducting polymers doped with chiral ions. Reported chiral biosensors include enzymatic devices and immunosensors. Especially pronounced enantioselectivity of enzymatic biosensors can be employed practically, e.g., in food control, showing chiral discrimination better than two orders of magnitude. Developed, so far, chemical sensors show enantioselectivity usually not exceeding two orders magnitude and require further investigation for any practical analytical applications.

Keywords: Chiral sensors, Biosensors, Ionophores, Conducting polymers, Immunosensors

DOI: 10.1002/elan.200804382

1. Introduction

Investigation of compounds having the asymmetric carbon in their structure have a fundamental significance for understanding the origin of life and all processes that occur in living organisms. Most of biochemical systems functioning in living organisms involve chiral interactions resulting from different stereochemistry of numerous biologically active compounds as amino acids, sugars, peptides, proteins and polysaccharides. All living organisms contain peptides in their cells and mostly chiral amino acids that are building blocks for peptides. Animal and human peptides consist of almost only left-handed (L) amino acids. According to one of theories of the origin of life amino acids were brought to Earth by meteorites [1]. A trip in space survived only left-handed amino acids in contrast to right-handed that were selectively destroyed by circularly polarized light emitted by neutron stars, which are one of final products of evolution of stars. The determination of amino acids in extracts of meteorites proves the majority of L-forms [2]. The origin of life is still important and fascinating subject of research.

A huge interest in chirality results also from the fact that present pharmaceutical and chemical industry to large extent is based on the synthesis of enantiomeric compounds. Although they do not possess any physical differences, they can have a different influence on living organisms. In such case it is extremely important to use the particular compound as a pure enantiomer. The fact how the chiral purity is important for pharmaceutical industry,

can be illustrated by the worldwide sale of chiral components as a racemate, which decreased from 35% in 1983 practically to zero in 2001. The prognosis of revenues from chiral technology show over triple growth during 10 years and predict amount of 15 billions of dollars in 2009 [3].

Many examples showing that enantiomers differ in activity, rate of reaction or time of dissolution can be found in the literature. The example of difference in time of dissolution can be fungicide metalaxyl [4]. The *R* enantiomer in its active form shows over 4 times higher dissolution constant than *S* enantiomer in a neutral pH. From the pair of enantiomers of organophosphorus pesticide methamidophos, (*R*)-(+)-isomer reveals the higher insecticidal activity against flies than other enantiomer and racemate [5]. On the other hand (*S*)-(–)-methamidophos is more toxic against German cockroaches in a short time. During first 5 hours the same dose of *S* enantiomer caused death of 75% of insects whereas *R* enantiomer caused death of only 20%.

Enantiomers can be used also to trace sources of water contamination. It was recently demonstrated that propranolol exists in untreated sewage as a racemate. During successive steps of sewage treatment the amount of *R* enantiomer in relation to both enantiomers decreased to even less than 40% regardless the concentration of compound. Determination of enantiomeric fraction (the ratio of the concentration of one of the isomers to the total concentration) can be useful indicator to evaluate if examined water is significantly affected by untreated sewage, for example as a result of leaking sewers and to

apportion the contribution of treated and untreated sewage into surface waters [6].

2. Chiral Analysis

Enantioselective sensors can be potentially employed for monitoring of different physiological and pharmaceutical interactions of enantiomers, investigating toxicological effects of particular enantiomers, determination of chiral purity in production of chirally pure pharmaceuticals and pesticides. They can enable to study different environmental impact of enantiomers, and can be useful in measuring of enantiomeric fraction for identification of sewage discharges for environmental protection.

In contemporary analytical chemistry the main methods commonly employed for chiral analysis are GC and LC high-performance chromatography with chiral stationary phases, with chiral selectors in mobile phase, or with in-line flow through reactors with enantioselective derivatization as well as also high-performance electromigration techniques with chiral selectors. The other methods employed are high resolution mass spectrometry with soft ionization methods, NMR for study a chiral molecular recognition and some spectroscopic techniques as circular dichroism, vibrational circular dichroism, optical rotation and X-ray scattering for interpreting absolute configuration, and also gas-phase separation by ion mobility spectrometry.

The enantioselectivity for analytical purposes can be exhibited in the presence of chiral selector that forms diastereomeric complex with an analyte. The equilibrium constant for formation or dissolution of the complex has to be different for both enantiomers [7]. There are a lot of types of compounds that can be used as chiral selectors. The naturally occurred chiral selectors are proteins, polysaccharides, cyclodextrins, macrocyclic glycopeptides and alkaloids. Synthetic selectors are ligand exchangers, ligands that form π - π complexes, molecularly imprinted polymers, chiral crown ethers and doped polymers. All of them form complexes on the base of similar mechanisms using various interactions such as formation of hydrogen bonds, coulomb and ion-dipole interactions, and also selective shape interaction and combination of all above mentioned [8].

The chiral separations can be modeled and optimized based on the so called three-point contact model. The privileged enantiomers by the asymmetric carbon possess three groups that can match exactly three sites of the selector. The other enantiomer after all possible rotations can provide maximum two groups able to interact with only two sites of the selector; hence such interaction will be always weaker than that one of privileged enantiomer [8]. The more complicated and possessed functional groups selector is the more possible interaction analyte-selector can occur. The same interactions might be employed in design of chemical or biochemical enantioselective sensors. In separation techniques however, beside of the choice of proper selector other variables can be also optimized. For example in capillary electrophoresis it can be pH of the buffer,

addition of surfactants or other selectors and modification of the high-voltage for separations. For instance, in capillary electrophoresis in optimized conditions the base-line separation of four diastereomers of ephedrine was possible with the use of 20 mM carboxymethyl- β -cyclodextrin as chiral selector in background electrolyte [9].

As example of correlation between the chiral selectivity observed in separation techniques and the signal from the sensor one can find application of the same selectors in stationary phase in HPLC as well as deposited as a self-organized monolayer at the surface of gold electrode of piezoelectric sensor [10]. The difference in frequency changes of the sensor well corresponds with the resolution observed in HPLC measurements for examined pairs of analyte-selector. It allows predicting the resolution in HPLC and even the order of elution of the enantiomers from the column, by method that is faster and using less amount of selector. Therefore, enantioselective electrochemical sensors can be a potential source of information concerning the possibility of using new chiral selectors in separation techniques, particularly at the initial stage of optimization of analytical procedure.

