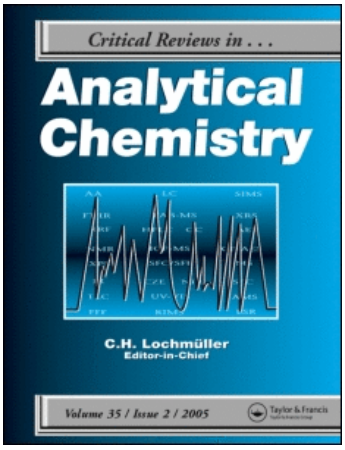

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

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Online publication date: 03 June 2010

To cite this Article Aboul-Enein, Hassan Y. and Stefan, Raluca-Ioana(1998) 'Enantioselective Sensors and Biosensors in the Analysis of Chiral Drugs', Critical Reviews in Analytical Chemistry, 28: 3, 259 — 266
To link to this Article: DOI: 10.1080/10408349891194199
URL: http://dx.doi.org/10.1080/10408349891194199

Critical Reviews in Analytical Chemistry, 28(3):259–266 (1998)

Enantioselective Sensors and Biosensors in the Analysis of Chiral Drugs

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ABSTRACT: The opportunity to use electrochemical sensors in enantioselective analysis is discussed. Chiral selectors such as crown ethers, cyclodextrins, and enzymes had been used for both chromatographic enantioseparation methods and enantioselective electrochemical sensors and biosensors. New possibilities of membranes construction, like molecular imprinting-based enantioselectivity sensors are also discussed. The advantages of use enantioselective sensors and biosensors over separation methods (HPLC, MEKC, CZE) are presented. The reliability of chiral drugs enantioselective assay is improved by using enantioselective sensors and biosensors.

KEY WORDS: enantioselective analysis, chiral drugs, electrochemical sensors, electrochemical biosensors.

I. INTRODUCTION

Enantioselective analysis of drugs requires reliable methods. The sampling process can introduce a lot of uncertainties, especially when a separation method for the enantiomers, using nonadequate chiral selectors, is proposed.[1] There are several separation methods, such as high-performance liquid chromatography (HPLC) or capillary zone electrophoresis (CZE), applied for enantiomers discrimination. The main problem with the enantioselective analysis is the detection system. By assuring an enantioselective detection system, the separation step can be canceled from the sampling process. One of the most reliable enantioselective detection systems are enantioselective membrane electrodes and electrochemical biosensors. The enantioselective membrane electrodes contained in the membrane as chiral selectors crown ethers and α, β, and γ cyclodextrins had been used. However, the best enantioselective electrochemical biosensors are the amperometric ones due to the coupling between the enantioselectivity of the enzyme and the sensitivity of the amperometric devices.

For the enantioselective analysis using electrochemical sensors and biosensors, the sampling process means only sample dissolution in water or buffer. This fact rendered the analytical information of high precision,

especially if the chiral selector formed a high stability complex with one of the enantiomer.

II. ENANTIOMERS SEPARATION METHODS USED IN SAMPLING PROCESS

One of the main problems of the enantioseparation technique is the selection of the chiral selector. The first class of compounds used as chiral selectors is the crown ethers. This class of organic selectors did not resolve the enantiomeric pairs adequately and the accuracy of the separation was poor.

Cyclodextrins assure the best reliability of the separation methods due to their better interactions with one of the enantiomers. The best chiral selectors in the cyclodextrin class of compounds is β-cyclodextrin and its derivatives. High-performance liquid chromatography (HPLC), capillary zone electrophoresis (CZE), and micellular electrokinetic chromatography (MEKC) are frequently used separation techniques for enantiomeric resolution.

A. HPLC

To predict the chiral chromatographic separation, a multivariate regression analysis is proposed, combined with multilayer feed-forward neural networks trained with error back-propagation, to model the enantioselective chromatographic retention behavior of a series of aromatic acids and amides as a function of nonempirical solute descriptors.[2] Combinations of charge transfer, electrostatic, lipophilic, and dipole interactions, identified by multivariate regression, were found to describe retention and enantioselectivity, with highly predictive models being generated by the training of back-propagation neural networks.

Macrocyclic glycopeptides antibiotics are the newest class of chiral selectors for the chromatographic and electrophoretic separation of enantiomers. The two most successful macrocylic antibiotics used for separations are vancomycin and teicoplanin.[3,4] Teicoplanin is a stable glycopeptide that assures, as chiral selector, the integrity of the chiral stationary phase (CSP). Teicoplanin chiral stationary phase appears to have excellent enantioselectivity for native amino acids, peptides, α-hydroxycarboxylic acids, and a variety of neutral analytes including cyclic amides and amines.[5]

The best chiral selectors for enantiomers separation are cyclodextrins. Taking into account that for chiral drugs, the reliability must have the maximum value, and that the enantiomers separation is one of the most difficult problems in chemistry, and is of great importance in fields such as chiral synthesis, kinetics, biochemistry, pharmacology, and medicine. The use of cyclodextrins as chiral selectors improve the quality and reliability of the separation when HPLC is used as a separation technique. Cyclodextrins and modified cyclodextrins can be used as either the chiral stationary phases, as chiral mobile phase additives, or as chiral counterions. Also, cyclodextrins stationary phases are quite versatile when compared with other chiral stationary phases as they have been used in three different chromatographic modes (normal, reversed, and polar organic). For this reason, they have been termed a multimodal chiral stationary phase.[6]

