# Enantioselective sensors and biosensors for clinical analysis

by

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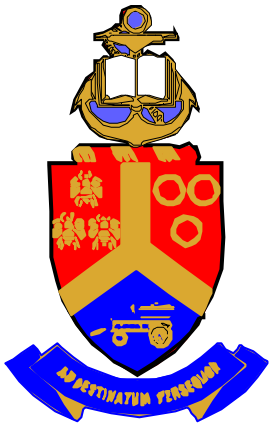

# Dedications

*In deep appreciations, I would like to dedicate this work to: my lovely wife, Grace, for her patience in the durable era of the country state, and for her extensive encouragement and incredible endurance; my children, Aysha, Ameera, Khaldon and Mohamad of being apart of them, promise I will compensate you all the times you missed me; my parents, Mahmoud and Aysha, for their blessings and pray for my success; my brother, Moien, for his full support to my dreams, dreams become true; and my brothers and sisters for continuous support.*

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## SYNOPSIS

The enantioanalysis of compounds of biological importance with a chiral moiety is very important because each enantiomer is a marker for a different disease. Accordingly, very reliable methods of enantioanalysis should be employed for the correct diagnosis of the diseases. The utilization of amperometric biosensors and enantioselective, potentiometric membrane electrodes made the assay of a single enantiomer faster, easier and more reliable if one compare with the chromatographic techniques which are widely proposed for this kind of analyses.

Monocrystalline diamond was proposed as matrix for amperometric electrodes and amperometric biosensors design. The advantages of using such material for electrode design are: (a) lower background currents and noise signals, which lead to improve S/B and S/N ratios, and lower detection

limits; (b) good electrochemical activity (pre-treatment is not necessary); (c) wide electrochemical potential window in aqueous media; (d) very low capacitance; (e) extreme electrochemical stability; and (f) high reproducibility of analytical information.

The design selected for the electrodes is simple, fast and reproducible. The carbon or diamond powders were mixed with paraffine oil to give the carbon or diamond paste which can act alone as electroactive material in the electrodes or it can be modified with a chiral selector (e.g., cyclodextrins, maltodextrins or antibiotics) or enzyme (e.g., L(D)-aminoacid oxidase, L-lysine oxidase). The results obtained by employing the amperometric electrodes and biosensors and the enantioselective, potentiometric membrane electrodes proved a high sensitivity, selectivity, accuracy and high reliability. These characteristics made them suitable to be used for the enantioanalysis of different compounds of biological importance (e.g., pipecolic acid, glyceric acid, 2-hydroxyglyceric acid, fucose, L-vesamicol and L-lysine) in serume and/or urine samples.

The features of the proposed enantioselective, amperometric and potentiometric electrodes proposed in this thesis are their utilization for *in vivo* measurements and as detectors in flow systems (flow injection analysis or/and sequential injection analysis). This will simplify the enantioanalysis and will improve considerable the reliability of the analytical imformation favorazing a fast and accurate diagnosis of the diseases associated with the marker determined.

# Enantioselective sensors en biosensors vir kliniese analise

**deur**

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## SAMEVATTING

Die enantioanalise van verbindings van biologiese belang met 'n chirale deel is baie belangrik omdat elke enantiomeer 'n merker vir verskillende siektes is. Gevolglik moet uiters betroubare enantioanalitiese metodes gebruik word vir die korrekte diagnose van die siektes. Die aanwending van amperometriese biosensors en enantioselektiewe, potensiometriese membraanelektrodes maak die essai van 'n enkele enantiomeer vinniger, makliker en meer betroubaar as dit met chromatografiese tegnieke, wat algemeen vir die tipe analises voorgestel word, vergelyk word.

Monokristallyne diamant is as matrys vir die ontwerp van amperometriese elektrodes en amperometriese biosensors voorgestel. Die voordele om sulke materiaal vir elektrode-ontwerp te gebruik, is: (a) laer agtergrondstroom en geraas seine, wat lei tot verbeterde S/B en S/N

verhoudings, en laer deteksielimiete; (b) goeie elektrochemiese aktiwiteite (voorafbehandeling is nie nodig nie); (c) 'n wye elektrochemiese potensiaalvenster in waterige media; (d) baie lae kapasitansie; (e) ekstreem elektrochemiese stabiliteit; en (f) hoë reproduseerbaarheid van analitiese inligting.