Enantioselective electrochemical sensors similarly to other methods of chiral analysis can be based on different mechanisms. The most important are presented below.

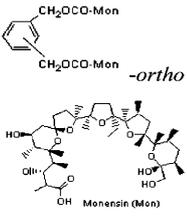
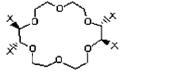
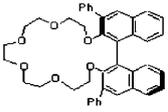
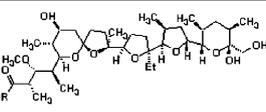
3. Chiral Electrochemical Sensors

3.1. Ion-Selective Electrodes with Chiral Ionophores

First reports on electrochemical enantioselective sensors have been published in 1970-ties [11]. The construction of potentiometric electrodes with the plasticized membrane was described, where ion-selective membrane contained chiral ionophore – a derivative of crown ether. As the model chiral analyte the phenylethylamine cation was used. The obtained results of small chiral resolution did not allow any application of the sensor but it initiated the new field of research in area of electrochemical sensors. A similar construction of the electrode was used in numerous later works for examination of different chiral selectors. The membranes consisted of polyvinyl chloride, plasticizer and alternatively addition of lipophilic anion. The results of numerous studies are collected on Web page <http://www.chem.s.u-tokyo.ac.jp/~analyt/En3.htm>. The derivatives of antibiotic monensin were used in several works as a chiral selector [12]. The best results were obtained for enantiomers of phenylmethyl esters, with the values of selectivity coefficient 0.12. The derivatives of crown ethers were also applied with the best results obtained for phenylethylamine hydrochloride [13], and phenylglycine methyl ester [14–15]. For the similar group of enantiomers the polyether antibiotic was examined with the best results for phenylglycine methyl ester [16]. Data on mentioned selected examples are shown in Table 1.

The identical method of membrane preparation was used with different cyclodextrin derivatives as chiral ionophores,

Table 1. Examples of potentiometric membrane electrodes based on enantioselective ionophores.

| Chiral selector | Membrane composition | Analyte, Selectivity coefficient K_{SR} | Reference |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------|
|  Monensin (Mon) | Selector- substituted antibiotic monensin (3 wt%), dibenzyl ether (66 wt%), PVC (30 wt%), KTpCIPB (39 mol%) | Phe-OMe (<i>R</i>) 8.6 | 12 |
|  $X = \text{CONH}-(S)\text{-CHCH}_2\text{C}_6\text{H}_5$ $\quad\quad\quad $ $\quad\quad\quad \text{CO}_2\text{CH}_3$ | Selector -crown ether derivative 1.2 wt%), DPP (69.8 wt%), PVC (29.1 wt%) | Phenylethylamine hydrochloride (<i>R</i>), 0.67 | 13 |
|  | Selector - crown ether derivative (3 wt%), DBE (66 wt%), PVC (30 wt%), KTpCIPB (31 mol%) | Phe-Gly-OMe (<i>R</i>) 18.4 | 14, 15 |
|  $R = \text{CH}_2\text{C}_6\text{F}_5$ | Selector- polyether antibiotic (3 wt%), DBE (66 wt%), PVC (30 wt%), KTpCIPB (36 mol%) | Phe-Gly-OMe (<i>R</i>) 4.4 | 16 |

and with various plasticizers for investigation of enantioselective response for enantiomers of nitrophenylethylamine [17]. The best results, affected by the kind of plasticizer employed, were obtained for per-methoxylated- β -cyclodextrin. For ethylhexylsebacate (DOS) the enantioselectivity coefficient obtained by separate solution method was 130, and for *o*-nitrophenyloctyl ether (*o*-NPOE) 19 [17]. The possibility of selective discrimination of compound of a biological importance, L-carnitine, was also examined. L-carnitine, widely used as nutritional supplement and slimming agent is an amino acid that is responsible for transport of fatty acids into cell's mitochondria and helps to transport the toxic compounds generated out of the cellular organelle to prevent their accumulation. The macrocyclic antibiotic, teicoplanin, was used as a chiral selector in this case. Teicoplanin widely used as chiral selector in HPLC, TLC or GC, possess as many as 23 chiral centers. Two types of electrode construction were employed, one with PVC plasticized membrane and another with graphite paste. Because teicoplanin, containing numerous hydrophilic groups, is poorly soluble in organic solvent used for preparation of PVC membrane, it has been modified to more hydrophobic derivative by esterification of carboxylic groups and reaction of amino groups with BOC. In the case of graphite paste the non-modified selector was used. The obtained enantioselectivity coefficient for graphite paste electrode was 2.6, and for the plasticized membrane 2.4. Electrode with modified teicoplanin in a plasticized membrane has a significantly longer life-time and much better reproducibility. The same electrode exhibits also differences

in a dynamic response of the sensor in the presence of enantiomers.

The carnitine optical isomers, derivatized with 9-fluorenylmethoxycarbonyl chloride (Fmoc), can be resolved by capillary electrophoresis with the use of BGE containing 20 mM γ -cyclodextrin in phosphate buffer (pH 3.4). Electrophoretic determination in mentioned conditions was compared with the results of potentiometric determination with the graphite paste electrode with γ -cyclodextrin. For derivatized enantiomers the obtained enantioselectivity coefficient was 2.3 [17].

Another example of the enantioselective sensor reported in the literature is the multichannel lipid membrane sensor with chiral alkaloid quinine in the PVC membrane, where the mechanism of enantioselectivity is also based on diastereomeric interaction between the analyte and the selector. The electrode was examined for selectivity towards tryptophan, aspartic acid and glutamic acid. The best results were obtained for two latter cases. The difference in response also depends on the plasticizer that is shown in Figure 1 [18].

3.2. Chiral Voltammetry

The electrochemical discrimination between enantiomers can be also based on the differences in Gibbs energy of transfer of chiral ion from aqueous phase to chiral organic phase. In the example presented in the literature, a graphite electrode with deposited drop of decamethylferrocene in chiral D- or L-octanol was used as working electrode [19].