B. MEKC

By using micellular electrokinetic chromatography (MEKC) with derivatized cyclodextrins as chiral selectors, the quality of the analytical information is improved. Resolution and migration time were found

to be strongly dependent on the cyclodextrins concentration of the background electrolyte and to a lesser extent on the concentration of the analyte.[7]

C. CZE

For chiral drugs analysis, the CZE using chiral selectors is the best analytical technique due to the fact that it does not need high solvent quantities and is a more reliable separation method.

By using cyclodextrins and their derivatives in CZE, high-quality analytical information is achieved.[8-14] Sulfobutyl ether-β-cyclodextrin as a chiral selector has a large countercurrent mobility, making it inherently advantageous as selectors when compared with neutral cyclodextrins;[15] it is an excellent chiral selector for a variety of structurally diverse drug compounds.

III. INSIGHTS INTO THE MECHANISMS OF ENANTIOSELECTIVITY BY CYCLODEXTRINS

To understand the mechanisms of enantioselectivity by cyclodextrins, ^{1}H NMR and ^{13}C NMR studies on chiral recognition have been examined.[16-19] The resolution of chromatographic methods is given by the apparent binding constants of enantiomers and by the chemical shift differences at saturation using ^{1}H NMR and ^{13}C NMR spectroscopies.[13]

Chankvetadze et al.[18] studied the effect of the nature and position of substituents of the chiral solute on the resolution using ^{13}C NMR. Sulfonated β-cyclo dextrins such as sulfobutyl and sulfoethyl ethers of β-cyclodextrins permit adequate enantio-separations at rather lower concentrations as chiral selectors in comparison with carboxymethyl-β-cyclodextrin and native β-cyclodextrin.

Bodenhöfer proposed a model for the chiral discrimination process by superimposing preferential and nonpreferential sorption mechanisms.[19] The model is applicable to any matrix containing preferential sorption sites. The "molecular recognition" effect can be observed by simply dissolving the supramolecular unit in an isotropic polymer.

IV. MOLECULAR IMPRINTING-BASED ENANTIOSELECTIVITY SENSORS

The molecular imprinting technology leads to highly stable synthetic polymers that possess selective molecular recognition properties because of recognition sites within the polymer matrix that are complementary to the analyte in the shape and positioning of functional groups. Some of these polymers have high selectivities and affinity constants comparable with naturally occurring recognition systems such as monoclonal antibodies or receptors, which make them especially suitable as constituents in chemical (biomimetric) sensors for analytical chemistry.

Chemical sensors provide an analytically powerful and inexpensive alternative to conventional technologies by enabling the identification of a target molecule in the presence of numerous interfering species. Methodologies have been developed for many different target species, including gaseous substances such as anesthetics, respiratory byproducts, compounds of biological importance (glucose, urea), hormones, steroids, drugs of abuse, and also drugs enantiomers discrimination.[20,21] A chemical sensor selectively recognizes a target molecule in a complex matrix and generates an output signal using a transducer that correlates to the concentration of the analyte.[22,23]

Rapid developments in electronics have led to microprocessors that are suitable for use in chemical sensors. Such microproces-

sors offer advanced signal-processing capability, and integrating the controller with the transducer could minimize noise and result in improved sensor performance. The problem of long response time (15 to 60 min) could be minimized by optimizing the kinetics and selectivity of polymers.

V. PIEZOELECTRIC SENSORS

The enantioselective analysis of drugs through sensors having piezoelectric devices as transducers assure high sensitivity.[25,26]

The piezoelectric sensor is usually coated with β-cyclodextrin or with a derivatives of β-cyclodextrin. The main advantage of this type of enantiomer selector is the possibility to be used for continuous on-line monitoring of the enantiomers.[26] Furthermore, α-cyclodextrins and β-cyclodextrins were also tested as coatings for piezoelectric sensors for chiral drug separations.[26]

VI. ENANTIOSELECTIVE MEMBRANE ELECTRODES

Enantioselective membrane electrodes are one of the best elecrochemical sensors used for chiral drug assay. The construction of these electrochemical sensors must take into account the stability constant of the complex formed between enantiomer and chiral selector. The main problem is the choice of the best chiral selector; that will assure the enantioselectivity of the analysis.

Crown ethers proved to be effective chiral selectors for basic drugs containing a primary amino group, including amino acids and derivatives.[27–30]

Horváth et al.[31] recently reported an enantioselective assay of ephedrinium ions using potentiometric sensors based on crown ethers.