Die ontwerp van die elektrodes is eenvoudig, vinnig en reproduseerbaar. Die koolstof - of diamantpoeiers word met paraffienolie gemeng om 'n koolstof of diamantpasta te gee wat alleen as elektroaktiewe materiaal in die elektrodes kan optree of gemodifiseer kan word met 'n chiraal selekteerder (byvoorbeeld, siklodekstriene, maltodekstriene of antibiotika) of ensiem (byvoorbeeld, L(D)-aminosuur oksidase, L-lisien oksidase). Die resultate wat met die amperometriese elektrodes en biosensors en die enantioselektiewe, potensiometriese membraanelektrodes verkry word toon 'n hoë sensitiwiteit, selektiwiteit, akkuraatheid en betroubaarheid. Hierdie kenmerke maak hulle uiters geskik in die gebruik van die enantioanalise van verskillende verbindings wat biologies belangrik is (byvoorbeeld, pipekoliensuur, gliseriensuur, 2-hidroksigliseriensuur, fukose, L-vesamikol en L-lisien) in serum en/of urienmonsters.

Die uitstaande kenmerke van die voorgestelde enantioselektiewe, amperometriese en potensiometriese elektrodes wat in hierdie tesis voorgestel word, is hulle toepassing vir *in vivo* metings en as detektore in vloeisisteme (vloei-inspuitanalise of/en sekwensiele inspuitanalise). Dit sal enantioanalise vereenvoudig en die betroubaarheid van analitiese inligting heelwat verbeter wat vinnige en akkurate diagnose van siektes wat met die merker bepaal word, bevoordeel.

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# Introduction

The availability of continuous monitoring of metabolic substances is very important in the intensive care units and can be a great aid to patients care. Chirality has been reported as an important issue for different compounds of biological importance. The presence of chiral compounds in human fluids (serum, urine, spinal fluids) as normal metabolites of human metabolism or drug metabolism give the vitality for monitoring levels of these molecules in biofluids. The existence of higher or lower levels of these specific molecules is a marker indicator of human body abnormalities. The normal concentration level changes of molecules in humans mostly referred to the deficiency of some enzymes. Amino acids, carbohydrates and urinary organic acids are excreted in human and their changes can cause different types of diseases such as inborn metabolic disorders and cancer.

Many diseases are caused by substances existing in enantiomeric form and each enantiomer causes a different disease, so to diagnose these illnesses it is very important to find an analytical method that can discriminate between the L- and D-enantiomers. These methods will be very helpful and should exhibit reliable analytical information, fast analysis and could be applied for the continuous monitoring of the enantiomers in biological fluids.

Molecular recognition plays the main role in chiral discrimination around an asymmetric center. Due to the importance of enantiomeric discrimination, there has been substantial need for the discovery of substances used in the enantioanalysis for the diagnosis,

preventation, and treatment of human diseases. These developments have resulted in increased demand for sensitive and specific analytical methods.

The instrumental methods for quantitation which are most commonly used in clinical enantioanalysis are structural analysis, chromatographic and electrochemical methods. Electrochemical sensors are a very good alternative for structural analysis because of their high reliability that is given by high precision, high reproducibility and rapidity. The precision obtained using electrochemical sensors is higher than that obtained using chromatographic methods due to the fact that electrochemical sensors can be used directly for measurements of the compounds in solution.

The aim of this thesis is to construct reliable enantioselective electrodes (amperometric electrodes, amperometric biosensors, enantioselective, potentiometric membrane electrodes) to be applied in diagnosis. Carbon and diamond pastes are proposed as matrices for the sensors' design. Chiral recognition principles based on selected binding as well as on catalyst selectivity must be considered for the selection of the best chiral selector or enzyme.

Differential pulse voltammetry, chronoamperometry and potentiometry can be used for the direct assay of enantiomers in the serum and/or urine samples. An analysis of the performances of the electrodes has shown that the selection of the type of the electrode and matrix of its membrane should be done in accordance with the complexity of the structure of the enantiomer to be determined. Also, the analytical information obtained in

the enantioanalysis using electrochemical sensors is more reliable than that obtained using conventional or chromatographic methods.