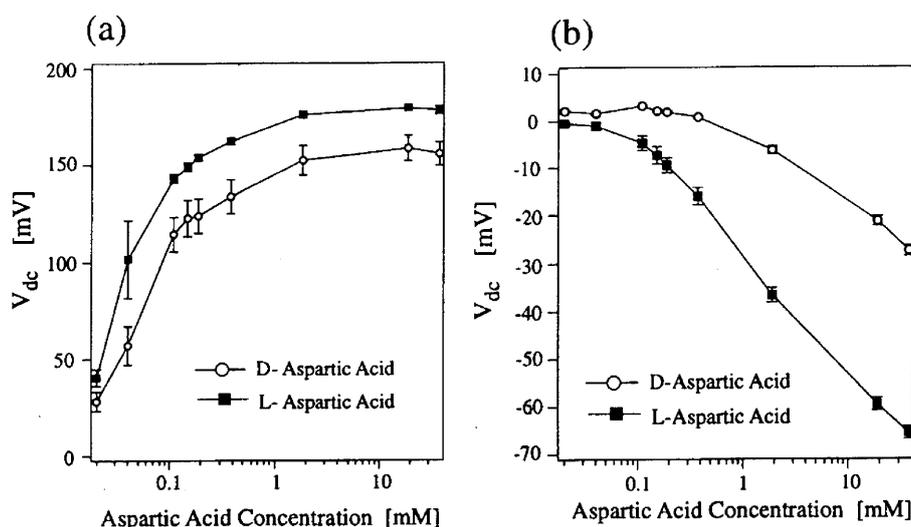


Fig. 1. The potentiometric response of a multichannel lipid membrane sensor to enantiomers of aspartic acid for sensors with chiral alkaloid quinine in the PVC membrane and different plasticizers: a) trioctylmethylammonium chloride, b) dioctylphenyl phosphate [18].

Figure 2 shows the voltammograms of decamethylferrocene oxidation in the chiral octanol coupled to the transfer of anions from water to organic solvent. In all examined configurations the interactions between L-compound and L-solvent and D-compound and D-solvent has a lower Gibbs energy than in the case of transfer L-compound to the D-solvent and D-compound to the L-solvent. The pair of enantiomer of lysine, tyrosine, phenylalanine and chlorpropionic acid were examined [19].

3.3. Enantioselectivity of Adsorption on Carbon Nanotubes

Nanotechnology exploits possibility to control and manipulate the chemical processes at the level of atoms, molecules or supramolecules. The main approaches of nanotechnology to chiral technology are nanoscale materials for asymmetric catalysis, nanoscale materials for enantiomeric analysis and for enantioselective separation [20]. The research for the materials and devices that benefits from achievements of chiral technology is focused on several main fields. They include molecular devices for example the molecular switches that can reversibly change between two stable states as an effect of external impact, chiral supramolecules, self-assembled nanotubes, chiral fullerenes, chiral carbon nanotubes and DNA nanotechnology [20]. One of the most popular examined nanostructures is carbon nanotubes. They possess the simplest chemical composition and are characterized by tremendous diversity and variety of structure, and as a result they exhibit variety of features. Single-walled carbon nanotubes are formed as hexagonal graphite sheet rolled up in a structure of cylinder. Depending on the lattice vectors along which the nanotubes were fold, they can possess chirality, and they can act as metals or as semiconductors [21]. It was shown that single-walled carbon nanotubes properly chosen with respect to length

and the angle of folding can be used to discriminate between amino acids, their enantiomers and in some cases diastereomers. The average energy of adsorption and average changes in frequency generated by amplifier under the influence of adsorption were reported for three pairs of enantiomers – alanine, aspartic acid, and threonine. In the latter case 3 diastereomers were considered [22].

3.4. Chiral Sensors Based on Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIP) are produced by the formation of polymer in the presence of target analyte and two forms of monomers, functional one that allows binding the analyte and another one acting as cross-linker. Functional monomers assembly complex with the target analyte prior to polymerization. This causes stable incorporation of their functional groups in the structure of polymer. The next step after the polymerization is the extraction of template and emptying the binding cavity that is well-fitting considering size and shape to analyte. When the template is a chiral compound, one can expect that obtained polymer can exhibit some chiral discrimination in uptake.

Colloid particles of polypyrrole imprinted with L-lactate were examined by HPLC for selective uptake of amino acids [23]. The ratio of absorption of enantiomers L- to D- for alanine in optimal conditions was 11. The dependence of uptake of amino acids by polymer imprinted with lactate on the difference of molecular volume of chosen amino acid and lactate was also demonstrated.

Molecularly imprinted polymers were also employed in the construction of chiral potentiometric sensors [24]. An octadecylsiloxane (ODS) layer was covalently bound onto indium-tin oxide (ITO) surface in the presence of chiral *N*-carboboxy-aspartic acid molecules. Interaction between amino acid and OTO is composed of two factors. One is

