Chapter 11

Conclusions

The application of electrochemical sensors and biosensors for enantioanalysis of compounds of biological importance (clinical analysis) is increasingly important. Such real-time measurements are highly desirable in intensive-care unit and surgery. The availability of more portable, precise and reliable measurements devices will inevitably be a further stimulus to the demands of the medical research establishment. The researchers involved in the development of biosensors and electrodes are approached for devices of high accuracy and precision for use in clinical and fundamental physiological research. The requirements for an ideal *in vivo* probe are high sensitivity, high selectivity, fast response time, long-term stability, and independence of variations in the biological sample (e.g., pH), biocompatibility, sterilizability and small size.

The aim of the thesis was to design new biosensors and enantioselective, potentiometric membrane electrodes; for the enantioanalysis of metabolites that are markers for different diseases. Three types of electrochemical techniques have been used for enantiomer recognition: amperometry, differential pulse voltammetry and potentiometry. L- and D-enantiomers of pipecolic acid, fucose, glyceric acid, 2-hydroxyglutaric acid, vesamicol and lysine are markers for different diseases. Therefore an enantioanalysis of these substances in serum or/and in urine samples is necessary. Pipecolic acid was analyzed using amperometric and potentiometric methods. Amperometric biosensor based on diamond and carbon pastes impregnated with L- and/or D-AAOD have been designed for the enantioanalysis of pipecolic acid in serum samples. The biosensors based on carbon

paste were utilized as detectors in sequential injection system. The amperometric biosensors described have excellent features in the assay of enantiomers in biological fluids.

The reliability obtained for the electrical properties of single-crystal diamond is encouraging for research in the electrochemical sensors based on the monocrystalline diamond. Monocrystalline diamond (natural diamond, synthetic-1 and synthetic-2) pastes were utilized for the design of electrochemical electrodes for the analysis of L- and D-pipecolic acids and L- and D-fucose using differential pulse voltammetry. An amperometric biosensor based on diamond paste impregnated with L-lysine oxidase has been designed and investigated for the assay of L-lysine in blood samples. The biosensor exhibits high reliability, low limits of detection, good stability and reproducibility due to the use of diamond paste. One of the features of the biosensor is its utilization for *in vivo* assay of L-lysine.

The results obtained in this work revealed the need to employ diamond paste based electrodes to diagnose the abnormal metabolites in biological fluids. One of the features is utilization of diamond paste electrodes for *in vivo* continuous monitoring of chiral molecules in biological fluids. Amperometric biosensor based on diamond paste impregnated with L-AAOD exhibited the lowest limit of detection for the analysis of L-pipecolic acid; for the assay of D-pipecolic acid, the lowest limit of detection was recorded when the amperometric biosensor based on carbon paste impregnated with D-AAOD and HRP was used. For L-fucose assay the amperometric electrode based on

natural diamond exhibited the lowest limit of detection when NaCl was used as electrolyte, while for the assay of D-fucose, amperometric electrode based on diamond synthetic-2 has the lowest limit of detection when $NaNO_3$ was used as electrolyte.

Enantioselective, potentiometric membrane electrodes based on carbon paste impregnated with maltodextrins (I, II, and II), cyclodextrins (α-CD, γ-CD β-CD, and 2-hydroxy-3-trimethylammoniopropyl-β-CD) and macrocyclic antibiotics (vancomycin, teicoplanin and teicoplanin modified with acetonitrile) have been designed for the analysis of the enantiomers of glyceric acid, 2-hydroxyglutaric acid and L-vesamicol. The proposed enantioselective, potentiometric membranes electrodes have excellent features in the real time enantioselective analyses of glyceric acid in biological fluids, e.g., serum and urine samples. The lowest limit of detection of L-glyceric acid determination was obtained using EPME based on α-cyclodextrin, while EPME based on 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin exhibited the lowest limit of detection for the assay of D-glyceric acid. EPME based on maltodextrin (I) has the lowest limit of detection for the analysis of L-2-hydroxyglutaric acid and EPME based on teicoplanin modified with acetonitrile exhibited the lowest limit of detection for the assay of D-2-hydroxyglutaric acid. EPMEs based on maltodextrins have only worked for the assay of L-2-HGA, while EPMEs based on antibiotics have worked only for the assay of D-2-HGA. In the determination of L-vesamicol, EPME based on β-CD exhibited the lowest limit of detection.

The construction of the electrodes is simple, fast and reproducible. One of the main advantages of the proposed methods is that the serum and urine samples did only need to

be buffered before the assay of any of the enantiomers and that makes the method simple, fast and highly reliable.

One can conclude, that the proposed amperometric (bio)sensors and enantioselective, potentiometric membrane electrodes can be directly used in clinical analysis. Application of these biosensors and electrodes for *in vivo* enantioanalysis is very important for fast and continuous assay of abnormal metabolites during surgery or in the intensive care units.