The chiral drug discrimination can also be also achieved by using cyclodextrins and the derivatives of cyclodextrins in membrane construction.[32] The enantioselectivity of the potentiometric sensor is improved by using these chiral selectors which are used for PVC (polyvinylchloride) matrix membrane construction. Functionalized α, β, and γ-cyclodextrins recognize a wide spectrum of onium ions of pharmaceutical importance.[32]

To assure the high reliability of membrane construction, liquid membranes impregnated with cyclodextrins must be used. A reliable construction of membrane electrodes for chiral drugs discrimination is the first step in the their accreditation. Now, this problem is solved by using biosensors based on immobilization of enzymes in carbon paste electrodes.

VII. ENANTIOSELECTIVE AMPEROMETRIC BIOSENSORS

The enantioselective amperometric biosensors assure the best enantioselectivity for chiral drugs discrimination. The main disadvantage is their short life time. To improve the life time, a novel design for an extended-life amperometric enzyme electrode is proposed.[33] The enzyme electrode is made by arranging the three components of a biosensor (an electrode material, an enzyme, and a stabilizer) on a shapable electroconductive (SEC) film (a polyanion-doped polypyrrole film) surface by a layer-after-layer approach. First, a thin and compact platinum black layer is prepared onto the SEC film by the heat-press method. Then, an ultrathin layer of enzyme (e.g., L-amino acid oxidase) is casted on the platinized SEC film, and after drying a thin gelatin layer is prepared on the enzyme-casted SEC film. Finally, the dried layer assembly is cross-linked by exposing it to a diluted substrate solution for a very short time. The sensor shows a shelf life of about 2 years when it is stored dry in a freezer at –18°C and about 1 year when it is stored at room temperature.

Of main importance for enantio-selective analysis are amperometric biosensors based on enzymes immobilized on carbon paste electrodes. The construction of this type of biosensors is reliable. Amperometric biosensors are shown for the first time to withstand prolonged high-temperature (>50°C) stress. Nearly full activity of some enzymes can be retained over periods of up 4 months of thermal stress at 60 to 80°C. Dramatic improvements in the thermostability are observed for L-amino acid oxidase. Such resistance to heat-induced denaturation is attributed to the conformational rigidity to these biocatalysts within the highly hydrophobic (mineral oil or silicon grease) pasting liquid. Besides their implications for electrochemical biosensors, such observations should lead to a new generation of thermoresistant enzyme reactors based on nonpolar semisolid supports.[34]

Several chiral drugs used for the treatment of hypertention are derivatives of amino acids. Biosensors based on L-amino acid oxidase (L-AAOD) and D-amino acid oxidase (D-AAOD) are proposed for L- and D-aminoacids assay.[35,36]

An amperometric biosensor based on L-AAOD in graphite paste as support is proposed for many chiral drug assay. By using this biosensor a high enantioselectivity level was assured — this fact made it reliable to use the amperometric biosensors for the enantio-purity analysis of active substance as well as for its assay in the pharmaceutical compounds. The limit of detection is low level μmol/L –pmol/L due to the high selectivity of the amperometric sensor (Ag/AgCl) used. This type of amperometric biosensor was tested for several angiotensin-converting enzyme (ACE) inhibitors: S-captopril,[37] S-enalapril, S-ramipril,[38] S-cilazapril, S-trandolapril, S-pentopril,[39] and S-perindopril.[40]

For the enantiopurity assay of (+)-3,3′,5-triiodo-L-thyronine (L-T_3) and L-thyroxine (L-T_4) which are used in treatment of hypothyroidism, the same construction for the amperometric biosensors is proposed [41].

VIII. ADVANTAGES AND DISADVANTAGES OF USING ELECTROCHEMICAL SENSORS AND BIOSENSORS VERSUS ENANTIOSEPARATION METHODS FOR THE ENANTIOSELECTIVE ANALYSIS

The enantioseparation by HPLC, CZE, or MEKC is very laborious and generally expensive and there are a lot of problems concerning the selective retention of one of the enantiomers on the column. It is not enough to obtain a high selective chiral selector. The working conditions must be improved for every enantiomeric pair.

Cyclodextrins are one of the best chiral selectors especially for chiral drug discrimination and were used for membrane electrode construction. The main advantages of the cyclodextrin-impregnated electrodes are the high reproducibility, the rapidity, and simplicity of the analysis. However, one of the greatest disadvantages of these constructed membrane electrodes is the construction nonreproducibility. The proposed enantio-selective membrane electrodes are based on PVC matrix membranes. The reproducibility of construction problem was solved by using the amperometric biosensors based on a modified graphite paste. They assure at the same time a high reproducibility of the assays, a high sensitivity and selectivity levels, and the best limits of detection. The main disadvantage of amperometric biosensors is their short life time.

IX. CONCLUSIONS

The enantioselective analysis of chiral drugs needs reliable methods. Separation methods such as HPLC can be used for the

separation of the enantiomers in order to obtain a high enantiopurity of chiral drug, but to assay its enantiopurity the use of an electrochemical sensor or biosensor is simpler, more reliable, and cost effective than HPLC. Through using electrochemical sensors and biosensors, the reliability of the enantiomers assay is improved. The high reproducibility for sensors construction was achieved by ampero-metric biosensors based on modified graphite paste. To improve the quality of plastic membranes, a lot of studies concerning the preparation and characterization of composite polymers were made that can serve for enantiomers discrimination in chiral drug analysis.[42]

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