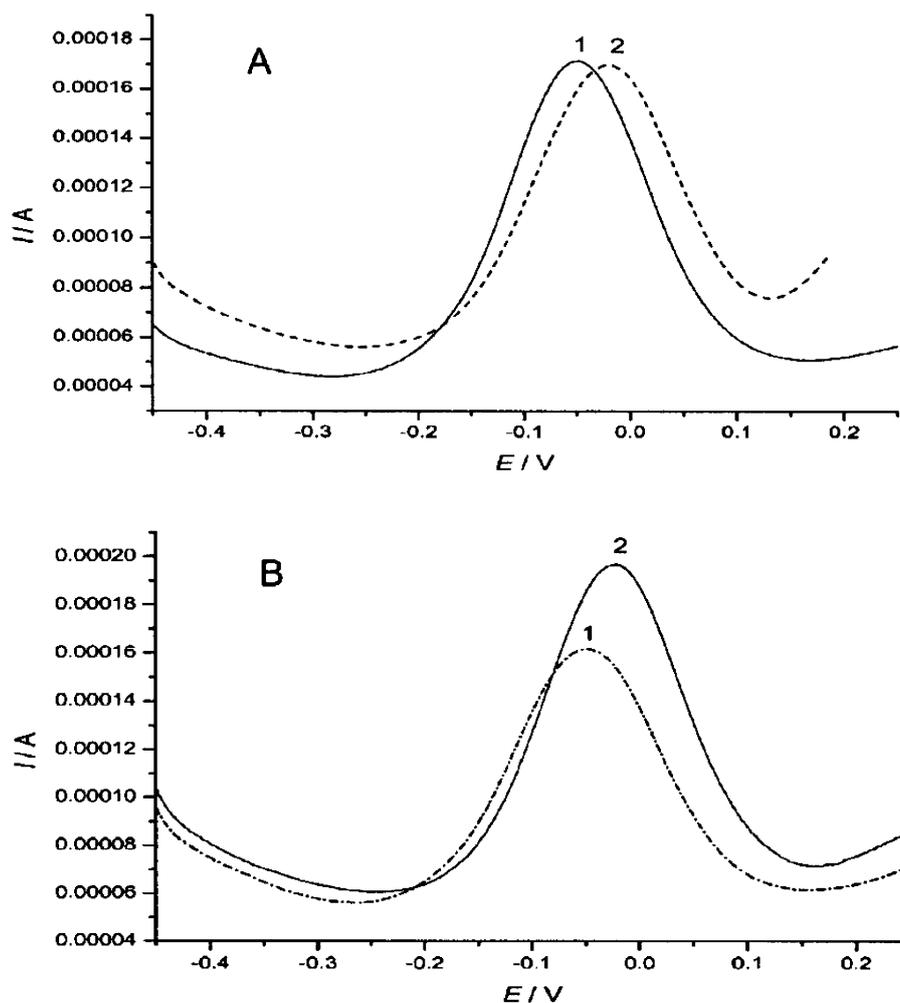


Fig. 2. A) Square-wave voltammograms of decamethylferrocene (dmfc) oxidation in the chiral octanol coupled to the transfer of anions of D-phenylalanine from water to D-octanol (1) and to L-octanol (2). B) Square-wave voltammograms of dmfc oxidation in the chiral octanol coupled to the transfer of anions of L-phenylalanine from water to L-octanol (1) and to D-octanol (2) [19].

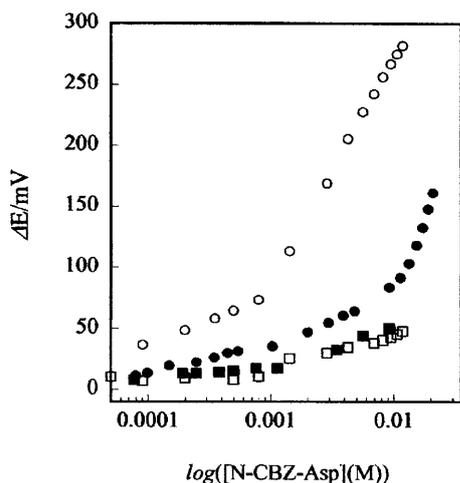


Fig. 3. Calibration curves for the potentiometric response of L- (●, ○) and D- (■, □) isomers of *N*-carbobenzoxy-aspartic acid (*N*-CBZ-L-Asp) for *N*-CBZ-L-Asp sensor. Open symbols for measurements in water, (pH 1.5–5.2), solid symbols for measurements in 0.1 M phosphate buffer with addition of 0.1 M KCl (pH 6.8) [24].

hydrophobic interaction with ODS layer that proves chiral selectivity, and another is electrostatic binding with surface oxides. Calibration curves presented in Figure 3 show the difference of potential for both enantiomers of carbobenzoxy-aspartic acid.

The other example reported in the literature is a field-effect transistor where the imprinting of chiral molecular recognition sites in TiO_2 films for detection of carboxylic acids was applied. Three pairs of enantiomers of methylferrocene carboxylic acid, 2-phenyl-butanoic acid and 2-propanoic acid were chosen to experiment. The obtained results show that response of the sensor is not only selective towards the other enantiomer (Fig. 4), but also specific for the chosen analyte even in the case of similar structure of compounds [25].

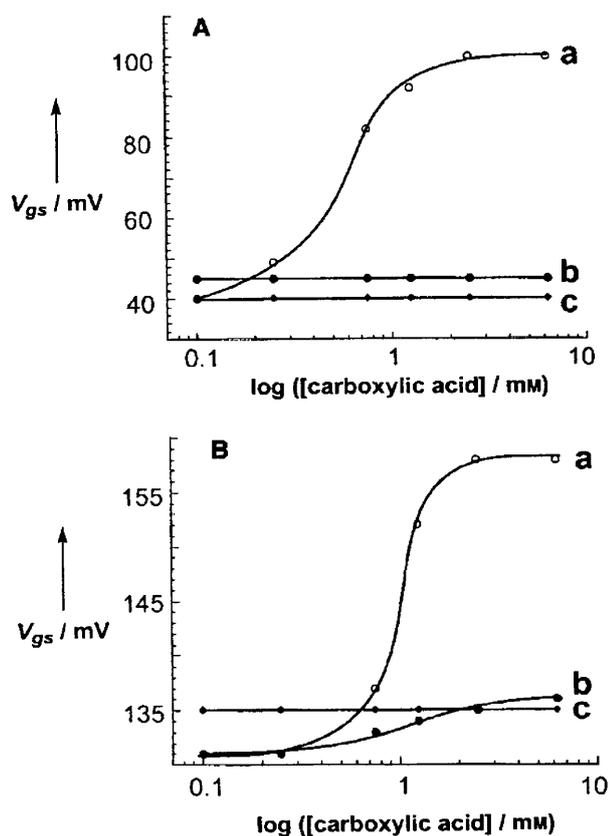


Fig. 4. The gate source voltage as a function of carboxylic acid concentration. A) Polymer imprinted with (*R*)-2-propanoic acid, a) response for (*R*)-2-propanoic acid, b) response for (*S*)-2-propanoic acid, c) response for (*R*)-2-phenylbutanoic acid. B) Polymer imprinted with (*S*)-2-propanoic acid, a) response for (*S*)-2-propanoic acid, b) response for (*R*)-2-propanoic acid, c) response for (*S*)-2-phenylbutanoic acid [25].

3.5. Chiral Sensors Based on Doped Electrodeposited Conducting Polymers

Electrodeposited conducting polymers are widely used in the construction of electrochemical sensors. They are based on the number of interactions with analytes such as oxidation and reduction, protonation and deprotonation, reactions with nucleophilic agents, ion-exchange, adsorption and complexation. The polymers are used for construction of potentiometric sensors of ions and molecules, amperometric and voltammetric sensors of electroinactive ions, voltammetric sensors of heavy metal ions, biosensors, conductometric sensors and redox switching sensors of solute species [26]. Because of the diversity of interactions the response of electrode based, e.g., on polypyrrole is often depended on number of factors and can be composed of diverse occurring processes.

Polypyrrole polymerized from the solution of monomer in the presence of salt is doped, and the polymer becomes ion exchanger and can be used as ion-selective membrane sensitive for doped ion [27]. It has been proved that thin film of polypyrrole doped with mandelate or tartate deposited

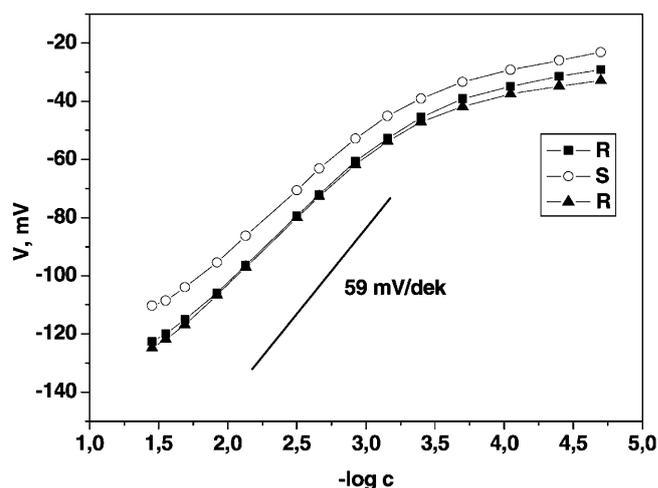


Fig. 5. Successive measurements of the potentiometric response of a platinum disk electrode with electrodeposited polypyrrole doped with (*R*)-camphorsulfonate for enantiomers of camphorsulfonate used as lithium salts [30].

on ITO-coated glass working electrodes exhibits certain circular dichroism, which is the evidence of chiral properties of polymer [28]. The doped polymer was also examined as a membrane for chiral potentiometric membrane electrode. Polypyrrole has been electrodeposited on the surface of platinum electrode from the solution of lithium mandelate and monomer by cyclic voltammetry in a potential range 0.5–1 V. Pure enantiomers of mandelic acid were used for polymerization and then examination of an electrode response. The electrode after assembling was conditioned in a solution of particular mandelate isomer. The significant difference in response of electrode for both enantiomers of lithium mandelate was observed, however measurements in buffers indicated predominant pH sensitivity. The similar attempt of doping polypyrrole by strong acid presenting chiral properties, namely camphorsulfonic acid, was made. The obtained electrodes calibrated with enantiomers of lithium camphorsulfonate exhibited the difference of potentiometric response with the selectivity coefficient 2.2 (Fig. 5).

The polypyrrole doped with camphorsulfonic acid was also examined as a sensor for enantiomers of phenylalanine. In that case the doped ion was rinsed out of the polymer layer by ammonia solution. Calibration curves for electrode with polypyrrole doped by (*R*)-camphorsulfonic acid in enantiomers of phenylalanine are shown in Figure 6.

Camphorsulfonates were also reported as a doping agent for the polymerization of aniline. The circular dichroism of polyaniline doped with camphorsulfonic acid was proved. The polymer was used to the construction of piezoelectric sensor selective towards L-phenylalanine [29]; hence, some attempts were made to use the polyaniline as a potentiometric membrane sensitive for phenylalanine. Polyaniline was electrodeposited from the solution of aniline, camphorsulfonic acid and DMSO. The best results were obtained by potentiostatic electrodeposition. Electrodes exhibit small

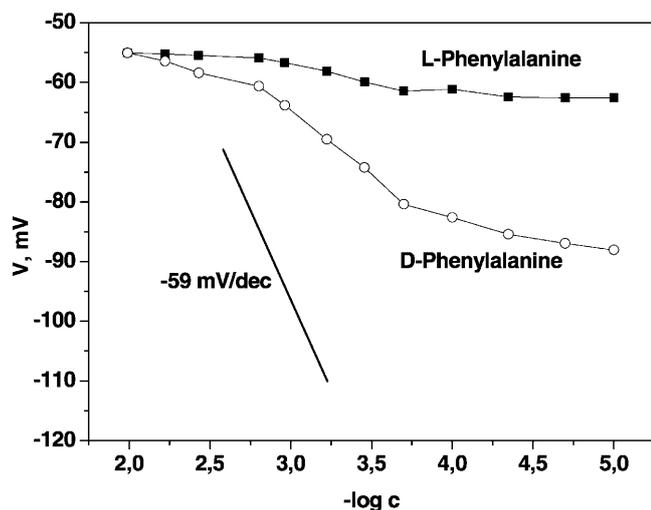


Fig. 6. Potentiometric response of a platinum disk electrode with electrodeposited polypyrrole doped with (*R*)-camphorsulfonate for enantiomers of phenylalanine [30].

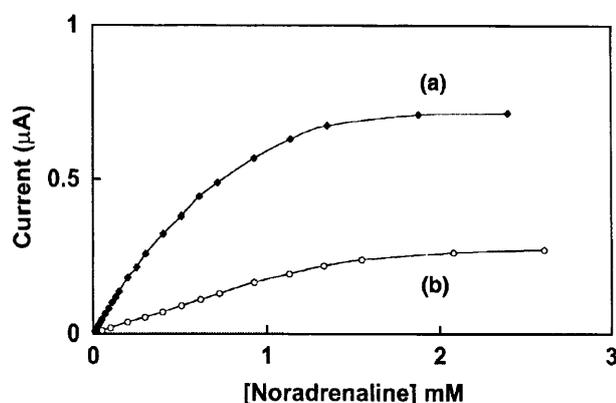


Fig. 7. Calibration curves obtained at electrode modified by six enzyme layers constructed by six cycles of deposition of avidin and then biotinylated PPO for D-norepinephrine (a), and L-norepinephrine (b) [31].

difference of signal for both enantiomers but it is not stable and decrease in time for consecutive measurements [30].

In the literature one can find also the interesting example of the design of biosensor with the layer of conducting polymer built from dicarbazol-biotin units. Biotinylated polyphenol oxidase coupled to biotinylated polymer by the use of avidin was applied as biocatalyst. The cross-linked biotinylated conducting polymer layer formed by cyclic voltammetry served as enantioselective barrier giving the possibility of selective determination of norepinephrine (Fig. 7) [31].

4. Chiral Electrochemical Biosensors

4.1. Enzymatic Biosensors

Enzymes are widely involved in metabolic processes of all living organisms taking part in reactions where a pure

enantiomer is a substrate. Enzymes can, however, often catalyze the reaction of both enantiomers and the crucial parameter is the kinetics of the reactions. In most cases the difference in kinetics is not sufficient to use the enzyme in the construction of enantioselective biosensors. Flavoproteins such as L- and D- amino acid oxidases are the enzymes that exhibit sufficient difference in velocity of biocatalytic reactions for both enantiomers. They catalyze the reaction of oxidation of L- or D- amino acids to corresponding iminoacids respectively, but they do not catalyze the reaction of opposite enantiomer. Literature contains a number of examples of the description of the biosensors with L-amino acid oxidase immobilized in graphite paste. That type of biosensor was used for example to enantioselective detection of (*S*)-captopril [32]. L-amino acid oxidase combined with horseradish peroxidase can be employed for detection of pipercolic acid [33]. The same enzymes combined also with glucose oxidase were used to detection of D- and L-methotrexate [34].

D-amino acid oxidase with horseradish peroxidase were employed in construction of biosensor selectively sensitive to enantiomers of methionine and leucine [35], and recently also for detection other D-amino acids [36]. In the latter example a screen-printed biosensor with D-amino acid oxidase with Prussian Blue as a mediator was reported for enantioselective determination of D-amino acids in natural samples of milk and juices. D-alanine is a product of degradation of bacterial cells. The presence of D-alanine in natural samples can be a marker of bacterial contamination. It can also give information on fermentation processes or can be an indicator of the presence of bacteria in milk from infected cows. Results of measurements for natural samples were compared with results of determination of D-alanine obtained by capillary electrophoresis with β -cyclodextrin as a chiral selector, and satisfactory correlation was obtained. Additionally, the attempt to modify a screen printed electrode by carbon nanotubes was reported, where carbon nanotubes were placed at the surface of electrode before deposition of mediator and enzyme immobilization. The modification causes significant extension of the biosensor life time; however a less stable response of electrode was reported.

Other examples of biosensors exhibiting the enantioselective response which were reported in the literature are the graphite porous electrode with lactate dehydrogenase for determination of enantiomers of lactic acid [37], and biosensor containing pairs of enzymes, esterase with α -chymotrypsin and esterase with lipase for enantioselective detection of (*S*)-phenylalanine methyl ester and β -hydroxy-acid esters, respectively [38]. Some examples of enantioselective biosensors are listed in Table 2.

All examples mentioned above present the enantioselectivity of biosensors originated from differences of catalytic properties for both enantiomers. Enzymes can be also differently inhibited by enantiomers. The ratio of inhibition of acetylcholinesterase by enantiomers of pesticide malaoxon depending on the origin of enzyme and temperature has a value greater than 1, and for enzyme originated from bovine

Table 2. Examples of enantioselective biosensors.

| Analyte | Enzyme | Enantioselectivity coefficient | Type of electrode | Reference |
|------------------------------|---------------------------------------------------------------|--------------------------------|-------------------|-----------|
| S-Captopril | L-Amino acid oxidase | 1.1×10^{-3} | Graphite paste | 32 |
| Pipecolic acid | L-Amino acid oxidase, Horseradish peroxidase | 1.5×10^{-4} | Graphite paste | 33 |
| D- and L-Methotrexate | L-Amino acid oxidase, glucose oxidase, Horseradish peroxidase | 8.1×10^{-4} | Graphite paste | 34 |
| Methionine, leucine | D-Amino acid oxidase, Horseradish peroxidase | Not determined | Graphite-Teflon | 35 |
| D-Amino acids | D-Amino acid oxidase | 1.1×10^{-2} | Screen-printed | 36 |
| Lactic acids | D-Lactate dehydrogenase or L-lactate dehydrogenase | 3.5×10^{-2} | Porous graphite | 37 |
| S-Phenylalanine methyl ester | Esterase, α -chymotrypsin | Not determined | pH-FET | 38 |
| β -Hydroxyacid esters | Esterase, lipase | Not determined | pH-FET | 38 |

erythrocytes it can be even 22.5 [39]. Therefore the possibility of potential adaptation of inhibition of the biological materials to construction of enantioselective biosensors can be expected, although any examples of such sensitivity can not be found in the literature, as yet.

4.2. Enantioselectivity of Immunochemical Interactions

Antibodies are another recognition biomolecules that find applications in separation methods as well as in chiral immunosensors. The example of chiral high performance liquid chromatography with antibody for separation of enantiomers of phenylalanine was reported, where anti-L-amino acid antibody was immobilized in the stationary phase [40]. The simple and inexpensive method of construction of the optical immunosensor that allows a quantitative analysis of chiral compounds up to 99.9% of enantiomeric excess was reported [41]. The mechanism of the sensor response was based on the competitive reaction between analyte and hepten analogue, derivatized by biotin for binding to reactive side of stereoselective antibody, immobilized on the surface of membrane. The complex of antibody with hepten analogue was detected by addition of peroxidase labelled avidin. The enzyme then converted a colorless substrate into an insoluble dye, and the color intensity was correlated with the amount of analyte in the sample.

Antibodies sensitive towards D-amino acids were used in immunosensing based on the surface plasmon resonance detection [42]. Streptavidin was covalently linked to carboxymethylated dextran on the gold surface. Isomers D- and L- of analyte were immobilized into separate channels of the sensor and the polyclonal rabbit antibody was injected. In the presence of compound D- antibodies were bound to the sensor which increased the signal from detector, while the L-isomer did not caused the binding. For the quantitative detection of amino acids by reported immunosensor, the antibody was added to the sample. The antibody was bound in the solution which resulted in decrease of signal from detector. The binding was specific for D-enantiomers, and

chiral, quantitative and extremely sensitive measurements for 3 pair of enantiomers were reported.

Another reported example of chiral immunosensors was based on antibodies bound on the surface of cantilevers [43]. The interaction of analyte with sensor caused the surface stress of the cantilevers which was a source of signal. The application of cantilevers allowed extremely sensitive direct detection of biological interactions. The antibodies to L-amino acids and the controlled immunoglobulin G were used. The enantioselectivity coefficients for examined enantiomers are 7.7 and 6.5 to tryptophan and phenylalanine, respectively. From the fact that immunosensors are specific and allow to discrimination of very small amounts of analyte one can expect the development of chiral electrochemical immunosensors in the nearest future.

4.3. Receptor Based Biosensors

As biomolecular recognition elements also receptors can be employed. A coulometric biosensor with glutamate

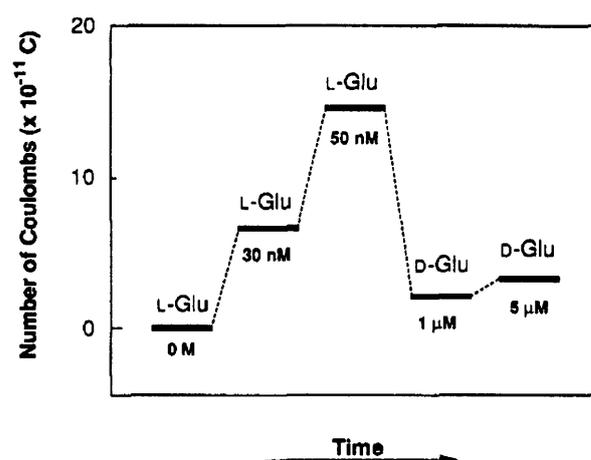


Fig. 8. Changes in the integrated channel current of ion-channel sensor with glutamate receptor protein immobilized in artificial bilayer lipid membrane. Measurements at potential 0.1 V for indicated concentrations of glutamate enantiomers [44].

receptor protein immobilized in artificial bilayer lipid membrane is a brand new type of biosensor, where two types of construction were presented [44]. The multi-channel type consisted of more than ten receptor units showed response as a composite of individual single-channel currents. The single-channel type of sensing membrane contained a sufficiently small number of receptor units and the response was observed as a series of pulse currents. Presented sensors possess the high selectivity for L-glutamate comparing with D-glutamate. Fig. 8 shows the response of multichannel biosensor to different concentrations of glutamate isomers.

5. Conclusions

Enantioselectivity of electrochemical sensors can be based on various principles of molecular recognition of target analytes. They may include mimicking biochemical mechanisms with synthetic selectors or they can employ natural biochemical recognition mechanisms. A broad knowledge about chiral selectors used in modern high-performance separation techniques can be potentially adapted for design of chiral electrochemical sensors. The possibility of the use of kinetic effects in chiral recognition is still interesting and not well known field for investigation with potential use in fast flow-injection measurements based on recording of transient signals. The main expected potential application area of chiral sensors seems to be first of all determination of unwanted enantiomer in monitoring of chiral purity of target compounds.

The chiral molecular recognition can be considered as the highest form of the molecular recognition, due to necessity of measuring of very small energetic differences between diastereomeric complexes. It is also one of the most difficult measurements. The basic difference in chiral sensing and chiral separations comes from the fact that in case of the direct chiral sensing devices there is only one single unitary process of separation that corresponds to one theoretical plate in chromatographic separations. On the other side, the enantioselectivity in high performance separation methods is a result of a multiple sequential separation unitary processes and has a large number of theoretical plates. This illustrates how serious challenge is a design of chiral electrochemical sensors and biosensors.

In most cases enantioselectivity obtained by electrochemical sensors is still not sufficient for practical applications in routine analysis to compete with high performance separation methods. The results obtained so far, however, seem to be very encouraging as chiral sensors might find potentially very wide applications and might be much more economic devices for chiral control in numerous cases. They are usually simpler in design, manufacturing and maintenance than high performance separation methods. Studies on chiral sensors might be also useful in search for new classes of chiral discrimination compounds that might be employed then for separation methods for design of new stationary phases for HPLC and as chiral selector in

capillary electrophoresis. Both natural antibodies and synthetic ones (molecularly imprinted polymers) are still not sufficiently explored for design of chiral sensors. A new stream of research in this field is design of electrodeposited polymers with enantioselective properties.

6. References

- [1] E. Rubenstein, W. A. Bonner, H. P. Noyes, G. S. Brown, *Nature* **1983**, *306*, 118.
- [2] L. D. Hutt, D. Glavin, J. L. Bada, R. A. Mathies *Anal. Chem.* **1999**, *71*, 4000.
- [3] A. M. Rouhi, *Chem. Eng. News* **2003**, *81*, 45.
- [4] I. J. Buerge, T. Poiger, M. D. Müller, H.-R. Buser, *Environ. Sci. Technol.* **2003**, *37*, 2668.
- [5] A. Miyazaki, T. Nakamura, M. Kawaradani, S. Marumo, *J. Agric. Food. Chem.* **1988**, *36*, 835.
- [6] L. J. Fono, D. L. Sedlak *Environ. Sci. Technol.* **2005**, *39*, 9244
- [7] K. A. Schug, W. Lindner, *J. Sep. Sci.* **2005**, *28*, 1932.
- [8] A. Berthod, *Anal. Chem.* **2006**, *78*, 2093.
- [9] W. Maruszak, M. Trojanowicz, M. Margasińska, H. Engelhardt, *J. Chromatogr. A* **2001**, *926*, 327.
- [10] S. Inagaki, J. Z. Min, T. Toyo'oka, *Anal. Chem.* **2008**, *80*, 1824.
- [11] A. P. Thoma, Z. Cimerman, U. Fiedler, D. Bedeković, M. Güggi, P. Jordan, K. May, E. Pretsch, V. Prelog, W. Simon, *Chimia* **1975**, *29*, 344.
- [12] H. Tsukube, H. Sohmiya, *Tetrahedron Lett.* **1990**, *31*, 7027.
- [13] Y. Yasaka, T. Yamamoto, K. Kimura, T. Shono, *Chem. Lett.* **1980**, 769.
- [14] K. Maruyama, H. Sohmiya, H. Tsukube, *J. Chem. Soc., Chem. Commun.* **1989**, 864.
- [15] K. Maruyama, H. Sohmiya, H. Tsukube, *Tetrahedron* **1992**, *48*, 805.
- [16] H. Tsukube, H. Sohmiya, *J. Org. Chem.* **1991**, *56*, 875.
- [17] M. Kaniewska, T. Sikora, R. Katak, M. Trojanowicz, *J. Biochem. Biophys. Meth.* **2008**, *70*, 1261.
- [18] H. Chibvongodze, K. Hayashi, K. Toko, *Sens. Mater.* **2001**, *13*, 99.
- [19] F. Scholz, R. Gulaboski *Faraday Discuss.* **2005**, *129*, 169.
- [20] J. Zhang, M. T. Albelda, Y. Liu, J. W. Canary, *Chirality* **2005**, *17*, 404.
- [21] H. Dai, *Acc. Chem. Res.* **2002**, *35*, 1035.
- [22] C. Girardet, D. Vardanega, F. Picaud, *Chem. Phys. Lett.* **2007**, *443*, 113.
- [23] H. Okuno, T. Kitano, H. Yakabe, M. Kishimoto, B. A. Deore, H. Siigi, T. Nagaoka, *Anal. Chem.* **2002**, *74*, 4184.
- [24] Y. Zhou, B. Yu, K. Levon, *Chem. Mater.* **2003**, *15*, 2774.
- [25] M. Lahav, A. B. Kharitonov, I. Willner, *Chem. Eur. J.* **2001**, *7*, 3992.
- [26] K. Maksymiuk, *Electroanalysis* **2006**, *18*, 1537.
- [27] Q. Pei, *Electrochim. Acta* **1992**, *37*, 1075.
- [28] V. Aboutanos, P. Akhtar, L. Kane-Maguair, G. Wallace, *Aust. J. Chem.* **2000**, *53*, 83.
- [29] J. Huang, V. M. Egan, H. Guo, J.-Y. Yoon, A. L. Briseno, I. E. Rauda, R. L. Garrell, C. M. Knobler, F. Zhou, R. B. Kaner, *Adv. Mater.* **2003**, *15*, 1158.
- [30] M. Kaniewska, M. Trojanowicz, unpublished results
- [31] S. Cosnier, A. Le Pellec, R. S. Marks, K. Périé, J.-P. Lellouche, *Electrochem. Commun.* **2003**, *5*, 973.
- [32] R. I. Stefan, C. Bala, H. Y. Aboul-Enein, *Sens. Actuators B.* **2003**, *92*, 228.
- [33] R. I. Stefan, R. M. Najem, J. F. van Staden, H. Y. Aboul-Enein, *Sens. Actuators B.* **2003**, *94*, 271.

- [34] R. I. Stefan, R. G. Bokretson, J. F. van Staden, H. Y. Aboul-Enein, *Talanta* **2003**, *60*, 983.
- [35] R. Dominguez, B. Serra, A. J. Reviejo, J. M. Pingarron *Anal. Biochem.* **2001**, *298*, 275
- [36] M. Wcislo, D. Compagnone, M. Trojanowicz, *Bioelectrochemistry* **2007**, *71*, 91.
- [37] J. Motonaka, Y. Katumoto, S. Ikeda, *Anal. Chim. Acta.* **1998**, *368*, 91.
- [38] T. Kullick, R. Ulber, H. H. Meyer, T. Scheper, K. Schlügerl, *Anal. Chim. Acta.* **1994**, *239*, 271.
- [39] O. P. Rodriguez, G. W. Muth, C. E. Berkman, K. Kim, C. M. Thompson, *Bull. Environ. Contam. Toxicol* **1997**, *58*, 171.
- [40] O. Hofstetter, H. Lindstrom, H. Hofstetter, *J. Chromatography A* **2004**, *1049*, 85.
- [41] O. Hofstetter, J. K. Hertweck, H. Hofstetter, *J. Biochem. Biophys. Meth.* **2005**, *63*, 91.
- [42] O. Hofstetter, H. Hofstetter, M. Wilchek, V. Schurig, B. S. Green, *Nature Biotechnol.* **1999**, *17*, 371.
- [43] P. Dutta, C. A. Tipple, N. V. Lavrik, P. G. Datskos, H. Hofstetter, O. Hofstetter, M. J. Sepaniak, *Anal. Chem.* **2003**, *75*, 2342.
- [44] H. Minami, M. Sugawara, K. Odashima, Y. Umezawa, *Anal. Chem* **1991**, *63*, 2787.



Marek Trojanowicz holds the position of a professor of chemistry in the Department of Chemistry, University of Warsaw, and also professor of Chemistry in Department of Analytical Chemistry in the Institute of Nuclear Chemistry and Technology in Warsaw. After obtaining a M.Sc. (1966), Ph.D. (1974) and D.Sc. (1981) degrees in the Department of Chemistry, University of Warsaw, in the field of Analytical Chemistry, he became head of the Laboratory for Flow Analysis and Chromatography in University of Warsaw (1989).

Professor Trojanowicz focuses his interests on flow analysis, chemical and biochemical sensors, liquid chromatography and capillary electrophoresis, application of chemical analysis in archaeometry, and use of ionizing radiation for decomposition of organic pollutants for environmental protection. He is author of almost 300 papers in scientific journals and two monographs on automation in analytical chemistry and flow injection analysis.



Marzena Kaniewska obtained a M.Sc. in 2003 in Department of Chemistry of University of Warsaw. She is currently a Ph.D. student in the Laboratory for Flow Analysis and Chromatography in the University of Warsaw. Her post-graduate work is focused on potentiometric enantioselective sensors, biosensors and separation techniques such as high performance liquid chromatography and capillary electrophoresis for selective determination of enantiomers.